International Union of Basic and Clinical Pharmacology. CX. Classification of Receptors for 5-hydroxytryptamine; Pharmacology and Function


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ABBREVIATIONS:

AA, arachidonic acid; AC, adenyl cyclase; AChE, Acetylcholinesterase; AD, Alzheimer disease; ADAR, RNA-specific adenosine deaminase; ADHD, attention deficit hyperactivity disorder; AIM, abnormal involuntary movement; AIWG, antipsychotic-induced weight gain; AngII, Angiotensin II; AP-1, activator protein 1; AP-2, activator protein 2; AP-MS, affinity purification-mass spectrometry; APP, amyloid precursor protein; ARF1, ADP-ribosylation factor 1; ASD, autism spectrum disorder; AT1, angiotensin II receptor type 1; Aβ, amyloid-β; BAC, bacterial artificial chromosome; BDNF, brain-derived neurotrophic factor; BNP, brain natriuretic peptide; BPAD, bipolar affective disorder; BSS, behavioral satiety sequence; CCI, chronic constriction injury; CD, cluster of differentiation; CDK5, cyclin-dependent kinase 5; cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; CHO, Chinese Hamster Ovary; CIPP, channel-interacting PDZ protein; eNOS, constitutive nitric oxide synthase; CNS, central nervous system; CpG, methyl-cytosine-phosphate-guanine; CRMP, collapsin response mediator protein; 5-CT, 5-carboxamidotryptamine; CXCL, chemokine ligand; CYP, cytochrome P450; DA, dopamine; DAG, diacylglycerol; DARPP32, dopamine and cAMP regulated phosphoprotein; DHE, dihydroergotamine; DOB, 2,5-Dimethoxy-4-methyltryptamine; DOCA, deoxycorticosterone; DOI, 2,5-Dimethoxy-4-iodoamphetamine; DRG, dorsal root ganglion; DRN, dorsal raphe nucleus; EB, embryoid body; EC, enterochromaffin cells; ECD, extracellular domain; ECL2, extracellular loop 2; EEG, electroencephalography; ENS, enteric nervous system; EPS, extrapyramidal side effects; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; E3, ubiquitine E3 ligase; FDA, Food and Drug Administration; Fmr1, fragile X mental retardation 1; FST, forced swim test; FXS, fragile X syndrome; GAGs, GPCR-associated sorting proteins; GEF, guanine nucleotide exchange factor; GH, growth hormone; GI, gastrointestinal; GIP, glucose-dependent insulinotropic peptide; GIP, GPCR interacting protein; GIRQ, G protein–coupled inwardly rectifying potassium channel; GLIC, gloeobacter ligand-gated ion channel; gp5-h15, guinea pig 5-h15 receptor; GPCR, G protein–coupled receptor; GR, glucocorticoid receptor; GSK3, glycogen synthase kinase-3; HB-EGF, heparin-binding EGF-like growth factor; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; 5-HIAA, 5-hydroxyindole acetic acid; HSC, hepatic stellate cells; 5-HT, 5-hydroxytryptamine; 5-HTBP, 5-HT binding protein; IBD, irritable bowel syndrome; IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; ICC, interstitial cells of Cajal; ICD, intracellular domain; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; INN, informal name; IP3, inositol-1,4,5-triphosphate; ISHH, in situ hybridization histochemistry; IUPHAR, International Union of Basic and Clinical Pharmacology; Jab, Jun activation domain–binding protein; JAK, Janus kinase; JCV, John Cunningham virus/polymavirus; KO, knockout; L-DOPA, levodopa; LD, L-DOPA–induced dyskinesia; LNk, ligand of numb protein X; LPS, lipopolysaccharide; LSD, lysergic acid diethylamide; LTD, long-term depression; M1, proinflammatory macrophage; M2, anti-inflammatory macrophage; MAG2, membrane-associated guanylate kinase with inverted domain structure 2; Man, mannoses; MAP, microtubule-associated protein; MAP1B-LC1, light chain 1 subunit of MAP1B protein; MAPK, mitogen-activated protein kinase; mCPP, M-chlorophenylpiperazine; MDA, 3,4-methylenedioxy methamphetamine; MDMA, 3,4-methylenedioxyamphetamine; McCP2, methyl-Cpg-binding protein 2; miRNA, microRNA; MK, mGluR5; MMP, matrix metalloproteinase; mPFC, medial prefrontal cortex; MPP, MAGUK p55 subfamily member; MPP3, MAGUK p55 subfamily member 3; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; MUPP1, multi-PDZ domain protein 1; NAc, nucleus accumbens; ND2, adaptor protein NADH dehydrogenase subunit 2; NF-αB, nuclear factor-αB; NHE-1, 1 sodium-proton exchanger; NHERF, Na+/H+ exchanger regulatory factor; NMDA, N-methyl-D-aspartic acid; NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal NOS; NOX, NADPH oxidase; NR1, NMDA receptor subunit 1; NREM, non–rapid eye movement sleep; OCD, obsessive-compulsive disorder; 8-OH DPAT, (±)-8-Hydroxy-2-(diR)-proplyaminotetralin; P2, purinergic 2 receptors; PAH, pulmonary arterial hypertension; PAM, positive allosteric modulator; PCD, phenylcyclohexyl carbamate; PCR, polymerase chain reaction; PD, Parkinson disease; PDE, phosphodiesterase; PDGFR, platelet-derived growth factor receptor; PDZ, multidomain PDZ protein; PDZ10, MUPP1 PDZ domain 10; PDZ, PSD-95/Disc large/Zonula occludens; pEC50, negative log of 50% effective concentration; PFT, protein expression; PFC, prefrontal cortex; P3K, phosphatidylinositol-3 kinase; PKA, protein kinase A; PKC, protein kinase C; PL-A, phospholipase A; PL-AC, phospholipase C; PLC, phospholipase D; POMC, pre-pro-opiomelanocortin; POV, postoperative vomiting; PPAR, peroxisome proliferator–activated receptors; PTP, PR, progressive ratio; PSD, post-synaptic marker; PSD-95, postsynaptic density-95; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PXX, polyhomeotic; qPCR, quantitative polymerase chain reaction; REM, rapid eye movement sleep; Rheb, Ras homolog enriched in brain; RGS, regulator of G protein signaling; RNS, reactive oxygen species; RSK2, R90 ribosomal S6 kinase 2; RT-PCR, reverse transcriptase polymerase chain reaction; RPP, rapid eye movement sleep; RRE, ras homolog enriched in brain; RGS, regulator of G protein signaling; ROS, reactive oxygen species; RSK2, R90 ribosomal S6 kinase 2; RT-PCR, reverse transcriptase polymerase chain reaction; RRT, Rett syndrome; SAP97, synapse-associated protein 97; SAPP, soluble amyloid precursor protein; SARR, structure activity relationship; SCFAs, short-chain fatty acids; SERT, serotonin transporter; Sf9, spodoptera frugiperda 9 cells; siRNA, small-interfering RNA; SN, substantia nigra; snRNA, small nuclear RNA; SNS, single-nucleotide polymorphism; SNX, sorting nexin family member; SRE, serum response element; SRI, serotonin reuptake inhibitor; SSSRI, selective serotonin reuptake inhibitor; STAT, signal transducer and activator of transcription; SW, slow wave; TASK-1, acid-sensitive potassium channel protein-1; TGF-β1, transforming growth factor beta 1; Tg, T-helper cells; THC, Δ9-tetrahydrocannabinol; TLR, Toll-like receptors; TMD, transmembrane domain; TNAP, tissue nonspecific alkaline phosphatase; TNG, tumor necrosis factor; TPH, tryptophan hydroxylase; TSC, tuberous sclerosis; TST, tail suspension test; VTA, ventral tegmental area; WAVE-1, Wiskott-Aldrich syndrome protein family verprolin homologous protein 1; WT, wild type; Yif1B, Yip1 interacting factor homolog B; 2C6, full length 5-HT1A receptor.
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Abstract—5-HT receptors expressed throughout the human body are targets for established therapeutics and various drugs in development. Their diversity of structure and function reflects the important role 5-HT receptors play in physiologic and pathophysiologic processes. The present review offers a framework for the official receptor nomenclature and a detailed understanding of each of the 14 5-HT receptor subtypes, their roles in the systems of the body, and, where appropriate, the (potential) utility of therapeutics targeting these receptors.

Significance Statement—This review provides a comprehensive account of the classification and function of 5-hydroxytryptamine receptors, including how they are targeted for therapeutic benefit.

I. Introduction

Classification of 5-HT receptors extends back to the middle of the last century when Gaddum and Picarelli (1957) suggested that the 5-HT–induced contraction of guinea pig ileum was mediated by two different receptors: a neurotropic “M” receptor located on parasympathetic ganglia (effect blocked by morphine and atropine; now known to be the 5-HT3 receptor) and a musculo-tropic “D” receptor located on smooth muscles (effect blocked by dibenzyline, lysergide, 2-bromolysergide, and dihydroergotamine; now known to be the 5-HT2A receptor). This original classification served well for around two decades, although, from time to time, it was reported that some 5-HT–induced effects (e.g., vasoconstriction in the canine carotid arterial bed) were not mediated by “M” or “D” but instead by “special” receptors (Saxena, 1974). Then, Bennett and Aghajanian (1974) reported the first successful radioligand binding study of 5-HT receptors using [3H]lysergide, and subsequent studies using [3H]5-HT, [3H]spiperone, and [3H]lysergide enabled Peroutka and Snyder (1979) to identify two 5-HT “receivers” named 5-HT1 (nanomolar affinity for 5-HT) and 5-HT2 (micromolar affinity for 5-HT). Subsequently, 5-HT “receivers” were subdivided pharmacologically into 5-HT1A and 5-HT1B receptors (Pedigo et al., 1981), and 8-OH-DPAT was designated as a selective 5-HT1A ligand (Gozlan et al., 1983; Middelmiss and Fozard, 1983). However, at these times, 5-HT receptors were being classified by various names (e.g., “D,” “M,” 5-HT1, 5-HT2, S1, S2), hence the clear need for uniform terminology. This effort culminated in the Bradley et al. (1986) publication, classifying 5-HT receptors into “5-HT1-like” (equivalent to some “D” or 5-HT1), 5-HT2 (equivalent to most “D” or 5-HT2), and 5-HT3 (equivalent to “M”) receptors. The authors emphasized that this classification was a “general framework,” which would be regularly updated with new findings. Indeed, with the explosion in new findings around the time, it was clear a new classification was required that gave rise to the 5-HT receptor IUPHAR subcommittee–sanctioned classification of 5-HT receptors into 5-HT1 (“5-HT1-like,” 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1e, and 5-HT1f), 5-HT2 (5-HT2A, 5-HT2B, and 5-HT2C), 5-HT3, 5-HT4, recombinant (5-htr5a/5b, 5-htr6, 5-htr7), and “orphan” receptors (Hoyer et al., 1994). This new classification scheme was based on the conjunction of structural (molecular structure), transductional (intracellular transduction mechanisms), and operational (selective agonists and antagonists and ligand binding affinities) criteria. This first IUPHAR review on 5-HT receptors (Hoyer et al., 1994) was a landmark for the then rather complex 5-HT receptor field and the associated diversity of nomenclature used by operators in the field. In the 1994 review, we noted that the authors had a cumulated 100 years of active 5-HT research to share. A number of our colleagues have, in the meantime, retired from active research or have moved to other professional priorities. The present review provides a comprehensive overview of each of the recognized 5-HT receptors (Table 1) as well as reviewing the roles of 5-HT receptors in the major organs. There is a lot of new “blood” on board to reflect the growing diversity of the research, which is currently performed in many different academic and industrial centers; the combined years in 5-HT research of the present authors has increased considerably, partly because of the expansion of authors to ensure a comprehensive review of 5-HT receptors guided by the IUPHAR subcommittee on 5-HT receptors, which is chaired by Nicholas Barnes and Danny Hoyer.

In the present review, we address each receptor separately, as was performed previously, and then have sections that deal with specific aspects in more detail, such as the structures of 5-HT receptors, their functions in the major systems, and translational/clinical outcomes arising from 5-HT research. Readers are also directed to a website (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=1) and the Concise Guide to Pharmacology (Alexander et al., 2019).

II. 5-HT1A Receptor

A. Introduction

5-HT1A receptors have attracted particular interest because of their negative feedback on 5-HT neurons,
thus inhibiting 5-HT release and having broad influence on 5-HT tone. Additionally, 5-HT1A receptors are widely distributed in terminal areas of the brain, where they are expressed as postsynaptic heteroreceptors in a variety of different brain regions, influencing a range of neuropsychopharmacological sequelae (Albert and Fiori, 2014). After outlining the molecular structure, tissue expression, and the tools that can aid in the delineation of 5-HT1A receptor function, the focus will be on the diverse therapeutic fields in which 5-HT1A receptors have become a target. Accordingly, substantial efforts have focused on targeting 5-HT1A receptors for pharmacotherapy of a variety of neurologic and psychiatric disorders, including major depressive disorder, anxiety, and schizophrenia. In addition, activation or blockade of 5-HT1A receptors has been implicated in control of diverse other effects, including cognition, pain, fear, substance use disorder, and Parkinson disease (PD), and, more recently, in emerging clinical opportunities such as female sexual dysfunction and the treatment of respiratory deficits. The complexity of the effects of 5-HT1A receptors presents both a challenge and a considerable opportunity for investigation of 5-HT function and for the potential identification of novel and improved therapeutic drugs.

B. 5-HT1A Receptor Identification and Expression

The introduction of tritiated [3H] receptor-binding techniques revealed the existence of 5-HT1 (and 5-HT2) receptor families in the prefrontal cortex (PFC) of the brain (Peroutka and Snyder, 1979), and extended studies indicated the existence of different 5-HT1 receptor populations, designated, for the first time, 5-HT1A and 5-HT1B receptors (Pedigo et al., 1981; Middlemiss and Fozard, 1983), leading to a much greater understanding of the pharmacological and functional role of the 5-HT1A receptor in health and disease.

The cloning of the 5-HT1A receptor from various species confirmed the existence of 5-HT1A receptors as distinct gene products that correlated with pharmacologically defined receptor responses (Table 2).

The 5-HT1A receptor has been located in a wide variety of peripheral and central targets. In the periphery, immunohistochemical studies have demonstrated that the receptor is located in human and rat kidney, including medulla and cortical ascending limbs, the convoluted tubules, connecting tubule cells, and the principal cells of the initial collecting tubule (Raymond et al., 1993), and murine peritoneal macrophages (Freire-Garabal et al., 2003). However, other techniques have revealed a wider distribution: Western blotting found the receptor in human benign and malignant prostate tissue (Dizeyi et al., 2004), whereas reverse transcriptase polymerase chain reaction (RT-PCR) demonstrated the presence of 5-HT1A receptors in rat taste buds (Kaya et al., 2004). However, the receptor is relatively poorly expressed in human coronary arteries, heart atrium, heart ventricles, and epicardium (Nilsson et al., 1999a,b). The brain and spinal cord have particularly dense populations of 5-HT1A receptors, consistent with the role of this receptor in neuropsychiatric disease. The use of 5-HT1A receptor agonists has been linked with the management of pain; accordingly, radioligand-binding and in situ hybridization studies have indicated the
presence in the human and rat dorsal and ventral horns (Pompeiano et al., 1992; Laporte et al., 1996) and rat superior cervical ganglia (Pierce et al., 1996). In the brain, a wide distribution of the receptor has been described in both terminal regions as postsynaptic sites and in the raphe nuclei, where it has a somatodendritic autoreceptor function (Jacobs and Azmitia, 1992; Fornal et al., 1994). Generally, there is much conservation in regional expression across species, although rat-human cortical and hippocampal differences in laminar organization were reported (Burnet et al., 1995; Barnes and Sharp, 1999). Within the brain, different techniques, including receptor binding, RT-PCR, in situ hybridization (Fig. 1), Western and Northern blotting, and immunohistochemistry, have localized the receptor to the septum, thalamus, hippocampus, entorhinal cortex, interpeduncular nucleus, olfactory bulb, amygdala, hypothalamic subnuclei, and subareas of the cortex and raphe nuclei (Gozlan et al., 1983; Hall et al., 1985; Pazos and Palacios, 1985; Weissmann-Nanopoulos et al., 1985; Dourish et al., 1986; Hoyer et al., 1986a; Vergé et al., 1986; Daval et al., 1987; Hamon et al., 1988; Albert et al., 1990; Hirose et al., 1990; Chalmers and Watson, 1991; Radja et al., 1991; Francis et al., 1992; Miquel et al., 1992; Pompeiano et al., 1992; Khawaja, 1995; Kung et al., 1995; Pike et al., 1995; Lemoine et al., 2010, 2012). More particularly, 5-HT1A receptors are located on septal cholinergic neurons, cortical and hippocampal glutamatergic pyramidal neurons and granule cells (Francis et al., 1992; Pompeiano et al., 1992; Burnet et al., 1995), and calbindin- and parvalbumin-positive neurons (Aznar et al., 2003).

C. Pharmacology

In view of the involvement of 5-HT1A receptors in a wide variety of physiologic responses, the pharmacological profile of these receptors has been investigated extensively using an impressive variety of ligands, with varying degrees of selectivity. These range from drugs preferentially targeting 5-HT1A receptors to nonsel ective compounds that have broad pharmacological activities. Examples of the latter are atypical antipsychotic drugs such as clozapine, ziprasidone, or aripiprazole, which interact with many receptor subtypes. Notably, there are currently no selective 5-HT1A receptor drugs approved for therapeutic use. This is somewhat surprising in view of the broad therapeutic interest of 5-HT1A receptors but likely reflects the difficulty of identifying chemical scaffolds that selectively engage this target. For example, the anxiolytic agent, buspirone, and its chemical analogs such as ipsapirone and gepirone lack selectivity over some other receptors (for example, buspirone displays submicromolar affinity for dopamine D2, D3, and D4 receptors; 5-HT2A, 5-HT2B, 5-HT2C, 5-HT6, and 5-HT7 receptors; and α1 adrenoceptors). Similarly, several antagonist ligands have been proposed, but few have proved to be selective “silent antagonists.” Nevertheless, some recent “full agonists” (notably befi radol) have been identified that exhibit good selectivity for 5-HT1A receptors and, as such, may constitute first-in-class therapeutic agents.

Tables 3 and 4 summarize the receptor-binding properties of many 5-HT1A receptor ligands that have been described over the last decades. It is also worth noting that even though certain compounds do display measurable receptor-binding affinity, this may be too low to induce functional responses at the 5-HT1A

![Fig. 1. In situ hybridization detection of 5-HT1A receptor mRNA expression in rat (A) and human brain (B) at the level of the hippocampus. CA1, dentate gyrus (DG) of the hippocampus, and parahippocampal gyrus (PHG) are shown. Adapted from Burnet et al. (1995) (with permission).](image-url)
receptor. Such an example is olanzapine, fails to elicit electrophysiological actions at the level of somatodendritic autoreceptors in contrast to ziprasidone and clozapine (Sprouse et al., 1999). Many of the ligands have been decisive in the operational definition of biochemical and pharmacological function at a basic science level and in key disease models. In addition to the receptor agonists and antagonists, there is some evidence for the existence of allosteric modulators, such as zinc, Galphimine-B, and RS-30199 (Spedding et al., 1998; Barrondo and Sallés, 2009; Jimenez-Ferrer et al., 2011).

The use of [35S]GTPγS binding, a nonhydrolysable analog of GTP that binds to agonist-activated G proteins, has proved useful for investigating 5-HT1A receptor signaling and pharmacology (Newman-Tancredi et al., 1996b, 1997b, 1998; Barr and Manning, 1997; Pauwels et al., 1997; Sim et al., 1997; Stanton and Beer, 1997; Dupuis et al., 1999a,b; Cosi and Koek, 2000; Gonzalez-Maeso et al., 2000; McLoughlin and Strange, 2000; Shen et al., 2002; Odagaki and Toyoshima, 2005a,b, 2007). Notably, the use of [35S]GTPγS binding enabled the investigation of both positive and negative efficacy ligands at 5-HT1A receptors. Thus, whereas a range of ligands efficaciously stimulated G proteins, other drugs, such as spiperone and methiothepin, markedly inhibited the [35S]GTPγS basal binding in both membranes prepared from 5-HT1A receptor–transfected Chinese Hamster Ovary (CHO) cells and native tissue, confirming the capacity of 5-HT1A receptors to elicit constitutive activation of G proteins in vitro (Newman-Tancredi et al., 1997a; Stanton and Beer, 1997; McLoughlin and Strange, 2000; Corradetti et al., 2005; Martel et al., 2007). In contrast to spiperone, WAY1000635 exhibited neither positive nor negative efficacy yet blocked the actions of both agonists and inverse agonists, consistent with “neutral antagonist” properties (Fletcher et al., 1996; Martel et al., 2007) also evident in vivo using electrophysiological procedures (e.g., Fornal et al., 1996). This was important because other compounds claimed as antagonists at 5-HT1A receptors, such as NAN190, BMY7378, SDZ216,525, and even WAY100135, were found to display partial agonist properties when tested in systems that exhibit high degrees of receptor reserve (Greuel and Glaser, 1992; Routledge, 1996); changes in receptor expression level can markedly affect functional responses, and this is important when considering the nature of ligand engagement and the notion that different brain areas exert distinct physiologic influence (Newman-Tancredi et al., 1997c). A threefold increase in receptor:G protein ratio almost doubled relative efficacy of the partial agonist eltoprazine (53%–93%), without a change in potency, whereas 5-HT exhibited a twofold increase in potency (decrease in EC50 value) (Newman-Tancredi et al., 1997c). In addition to these changes, the increase in 5-HT1A receptor:G protein ratio roughly doubled the negative efficacy of spiperone. These data therefore lead to the supposition that the targeting of agonist efficacy in vivo at different receptor populations is possible, which may offer therapeutic benefits.

D. Biased Agonism: Differential Activation of 5-HT1A Receptor Subpopulations

The term “biased agonism” (“functional selectivity” or “agonist-directed signaling”) (Berg and Clarke, 2006; Evans et al., 2010; Kenakin, 2010; Tzingounis et al., 2010) was coined to denote a pattern of agonist signaling that was distinct from the concept of “intrinsic activity.” Whereas the latter posits that receptor activation is an outcome of the “intrinsic” properties of the agonist, the concept of “biased agonism” is based on the capacity of agonists to preferentially mediate receptor signaling via specific pathways while not affecting, or even blocking, other secondary messenger pathways coupled to the same receptor. If the different signaling cascades mediate distinct functionality (e.g., therapeutic vs. side effects), then biased agonism will offer a strategy to potentially target different mechanisms with the opportunity to potentially develop more effective, better-tolerated drugs.

An early study of 5-HT1A receptors suggested that different agonists displayed differential Ga12 and Ga13 activation, determined using a photoactive TAP analog (4-azidoanilido-[α-32P]GTP) (Gettys et al., 1994). Rauwols-cine displayed similar EC50 values for activation of the two G protein subtypes; ipsapiron showed a nearly fourfold lower EC50 for Ga13 activation. 5-HT and 8-OH-DPAT had intermediate EC50 values (Gettys et al., 1994). In another study, the presence of anti-Ga13 antibodies almost completely suppressed G protein activation by pindolol, a 5-HT1A receptor partial agonist that preferentially elicits activation of Ga13, a property that may underlie its preferential occupancy of midbrain 5-HT1A autoreceptors (Hirani et al., 2000; Martinez et al., 2001; Newman-Tancredi et al., 2002). Drug differences were also seen in transduction experiments on native rat raphe; buspirone elicited Ga12-, Ga13-, and Gαo-mediated responses as well as inhibition of adenylyl cyclase (AC), whereas 8-OH-DPAT only elicited coupling to Ga13 and did not elicit the other responses (Valdizán et al., 2010).

Together, these data support that different 5-HT1A receptor agonists possess different G protein activation “fingerprints,” backing the biased agonist concept and hence suggesting that 5-HT1A receptor subpopulation targeting is possible (Fig. 2). Compounds such as the biased 5-HT1A receptor agonists, F15599 and F13714, reversed immobility in the rat forced swim test via actions at presumed postsynaptic receptors. Similarly, anxiolytic-like actions were seen in the rat ultrasonic vocalization test (De Vry et al., 1993; Assié et al., 2010). However, in animal tests related to side effects, F15599 exhibited a better profile compared with F13714 (Gaggi et al., 1997; Prinssen et al., 2000; Assié et al., 2010), further supporting the potential for improved therapeutics utilizing biased agonists to target the appropriate
TABLE 3
Receptor-binding characteristics of 5-HT<sub>1A</sub> receptor agonists
Data are extracted and adapted from Colpaert et al. (2002), Glennon et al. (2006), McCreary et al. (2007), Andrade et al. (2019), and McCreary and Newman-Tancredi (2019).

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Agonist Action</th>
<th>Affinity</th>
<th>Units</th>
<th>Clinical Utility</th>
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<td>8</td>
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<td>9.4–10.3</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
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</tr>
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<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
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<tr>
<td>8-OH-DPAT</td>
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<td>8.4–9.4</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>(R)-UH 301</td>
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<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
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<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
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<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>PD, erectile dysfunction</td>
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<td>Naratriptan</td>
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<td>7.1–7.6</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Migraine</td>
</tr>
<tr>
<td>Nemonapride</td>
<td>Partial</td>
<td>8.35</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Ocoperidone</td>
<td>Full</td>
<td>6</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>Full</td>
<td>5.6–5.8</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Pardoprunox (SLV308)</td>
<td>Full</td>
<td>8.5</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>PD</td>
</tr>
<tr>
<td>Pergolide</td>
<td>Partial</td>
<td>8.7</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>PD</td>
</tr>
<tr>
<td>Pribedil</td>
<td>Partial</td>
<td>6.4</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>PD</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>Full</td>
<td>6.5–6.6</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>Full</td>
<td>5.8</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Repinotan</td>
<td>Full</td>
<td>9.4</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Rizatriptan</td>
<td>Full</td>
<td>6.4</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Migraine</td>
</tr>
<tr>
<td>Roxindole</td>
<td>Partial</td>
<td>9.4–9.9</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>RU 24969</td>
<td>Full</td>
<td>9</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>S 16924</td>
<td>Partial</td>
<td>8.4</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>S-14506</td>
<td>Full</td>
<td>9.6–9.7</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>S-14671</td>
<td>Full</td>
<td>10.2–10.5</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>S-15535</td>
<td>Partial</td>
<td>9.2</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Sarizotan</td>
<td>Partial</td>
<td>8.65</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>PD dyskinesia</td>
</tr>
<tr>
<td>SB 216641</td>
<td>Partial</td>
<td>6.3</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Spiroxatrine</td>
<td>Full</td>
<td>8.8</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>SSR1181507</td>
<td>Partial</td>
<td>8.53</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Sumatriptan</td>
<td>Full</td>
<td>6</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Migraine</td>
</tr>
<tr>
<td>Tandospirone</td>
<td>Partial</td>
<td>8.2</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Anxiety</td>
</tr>
</tbody>
</table>
5-HT_{1A} receptor subpopulation (Table 5), which includes potential to improve the cognitive state of patients with schizophrenia (Depoortère et al., 2010; Horiguchi and Meltzer, 2012).

E. 5-HT_{1A} Receptor Intracellular Signal Transduction

The transfection (Fargin et al., 1988) and heterologous expression of 5-HT_{1A} receptors in various different cellular environments (including COS7, HeLa, CHO, NIH3T3, SF9, and Escherichia coli cells) enabled the study of their G protein coupling to secondary messenger systems (Raymond et al., 1999). A well characterized intracellular functional response is the inhibition of AC activity and has been extensively used to differentiate ligands for this receptor, their agonist and partial agonist actions, or their degree of antagonism (De Vivo and Maayani, 1986; Markstein et al., 1986; Bockaert et al., 1987; Shenker et al., 1987; Dumuis et al., 1988b; Fargin et al., 1989; Varrault and Bockaert, 1992; Raymond et al., 2006). 5-HT_{1A} receptors can also activate G protein inward rectifying potassium channels (GIRK), high-conductance anion channels to inhibit calcium conductance modulating intracellular calcium mobilization, and stimulate nitric oxide synthase (NOS) and an NADP oxidase-like enzyme (Adayev et al., 2003; Hsiung et al., 2006). The receptor can affect metabolism and arachidonic acid (AA) production (Raymond et al., 1999); activate protein kinase C production, Src kinase, and mitogen-activated protein kinases (MAPKs); and activate or inhibit phosphoinosititol hydrolysis and stimulate reactive oxygen species (ROS) production (superoxide and peroxide) (Raymond et al., 1999). Together, the elucidation of this diverse pattern has led to important developments in establishing test systems to probe receptor and drug function.

F. Function

1. Differential Function of 5-HT_{1A} Receptors at Cellular, Tissue, and In Vivo Levels. The functional properties of 5-HT_{1A} receptors have been extensively investigated. The overall conclusion from these studies is that subpopulations of 5-HT_{1A} receptors expressed in different brain regions exhibit specific patterns of receptor signaling, with differing impact on central function. These diverse properties indicate that separate subpopulations of 5-HT_{1A} receptors mediate particular responses and may constitute therapeutic targets in their own right (see also Fig. 2). For example, agonist activation of somatodendritic 5-HT_{1A} autoreceptors expressed on serotonergic neurons in the raphe elicits inhibition of 5-HT release in terminal regions such as the hippocampus. Conversely, 5-HT_{1A} receptors in the prefrontal cortex are activated by 5-HT released from the raphe, leading to cognitive enhancement.

### TABLE 3—Continued

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Agonist Action</th>
<th>Affinity</th>
<th>Units</th>
<th>Clinical Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAY-100135</td>
<td>Partial</td>
<td>8.5</td>
<td>pK_i</td>
<td>PD</td>
</tr>
<tr>
<td>Terguride</td>
<td>Full</td>
<td>9.7</td>
<td>pK_i</td>
<td>Depression</td>
</tr>
<tr>
<td>Vilazodone</td>
<td>Partial</td>
<td>9.7</td>
<td>pK_i</td>
<td>Depression</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>Partial</td>
<td>8.8</td>
<td>pK_i</td>
<td>Depression</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>Full</td>
<td>7.2</td>
<td>pK_i</td>
<td>Depression</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>Partial</td>
<td>7.9–8.9</td>
<td>pK_i</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Zolmitriptan</td>
<td>Full</td>
<td>6.6</td>
<td>pK_i</td>
<td>Migraine</td>
</tr>
</tbody>
</table>

**EMDT**, 2-Ethyl-5-methoxy-N,N-dimethyltryptamine.

### TABLE 4

Receptor-binding characteristics of 5-HT_{1A} receptor antagonists

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Affinity</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-butacalamol</td>
<td>6.4</td>
<td>pK_i</td>
</tr>
<tr>
<td>(-)-propranolol</td>
<td>7.5</td>
<td>pK_i</td>
</tr>
<tr>
<td>(+)-tertatolol</td>
<td>8.2</td>
<td>pK_i</td>
</tr>
<tr>
<td>(R)-fluorocarazolol</td>
<td>6.5</td>
<td>pK_i</td>
</tr>
<tr>
<td>(S)-fluorocarazolol</td>
<td>7.5</td>
<td>pK_i</td>
</tr>
<tr>
<td>(S)-UI 301</td>
<td>7.9</td>
<td>pK_i</td>
</tr>
<tr>
<td>[3H]p-MPPP</td>
<td>8.4</td>
<td>pK_i</td>
</tr>
<tr>
<td>[3H]robalzotan</td>
<td>9.8</td>
<td>pK_i</td>
</tr>
<tr>
<td>[3H]WAY100635</td>
<td>9.5</td>
<td>pK_i</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>6.2</td>
<td>pK_i</td>
</tr>
<tr>
<td>Cymemazine</td>
<td>6.3</td>
<td>pK_i</td>
</tr>
<tr>
<td>Fluspirilene</td>
<td>7.2</td>
<td>pK_i</td>
</tr>
<tr>
<td>GR 125,743</td>
<td>7.3</td>
<td>pK_i</td>
</tr>
<tr>
<td>GR 218,231</td>
<td>6.8</td>
<td>pK_i</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>5.7–5.8</td>
<td>pK_i</td>
</tr>
<tr>
<td>Iloperidone</td>
<td>6.8–7</td>
<td>pK_i</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>5</td>
<td>pK_i</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>7</td>
<td>pK_i</td>
</tr>
<tr>
<td>Methiothepin</td>
<td>7.8–8.1</td>
<td>pK_i</td>
</tr>
<tr>
<td>MPDT</td>
<td>5.8</td>
<td>pK_i</td>
</tr>
<tr>
<td>NAN 190</td>
<td>9.4</td>
<td>pK_i</td>
</tr>
<tr>
<td>[3H]FIMP+P</td>
<td>8.4</td>
<td>pK_i</td>
</tr>
<tr>
<td>p-MPPP</td>
<td>8.4</td>
<td>pK_i</td>
</tr>
<tr>
<td>Pimozide</td>
<td>6.8</td>
<td>pK_i</td>
</tr>
<tr>
<td>Pindolol</td>
<td>8.1</td>
<td>pK_i</td>
</tr>
<tr>
<td>Pipamperone</td>
<td>5.6</td>
<td>pK_i</td>
</tr>
<tr>
<td>Pizotifen</td>
<td>7.4</td>
<td>pK_i</td>
</tr>
<tr>
<td>Raclopride</td>
<td>5.2</td>
<td>pK_i</td>
</tr>
<tr>
<td>Rec 15/3079</td>
<td>9.7</td>
<td>pK_i</td>
</tr>
<tr>
<td>Risperidone</td>
<td>6.2–6.5</td>
<td>pK_i</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>6.2</td>
<td>pK_i</td>
</tr>
<tr>
<td>ritanserin</td>
<td>5.2–5.5</td>
<td>pIC_{50}</td>
</tr>
<tr>
<td>robalzotan</td>
<td>9.2</td>
<td>pK_i</td>
</tr>
<tr>
<td>SB 272183</td>
<td>8</td>
<td>pK_i</td>
</tr>
<tr>
<td>SB 649915</td>
<td>8.6</td>
<td>pK_i</td>
</tr>
<tr>
<td>SB 714785</td>
<td>6.5</td>
<td>pK_i</td>
</tr>
<tr>
<td>SBZ-216525</td>
<td>7.8–8.2</td>
<td>pIC_{50}</td>
</tr>
<tr>
<td>Sertindole</td>
<td>6.4–6.6</td>
<td>pK_i</td>
</tr>
<tr>
<td>Sipiprone</td>
<td>6.7–8.8</td>
<td>pK_i</td>
</tr>
<tr>
<td>Thiopiridazine</td>
<td>7</td>
<td>pK_i</td>
</tr>
<tr>
<td>Tio-piprazine</td>
<td>8.3</td>
<td>pK_i</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>7.9–9.2</td>
<td>pK_i</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>7.3</td>
<td>pK_i</td>
</tr>
<tr>
<td>Zotepine</td>
<td>6.5</td>
<td>pK_i</td>
</tr>
</tbody>
</table>

MPPF, 2'-methoxyphenyl-p-fluoro-benzamidoethyipiperazine.
as the hippocampus and cortex. In contrast, activation of postsynaptic cortical 5-HT1A heteroreceptors expressed on glutamatergic pyramidal cells and/or GABAergic interneurons elicits different neurochemical responses, including stimulation of dopamine release in the frontal cortex (Santana et al., 2004; Bortolozzi et al., 2010).

Activation of 5-HT1A autoreceptors induces anxiolytic activity in rodent behavioral tests (De Vry et al., 2004; Akimova et al., 2009), whereas antidepressant-like responses are seen upon activation of 5-HT1A heteroreceptors (De Vry et al., 2004). These data obtained in rat behavioral experiments are consistent with observations in transgenic mice overexpressing raphe 5-HT1A autoreceptors; accentuated depressive-like behavior was observed and diminished response to antidepressant treatment (Richardson-Jones et al., 2010). These data support the interpretation that desensitization of presynaptic 5-HT1A receptors is necessary before antidepressant efficacy may be achieved (Artigas et al., 2006; Millan, 2006), consistent with the relatively long latency (typically 3 to 4 weeks) to clinical responsiveness in patients with depression treated with 5-HT reuptake inhibitors.

Diverse responses to 5-HT1A receptor agonists are also observed in tests of cognition/memory function relevant to numerous neuropsychiatric diseases, including major depressive disorder, schizophrenia, Parkinson disease, and Alzheimer disease. Interestingly, the prototypical 5-HT1A receptor agonist, 8-OH-DPAT, facilitated rat passive avoidance at low doses, whereas higher doses impaired performance (Lüttgen et al., 2005; Madjid et al., 2006). This suggests that opposite responses are mediated by 5-HT1A receptor subpopulations (i.e., improved performance is elicited by 5-HT1A autoreceptors, whereas impairment is due to activation of hippocampal 5-HT1A heteroreceptors) (Ogren et al., 2008). This interpretation is supported by local administration experiments in which the 5-HT1A receptor weak partial agonist/antagonist S15535 was microinjected into the hippocampus. The compound reversed the memory deficit elicited by systemic injection of 8-OH-DPAT in a spatial discrimination task (Millan et al., 2004), indicating that activation of postsynaptic receptors in this brain region was detrimental to mnemonic performance.

Given that only a single 5-HT1A receptor gene has been identified in human and rat, and that it is intronless and hence without splice variants (Fargin

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**TABLE 5**

Comparison of properties of 5-HT1A receptor “biased agonists” F15599, F13714, and befaradol, and the reference agonists 8-OH-DPAT and 5-HT

<table>
<thead>
<tr>
<th>Agonist</th>
<th>In Vitro Affinity/Selectivity*</th>
<th>Cellular Transduction Pathways*</th>
<th>Target Brain Regions/†</th>
<th>Readout In Vivo‡</th>
<th>Relevant Therapeutic Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>F15599</td>
<td>Nanomolar/highly selective</td>
<td>Preferential pERK activation</td>
<td>Cortex, brain stem</td>
<td>Reverses PCP-induced cognitive deficits, active in antidepressant and anxiolytic tests. Normalizes breathing in MeCP2&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>Cognitive deficits, mood disorders, respiratory difficulties (Rett syndrome)</td>
</tr>
<tr>
<td>F13714</td>
<td>Subnanomolar/highly selective</td>
<td>Multiple (pERK, receptor internalization, G protein, cAMP, Ca&lt;sup&gt;2+&lt;/sup&gt; release)</td>
<td>Mid-brain, thalamus, hippocampus</td>
<td>Potently eliminates L-DOPA-induced AIMs, active in antidepressant and anxiolytic tests</td>
<td>Not clinically tested (research tool)</td>
</tr>
<tr>
<td>Befaradol</td>
<td>Nanomolar/highly selective</td>
<td>Multiple (pERK, receptor internalization, G protein, cAMP, Ca&lt;sup&gt;2+&lt;/sup&gt; release)</td>
<td>Mid-brain, cortex, thalamus, hippocampus</td>
<td>Potently eliminates L-DOPA-induced AIMs, active in antidepressant and anxiolytic tests</td>
<td>Dyskinesias in Parkinson disease, mood deficits, chronic pain</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>Nanomolar/binds 5-HT&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Preferential pERK activation</td>
<td>Hippocampus, mid-brain, cortex, thalamus, brain stem</td>
<td>Disparate effects on cognition tests, active in antidepressant and anxiolytic tests, reduces L-DOPA-induced AIMs</td>
<td>Not clinically tested (research tool)</td>
</tr>
<tr>
<td>5-HT</td>
<td>Nanomolar/nonselective</td>
<td>Multiple (pERK, receptor internalization, G protein, cAMP, Ca&lt;sup&gt;2+&lt;/sup&gt; release)</td>
<td>All 5-HT projection areas</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

* N/A, not applicable.
*<sup>+</sup>Colpaert et al., 2002; Newman-Tancredi et al., 2009a.
*<sup>+</sup>Colpaert et al., 2002; Pauwels and Colpaert, 2003; Buritova et al., 2009; Newman-Tancredi et al., 2009b.
*<sup>+</sup>Lemoine et al., 2010, 2012; Lladó-Pelfort et al., 2010, 2012; Vidal et al., 2014.
*<sup>+</sup>Assié et al., 2010; Depourret et al., 2010; Levitt et al., 2015; Iderberg et al., 2015; van Goethem et al., 2015.
et al., 1988; Albert et al., 1990; Kobilka et al., 1987), the variety of responses described above are likely attributable to regional “receptor interactome” differences, including coupling to distinct G protein subtypes (see below) (Mannoury la Cour et al., 2006), regulators of G protein signaling (RGS) (Talbot et al., 2010), or transcriptional regulation.

At a molecular level, 5-HT\textsubscript{1A} receptor inactivation studies using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline demonstrated the existence of receptor reserve in the raphe for inhibition of 5-HT synthesis (Meller et al., 1990). In contrast, receptor reserve was not evident in the hippocampus for the inhibition of adenylyl cyclase or for control of hypothermia (Meller et al., 1992; Yocca et al., 1992).

The agonist radioligand, \textsuperscript{3}H\textsuperscript{8}-OH-DPAT, which preferentially recognizes 5-HT\textsubscript{1A} receptors when coupled to G proteins, displayed fivefold higher affinity for hippocampal compared with raphe binding sites (Johnson et al., 1997b), supporting differing receptor–G protein coupling state between the two brain regions. Furthermore, whereas 5-HT\textsubscript{1A} receptors are coupled to inhibition of adenylyl cyclase in hippocampus, such coupling was not detected in raphe homogenates (Clarke et al., 1996). Further support that 5-HT\textsubscript{1A} receptors couple to different G protein subtypes depending on brain region arises from immunoprecipitation studies in which raphe 5-HT\textsubscript{1A} receptors couple preferentially to Go\textsubscript{i3} subtypes, whereas they couple preferentially to Go\textsubscript{a} in hippocampus and to a combination of G proteins in cortex and hypothalamus (Mannoury la Cour et al., 2006).

Functional ([\textsuperscript{35}S]GTP\_S) autoradiography experiments also support the contention that regional variations exist in native brain activation of G proteins by 5-HT\textsubscript{1A} receptors. Indeed, whereas 5-HT\textsubscript{1A} receptor density is similar in the raphe and hippocampus, agonist-induced ([\textsuperscript{35}S]GTP\_S) labeling was markedly lower in the former (Hensler, 2003; see also Newman-Tancredi et al. (2003) for relevant evidence).

An additional level of complexity of 5-HT\textsubscript{1A} receptor signaling has been reported (i.e., the existence of receptor homo-, hetero-, and potential trimers with a variety of targets). First, 5-HT\textsubscript{1A} homodimers may be formed constitutively (Łukasiewicz et al., 2007; Renner et al., 2012) and are affected by the presence of selective ligands such as 8-OH-DPAT, which enhanced dimerization, whereas methysergide reduced dimer formation potentially via a mechanism modulated by Go\textsubscript{a} subunits. The 5-HT\textsubscript{7} receptor, like the 5-HT\textsubscript{1A} receptor, has been reported to play a role in depression and form homodimers; but it has also heterodimerized with the 5-HT\textsubscript{2A} receptor and may have functional consequences insofar as 5-HT\textsubscript{1A}–5-HT\textsubscript{7} heterodimerization reduces GIRK currents in a heterologous cell system, potentially affecting 5-HT\textsubscript{1A} receptor internalization in the hippocampus (Renner et al., 2012; Naumenko et al., 2014). Heterodimerization with a novel negative response element of 5-HT\textsubscript{1A} receptors has been suggested with glucocorticoid and mineralocorticoid receptors, which may also be key players in depression (Ou et al., 2001). Galanin receptors form heteromers with a variety of targets, including galanin receptor–5-HT\textsubscript{1A} heteromers and trimers (Fuxe et al., 2012). With potential relevance to the influence of 5-HT\textsubscript{1A} receptors in ascending and central pain perception, heterodimerization has also been demonstrated with 5-HT\textsubscript{1A} receptors and \(\mu\)-opioid receptors in vitro, and further data suggested that both receptors could exert effects on extracellular signal–regulated kinase (ERK)1/2 phosphorylation (Cussac et al., 2012).

5-HT\textsubscript{1A} receptors in depression and anxiety. The key role of 5-HT\textsubscript{1A} receptors in major depression and anxiety has been recognized for nearly four decades [see Barnes and Sharp (1999); Albert et al. (2014)]. Accordingly, animal behavioral models of fear, anxiety, depression, and cognition have been used to identify potential antidepressant and clinically active anxiolytics, such as the partial agonists buspirone, gepirone, ipsapirone, and tandospirone (Peroutka, 1985; Taylor et al., 1985; Gilbert and Dourish, 1987). Buspirone and tandospirone were both clinically developed and received marketing approval for treating anxiety. However, their azapirone chemical structures are associated with only limited selectivity (e.g., vs. \(\alpha\textsubscript{1}\) adrenoceptors and D\textsubscript{2} dopamine receptors), and they also exhibit relatively poor metabolic stability and generate pharmacologically active metabolites, such as 1-(2-pyrimidinyl)-piperazine, which is an \(\alpha\textsubscript{2}\) adrenoceptor antagonist (Garattini et al., 1982; Cao and Rodgers, 1997; Zuiderveld et al., 2002; Sugimoto et al., 2005; Wong et al., 2007). Consequently, the therapeutic usefulness of selective 5-HT\textsubscript{1A} receptor agonists still remains to be determined.

Indeed, data suggest that the phenotypic expression of normal behavior, anxiety, or depression may be influenced by the differential 5-HT\textsubscript{1A} receptor–sensitive circuitry at the level of the PFC; the balance between 5-HT\textsubscript{1A} receptor stimulation of glutamatergic pyramidal cells and GABAergic interneurons may impact the expression of anxiety (Goodfellow et al., 2009; Albert et al., 2014), although juvenile development processes play a key role in determining vulnerability to mood disorders (Leonardo and Hen, 2008; Donaldson et al., 2014; Garcia-Garcia et al., 2014). In depression, the neurobiology appears different. Therefore, activation of pyramidal neurons by stimulation of 5-HT\textsubscript{1A} receptors expressed on GABAergic interneurons disinhibits the “antidepressive” pyramidal neurons (Albert et al., 2014). It is interesting to note that the rapid antidepressant activity of ketamine appears to be partly mediated via 5-HT\textsubscript{1A} receptor activation. Indeed, ketamine inhibits 5-HT reuptake in vivo (Martin et al., 1982; Martin and Smith, 1982) and elicits its prolonged antidepressant-like effects in rodents via a 5-HT–dependent mechanism (Gigliucci et al., 2013). This is likely to involve indirect activation of 5-HT\textsubscript{1A} receptors,
as exemplified by the fact that the effects of ketamine in the novelty-suppressed feeding test are blocked by a 5-HT_{1A} receptor antagonist (Fukumoto et al., 2014).

Additional evidence that 5-HT_{1A} receptors are involved in affective disorders comes from genetic studies. The expression of 5-HT_{1A} receptors is differentially regulated by a single-nucleotide polymorphism (SNP) in the promoter region of the 5-HT_{1A} receptor gene (C-1019G substitution) (Lesch and Gutknecht, 2004; Albert and Francois, 2010). This SNP impairs repression of the 5-HT_{1A} promoter by the nuclear DEAF-1-related/drosophila deformed epidermal autoregulatory factor-1 transcription factors in raphe cells, consistent with overexpression of presynaptic 5-HT_{1A} receptors (Lemonde et al., 2004; Parsey et al., 2006). Thus, C-1019G polymorphism is associated with higher levels of symptom remission and suicidal behavior in patients with depression (Lemonde et al., 2003), consistent with impaired antidepressant efficacy caused by excessive feedback inhibition by presynaptic 5-HT_{1A} receptors.

Taken together, the above considerations indicate that 5-HT_{1A} receptors remain promising targets for the pharmacotherapy of affective disorders, both as a somatodendritic and postsynaptic receptor target in the brain. Accordingly, various efforts have been made to incorporate 5-HT_{1A} receptor activity in antidepressant/anxiolytic drug candidates. For example, SB-649915-B is a 5-HT reuptake inhibitor (SSRI) that also acts as a 5-HT_{1A} receptor antagonist (Hughes et al., 2007; Starr et al., 2007) based on the rationale that accelerated antidepressant response may be achieved by avoiding feedback inhibition of terminal 5-HT release by blocking the activation of 5-HT_{1A} autoreceptors (Gartside et al., 1999; Artigas et al., 2006; Portella et al., 2011). However, though antidepressant efficacy may be enhanced by 5-HT_{1A} autoreceptor antagonism, the blockade of postsynaptic 5-HT_{1A} receptors likely opposes antidepressant activity (De Vry et al., 2004; Berrocoso and Mico, 2009). Accordingly, a clinical trial in which a selective 5-HT_{1A} receptor antagonist was administered as adjunct to fluoxetine did not show any acceleration of antidepressant onset of efficacy (Scorza et al., 2012), likely because of its concurrent blockade of both pre- and postsynaptic 5-HT_{1A} receptors. In contrast, adjunct treatment with pindolol, which preferentially occupies 5-HT_{1A} autoreceptors (Martinez et al., 2001), appears to reliably elicit acceleration of antidepressant efficacy (Artigas et al., 1996, 2006; Portella et al., 2011). Compounds such as vilazodone, vortioxetine, and VN2222 are SRIs possessing partial agonist actions at 5-HT_{1A} receptors (Romero et al., 2003; Dawson and Watson, 2009; Mork et al., 2009; Alvarez et al., 2012) that might assist in engaging diverse frontal circuitry, leading to better treatment of the disease.

b. 5-HT_{1A} receptor activation for improved antipsychotic action. A noteworthy development in the study of 5-HT_{1A} receptors has been the increasing therapeutic interest for this target in psychotic disorders. This has stemmed from extensive clinical and preclinical observations [see McCreary and Newman-Tancredi (2015) for review].

Schizophrenia, which shares some symptoms with other neuropsychiatric diseases, includes positive symptoms (auditory and visual hallucinations, delusions, conceptual disorganization, thought disorders, and some motor disturbances); negative symptoms (affective blunting, social withdrawal, anhedonia, avolition, and poverty of thought and speech); and cognitive impairments, such as working-memory abnormalities, deficits of cognitive processing, and attention and affective disorders (depression and anxiety) (Meltzer, 1999). 5-HT_{1A} receptors appear involved both in the pathophysiology and in functionality of potential novel treatments. Thus, the newer generation antipsychotics clozapine, ziprasidone, quetiapine, aripiprazole, lurasidone, and cariprazine possess (partial) agonist effects at 5-HT_{1A} receptors; however, interestingly, risperidone and olanzapine do not (McCreary and Newman-Tancredi, 2015; Newman-Tancredi et al., 1996a, 2005). In patients, changes in 5-HT_{1A} receptor binding or functional activity have been identified (Burnet et al., 1996; Kasper et al., 2002; Yasuno et al., 2003; Bantick et al., 2004; Frankie et al., 2006; Lerond et al., 2013; Billard et al., 2014) along with SNPs at loci ss212928868 and rs6294, which are associated with the clinical outcome in women with paranoid schizophrenia (Zhou et al., 2013). Polymorphisms were also associated with much of the depression and negative treatment outcomes (Reynolds et al., 2006; Newman-Tancredi and Albert, 2012). Preliminary studies assessing cytosine methylation at a site close to this rs6295 polymorphism suggested that this was associated with a lower incidence of negative symptoms (Reynolds et al., 2006; Tang et al., 2014b), reinforcing the importance of this site in the negative symptoms of schizophrenia. Taken together, these accumulated data support the assertion that there is involvement of 5-HT_{1A} receptors in the pathophysiology and treatment-related facets of the disease, particularly negative symptomatology.

A net hypofunctionality of the PFC, a brain area key in working memory, decision, and attentional processing, has been proposed in schizophrenia (Weinberger and Lipska, 1995; McCreary et al., 2007). It is therefore interesting that many atypical antipsychotic drugs may impact this deficit (McCreary and Newman-Tancredi, 2015). It may therefore be relevant that the 5-HT_{1A} receptor agonist agents possessing antipsychotic properties (SSR181507, adoprazine, and lurasidone) augment extracellular microdialysate dopamine and acetylcholine levels in the PFC to “normalize” hypofrontal tone (Claustre et al., 2003; McCreary et al., 2007; Huang et al., 2014b) and promote potential therapeutic outcomes. This is supported by preclinical evidence (Depoortere et al., 2007) and clinical evidence with the partial agonist, tandospirone, which improved cognitive symptoms in patients with schizophrenia treated with
neuroleptics (Sumiyoshi et al., 2001a,b, 2007; Meltzer and Sumiyoshi, 2008). Additionally, blonanserin, tandospirone, lurisdione, and buspirone reduced MK-801–induced novel object recognition deficits (Horiguchi and Meltzer, 2012; Horiguchi and Meltzer, 2013), and PCP-induced reversal learning was attenuated by 5-HT1A receptor activation (McLean et al., 2009b). In the social interaction test, a model for negative symptoms, aripiprazole, SSR181507, and F-15063 induced a 5-HT1A receptor–dependent performance improvement (Boulay et al., 2004; Bruins Slot et al., 2005; Depoortère et al., 2007; Snigdha and Neill, 2008). In addition, administration of 5-HT1A receptor (partial) agonists reversed PCP–induced decreases of ticking-induced 50-kHz ultrasound vocalization in juvenile rats, a model for negative symptoms, and improved attentional processing in a five-choice serial reaction time task (Winstanley et al., 2003; Boulay et al., 2013). In conclusion, data from preclinical and clinical findings support that 5-HT1A receptor activation will benefit the treatment of cognitive, attentional, and negative symptom domains.

An additional complication of antipsychotic treatment is so-called extrapyramidal side effects induced by the typical antipsychotics, such as haloperidol, which can reduce striatal output and lead to a parkinsonian phenotype. Such symptoms in preclinical models can be reduced by 5-HT1A receptor agonists (McCreary et al., 2007). Compounds such as adropazine, bifeprunox, and F-15063 elicit less catalepsy than neuroleptics such as haloperidol. However, treatment, with WAY-100635 unmasked this blockade of catalepsy, indicating a key role of 5-HT1A receptors (Kleven et al., 2005; Bardin et al., 2006). Consistently, mesolimbic selectivity, and therefore the ability to treat the positive symptoms, was supported with electrophysiological studies demonstrating that depolarization block of VTA, but not substantia nigra pars compacta, dopaminergic neurons was mediated by 5-HT1A receptor agonists (Nakamura et al., 2006; McCreary et al., 2007) and that PFC 5-HT1A receptors influenced VTA cell firing by indirectly affecting pyramidal cell afferents to the VTA, thereby increasing dopamine cell firing (Lladó-Pelfort et al., 2012; Santana et al., 2013). Such mechanisms may indirectly influence mesocuellar dopaminergic output and impact positive symptoms. Some clinical meta-analytical studies support this assertion and suggest a trend for improved cognitive symptoms following the addition of 5-HT1A receptor partial agonists, together with a trend for improved positive symptoms (Kishi et al., 2013), but more extensive clinical studies are warranted. It is interesting to speculate that fully efficacious agents might offer added benefit. Moreover, benefit in other symptom domains might be expected, particularly mood. Accordingly, bifeprunox, SSR181507, and adropazine (SLV313) all demonstrated anxiolytic-like and antidepressive-like properties (Depoortere et al., 2003), and 5-HT1A receptors appear to mediate the antidepressant effects of ketamine and metabolotropic glutamate (2/3) receptor antagonists (Fukumoto et al., 2014). Moreover, 5-HT1A gene loci polymorphism linkage studies support this in schizophrenic patients with depression (Albert, 2012).

Taken together, these data support a role for the 5-HT1A receptor in schizophrenia. This is particularly interesting in light of the clinical development and marketing approval of lurisdione and cariprazine, which possess dopamine D2 and 5-HT1A receptor agonist action (Ishibashi et al., 2010; Kiss et al., 2010). Indeed, pharmacodynamic studies support the described 5-HT1A receptor–mediated mechanisms in the actions of lurisdione on augmented PFC dopamine and acetylcholine levels and cognitive actions (Horiguchi and Meltzer, 2012; Huang et al., 2012, 2014). Consistently, clinical benefit in a variety of symptom domains was evident (Veselínović et al., 2013; Citrome et al., 2014; Durgam et al., 2014; Loebel et al., 2014a,b).

G. 5-HT1A Receptors and Some Emerging Treatment Areas

1. Parkinson Disease. Parkinson disease is characterized by a loss of nigrostriatal dopaminergic neurons, resulting in the cardinal motor symptoms (Schapira et al., 2006). Symptomatic treatment ultimately relies on the gold-standard medication and dopamine precursor levodopa (L-DOPA) (Jenner et al., 2011). However, over time, the effects of L-DOPA are prone to wearing off (i.e., there is a tolerance to the actions of L-DOPA), and patients develop dose-limiting dyskinesia (Jenner et al., 2011). The treatment of L-DOPA–induced dyskinesia (LID) has been hampered by a lack of approved medications. Recently, the 5-HT system has emerged as a key player in the induction of LID. 5-HT neurons possess the enzymes necessary to convert exogenous L-DOPA to dopamine (DA) and mediate its vesicular storage and “false neurotransmitter” release. However, 5-HT neurons lack appropriate control mechanisms to regulate synaptic DA levels (e.g., via presynaptic D2 receptors or dopamine transporters), resulting in excessive DA release and pulsatile (over) stimulation of postsynaptic dopamine receptors that generate dyskinesia. Theoretically, it might be possible to mitigate dopamine release from serotonergic neurons by suppressing serotonergic tone by the application of 5-HT1A (or 5-HT1B) receptor agonists, which suppress neurotransmission by influencing the negative feedback somatodendritic (or terminal autoreceptors). Indeed 5-HT1A receptor agonist treatment does reduce LID in both rat and nonhuman primate models (Bibbiani et al., 2001; Eskow et al., 2007, 2009; Munoz et al., 2009; Huot, 2015; Iderberg et al., 2015) and appears to translate in clinical studies using the partial agonists buspirone and the mixed 5-HT1A/5-HT1B agonist eltoprazine (Svenningsson et al., 2015). However, other clinical attempts to target the 5-HT1A receptor have been disappointing,
with compounds such as sarizotan and tandospirone also impairing the antiparkinsonian activity (Bonifati et al., 1994; Kannari et al., 2002; Olanow et al., 2004; Goetz et al., 2007), whereas eltoprazine showed only modest effects (Svenningsson et al., 2015). Together, this suggests that although 5-HT1A receptors can reduce dyskinesia, compounds tested to date may be less than optimal (Hamik et al., 1990; Newman-Tancredi et al., 1997c, 1998, 2003). Interestingly, only full agonists succeed in completely reversing haloperidol-induced catalepsy, whereas partial agonists failed to do so (Prinssen et al., 2002), suggesting that maximal efficacy may be required. The selective 5-HT1A receptor “biased agonist” F13714, which preferentially targets raphé 5-HT1A autoreceptors (Assié et al., 2006), completely abolished abnormal involuntary movements (AIMs) along with inhibiting 5-HT release (Iderberg et al., 2015). Comparable findings were evident with Befiradol (McCreary and Newman-Tancredi, 2015).

In addition, “full agonist” activity at 5-HT1A receptors may also provide beneficial influence on nonmotor symptoms of PD, such as the mood deficits likely elicited by deficient 5-HT neurotransmission (Eskow Jaunarajs et al., 2010; Politis, 2010). Indeed, whereas treatment of depressive symptoms in PD using 5-HT reuptake inhibitors is poorly effective, direct activation of postsynaptic (cortical) 5-HT1A receptors is associated with potent antidepressant actions (Celada et al., 2004). In restless legs syndrome, another movement disorder typically managed with low doses of dopamine receptor agonists or L-DOPA, 5-HT1A receptor agonists may also display clinical benefit (Shioda et al., 2006).

2. Pain. There is good evidence for the involvement of the 5-HT system in chronic pain (Millan, 2002), which is not surprising given their expression by descending pathways of the dorsal horn and other relevant structures. The receptors of the dorsal horn appear pivotally involved in the pronociceptive effects (Fasmer et al., 1986; Millan, 1994, 2002; Millan et al., 1996; You et al., 2005; Colpaert, 2006; Avila-Rojas et al., 2015; Sagalajev et al., 2015) and may also influence antinociception (Millan et al., 1996). Recent evidence suggests that the newer generation antipsychotic agent (e.g., aripiprazole), which possesses 5-HT1A receptor partial agonist actions, displays antinociceptive effects (Fei et al., 2012; Almeida-Santos et al., 2015). Moreover, the ability of 5-HT1A receptors to form heterodimers with µ-opioid receptors (Cussac et al., 2012) suggests 5-HT1A receptor targeting as an adjunct to opioid strategies may be useful.

3. Attention Deficiency Hyperactivity Disorder. In animal models of impulse control, 5-HT1A receptor stimulation reduced the impulsivity, suggesting potential benefit in diseases such as attention deficiency hyperactivity disorder (ADHD; Winstanley et al., 2003). Furthermore, in an isolation rearing model, which models some components of ADHD, 5-HT1A receptor binding sites were altered in a region-specific manner (Preece et al., 2004). Pharmacological study using the agonists SSR181507 (Terranova et al., 2005) and sarizotan (Danysh et al., 2015) suggest efficacy in animal models of ADHD. It is also relevant that a HTR1A rs10042486 polymorphism is associated with ADHD (Park et al., 2013). Indeed, buspirone may benefit ADHD management (Levin, 2015), though to a lesser extent than methylphenidate (Mohammadi et al., 2012).

4. Autism Spectrum Disorder. Preclinical studies reveal altered central 5-HT1A receptor activity, in a rat valproate model of autism (Wang et al., 2013b) and BTBR mice (BTBR T^Itpr3^+/J mouse), which have a phenotype paralleling that of autism spectrum disorder, elevated [35S]GTPγS binding is evident, corresponding to enhanced 5-HT1A receptor functional activity that potentially contributes to poor social behavior (Gould et al., 2011). Clinical data are limited, but anti–5-HT1A receptor antibodies have been identified in the blood of an autistic boy (Todd and Ciarnello, 1985). Furthermore, a HTR1A C-1019G polymorphism in autism may influence clinical outcomes (Egawa et al., 2012).

5. Respiratory Control. 5-HT1A receptor agonists increased respiration in rats and cats (Edwards et al., 1990; Rose et al., 1995), and morphine-induced ventilatory depression was reduced by the 5-HT1A receptor agonist repinotan (Guenther et al., 2010). Electrophysiological studies support a modulatory role of the 5-HT1A receptor in the bursting activity of respiratory neurons (Onimaru et al., 1998), and 5-HT1A receptors activate bronchioconstrictor vagal preganglionic neurons and phrenic nerve neurons (Boottle et al., 1998; Valic et al., 2008). These and other data have led to the suggestion that 5-HT1A receptor agonists display potential to treat sleep apnea (Futuro-Neto et al., 1993; Khater-Boidin et al., 1996, 1999; Dando et al., 1998; Sahibzada et al., 2000) that may translate to the clinic given an evident reduction in apnea evoked by buspirone (Wilken et al., 1997). In addition, activation of 5-HT1A receptors may be beneficial to reverse compromised respiration; for instance, in a transgenic mouse model of Rett syndrome that also models disordered breathing, (+)-8-OH-DPAT and sarizotan reduced the apneic frequency to restore the respiratory pattern (Abdala et al., 2010, 2014a,b; Levitt et al., 2013). Furthermore, the 5-HT1A receptor–biased agonist, F15599, impacts apnea and respiration frequency in MECP2-null male and heterozygous female mice (Levitt et al., 2013). Clinical experiences investigating the 5-HT1A receptor role in Rett syndrome are limited, but buspirone administered with fluoxetine reduced the frequency of hyperventilation and apneic attacks (Gokben et al., 2012).

6. Sexual Dysfunction. 5-HT1A receptors may be a promising target in the treatment of sexual dysfunction. The 5-HT1A receptor agonist fibanserin (which also possesses 5-HT2A receptor antagonist and dopamine D4 receptor partial agonist properties; Mendelson...
In vivo imaging studies suggest 5-HT1A receptor agonists may help treat patients with eating disorders receptor activation induces hyperphagia, suggesting agonists may help treat patients with eating disorders such as bulimia and/or anorexia nervosa (Dourish et al., 1987). In vivo imaging studies suggest 5-HT1A receptor binding increases in cortical and limbic structures of the brain of patients with anorexia and/or bulimia, consistent with a potential role in anxiety, behavioral inhibition, and body ideation (Kaye et al., 2005; Bail et al., 2007, 2011; Galusca et al., 2008; Bail and Kaye, 2011). Although clinical pharmacology studies are limited, and restricted to case studies, the partial agonist tandospirone improved the weight gain of patients with anorexia nervosa (restricting and binge-eating/purging subtypes) and also improved scores on the Eating Disorder Examination Questionnaire following treatment of up to 6 months (Okita et al., 2013). The mechanistic basis for this may involve control of mood: the anxiolytic effects of 5-HT1A receptor agonists are likely to be beneficial (Crow and Mitchell, 1994) and potentially contribute to treatment outcome.

8. Aggressive Behavior. 5-HT1A receptor activation appears to reduce aggressive behavior in preclinical and clinical (buspirone) settings (Olivier and Mos, 1992; Bell and Hobson, 1994; Takahashi et al., 2012) with animal models, indicating impact at the level of the dorsal raphe, and hence a reduction in 5-HT neurotransmission, may underlie the response (Mos et al., 1993). This is supported by results generated with S15535, a preferential autoreceptor agonist and, possibly, via blockade of hypersensitive postsynaptic 5-HT1A heteroreceptors (Millan et al., 1997; de Boer et al., 2000). Indeed, elevated postsynaptic 5-HT1A heteroreceptors in the forebrain are associated with aggressive behavior (Korte et al., 1996), although direct administration of F15599 into ventral orbital PFC reduces aggression in male mice (Stein et al., 2013).

9. Neuroplasticity and Neuroprotection. 5-HT1A receptor agonists evoke neurogenesis and synaptogenesis in the adult hippocampus, thereby improving cognitive performance in this structure that is important for mnemonic function (Mogha et al., 2012; Vines et al., 2012; Schreiber and Newman-Tancredi, 2014). Moreover, 5-HT1A receptor stimulation can lead to long-term potentiation or depression (Meunier et al., 2013) with consequent elevated BDNF expression to influence neurogenesis (Luoni et al., 2013; Quesseveur et al., 2013).

In addition to the effects of 5-HT1A receptor agonists on neuroplasticity, targeting this receptor may also have a beneficial role in neuroprotection. Indeed, there is considerable data supporting this assertion: repinotan reduced staurosporine-induced apoptosis (Suchanek et al., 1998), and 8-OH-DPAT reduced the impact of excitotoxic doses of NMDA in vivo (Oosterink et al., 1998) and, further, may protect neurons via protective effects of astrocytes; conversely, 5-HT1A receptor antagonism by WAY100635 increased damage (Ramos et al., 2004). Similarly, the selective 5-HT1A receptor agonist F13714 and the antipsychotic drugs clozapine, ziprasidone, and aripiprazole attenuated kainic acid–induced lesion volume in the striatum—effects that were reversed by WAY100635 (Così et al., 2005).

In models of Parkinson disease, 5-HT1A receptor agonists may slow neuronal damage (Bezard et al., 2006) and limit astrogliosis (Miyazaki et al., 2013). In the experimental autoimmune encephalopathy model of multiple sclerosis and in vitro cell-based models, the efficacy of a novel arylpiperazine D2/5-HT1A receptor ligand suggested this was due to combined action of the compound to limit inflammation and neuroprotective actions (Popovic et al., 2015), and buspirone appears to exert some efficacy against apneusis in multiple sclerosis (OSullivan et al., 2008). Interestingly, repinotan was developed for activity in ischemic stroke and traumatic brain injury (Lutsep, 2002; Berends et al., 2005; Mauler and Horváth, 2005; Guenther et al., 2010), therapeutic areas that are historically very difficult for drug development. However, repinotan failed to show efficacy in acute ischemic stroke, and its development was discontinued (Teal et al., 2009).

III. 5-HT1B Receptors

A. Introduction

The 5-HT1B receptor and its counterpart the 5-HT1D receptor have experienced a complex and debated history (Fig. 3) that is explained here. The two receptors are clearly closely related and result probably from gene duplication, which explains that in most species, their pharmacological profiles are almost indistinguishable (however, this is less evident in some species such as rat, mouse, hamster, or opossum; see below). In addition, 1) expression levels of the 5-HT1D receptor are very low compared with those of the 5-HT1B receptor, 2) the two receptors tend to be expressed together in many brain regions (although not in the periphery; Fig. 4), and 3) 5-HT1B and 5-HT1D receptors are coexpressed and...
may form heterodimers in certain brain cells. In essence, the 5-HT1B receptor is predominant, and, in the absence of selective compounds, it is very challenging to identify a separate population of 5-HT1D receptors in the brain. Except in rodents, hamster, and opossum, in which both receptors display somewhat different pharmacological profiles, the 5-HT1B Receptor is still largely predominant in terms of expression and function.

The 5-HT1B receptor was originally defined according to operational and transductional criteria, and it was initially thought to be a rodent-specific receptor (for references, see Hoyer et al. (1994)). In the 1970s, Peroutka and Snyder (1979) and others postulated that whereas $[\text{3H}]$-5-HT labeled 5-HT1 binding sites, $[\text{3H}]$-spiperone (and later $[\text{3H}]$-ketanserin) labeled 5-HT2 binding sites, and $[\text{3H}]$-LSD labeled both 5-HT1 and 5-HT2 binding sites. In 1981, Nelson and colleagues (Pedigo et al., 1981) proposed that 5-HT1 binding sites were a heterogeneous population, as $[\text{3H}]$-5-HT was displaced biphasically by spiperone; accordingly, the high affinity site for spiperone was called 5-HT1A, and the low affinity was 5-HT1B. Middlemiss et al. (1977) had reported earlier that certain indole $\beta$-blockers displayed high affinity for some 5-HT receptors. In 1982/1983, a breakthrough was reached when Hjorth et al. (1982) and Middlemiss and Fozard (1983) described 8-OH-DPAT as a selective 5-HT1A agonist. Furthermore, Gozlan et al. (1983) reported the selective labeling of 5-HT1A sites using $[\text{3H}]$-8-OH-DPAT. This allowed a clear definition of the 5-HT1A pharmacological profile and, by extension, of the features of non 5-HT1A/1B/1C receptor binding sites. In 1982/1983, a breakthrough was reached when Hjorth et al. (1982) and Middlemiss and Fozard (1983) described 8-OH-DPAT as a selective 5-HT1A ligand. Furthermore, Gozlan et al. (1983) reported the selective labeling of 5-HT1A sites using $[\text{3H}]$-8-OH-DPAT. This allowed a clear definition of the 5-HT1A pharmacological profile and, by extension, of the features of non 5-HT1A sites (Pazos et al., 1984a,b; Hoyer et al., 1985a,b). Thus, Palacios and Hoyer and colleagues (Hoyer et al., 1985b) at Sandoz in Basel characterized $[\text{3H}]$-mesulergine binding in the choroid plexus (Pazos et al., 1984a), which 5-HT competed for with high affinity, but the relatively low affinity of ketanserin and spiperone suggested a 5-HT1 receptor pharmacology. The features of $[\text{3H}]$-mesulergine-labeled sites were different from classic 5-HT2 binding sites labeled with, for example, $[\text{3H}]$-ketanserin. The novel $[\text{3H}]$-mesulergine-labeled binding site was named 5-HT1C (now 5-HT2C). Indeed, $[\text{3H}]$-mesulergine binding was also markedly different from 5-HT1B binding as evidenced in radioligand binding and autoradiographic studies (Hoyer et al., 1985a,b, 1986a,b; Pazos and Palacios, 1985; Pazos et al., 1985, 1987a,b). More specifically in rodents, 5-HT1B binding sites were characterized extensively with the iodinated version of cyanothipindolol, $[^{125}]$ICYP (Engel et al., 1981), a potent $\beta$-blocker with high affinity for 5-HT1B binding sites. These sites displayed high affinity for 5-HT, 5-carboxamidotryptamine (5-CT), some $\beta$-blockers, some ergolines, lysergic acid diethylamide (LSD), and RU24969 (Hoyer et al., 1985a, 1986a; Engel et al., 1986). Species differences in receptor pharmacology soon became evident with $[\text{3H}]$mesulergine, which had different binding profiles in rodents, pigs, and humans; this pattern would repeat itself with a number of 5-HT receptors, most prominently with the 5-HT1B receptor (Hoyer et al., 1988; Waebler et al., 1988a,b). The Sandoz group used rat, mouse, hamster, rabbit, guinea pig, cat, dog, bovine, human, and more atypical for research species such as pigeons, opossum, and trout (e.g., Waebler et al., 1988a,b, 1989a,b) to investigate the pharmacology of 5-HT1A, 1B, 1C, and 5-HT2 receptor–binding sites. In addition, the pharmacology, transduction, and distribution of non 5-HT1A/1B/1C receptor binding sites, identified initially in calf and human brain and then most other species investigated, was termed 5-HT1D receptor binding sites (Hoyer and Schoeffter, 1988; Schoeffter et al., 1988; Waebler et al., 1988a,b; Hoyer et al., 1988). Although the pharmacology of 5-HT1B and 5-HT1D binding sites/receptors displayed some distinct differences, their distribution pattern in brain was similar (if not overlapping), and they shared transductional and functional responses (Hoyer and Schoeffter, 1988; Schoeffter and Hoyer, 1989a, 1990). Therefore, rodent “5-HT1B” and nonrodent “5-HT1D” receptors were proposed initially to represent species homologs (Hoyer and Middlemiss, 1989), a view that was unequivocally confirmed when genetic and structural information became available with the cloning of these receptors (Voigt et al., 1991; Adham et al., 1992; Hamblin et al., 1992a,b; Hartig et al., 1992; Levy et al., 1992b; Maroteaux et al., 1992; Mochizuki et al., 1992).
However, matters were further complicated when Weinshank et al. (1992) identified two structurally distinct genes encoding human 5-HT$_1$ receptors with, at the time, almost overlapping pharmacological profiles, both resembling the 5-HT$_{1D}$ receptor. Earlier on, a canine “orphan” clone called RDC4 (later named 5-HT$_{1Da}$) had been reported to display a 5-HT$_{1D}$-like pharmacological profile (Libert et al., 1990; Hamblin and Metcalf, 1991; Maenhaut et al., 1991; Zgombick et al., 1991). A human receptor, initially called S12, was cloned independently and differed in sequence from the canine RDC4, yet it displayed 5-HT$_{1D}$-like pharmacology (Levy et al., 1992b). Since the operational profiles of these two new receptors were mostly indistinguishable, they were called 5-HT$_{1Da}$ (canine RDC4 and species homologs) and 5-HT$_{1Db}$ receptors (human S12 and species homologs). It soon became evident, however, that in spite of some fundamental differences in their pharmacological profiles (see below), the 5-HT$_{1Db}$ receptor was a human homolog of the rodent 5-HT$_{1B}$ receptor (displaying 96% overall sequence homology; Adham et al., 1992). The subsequent identification of the 5-HT$_{1Da}$ gene in rats confirmed that 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors represent just two different receptor classes (Hartig et al., 1992), which prompted a realignment of 5-HT receptor nomenclature to recognize primacy (preeminence) of the human genome (Hartig et al., 1996). As a result, the 5-HT$_{1Db}$ receptor was renamed 5-HT$_{1B}$ (subsuming the rodent 5-HT$_{1B}$ receptor), whereas the 5-HT$_{1Da}$ nomenclature was abandoned for 5-HT$_{1D}$ in recognition of the fact that this gene product encodes the 5-HT$_{1D}$ receptor (see Fig. 3; Hartig et al., 1996). This nomenclature for 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors has been used since 1996 and remains to date.

![Fig. 4. In situ hybridization detection of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor mRNA in rat brain (and 5-HT$_{1B}$ receptor mRNA in the posterior communicating artery [reverse autoradiogram]; K)]. 5-HT$_{1B}$ (B–K) and 5-HT$_{1D}$ (B’–J’) receptor mRNA. Ace, nucleus accumbens; AON, anterior olfactory nucleus; Arc, arcuate hypothalamic nucleus; AV, anteroventral thalamic nucleus; BL, basolateral amygdaloid nucleus; CA1, CA1 region of the hippocampus; CgCx, cingulate cortex; CPu, caudate putamen; DK, nucleus of Darkschewitsch; FrCx [layer VI], frontal cortex; IP, interpeduncular nucleus; IPIP, inner posterior subnucleus of the interpeduncular nucleus; layers III and V, parietal motor cortex; MVe, medial vestibular nucleus; PCA, posterior communicating artery; PO, primary olfactory cortex; Pur, Purkinje cells of the cerebellum; R, red nucleus; Re, reuniens nucleus; STh, subthalamic nucleus; SuG, superficial gray layer of the superior colliculus; Tu, olfactory tubercle. Scale bar, 5 mm (except K, where it is 0.5 mm). Adapted from Bruinvels et al. (1994a) (with permission).
The 5-HT$\textsubscript{1B}$-like receptor mediating smooth muscle contraction and inhibition of noradrenaline release showed close similarities to the 5-HT$\textsubscript{1A}$ and/or 5-HT$\textsubscript{1D}$ receptors; however, the lack of selective ligands at these receptors made it difficult to distinguish these receptors with confidence, hampering research for quite some time (Hoyer, 1988a; Hoyer et al., 1994). Clitherow et al. (1994) reported the properties of several compounds, including a piperazinylbenzanilide derivative, GR127935, which shows a high affinity for and selective antagonist activity at 5-HT$\textsubscript{1B/1D}$ receptors. But more importantly, the subsequent identification of potent and relatively selective antagonists at either the 5-HT$\textsubscript{1B}$ (SB224289; Hagan et al., 1997; Gaster et al., 1998) or 5-HT$\textsubscript{1D}$ (BRL15572; Price et al., 1997) receptors allowed responses to be attributed to either 5-HT$\textsubscript{1B}$ or 5-HT$\textsubscript{1D}$ receptors; for example, the sumatriptan-induced contraction of vascular smooth muscle was mediated via the 5-HT$\textsubscript{1B}$ receptor (e.g., De Vries et al., 1998, 1999; Verheggen et al., 1998, 2004).

Despite the 96% amino acid sequence homology in the transmembrane regions (Adham et al., 1992), the rodent 5-HT$\textsubscript{1B}$ receptor displays a distinct pharmacology compared with the 5-HT$\textsubscript{1B}$ receptor in other species (Hartig et al., 1996). The differences in the pharmacology of these species homologs are largely attributed to the mutation of a single amino acid in the transmembrane spanning region Asp$^{123}$ to Arg$^{123}$ (Adham et al., 1994a). Thus, CP93129 is a selective agonist at the rodent 5-HT$\textsubscript{1B}$ receptor, whereas some $\beta$-adrenergic antagonists, such as cyanopindolol, (–)pindolol, and (–)propranolol, are selective antagonists at the rodent 5-HT$\textsubscript{1B}$ receptor but not in other species. Unfortunately, no selective agonist is thus far available for the nonrodent 5-HT$\textsubscript{1B}$ receptor.

C. Receptor Structure and Transduction

The 5-HT$\textsubscript{1B}$ receptor gene is intronless, encoding for a 386-amino-acid protein in rat and mouse and 390-amino-acid protein in humans that displays the typical structure of a seven-transmembrane–spanning GPCR. The human, mouse, and rat 5-HT$\textsubscript{1B}$ receptor genes are located on chromosomes 6q13, 9E1, and 8q31, respectively. The rat receptor has 96% homology in the TMR with the human receptor, but the rat and mouse receptor (Voigt et al., 1991; Adham et al., 1992; Maroteaux et al., 1992) exhibit the typical 5-HT$\textsubscript{1B}$ receptor operational profile in contrast to the human receptor, which is close to the 5-HT$\textsubscript{1D}$ receptor operational profile (Levy et al., 1992b; Weinshank et al., 1992).

The 5-HT$\textsubscript{1B}$ receptor couples negatively to adenyl cyclase (Bouhelal et al., 1988; Hoyer and Schoeffter, 1988, 1991; Adham et al., 1992; Levy et al., 1992b; Maroteaux et al., 1992). Native 5-HT$\textsubscript{1B}$ receptors expressed in opossum kidney cells also mediate elevation of intracellular calcium (Zgombick and Branchek, 1998).

It is noteworthy that 5-HT$\textsubscript{1B}$ (and 5-HT$\textsubscript{1D}$) receptors have been crystallized (Wang et al., 2013; Wacker et al., 2013; McCory and Roth, 2015; see section XVI. A. 5-HT GPCRs), which greatly increases knowledge of the structure pharmacology of the receptor. Indeed, the conformation of a number of agonists is different when bound to 5-HT$\textsubscript{1B}$ or 5-HT$\textsubscript{1D}$ receptors, in spite of very similar orthosteric binding sites (Wacker et al., 2013; Wang et al., 2013; McCory and Roth, 2015). Sumatriptan and a range of other triptans fit well into the orthosteric pocket of the human 5-HT$\textsubscript{1B}$ receptor (in contrast to the 5-HT$\textsubscript{1D}$ receptor), thus confirming the high affinity and potency reported for the triptans at 5-HT$\textsubscript{1B}$ (and 5-HT$\textsubscript{1D}$) receptors. Some ergolines [LSD, metergoline, dihydroergotamine (DHE), ergotamine] bind to an accessory, possibly allosteric, site, which is located outside of the orthosteric pocket. It has been proposed that a short peptide, 5-HT-moduline, is a negative allosteric modulator of both 5-HT$\textsubscript{1B}$ and 5-HT$\textsubscript{1D}$ receptors (Roussel et al., 1998). Research concerning this peptide appears to have waned in recent years; the interested reader is directed to previous reviews on the subject (Fillion, 2000; Moret et al., 2003).

D. Distribution and Function

Autoradiographic studies performed in various species showed that both 5-HT$\textsubscript{1A}$ and 5-HT$\textsubscript{1C}$ (now named 5-HT$\textsubscript{2C}$) receptor binding was evident, in addition to 5-HT$\textsubscript{2}$ receptor binding. However, what was then called 5-HT$\textsubscript{1B}$ binding site was apparently absent in pig, calf, and human brain in contrast to rodent brain. This observation was extended to the guinea pig and then to an increasing number of other species (Hoyer at al., 1988; Waeb et al., 1988a,b; Hoyer and Middlemiss, 1989). Eventually, it was found that only rat, mouse, hamster, and opossum had a 5-HT$\textsubscript{1}$ receptor with a classic 5-HT$\textsubscript{1B}$ profile [see Hoyer et al. (1985a,b)]. By contrast, other species expressed what was called 5-HT$\textsubscript{1D}$ receptors in the brain (e.g., guinea pig, bovine, dog, rabbit, monkey, and humans) (see Waeb et al., 1988a, 1989a,b; Hoyer and Schoeffter, 1991; Hoyer et al., 1992). It was subsequently shown that $[^3$H]sumatriptan and a number of other triptans label both 5-HT$\textsubscript{1B}$ and 5-HT$\textsubscript{1D}$ sites. However, they may also label 5-HT$\textsubscript{1F}$ sites (Waeb and Moskowitz, 1995b). It also became evident when using selective antagonists that both 5-HT$\textsubscript{1B}$ and 5-HT$\textsubscript{1D}$ receptors could be detected in a single species (Bruinvels et al., 1993a,b, 1994a; Doménech et al., 1997; Bonaventure et al., 1997; Napier et al., 1999; Varnäs et al., 2001), but 5-HT$\textsubscript{1D}$ receptor levels were minor when compared with the 5-HT$\textsubscript{1B}$ receptor.

An elegant study demonstrated the rat brain autoreceptors mediating inhibition of 5-HT release displayed the pharmacology of the 5-HT$\textsubscript{1B}$ receptor (Engel et al., 1986). In various other species, including humans, inhibitory autoreceptors displayed 5-HT$\textsubscript{1D}$ receptor pharmacology (Schlicker et al., 1989). 5-HT$\textsubscript{1B}$ receptors were also reported to mediate inhibition of GABA, cholinergic, and glutamatergic neurotransmission (Maura and Raiteri, 1986; Johnson et al., 1992; Singer et al., 1996;
Chadha et al., 2000; Morikawa et al., 2000). The 5-HT$_{1B}$ receptor is highly concentrated in the substantia nigra (SN) and was shown to be negatively coupled to adenylyl cyclase activity (Bouhelal et al., 1988; Hoyer and Schoeffter, 1988, 1991).

Both 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors have a neuronal localization (Waebcr et al., 1990a,b; Bruinvels et al., 1991, 1992a,b, 1993a,b, 1994a,b; Sari et al., 1999), including in the trigeminal ganglia (Bruinvels et al., 1992a,b, 1994a,b; Hou et al., 2001; Ma, 2001; Potrebic et al., 2003). There is also evidence that both receptors colocalize and may form heterodimers (Xie et al., 1999; Ma, 2001).

Evidence from radioligand binding experiments using 5-HT neuronal lesions is equivocal regarding the location of the rat 5-HT$_{1B}$ receptor, with some studies finding that the lesion causes an upregulation of 5-HT$_{1B}$ binding sites and others finding a downregulation in the same areas. However, it is now clear that, like the 5-HT$_{1A}$ receptor, the 5-HT$_{1B}$ receptor functions as a presynaptic autoreceptor (see also section XVIII. 5-HT Receptors and the Brain). In situ hybridization studies have located mRNA encoding the 5-HT$_{1B}$ receptor in the dorsal and median raphe nuclei (Bruinvels et al., 1994a). Furthermore, 5-HT$_{1B}$ receptor mRNA in the raphe nuclei is markedly reduced by a 5-HT neuronal lesion. Together, these data suggest that 5-HT$_{1B}$ receptors are located both presynaptically (inhibitory autoreceptor) and postsynaptically (heteroreceptor) relative to 5-HT neurons [see Waebcr et al. (1990b)]; as an example of the latter, 5-HT$_{1B}$ heteroreceptors inhibit CGRP release from sensory perivascular nerves in the rat systemic vasculature (González-Hernández et al., 2010).

5-HT$_{1B}$ receptors are also located on cerebral arteries and other vascular tissues mediating direct vasoconstriction [see Villalón et al. (2003) and Villalón and Centurión (2007)]. Furthermore, it seems that the receptor may be “silent” in a number of vascular preparations, becoming responsive in conditions such as atherosclerosis or when costimulated with “priming” factors (Sahin Erdemli et al., 1991; Kaumann et al., 1993, 1994). Other peripheral effects have also been described, such as 1) inhibition of noradrenaline release from sympathetic nerves in vena cava (Göthert et al., 1986) and systemic vasculature (Villalón et al., 1998) and 2) inhibition of plasma extravasation produced by trigeminal ganglion stimulation (Buzzi and Moskowitz, 1991). 5-HT$_{1B}$ receptors also mediate vasoconstriction in the rat caudal arteries (Craig and Martin, 1993) and the canine external carotid circulation (De Vries et al., 1998) or guinea pig iliac artery (Sahin Erdemli et al., 1991), although endothelium-mediated relaxation has also been reported (Schoeffter and Hoyer, 1989, 1990). Interestingly, 5-HT$_{1B}$ receptor mRNA is more abundant within vascular smooth muscle cells compared with 5-HT$_{1D}$ receptor mRNA (Bouchelet et al., 1996; Sgard et al., 1996). The latter was reinforced by evident 5-HT$_{1B}$ but not 5-HT$_{1D}$ receptor immunoreactivity in cranial blood vessels (Longmore et al., 1997). Consistent with these findings, the subsequent advent of the potent and relatively selective antagonists at either the 5-HT$_{1B}$ (SB224289; Hagan et al., 1997; Gaster et al., 1998) or 5-HT$_{1D}$ (BRL15572; Price et al., 1997) receptors made it possible to demonstrate that the 5-HT$_{1B}$, but not the 5-HT$_{1D}$, receptor mediates the sumatriptan-induced contraction of vascular smooth muscle (e.g., De Vries et al., 1998, 1999; Verheggen et al., 1998).

E. Radioligand Binding

Autoradiographic studies using $[^3]$H-5-HT (in the presence of 8-OH-DPAT), $[^1]^{25}$IICYP (in the presence of isoprenaline), or $[^1]^{25}$I-carboxymethylglycyl iodotyrosinamide demonstrated a high density of 5-HT$_{1B}$ sites in the rat basal ganglia (particularly the substantia nigra, globus pallidus, ventral pallidum, and entopeduncular nucleus) but also in many other regions (Hoyer, 1988; Palacios et al, 1992; Hoyer et al., 1994; Mengod et al., 2010). The discrimination of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors in both rodent and nonrodent species has become more straightforward with the availability of a new 5-HT$_{1RD}$ radioligand, namely, $[^3]$H-GR-125743 (Doménech et al., 1997; Varnäs et al., 2001), or various triptans (Leyssen et al., 1996; Bonaventure et al., 1997; Napier et al., 1999) as well as cold ligands, which discriminate 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Price et al., 1997; Middlemiss et al., 1999).

IV. 5-HT$_{1D}$ Receptors

A. Introduction

To recap, following the cloning of 5-HT$_{1D}$a and 5-HT$_{1D}$b receptor genes in various species, 5-HT$_{1Da}$ was renamed the 5-HT$_{1D}$, and 5-HT$_{1Db}$ became the 5-HT$_{1B}$ receptor, keeping in mind that 5-HT$_{1D}$ expression levels are generally low compared with the 5-HT$_{1B}$ receptor (see section III. 5-HT$_{1B}$ Receptors for more detail).

B. Pharmacology

As noted in III. 5-HT$_{1B}$ Receptors, the pharmacological distinction of 5-HT$_{1B}$ from 5-HT$_{1D}$ receptors was a challenge until the advent of selective and silent antagonists (devoid of intrinsic activity) for 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Hagan et al., 1997).

A series of isochroman-6-carboxamide derivatives, including PNU109291 (Ennis et al., 1998), PNU142633 (McCah, 1997; McCall et al., 2002), and L775606 (MacLeod et al., 1997), have been reported to be selective 5-HT$_{1D}$ receptor agonists, although they display low intrinsic efficacy at primate 5-HT$_{1D}$ receptors in GTP-γS binding assays (Pregenzer et al., 1999).

The 5-HT$_{1D}$ receptor is potently antagonized by the 5-HT$_{1B,1D}$ receptor antagonist GR127935 (Clitherow et al., 1994; Skingle et al., 1996) and by the selective 5-HT$_{1D}$ receptor antagonist BRL15572 (Price et al., 1997). Additionally, some 5-HT$_{2}$ receptor antagonists (e.g.,
ketanserin and ritanserin) can discriminate the 5-HT1D receptor from 5-HT1B and 5-HT1F receptors (Hoyer et al., 1994), although this is highly species-dependent (see Branchek et al., 1995; Zgombick et al., 1995, 1997). Sumatriptan and the second-generation triptans are potent agonists at the 5-HT1D receptor (but also interact with 5-HT1B and 5-HT1F Receptors; Villalón et al., 2003; Table 6). It has been demonstrated that the 5-HT1D receptor is located preferentially on neuronal, rather than vascular, tissues (Ullmer et al., 1995; Sgard et al., 1996; Longmore et al., 1997).

Given the cardiovascular liabilities of triptans potentially via 5-HT1B receptors expressed by vasculature (Nilsson et al., 1999a,b), which is not the case for 5-HT1D receptors (Nilsson et al., 1999b), it was hypothesized that selective 5-HT1D receptor agonists may treat migraine, with reduced cardiovascular side effects. Unfortunately, this has not translated to the clinic; the 5-HT1D receptor agonist PNU-142633 was ineffective in the acute treatment of migraine (Gómez-Mancilla et al., 2010), although the intrinsic activity of this compound may complicate interpretation.

C. Receptor Structure and Transduction

The 5-HT1D receptor gene, like the 5-HT1B receptor gene, is intronless. The human 5-HT1D receptor gene is located on chromosome 1p34.3-p36.3, codes for a 377-amino-acid protein, and possesses 63% overall structural homology with the 5-HT1B receptor; the mouse and rat receptor genes are located on chromosomes 4D3 and 5q36 and code for 374-amino-acid proteins. These receptors are made of a single polypeptide chain that spans the membrane seven times, with the amino terminus being extracellular and the carboxyl terminus intracellular in the manner typical of GPCRs (Hamblin and Metcalf, 1991; Hamblin et al., 1992; Weinshank et al., 1992; Weydert et al., 1992). The receptor is negatively coupled to adenyl cyclase activity (Weinshank et al., 1992).

D. Distribution and Function

The distribution of 5-HT1D receptors is known but understood with less confidence because protein levels are low along with the relative difficulty of radio-ligands to discriminate this receptor from the 5-HT1B receptor. Receptor autoradiographic studies in rat (CP93129-insensitive [125I]carboxymethylglycyl iso-tirosinamide binding) or human (ketanserin-insensitive [3H]-sumatriptan binding) brain clearly indicate 5-HT1D receptor site is expressed in the basal ganglia (globus pallidus, substantia nigra, and caudate putamen) and also the hippocampus and cortex (Pineyro et al., 1995; Hou et al., 2001; Potrebic et al., 2003; Mengod et al., 2010).

### TABLE 6

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gp, guinea pig; m, mouse.

<sup>a</sup>Data taken from Leysen et al. (1996).
<sup>b</sup>Data taken from Napier et al. (1999).
<sup>c</sup>Data taken from Newman-Tancredi et al. (1997b).
<sup>d</sup>Data taken from Bou et al. (1997).
<sup>e</sup>Data taken from Brown et al. (1998c).
<sup>f</sup>Data taken from John et al. (1999).
<sup>g</sup>Data taken from Martin (1997).
<sup>h</sup>Data taken from Connor et al. (1997).
<sup>i</sup>Data taken from Wuch et al. (1997).
<sup>j</sup>Data taken from Beuer et al. (1998).
<sup>k</sup>Data taken from P. J. Pauwels, personal communication.
<sup>l</sup>Data taken from Adham et al. (1993).
<sup>m</sup>Data taken from P. Gupta, personal communication.
In situ hybridization experiments allow a greater confidence of gene expression, albeit not at the level of protein. Thus, 5-HT_{1D} mRNA is present in rat brain, including the caudate putamen, nucleus accumbens (NAC), olfactory cortex, dorsal raphe nucleus, and locus coeruleus (e.g., Bruinvels et al., 1994a; Mengod et al., 2010; Fig. 4). The mRNA shows low abundance in all regions and was undetectable in the globus pallidus, ventral pallidum, and substantia nigra where, as noted above, 5-HT_{1D} receptor sites appear to be present, which is perhaps indicative of the 5-HT_{1D} receptor being located predominantly on axon terminals of both 5-HT and non–5-HT neurons.

In the periphery, the presence of 5-HT_{1D} receptors is rather limited with evidence of presence in autonomic and trigeminal nerve terminals/ganglia (Molderings et al., 1996; Villalón et al., 1998).

The function of the 5-HT_{1D} receptor still remains, to some extent, enigmatic. There is little evidence supporting the role of the 5-HT_{1D} receptor in any pathology. The availability of suitable tools for investigation in vivo has limited the investigations into the importance of 5-HT_{1D} receptors; they have been identified as autoreceptors in the dorsal raphe (Pineyro et al., 1995) or terminal brain regions. Thus, given their autoreceptor activity, 5-HT_{1D} receptor antagonists may have antidepressant potential, and to maximize 5-HT release in terminal brain regions. Thus, given their autoreceptor activity, 5-HT_{1D} receptor antagonists may have antidepressant potential, and to maximize 5-HT release in terminal brain regions, 5-HT_{1D}, 5-HT_{1B}, and 5-HT_{1A} receptors must be blocked simultaneously.

Operationally, 5-HT_{1D} receptors mediate inhibition of noradrenaline release in human atrium. Additionally, the 5-HT_{1D} receptor seems to be involved in the inhibition of guinea pig dural plasma protein extravasation (Ennis et al., 1998) and the central trigeminal inhibitory effects by some antimigraine compounds (Mills and Martin, 1995; Cumberbatch et al., 1998; De Vries et al., 1999a,b; Villalón et al., 2003).

It has been proposed that the 5-HT_{1D} receptor modulates growth hormone release (Mota et al., 1995; Whale et al., 1999), although this requires clearer pharmacological verification.

V. 5-ht_{1E} Receptors

A. Introduction

There has been relatively limited research on the 5-ht_{1E} receptor, with an apparent lack of expression in rodents complicating preclinical studies. The lack of functional data concerning natively expressed 5-ht_{1E} receptors means by convention lower case appellation is still used for nomenclature.

With hindsight, the 5-ht_{1E} receptor was likely discovered by virtue of an atypical pharmacology of a [^3]H5-HT binding site in human frontal cortex (Leonhardt et al., 1989), which was sensitive to guanyl nucleotides, suggesting association with the GPCR family. The high affinity [^3]H5-HT displayed for the binding sites and the low affinity of drugs displaying affinity for the 5-HT_{2} receptor (e.g., mesulergine) supported membership of the 5-HT_{1} receptor family. However, the low affinity of 5-CT, the prototypical 5-HT_{1} receptor agonist, and detailed pharmacological characterization of the new [^3]H5-HT binding site in human and bovine cortical homogenates highlighted that this site likely represented a further member of the 5-HT_{1} family, and hence it was given that next available name: 5-HT_{1E} (Leonhardt et al., 1989; now reclassified as 5-ht_{1E} until a functional response in native tissue/cell preparation can be attributed).

B. Cloning and Distribution of 5-ht_{1E} Receptors

Soon after the 5-ht_{1E} receptor binding site was pharmacologically characterized by radioligand binding in human and bovine brain tissue, a human GPCR gene, termed S31, was cloned (Levy et al., 1992a; see h5-ht_{1E} in Fig. 5 for sequence) and assigned to human chromosome 6q14-q15 (Levy et al., 1994). When S31 was expressed in cell lines, the gene product was found to have pharmacological properties similar to the tissue-expressed 5-ht_{1E} receptor binding site, and the conclusion was that this gene encodes the protein for the receptor binding site discovered by Leonhardt et al. (1989) (McAllister et al., 1992; Zgombick et al., 1992; Guerdemann et al., 1993). However, the 5-HT_{1F} receptor, discovered subsequent to these early reports on the 5-ht_{1E} receptor, shares a high degree of sequence homology with the 5-ht_{1E} receptor compared with other 5-HT receptors (see Fig. 5 for h5-ht_{1E} and h5-HT_{1F} amino acid sequence alignment) and bears a pharmacological profile very similar to the 5-ht_{1E} receptor (Adham et al., 1993a,b; Lovenberg et al., 1993b). A careful examination of the binding data presented in the original report on the 5-ht_{1E} receptor (Leonhardt et al., 1989) suggests the binding site identified in this report is likely a composite of both 5-ht_{1E} and 5-HT_{1F} receptor binding sites. Drugs that can discriminate between these receptor subtypes were not identified until after a number of studies were published that attempted to identify the distribution of 5-ht_{1E} receptors via radioligand binding and autoradiography methodologies (Miller and Teitler, 1992; Beer et al., 1993; Barone et al., 1994; Stanton et al., 1996; Fugelli et al., 1997). This resulted in reports that incorrectly attributed [^3]H5-HT radioligand binding to the 5-ht_{1E} receptor in both rat and mouse brain tissue, species that were later identified to lack the 5-ht_{1E} receptor gene (Bai et al., 2004). Even those reports that used tissue from species that do express a 5-ht_{1E} receptor gene (e.g., humans, monkeys, guinea pigs, and bovine) were, in hindsight, confounded by the labeling of 5-HT_{1F} receptors and thus need to be viewed as data that reflects a mixture of 5-ht_{1E} and 5-HT_{1F} receptor populations. Subsequent pharmacological isolation of 5-ht_{1E} receptors (Klein and Teitler, 2012) has shown the following pattern of expression of the
5-HT$_{1e}$ receptor binding sites: olfactory bulb > frontal cortex > hypothalamus = cerebellum > brainstem-thalamus = striatum (Fig. 6). Issues impacting the ability to define with confidence the distribution of the 5-HT$_{1e}$ receptor were to some extent overcome with the development of an antibody recognizing the 5-HT$_{1e}$ receptor protein (Klein and Teitler, 2012), allowing protein expression to be revealed in native tissue. Such immunohistochemical studies revealed that the 5-HT$_{1e}$ receptor immunoreactivity was expressed in the olfactory bulb (glomerula cells), whereas in the hippocampus, expression is limited to the dentate gyrus (Klein and Teitler, 2012). Interestingly, 5-HT$_{1e}$ receptor immunoreactivity was also expressed in cerebral arteries (guinea pig; Klein and Teitler, 2012).

### C. Pharmacology

Bai et al. (2004) demonstrated that the rhesus monkey, pig, rabbit, and guinea pig express a homolog of the human 5-HT$_{1e}$ receptor gene. Because of the relative utility in preclinical models, the guinea pig 5-HT$_{1e}$ receptor (gp5-HT$_{1e}$) sequence was cloned for further study. The guinea pig homolog shares 88% nucleic acid and 95% amino acid sequence homology with the human 5-HT$_{1e}$ receptor. The pharmacological properties of the guinea pig recombinant 5-HT$_{1e}$ receptor correlate well with the human counterpart in terms of affinity ($R^2 = 0.99$) and potency ($R^2 = 0.96$), indicating a high degree of evolutionary conservation for the receptor.

Quantitative RT-PCR of guinea pig brain regions revealed high levels of gp5-HT$_{1e}$ receptor mRNA in the cortex, hippocampus, and olfactory bulb and moderate expression in some other regions, similar to the expression pattern in the human brain (Bai et al., 2004). Thus, the structural and pharmacological similarities of the human and guinea pig receptors, along with comparable patterns of expression in gross brain regions, lend a great deal of support to the guinea pig as a valid model to study the functionality of the h5-HT$_{1e}$ receptor.

Some attempts have been made to develop selective pharmacological tools for the h5-HT$_{1e}$ receptor (Dukat et al., 2004) but failed to identify 5-HT$_{1e}$ receptor ligands with affinities substantially higher than 5-HT. A relatively selective high-affinity 5-HT$_{1e}$ receptor ligand has been identified, BRL54443 (Brown et al., 1998); this drug displays similar affinities for the h5-HT$_{1e}$ and h5-HT$_{1F}$ receptors but, more usefully, at least 60-fold lower affinities for other 5-HT, dopamine, and adrenergic receptors. Few published reports exist regarding the pharmacology of this compound, and the reports of BRL54443 action in vivo have used species that do not express the 5-HT$_{1e}$ receptor (mice and rats; Adham et al., 1994; Brown et al., 1998; McKune and Watts, 2001; Watts et al., 2001; Hisadome et al., 2009; Granados-Soto et al., 2010).

A high throughput screening study conducted at the Scripps Research Institute’s Molecular Screening Center in collaboration with Milt Teitler’s laboratory, with the aim of identifying highly potent, selective agonists or antagonists for the 5-HT$_{1e}$ receptor, has been performed [PubChem BioAssay Database, AID (accession #): 567; 574; 613; 718; 726; 730]. Nearly 65,000 compounds from a broad range of structural classes were screened for agonist and antagonist properties at the h5-HT$_{1A}$ receptor and counter-screened at the 5-HT$_{1A}$ receptor as an assessment of selectivity. Though none of the compounds were highly selective for the h5-HT$_{1e}$ receptor, a number of high-potency agonists (EC$_{50}$ low nanomolars) were identified that displayed some structural similarity to BRL54443. In a more recent study comparing 51 tryptamine-based compounds for affinities at the human 5-HT$_{1e}$ and 5-HT$_{1F}$ receptors, no drugs were identified that showed a significant preference for the 5-HT$_{1e}$ receptor over the
5-HT1F receptor, again demonstrating the difficulties in attempting to identify 5-HT1e receptor–selective drugs (Klein et al., 2011).

D. Functions

Recombinant expression of the 5-HT1e receptor in cell lines demonstrates the coupling to G\textsubscript{i/o} signaling pathways (Levy et al., 1992a; Gudermann et al., 1993; Adham et al., 1994) although no signaling pathways have been identified in native tissues, and in the absence of a recognized functional response, the lower case appellation nomenclature is retained.

VI. 5-HT\textsubscript{1F} Receptors

A. Introduction

Although the first published reports for the 5-HT\textsubscript{1F} receptor occurred in 1992 and 1993 (Amlaiky et al., 1992; Adham et al., 1993b; Lovenberg et al., 1993), there is still only limited information about this receptor. Much of the current literature centers on possible roles for the 5-HT\textsubscript{1F} receptor in the treatment of migraine despite the 5-HT\textsubscript{1F} receptor displaying a broad distribution within the central nervous system (CNS), and it also appears to be expressed in the periphery.

B. Cloning and Structure

The 5-HT\textsubscript{1F} receptor was discovered as the result of homology cloning strategies. The first report of the cloning of the human 5-HT\textsubscript{1F} receptor was in a patent application by Synaptic Pharmaceuticals, Inc., (Weinshank et al. 1994, U.S. patent number 5,360,735, filed in 1992, issued in 1994). Amlaiky et al. (1992) reported the cloning of the mouse receptor (initially called 5-HT\textsubscript{1E}) that same year, followed by a peer-reviewed report on the human receptor (Adham et al., 1993b) and the cloning of both the rat and human versions (initially called 5-HT\textsubscript{1E-like}) of the receptor in 1993 (Lovenberg et al., 1993) (Table 7).

The 5-HT\textsubscript{1F} receptor gene is intronless, coding for a GPCR of 366 amino acids that conform to the classic GPCR structure, and has been sequenced in a number of species (mouse, rat, guinea pig, pig, human; Amlaiky et al., 1992; Adham et al., 1993b, 1997; Lovenberg et al., 1993; Bhalla et al., 2002b). In mouse and rat, it appears that the 5-HT\textsubscript{1F} receptor is encoded by three different mRNA transcripts (Guptan et al., 1997), which differ in their 3′ untranslated regions.

C. Distribution

1. mRNA. The initial studies of 5-HT\textsubscript{1F} receptor distribution located 5-HT\textsubscript{1F} receptor mRNA, either by RT-PCR or in situ hybridization (Fig. 7; Table 8). 5-HT\textsubscript{1F} receptor mRNA has a rather broad distribution within the brain; the cerebral cortex shows a relatively dense band of expression within the internal layers (approximately layers IV–VI), and hippocampal areas CA1–CA3 display relatively high expression, as do the thalamus and striatum.

Documentation of the presence of 5-HT\textsubscript{1F} receptor mRNA in peripheral tissues is limited to a few single reports in different species, including human, bovine, pig, rat, and rabbit (Table 8). Overall, there is still a need for a systematic mapping of the peripheral distribution of 5-HT\textsubscript{1F} receptors. Peripheral blood vessels such as the coronary artery have been reported to express 5-HT\textsubscript{1F} message, although the findings in human coronary appear variable, with Ishida et al. (1999) finding none, Nilsson et al. (1999b) reporting a strong signal, and Bouchet et al. (2000) reporting a weak signal in about 40% of patients. Bhalla et al.
(2002b) found a 5-HT_{1F} receptor mRNA signal in porcine coronary artery. Regardless, the 5-HT_{1F} receptor agonist LY334370 did not elicit contractions in human coronary artery (Nilsson et al., 1999b). 5-HT_{1F} receptor transcripts are also present in vascular preparations from the CNS (Table 8). However, when the brain microvessel preparations were treated to yield cultures of either smooth muscle or endothelial cells, no 5-HT_{1F} receptor mRNA was detected (Cohen et al., 1999).

In contrast to peripheral tissues, evidence for the presence of 5-HT_{1F} receptor mRNA in the peripheral nervous system is clear; thus, multiple studies in trigeminal ganglia and dorsal root ganglia (fresh or cultured) have identified 5-HT_{1F} receptor mRNA (Table 8).

2. Radioligand Binding. The first localization studies of 5-HT_{1F} receptor binding sites used [3H]sumatriptan, which displays high affinity for 5-HT_{1F} receptors as well as 5-HT_{1B/1D} receptors. This radioligand has been used to map the 5-HT_{1F} receptor in a variety of species (including “cold” competing ligands such as 5-CT or methiothepin prevents radiolabeling of 5-HT_{1B} and 5-HT_{1D} receptors; Waeber and Moskowitz, 1995b; Mengod et al., 1996; Scarr et al., 2004; Dean et al., 2006). Although [3H]5-HT can be used to label heterologous expression of 5-HT_{1F} receptors in cultured cells (Adham et al., 1993b), lack of selectivity makes 5-HT_{1F} receptor localization studies with [3H]5-HT challenging (Fugelli et al., 1997). A second useful radioligand to label the 5-HT_{1F} receptor is [3H]LY334370, albeit in the presence of 5-HT_{1A} receptor ligands such as 8-OH-DPAT to prevent labeling of the latter receptor (Wainscott et al., 2005). It is probably noteworthy that with all 5-HT_{1F} receptor–labeling studies, the lack of a selective radioligand necessitating the use of “cold” blocking drugs to better isolate radioligand binding to the 5-HT_{1F} receptor may underestimate the reported levels of 5-HT_{1F} receptors, or conversely, the specific radioligand binding signal consists of a heterogeneous population of sites that includes the 5-HT_{1F} Receptor; either way, interpretation should be made with caution.

3. Immunoreactivity. Relatively few studies have used antibodies to localize 5-HT_{1F} receptors (Table 9). The antibody studies of Ma (2001) and Classey et al. (2010) are consistent with investigations showing 5-HT_{1F} receptor mRNA in trigeminal ganglia and dorsal root ganglia (Table 8). Classey et al. (2010) describe 5-HT_{1F} receptor–like immunoreactivity in dorsal root ganglia

**TABLE 7**

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<td>Mouse</td>
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*Maassen Vandenbrink et al. (1998).*

![Fig. 7. In situ hybridization detection of 5-HT_{1F} receptor mRNA expression in guinea pig brain. (A) Frontal cortex (FRCX), anterior olfactory nucleus (AON). (B) Cingulate cortex (CGCX), septo-hippocampal nucleus (SHI), olfactory tubercle (TU), primary olfactory cortex (PO). (C) Claustrum (CL), medial amygdaloid nucleus (ME), supraoptic hypothalamic nucleus (SO). (D) Layer IV of the parietal motor cortex (IV), dentate gyrus (DG), and CA1-3 field (CA1-3) of the hippocampus are shown. Adapted from Bruinvels et al. (1994) (with permission).*
The 5-HT1F receptor, like the other members of the 5-HT family, has high affinity for 5-HT itself. This characteristic has driven much of the structural work devoted to developing high-affinity, selective 5-HT1F receptor agonists. Compared with 5-HT1A, 5-HT1B, and 5-HT1D receptors, the pharmacology described for 5-HT1F receptors is rather sparse. Because of a potential link between 5-HT1F receptor activation and the treatment of migraine (see below), most of the effort to develop selective compounds has focused on the development of orthosteric agonists. No selective orthosteric 5-HT1F receptor antagonists have been reported. Likewise, there are no literature descriptions of allosteric 5-HT1F receptor ligands.

Almost all of the structure activity relationship (SAR) work has come from the laboratories of Eli Lilly and Company, which had exclusive rights to the 5-HT1F receptor through a collaborative agreement with the patent holder, Synaptic Pharmaceutical Corporation. Table 10 summarizes published named compounds showing selectivity for the 5-HT1F receptor. Lasmiditan (COL-144, LY573144) and LY344864 display very good selectivity for the 5-HT1F receptor relative to all other 5-HT receptors, as does LY334370, except that it is only about eight- to ninefold selective over the 5-HT1A receptor. LY302148 was an early molecule that showed selectivity for the 5-HT1F receptor. Lasmiditan (COL-144, LY573144) is a departure from the other structures in that it contains no indole nucleus. The progression from a bicyclic aromatic nucleus (indole)
to the monocyclic nucleus of lasmiditan apparently involved some serendipity, as Zhang et al. (2015) describe how, in the process of producing a homolog of LY334370, a monocyclic intermediate was formed that had moderately good affinity for the 5-HT\(_{1\mathrm{F}}\) receptor. Expanding an SAR around this finding, they discovered several compounds that had high affinity and good selectivity for the 5-HT\(_{1\mathrm{F}}\) receptor (Zhang et al., 2015). Replacing the indole to eventually generate lasmiditan resulted in a highly selective 5-HT\(_{1\mathrm{F}}\) receptor agonist.

Several additional studies have generated significant SAR for 5-HT\(_{1\mathrm{F}}\) receptor agonists (Xu et al., 2001; Filla et al., 2003; Mathes et al., 2004; Zhang et al., 2004). The most potent and selective compounds are included in Table 11. These molecules represent riffs on the indoleethylamine core of 5-HT. For example, compound A (Table 11) employs N,N-dimethyltryptamine as its core, resulting in a molecule that has slightly lower affinity for the 5-HT\(_{1\mathrm{F}}\) receptor compared with LY334370 but overall greater selectivity. Compounds B, C, D, and E illustrate that the indole nucleus can be replaced with other bicycles (e.g., azaindole, indazole, and indoline), resulting in compounds with very good 5-HT\(_{1\mathrm{F}}\) receptor affinity and selectivity.

2. Partial Agonists and Antagonists. There are no published SAR studies for 5-HT\(_{1\mathrm{F}}\) receptor antagonists. Methiothepin has been used in vitro functional studies, but it is nonselective and of only moderate affinity for the 5-HT\(_{1\mathrm{F}}\) receptor (Adham et al., 1993b). 1-naphthylpiperazine and metergoline were described as partial agonists to inhibit adenylyl cyclase activity (Adham et al., 1993a). Other compounds have been reported as partial agonists at 5-HT\(_{1\mathrm{F}}\) receptor–stimulated \([\text{S}^{35}\text{S}]\text{GTP}\gamma\text{S}\) binding, such as dihydroergotamine (68% of 5-HT; Wainscott et al., 1998), LY302148 (17% of 5-HT; Wainscott et al., 1998), frovatriptan (46% of 5-HT; Nelson et al., 2010), 2-thienyl-LYX (61% of 5-HT; Filla et al., 2003), and 2-pyridyl-LYX (63% of 5-HT; Filla et al., 2003).

The prototypical triptan, sumatriptan, has relatively high affinity for the 5-HT\(_{1\mathrm{F}}\) receptor, and for comparison with the selective 5-HT\(_{1\mathrm{F}}\) compounds, Table 6 lists the affinities of a number of triptans across the 5-HT receptor subtypes. In efficacy studies using a \([\text{S}^{35}\text{S}]\text{GTP}\gamma\text{S}\) binding system to measure functional activity at the 5-HT\(_1\) family of receptors, naratriptan, rizatriptan, sumatriptan, and zolmitriptan were found to act as full agonists at all the 5-HT\(_1\) receptors that they stimulated. Frovatriptan, the only carbazole (i.e., a three-ring system containing indole), was a full agonist at 5-HT\(_{1\mathrm{B}}\) and 5-HT\(_{1\mathrm{D}}\) receptors but only a partial agonist at 5-HT\(_{1\mathrm{A}}\) and 5-HT\(_{1\mathrm{F}}\) receptors (Nelson et al., 2010). Hence, several triptans have high affinity for the 5-HT\(_{1\mathrm{F}}\) receptor and may activate 5-HT\(_{1\mathrm{F}}\) receptors in vivo at doses sufficient to also activate 5-HT\(_{1\mathrm{B}}\) and 5-HT\(_{1\mathrm{D}}\) receptors (as well as other receptors; Table 6).

E. Signal Transduction

The 5-HT\(_{1\mathrm{F}}\) receptor inhibits forskolin-stimulated adenylyl cyclase activity in recombinant cell systems

---

**TABLE 9**

Localization of 5-HT\(_{1\mathrm{F}}\) receptor protein using antibodies

<table>
<thead>
<tr>
<th>Tissue or Cells</th>
<th>Species</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trigeminal ganglion neurons</td>
<td>Rat</td>
<td>Immunohistochemistry</td>
<td>Ma, 2001</td>
</tr>
<tr>
<td>Superior, lateral, spinal, and medial vestibular nuclei</td>
<td>Rat</td>
<td>Immunohistochemistry</td>
<td>Ahn et al., 2009</td>
</tr>
<tr>
<td>Neurons of trigeminal ganglia and dorsal root ganglia (C2, C3, T5, and L6 levels)</td>
<td>Rat</td>
<td>Immunohistochemistry</td>
<td>Classy et al., 2010</td>
</tr>
<tr>
<td>Renal proximal tubule cells</td>
<td>Rabbit</td>
<td>Immunoblot</td>
<td>Garrett et al., 2014</td>
</tr>
</tbody>
</table>

---

**TABLE 10**

Affinities of selective 5-HT\(_{1\mathrm{F}}\) receptor agonists at cloned human 5-HT receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>LY392148</th>
<th>LY306258</th>
<th>LY344864(^a)</th>
<th>LY397584</th>
<th>Lasmiditan(^b) (COL-144, LY573144)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT(_{1\mathrm{F}})</td>
<td>(K_i), nM</td>
<td>(K_i), nM</td>
<td>(K_i), S.E.M., nM</td>
<td>(K_i), nM</td>
<td>(K_i), S.E.M., nM</td>
</tr>
<tr>
<td>5-HT(_{1\mathrm{B}})</td>
<td>12.0</td>
<td>557.0</td>
<td>16.4 ± 2.7 (9)</td>
<td>530</td>
<td>1046 ± 25 (10)</td>
</tr>
<tr>
<td>5-HT(_{1\mathrm{D}})</td>
<td>53.7</td>
<td>1698</td>
<td>189 ± 25 (10)</td>
<td>549</td>
<td>724 ± 15 (9)</td>
</tr>
<tr>
<td>5-HT(_{1\mathrm{A}})</td>
<td>2.9</td>
<td>794.3</td>
<td>281 ± 58 (10)</td>
<td>575</td>
<td>724 ± 15 (9)</td>
</tr>
<tr>
<td>5-HT(_{1\mathrm{C}})</td>
<td>50.1</td>
<td>73.6</td>
<td>176 ± 34 (10)</td>
<td>1415</td>
<td>776 ± 15 (9)</td>
</tr>
<tr>
<td>5-HT(_{2\mathrm{A}})</td>
<td>2.5</td>
<td>10.2</td>
<td>1.87 ± 0.34 (10)</td>
<td>6</td>
<td>5.5 ± 0.34 (10)</td>
</tr>
<tr>
<td>5-HT(_{3\mathrm{B}})</td>
<td>5.89</td>
<td>1072</td>
<td>1530 ± 200 (3)</td>
<td>3955</td>
<td>3890 ± 200 (3)</td>
</tr>
<tr>
<td>5-HT(_{3\mathrm{C}})</td>
<td>6.17</td>
<td>1072</td>
<td>1280 ± 90 (3)</td>
<td>1695</td>
<td>6166 ± 200 (3)</td>
</tr>
<tr>
<td>5-HT(_{3\mathrm{C}})</td>
<td>13.5</td>
<td>813</td>
<td>3250 ± 930 (3)</td>
<td>3499</td>
<td>309 ± 200 (3)</td>
</tr>
<tr>
<td>5-HT(_{6})</td>
<td>&gt;3 μM (2)</td>
<td>&gt;3 μM (2)</td>
<td>&gt;3 μM (2)</td>
<td>&gt;3 μM (2)</td>
<td>&gt;3 μM (2)</td>
</tr>
<tr>
<td>5-HT(_{7})</td>
<td>1550 ± 260 (5)</td>
<td>4851</td>
<td></td>
<td></td>
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</tbody>
</table>

\(^a\)Taken from Nelson et al. (2010).

\(^b\)Taken from Thebus et al. (1997).

\(^c\)Taken from Johnson et al. (2005).

\(^d\)Taken from Ramadan et al. (2003) (structure not reported).

\(^e\)Taken from Johnson et al. (1997).

\(^f\)Taken from Flaugh et al. (1998).
(Amlaiky et al., 1992; Adham et al., 1993b), with later studies suggesting coupling through Gi/Go proteins based on pertussis toxin sensitivity (Adham et al., 1993a). The human 5-HT1F receptor can also mediate inositol phosphate production and calcium flux through a pertussis toxin–sensitive mechanism in a recombinant system (Adham et al., 1993a). There are no reports on the transduction mechanisms affected by the 5-HT1F receptor in native tissues.

**F. Function**

As noted above, signal transduction studies with heterologously expressed 5-HT1F receptor readily identify responses, such as inhibition of forskolin-stimulated adenylyl cyclase activity (Amlaiky et al., 1992; Adham et al., 1993b), stimulation of inositol phosphate production and cellular calcium flux (Adham et al., 1993a), and stimulation of [35S]GTPγS binding (Wainscott et al., 1998).

Neurogenic dural inflammation has been used as a model for the development of antimigraine drugs, in which the triptans display efficacy. Early dogma considered the mechanism of action of the triptans to be selective agonists inhibit neurogenic inflammation in the guinea pig (Amrutkar et al., 2012). Additionally, LY344864 inhibited activation of second-order neurons in the trigeminal nucleus caudalis elicited by electrical stimulation of the dura mater in rats (Shepheard et al., 1999).

Little work has been devoted to potential peripheral actions of 5-HT1F receptors. Granados-Soto et al. (2010) have suggested that 5-HT1F receptors are involved in peripheral pain mechanisms, as LY344864 blocked nociception induced by formalin injection into the rat paw. In a more direct examination of peripheral tissues LY334370 and LY344864 stimulated the production of markers of mitochondrial biogenesis in isolated rabbit renal proximal tubules, which was diminished when the tubule preparation was subject to 5-HT1F receptor knockdown by siRNA transfection (Garrett et al., 2014). Administration of LY344864 in vivo to mice also led to an increase in a panel of markers for mitochondrial biogenesis in renal cortex, heart, and liver (Garrett et al., 2014).

**TABLE 11**

Affinities of selective 5-HT1F receptor agonists from SAR studies at cloned human 5-HT receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Compound A</th>
<th>Compound B</th>
<th>Compound C</th>
<th>Compound D</th>
<th>Compound E</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT1A</td>
<td>265 ± 99</td>
<td>620 ± 37</td>
<td>1000 ± 270</td>
<td>870</td>
<td>240</td>
</tr>
<tr>
<td>5-HT1B</td>
<td>1060 ± 204</td>
<td>270 ± 47</td>
<td>720 ± 220</td>
<td>2300</td>
<td>&gt;10 μM</td>
</tr>
<tr>
<td>5-HT1D</td>
<td>1620 ± 100</td>
<td>250 ± 38</td>
<td>720 ± 55</td>
<td>3100</td>
<td>2000</td>
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<tr>
<td>5-HT2A</td>
<td>8.2 ± 1.2</td>
<td>5.0 ± 0.5</td>
<td>5.5 ± 0.6</td>
<td>3.9</td>
<td>9</td>
</tr>
<tr>
<td>5-HT2B</td>
<td>2000 ± 390</td>
<td>1900 ± 1600</td>
<td>900 ± 41</td>
<td>2400 ± 740</td>
<td>Not determined</td>
</tr>
<tr>
<td>5-HT2C</td>
<td>4000 ± 350</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>Not determined</td>
</tr>
<tr>
<td>5-HT3</td>
<td>1770 ± 130</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

**Note:**

1. N-[3-(2-dimethylamino)-ethyl]-2-methyl-1H-indol-5-yl)-4-fluorobenzamide, from Xu et al. (2001).
4. 4-Fluoro-N-[3-(1-methyl-4-piperidinyl)-1H-indol-5-yl]benzamide, from Zhang et al. (2004).
5. 4-Fluoro-N-[3-(1-methyl-4-piperidinyl)-2,3-dihydro-1H-indol-5-y]benzamide, from Zhang et al. (2004).
Extensive study has failed to demonstrate that 5-HT_{1F} receptors contract blood vessels (e.g., Johnson et al., 1997; Cohen and Schenck, 1999, 2000; Razzaza et al., 1999; Shepheard et al., 1999; Bouchelet et al., 2000; Nelson et al., 2010). As detailed in *III. 5-HT_{1B} Receptors*, this contrasts functions associated with the 5-HT_{1B} receptor, through which triptans act to contract certain vascular tissues, including coronary artery, that can present serious adverse effects for patients, hence the optimism for 5-HT_{1F} receptor agonists as treatments for migraine with a reduced side-effect profile (see below).

**G. Clinical Relevance and Therapeutics**

As discussed above, because 5-HT_{1F} receptor agonism correlates with the pharmacology of the inhibition of a model of neurogenic dural inflammation—combined with the apparent absence of the 5-HT_{1F} receptor to contract vasculature—much of the translatable work concerning the 5-HT_{1F} receptor has centered on the treatment of migraine. This led, ultimately, to the development of LY334370, the first selective 5-HT_{1F} receptor agonist examined clinically for which efficacy to treat the pain of acute migraine attacks was noted (Goldstein et al., 2001). However, development of LY334370 was terminated because of safety concerns identified in animal toxicology studies (Ramadan et al., 2000; Shepheard et al., 1999; Ramadan and Buchanan, 2000). Further efforts to search for a more selective 5-HT_{1F} receptor agonist with preclinical toxicology issues identified LY573144. This molecule was subsequently out-licensed to CoLucid Pharmaceuticals (becoming COL-144; lasmiditan; Table 10), although the acquisition of CoLucid in 2017 by Eli Lilly returned the molecule to the parent company.

Lasmitidan is efficacious in alleviating symptoms of acute migraine in clinical trials. The first peer-reviewed published trial was a proof-of-concept investigation that demonstrated the efficacy of lasmiditan given intravenously (Ferrari et al., 2010). The primary efficacy measure was headache relief 2 hours after administration; a significant dose-response effect on efficacy separated lasmiditan from placebo. A trial using oral lasmiditan used the same primary efficacy measure, and all doses significantly improved headache response at 2 hours compared with placebo (Färkkilä et al., 2012). The most common side effects were dizziness, fatigue, vertigo, and paresthesia. Positive results from two pivotal phase III trials of lasmiditan (Kuca et al., 2018; Luo et al., 2019) led to subsequent marketing approval in 2019.

**VII. 5-HT_{2A} Receptors**

**A. Introduction**

The 5-HT_{2A} receptor (formerly 5-HT_{2}) was first identified as a binding site in rat brain with high (nanomolar) affinity for \[^{3}H\]spiperone and \[^{3}H\]ketanserin and low (micromolar) affinity for 5-HT (Peroutka and Snyder, 1979; Leysen et al., 1981). Soon after its discovery, the 5-HT_{2A} receptor was found to mediate several effects of 5-HT in the periphery, including platelet aggregation (De Clerck et al., 1982) and smooth muscle contraction (Cohen et al., 1981; Maayan et al., 1984; Engel et al., 1985). The peripheral 5-HT_{2A} receptors were originally classified as “D-type” 5-HT receptors based on pharmacological evidence (Bradley et al., 1986). The 5-HT_{2A} receptor was also the first 5-HT receptor found to couple to stimulate phosphatidyl inositol hydrolysis (Conn and Sanders-Bush, 1984).

**B. Cloning of the Gene**

The first 5-HT_{2A} receptor clone was isolated from rat brain cDNA libraries by homology screening based on the sequence of structurally related 5-HT_{2C} receptor (Pritchett et al., 1988; Julius et al., 1990). Functional expression of the cloned receptor confirmed coupling to phosphoinositide hydrolysis and Ca^{2+} mobilization. The human 5-HT_{2A} receptor was subsequently cloned by Saltzman et al. (1991) and displayed 87% homology with the rat receptor. The receptor contains 471 amino acids, with five potential glycosylation sites in the N-terminal extracellular domain and 11 potential phosphorylation sites in the C-terminal intracellular domain. The HTR2A gene encoding the human 5-HT_{2A} receptor has been mapped to chromosome 13q14–q21 (Sparkes et al., 1991). Analysis of the genomic structure of the human 5-HT_{2A} receptor revealed that it contains three exons separated by two introns, spanning more than 20 kb (Chen et al., 1992; Stamm et al., 1992). Other species from which the 5-HT_{2A} receptor has been cloned include hamster (Van Obberghen-Schilling et al., 1991), mouse (Yang et al., 1992), and pig and rhesus monkey (Johnson et al., 1995) (Table 12). Sequence alignments for the 5-HT_{2A} receptor from eight species are shown in Fig. 8.

1. **Regulation of 5-HT_{2A} Receptor Gene Expression**

The structure of the 5-HT_{2A} promoter region has been characterized in humans, rats, and mice; the promoters lack canonical TATA or CAAT boxes. Fragments of a 1.6-kb segment from the 5’ flanking region of the human gene showed promoter activity when transfected into receptor-expressing human cell lines (Zhu et al., 1995). The human promoter sequence contains multiple transcription initiation sites, along with several binding sites for transcription factors, including simian virus 40 promoter factor 1, polyomavirus enhancer activator 3, CAMP response element, and E-box binding proteins. There was also evidence that the 5’ flanking sequence contains an alternative promoter as well as a silencing element upstream from the translation start codon. Falkenberg et al. (2011) subsequently demonstrated that the human promoter contains a glucocorticoid receptor (GR) binding site at position −1420. Furthermore, the A-allele of the −1438G/A (rs6311) polymorphism is believed to create a binding site for the
transcription factor Th1/E47, which reportedly increases promoter activity (Smith et al., 2008).

Multiple cis elements in the mouse 5-HT2A promoter act in a dynamic manner to regulate transcription. The 5’ flanking region of the mouse 5-HT2A receptor gene contains a basal promoter (located −0.6 to −2.3 kb from the translational start site), which includes 11 transcription initiation sites, and binding sites for AP-2 (activating protein 2), polyomavirus enhancer activator 3, and simian virus 40 promoter factor 1 transcription factors (Ding et al., 1993; Toth et al., 1994). The activity of the basal promoter is attenuated in nonneuronal cells by two upstream repressor elements (extending from −2.3 to −4.2 kb), domains that presumably contain binding sites for transcription-inhibiting factors present in nonneuronal but not in neuronal cell types. Repressed genes can be reactivated in particular cell types via cell-specific activators, which may be responsible for 5-HT2A receptor expression in certain nonneuronal cells. For example, Ding et al. (1993) identified a domain located upstream (−4.2 to −5.6 kb) from the repressor elements that reactivates transcription of the 5-HT2A gene in C6 glioma cells.

The organization of the rat 5-HT2A promoter is similar to that of mice, containing multiple negative and positive regulatory elements. Garlow et al. (1994) identified a primary transcription initiation site at −1173 from the translational start site and a minimal promoter sequence in the 0.2-kb sequence immediately upstream from the primary initiation site. The activity of the minimal promoter is enhanced by proximal positive transitional elements 0.2–1.1 kb from the initiation site and attenuated by two distal negative domains located further upstream (1.1–2.2 and 2.3–2.5 kb from the initiation site, respectively). Analysis of the promoter and enhancer sequences revealed the presence of binding sites for the transcription factors nuclear factor 1, AP-1, AP-2, and Egr 1 as well as a GR element (Garlow et al., 1994; Garlow and Ciaranello, 1995). Experiments with transfected promoter-reporter plasmids showed that dexamethasone and AP-1 affected transcription of the promoter (Garlow and Ciaranello, 1995). The GR element appears to regulate 5-HT2A receptor transcription in rat brain, as evidenced by the significant increase in 5-HT2A mRNA expression induced by GR knockdown (Islam et al., 2004). AP-1 may play a role in agonist-induced upregulation of the 5-HT2A receptor in rat cerebellar granule cells (Chalecka-Franaszek et al., 1999). In contrast, Du et al. (1994, 1995) reported that the primary 5-HT2A transcriptional start site in rat myometrial smooth muscle cells is located at position −1120 from the translational start site. They also identified a basal promoter and two upstream repressor domains, but no enhancer region was detected. These discrepant findings may reflect cell type–specific differences in the function of the rat 5-HT2A promoter.

### C. Distribution

Many cell types in peripheral tissues express 5-HT2A receptors, including platelets, fibroblasts, lymphocytes, and myocytes. In the CNS, neurons are the main site of localization, although the presence of 5-HT2A receptors on nonneuronal cells types (glia, astrocytes) has also been reported (see below). The localization of 5-HT2A receptors in the brain has been mapped by a combination of receptor autoradiography, in situ hybridization, immunochemistry, and, more recently, PET neuroimaging. Receptor autoradiography studies using [3H]spiperone, [3H]ketanserin, [125I]DOI, and [3H]MDL 100907 as radioligands have revealed high levels of 5-HT2A receptor binding sites in many forebrain regions, including cortical and hippocampal areas, the basal ganglia, and olfactory tubercle, and the pattern is similar across species (e.g., Pazos et al., 1987b and López-Giménez et al., 1997). The distribution of 5-HT2A receptor binding sites agrees well with that of 5-HT2A mRNA in the cerebellum (Chalecka-Franaszek et al., 1999). In contrast, Du et al. (1994, 1995) reported that the primary 5-HT2A transcriptional start site in rat myometrial smooth muscle cells is located at position −1120 from the translational start site. They also identified a basal promoter and two upstream repressor domains, but no enhancer region was detected. These discrepant findings may reflect cell type–specific differences in the function of the rat 5-HT2A promoter.

## 5-HT2A receptor genes, transcripts, and proteins

<table>
<thead>
<tr>
<th>Organism</th>
<th>Location</th>
<th>Ensembl Gene ID</th>
<th>mRNA Transcript</th>
<th>Receptor Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bos taurus</strong></td>
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<td>ENSBTAG000000013498</td>
<td>NCBI RefSeq ID</td>
<td>Amino Acids (aa)</td>
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<td><strong>Equus caballus</strong></td>
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**TABLE 12**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Location</th>
<th>Ensembl Gene ID</th>
<th>mRNA Transcript</th>
<th>Receptor Protein</th>
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<td><strong>Sus scrofa</strong></td>
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<td>ENSSSCG00000009406</td>
<td>NM_214217</td>
<td>1432 bp</td>
</tr>
</tbody>
</table>
intracellular reserve of the 5-HT\textsubscript{2A} receptors and could be useful for the dynamic insertion of these receptors into the membrane.

A combination of immunocytochemical and in situ hybridization studies have investigated the cell types expressing the 5-HT\textsubscript{2A} receptor in cerebral cortex (Fig. 9). Early data demonstrated the presence of 5-HT\textsubscript{2A} receptors in cortical glutamatergic pyramidal (projection) neurons (Burnet et al., 1995), which have subsequently been mapped to specific cortical pathways (Vázquez-Borsetti et al., 2009; Mocci et al., 2014). Most such studies indicate that these cortical 5-HT\textsubscript{2A}

<table>
<thead>
<tr>
<th>MOUSE</th>
<th>HՀ:\Hկերահայք</th>
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<th>Տղաներ</th>
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<td>նույն</td>
<td>82</td>
<td>153</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 8. Primary structure of 5-HT\textsubscript{2A} receptors from various species.

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Barnes et al.
receptors are predominantly postsynaptic and localized to either the apical dendrites or soma of pyramidal neurons. However, 5-HT$_{2A}$ receptors have also been detected in GABAergic interneurons in the cortex (Morilak et al., 1994; Burnet et al., 1995; Mengod et al., 2015) and amygdala. There has also been an immunohistochemical analysis of 5-HT$_{2A}$ receptor localization in the ventral tegmental area, and the majority of immunolabeling was colocalized with tyrosine hydroxlyase, suggesting that the receptors are expressed on dopaminergic neurons; however, there is also evidence for localization on VTA GABA neurons (Doherty and Pickel, 2000; Nocjar et al., 2002).

More recently, 5-HT$_{2A}$ receptor localization has been mapped using bacterial artificial chromosome (BAC) transgenic mice engineered to express a fluorescent reporter (enhanced green fluorescent protein) under the control of the 5-HT$_{2A}$ receptor promoter, thus revealing 5-HT$_{2A}$ expression (Weber and Andrade, 2010). These data show a striking pattern of 5-HT$_{2A}$ receptor distribution at the regional and cellular levels. Mapping within the cortical microcircuitry revealed 5-HT$_{2A}$ receptor expression in specific lamina and in both pyramidal and interneurons. Interestingly, and in agreement with previous observations (Puig et al., 2010), expression was marked in cortical parvalbumin-positive interneurons, which underpin the formation of certain network oscillations (gamma frequency) thought critical for sensory information processing. The BAC transgenic mouse study and previous immunocytochemical studies (Stein et al., 2000; Weber and Andrade, 2010) also found 5-HT$_{2A}$ receptors are located on parvalbumin-containing interneurons in the basolateral nucleus of the amygdala. This finding is consistent with data from electrophysiological studies showing that in the amygdala, 5-HT acts on 5-HT$_{2A}$ receptors to potentiate GABAergic inhibition, including the GABA input to pyramidal neurons in this region (Jiang et al., 2009; Bocchio et al., 2015).

The study of BAC transgenic mice with enhanced green fluorescent protein under the control of the 5-HT$_{2A}$ receptor promoter (Weber and Andrade, 2010) did not report the presence of 5-HT$_{2A}$ receptors in nonneuronal cells, as suggested in earlier immunocytochemical studies (Xu and Pandey, 2000); however, further confirmation is awaited. Colocalization of 5-HT$_{2A}$ receptors with other 5-HT receptor subtypes has been reported (5-HT$_{1A}$, 5-HT$_{2C}$; e.g., Puig et al., 2010; Stephens et al., 2014; Mengod et al., 2015; Nocjar et al., 2015; Tian et al., 2016), providing further evidence of potential crosstalk in 5-HT signaling at the receptor level.

The development of a number of 5-HT$_{2A}$ receptor–selective radioligands has been useful for research tools, such as the imaging of 5-HT$_{2A}$ receptors in humans, with the most successful including the single-photon emission computerized tomography radioligand [123I] R91150 and the PET radioligands [18F]setoperone, [18F]altanserin, and [11C]MDL 100907 (Paterson et al., 2013; Herth and Knudsen, 2015). The first 5-HT$_{2A}$ receptor agonist PET ligand, [11C]N-(2-methoxybenzyl)-2,5-dimethoxy-4-bromophenethylamine ([11C]Cimbi-36), has recently been reported (Ettrup et al., 2014) and raised

**Fig. 9.** In situ hybridization detection of 5-HT$_{2A}$ receptor mRNA expression in rat and human brain. Reverse autoradiograms of the rat (A) and human brain (B–F). Human section: hippocampus and surrounding cortex (B), orbitofrontal cortex (Brodmann area 11) (C), striate cortex (Brodmann area 17) (D), superior temporal gyrus (Brodmann area 22) (E), and brainstem at the level of the raphe nucleus (F); no lack of 5-HT$_{2A}$ receptor mRNA was evident. Adapted from Burnet et al. (1995) (with permission).
the interesting possibility that this may be displaceable by endogenous 5-HT and therefore provide an index of 5-HT release. \[^{18}F\]Altanserin PET has also been used to quantify 5-HT release (Quednow et al., 2012). A potential confound for the development of 5-HT\(_{2A}\) receptor PET ligands is the reported high levels of 5-HT\(_{2A}\) receptors in the intracellular compartment (see above). If the significant levels of PET binding are intracellular, then it is less likely to be in a position to be displaced by endogenous 5-HT. However, collectively, these imaging studies confirm the cross-species localization of 5-HT\(_{2A}\) receptor and, more importantly, have opened the way for investigations of 5-HT\(_{2A}\) receptors in disease states.

**D. Post-translational Modifications and Impact**

\[^{N}\]-Glycosylation is known to regulate the intracellular sorting, surface expression, ligand binding, and signal transduction of GPCRs (Couvineau et al., 1996; Michineau et al., 2004). The extracellular N-terminus of the 5-HT\(_{2A}\) receptor contains five potential N-glycosylation sites; glycosylation is apparently required for the 5-HT\(_{2A}\) receptor to be targeted to the cell surface (Maginnis et al., 2010). Multiple proteins have been shown to interact with the 5-HT\(_{2A}\) receptor (Table 13; see also XVII. 5-HT GPCRs and their Interacting Proteins).

**E. Pharmacology**

The three members of the 5-HT\(_2\) receptor family share significant sequence homology (Fig. 10). Depending on the species examined, the seven transmembrane domains of 5-HT\(_{2A}\) and 5-HT\(_{2C}\) receptors display 79%–80% amino acid sequence conservation. Because of the high degree of structural homology, not surprisingly, 5-HT\(_{2A}\) and 5-HT\(_{2C}\) receptor binding affinities are highly correlated (Glennon et al., 1992a,b, 1994; Nelson et al., 1999). It is now recognized that most of the antagonists that have traditionally been used to block 5-HT\(_{2A}\) receptors, including \[^{N}\]-alkylpiperidines (e.g., ketanserin, ritanserin, pirenperone, and altanserin), ergolines (e.g., methysergide, metergoline, and LY53857), and tricyclic benzocycloheptenes (e.g., cyproheptadine and pizotifen), are also active at 5-HT\(_{2C}\) receptor (Newton et al., 1996; Hoyer, 1988a,b). For example, altanserin is only 20-fold selective for 5-HT\(_{2A}\) versus 5-HT\(_{2C}\) receptor sites (Table 14). Ketanserin has been used extensively for reported pharmacological definition of 5-HT\(_{2A}\) receptor responses and does show some selectivity for 5-HT\(_{2A}\) receptor (\(p_K = 8.7\)) compared with 5-HT\(_{2B}\) (\(p_K = 6.4\)) and 5-HT\(_{2C}\) (\(p_K = 6.8\)) receptors (Wainscott et al., 1996). However, ketanserin also has moderate affinity for adrenergic (\(\alpha_1\)) and histaminergic (H\(_1\)) receptors as well as 5-HT\(_{1D}\) receptors and the vesicular monoamine transporter (Érickson et al., 1996; Leysen et al., 1996; Bucholtz et al., 1999; Yoshio et al., 2001), which can complicate interpretation of arising data. Ritanserin is even less selective for 5-HT\(_{2A}\) versus 5-HT\(_{2C}\) receptors and also interacts with 5-HT\(_{D}\), 5-HT\(_{2B}\), 5-HT\(_{7}\), D\(_2\), D\(_3\), D\(_4\), H\(_1\), and \(\alpha_1\) sites (Bard et al., 1993; Monsma et al., 1993; Shen et al., 1993; Leysen et al., 1996; Seeman and Tallerico, 1998; Yoshio et al., 2001). The butyrophenone neuroleptic spiperone displays 500- to 2000-fold selectivity for 5-HT\(_{2A}\) versus 5-HT\(_{2C}\) and has often been used to discriminate those receptors, but it binds to numerous other receptors, including dopaminergic D\(_2\), D\(_3\), and D\(_4\) adrenergic \(\alpha_1\) and \(\alpha_2\); and 5-HT\(_{1A}\) and 5-HT\(_{7}\) receptors (Ruat et al., 1993b; Tang et al., 1994; Metwally et al., 1998; Corradetti et al., 2005). The spiperone derivative AMI-193 (8-[3-(4-fluorophenoxy)propyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one) is twice as selective as spiperone for 5-HT\(_{2A}\) versus 5-HT\(_{2C}\) but retains nanomolar affinity for D\(_2\) and 5-HT\(_{1A}\) (Ismaiel et al., 1993). Atypical antipsychotics such as risperidone, olanzapine, and clozapine block 5-HT\(_{2A}\) receptors with high affinity but generally have limited selectivity versus 5-HT\(_{2C}\) and dopamine receptors. By contrast, haloperidol and other typical antipsychotics have higher affinity for dopamine D\(_2\) receptors than for 5-HT\(_{2A}\) receptors.

The 4-carbinolpiperidines volinaserin (MDL 100907, M100907) and glemaserin (MDL 11939) were the first truly selective 5-HT\(_{2A}\) receptor antagonists. Volinaserin

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**TABLE 13**

Proteins reported to interact with the 5-HT\(_{2A}\) receptor

<table>
<thead>
<tr>
<th>Interacting Protein</th>
<th>Region of the 5-HT(_{2A}) Receptor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP-ribosylation factor 1 (Arl1)</td>
<td>C-terminus (NPxxY motif)</td>
<td>Robertson et al., 2003; Johnson et al., 2006</td>
</tr>
<tr>
<td>(\beta)-arrestin</td>
<td>ICL3, C-terminus (ASK motif)</td>
<td>Gelber et al., 1999; Bhattacharya et al., 2010</td>
</tr>
<tr>
<td>Calmodulin (CaM)</td>
<td>ICL2, C-terminus</td>
<td>Turner and Raymond, 2005</td>
</tr>
<tr>
<td>Caveolin-1 (Cav-1)</td>
<td>ND</td>
<td>Bhatnagar et al., 2004</td>
</tr>
<tr>
<td>Glutamine synthetase</td>
<td>ICL3</td>
<td>Sheffler et al., 2006</td>
</tr>
<tr>
<td>Jak2 kinase</td>
<td>ND</td>
<td>Guillet-Deniaux et al., 1997</td>
</tr>
<tr>
<td>Microtubule-associated protein 1A (MAP1A)</td>
<td>ICL3</td>
<td></td>
</tr>
<tr>
<td>Multi-PDZ domain protein 1 (MUPP1)</td>
<td>C-terminus (PDZ domain)</td>
<td>Jones et al., 2009</td>
</tr>
<tr>
<td>Na(^+)/H(^+) exchange regulatory factor 3 (NHERF3)</td>
<td>ND</td>
<td>Walther et al., 2015</td>
</tr>
<tr>
<td>Nucleoside-diphosphate kinase 3 (NME3)</td>
<td>ICL3</td>
<td>Shreffler et al., 2006</td>
</tr>
<tr>
<td>Paraoxonase 2 (PON2)</td>
<td>ICL3</td>
<td></td>
</tr>
<tr>
<td>Postsynaptic density protein 95 kDa (PSD-95)</td>
<td>C-terminus (PDZ domain)</td>
<td>Xia et al., 2003</td>
</tr>
<tr>
<td>Protein phosphatase 5 (PP-5)</td>
<td>ICL3</td>
<td>Shreffler et al., 2006</td>
</tr>
<tr>
<td>Ribosomal S6 kinase 2 (RSK2)</td>
<td>ICL3</td>
<td>Shreffler et al., 2006</td>
</tr>
<tr>
<td>Synapsin-associated protein 97 (SAP97)</td>
<td>C-terminus (PDZ domain)</td>
<td>Dunn et al., 2014</td>
</tr>
</tbody>
</table>

C-terminus, carboxyl-terminus; ICL2, second intracellular loop; ICL3, third intracellular loop; ND, not determined.

\(^{a}\)Specific to the human and monkey 5-HT\(_{2A}\) receptor.
has subnanomolar affinity for 5-HT_{2A} and 50- to 100-fold lower affinity for 5-HT_{2C} and α_{1} receptors, with negligible affinity for other investigated sites (Palfreyman et al., 1993; Kehne et al., 1996). In contrast to most 5-HT_{2A} receptor antagonists, the selectivity of M100907 for 5-HT_{2A} versus 5-HT_{2C} receptors has been verified in mice (Canal et al., 2013). Compared with volinaserin, glemaserin displays even greater selectivity for 5-HT_{2A} receptor (K_{i} = 2.893 nM) versus 5-HT_{2B} (K_{i} = 1419 nM), 5-HT_{2C} (K_{i} = 853.6 nM), and α_{1} (K_{i} = 588 nM) receptor sites (Dudley et al., 1988; Pehek et al., 2006). Like most drugs that block 5-HT_{2A} receptor responses, volinaserin and glemaserin were initially thought to be neutral antagonists but are now known to act as inverse agonists (Weiner et al., 2001; Aloyo et al., 2009). Although it was previously difficult to conclusively discriminate responses mediated by individual 5-HT_{2} receptor subtypes in vitro and in vivo, volinaserin or glemaserin at appropriate concentrations in combination with selective 5-HT_{2B} and 5-HT_{2C} receptor antagonists, such as RS-127445 and SB-242084, respectively, allow such pharmacological investigations.

In contrast to initial reports, it is now recognized that the 5-HT_{2A} receptor has high affinity for 5-HT. For example, 5-HT competes for the agonist radioligand [^{3}H]DOB with a K_{i} of 6.13 nM (Titeler et al., 1985), and [^{3}H]5-HT reportedly radiolabels the 5-HT_{2A} receptor with K_{d} = 1.3 nM (Sleight et al., 1996). In contrast, competition binding experiments with antagonist radioligands tend to underestimate the affinity of 5-HT_{2A} receptor agonists; 5-HT_{2A} receptors exist in low-affinity and high-affinity agonist binding conformations depending on whether they are coupled to G proteins, and only a small fraction of 5-HT_{2A} receptors are in the G protein–coupled, agonist high-affinity conformation at any given time. 5-HT_{2A} receptor antagonists bind to both conformations with equal affinity (Lyon et al., 1987; Glennon et al., 1988). Therefore, the apparent affinity of 5-HT_{2A} receptor agonists varies depending on the intrinsic activity of the radioligand used to label the receptor, with agonists displaying 10- to 100-fold higher affinity for agonist-labeled receptors versus antagonist-labeled receptors.

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Fig. 10. Primary structure of human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors.
which are nonselective for 5-HT2A versus 5-HT2C receptors but do not discriminate between the three 5-HT2 receptor subtypes. In contrast to most phenylalkylamines, receptors nonselectively and can inhibit the 5-HT transporter at micromolar concentrations (Ismaiel et al., 1990; Nagai et al., 2007; Blough et al., 2014). 2,5-Dimethoxy-4-bromobenzyl)-6-(2-methoxyphenyl)piperidine, DOB, and (4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)-2,5-dimethoxy-4-methylphenyl)-2-aminopropane, DOB, iodoamphetamine (DOI) and its structural analogs

Numerous 5-HT2A receptor agonists are available (Table 15). Tryptamines such as α-methyl-5-HT and 5-methoxy-N,N-dimethyltryptamine are widely used as 5-HT2A receptor agonists, but they tend to activate 5-HT receptors nonselectively and can inhibit the 5-HT transporter at micromolar concentrations (Ismaiel et al., 1990; Nagai et al., 2007; Blough et al., 2014). 2,5-Dimethoxy-4-iodoamphetamine (DOI) and its structural analogs can activate 5-HT receptors nonselectively and can inhibit the 5-HT transporter at micromolar concentrations (Ismaiel et al., 1990; Nagai et al., 2007; Blough et al., 2014). 2,5-Dimethoxy-4-bromobenzyl)-6-(2-methoxyphenyl)piperidine, DOB, and (4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)-2,5-dimethoxy-4-methylphenyl)-2-aminopropane, DOB, iodoamphetamine (DOI) and its structural analogs can activate 5-HT receptors nonselectively and can inhibit the 5-HT transporter at micromolar concentrations (Ismaiel et al., 1990; Nagai et al., 2007; Blough et al., 2014).

In addition to the classic signaling mediated by the PLC-IP3 cascade, the 5-HT2A receptor can activate a variety of other effector mechanisms. For example, the 5-HT2A receptor has been shown to stimulate phospholipase A2 (PLA2), resulting in increased production of the second messenger AA (Felder et al., 1990; Berg et al., 1996). The 5-HT2A receptor also increases release of the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006).

F. Function

1. Signaling. The Gaq–PLCβ cascade is the canonical signaling pathway coupled to 5-HT2A receptor activation (Conn and Sanders-Bush, 1984; Kendall and Nahorski, 1985), resulting in the hydrolysis of membrane phospholipids to inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). In turn, IP3 elevates the concentration of Ca2+ in the cytosol by releasing it from the endoplasmic reticulum (ER), whereas DAG activates protein kinase C (PKC) and facilitates its translocation from the cytosol to the membrane. Both PKC and Ca2+ are known to have widespread and far-reaching influences on intracellular signaling; PKC phosphorylates various target proteins, whereas Ca2+ is known to modulate the activity of enzymes (e.g., Ca2+/calmodulin-dependent kinases) and ion channels.

In addition to the classic signaling mediated by the PLC-IP3 cascade, the 5-HT2A receptor can activate a variety of other effector mechanisms. For example, the 5-HT2A receptor has been shown to stimulate phospholipase A2 (PLA2), resulting in increased production of the second messenger AA (Felder et al., 1990; Berg et al., 1996). The 5-HT2A receptor also increases release of the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006). The 5-HT2A receptor can also couple to the endocannabinoid 2-arachidolylglycerol (Parrish and Nichols, 2006).

Some of the aforementioned signaling pathways are coupled to the 5-HT2A receptor in a Gaq-independent manner. The ability of the 5-HT2A receptor to stimulate PLD is mediated by the monomeric G protein ADP-ribosylation factor-1 (ARF1), which interacts directly with the receptor (Barclay et al., 2011). The coupling of the 5-HT2A receptor to PLA2 in NIH3T3 cells appears to

<table>
<thead>
<tr>
<th>Ligand</th>
<th>h5-HT2A K (nM)</th>
<th>r5-HT2A K (nM)</th>
<th>h5-HT2C K (nM)</th>
<th>r5-HT2C K (nM)</th>
<th>h5-HT2A K (nM)</th>
<th>r5-HT2A K (nM)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>DOI</td>
<td>3.2</td>
<td>19.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Canal et al., 2013</td>
</tr>
<tr>
<td>Cimbi-5 (25I-NBOMe)</td>
<td>0.52</td>
<td>0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nichols et al., 2015</td>
</tr>
<tr>
<td>Cimbi-36 (25B-NBOMe)</td>
<td>0.19</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Juncoisa et al., 2013</td>
</tr>
<tr>
<td>(+)-(2S,6S)-DMBMPP</td>
<td>2.5</td>
<td>310</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Juncosa et al., 2013</td>
</tr>
<tr>
<td>25CN-NBOH (NBOH-2C-CN)</td>
<td>2.2</td>
<td>49.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Halberstadt et al., 2016</td>
</tr>
</tbody>
</table>

*Alternative names are shown in parentheses.
be mediated by two independent signaling cascades. In the first case, activation of Src by $G_{a_{i/o}}$-associated $G_{i/o}$ $G_{q}$ subunits results the formation of a ternary complex between SHC/GRB/SOS, which in turn activates the Ras–Raf–MEK–ERK1/2 cascade. In the second, activation of $R_{a_{12/13}}$ by $G_{a_{i/o}}$ stimulates p38 MAPK (Kurrasch-Orbaugh et al., 2003a). Reports also indicate PLC and PKC are not involved in the ERK activation produced by 5-HT2A receptor agonists in PC12 and vascular smooth muscle cells (Florian and Watts, 1998; Banes et al., 1999; Quinn et al., 2002). Nevertheless, in certain cell types, the coupling of ERK1/2 to 5-HT2A receptors is dependent on $G_{a_{q}}$. Activation of the Ras–Raf–MEK–ERK1/2 cascade by 5-HT2A receptors in tracheal smooth muscle cells and mesangial cells is downstream from $G_{a_{q}}$ and dependent on PKC (Hershenson et al., 1995; Watts, 1996; Greene et al., 2000). The coupling of the 5-HT2A receptor to MAPK may actually be ligand-specific. In mouse embryonic fibroblasts transfected with the 5-HT2A receptor, the stimulation of ERK1/2 by DOI is dependent on PLC, whereas the stimulation by 5-HT is PLC-independent and requires $\beta$-arrestin2 (Schmid et al., 2008).

The 5-HT2A receptor was one of the first receptors shown to display functional selectivity. According to Berg et al. (1998a,b), 5-HT2A receptors activate PLC and PLA$_2$ independently in CHO cells, and the relative efficacies of agonists differ depending on which response is measured. Subsequent studies confirmed the PLC–IP$_3$ and PLA$_2$–AA pathways coupled to 5-HT2A receptor in NIH3T3 cells have different receptor reserves, indicating they are activated independently (Kurrasch-Orbaugh et al., 2003b). It has been proposed that 5-HT2A receptor functional selectivity (biased signaling) may explain why certain 5-HT2A receptor agonists produce hallucinogenic effects, whereas other agonists such as lisuride are non-hallucinogenic (González-Maeso et al., 2007). Specifically, although both hallucinogenic and non-hallucinogenic 5-HT2A receptor agonists increase the expression of the immediate-early gene $c$-fos by activating $G_{a_{q}}$, only agonists with hallucinogenic effects increase the cortical expression of the immediate-early gene ($e.g.$, $r$-2) by activating $G_{a_{i/o}}$ and Src. Another group has reported that the activation of the 5-HT2A receptor by 5-HT and hallucinogens results in vastly different downstream signaling responses (Schmid and Bohn, 2010). The behavioral response to 5-HT in mice requires Akt phosphorylation and formation of a complex between Akt, Src, and $\beta$-arrestin2, whereas the response to hallucinogens is independent of Akt and $\beta$-arrestin2.

It is now apparent that many 5-HT2A receptor antagonists act in a functionally selective manner. Like most GPCRs, the 5-HT2A receptor is downregulated by exposure to agonists. Somewhat paradoxically, however, prolonged exposure to certain 5-HT2A receptor antagonists also induces 5-HT2A receptor downregulation (Leysen et al., 1986; Eison et al., 1989; Pranzatelli, 1991; Moreno et al., 2013). The anomalous downregulation of 5-HT2A receptors induced by antagonists is not a consequence of altered gene transcription (Roth and Ciaramello, 1991) but is likely caused by redistribution of the receptor from the cell surface to intracellular compartments (Willins et al., 1998, 1999; Bhatnagar et al., 2001). Interestingly, although the 5-HT2A receptor antagonists ketanserin, clozapine, and olanzapine reduce 5-HT2A immunoreactivity and [$^{3}$H]ketanserin binding in mouse frontal cortex, M100907, MDL 11939, and altanserin do not alter receptor expression (Yadav et al., 2011). These findings indicate that certain 5-HT2A receptor antagonists have agonist-like effects on the signaling pathways responsible for promoting receptor internalization. For example, although ketanserin and risperidone act as inverse agonists on the 5-HT2A–PLC and 5-HT2A–ERK pathways, they also act as 5-HT2A receptor agonists by stimulating $\beta$-arrestin translocation (Clarke et al., 2013). Nevertheless, it appears that 5-HT2A receptor antagonists can promote 5-HT2A receptor internalization through multiple mechanisms because clozapine does not recruit $\beta$-arrestin (Schmid et al., 2014). Clozapine does act in a functionally selective manner—it has been shown to induce Akt phosphorylation in cortical neuronal cultures via the 5-HT2A receptor (Schmid et al., 2014); but the mechanism by which clozapine promotes receptor downregulation remains to be determined.

Adding to the complexity of 5-HT2A receptor signaling is the discovery that it can oligomerize with other GPCRs, potentially allowing the 5-HT2A receptor to couple to an even wider range of effector mechanisms. Gonzalez-Maseo et al. (2008) have reported that 5-HT2A and mGlur$_2$ receptors form heterocomplexes, providing a mechanism whereby 5-HT2A receptors can modulate $G_{a_{i/o}}$ signaling. Heteromers between 5-HT2A and D$_2$ receptors (Borrot-escuela et al., 2010; Lukasiewicz et al., 2010) and 5-HT2A and CB$_3$ receptors (Vinâls et al., 2015) have also been detected in transfected cells, but the functional significance of these interactions and the extent to which they occur in native tissues is unclear.

2. Action in Cells, Tissues, and In Vivo. 5-HT2A receptor activation produces a variety of physiologic effects in peripheral tissues and in the CNS. There is evidence for 5-HT2A receptor involvement in the proliferation, differentiation, and contraction of vascular and extravascular smooth muscle (Maayani et al., 1984; Watts and Cohen, 1992; Fanburg and Lee, 1997; Shum et al., 2002; Itoh and Kajikuri, 2011) as well as increased contractility of cardiac muscle, where it is expressed in neonatal and reexpressed in failing and hypertrophic heart (Qvigstad et al., 2005c; Birkeland et al., 2007b; Brattelid et al., 2007a,b, 2012; Levy et al., 2008). Similarly, the 5-HT2A receptor increases the proliferation and synthesis of extracellular matrix proteins by glomerular mesangial cells (Kasho et al., 1998; Gööz et al., 2006). 5-HT2A receptor antagonists inhibit the platelet...
aggregation and shape change induced by 5-HT, DOB, and DOI (de Clerck et al., 1982, 1984; Seggel et al., 1987). The neuroendocrine effects of DOI in rats, including increases in the release of adrenocorticotropic hormone, corticosterone, oxytocin, renin, and prolactin, appear to be mediated by 5-HT_{2A} (Calogero et al., 1990; van de Kar et al., 2001; Zhang et al., 2002). 5-HT_{2A} receptor activation produces sympathoexcitatory effects and increases arterial pressure through a combination of central and peripheral effects (Tadepalli et al., 1975; McCall et al., 1987; Alper, 1990; Dedeoglu and Fisher, 1991; Chaouche-Teyara et al., 1994).

5-HT_{2A} receptor activation produces long-lasting increases in the excitability and firing rate of glutamatergic and GABAergic neurons, resulting in increased excitatory and inhibitory network activity (McCormick and Wang, 1991; Sheldon and Aghajanian, 1991; Cumming-Hood et al., 1993; Pessia et al., 1994; Marek and Aghajanian, 1996; Aghajanian and Marek, 1997; Shen and Andrade, 1998; Zhou and Hablitz, 1999; Lambe and Aghajanian, 2006, 2007; Béïque et al., 2007; Benekareddy et al., 2010; Avesar and Gullidge, 2012; but see also Carr et al., 2002; Tian et al., 2016). These electrophysiological effects are absent in Htr2A-knockout mice and are restored upon conditional cortical rescue of 5-HT_{2A} receptors (Weisstaub et al., 2006). Reductions in resting K⁺-conductances are thought to contribute to many of the excitatory effects of 5-HT_{2A} receptor activation. For example, 5-HT_{2A} receptor activation induces membrane depolarization, reduces afterhyperpolarization, and evokes a slow afterdepolarization in layer V pyramidal neurons in the PFC, effects thought to be mediated by inhibition of the Ca^{2+}-activated K⁺-current I_{K(Ca)} and activation of the Ca^{2+}-dependent nonselective cation current I_{CAN} (Araneda and Andrade, 1991; Tanaka and North, 1993; Villalobos et al., 2005, 2011; Zhang and Arsenault, 2005). The ability of 5-HT_{2A} receptor activation to enhance motoneuron excitability is mediated by inhibition of a leak K⁺-current I_{K(leak)} and enhancement of the hyperpolarizing-activated nonselective cation current (Garratt et al., 1993; Hsiao et al., 1997; Larkman and Kelly, 1998; Xu et al., 2009). TASK-1 and TASK-3 two-pore domain K⁺-channels are believed to be responsible for I_{K(leak)} in motoneurons (Talley et al., 2000; Larkman and Perkins, 2005). Likewise, the 5-HT_{2A} receptor-mediated depolarization produced by 5-HT in entorhinal cortex interneurons occurs as a consequence of TASK-3 channel inhibition (Deng and Lei, 2008). 5-HT_{2A} has been shown to depolarize nucleus accumbens medium spiny neurons by inhibiting an inwardly rectifying K⁺-current (North and Uchimura, 1989). There is also evidence that 5-HT_{2A} receptor–induced arterial constriction is mediated by inhibition of Kv channels (Cologludo et al., 2006; Sung et al., 2013). Other ion channels known to couple to the 5-HT_{2A} receptor include rapidly inactivating and persistent voltage-dependent Na⁺ channels (Carr et al., 2002), CaV 1.2 L-type Ca^{2+} channels (Day et al., 2002), Kv1.2 K⁺ channels (Lambe and Aghajanian, 2001), and voltage-independent Ca^{2+} channels (Hagberg et al., 1998).

### G. Clinical Relevance

5-HT_{2A} receptors are clinically relevant to numerous CNS disorders, ranging from mood disorder and schizophrenia to drug dependence. The links between the 5-HT_{2A} receptor and the causes of such CNS disorders are not well understood. Early life stress has been shown trigger strong upregulation of the functional effects of 5-HT_{2A} receptors on electrophysiology, signaling, and gene expression in prefrontal cortex (Benekareddy et al., 2010). Emerging findings regarding 5-HT_{2A} gene polymorphisms and epigenetic regulation of 5-HT_{2A} receptor expression are also of great interest. The association between the 5-HT_{2A} receptor and successful treatment of certain CNS disorders is strong, and there is an ongoing active research interest in the therapeutic potential of 5-HT_{2A} receptor ligands. The majority of these studies have had a particular focus on schizophrenia and depression and the actions of antipsychotic and antidepressant drugs.

In schizophrenia, there are reports of reduced 5-HT_{2A} receptor binding in frontal cortex of postmortem brains, and there is supporting evidence from PET imaging studies for similar changes, as recently reviewed (Selvaraj et al., 2014). However, given that not all the PET studies used 5-HT_{2A} receptor–selective radiotracers and that there are inconsistencies in the postmortem studies, the extent to which impaired cortical 5-HT_{2A} receptor function contributes to the symptoms of schizophrenia is unclear.

The psychotomimetic effects of hallucinogens such as LSD and psilocybin are undoubtedly linked to activation of 5-HT_{2A} receptors (Halberstadt, 2015). Furthermore, commonly used second-generation antipsychotics such as clozapine, risperidone, and sertindole are potent 5-HT_{2A} receptor antagonists in addition to having affinity at other 5-HT receptor subtypes and receptors for other neurotransmitters (Meltzer, 2012). 5-HT_{2A} receptor blockade has been linked to the antipsychotic efficacy of second-generation antipsychotics as well as a reduced side effect profile (Meltzer and Massey, 2011). This provided support for the idea that selective 5-HT_{2A} receptor antagonists might be useful as a monotherapy for some psychoses and as antipsychotic augmenting agents, although schizophrenia trials with the selective 5-HT_{2A} receptor antagonist MDL 100907 were disappointing. Nevertheless, the subsequent discovery that 5-HT_{2A} receptor inverse agonists have antipsychotic actions in preclinical models (Weiner et al., 2001) suggests an alternative way to treat psychosis. This thinking advanced to the clinical development of pimavanserin (Vanover et al., 2006) for the treatment of psychosis in patients with Parkinson disease (Meltzer and Roth, 2013). In 2016, pimavanserin was approved by the U.S. FDA as a treatment for hallucinations and delusions associated with Parkinson disease. Although the degree
of inverse agonism required for an antipsychotic effect is uncertain, 5-HT2A receptor inverse agonists are currently considered as a potential new generation of antipsychotic agents.

In postmortem studies of suicide victims, there is evidence of an upregulation in 5-HT2A receptors in cortex [for review, see Meyer (2013)]. This finding parallels results from PET imaging studies that report increased 5-HT2A receptor binding in patients with depression, particularly in association with severe pessimism (Meyer et al., 2003; Bhagwagar et al., 2006). The latter observations fit with findings in healthy subjects, in that individuals with high scores for pessimistic personality had higher cortical 5-HT2A receptor binding (Frokjaer et al., 2008). It has been hypothesized that the increase in cortical 5-HT2A receptor binding in depression is associated with severe pessimism and arises as an adaptive response to chronic 5-HT deficiency (Meyer, 2013).

The above findings in patients with depression link to evidence that certain antidepressants, and particularly tricyclics, have high affinity for 5-HT2A receptors (e.g., Millan, 2006). Though tricyclic antidepressants have high affinity for multiple receptors, it can be argued that evidence of the superior therapeutic efficacy of tricyclic antidepressant drugs over other antidepressant drug classes, particularly in the treatment of severe melancholic depression, is linked in part to their ability to block 5-HT2A receptors. Thus, 5-HT2A receptor antagonists augment the effect of 5-HT uptake inhibitors in preclinical models (Marek et al., 2005; Boothman et al., 2006), and drugs with 5-HT2A receptor antagonist properties are advocated as an add-on to antidepressant therapy in treatment-resistant cases (Marek et al., 2003); all are a possible link to evidence of an inhibitory 5-HT2A receptor-mediated feedback on 5-HT neurons (Sharp et al., 2007).

Aside from schizophrenia and depression, 5-HT2A receptors are relevant to the pathophysiology of many other CNS disorders. Currently, there is large and ongoing research effort to understand the importance of polymorphic variation in the 5-HT2A receptor gene to a large variety of psychiatric disorders (e.g., Smith et al., 2013; Paquette and Marsit, 2014). Despite the many reports of associations between 5-HT2A gene polymorphisms and neuropsychiatric disorders, some of which are subject to careful meta-analysis, effect sizes are at best modest, and the role of genetic 5-HT2A variability in mental health continues to be uncertain. This work is expanding to incorporate recent findings of epigenetic variation of the 5-HT2A receptor gene and specifically methylation sites near the gene promoter region [for review, see Paquette and Marsit (2014)].

Ligands for 5-HT2A receptors have a current untapped potential for the management of cognitive dysfunction. There are well established links between the receptor and the formation of different memory types, including recognition (Morici et al., 2015) and fear memories (Bombardi and Di Giovanni, 2013; Zhang and Stackman, 2015). The latter connects with the considerations of ecstasy and other similar psychotomimetics for the management of post-traumatic stress disorder (Smith et al., 2014; Sessa and Nutt, 2015). There are also very interesting links between the 5-HT2A receptor and impulse control; in animal models, 5-HT2A receptor antagonists consistently reduce measures of impulsivity (Higgins et al., 2003; Winstanley et al., 2004; Fletcher et al., 2007; Winstanley, 2011) and attenuate abuse-related effects of cocaine (Howell and Cunningham, 2015). This raises the possibility that 5-HT2A antagonists/inverse agonists may have utility in the control of addictions and disorders of impulse control more generally.

5-HT2A receptors are required for entry of JC polyomavirus (JCV) into cells (Elphick et al., 2004). JCV causes progressive multifocal leukoencephalopathy, a fatal demyelinating disease. JCV attaches to the sialic acid receptor motif α2,6-linked lactoseries tetrascarhide c on the cell surface and then undergoes endocytosis with the 5-HT2A receptor (Assetta et al., 2013). 5-HT2A antagonists have been found to inhibit infection of cell by JCV (O’Hara and Atwood, 2008). Furthermore, treatment with mirtazapine, an antidepressant that acts as a 5-HT2A receptor antagonist, has proven beneficial in patients infected with JCV (Verma et al., 2007; O’Hara and Atwood, 2008; Cettomai and McArthur, 2009; Park et al., 2011a).

5-HT2A receptor agonists have ocular hypotensive effects. R-(-)-DOI, 5-methoxy-N,N-dimethyltryptamine, and α-methyl-5-HT have been shown to lower intraocular pressure in a cynomolgus monkey model of ocular hypertension (May et al., 2003). 5-HT2A receptors have been identified in tissues involved in the regulation of aqueous humor dynamics, including ciliary muscle (Sharif et al., 2006a), ciliary epithelium (Inoue-Matsuhisa et al., 2003), and trabecular meshwork (Sharif and Senchyna, 2006; Sharif et al., 2006b). Studies have not completely elucidated the mechanism for the reduction of intraocular pressure by 5-HT2A receptor agonists, but increased uveoscleral outflow may play a role (Gabelt et al., 2005). This effect led to the evaluation of 5-HT2A receptor agonists as potential treatments for glaucoma. To reduce the potential for psychotropic side effects, much of the work to develop 5-HT2A receptor agonists as ocular hypotensive agents has focused on compounds with limited blood-brain barrier permeability. Several 5-HT2A receptor agonists with limited blood-brain barrier permeability have been shown to lower intraocular pressure following topical ocular administration to monkeys, including AL-34662 (1-[(2S)-2-aminopropyl]-1H-indazol-6-ol) (Sharif et al., 2007), (8R)-1-[(2S)-2-aminopropyl]-8,9-dihydro-7H-pyrano[2,3-g]indazol-8-ol (May et al., 2015), phenylisopropylamines incorporating α-hydroxy or α-methoxy substituents (Glennon et al., 2004), and benzodifuran derivatives (Feng et al., 2007).
Ketanserin can reportedly lower interocular pressure in patients with glaucoma (Costagliola et al., 1993; Mastropasqua et al., 1997). However, the ocular hypotensive effect of ketanserin is attenuated in unilaterally sympathectomized rabbits and therefore is thought to be mediated by a blockade of α1-adrenoceptors (Chang et al., 1985).

VIII. 5-HT2B Receptor

A. Introduction

5-HT–induced contractions of rat stomach fundus strips were used as a sensitive bioassay for 5-HT long before more specific analytical assays became available (Vane, 1957); however, the 5-HT receptor was only defined more than three decades later as the 5-HT2B receptor. Early pharmacological studies suggested a strong similarity to the 5-HT2C receptor, in line with the high potency of 5-HT and blockade by 5-HT2 receptor antagonists. However, 5-HT2C receptor mRNA was absent, and molecular cloning identified the new receptor, first in rat and mouse (Foguet et al., 1992a,b; Kursar et al., 1992; Loric et al., 1992; Wainscott et al., 1993) and then in humans (Choi et al., 1994; Kursar et al., 1994; Schmuck et al., 1994; Wainscott et al., 1996). Initially, given the tissue associated primarily with the functional receptor, the receptor was named the 5-HT2b receptor, but it was reclassified subsequently to the 5-HT2B receptor to better fit 5-HT receptor nomenclature. The pharmacological characterization of this receptor in various species confirmed its close relationship to both 5-HT2C and 5-HT2A receptors, as expected from their closely related structural and transductional features. The physiological and pathological functions of the 5-HT2B receptor both in the peripheral and central nervous systems are rather unique.

B. Expression Profile

1. Peripheral Expression.

5-HT2B receptor mRNA and/or protein are detected in the stomach fundus, intestine, liver, kidney, pancreas, spleen, lung, and heart in rats, mice, and humans (Kursar et al., 1992; Choi et al., 1994; Kursar et al., 1994; Choi and Maroteaux, 1996). Functionally active 5-HT2B receptors are present in human uterine smooth muscle (Kelly and Sharif, 2006). The 5-HT2B receptor is also expressed in the vasculature, in various smooth muscle cells (Ullmer et al., 1995), endothelial cells of pig pulmonary arteries (Glusa and Pertz, 2000), human meningeal blood vessels (Kursar et al., 1992; Choi et al., 1994; Kursar et al., 1994; Choi and Maroteaux, 1996). 5-HT2B receptor mRNA was reported in rat cultured astrocytes (Sanden et al., 2000; Osredkar and Kržan, 2009). Microglial expression of 5-HT2B receptors was documented in primary cultured and acutely isolated adult microglia, using two-photon microscopy on brain slices (Kolodziejczak et al., 2015) and patch-clamp studies in cultured microglia (Krabbe et al., 2012), suggesting 5-HT2B receptor may mediate modulation of microglial functions such as phagocytosis and migration, synaptogenesis, and neuronal death.

The 5-HT2B receptor modulates the release of rat growth hormone (GH) in the pituitary (Papageorgiou and Denef, 2007). The 5-HT2B receptor is expressed in the spinal cord (Helton et al., 1994; Holohan and Hackman, 2004), the rat organ of Corti lateral wall, and spiral ganglion subfractions (Oh et al., 1999) and is upregulated in ageing mice cochlea (Tadros et al., 2007). 5-HT2B receptor mRNA is expressed predominantly in the human retina, ciliary body, ciliary epithelium, choroid, conjunctiva, and iris. 5-HT2B receptor mRNA was also documented in optic nerve tissue of human donor trabecular meshwork cells (Sharif and Senceryna, 2006).

C. Post-translational Modifications and Impact


The 5-HT2B receptor gene is composed of four Exons, including one 5’ noncoding exon and three coding exons in all vertebrates that have been sequenced.

2. Gene Regulation.

The transcriptional regulation of the 5-HT2B receptor gene is not well understood. In breast tumors, the c-Myc transformation induces an increased 5-HT2B receptor expression (Pai et al., 2009). In human umbilical endothelial cells, Wnt2 downstream targets the 5-HT2B receptor gene (Klein et al., 2009). The 5-HT2B receptor expression is downregulated via nuclear factor-κB (NF-κB) (RelAp65-p52) in C-reactive protein–stimulated pulmonary arterial endothelial cells (Wynants et al., 2013). In pulmonary
artery smooth muscle cells. 5-HT induces 5-HT$_{2B}$ receptor mRNA expression, which is inhibited by peroxisome proliferator–activated receptor (PPAR)$\gamma$ activation and suggests the suppression of AP-1 activity (Liu et al., 2012b; Maroteaux, 2013). The presence of retinoic acid response elements in the 5-HT$_{2B}$ receptor promoter suggests a negative regulatory relationship between retinoic acid and 5-HT signaling at sites of epitheliomesenchymal interaction (Bhasin et al., 2004).

3. Receptor Isoforms. Only a few studies have investigated putative splice variants in the 5-HT$_{2B}$ receptor. In puffer fish, 5-HT$_{2B}$ receptor splicing variants have been identified (De Lucchini et al., 2001). Splice variant 1 contains a 136-bp deletion that eliminates a portion of exon 2 by alternative splice sites located within transmembrane regions I and II, which results in a premature stop codon to produce a very short truncated 31-amino-acid protein. Splice variant 2 contains a 201-bp deletion because of an exon-skipping mechanism that eliminates exon 3 (which is also found in human and mice). The resulting RNA retains the same open reading frame. Splice variant 3 contains a 19-bp deletion, probably by an alternative 5’ splice site upstream of the canonical 5’ splice site of intron C; this leads to a frame shift and a premature termination codon, which was also found in human and mice. This short variant results in a putative 177-amino-acid protein, with 28 specific residues at the carboxyl terminus. The functions of these splice variants remain to be defined. Finally, an exon-skipping mechanism that eliminates exon 3 was found in fish, humans, and rodents; this leads to a truncated receptor containing only the first transmembrane domain.

D. Protein Structure

The 5-HT$_{2B}$ receptor displays the characteristic structure of a GPCR receptor, with a relatively long N-terminus of about 55 amino acids. The human 5-HT$_{2B}$ receptor has 481 amino acids (479 amino acids in rat or mouse), with 79% and 82% homology for human versus rat and mouse, respectively. The 5-HT$_{2B}$ receptor N-terminus may act as a negative modulator, affecting both constitutive and agonist-stimulated activity (Belmer et al., 2014).

The ergotamine-bound 5-HT$_{2B}$ receptor crystal structure exhibits some conformational features of both the active and inactive states: an active-like state in the helix VII conformation but only partial changes in helix VI, which mirror the strong $\beta$-arrestin bias of ergotamine seen in functional assays (Wacker et al., 2013; Wang et al., 2013). A structural explanation for the distinct conformational features and the biased pharmacology of ergotamine can be seen in the extracellular loop 2 (ECL2) junction with helix V, E212-R213-P214 forming an additional helical turn stabilized by a structured water molecule at the extracellular tip of helix V. The segment of ECL2 connecting helices III and V via the conserved disulfide bond is, therefore, shortened and creates a conformational constraint on the extracellular tip of helix V (Martí-Solano et al., 2014). However, this structured water molecule involved in ECL2 junction with helix V has been challenged, as differential interactions of ergotamine with the top of helices V and VI could determine the rotational freedom of helix VI (Liu et al., 2013a). For more discussion of the crystal structure, see XVI. A. 5-HT GPCRs.

E. Heteromeric Receptor Associations

In cardiac fibroblasts, angiotensin AT1 receptors and 5-HT$_{2B}$ receptors, which share common signaling pathways, could exist in heterodimeric complexes as shown by coimmunolocalization and a pull-down assay (Jaffre et al., 2009), but experimental confirmation is lacking.

F. Pharmacology

1. Agonists. Biased agonism is evident with a range of drugs that impact the 5-HT$_{2B}$ receptor, and the influence of this phenomenon on the pharmacological profile of agonists can be profound (see, for example, Huang et al., 2009). Hence, when defining the action of agonists at the 5-HT$_{2B}$ receptor—at least in terms of their potency and efficacy—the particular readout (e.g., [Ca$^{2+}$]), arrestin, ERK, IP$_3$, and the nature of the receptor preparation needs to be taken into account. Against this background, the pharmacology of agonists will be described.

BWT23C86, 1-methyl-2-[5-(2-thienylmethoxy)-1H-indole-3-yl] ethylamine hydrochloride, has 10- and 100-fold selectivity for the human 5-HT$_{2B}$ receptor over the human 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors, respectively. (Recommended use: <100 nM concentration or <3 mg/kg i.p. in rodents; Porter et al., 1999; Jerman et al., 2001; Knight et al., 2004; Cussac et al., 2008). $\alpha$-Methyl-5-HT is a full agonist with high potency for the 5-HT$_{2B}$ receptor (pEC$_{50}$ = 8.4) and lower potency for both 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors. 5-Methoxytryptamine is also 25- and 400-fold selective over the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, respectively. Nordexfenfluramine (metabolite of dexfenfluramine), methylergonovine (metabolite of methysergide), and Ro 60-0175 (2(S)-1-(6-chloro-5-fluoro-1H-indol-1-yl)-2-propanamine fumarate) are all somewhat preferential 5-HT$_{2B}$ receptor agonists with about 10-fold selectivity over 5-HT$_{2C}$ receptor.

The 5-HT$_{2B}$ receptor displays high affinity to 5-HT (Kd ~10 nM) and many nonselective 5-HT$_2$ receptor active compounds, including some metabolites of therapeutics and drugs of abuse. Such agonists include MDA (3,4-methylenedioxyamphetamine-MDA, a metabolite of 3,4-methylenedioxy methamphetamine-MDMA) (Setola et al., 2003), MDMA ("ecstasy") itself, tryptamine, and LSD. DOI is a nearly full agonist at 5-HT$_{2B}$ Receptors but with similar affinity to 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (Porter et al., 1999; Jerman et al., 2001; Knight et al., 2004; Cussac et al., 2008).
Many substances known as “legal highs” display notable affinity for 5-HT_{2B} receptors, including 5-APB, commonly “marketed” as “benzofury” (K_i = 14 nM) and 6-APB (K_i = 3.7 nM), and 5-iodo-aminodindane (K_i = 70 nM). 5-APB and 6-APB act as potent (i.e., nanomolar EC_{50} values) full agonists at 5-HT_{2B} receptors (Iversen et al., 2013; Rickli et al., 2015). 5-APB contracts the rat stomach fundus and is antagonized by the 5-HT_{2B} receptor antagonist, RS127445 (Dawson et al., 2014). Other such drugs show submicromolar affinities for the 5-HT_{2B} receptor (mephedrone, naphyrone, 1-naphyrone, and methylenedioxy-aminotetralin). Indeed, there is a correlation in a series of phenylisopropylamines between hallucinogenic activity and affinity for the 5-HT_{2B} receptor agonist (Nelson et al., 1999), although 5-HT_{2A} receptor agonism is still also considered to play a major role in “psychedelics,” such as LSD or psilocybin. Activation of the 5-HT_{2B} receptor appears to play a key role in the behavioral stimulant and 5-HT releasing effects of MDMA (Doly et al., 2008) and in the reinforcing effects of MDMA in mice (Doly et al., 2009).

2. Antagonists. The first highly 5-HT_{2B} selective antagonist is LY266097, 1-(2-chloro-3,4-dimethoxybenzyl)-6-methyl-1,2,3,4-tetrahydro-9H-pyrido [3,4-b]indole hydrochloride, with a K_i of 9.7 for the human cloned 5-HT_{2B} receptor and a 100-fold greater selectivity over human 5-HT_{2C} and 5-HT_{2A} receptor binding sites (recommended use: 20 nM concentration in vitro or 0.5 mg/kg i.p. in rodents; Audia et al., 1996). SB204741, N-(1-methyl-5-indolyl)-N’-(3-methyl-5-isothiazolyl)urea, is another selective 5-HT_{2B} receptor antagonist with approximately 100-fold selectivity over the 5-HT_{2C} and 5-HT_{2A} sites but with rather low potency (K_i around 100 nM) (recommended use: 500 nM concentration in vitro or 10 mg/kg i.p. in rodents; Bonhaus et al., 1995). The tetrahydro-β-carboline, LY272015 (6-chloro-5-methyl-N-(5-quinolinyl)-2,3-dihydro-1H-indole-1-carboxamide) is also a fairly selective and highly potent antagonist (recommended use: 50 nM concentration in vitro or 1.0 mg/kg i.p. in rodents; Cohen et al., 1996). RS127445, 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine, has subnanomolar affinity for the 5-HT_{2B} receptor (pK_i = 9.5) and 1000-fold selectivity compared with numerous other receptors and ion channels, and it appears as the most selective, high-affinity 5-HT_{2B} receptor antagonist suitable now (Bonhaus et al., 1999; recommended use: 20 nM concentration or 0.25 mg/kg i.p. in rodents). The methoxymethoxanthene BF-1 is a highly selective and potent 5-HT_{2B} receptor antagonist lacking high affinities for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, histamine H_{1}, dopamine D_{1}, and D_{2} as well as muscarinic M_{3} and M_{5} receptors (Schmitz et al., 2015). S33526, 6-chloro-2,3,4,9-tetrahydro-1H-b-carbolin-1-yl)-phenyl-acetic acid ethyl ester, is a high-affinity and relatively selective antagonist at 5-HT_{2B} receptors (Cussac et al., 2002). SB215505, 6-chloro-5-methyl-N-(5-quinolinyl)-2,3-dihydro-1H-indole-1-carboxamide, behaves as a high-affinity and preferential inverse agonist at 5-HT_{2B} receptors. SB206553, 5-methyl-N-(3-pyridyl)-1,2,3,5-tetrahydrobenzo[1,2-b,4,5-b’]dipyrrrole-1-carboxamide, is a mixed 5-HT_{2C}/5-HT_{2B} receptor inverse agonist with 50- to 100-fold lower affinity for the 5-HT_{2A} and other sites.

Nonselective 5-HT_{2} receptor antagonists such as ritalserin and metergoline antagonize 5-HT_{2B} receptor-mediated effects. Furthermore, the α_{2} adrenergic receptor antagonists yohimbine and rauwolscine are potent 5-HT_{2B} antagonists, with low affinity for the 5-HT_{2C} and 5-HT_{2A} receptors. Atypical antipsychotics have also fairly high affinity for 5-HT_{2B} receptors, including clozapine, asenapine, or cariprazine (Wainscott et al., 1996; Millan et al., 2003; Shahid et al., 2009; Kiss et al., 2010). Aripiprazole (OPC-14597) is a novel atypical antipsychotic, with high antagonist affinity (IC_{50} = 11 nM) for the human 5-HT_{2B} receptor (Shapiro et al., 2003).

3. Allosteric Modulators. No selective 5-HT_{2B} allosteric modulator has been definitely identified. In the crystal, ergotamine binds to two distinct sites at the 5-HT_{2B} receptor, the orthosteric site, where the indole nucleus of ergotamine resides, and the “extended” binding site, to which the tripeptide portion of the ergoline binds. This potential allosteric site is also present in the muscarinic M2 receptor at the same extracellular region. The similarities in both the M2 and 5-HT_{2B} receptors suggest that the location of the extracellular allosteric site for class A GPCRs is rather similar and conserved; these common features suggest that ergotamine and other ergolines may function as “bitopic” ligands, acting at both the orthosteric and the putative extracellular allosteric site in the 5-HT_{2B} receptor. It is thought that a sodium ion allosterically modulates the binding pocket to reduce G protein signaling, thus favoring β-arrestin recruitment (McCory and Roth, 2015).

G. Transduction System(s)

1. Transfected Cells. 5-HT_{2B} receptors expressed in mouse fibroblast L-cells stimulate GTPase activity and inositol 1,4,5-triphosphate production upon agonist stimulation. This GTPase activation is mediated by G_q/11 but not by G_{as} or G_{ai}. The GTPase activation was also blocked by anti-β1-4 or anti-γ2 subunit antibodies. The 5-HT_{2B} receptor couples to phospholipase A2 (PLA2)-mediated release of arachidonic acid (Tournois et al., 1998). In addition, stimulation of the 5-HT_{2B} receptor triggers intracellular cyclic guanosine monophosphate (cGMP) production through dual activation of constitutive nitric-oxide synthase (cNOS) and inducible NOS. The group I PDZ motif at the carboxy terminus of the 5-HT_{2B} receptor is required for cNOS transduction pathways, whereas inducible NOS stimulation is G_{α13}-dependent (Manivet et al., 2000). The 5-HT_{2B} receptor shares the C-terminal
E-X-V1-S-X-V sequence with the 5-HT2C receptors and binds MUPPI-PDZ domains in vitro (Becamel et al., 2001). agonist-induced stimulation of the 5-HT2B receptor promotes rapid and transient activation of the proto-oncogene product p21ras, as measured by an increase in GTP-bound Ras (Launay et al., 1996). 5-HT2B receptor stimulation activates the MAPks p42mapk/p44mapk as well as ERK2/ERK1. It results in the formation of foci and to the formation of tumors from these foci in nude mice (Launay et al., 1996). The 5-HT2B receptor–dependent cell-cycle progression happens through retinoblastoma protein hyperphosphorylation and the activation of both cyclin D1/cdk4 and cyclin E/cdk2 kinases. The induction of cyclin D1, but not that of cyclin E expression, is under MAPK control, indicating an independent regulation of these two cyclins in 5-HT2B receptor–induced mitogenesis. Similarly, platelet-derived growth factor receptor (PDGFR) kinase activity is essential for 5-HT2B-triggered MAPK/cyclin D1, but not cyclin E, signaling pathways. 5-HT2B receptor activation also increases activity of the Src family kinases c-Src, Fyn, and c-Yes. Strikingly, c-Src, but not Fyn or c-Yes, is the crucial link between the Gq protein–coupled 5-HT2B receptor and the cell-cycle regulators (Nebigil et al., 2000b). Inhibition of c-Src activity is sufficient to abolish 5-HT–induced PDGFR tyrosine kinase phosphorylation and MAPK activation, cyclin D1 and cyclin E expression levels, and thymidine incorporation. Thus, c-Src activation by the 5-HT2B receptor controls cyclin E induction and, in concert with PDGFR, also induces cyclin D1 expression via the MAPK/ERK pathway (Nebigil et al., 2000b). The 5-HT2B signal transduction pathways are thus quite diverse and are similar to those of 5-HT2A receptors. On the other hand, the NOS pathway seems to be 5-HT2B–receptor–specific.

2. Primary Cell Cultures. In cultivated cardiac fibroblasts, angiotensin II (AngII)– or 5-HT– dependent cytokine release is critical for the expression of HB-EGF and Src activity via endogenous AT1 and 5-HT2B receptors (Jaffre et al., 2009). Matrix metalloproteinases (MMPs) are responsible for HB-EGF shedding and subsequent EGF-receptor transactivation induced by AngII or 5-HT. Tumor necrosis factor-α (TNF-α)–converting enzyme controls HB-EGF shedding in fibroblasts and is directly regulated by 5-HT2B Receptors (Pietri et al., 2005). Blockade of one of the two receptors prevents cytokine release induced by the other receptor (Jaffre et al., 2009). These findings also indicate that AT1 and 5-HT2B receptors share common EGF receptor– dependent signaling pathways in adult cardiac fibroblasts, and the two receptors were shown to interact in a common cell compartment. Together, the data support AT1 and 5-HT2B receptors exist as heterodimers that may play a key role in receptor maturation and trafficking to the plasma membrane and/or signaling (Bulenger et al., 2005) to drive common signaling regulating hypertrophic factors in the heart (Jaffre et al., 2009).

5-HT2B receptor stimulation in hepatic stellate cells (HSC) activates the expression of TGFβ1 (a powerful suppressor of hepatocyte proliferation) via ERK/JunD signaling. 5-HT2B receptor antagonists decrease the mRNA levels of TGFβ1, connective growth factor, plasminogen activator inhibitor-1, Smad-3, and JunD in lung and skin fibroblasts (Dees et al., 2011). 5-HT2B receptor activation leads to sustained phosphorylation of two downstream targets of mTOR, p70S6K and 4E-BP1, thereby facilitating survival and inhibiting autophagy of hepatocellular carcinomas (Soll et al., 2010). The 5-HT2B receptor protects newborn postmitotic cardiomyocytes against serum deprivation–induced apoptosis as manifested by DNA fragmentation, nuclear chromatin condensation, and terminal deoxynucleotidyl transferase dUTP nick end labeling. 5-HT prevents cytochrome c release and caspase-9 and -3 activation after serum deprivation via crosstalk between phosphatidylinositols-3 kinase (PI3K/Akt and ERK1/2) signaling pathways. 5-HT2B receptor–activated ERK kinases inhibit Bax expression induced by serum deprivation. 5-HT activates NF-κB via PI3K/Akt required for the regulation of the mitochondrial adenine nucleotide translocator and mitochondrial permeability. Thus, 5-HT via the 5-HT2B receptor is a novel survival factor targeting mitochondria (Nebigil et al., 2003). Interestingly, NF-κB regulation by 5-HT2B receptors is confirmed in a large screen for genes regulating NF-κB and the MAPK pathways (Matsuda et al., 2003).

Primary osteoblasts from mutant 5-HT2B receptor KO mice show reduced proliferation and delayed differentiation; calcium incorporation is markedly reduced in osteoblasts after 5-HT2B receptor inactivation (by genetic invalidation or by pharmacological inhibition; Collet et al., 2008). A functional link between the 5-HT2B receptor and the activity of the tissue-non-specific alkaline phosphatase (TNAP) was established in an osteoprogenitor C1 cell line (Baudry et al., 2010a). During osteogenic differentiation, both 5-HT2B receptor and TNAP mRNA translations are delayed with respect to extracellular matrix deposition. Once the receptor is expressed, it constitutively controls TNAP activity at a post-translational level along the entire period of mineral deposition. The lack of 5-HT2B receptors is associated with a 10-fold overproduction of prostacyclin in osteoblast primary cultures. A specific prostacyclin synthase (CYP8A1) inhibitor (U51605) totally rescued osteoblast aggregation and matrix mineralization in 5-HT2B receptor KO osteoblasts without any effect on WT osteoblasts. Prostacyclin is the endogenous ligand of the nuclear receptor PPAR-β/δ, and its inhibition in 5-HT2B KO cells totally rescued the alkaline phosphatase TNAP and osteopontin SPP1 mRNA levels, cell-cell adhesion, and matrix mineralization. The absence of 5-HT2B Receptors leads to the overproduction of prostacyclin, inhibiting osteoblast differentiation because of PPAR-β/δ–dependent target regulation and defective
cell-cell adhesion and matrix mineralization (Chabbi-Achengli et al., 2013), supporting a physiologic negative control of prostacyclin synthase by 5-HT\textsubscript{2B} receptors. Thus, endogenously expressed 5-HT\textsubscript{2B} receptors can modulate various transduction pathways, including Src, MMPs, and PLA2 activities in a cell type-dependent manner.

\textbf{H. Regulatory Mechanisms}

1. \textit{Internalization.} In transfected cells, prior exposure to 5-HT results in a rapid and considerable (up to 80\%) 5-HT\textsubscript{2B} receptor desensitization (Porter et al., 2001). Internalization of 5-HT\textsubscript{2B} receptors is caveolin1-dependent and clathrin- and \(\beta\)-arrestin2-dependent (Janoshazi et al., 2007).

Some ergot derivatives are “slow” 5-HT\textsubscript{2B} receptor binders, with very slow association and dissociation rates. The compounds have apparent lower potency to increase intracellular concentrations of calcium ions relative to inositol phosphate accumulation assays. Similarly, the potency of ergolines to activate ERK1/2 is highly time-dependent. In addition, a number of ergot derivatives produce “wash-resistant” 5-HT\textsubscript{2B} receptor signaling that persists for hours without appreciable loss of potency, which is not explained simply by slow receptor-dissociation kinetics. Thus, this persistent signaling has been proposed to originate from internalized or sequestered receptors (Unett et al., 2013). The 5-HT\textsubscript{2B} receptor crystal structure (Huang et al., 2009) reveals an intermediate state of activation stabilized by the extracellular-facing tripeptide portion of ergotamine, which likely drives \(\beta\)-arrestin bias and is not seen in unbiased ligands such as 5-HT itself. Thus, the long duration of action of some ergolines may be explained by a combination of very slow kinetics at the receptor, coupled with persistent intracellular signaling.

2. \textit{Interacting Proteins.} Proteins known to interact with the 5-HT\textsubscript{2B} receptor include constitutive and inducible NOS; Geq, Go11, and Go13, involved in signaling of the receptor; and MUPP1, a multivalent PDZ scaffolding protein. (Becamel et al., 2001).

For more details on proteins that interact with the 5-HT\textsubscript{2B} receptor, see XVII. B. 4. 5-HT\textsubscript{2B} Receptor.

\textbf{I. Function at Cellular, Tissue, and In Vivo Level}

1. \textit{Hematopoiesis.} 5-HT promotes megakaryocyte (MK) proliferation and reduces cell apoptosis via activation of the 5-HT\textsubscript{2B} receptor and Akt pathway (Liu and Yang, 2006). 5-HT\textsubscript{2B} increases proplatelet-bearing MKs and polymerizes actin via ERK1/2 (Ye et al., 2014). Tph1\textsuperscript{–/–} mice are deficient in peripheral 5-HT and display features of ineffective erythropoiesis. The central event starts in the bone marrow where the absence of 5-HT inhibits the terminal differentiation of erythroid precursors expressing 5-HT\textsubscript{2A} and 5-HT\textsubscript{2B} receptors. In addition, red blood cells from 5-HT-deficient mice are more sensitive to macrophage phagocytosis and have a shortened in vivo half-life (Amireault et al., 2011). In addition, the 5-HT\textsubscript{2B} Receptor is expressed in c-kit\textsuperscript{+} bone marrow cells (Launay et al., 2012). The 5-HT\textsubscript{2B} receptor antagonist RS127445 decreases colony-forming capacity, with inhibition of both early stem/progenitors and erythroid burst-forming unit formation attributed to a reduction of cell proliferation and/or an apoptotic effect. By contrast, 5-HT significantly enhances the expansion of CD34\textsuperscript{+} cells to early stem/progenitors and committed progenitors (erythroid burst-forming units) (Yang et al., 2007).

In human macrophages, 5-HT inhibits the LPS-induced release of proinflammatory cytokines to upregulate the expression of M2 polarization–associated genes and to reduce the expression of M1-associated genes. 5-HT\textsubscript{2B} receptors mediate the pro-M2 skewing effect of 5-HT. Blockade of this receptor during in vitro monocyte-to-macrophage differentiation preferentially regulates the acquisition of M2 polarization markers. 5-HT\textsubscript{2B} receptor mRNA is preferentially expressed by anti-inflammatory M2 (macrophage colony-stimulating factor) macrophages and is detected in vivo in liver Kupffer cells and in tumor-associated macrophages (de Las Casas-Engel et al., 2013). 5-HT\textsubscript{2B} receptor expression is found in postnatal microglia, suggesting that 5-HT participates in microglial functions (Kolodziejczak et al., 2015). 5-HT\textsubscript{2B} receptor mRNA expression is evident in spleen, thymus, and peripheral blood lymphocytes (Stefulf et al., 2000). Immature dendritic cells express 5-HT\textsubscript{2B} receptor mRNA, and 5-HT\textsubscript{2B} receptor stimulation induces intracellular Ca\textsuperscript{2+} mobilization in immature, but not mature, dendritic cells. Thus, 5-HT stimulates, in a maturation-dependent manner, different signaling pathways in dendritic cells (Idzko et al., 2004).

A proper balance between different T-helper (Th) cell subsets is necessary for normal functioning of the adaptive immune system. Th cells (from human umbilical cord blood) differentiated in vitro into Th1 or Th2 cells reveal the latter express 5-HT\textsubscript{2B} receptor mRNA (Aijö et al., 2012). In gene expression profiles during human CD41 T-cell differentiation, 5-HT\textsubscript{2B} receptor mRNA was found to be SP4-specific (−10-fold) among the 16 transcripts expressed in SP4 thymocytes at levels threefold or higher than in any other isolated T-cell subpopulation (Lee et al., 2004b).

Treatment with aggregated (1–40 or 1–42) and oligomeric (1–42) amyloid \(\beta\) (A\(\beta\), found in Alzheimer disease) promoted differentiation of bone marrow–derived mesenchymal stem cells without toxic effects. The effect of A\(\beta\) was shown to be mediated by the neuropeptide Y1 receptor and the 5-HT\textsubscript{2B} receptor via PI3K-dependent activation of the MAPK/ERK1/2 pathway (Jin et al., 2009). Thus, the 5-HT\textsubscript{2B} receptor, among others, mediates the balance among various hematopoietic lineages.

2. \textit{Pancreas.} A strong lactogen-dependent upregulation of 5-HT biosynthesis takes place in a subpopulation of
mouse islet β-cells during pregnancy (Schraenen et al., 2010). Pancreatic islet cells express the genes encoding all of the products necessary for synthesizing, packaging, and secreting 5-HT, including both isoforms of the 5-HT synthetic enzyme tryptophan hydroxylase (TPH) and the archetypal 5-HT transcription factor Pet1. In β-cells, Pet1 can bind to the 5-HT–relevant genes but also to a conserved insulin gene regulatory element. Mice lacking Pet1 display reduced insulin production and secretion and impaired glucose tolerance (Ohta et al., 2011). Inhibition of 5-HT synthesis blocks β-cell expansion and induced glucose intolerance in pregnant mice without affecting insulin sensitivity. Expression of the 5-HT2B receptor in maternal islets has been reported to increase during pregnancy and to normalize just before parturition. Blocking 5-HT2B receptor signaling in pregnant mice may also block β-cell expansion and cause glucose intolerance (Kim et al., 2010).

3. Adipocytes. By inhibiting 5-HT2B receptor signaling during adipogenesis using RS127445, an increased fat accumulation was observed similar to the knockdown phenotype (Söhle et al., 2012). In adipocytes, 5-HT2B receptors favor lipolysis by increasing phosphorylation and activity of hormone-sensitive lipase (Sumara et al., 2012).

4. Cardiovascular and Pulmonary Systems. 5-HT2B receptor inactivation in mice leads to partial embryonic lethality caused by major defects in heart development (Monassier et al., 2010). Neonates exhibit a second wave of partial lethality because of cardiac dilation resulting from contractility deficits and structural deficits at the intercellular junctions between cardiomyocytes. Echocardiography and electrocardiography studies in animals that live past the first week and survive until adulthood confirm the presence of left-ventricular dilation and decreased systolic function. 5-HT, via the 5-HT2B receptor, regulates heart differentiation and proliferation during development as well as cardiac structure and function in adults (Nebigil et al., 2000a). The 5-HT2B receptor is functionally coupled to ROS synthesis through NADPH oxidase (NOX) stimulation in 1C11 cells (Schneider et al., 2006) and in angiotensin II and isoproterenol-induced cardiac hypertrophy (Monassier et al., 2008). In human atrial myocytes, 5-HT reduces the amplitude of L-type calcium currents and affects the strength of gap junctional intercellular communication, which is markedly reduced by blocking receptors, showing that activation of 5-HT2B receptors inhibit gap junctional intercellular communication (Derangeon et al., 2010). Upon pulmonary artery branding, the 5-HT2B receptor antagonist SB204741 reduces right-ventricular fibrosis and improves heart function in mice (Janssen et al., 2015).

A model in which 5-HT2B receptor signaling promotes cardiac hypertrophy by stimulating calcineurin/nuclear factor of activated T cells signaling suggests the recruitment of histone acetyl transferases to regulatory regions of nuclear factor of activated T-cells target genes. 5-HT2B receptor agonist–induced hypertrophy of cardiac muscle cells results from a signaling pathway involving calcineurin and a kinase-dependent mechanism that inactivates class II histone deacetylases (HDAC), which act as repressors of cardiac growth (Bush et al., 2004). Because it also stimulates nuclear export of class II HDACs, myocyte enhancer factor-2 protein may play a role in the mechanism by which 5-HT2B receptor signaling triggers cardiac remodeling (McKinsey and Olson, 2005). A cDNA encoding the 5-HT2B Receptor was found in a screen for genes encoding HDAC5 modulators, and the ability of 5-HT2B receptors to promote HDAC5 phosphorylation and cardiomyocyte hypertrophy was confirmed (McKinsey and Olson, 2005). The 5-HT2B Receptor–triggered intracellular calcium ion release and PKC activation accounts, at least in part, for the overexpressed receptor-induced HDAC5 phosphorylation (Chang et al., 2005).

5. Endothelial Cells. In human pulmonary artery endothelial cells, 5-HT2B receptors stimulate calcium ion release from intracellular stores (Ullmer et al., 1996a). 5-HT2B receptors mediate the endothelium-dependent relaxation of rat jugular vein (Ellis et al., 1995) and pig pulmonary artery (Glusa and Pertz, 2000). Activation of 5-HT2B receptor by 25%, of inhibitor of NF-κB kinase subunit epsilon by 30%, and of toll-like receptor-4 and -6 by 18% and 39%, respectively (Wynants et al., 2013). A cardioprotective function of the 5-HT2B receptor in an integrated model of heart failure with preserved ejection fraction can be explained by a contribution of the endothelial 5-HT2B Receptors to coronary vasodilatation (Ayme-Dietrich et al., 2015).

6. Aorta. In normotensive rats, 5-HT–induced contraction of the aorta is primarily 5-HT2A receptor–dependent; however, in hypertensive rats, it is mediated by both 5-HT2A and 5-HT2B receptors. The endothelium-denuded isolated superior mesenteric artery of hypertensive (DOCA-salt) rat displays a marked increase in maximum arterial contraction to 5-HT2B receptor agonists when compared with control rats, confirming that the 5-HT2B receptor plays a greater role in 5-HT–induced contraction in arteries from hypertensive rats (Watts and Fink, 1999; Banes and Watts, 2003).

7. Liver. 5-HT is a potent growth factor for liver development and regeneration. The expression of both 5-HT2A and 5-HT2B receptors in the liver increases following hepatectomy. 5-HT2 receptor inhibition by ketanserin blocks liver regeneration when administered close to the G1/S transition point, suggesting 5-HT as a cofactor for DNA synthesis (Papadimas et al., 2006). In Tph1−/− mice, the failure of liver regeneration
is rescued by reloading 5-HT–free platelets with a 5-HT precursor molecule (Lesurtel et al., 2006). Elderly mice have decreased ability of the liver to restore normal volume after partial hepatectomy. The 5-HT₂ receptor agonist DOI reverses the age-related pseudocapillarization of old liver and improves hepatosinusoidal blood flow (Furrer et al., 2011); it also enhances hepatocyte proliferation after liver transplantation in mice. 5-HT₂B receptor activation significantly improves survival in recipients of a small, otherwise nonviable graft by enhancing liver regeneration and hepatic microcirculation, thereby reducing ischemia/reperfusion injury (Tian et al., 2011). 5-HT protects the liver in an IL-6–independent manner. The protective effects of DOI is lost in animals treated with SB206553, a 5-HT₂B/2C receptor antagonist. DOI may thus preserve microcirculation and accelerate liver regeneration via 5-HT₂B Receptors, thus preventing the liver parenchyma from further injury (Tian et al., 2011). However, in a pathophysiological setting, the regenerative influence of 5-HT acting through 5-HT₂A receptors on hepatocytes may be subjected to opposite antiregenerative effects arising from 5-HT acting through 5-HT₂B receptors in hepatic stellate cells (Ebrahimkhani et al., 2011). In hepatocytes, signaling through 5-HT₂B receptors also promotes gluconeogenesis (Sumara et al., 2012).

8. Gut. The 5-HT₂B receptor was initially characterized as the receptor contracting the rat stomach fundus (Vane, 1957). The 5-HT₂B receptor is expressed by smooth muscles in the small intestine, the stomach, and by enteric neurons. 5-HT, stimulating 5-HT₂B receptors, affects the fate of the large subset of enteric neurons that arises after the development of endogenous sources of 5-HT (Fiorica-Howells et al., 2000). High levels of both 5-HT₂B receptor mRNA and protein are found predominantly in the muscle layers and in the myenteric nerve plexus throughout the colon, where they cause neurally mediated contractions of longitudinal muscle (Borman et al., 2002) and tonically regulate colonic motility (Bassil et al., 2010).

Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011). Ghrelin, an orexigenic peptide present in the stomach, has gastroprotective properties. In vivo, the ghrelin receptor antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents. D-Lys(3)-GHRP-6 contracts stomach strips, an effect blocked by methysergide and yohimbine, suggesting an interaction with 5-HT₂B receptors (Depoortere et al., 2011).
the selective 5-HT2B receptor antagonist LY202715 significantly reduced the excitatory actions of BW723C86 on “intermediate” and “polysynaptic” cells (Sévoz-Couche et al., 2000).

Multiple, as opposed to single, applications of α-methyl-5-HT cause a long-lasting inhibition of both spontaneous and fictive inspiratory-related GABAergic neurotransmission to cardioinhibitory vagal neurons, which are prevented by the 5-HT2B receptor antagonist SB204741 (Dergacheva et al., 2008). BW723C86 reversibly increases both the frequency and amplitude of miniature excitatory postsynaptic currents in cardiac vagal neurons. The facilitation evoked by α-methyl-5-HT is blocked by the 5-HT2B receptor antagonist SB206553. Interestingly, the blockade of both NMDA and non-NMDA glutamatergic receptors did not prevent α-methyl-5-HT-evoked facilitation of miniature excitatory postsynaptic currents; however, the responses were blocked by P2 receptor antagonists (Dergacheva et al., 2008). These results indicate that activation of 5-HT2 receptors facilitates excitatory purinergic, but not glutamatergic, neurotransmission to cardiac vagal neurons.

11. Amygdala and Anxiety. Animals administered the 5-HT2B receptor agonist BW723C86 exhibit anxiolytic-like behavior in both the rat social interaction test and two conflict models of anxiety, the rat Geller-Seifter and marmoset conflict test (Kennett et al., 1995). Adult rat neurons in the medial amygdaloid nucleus express 5-HT2B receptors protein (Fig. 11), where local application of a 5-HT2B receptor agonist displays anxiolytic activity in the social-interaction model but has little effect on behavior in a punished conflict model of anxiety (Kennett et al., 1996a,b). This 5-HT2B receptor agonist also increased the time spent in feeding behavior of freely fed rats in observation cages over 15 minutes. The effect was also likely to be 5-HT2B receptor–mediated, as no response to BW723C86 was evident in freely fed rats pretreated with the 5-HT2C/2B receptor antagonist SB206553. BW723C86 also reduced the frequency of grooming bouts of rats in observation cages (Kennett et al., 1997a). Finally, BW723C86 increased the number of punishments accepted in a rat Vogel drinking conflict paradigm over 3 minutes, as do anxiolytic benzodiazepine drugs. The antipunishment effect of BW723C86 was blocked by the 5-HT2B/2C receptor antagonists SB206553 or SB215505 but not by the selective 5-HT2C receptor antagonist SB242084. Thus, the antipunishment action of BW723C86 is likely to be 5-HT2B receptor–mediated (Kennett et al., 1998).

12. Sleep. 5-HT2A and 5-HT2C receptors modulate deep [slow wave (SW)] sleep and low-frequency EEG power in humans and rodents. Antagonists of 5-HT2A and/or 5-HT2C receptors have a well known slow-wave sleep–enhancing effect. In contrast, blockade of 5-HT2B receptors increases motor activity and wakefulness along with decreased theta activity during wakefulness and REM sleep. The 5-HT2B receptor antagonist SB215505 dose-dependently increases wakefulness at the expense of the intermediate stage of sleep, REM sleep, and slow-wave sleep and reduces low-frequency (<8 Hz) EEG power. In REM sleep, the 5-HT2B receptor antagonist SB215505 dose-dependently decreases EEG power solely in the theta (6–9 Hz) band, primarily affecting the peak power value (7 Hz) (Kantor et al., 2004). 5-HT exerts a 5-HT2B receptor–mediated facilitation of NREM sleep and an influence that is, respectively, inhibitory on NREM sleep and facilitatory on sleep apnea generation via 5-HT2A receptors (Popa et al., 2005). Htr2B−/− mice exhibited significantly increased wakefulness, with less NREM sleep, whereas REM sleep is not affected. Chronic oral intake of haloperidol restored the balance between wakefulness and NREM sleep in Htr2B−/− mice (Pitychouts et al., 2015). Activation of 5-HT2B receptors may thus contribute to initiation of sleep and to theta generation during wakefulness and REM sleep under physiologic conditions. However, it should be kept in mind that, clinically, there have been no successes in insomnia/sleep disorders with 5-HT2 receptor antagonists in spite of multiple attempts, at least with 5-HT2A receptor antagonists.

J. Clinical Relevance

1. Feeding and Anorexigen. The hypophagic response to the anorexigen and 5-HT releaser, dexfenfluramine, observed in wild-type mice is absent in Htr2B−/− mice or in wild-type mice treated with RS127445. The dexfenfluramine-induced hypothalamic peak of 5-HT release is strongly reduced in Htr2B−/− awake mice.

**Fig. 11.** 5-HT2B receptor immunoreactivity in rat brain. Immunohistochemical detection of 5-HT2B receptor immunoreactivity within Purkinje cells in the cerebellum (A), multipolar neurons in the lateral septum (B), multipolar and bipolar neurons in the medial amygdala (C), and cells in the dorsal hypothalamic nucleus (D). In each case, the staining was abolished in adjacent sections by coincubation with synthetic 5-HT2B receptor peptide (data not shown). Adapted from Duxon et al. (1997) (with permission).
compared with control mice. Dexfenfluramine-induced 5-HT release is observed in synaptosomal preparation from wild-type mice but absent in Htr2B-/- mice (Banas et al., 2011). 5-HT2B receptor–induced NO production phosphorylates SERT and maximizes 5-HT uptake in raphe neuron primary culture. 5-HT2B receptor–PKC coupling promotes additional phosphorylation of both SERT and Na+, K+-ATPase α-subunit, impairing the electrochemical gradient necessary for 5-HT uptake. Such 5-HT2B receptor–mediated control of SERT activity operated in primary neurons from the raphe nuclei (Launay et al., 2006). Thus, activation of 5-HT2B receptors is a limiting step in the SERT-dependent releasing effect of dexfenfluramine, whereas other 5-HT receptors may act downstream with respect to feeding behavior.

2. Raphe and Antidepressant Activity. 5-HT neurotransmission is tightly regulated by autoreceptors that fine-tune 5-HT neurotransmission through negative feedback inhibition at the cell bodies (predominantly 5-HT1A) or at the axon terminals (predominantly 5-HT1B), although 5-HT2B receptors may play different roles (McDevitt and Neumaier, 2011). The therapeutic effects induced by 5-HT–selective reuptake inhibitor (SSRI) antidepressants are initially triggered by blocking the SERT and rely on long-term adaptations of pre- and postsynaptic receptors. Long-term behavioral and neurogenic SSRI effects were abolished after either genetic or pharmacologic inactivation of 5-HT2B receptors (Diaz et al., 2012). Conversely, direct agonist stimulation of 5-HT2B receptors induced an SSRI-like response in behavioral and neurogenic assays. The 5-HT2B receptor is expressed in raphe serotonergic neurons. The SSRI-induced increase in hippocampal extracellular 5-HT concentration is strongly reduced in the absence of functional 5-HT2B receptors; hence, selective 5-HT2B activation mimics SSRI effects (Diaz et al., 2012). Thus, the 5-HT2B receptor modulates 5-HT neuron activity and may be required for the therapeutic actions of SSRIs.

3. Dopamine and Antidepressant Activity. Agomelatine, a potent melatonin MT1/MT2 receptor agonist, is an effective antidepressant and a potent 5-HT2B receptor antagonist (Millan et al., 2003). Twice daily melatonin increases the number of spontaneously active dopamine neurons without affecting noradrenergic neurons. Long-term administration of either melatonin or the 5-HT2C receptor antagonist SB242084 has no effect on the firing rate and burst parameters of 5-HT and dopamine neurons, whereas their combination enhances the number of spontaneously active dopamine neurons while leaving the firing of 5-HT neurons unchanged. The addition of the 5-HT2B receptor antagonist LY266097 to the previous regimen, which by itself is devoid of effect, increases the activity of dopamine neurons (Chenu et al., 2014). Hence, the combination of melatonin receptor activation, 5-HT2B, and 5-HT2B receptor blockade results in a disinhibition of dopamine neurons, reproducing the antidepressant effect of agomelatine.

4. Serotonin (5-HT) Syndrome. The 5-HT syndrome occurs in humans after a combination of drugs inducing a massive increase in extracellular 5-HT or direct targeting of several 5-HT receptor subtypes [see Scotton et al. (2019)]. Htr2B-/- mice are more prone to develop the 5-HT syndrome symptoms after administration of high dose of SSRI or the 5-HT precursor, 5-hydroxytryptophan, although increases in 5-HT plasma levels are similar in both genotypes (Diaz and Maroteaux, 2011).

5. Drug of Abuse. The “club drug” MDMA (“ecstasy”) inhibits SERT activity, releasing 5-HT stores from nerve terminals. The subsequent activation of postsynaptic 5-HT receptors by released 5-HT is critical for the unique psychostimulatory effects of MDMA. Acute pharmacological inhibition or genetic ablation of the 5-HT2B receptor in mice completely abolishes MDMA-induced hyperlocomotion and 5-HT release in nucleus accumbens and ventral tegmental area. The MDMA-stimulated release of endogenous 5-HT from superfused midbrain synaptosomes is 5-HT2B receptor–dependent (Doly et al., 2008). Htr2B-/- mice show no behavioral sensitization or conditioned place preference following MDMA. In addition, MDMA-induced reinstatement of conditioned place preference and locomotor sensitization are both abolished by RS127445 in mice, whereas MDMA-induced dopamine D1 receptor–dependent phosphorylation of extracellular regulated kinase in nucleus accumbens is abolished in mice lacking functional 5-HT2B receptors. These results underpin the importance of 5-HT2B receptors in the reinforcing properties of MDMA (Doly et al., 2009).

The selective 5-HT2B receptor antagonist LY266097 reduces dopamine outflow in the ventral striatum/nucleus accumbens but not in the dorsal striatum (Auclair et al., 2010). The locomotor response of Htr2B-/- mice to both dizocilpine and amphetamine is significantly enhanced compared with control mice (Pitychoutis et al., 2015). 5-HT2B receptor antagonists reduce cocaine-induced hyperlocomotion independently of changes of subcortical dopamine outflow, supporting a regulatory control exerted by this receptor on ascending dopamine pathways (Devroye et al., 2015). Thus, 5-HT2B receptors may also exert, in addition to 5-HT neurons, a facilitatory control on mesoaccumbens dopamine pathway activity.

6. Impulsivity. A functional stop codon in the human 5-HT2B receptor gene enhances impulsive behavior (Bevilacqua et al., 2010). Especially under conditions in which control is impaired, the carriers of the stop codon are more vulnerable to alcohol and more impulsive upon drinking. Similarly, Htr2B-/- mice display more impulsive choice in delayed discounting tasks, sought novelty, and are more active after receiving a D1 dopamine receptor agonist (Bevilacqua et al., 2010). Interestingly, early onset schizophrenia is more prevalent in human
5-HT$_{2B}$ receptor gene Q*20 carriers. Domains related to the positive, negative, and cognitive symptom clusters of schizophrenia are affected in Htr$_{2B}^{-/-}$ mice, with deficits in sensorimotor gating, selective attention, social interactions, learning, and memory processes. Htr$_{2B}^{-/-}$ mice show enhanced locomotor response to dizocilpine and amphetamine and alterations in sleep architecture. 5-HT$_{2B}$ receptor ablation induces a region-selective decrease of dopamine and glutamate concentrations in the dorsal striatum. Importantly, selected schizophrenic-like phenotypes and endophenotypes are rescued by chronic haloperidol treatment (Pitychoutis et al., 2015). The phenotypes of Htr$_{2B}^{-/-}$ mice result from a combination of both the direct absence of 5-HT$_{2B}$ receptor signaling and the resultant neural adaptations.

7. Fragile X Syndrome. Fragile X syndrome, caused by the loss of Fmr1 gene function, is the most common form of inherited mental retardation, with no current effective treatment. 5-HT$_{2B}$ receptor activation moderately enhances Ras-PI3K/PKB signaling input, GluA1-dependent synaptic plasticity, and learning in Fmr1 knockout mice without causing anxiety-related side effects (Lim et al., 2014).

8. Cardiovascular Diseases. 5-HT$_{2B}$ receptors are overexpressed in heart tissue from patients with congestive heart failure, in parallel to increased cytokine and norepinephrine plasma levels (Jaffre et al., 2009). 5-HT plasma levels are also increased in patients with heart failure and in rodents with cardiac hypertrophy induced by aortic constriction. Thus, 5-HT–induced cardiac hypertrophy and/or heart failure may be 5-HT$_{2B}$ receptor–dependent or at least have responses likely exacerbated via the lack of 5-HT$_{2B}$ receptor. In support of which, 5-HT$_{2B}$ receptor KO mice do not develop isoproterenol-induced left-ventricular hypertrophy (Jaffré et al., 2004). Mice expressing the 5-HT$_{2B}$ receptor exclusively in cardiomyocytes, similarly to global 5-HT$_{2B}$ Receptor–null mice, are resistant to isoproterenol-induced cardiac hypertrophy and dysfunction as well as to isoproterenol-induced increases in plasma cytokine levels (Jaffré et al., 2009). In primary culture of cardiac fibroblasts, angiotensin II– and isoproterenol-stimulated NOX activity is prevented by the selective 5-HT$_{2B}$ receptor antagonist (SB215505). SB215505 prevents the increase in cardiac superoxide generation and hypertrophy in two models of cardiac hypertrophy (i.e., angiotensin II and isoproterenol infusions in mice) (Monassier et al., 2012). A functional interaction between AT$_1$ and 5-HT$_{2B}$ receptors via a transinhibition mechanism may involve heterodimeric receptor complexes and trigger cytokine release in cardiac fibroblasts (Jaffré et al., 2009).

The 5-HT$_{2B}$ receptor is involved in cardiac hypertrophy by acting directly on cardiac myocytes. After 2 weeks of aortic banding surgery, 5-HT$_{2B}$ receptor mRNA and protein expression are increased. SB215505 significantly reduces the arising increase in heart weight, heart wall thickness, left-ventricular mass, and expression of the brain natriuretic peptide (BNP) but does not attenuate the upregulation of 5-HT$_{2B}$ receptor protein expression in rats after aortic banding. Following in vitro mechanical stretch of cardiomyocytes and incubation with 5-HT, the level of 5-HT$_{2B}$ receptors and BNP protein increases time-dependently. 5-HT$_{2B}$ receptor siRNA applied to cardiomyocytes reversed both the increase of NF-κB translocation and BNP protein induced by 5-HT incubation plus mechanical stretch (Liang et al., 2006). 5-HT$_{2B}$ receptors are involved in the generation of apoptotic events associated with cardiac remodeling during increased adrenergic stimulation (Bai et al., 2010). Thus, there is a dual role for 5-HT$_{2B}$ receptors on both cardiomyocytes and cardiac fibroblasts in regulating cardiac hypertrophy in vivo.

9. Pulmonary Arterial Hypertension. Pulmonary arterial hypertension (PAH) is a progressive and often fatal disorder that results from increased pulmonary blood pressure associated with abnormal vascular proliferation. 5-HT is associated with the pathogenesis of PAH (Chan and Loscalzo, 2008). Therapeutics with PAH as a side effect (e.g., dexfenfluramine) are potent 5-HT releasers acting at SERT and/or 5-HT$_{2B}$ receptor agonists (Weir et al., 2008). The blockade of 5-HT$_{2B}$ receptors, either by genetic (Htr$_{2B}^{-/-}$) or pharmacologic inactivation (5-HT$_{2B}$ receptor antagonist RS127445), completely prevents the development of hypoxia-induced pulmonary hypertension in mice and lung remodeling including the increase in vascular proliferation, elastase activity, and TGFβ1 levels (Launay et al., 2002). In the monocrotaline-induced pulmonary hypertension model, a number of 5-HT$_{2B}$ receptor antagonists (terguride, PRX-08066, or C-122) reduce pulmonary pressure, arterial wall thickening, and lumen occlusion but maintained cardiac function (Porvasnik et al., 2010; Dumitrascu et al., 2011; Zopf et al., 2011). Pulmonary hypertension is associated with a substantial increase in 5-HT$_{2B}$ receptor expression in the pulmonary arteries of both rodents and humans (Launay et al., 2002; Dumitrascu et al., 2011). Activation of 5-HT$_{2B}$ receptors appears to be, therefore, a limiting step in the development of pulmonary hypertension. However, the restricted expression of 5-HT$_{2B}$ receptors to bone marrow cells is necessary and sufficient for pulmonary hypertension to develop via an action at hematopoietic stem cell differentiation (Launay et al., 2012). There appears to be a limiting role of 5-HT$_{2B}$ receptors in PAH development; thus, the contribution of 5-HT to PAH may be an extrapulmonary, and specifically hematopoietic, event.

10. Vascular Hypertension. Mesenteric arteries from DOCA-salt hypertensive rodents predominantly contract via 5-HT$_{2B}$ receptors; they display an increase in 5-HT$_{2B}$ receptor mRNA and receptors (Watts et al., 1996). The isolated endothelium-denuded superior mesenteric artery of DOCA-salt rats displays a marked
increase in the maximal contraction to the 5-HT\textsubscript{2B} receptor agonist BW723C86. In chronically instrumented rats, the 5-HT\textsubscript{2B} receptor antagonist LY272015 significantly reduces mean blood pressure (Watts and Fink, 1999). LY272015 also inhibited 5-HT–induced contractions in aorta from rats made hypertensive by exposure to the nitric-oxide synthase inhibitor \(\text{N(omega)}\)-nitro-L-arginine, whereas ketanserin was inactive (Russell et al., 2002). Thus, the 5-HT\textsubscript{2B} receptor appears to play an important role in 5-HT–induced contraction in hypertensive rodent arteries.

## 11. Fibrosis

5-HT increases proliferation and collagen synthesis of lung fibroblasts. 5-HT concentrations in lung homogenates increase significantly following bleomycin-induced fibrosis, paralleled with an increased expression of 5-HT\textsubscript{2A} and 5-HT\textsubscript{2B} receptors (Königshoff et al., 2010). Blockade of 5-HT\textsubscript{2B} receptors by SB215505 reduces bleomycin-induced lung fibrosis, with reduced lung collagen levels and procollagen 1 and 3 mRNA expression. 5-HT\textsubscript{2B} receptor antagonists decrease levels of lung TGF\(\beta\)1 mRNA, connective tissue growth factor and plasminogen activator inhibitor-1, and JunD mRNA, consistent with their antifibrotic activity. Interestingly, the 5-HT\textsubscript{2B} receptor is strongly overexpressed in fibroelastic foci in human idiopathic pulmonary fibrosis (Fabre et al., 2008). Thus, it is likely that 5-HT–induced lung fibrosis is controlled by 5-HT\textsubscript{2B} receptors regulating TGF\(\beta\)1 levels.

In the liver, fibrogenic HSC, which are negative regulators of hepatocyte regeneration, are known to express both 5-HT\textsubscript{2A} and 5-HT\textsubscript{2B} receptors, which may regulate TGF\(\beta\)1 and Smad signaling (Li et al., 2006). HSCs play a key role in hepatic wound healing and fibrosis. After HSC activation, expression of 5-HT\textsubscript{2A} and 5-HT\textsubscript{2B} receptors is around 100- and 50-fold that of quiescent cells, respectively. The 5-HT\textsubscript{2B} receptor expression is strongly associated with fibrotic tissue in diseased liver. 5-HT\textsubscript{2} receptor antagonist–treated HSCs display suppressed proliferation and increased apoptosis. 5-HT synergizes with platelet-derived growth factor to stimulate HSC proliferation (Ruddell et al., 2006). In contrast to quiescent cells, activated HSCs exhibit \([\text{Ca}^{2+}]\text{i}\) transients following treatment with 5-HT, which is inhibited by ritanserin. Expression of type 1 inositol-5’-triphosphate receptor and type 2 sarcoplasmic/endoplasmic reticulum \(\text{Ca}^{2+}\) ATPase is also increased during activation of HSCs and serves as the major isotype for ER \(\text{Ca}^{2+}\) storage and release in activated HSCs. ER \(\text{Ca}^{2+}\)-binding chaperone proteins, including calreticulin, calnexin, and calsequestrin, are upregulated following activation of HSCs (Park et al., 2011). 5-HT\textsubscript{2B} receptor stimulation of HSCs increases the expression of TGF\(\beta\)1 (a powerful suppressor of hepatocyte proliferation) via ERK/JunD signaling. Similar effects are evident in mice lacking 5-HT\textsubscript{2B} receptor or JunD or when HSCs have been selectively depleted. 5-HT\textsubscript{2B} receptor blockade attenuates fibrogenesis and improves liver function in disease models in which fibrosis is pre-established and progressive (Ebrahimkhani et al., 2011). Therefore, the hepatic 5-HT\textsubscript{2B} receptor appears to have a dual role, promoting regeneration in physiologic conditions and fibrosis in pathologic conditions.

Dermal fibrosis is reduced in \(\text{Htr2B}^{-/-}\) mice using both inducible and genetic models of fibrosis. Pharmacologic inactivation of the 5-HT\textsubscript{2B} receptor effectively prevents the onset of experimental fibrosis and ameliorates established fibrosis by decreasing mRNA levels of TGF\(\beta\)1, connective growth factor, plasminogen activator inhibitor-1, and Smad-3 (Dees et al., 2011). Moreover, inhibition of platelet activation prevents fibrosis in models of skin fibrosis. In TPH1-deficient mice, the rate-limiting enzyme for 5-HT production outside the central nervous system shows reduced experimental skin fibrosis (Dees et al., 2011). Skin fibrosis is thus controlled by 5-HT\textsubscript{2B} receptors via regulation of TGF\(\beta\)1 levels.

Treatment of neonatal rat cardiac fibroblasts with 5-HT increases the expression of smooth muscle \(\alpha\)-actin, a marker of fibroblast differentiation into myofibroblasts and stimulated cardiac fibroblast migration. 5-HT enhances secretion of TGF\(\beta\)1 and expression of MMPs in cardiac fibroblasts (Yabanoglu et al., 2009). 5-HT– or AngII-stimulated cytokine release and secretion of TGF\(\beta\)1 in adult cardiac fibroblasts is sensitive to 5-HT\textsubscript{2B} receptor blockade. Treatments with epidermal growth factor receptor (ErbB1/4)-selective inhibitors or with selective inhibitors of MMPs also abolished AngII and 5-HT–induced cytokine release. Finally, the use of \(\text{HB-EGF}^{-/-}\) cardiac fibroblasts confirmed that epidermal growth factor receptor stimulation is absolutely required for AngII- and 5-HT–dependent cytokine release (Jaffre et al., 2009). Collectively, these results highlight that a convergent action of norepinephrine, AngII, and 5-HT via interactions between AT\(_1\) and 5-HT\textsubscript{2} receptors coexpressed by noncardiomyocytes is limiting key events in cardiac hypertrophy.

## 12. Valvular Heart Disease

Valvular heart disease occurs in over 65% of patients with carcinoid syndrome and is characterized by fibrous thickening of cardiac valves, leading to heart failure [for review, see Roth (2007)]. High plasma 5-HT levels correlate with valvular abnormalities detected by cardiac catheterization and echocardiography (Robiolio et al., 1995). The similarity of lesions in carcinoid heart disease and in methysergide-associated valvular disease suggested a direct stimulation of myofibroblast growth by an unknown 5-HT receptor agonism (Hendrikkx et al., 1996). The increase in fenfluramine-associated valvular heart disease raised concerns that other 5-HT–relevant medications might also increase the risk of valvular heart disease (Connolly et al., 1997). Dexfenfluramine had been approved in the United States for long-term use as an appetite suppressant until it was associated with valvular heart disease. The valvular changes...
(myofibroblast proliferation) are histopathologically indistinguishable from those observed in carcinoid disease or following long-term exposure to 5-HT2 receptor–preferring ergot drugs (ergotamine, methysergide). The amphetamine derivative, MDMA ("ecstasy"), and its N-demethylated metabolite, 3,4-methylenedioxyamphetamine (MDA), both preferentially bind to and activate human recombinant 5-HT2B receptors. Like fenfluramine and norfenfluramine, they elicit mitogenic responses in human valvular interstitial cells via likely activation of 5-HT2B receptors (Setola et al., 2003). Based on these strikingly similar echocardiographic and histopathologic features, it is now considered that ergot-derived dopamine agonists (e.g., pergolide and cabergoline) cause a valvular heart disease nearly identical to that seen in patients with carcinoid syndrome (Horvath et al., 2004). Population studies of patients with Parkinson disease compared with non-Parkinsonian controls show that pergolide and cabergoline have a similar risk of inducing fibrotic changes in cardiac valve leaflets. Pergolide and cabergoline have high affinity for the 5-HT2B receptors. The frequency of moderate-to-severe regurgitation in at least one heart valve was higher in patients receiving cabergoline or pergolide compared with patients taking nonergot agonists or controls, and the incidence of new-onset valvulopathy was relatively high in patients taking the ergot-derived drugs (Antonini and Poewe, 2007; Roth, 2007). A simultaneous mitral bioprosthesis hypertrophic scarring and native aortic valve fibrosis was recently reported following benfluorex therapy. The bioprosthesis and aortic valves exhibit similar histopathological lesions. Thickening and plaque deposits made by smooth muscle α-actin– and vimentin-positive cells in a glycosaminoglycan matrix were observed, supporting that activation of the 5-HT2B receptor by norfenfluramine may trigger the development of drug-induced heart disease (Aymed-Dietrich et al., 2012).

5-HT2B and 5-HT2A receptor transcripts are reported to be present in heart valves, whereas no 5-HT2C receptor transcript is detectable. Preferential stimulation of valvular 5-HT2B receptors (with or without accompanying 5-HT2A receptor activation) may contribute to valvular fibroplasia in humans (Fitzgerald et al., 2000). Mitral valve regurgitation has been associated with increased mRNA expression of valvular 5-HT2B receptors and SERT in pigs (Cremer et al., 2015b). Canine myxomatous mitral valve disease was associated with higher expression of 5-HT2B receptors in mitral valve (Cremer et al., 2015a).

These findings suggest that 5-HT2B receptor signaling links vascular damage and platelet activation to tissue remodeling and identify the 5-HT2B Receptor as a novel potential therapeutic target to treat valvular heart diseases. As a result of these investigations, the development of 5-HT2B receptor agonists have been banned by the FDA.
postsynaptic marker PSD-95. Delivery of SB204741 bilaterally attenuates thermal and mechanical allodynia occurring after spinal nerve ligation, at early time periods, day 2 postinjury. The transient activation of the PKC&g/NMDA receptor pathway is critically involved in 5-HT_{2B} receptor–mediated facilitation in the spinal nerve ligation model (Aira et al., 2013). The adaptor protein NADH dehydrogenase subunit 2 (ND2) is involved in NR1 phosphorylation and spinal hyperexcitability secondary to peripheral nerve injury. Spinal nerve ligation is followed by increased colocalization of ND2 with pNR1. C-fiber–evoked dorsal horn field potentials are increased after spinal nerve ligation by superfusion with an NMDA receptor agonist. This increased postsynaptic upregulation of ND2/pNR1 can be prevented by prior administration of selective 5-HT_{2B} antagonist SB204741 (Aira et al., 2014). Thus, NMDA receptor phosphorylation is instrumental in coupling 5-HT_{2B} receptor–mediated input to NMDA receptor expressing synapses in spinal hyperexcitation involved in pain.

15. Neuropathic Pain. 5-HT was also implicated in a rat model of neuropathic pain evoked by chronic constriction injury (CCI) of the sciatic nerve. 5-HT_{2B} receptor activation has been reported to both prevent and reduce CCI-induced allodynia at 3 weeks postinjury. Intrathecal administration of the 5-HT_{2B} receptor agonist BW723C86 attenuated established mechanical and cold allodynia, an effect prevented by coinjection of RS127445. A single application of BW723C86 on the sciatic nerve concomitantly to CCI dose-dependently prevented mechanical allodynia and reduced cold allodynia 17 days after CCI. This behavioral effect is accompanied with a marked decrease in macrophage infiltration into the sciatic nerve and, in the DRG, with an attenuated abnormal expression of several markers associated with local neuroinflammation and neuropathic pain. CCI results in a marked upregulation of 5-HT_{2B} receptor expression in sciatic nerve and DRG. In the latter structure, it is biphasic, consisting of a transient early increase 2 days after surgery (around 23-fold) before neuropathic pain emergence, followed by a steady (around fivefold) increase that remains relatively constant until the pain disappeared. In DRG and sciatic nerve, 5-HT_{2B} receptors are immunolocalized on sensory neurons and infiltrating macrophages (Urtilkova et al., 2012).

It thus appears that 5-HT_{2B} receptor involvement in pain takes place at various sites and time periods; early events are more pronociceptive, whereas at later stages, this receptor contribution may be more antinociceptive, although this varies according to the animal models.

16. Spasticity in Amyotrophic Lateral Sclerosis. Spinal cord injury leads to an initial phase of hyporeflexia followed by hyperreflexia, often referred to as spasticity. Spasticity is a common and disabling symptom also observed in patients with amyotrophic lateral sclerosis, a disease that can affect both upper and lower motor neurons. A rat tail spasticity model with a caudal spinal transection demonstrates 5-HT_{2B} receptor downregulation at 21 days postinjury (Wienecke et al., 2010). Motoneurons, which recover from denervation, function autonomously, exhibiting large persistent calcium currents that help with functional recovery and contribute to uncontrolled muscle spasms. Application of agonists relatively selective to 5-HT_{2B} receptors (including BW723C86) increase persistent calcium currents. 5-HT_{2B} receptors on motoneurons ultimately contribute to recovery of motoneuron function and emergence of spasms (Murray et al., 2011). In amyotrophic lateral sclerosis, spasticity is traditionally thought to be the result of degeneration of the upper motor neurons in the cerebral cortex, although degeneration of other neuronal types, particularly 5-HT neurons, might also underlie the spasticity. In superoxide dismutase 1 (G86R) mice, a transgenic model of amyotrophic lateral sclerosis, 5-HT levels are decreased in brainstem and spinal cord before onset of motor symptoms. Furthermore, there is noticeable atrophy of 5-HT neuronal cell bodies along with neuritic degeneration at disease onset. In superoxide dismutase 1 (G86R) mice, tail muscle spastic-like contractions occur at end-stage. Importantly, they are abolished by the 5-HT_{2B/2C} receptors inverse agonist, SB206553. In keeping with this, 5-HT_{2B} receptor expression is strongly increased at disease onset (Dentel et al., 2013). In summary, 5-HT_{2B} receptors on motoneurons can become constitutively active after injury and ultimately contribute to some recovery of motoneuron function and emergence of spasms.

17. Migraine. A role for 5-HT in migraine is supported by changes in circulating levels of 5-HT and its metabolites during the phases of a migraine attack. A migraine headache is thought to be transmitted by the trigeminal nerve from the meninges and their associated blood vessels. Correlation of the receptor affinities with the potencies of drugs used in migraine prophylaxis demonstrates correlations with the 5-HT_{2B} receptor, and various human meningeal tissues express 5-HT_{2B} mRNAs (Schmuck et al., 1996). The 5-HT_{2B} receptor can activate the release of the smooth muscle relaxant NO and induce relaxation of the cerebral arteries and the jugular vein. 5-HT_{2B} receptors expressed by endothelial cells of meningeal blood vessels may trigger migraine headache through the formation of NO, which results in the dilation of cerebral blood vessels and the concomitant activation of sensory trigeminovascular afferents, thus initiating the manifestation of head pain (Johnson et al., 2003). In addition, a genetic study identified 5-HT_{2B} receptors as a susceptibility gene to migraine (Corominas et al., 2010). Endothelial 5-HT_{2B} receptors may thus trigger dilation of meningeal blood vessels, which by activating sensory trigeminovascular afferents, induces head pain.
18. Visceral Pain. 5-HT_{2B} receptors are involved in signaling from the colon in rats, in which there is visceral hypersensitivity. Oral administration of RS127445 inhibits visceral hypersensitivity provoked by restraint stress without significant effect on the visceral nociceptive threshold of naive rats (Ohashi-Doi et al., 2010). Moreover, when administered intracerebroventricularly, RS127445 also decreases the number of pain behaviors during noxious colorectal distension. A selective 5-HT_{2B} receptor antagonist has thus been proposed to have therapeutic potential for the treatment of gut disorders characterized by visceral hypersensitivity (O’Mahony et al., 2010). The 5-HT_{2B} receptor appears thus to be involved in regulating sensory pathways but only under hyperalgesic conditions, suggesting the possible utility of 5-HT_{2B} receptor antagonism in reducing visceral hypersensitivity in patients with irritable bowel syndrome and other hypersensitivity conditions.

19. Bones. 5-HT_{2B} receptor mRNA, which is undetectable in anaplastic osteoblasts, appears in differentiated and matured osteoblasts (Bliziotis et al., 2001; Westbroek et al., 2001). The differentiation and maturation of osteoblasts might thus be regulated by 5-HT_{2B} receptor activation (Hirai et al., 2009). Of interest, Htr2B^{−/−} female mice display reduced bone density from the age of 4 months that intensifies by 12 and 18 months. This histomorphometrically confirms that osteopenia is due to reduced bone formation (Collet et al., 2008). Using the osteoprogenitor cell line C1, blockade of 5-HT_{2B} receptor intrinsic activity affects the efficiency of mineralization by decreasing calcium incorporation. Optimal bone matrix mineralization involves both NO and PLA2 signaling pathways, and the 5-HT_{2B} receptor promotes prostaglandin E2 production through cyclooxygenase activation. When C1 osteoblasts undergo conversion into osteocyte-like cells, cyclooxygenase activity is quenched. The 5-HT_{2B} Receptor contributes in an autocrine manner to osteogenic differentiation (Locke et al., 2006). There is a functional link between the 5-HT_{2B} receptor and the activity of the tissue nonspecific alkaline phosphatase (TNAP). Agonist stimulation of the receptor increases TNAP activity during the initial mineralization phase, whereas inhibition of 5-HT_{2B} receptor intrinsic activity prevents TNAP activation. In contrast, agonist stimulation of the receptor further increased TNAP activity during the initial mineralization phase. The 5-HT_{2B} receptor couples to PLA2 pathway and prostaglandin production at the beginning of mineral deposition. The 5-HT_{2B} receptor also controls leukotriene synthesis via PLA2 at the terminal stages of differentiation. These two 5-HT_{2B} receptor–dependent eicosanoid productions delineate distinct time windows of TNAP regulation during the osteogenic program. Finally, prostaglandins or leukotrienes relay the post-translational activation of TNAP via stimulation of the phosphatidylinositol-specific phospholipase C. In agreement with this, primary calvarial osteoblasts from Htr2B^{−/−} mice exhibit defects in TNAP activity (Baudry et al., 2010). Brain 5-HT may indirectly favor bone mass accrual following activation of 5-HT_{2C} receptors on ventromedial hypothalamic neurons and 5-HT_{2B} receptors on arcuate neurons (Yadav et al., 2009). Compared with control osteoblasts, the lack of 5-HT_{2B} receptors is associated with a 10-fold overproduction of prostacyclin, and the specific prostacyclin synthase inhibitor (U51605) totally rescues osteoblast aggregation and matrix mineralization in Htr2B^{−/−} osteoblasts without effect on WT osteoblasts. Prostacyclin is the endogenous ligand of PPAR-β/δ, and its inhibition in Htr2B^{−/−} cells totally rescues the alkaline phosphatase and osteopontin mRNA levels, cell-cell adhesion, and matrix mineralization. The absence of 5-HT_{2B} receptors leads to the overproduction of prostacyclin, inducing reduced osteoblast differentiation because of PPAR-β/δ–dependent target regulation and defective cell-cell adhesion and matrix mineralization (Chabbi-Achengli et al., 2013). Of relevance, the 5-HT_{2A} receptor is expressed only in osteoblasts, whereas 5-HT_{2B} receptor expression increases from precursor to mature osteoclasts (Hodge et al., 2013b). The 5-HT_{2B} receptor therefore appears to contribute in an autocrine manner to osteogenic differentiation.

20. Teeth Development. Periodontal diseases occur in patients treated with antidepressants such as SSRIs (e.g., fluoxetine). In the molar teeth of Htr2B^{−/−} mice, rod curvatures and twisting are altered compared with WT mice, suggesting involvement of the 5-HT_{2B} receptor at early stages of enamel formation. The volume of the enamel layer in Htr2B^{−/−} mice is also reduced, with smaller crystallite thickness. The outer aprismatic enamel border is 1.5- to twofold larger in Htr2B^{−/−} compared with WT mice. Finally, although no noticeable difference is observed in dentin, the micro-CT three-dimensional pulp reconstruction reveals a decrease in both length and width of dentin formation in the root canals of the Htr2B^{−/−} mice (Dimitrova-Nakov et al., 2014). Therefore, 5-HT_{2B} receptors may mediate some harmful effects of long-term use of SSRIs on bone and teeth regeneration.


a. Carcinoid tumors. 5-HT_{2B} receptor expression is observed in spontaneous human carcinoid tumors, along with coupling to p21ras activation (Launay et al., 1996). The tumor proliferative activity of small intestinal neuroendocrine tumors (including cell growth and the development of desmoplasia) is associated with the particular microenvironment in the peritoneum, and tumor cells support this necessary milieu through the secretion of profibrotic/angiogenetic factors (Svejda et al., 2010).

b. Breast tumors. Increased 5-HT biosynthetic capacity accompanied by multiple changes in 5-HT receptor expression and signaling favor malignant progression of
human breast cancer cells. Among them, expression of 5-HT2B receptors is increased (Pai et al., 2009). 5-HT2B receptor mRNA expression is lower in basal estrogen receptor–negative tumors compared with luminal tumors, which are most commonly estrogen receptor–positive. 5-HT2B receptor mRNA is elevated in carcinomas, increased with tumor stage, and higher in lymph node–positive tumors compared with node-negative tumors. c-Myc transformation induces an increase in 5-HT2B receptor expression (Pai et al., 2009). In human breast cancer, there is a significant correlation of 5-HT2B receptor expression with estrogen receptor-α expression (Kopparapu et al., 2013).

c. Melanoma. Uveal (ocular) melanoma is an aggressive cancer that often forms undetectable micrometastases before diagnosis of the primary tumor. High increases in 5-HT2B receptor mRNA are evident in all uveal melanomas with monosomy 3 compared with low expression in all tumors with disomy 3. As monosomy 3 is associated with metastatic disease, 5-HT2B receptor expression has been proposed as a marker to identify patients with poor prognosis (Tsentscher et al., 2003). The 5-HT2B receptor gene is among the genes showing the highest overexpression in class 2 uveal melanoma (van Gils et al., 2008). A PCR-based 15-gene assay comprising 12 discriminating genes, including the 5-HT2B receptor gene, is now part of a prognostic assay, which provides an important addition to help manage patients with uveal melanoma (Onken et al., 2010) by distinguishing whether uveal melanomas contain liver metastases and thus aid in the diagnosis and prevention of uveal melanoma liver metastases based on their different features (Zhang et al., 2014a).

d. Prostate cancer. Prostate cancer is the most commonly diagnosed noncutaneous cancer in men. Despite this fact, many of the genetic changes that coincide with prostate cancer progression remain enigmatic. The 5-HT2B receptor has been shown to be upregulated in tumors (Magee et al., 2001). Overexpression of receptors to neuromodulatory and endocrine cell products may contribute to development of hormone-refractory prostate cancer. Immunostaining for the 5-HT2B receptor is evident in low-grade and high-grade tumors, prostatic intraepithelial neoplastic and benign prostatic hyperplasia cells, and vascular endothelial cells. Antagonists for the 5-HT2B receptor inhibit proliferation of prostate cancer cells in a dose-dependent manner (Dizeyi et al., 2005).

e. Adrenocortical carcinoma. Gene expression profiles of adrenocortical tumors demonstrate underexpression of 5-HT2B receptor mRNA as a marker of malignant adrenocortical carcinoma (Fernandez-Ranvier et al., 2008). Analysis of biomarkers of malignancy of adrenocortical cancers in a meta-analysis suggests the combination of overexpressed anillin and underexpressed 5-HT2B receptor mRNA to be the best predictor of malignancy (Ziippai et al., 2011). However, in adrenocorticotropin-dependent adrenal hyperplasia, the mechanisms responsible for the ectopic adrenal expression of glucose-dependent insulinotropic peptide (GIP) receptor in GIP-dependent Cushing’s syndrome are unknown. Chronic adrenal stimulation by GIP in GIP-dependent adrenocorticotrophic hormone–independent macronodular adrenal hyperplasia leads to the significant induction of genes for the GPR54, 5-HT2B, GPR4, and endothelial differentiation sphingolipid EDG8 receptor (Lampron et al., 2006).

f. Hepatocellular carcinoma. Among 64 genes for which mRNA expression differed between non–hepatitis B and non–hepatitis C compared with hepatitis C–type hepatocellular carcinoma (HCC), the most affected is the gene for the 5-HT2B receptor (Iizuka et al., 2004). The function of 5-HT as a survival factor of HCC cells has been demonstrated; activation of the 5-HT2B receptor leads to sustained phosphorylation of two downstream targets of mTOR, p70S6K and 4E-BP1, thereby facilitating survival and inhibiting autophagy. Inhibiting the 5-HT2B receptor reduces cancer cell growth in vitro and in vivo. The presence of 5-HT2B receptors in HCC and the activation of autophagy-related mechanisms provide novel insights of 5-HT in cancer biology and propose 5-HT–mediated signaling as a therapeutic target (Soll et al., 2010). 5-HT1B and 5-HT2B receptors are expressed, respectively, in around 32% and 35% of the patients with hepatocellular cancer. Both receptors are associated with an increased proliferation index (Soll et al., 2012). The 5-HT2B receptor mediates 5-HT–induced proliferation in the serum-deprived HCC Huh7 cells. Additionally, selective 5-HT2B receptor antagonism using SB204741 in Huh7 cells decreases the expression of FOX03a (Liang et al., 2013).

g. T-cell leukemia. A proteasome inhibitor, bortezomib, is a potential therapeutic agent to treat adult T-cell leukemia. A network including the 5-HT2B receptor was identified that converges upon the secreted protein acidic and rich in cysteine gene, a tumor-invasiveness–related gene, which may act as a modulator of bortezomib-induced cell death in adult T-cell leukemia cells (Ohyashiki et al., 2008).

h. Myosarcoma. In the pathogenesis of uterine leiomyosarcoma, there is a fourfold overexpression of the 5-HT2B receptor gene, and it is one of the most overexpressed genes (Arslan et al., 2005; Matsumura et al., 2006).

i. Tumor angiogenesis. 5-HT does not enhance colon cancer tumor cell proliferation but may act as a regulator of angiogenesis by reducing the expression of MMP-12 and lower levels of angiostatin—an endogenous inhibitor of angiogenesis (Nocito et al., 2008). 5-HT stimulates the phosphorylation of ERK1/2 in bovine endothelial cells, and the 5-HT2B receptor plays a role in the activation of eNOS in human endothelial cells. In SB204741-treated mice, the selective blockade of the 5-HT2B receptor results in the reduction of tumor angiogenesis and growth through the inhibition effect of ERK1/2 and eNOS (Asada et al., 2009). Therefore, the 5-HT2B receptor may participate in tumor angiogenesis.
22. Clinically Relevant Knowledge Gained from the Gene.

a. The human 5-HT$_{2B}$ receptor gene. The human 5-HT$_{2B}$ receptor gene (MIM 601122) is located on chromosome 2q37.1 (Le Coniat et al., 1996). The human 5-HT$_{2B}$ receptor gene rate of evolution displays high conservation in primates (Andrés et al., 2007). Within the 23 sites that are highly conserved among primates and that have changed on the modern human lineage after separation from Denisovan ancestors, one (D216N) is found in ECL2 of the 5-HT$_{2B}$ receptor, suggesting that crucial aspects of synaptic transmission involving the receptor may have changed in modern humans (Meier et al., 2012). There is evidence of the contribution of 5-HT$_{2B}$ receptor gene variants to intelligence quotient, intellectual disability, and language onset delay in patients with autism spectrum disorders (ASD) (Hervas et al., 2014).

The 5-HT$_{2B}$ receptor gene was identified as a susceptibility gene for impulsivity disorders; one single-nucleotide polymorphism (SNP) introducing a stop codon after amino acid 21 was found more frequently in severely impulsive individuals presenting suicidal behavior (Bevilacqua et al., 2010). In the same cohort, early-onset schizophrenia was more prevalent in 5-HT$_{2B}$ receptor gene Q$^+$20 carriers. Other work testing SNPs within the 5-HT$_{2B}$ receptor gene for potential associations with the behavioral inhibition system and the three components of the behavioral approach system (fun-seeking, drive, and reward responsiveness) in a Han Chinese sample found four 5-HT$_{2B}$ receptor gene SNPs significantly associated with behavioral approach system fun-seeking (Zhu et al., 2012a).

Several SNPs confer a double-mutant R66G/E42G of the receptor protein associated with drug abuse, suggesting that 5-HT$_{2B}$ receptor contributes to pathways that are involved in drug dependence (Lin et al., 2004). Peripheral blood DNA methylation levels of CpGs in the promoter regions were examined in African Americans and European Americans. In European Americans, six CpGs in the 5-HT$_{2B}$ receptor gene promoter are significantly hypermethylated in alcohol-dependent cases (Zhang et al., 2013a).

An association study on susceptibility to migraine in a Spanish population supports the involvement of the 5-HT$_{2B}$ receptor gene and the monoamine oxidase A gene in the genetic predisposition to migraine without aura (Corominas et al., 2010). Finally, by investigating the 5-HT$_{2B}$ receptor gene in patients who developed pulmonary hypertension after use of fenfluramine, a heterozygous mutation was identified in one female patient who, 5 years earlier, had followed a 9-month anorexigen treatment (Blanpain et al., 2003). This heterozygous mutation R393X in the 5-HT$_{2B}$ receptor generates a carboxy terminus-truncated receptor characterized by a switch of coupling from G$_{q/11}$ to Ga$_{13}$, reduced NOS activation, and an increase in cell proliferation, modifications relevant to pathophysiological vasoconstriction (Deraet et al., 2005).

IX. 5-HT$_{2C}$ Receptor

A. Introduction

The 5-HT$_{2C}$ receptor is a G protein–coupled receptor (GPCR) with the characteristic seven-transmembrane domain structure with an extracellular N terminus and intracellular C terminus. Binding of 5-HT to the 5-HT$_{2C}$ receptor results in a conformational change that catalyzes the diffusion of multiple second messenger effectors. The canonical G protein–dependent signaling through the 5-HT$_{2C}$ receptor is engendered by 5-HT–stimulated coupling predominantly to Ga$_{q/11}$ to activate the enzyme phospholipase C (PLC), which generates phosphoinositide hydrolysis and intracellular calcium (Ca$^{2+}$) mobilization (Conn and Sanders-Bush, 1986a; Hoyer et al., 1989c; Chang et al., 2000). The 5-HT$_{2C}$ receptor also signals through other second messengers, which can include phospholipase D (PLD) and phospholipase A$_2$ (PLA$_2$), cyclic nucleotides, and extracellular signal–regulated kinases (Berg et al., 1994, 1998b; Kaufman et al., 1995; Werry et al., 2005). Landmarks in the progress of 5-HT$_{2C}$ receptor research are illustrated in Fig. 12 (see also Palacios et al. (2017)).

The gene for the human 5-HT$_{2C}$ receptor (HTR2C) was cloned and localized to the X chromosome (Xq24) in the early 1990s (Yu et al., 1991; Milatovich et al., 1992; Stam et al., 1994; Xie et al., 1996). The genomic DNA for the 5-HT$_{2C}$ receptor is divided into six exons with five introns (Xie et al., 1996), whereas the coding region contains four exons with three introns (Stam et al., 1994).

The gene encodes a 458-amino-acid protein in humans and 460-amino-acid protein in rats, which share 90% amino acid homology (Saltzman et al., 1991). The 5-HT$_{2C}$ receptor is the only GPCR known to undergo RNA editing, resulting in the functional expression of multiple isoforms of the receptor that differ in their distribution, pharmacology, and signaling capabilities.

The 5-HT$_{2C}$ receptor was first identified in choroid plexus tissue by receptor autoradiography as a highly expressed binding site with high affinity for radioligands that bind to the 5-HT$_1$ receptor family (Pazos et al., 1984; Yagaloff and Hartig, 1985). This binding site displayed a different pharmacological profile from the known 5-HT$_{1A}$ receptor and 5-HT$_{1B}$ receptor and thus was originally named 5-HT$_{1C}$ receptor. It was cloned by functional expression of choroid plexus RNA injected into Xenopus oocytes (Lübbert et al., 1987; Julius et al., 1988). The genomic organization of the 5-HT$_{1C}$ receptor (with the presence of introns), a 50% overall homology to the 5-HT$_{2A}$ receptor and similar signal transduction mechanisms as 5-HT$_{2A}$ receptor and 5-HT$_{2B}$ Receptor, indicated that the 5-HT$_{1C}$ receptor was more similar to members of the 5-HT$_2$ receptor family than the intronless, adenyl cyclase–coupled members of...
the 5-HT₁ receptor family. Therefore, the 5-HT₁C receptor was reclassified as a member of the 5-HT₂C receptor family and renamed 5-HT₂C receptor (Hoyer et al., 1994).

Early autoradiography studies reported a predominant localization of this receptor in choroid plexus tissue, whereas subsequent in situ hybridization studies revealed a widespread distribution throughout the basal ganglia, limbic system, and prefrontal cortex (Hoyer et al., 1986; Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990; Fig. 13). In fact, 5-HT₂C receptor mRNA has been reported to be more abundant and widespread throughout the CNS than mRNA of the closely related 5-HT₂A receptor (Pompeiano et al., 1994; Wright et al., 1995). Thus, the 5-HT₂C receptor, largely thought of as “the choroid plexus receptor,” is actually well positioned throughout the CNS to mediate many of the central actions of 5-HT, including regulation of appetite, cognition, mood, movement, and sleep [for reviews, see Berg et al. (2008a) and Di Giovanni et al., 2010]. The 5-HT₂C receptor has been implicated in addiction, anxiety, depression, epilepsy, schizophrenia, and obesity. Therefore, the 5-HT₂C receptor is a therapeutic target of great interest [for reviews, see Bubar and Cunningham (2006); Howell and Cunningham (2015); Sullivan et al. (2015); Di Giovanni and De Deurwaerdere (2016)].

B. Expression Profile

The 5-HT₂C receptor mRNA is differentially overexpressed in neurons relative to astrocytes and oligodendrocytes in the postnatal mouse forebrain (Cahoy et al., 2008). Regional analyses indicate that the 5-HT₂C receptor mRNA is present in high levels in the choroid plexus, hippocampus, and the subthalamic and lateral habenular nuclei (Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990). Brain regions with moderate to high levels of 5-HT₂C receptor mRNA include the amygdala, nuclei of the basal ganglia (NAc, striatum, VTA, SN, and internal globus pallidus), cortex, hypothalamus, dorsal raphe, brain stem, and spinal cord (Hoffman and
Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990). The 5-HT$_{2C}$ receptor mRNA is found in regions containing the major dopaminergic cell bodies, including the SN and the VTA (Mengod et al., 1990; Pompeiano et al., 1994; Eberle-Wang et al., 1997; Bubar and Cunningham, 2007; Bubar et al., 2011); cholinergic cell bodies (Lopez-Gimenez et al., 2001); encephalin-, substance P-, and dynorphin-containing neuropeptidergic neurons in the dorsal and ventral striatum (Ward and Dorsa, 1996); and neuropeptide Y–containing neurons in the lateral and basolateral amygdala (Bonn et al., 2013). The 5-HT$_{2C}$ receptor mRNA is not present in neurons expressing 5-HT transporter (SERT) mRNA in the raphe nuclei but is localized to GABA interneurons in this region (Serrats et al., 2005).

There is good agreement between mRNA and protein distribution in the majority of brain regions, suggesting that the 5-HT$_{2C}$ receptor is predominantly localized in somatodendritic compartments (Mengod et al., 1990; Abramowski et al., 1995; Abramowski and Staufenbiel, 1995; Eberle-Wang et al., 1997; Clemett et al., 2000; Anastasio et al., 2010). The exceptions are the subthalamic nucleus, in which 5-HT$_{2C}$ receptor mRNA expression is high while its protein levels are low, and the external globus pallidus where the 5-HT$_{2C}$ receptor is present but 5-HT$_{2C}$ receptor mRNA is absent.

Early radioligand binding studies suggested that the 5-HT$_{2C}$ receptor is located on the apical surface of epithelial cells in the choroid plexus (Hartig et al., 1990). More recently, confocal microscopy and fluorescence correlation spectroscopy were used to directly visualize native 5-HT$_{2C}$ receptors and reveal their expression as homodimers on the apical surface of choroid epithelial cells (Herrick-Davis et al., 2015).

The 5-HT$_{2C}$ receptor is widely distributed throughout the basal ganglia and limbic-corticostriatal circuit. The 5-HT$_{2C}$ receptor is located postsynaptic to serotonergic neurons on GABAergic, glutamatergic, dopaminergic, neuropeptidergic, and cholinergic neurons. For example, the 5-HT$_{2C}$ receptor is expressed on GABAergic interneurons in the raphe nuclei (Serrats et al., 2005) and on GABAergic projection neurons in the NAc and striatum (Alex and Pehek, 2007). They are also found on GABAergic neurons in the substantia nigra (Eberle-Wang et al., 1997; Invernizzi et al., 2007) and VTA (Di Giovanni et al., 2001; Bubar and Cunningham, 2007; Bubar et al., 2011). Because the 5-HT$_{2C}$ receptor stimulates IP turnover (Conn and Sanders-Bush, 1986a; Hoyer et al., 1989; Chang et al., 2000) and increases Ca$^{2+}$ levels resulting in membrane depolarization and neuronal firing (Stanford et al., 2005), activation of the 5-HT$_{2C}$ receptor on GABAergic neurons would be expected to increase the firing of these neurons, which have an inhibitory influence in that region. For example, stimulation of the 5-HT$_{2C}$ receptor in the VTA increases the firing rate of GABAergic interneurons, resulting in a decreased firing rate of dopaminergic neurons (Prisco et al., 1994; Di Giovanni et al., 2001). Conversely, 5-HT$_{2C}$ receptor antagonism has been reported to increase dopaminergic neurotransmission and dopamine levels in the NAc and prefrontal cortex (Di Giovanni et al., 1999; Di Matteo et al., 1999; Gobert et al., 2000). However, the story is likely to be more complicated, as the 5-HT$_{2C}$ receptor has also been shown to be expressed on a subset of dopaminergic neurons in the VTA, with higher expression in the middle relative to the rostral and caudal regions (Bubar and Cunningham, 2007; Bubar et al., 2011). These findings suggest the possibility that activation of the 5-HT$_{2C}$ receptor may directly enhance dopamine neurotransmission within specific subnuclei of the VTA in a region-specific manner.

5-HT neurons from the raphe terminate in layers V and VI of the medial prefrontal cortex, adjacent to GABAergic interneurons that express the 5-HT$_{2C}$ receptor (Liu et al., 2007; Nocjar et al., 2015). In turn, the GABAergic interneurons terminate on pyramidal efferents (likely glutamatergic), which may also express the
5-HT\textsubscript{2C} receptor (Vysokanov et al., 1998; Carr et al., 2002; Liu et al., 2007; Nocjar et al., 2015). In the prefrontal cortex, the 5-HT\textsubscript{2C} receptor associates with PSD-95 in postsynaptic densities (Anastasio et al., 2010) and colocalizes with the 5-HT\textsubscript{2A} receptor on GABAergic interneurons (Nocjar et al., 2015). In the hypothalamus, the 5-HT\textsubscript{2C} receptor is expressed on pro-opiomelanocortin (POMC) neurons in the arcuate nucleus, where they stimulate the release of the anorectic peptide α-melanocyte-stimulating hormone (Heisler et al., 2002).

Previous studies provide little evidence that the 5-HT\textsubscript{2C} receptor is expressed outside the CNS. However, portions of the HTR2C gene are expressed outside the CNS. Exons I and II of the HTR2C gene are located in the 5′ untranslated region, and the translational start site for the 5-HT\textsubscript{2C} receptor protein is located in the middle of exon III (Xie et al., 1996). Exons I–III of the HTR2C gene have been reported to be expressed in non-neuronal cells, along with four different micro-RNAs (miRNA) generated from intron II (Zhang et al., 2013c). Expression of these miRNAs is regulated by the small nucleolar RNAs (snoRNA) SNORD 115/MBII-52 and SNORD 116/MBII-85. Patients with Prader-Willi syndrome lack MBII-52 and MBII-85 and express different levels of the 5-HT\textsubscript{2C} receptor miRNAs than control subjects (Zhang et al., 2013c). Although the function of these miRNAs is not fully understood, it is possible that their dysregulation may contribute to the Prader-Willi syndrome phenotype as explored in 5-HT\textsubscript{2C} receptor transgenic mouse models.

Because the 5-HT\textsubscript{2C} receptor mRNA in human brain includes exons I–VI, and the mRNA found outside the CNS contains exons I–III, it was hypothesized that a transcriptional termination signal prior to exon IV may be employed in non-neuronal cells outside the CNS (Zhang et al., 2013c). In contrast, a 185-base-pair fragment of 5-HT\textsubscript{2C} receptor mRNA was identified by quantitative RT-PCR from rat adipocyte visceral tissue (Stunes et al., 2011). The expression level of the 5-HT\textsubscript{2C} receptor was significantly higher in adipocytes than in brain (Stunes et al., 2011). However, expression of 5-HT\textsubscript{2C} receptor mRNA and protein expression could be induced in cultured adipocytes. In addition, low levels of 5-HT\textsubscript{2C} receptor mRNA and protein have been reported in rat pancreatic islet cells (Zhang et al., 2013b). Further research is required to elucidate the physiologic role of the 5-HT\textsubscript{2C} receptor in the function of adipocytes (Stunes et al., 2011) and potentially pancreatic β-cells (Zhang et al., 2013b).

C. Post-transcriptional and Post-translational Modifications

1. RNA Editing. The 5-HT\textsubscript{2C} receptor is the only GPCR reported to undergo RNA editing. The 5-HT\textsubscript{2C} receptor pre-mRNA is unique in containing a region of exonic and intronic sequence complementarity between the distal half of exon V and the beginning of intron V (Burns et al., 1997). This results in base pairing between the exonic and intronic sequences, giving the pre-mRNA a double-stranded, stem-loop structure. The secondary structure of the 5-HT\textsubscript{2C} receptor pre-mRNA influences the pattern of editing (Fukuda et al., 2015) as well as splicing (Shen et al., 2013). The double-stranded RNA is a substrate for adenosine deaminases that act on RNA and RNA-specific adenosine deaminase (ADAR) 1 and ADAR2 (Burns et al., 1997; Liu et al., 1999). These enzymes catalyze the deamination of adenosine to inosine, which is read as guanosine when the RNA is translated into protein. Adenosine to inosine RNA editing can occur at up to five different locations (termed A–E) within exon V (Burns et al., 1997; Fitzgerald et al., 1999; Niswender et al., 1999; Wang et al., 2000a) and one site located in intron V (Flomen et al., 2004). The editing sites in exon V are located in the second intracellular loop of the receptor in close proximity to the highly conserved “DRY” motif at the base of the transmembrane domain III. The resulting adenosine to guanosine conversions change the coding potential of amino acids 1156, N158, and N160 from INI (isoleucine, asparagine, isoleucine) in the unedited isoform to VSV (valine, serine, valine) or VGV (valine, glycine, valine) in the fully edited isoforms.

The RNA editing of the HTR2C gene can produce up to 32 different 5-HT\textsubscript{2C} receptor pre-mRNAs encoding 24 different proteins. There are seven predominant 5-HT\textsubscript{2C} receptor isoforms that are expressed in a region-specific manner in human and rodent brain (Burns et al., 1997; Fitzgerald et al., 1999; Niswender et al., 1999; Wang et al., 2000; Dracheva et al., 2009; Abbas et al., 2010; Morabito et al., 2010b). The more highly edited isoforms are the predominant isoforms expressed in whole brain, with VSV and VNV being the most prominent. Regions such as the hypothalamus, hippocampus, striatum, and cortex predominantly express the edited VNV, VSV, and VSI isoforms, whereas the choroid plexus and cerebellum express significantly higher levels of the unedited INI and partially edited INV and ISV isoforms.

Because RNA editing occurs in the second intracellular loop of the receptor, a region known to play an important role in G protein activation, it has profound effects on the pharmacology and signaling capabilities of the 5-HT\textsubscript{2C} receptor. RNA editing decreases agonist binding affinity and G protein coupling efficiency (Burns et al., 1997; Fitzgerald et al., 1999; Herrick-Davis et al., 1999; Niswender et al., 1999; Wang et al., 2000; Berg et al., 2001). In these studies, the fully edited VSV and VGV isoforms displayed a 5- to 40-fold decrease in 5-HT binding affinity and potency for stimulating phosphoinositide production compared with the unedited INI isoform, and they displayed decreased affinity and potency for a variety
of different agonists. When expressed in COS-7 cells at levels similar to native 5-HT$_{2C}$ receptor in choroid epithelial cells, the unedited INI isoform displayed high constitutive activity that was successively diminished by RNA editing to very low levels of constitutive activity in the fully edited VSV and VGV isoforms (Herrick-Davis et al., 1999; Niswender et al., 1999). In addition to regulating the level of basal activity, RNA editing alters the specificity of G protein coupling and second messenger activation. For example, the unedited INI isoform can signal through G$_{q}$, G$_{13}$, and G$_{15}$, but the fully edited VSV and VGV isoforms predominantly couple to and signal through G$_{q}$ (Price et al., 2001; McGrew et al., 2002, 2004). The 5-HT$_{2C}$ receptor has been shown to signal in an agonist-specific manner to differentially regulate phosphoinositide and arachidonic acid production (Berg et al., 1998b) and the phosphorylation of ERK$_{1/2}$ (Werry et al., 2005), effects which are diminished following RNA editing (Berg et al., 2001, 2008b; Werry et al., 2008). In addition to impacting signaling, RNA editing also influences receptor desensitization and trafficking (Marion et al., 2004).

The 5-HT$_{2C}$ receptor pre-mRNA editing is regulated by ADAR1 and ADAR2 (Burns et al., 1997). Both in vitro and in vivo studies have reported that RNA editing at amino acid 156 of the 5-HT$_{2C}$ receptor is predominantly accomplished by ADAR1, editing at amino acid 160 requires ADAR2, and both enzymes participate in editing at amino acid 158 (Liu et al., 1999; Wang et al., 2000, 2004; Hartner et al., 2004). ADAR1 and ADAR2 may act in a concerted manner whereby ADAR1 editing at amino acid 156 enhances ADAR2 pre-mRNA binding and subsequent editing at amino acids 160 and 158 (Carmel et al., 2012). In addition, factors that regulate the alternative splicing of the ADAR1 and ADAR2 pre-mRNAs have been reported to influence 5-HT$_{2C}$ receptor RNA editing (Liu et al., 1999; Schmauss et al., 2010). Alterations in ADAR activity or pattern of expression can result in altered 5-HT$_{2C}$ receptor isoform expression. Studies using neuronal cell cultures showed that factors that increase ADAR1 expression, such as treatment with interferon, increase 5-HT$_{2C}$ receptor editing at amino acid 156 (Yang et al., 2004) and that reduced ADAR1 expression abolished editing at amino acid 156 (Sukma et al., 2005). In vivo evidence supporting this hypothesis is provided by a recent study reporting a downregulation in ADAR2 expression following spinal cord injury in rats, which resulted in decreased 5-HT$_{2C}$ receptor RNA editing at amino acid 160 (Di Narzo et al., 2015). Conversely, RNA editing has been reported to be increased in transgenic mice overexpressing ADAR2, which resulted in an increase in the expression of the more fully edited, and a decrease in expression of the unedited, 5-HT$_{2C}$ receptor isoforms (Singh et al., 2011). The results of these studies indicate that alterations in ADAR activity and expression patterns alter the degree and pattern of 5-HT$_{2C}$ receptor editing, resulting in changes in 5-HT$_{2C}$ receptor isoform expression and signal transduction.

2. RNA Splicing. Three different variants of the 5-HT$_{2C}$ receptor are produced by RNA splicing, only one of which is expressed on the plasma membrane and is fully functional. The full-length functional 5-HT$_{2C}$ receptor (2Cfl) is generated following splicing at the traditional exon/intron boundaries to remove introns III–V (Xie et al., 1996). There are two alternative donor splice sites flanking the traditional donor splice site located at the exon V intron V boundary: one located in the middle of exon V (Canton et al., 1996; Xie et al., 1996) and an infrequently used site located within intron V (Wang et al., 2000; Flomen et al., 2004). The splice sites are conserved across rat, mouse, and human species. Splicing at either one of the alternative splice sites produces an RNA that encodes a prematurely truncated protein. Use of the donor splice site located in the middle of exon V results in the deletion of the last 95 nucleotides of exon V, including the RNA editing sites, and causes a frame shift mutation with stop codon. The result is a truncated protein (2Ctr) containing the N terminus and first three transmembrane domains followed by 96 unique amino acids (Canton et al., 1996; Xie et al., 1996; Wang et al., 2000). In human brain tissue, 2Ctr mRNA is found in all brain regions containing 2Cfl mRNA. Though Western blots of membrane extracts from 2Ctr-transfected NIH3T3 cells revealed immunoreactive bands the predicted size of 2Ctr protein, radioligand binding and phosphoinositide production were not observed in the transfected cells (Canton et al., 1996; Wang et al., 2000). The 2Ctr protein is not expressed on the plasma membrane but is retained within the endoplasmic reticulum where the 2Ctr can form heterodimers with 2Cfl (Herrick-Davis and Farrington, 2011; Martin et al., 2013). Thus, one possible function of 2Ctr is to regulate 5-HT$_{2C}$ receptor signaling by forming heterodimers with 2Cfl and trapping 2Cfl in the endoplasmic reticulum (Herrick-Davis and Farrington, 2011; Martin et al., 2013).

Factors that influence splice site selection will impact 5-HT$_{2C}$ receptor function and signaling by altering the relative balance between 2Cfl and 2Ctr within a given brain region. RNA editing and factors that influence RNA editing, such as ADAR1 and ADAR2 activity, have been shown to influence splice site selection (Rueter et al., 1999; Maas et al., 2001; Flomen et al., 2004; Tohda et al., 2004; Dracheva et al., 2008a). Increased editing of the 5-HT$_{2C}$ receptor pre-mRNA, generating the more fully edited and less active isoforms, promotes the use of the traditional donor splice site at the exon V intron V boundary and favors the generation of 2Cfl over 2Ctr (Flomen et al., 2004). On the other hand, the unedited and highly active INI isoform is associated with increased use of the alternative donor splice site in the middle of exon V, increasing the production of 2Ctr. The preferred splicing of the INI isoform into 2Ctr (Flomen et al., 2004) may explain the higher plasma
membrane expression levels of 2Cfl observed in transgenic mice expressing the VGV isoform compared with mice expressing the INI isoform of the 5-HT₂R (Kawahara et al., 2008). These results are consistent with the original studies in which 2Ctr was first identified and was reported to be most abundant in the choroid plexus (Canton et al., 1996; Xie et al., 1996), the same brain region that was subsequently reported to express the highest levels of unedited and partially edited isoforms of the 5-HT₂₃C receptor (Burns et al., 1997; Niswender et al., 1999; Wang et al., 2000).

Several studies have provided evidence for a link between 5-HT₂₃C receptor RNA editing and splicing in vivo. In postmortem brain samples from patients who committed suicide, RNA editing was increased along with the ratio of 2Cfl to 2Ctr, with increased expression of the less-active 5-HT₂₃C receptor isoforms (Dracheva et al., 2008a). In malignant gliomas from human brain, decreased ADAR2 activity (predicted to reduce RNA editing) was positively correlated with increased alternative splicing and the production of 2Ctr (Maas et al., 2001). In several of the gliomas examined and in glioma-derived cells lines, 2Ctr was predominant and 2Cfl was mostly absent (Maas et al., 2001; Tohda et al., 2004). In light of these findings, it is interesting to note that patients with glioblastoma have an increased incidence of seizures, as do 5-HT₂₃C receptor knockout mice.

noRNAs also regulate 5-HT₂₃C receptor RNA splicing. The snoRNA MBII-52 binds to exon V of the HTR2C gene in the region containing the alternative splice site and the RNA editing sites (Kishore and Stamm, 2006; Kishore et al., 2010). MBII-52 reduces RNA editing and the use of the alternative splice site, thereby favoring the production of the full-length, unedited, and more active 5-HT₂₃C receptor isoforms. In mice lacking MBII-52, RNA editing is increased, leading to increased expression of the edited and less-active 5-HT₂₃C receptor isoforms (Doe et al., 2009). These mice display phenotypic and behavioral changes similar to those observed in Prader-Willi syndrome (Doe et al., 2009). Consistent with these findings, transgenic mice expressing only the fully edited VGV isoform display characteristics of Prader-Willi syndrome (Morimoto et al., 2010a). The human homolog HBII-52 has been shown to be absent in patients with Prader-Willi syndrome, and 5-HT₂₃C receptor RNA editing is increased in patients with Prader-Willi syndrome (Kishore and Stamm, 2006). These studies highlight the important roles that RNA editing and splicing play in the regulation of 5-HT₂₃C receptor activity and demonstrate how deregulation of this system can have profound phenotypic and behavioral consequences.

3. Single-Nucleotide Polymorphisms. The 5-HT₂₃C receptor not only achieves diversity through RNA editing and splicing but also through the incorporation of SNPs, substitutions of a novel nucleotide for a wild-type nucleotide, a common type of genetic variation (Wang et al., 1998). These SNPs can produce an unstable conformation that alters the protein structure of a GPCR and affects its ultimate functional properties (Wenkert et al., 1996). A number of HTR2C SNPs have been reported in the literature: three polymorphisms as well as a GT nucleotide repeat variation have been identified in the promoter (Xie et al., 1996); three polymorphisms have been reported within intronic regions (Gibson et al., 2004); one polymorphism has been reported in the coding region, resulting in the replacement of cysteine with serine at amino acid 23 (C23S) in the amino-terminus of the receptor (Lappalainen et al., 1995); and there is one polymorphism in the 3′ untranslated region (Song et al., 1999). Few studies are reported that have ascertained the impact of these HTR2C SNPs on function at either the cellular or whole organism level. The impact of the S23 variant, when expressed in insect cells, was reported to have slightly higher affinity for 5-HT than the C23 variant and to alter 5-HT₂₃C receptor desensitization/resensitization mechanisms (Okada et al., 2004; Walstab et al., 2011). However, this finding remains controversial, as other studies have reported no difference between the C23 and S23 variants with respect to agonist-binding affinity or potency, G protein coupling, constitutive activity, and homodimerization (Lappalainen et al., 1995; Fentress et al., 2005). Given suggestions that the C23 variant may have clinical implications (Lappalainen et al., 1995; Okada et al., 2004; Piva et al., 2011; Walstab et al., 2011), further studies of this and other HTR2C SNPs are required.

4. Glycosylation. The rat 5-HT₂₃C receptor is reported to be glycosylated, and bands with different molecular weights were observed on Western blot of brain and cells expressing the receptor (Abramowski and Staufenbiel, 1995). Antibodies raised against the third and fourth cytoplasmic domain of the 5-HT₂₃C receptor identified an N-glycosylated polypeptide with a 60-kDa apparent molecular mass. Polypeptides were detected in immunoprecipitates from extracts of pig choroid plexus upon Western blot and binding assays with [³H]-mesulergine. A signal sequence was cleaved during membrane insertion, resulting in a 38-kDa polypeptide. During further maturation, the receptor was N-glycosylated at two sites via a 48-kDa intermediate, which was more abundant in choroid plexus than in hippocampus. However, there may be more glycosylated species, as following transfection of 5-HT₂₃C receptor cDNAs into cultured cells, polypeptides were observed that differed from the ones found in the brain. Similarly, the N-glycosylated 5-HT₂₃C receptor was identified in solubilized extracts from cell lines and rat brain (Backstrom et al., 1995). Extracts from NIH3T3 fibroblasts stably expressing rat 5-HT₂₃C receptor contained immunoreactive proteins of 51 to 52 kDa and 58 to 68 kDa.

In the brain, immunoreactive proteins were identified from choroid plexus extracts with masses of 51 kDa and...
58–62 kDa. On the other hand, the major 58- to 62-kDa and minor 51-kDa proteins were not detected in extracts prepared from the hippocampus, striatum, or frontal cortex prepared under the same conditions. The association of asparagine-linked (N-linked) oligosaccharides with the receptor was also examined. Cells grown in the presence of tunicamycin to inhibit N-linked glycosylation resulted in proteins with masses of 40 and 41 kDa. Extracts prepared from NIH3T3 cells and choroid plexus incubated with N-glycosidase F showed proteins of 41 and 42 kDa from NIH3T3 cells and 41 kDa from choroid plexus. Neuraminidase treatment, to cleave sialic acid, reduced the mass of the 51-kDa and 58- to 62-kDa proteins from the choroid plexus to 50 kDa and 54–58 kDa, whereas the proteins from NIH3T3 cells were not affected by neuraminidase. Altogether, the 5-HT2C receptor contains N-linked sugars, and it is suggested that sialic acid residues associate with the receptor from the choroid plexus but not from other brain regions. These oligosaccharide moieties contribute up to approximately 30% of the relative mass of the receptor and may affect the functional properties of the 5-HT2C receptor.

5. Phosphorylation. The constitutively active 5-HT2C receptor is phosphorylated under basal conditions, and phosphorylation is increased by agonist treatment (Westphal et al., 1995). Pretreatment of cells with 5-HT resulted in 5-HT2C receptor desensitization, which was blocked by calcineurin (presumably by its phosphatase activity) (Boddeke et al., 1993). It was subsequently shown that S458 and S459 are phosphorylated (Backstrom et al., 2000). Phosphorylation of a mutant 5-HT2C receptor that lacks the carboxyl-terminal PDZ recognition motif [Ser(458)-Ser-Val-COOH; δPDZ] was not detectable, although these cells produced similar amounts of phosphoinositide and Ca2+ with similar kinetics as wild-type cells. Alanine mutations S458A or S459A decreased phosphorylation to 50% of wild-type receptor levels. Subsequent Ca2+ responses of S459A receptors were diminished relative to S458A and wild-type receptors. Thus, desensitization may occur in the absence of 5-HT2C receptor phosphorylation, suggesting that receptor phosphorylation at S459 enhances resensitization of 5-HT2C receptor responses. Agonist-induced phosphorylation of the 5-HT2C receptor was later established to regulate the receptor interaction with multiple PDZ protein 1 (Parker et al., 2003), also known as MUPP1 (Ullmer et al., 1998). MUPP1 is a putative scaffolding protein containing 13 PSD-95, Dlg, ZO-1 (PDZ) domains, identified by a yeast two-hybrid screen as one of a series of 5-HT2C receptor–interacting proteins. The MUPP1 PDZ domain 10 (PDZ 10) associates with Ser458-Ser-Val of the 5-HT2C receptor. An Asp mutation at Ser458 significantly decreased receptor interaction with PDZ 10. Also, 5-HT treatment of 5-HT2C receptor–NIH3T3 cells reduced receptor interaction with PDZ 10, an effect that was blocked by a 5-HT2C receptor antagonist.

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) interacts within several amino acids of the third intracellular loop (termed 3L4F) of the 5-HT2C receptor (Ji et al., 2006). The tumor suppressor PTEN is widely distributed in the brain (Lachyankar et al., 2000). The PTEN and 5-HT2C receptor proteins coimmunoprecipitate in fractions of the VTA (Ji et al., 2006; Anastasio et al., 2013). Employing a proximity ligation assay, the assembly of the 5-HT2C receptor:PTEN complex, and the ability of 3L4F to disrupt the complex, was validated under native conditions within intact live cells (Anastasio et al., 2013). A peptide fragment of the 5-HT2C receptor third intracellular loop (3L4F), labeled with the cell-penetrating peptide TAT, disrupts the 5-HT2C receptor:PTEN complex and PTEN-mediated dephosphorylation of the 5-HT2C receptor in PC12 cells (Ji et al., 2006) as well as enhances 5-HT–mediated i Ca2+ release in CHO cells stably transfected with the human 5-HT2C-β3 receptor (but not the 5-HT2A receptor) (Anastasio et al., 2013). Interestingly, the cell-adhesion molecule close homology of L1 protein also binds to the third intracellular loop of the 5-HT2C receptor (amino acids 292–304) and may regulate the 5-HT2C receptor association with PTEN and β-arrestin2 to control its phosphorylation (Kleene et al., 2015).

Systemic administration of the TAT-3L4F peptide suppresses the Δ9-tetrahydrocannabinol (THC)-induced increase in firing rate of VTA dopaminergic neurons in the rat (Ji et al., 2006) and suppresses the place association conditioned to Δ9-THC and nicotine in rats (Ji et al., 2006). In more recent studies, whereas TAT-3L4F had no effect alone, the combination of ineffective doses of TAT-3L4F plus WAY163909 synergizes to suppress motor impulsivity, a primary symptomatic element of multiple neuropsychiatric disorders (American Psychiatric Association, 2013) that is consistently suppressed by pretreatment with a selective 5-HT2C receptor agonist (Winstanley et al., 2004; Fletcher et al., 2007; Fletcher et al., 2011; Anastasio et al., 2013; Cunningham et al., 2013). In addition, TAT-3L4F augments 5-HT2C receptor agonist–mediated suppression of spontaneous locomotor activity, effects that are consistent with positive allosteric modulation of 5-HT2C receptor function (Anastasio et al., 2013; Wild et al., 2014, 2019). Together, coupled with the findings that Tat-3L4F did not affect spatial learning and memory (Maillet et al., 2008) or generate the total behavioral profile of 5-HT2C receptor agonists (Ji et al., 2006; Anastasio et al., 2013), inhibition of the PTEN:5-HT2C receptor interface and, thus, 5-HT2C receptor dephosphorylation is a novel pharmacological approach with therapeutic potential in substance use disorders (Ji et al., 2006; Muller and Carey, 2006; Maillet et al., 2008; Anastasio et al., 2013; Cunningham and Anastasio, 2014).

6. Dimerization. Homodimerization for many GPCRs is thought to be a post-translational event that occurs within the endoplasmic reticulum as a prerequisite for the transport and expression of functional receptors on the
plasma membrane [for review, see Milligan (2010)]. Many members of the 5-HT receptor family, including the 5-HT$_{2C}$ receptor, have been reported to form homodimers [for review, see Herrick-Davis (2013)]. Using a confocal microscopy-based resonance energy-transfer technique, 5-HT$_{2C}$ receptor homodimer formation was visualized within the endoplasmic reticulum during receptor biosynthesis (Herrick-Davis et al., 2006). The 5-HT$_{2C}$ receptor is transported through the Golgi complex to the plasma membrane as a homodimer.

The 5-HT$_{2C}$ receptor forms detergent-sensitive homodimers (Herrick-Davis et al., 2004) that do not dissociate or associate to form higher-order complexes following agonist or inverse agonist treatment (Herrick-Davis et al., 2007). Coexpression of wild-type receptors with inactive, mutant receptors provided evidence that the 5-HT$_{2C}$ receptor homodimer interacts with a single G protein, that both protomers participate in signaling, and that both protomers must be functional in order for signaling to occur (Herrick-Davis et al., 2005). Time-lapse fluorescence confocal microscopy provided direct visualization of $\beta$-arrestin$_2$ recruitment to the plasma membrane following 5-HT binding to the homodimer (Herrick-Davis et al., 2007; Herrick-Davis et al., 2012). Homodimerization was observed for both the unedited INI and the fully edited (VSV and VGV) isoforms (Herrick-Davis et al., 2007; Herrick-Davis et al., 2012).

Advanced imaging techniques with near-single-molecule sensitivity have been employed more recently in an attempt to distinguish between dimers and higher-order oligomers. Fluorescence correlation spectroscopy studies report that the 5-HT$_{2C}$ receptor expressed in HEK293 cells exists as homodimers, with no evidence for monomers, tetramers, or higher-order oligomers (Herrick-Davis et al., 2012). Spatial intensity distribution analysis was used to monitor the oligomer status of the 5-HT$_{2C}$ receptor over a wide range of receptor-expression levels (Ward et al., 2015). In this study, the 5-HT$_{2C}$ receptor was expressed as a mixture of monomers, dimers, and tetramers; treatment with a 5-HT$_{2C}$ receptor antagonist for 90 minutes converted the majority of dimers and tetramers to monomers. However, it should be noted that tetramers were prominent only when 5-HT$_{2C}$ receptor expression exceeded 100 receptors/µm$^2$ (Ward et al., 2015), greatly in excess of physiologic expression levels for native GPCRs (Hegener et al., 2004; Herrick-Davis et al., 2015). Similarly, the homodimer was the predominant species observed for biogenic amine receptors when expressed within their normal physiologic range in HEK293 cells (Herrick-Davis et al., 2013).

Only one study to date has examined the 5-HT$_{2C}$ receptor endogenously expressed in its native cellular environment. The native 5-HT$_{2C}$ receptor, endogenous to the choroid plexus, is expressed as homodimers on the apical surface of the epithelial cells at a density of 32 receptors/µm$^2$ (Herrick-Davis et al., 2015). Though this is similar to a reported density of 20 receptors/µm$^2$ for native $\beta_2$-adrenergic receptors in alveolar epithelial cells, expression levels in neurons were much lower at 4.5 receptors/µm$^2$ (Hegener et al., 2004). The study by Herrick-Davis et al. (2015) found no evidence for monomers or tetramers of the native 5-HT$_{2C}$ receptor in choroid epithelial cells or when the 5-HT$_{2C}$ receptor was expressed at normal physiologic levels in HEK293 cells. In this study, the signaling properties of the 5-HT$_{2C}$ receptor homodimer were investigated using agonists that bind in a wash-resistant manner to one or both protomers of the 5-HT$_{2C}$ receptor homodimer. Agonist binding to one protomer stimulated a half-maximal phosphoinositide response, whereas binding to both protomers was required to produce a maximal response (Herrick-Davis et al., 2015). These experiments provide pharmacological evidence supporting the hypothesis that the 5-HT$_{2C}$ receptor functions as a homodimer.

Heterodimers can form between the different isoforms of the 5-HT$_{2C}$ receptor generated by RNA editing (Herrick-Davis and Farrington, 2011). In HEK293 cells, positive resonance energy transfer was observed between the INI/VSV, INI/VGV, and VSV/VGV isoform pairs. However, the influence of heteromer pairs of edited 5-HT$_{2C}$ receptor isoforms on receptor signaling and neural function in vivo are yet to be demonstrated. In terms of heterodimers between the 5-HT$_{2C}$ receptor and other members of the 5-HT receptor family, the 5-HT$_{2A}$ receptor would be the most likely candidate given the similarity in structure (for review, see Hannion and Hoyer, 2008a,b). Immunohistochemical analyses indicate that the 5-HT$_{2A}$ receptor and 5-HT$_{2C}$ receptor protein colocalize in the same GABAergic neurons as well as in a population of pyramidal projection neurons in the rat medial prefrontal cortex (mPFC) (Nocjar et al., 2015). Coimmunoprecipitation studies suggest that the 5-HT$_{2A}$ receptor and 5-HT$_{2C}$ receptor are found in the same protein complex in the rat mPFC (Anastasio et al., 2015). Further analyses of the structural and biologic significance of a possible heteromeric protein complex incorporating both the 5-HT$_{2A}$ receptor and 5-HT$_{2C}$ receptor are required to understand its potential role in behavior and neuropsychiatric disorders.

Selective 5-HT$_{2A}$ receptor antagonists and selective 5-HT$_{2C}$ receptor agonists have been noted to suppress a wide range of behaviors that are particularly well studied in preclinical models of addictive disorders (Bubar and Cunningham, 2008; Cunningham and Anastasio, 2014; Howell and Cunningham, 2015). The combination of low doses of the selective 5-HT$_{2A}$ receptor antagonist M100907 plus the preferential 5-HT$_{2C}$ receptor agonist MK212 evoked modest effects but resulted in an approximately additive suppression of cocaine-evoked hyperlocomotion and Fos expression in the caudate putamen (Pockros et al., 2012). In a second study, the combination of subthreshold doses of M100907 plus the selective 5-HT$_{2C}$ receptor agonist WAY163909
Knockdown of 5-HT2C receptor in the mPFC resulted by coimmunoprecipitation (Anastasio et al., 2015). This led to compensatory upregulation of 5-HT2A receptor protein in increased motor impulsivity and triggered a competitive inhibition of M100907 to suppress impulsive behavior (Anastasio et al., 2015). These data further support the concept that an interactive relationship between the mPFC 5-HT2A receptor and 5-HT2C receptor is behaviorally relevant. The manner in which a potential 5-HT2A receptor and 5-HT2C receptor heteromeric protein complex in the mPFC contributes to high levels of inherent motor impulsivity remains to be uncovered.

The 5-HT2C receptor has also been reported to form heterodimers with ghrelin GHS-R1a receptor when overexpressed in HEK293 cells and to colocalize with the GHS-R1a in cultured primary hypothalamic and hippocampal neurons from the rat (Schellekens et al., 2015). In this study, activation and blockade of 5-HT2C receptor in vivo attenuated and potentiated, respectively, the orexigenic effects of ghrelin. Heterodimers between 5-HT2C receptor and melatonin MT2 receptors have also been reported in transfecte cells as well as human cortex and hippocampus (Kamal et al., 2015).

Interestingly, the novel antidepressant agomelatine demonstrated biased signaling and displays 5-HT2C receptor and MT2 receptor agonist properties, suggesting the heterodimer as a potential target for the development of a novel class of therapeutics for the treatment of psychiatric disorders as well as eating disorders and obesity.

Immunohistochemical studies demonstrated that the NMDA receptor GluN2A colocalizes with the 5-HT2C receptor in rat spinal cord neurons, whereas coimmunoprecipitation analysis of synaptosomal fractions suggests formation of a 5-HT2C receptor and GluN2A protein complex (Bigford et al., 2012). Stimulation of the 5-HT2C receptor enhanced NMDA-evoked motoneuron depolarization through involvement of Src tyrosine kinase (Bigford et al., 2012). These results support the assembly of a functionally relevant 5-HT2C receptor and NMDA receptor complex in the spinal cord, although the presence of this complex in the brain and its involvement in higher-order neural function is unexplored.

D. Pharmacology

For the 5-HT2C receptor, the identification of selective orthosteric ligands relative to the close family members 5-HT2A receptor and 5-HT2B receptor is challenged by their ~50% overall homology, which rises to ~80% in the transmembrane domains, comprising the orthosteric receptor–binding pocket (Hoyer et al., 2002). Because of this challenge, the earliest preclinical and clinical research employed “preferential” 5-HT2C receptor ligands, which frequently displayed affinity (agonists, antagonists) and/or efficacy (agonists) at the 5-HT2A receptor and 5-HT2B receptor. Because of the lack of selectivity of available agonists [e.g., MK212 and m-chlorophenylpiperazine (mCPP)] and antagonists (e.g., ketanserin), experimental outcomes with such compounds initially led to ambiguous conclusions concerning the biologic roles for the 5-HT2C receptor, particularly in vivo. Furthermore, with the understanding that 5-HT2A receptor or 5-HT2B receptor agonists may evoke hallucinations (Nichols, 2004) or cardiac valvulopathy (Fitzgerald et al., 2000; Roth, 2007), respectively, the need for 5-HT2C receptor orthosteric agonists that lack demonstrable efficacy at 5-HT2A receptor or 5-HT2B receptor is recognized. In 1997, the chemists at SmithKline Beecham synthesized and characterized SB242084 as the first selective 5-HT2C receptor antagonist (Bromidge et al., 1997; Kennett et al., 1997). Because the 5-HT2C receptor exhibits constitutive activity dependent on the edited isoform and the in vitro and in vivo conditions employed for analyses, SB242084 and other compounds in this series (e.g., SB206553 and SB243213) (Bromidge et al., 1997; Kennett et al., 1997) have been noted to act as inverse agonists to attenuate constitutive 5-HT2C receptor activity (for reviews, see Aloyo et al., 2009; Sullivan et al., 2015). Atypical antipsychotic drugs are also reported to act as inverse agonists with the ability to inhibit 5-HT2C receptor constitutive activity (Herrick-Davis et al., 2000; Rausser et al., 2001) and Spampinato and colleagues have identified the role synergistically suppressed inherent and cocaine-evoked motor impulsivity as well as cocaine-induced hyperactivity and cocaine-seeking behavior (Cunningham et al., 2013). These data raise the possibility that the 5-HT2A receptor and 5-HT2C receptor may act in concert to regulate the neural bases for behavior. Further analyses of the structural and functional interactions between the 5-HT2A receptor and 5-HT2C receptor are necessary to disentangle the manner in which these GPCRs interact at neuronal and circuit levels.

Microinfusion of the preferential 5-HT2A receptor agonist DOI into the mPFC enhances (Wischhof et al., 2011), whereas intra-mPFC infusion of M100907 (Winstanley et al., 2003) suppresses, motor impulsivity. The density of 5-HT2A receptor (Fink et al., 2015) as well as 5-HT2C receptor protein expression in the mPFC (Anastasio et al., 2014b) predicts the level of motor impulsivity in outbred rats. Highly impulsive rats exhibit a greater 5-HT2A receptor–mediated head twitch response and are more sensitive to the suppressive effects of the selective 5-HT2A receptor antagonist M100907 on motor impulsivity (Fink et al., 2015). The levels of 5-HT2A receptor and 5-HT2C receptor protein predicted the intensity of motor impulsivity, and the ratio of the 5-HT2A receptor to 5-HT2C receptor protein in mPFC positively correlated with levels of motor impulsivity in individual outbred rats (Anastasio et al., 2015). High phenotypic motor impulsivity was associated with a diminished mPFC synaptosomal 5-HT2AR:5-HT2C receptor protein:protein interaction assessed by coimmunoprecipitation (Anastasio et al., 2015). Knockdown of 5-HT2C receptor in the mPFC resulted in increased motor impulsivity and triggered a compensatory upregulation of 5-HT2A receptor protein expression in mPFC and a leftward shift in the potency of M100907 to suppress impulsive behavior (Anastasio et al., 2015). These data further support the concept that an interactive relationship between the mPFC 5-HT2A receptor and 5-HT2C receptor is behaviorally relevant. The manner in which a potential 5-HT2A receptor and 5-HT2C receptor heteromeric protein complex in the mPFC contributes to high levels of inherent motor impulsivity remains to be uncovered.
of 5-HT$_{2C}$ receptor constitutive activity in the control of dopamine corticoaccumbens function (Aloyo et al., 2009; Leggio et al., 2009b).

A body of knowledge has been developed around several useful 5-HT$_{2C}$ Receptor agonists that have been available to scientists for the last 10 years. The compound RO60-0175 (Knight et al., 2004) has generated a great deal of information concerning the biologic role of the 5-HT$_{2C}$ receptor (Millan et al., 1998; Di Matteo et al., 2000; Grottick et al., 2000; Filip and Cunningham, 2002; Tomkins et al., 2002; Leggio et al., 2009a; Fletcher et al., 2012). RO60-0175 exhibits affinity and efficacy for all three 5-HT$_2$ receptor subtypes, although many of its effects in vivo are blocked by SB242084 (Martin et al., 1998; Porter et al., 1999; Knight et al., 2004). Lorcaserin is a selective, high-efficacy 5-HT$_{2C}$ receptor agonist that was marketed as Belviq for weight reduction in patients with a body mass index $>$30 or with a body mass index $>$27 comorbid with type 2 diabetes, hypertension, or dyslipidemia (www.us.eisai.com/). The availability of lorcaserin for clinical research has prompted a growing number of studies, particularly focused on addictive disorders (Rezvani et al., 2014; Higgs et al., 2016; Harvey-Lewis et al., 2016; for review, see Higgins et al., 2020). The preliminary results of a clinical trial (http://www.eisai.com/news/news201465.html) demonstrated the efficacy of lorcaserin to increase abstinence from nicotine, a highly abused psychostimulant, whereas preclinical studies continue to promote the prospects of lorcaserin as a tool to reduce substance use disorders (Levin et al., 2011; Higgins et al., 2016; for review, see Higgins et al., 2020). The role of the 5-HT$_{2C}$ receptor (Millan et al., 1998; Di Matteo et al., 2000; Filip and Cunningham, 2002; Tomkins et al., 2002; Leggio et al., 2009a; Fletcher et al., 2012). RO60-0175 exhibits affinity and efficacy for all three 5-HT$_2$ receptor subtypes, although many of its effects in vivo are blocked by SB242084 (Martin et al., 1998; Porter et al., 1999; Knight et al., 2004). Lorcaserin is a selective, high-efficacy 5-HT$_{2C}$ receptor agonist that was marketed as Belviq for weight reduction in patients with a body mass index $>$30 or with a body mass index $>$27 comorbid with type 2 diabetes, hypertension, or dyslipidemia (www.us.eisai.com/). The availability of lorcaserin for clinical research has prompted a growing number of studies, particularly focused on addictive disorders (Rezvani et al., 2014; Higgs et al., 2016; Harvey-Lewis et al., 2016; for review, see Higgins et al., 2020). The preliminary results of a clinical trial (http://www.eisai.com/news/news201465.html) demonstrated the efficacy of lorcaserin to increase abstinence from nicotine, a highly abused psychostimulant, whereas preclinical studies continue to promote the prospects of lorcaserin as a tool to reduce substance use disorders (Levin et al., 2011; Cunningham and Anastasio, 2014; Rezvani et al., 2014; Howell and Cunningham, 2015; Harvey-Lewis et al., 2016; Higgins et al., 2012; for review, see Higgins et al., 2020). Strikingly, Di Giovanni's group has also recently shown that lorcaserin is very effective as an anticonvulsant in both animal models of generalized nonconvulsive absence epilepsy and temporal lobe epilepsy (Orban et al., 2014; Venzi et al., 2016).

A compound series developed at Wyeth Research includes vabicaserin (SCA-136), which acts as a selective 5-HT$_{2C}$ receptor full agonist ($K_i = 3$ nM; efficacy 100% relative to 5-HT), a 5-HT$_{2B}$ receptor antagonist ($IC_{50} = 29$ nM), and a weak 5-HT$_{2A}$ receptor antagonist ($IC_{50} = 1650$ nM) (Rosenzweig-Lipson et al., 2007a; Tong et al., 2010; Dunlop et al., 2011). A randomized, double-blind, placebo-controlled study suggested the efficacy, safety, and tolerability of vabicaserin in the treatment of acute schizophrenia (Shen et al., 2014), although few preclinical analyses of this compound are published. WAY163909 is chemically similar to vabicaserin with high affinity ($K_i = 10.5$ nM) and full efficacy (90% relative to 5-HT) at the 5-HT$_{2C}$ receptor. WAY163909 exhibits a lower affinity ($K_i = 212$ nM) and no efficacy at the 5-HT$_{2A}$ receptor and is a weak partial agonist at the 5-HT$_{2B}$ Receptor (Dunlop et al., 2005). WAY163909 has been employed as a tool compound to test hypotheses related to the involvement of the 5-HT$_{2C}$ receptor in animal models of addiction, depression, impulsivity, and schizophrenia (Dunlop et al., 2006; Marquis et al., 2007; Rosenzweig-Lipson et al., 2007b; Navarra et al., 2008; Cunningham et al., 2011, 2013; Anastasio et al., 2013; Navailles et al., 2013b; Anastasio et al., 2014a). More recently, rational medicinal chemistry approaches have been employed to craft novel, highly selective 5-HT$_{2C}$ receptor agonists (Storer et al., 2014; Rouquet et al., 2015; Cheng et al., 2016). Some of these newer 5-HT$_{2C}$ receptor agonists have been made commercially available (e.g., PF-3246799 and PF-4479745 from Pfizer) (Storer et al., 2014), which will allow their increasing employment in in vitro and in vivo studies and provide greater breakthroughs in our understanding of 5-HT$_{2C}$ receptor biology.

The orthosteric site of a GPCR at which the endogenous agonist binds has been the traditional target for ligand discovery, but the chemical space for GPCR neuroprobes and therapeutics has greatly expanded with the discovery of allosteric ligands for many GPCR subfamilies. An allosteric modulator is a ligand that binds to a spatially distinct allosteric site and alters the receptor conformation to modulate its interaction with other ligands and/or signal transduction molecules [for reviews, see Conn et al. (2009) and Christopoulos et al. (2014)]. Allosteric sites are expected to exhibit higher sequence divergence across receptor subtypes relative to the highly conserved orthosteric domain (Kenakin, 2009; Kenakin and Miller, 2010). For example, a positive allosteric modulator (PAM) can increase the affinity and/or efficacy of the orthosteric ligand (Conn et al., 2009) and, thus, has the potential to improve its therapeutic index and diminish negative side effects. Such allosteric modulation is saturable (comes to a finite magnitude when the allosteric site is fully occupied) and probe-dependent (varies dependent on the orthosteric ligand) with the prospects for separate control of affinity and efficacy, making allosteric ligands intriguing therapeutic chemical targets (Kenakin, 2010). At present, allosteric modulators are defined operationally as positive (PAM), negative allosteric modulators, or neutral allosteric ligands, with the possibility of the additional property of allosteric agonism (agonist effects consequent to binding to allosteric sites; Christopoulos et al., 2014). Adron Harris and colleagues were the first to identify the effects of the fatty acid amide oleamide to positively modulate 5-HT$_{2C}$ receptor–mediated activity in Xenopus oocytes (Huidobro-Toro et al., 1996a), and further analyses have identified oleamide as a member of a family of amphipathic lipid metabolites that allosterically promote 5-HT receptor signaling through other receptors (e.g., 5-HT$_{2A}$ receptor and 5-HT$_7$ receptor; Thomas et al., 1997; Thomas et al., 1998; Alberts et al., 2001) but also exhibit a myriad of additional effects on receptor signaling (Leggett et al., 2004). In 2003,
chemical library screening at Pharmacia (now Pfizer) resulted in the discovery and characterization of PNU-69176E as a PAM highly selective for the 5-HT\textsubscript{2C} receptor over the 5-HT\textsubscript{2A} receptor, 5-HT\textsubscript{2B} receptor, 5-HT\textsubscript{7} receptor, and dopamine receptors (Im et al., 2003). In 2012, Zhou and colleagues optimized the synthetic route to generate PNU-69176E and its diastereomer (Ding et al., 2012). A series of new molecules based on the 4-alkylpiperidine-2-carboxamide scaffold were designed, synthesized, and pharmacologically evaluated as 5-HT\textsubscript{2C} receptor PAMs (Wild et al., 2019). Several analogs, potentiated 5-HT–evoked Ca\textsuperscript{2+} in 5-HT\textsubscript{2C} receptor CHO cells but not in 5-HT\textsubscript{2A} receptor CHO cells; one compound was further evaluated in vivo and exhibited a favorable overall pharmacokinetic and behavioral profile in rats (Wild et al., 2018). In addition, two predicted allosteric sites were identified by molecular docking to a 5-HT\textsubscript{2C} receptor homology model (Wild et al., 2018). Taken together, these data provide proof of concept that allosteric modulation of 5-HT\textsubscript{2C} receptor may be a viable strategy toward the discovery of novel neurotherapeutics. Recent preclinical indications of the efficacy of allosteric modulators in disease models, coupled with the launch of cinacalcet and maraviroc as the first marketed GPCR allosteric modulators, provide strong validation of the potential clinical utility of allosteric modulators (Conn et al., 2009). Ultimately, further analyses of novel allosteric modulators will improve understanding of 5-HT\textsubscript{2C} receptor function and how allosteric modulators may provide gain (or loss) of function in this system.

**E. Signal Transduction**

Intracellular signaling cascades stimulated by the 5-HT\textsubscript{2C} receptor have been assessed predominantly in recombinant cellular models and to a lesser extent within natural cellular environments (e.g., choroid plexus epithelial cells). Multiple G proteins (e.g., Ga\textsubscript{q11}, Ga\textsubscript{12/13}, and Ga\textsubscript{16}) and activation of second messengers such as phospholipases, cyclic nucleotides, and ERK\textsubscript{1/2} are essential mediators of 5-HT\textsubscript{2C} receptor actions in cells. By the late 1990s, structurally diverse 5-HT\textsubscript{2C} receptor agonists (Berg et al., 1994, 1998a; Moya et al., 2007) were noted to differentially activate intracellular signaling pathways, variably referred to as “agonist-directed trafficking of receptor stimulus,” biased agonism (signaling), stimulus trafficking, collateral efficacy, and functional selectivity (Berg and Clarke, 2009; Whalen et al., 2011; Kenakin and Christopoulos, 2013). Recent modeling studies suggest that 5-HT\textsubscript{2C} receptor ligands with fewer docking poses may stabilize a structural conformation contributory to a specific signaling pathway (Canal et al., 2011). Thus, distinct conformational states may differentially modulate the receptor interaction with immediate effectors (e.g., G proteins vs. \(\beta\)-arrestins), resulting in biased intracellular signal transduction patterns depending on the recruited effector (Gesty-Palmer et al., 2006; Whalen et al., 2011; Kenakin and Christopoulos, 2013). For example, G protein–dependent signaling pathways result in activation of specific downstream signaling effectors (e.g., pERK\textsubscript{1/2}), whereas \(\beta\)-arrestin\textsubscript{2}–dependent signaling can result in a different subset of downstream effectors as well as subcellular distribution of shared effectors (e.g., cytoplasmic vs. nuclear pERK\textsubscript{1/2}). These signaling profiles can then lead to distinct overall effects of GPCR activation (Gesty-Palmer et al., 2006). Targeting G protein– versus \(\beta\)-arrestin\textsubscript{2}–dependent mechanisms can allow for selectively inducing certain outcomes of receptor activation and perhaps not only reducing undesired side effects but also providing new therapeutic possibilities (Luttrel et al., 2015), a hypothesis that is supported for the 5-HT\textsubscript{2C} receptor system (Berg and Clarke, 2009) as well as for other GPCR signaling systems (Gesty-Palmer et al., 2006; Masri et al., 2008; Allen et al., 2011; Lovell et al., 2015; Martí-Solano et al., 2015).

1. **Phospholipase C.** The 5-HT\textsubscript{2C} receptor is characterized to stimulate phospholipase C (PLC) signaling through pharmacological analyses in heterologous expression systems (Westphal and Sanders-Bush, 1996; Briddon et al., 1998; Herrick-Davis et al., 1999; Rosendorff et al., 2000; Devlin et al., 2004), choroid plexus cells (Conn and Sanders-Bush, 1986b; Conn et al., 1986; Sanders-Bush and Conn, 1986), and corticostriatal regions of the brain (Wolf and Schutz, 1997). In this regard, the 5-HT\textsubscript{2C} receptor is largely thought to act through pertussis toxin–insensitive Ga\textsubscript{q11} proteins (Conn et al., 1986; Berg et al., 1994; Chang et al., 2000), although there is evidence that pertussis toxin–sensitive Ga\textsubscript{q10} G proteins may couple the 5-HT\textsubscript{2C} receptor to PLC in Xenopus laevis oocytes (Chen et al., 1994) and HEK293 cells (Alberts et al., 1999). Thus, the canonical G protein–dependent signaling through the 5-HT\textsubscript{2C} receptor is engendered by 5-HT–stimulated coupling to Ga\textsubscript{q11} to activate the enzyme phospholipase C\(_{\beta}\) (PLC\(_{\beta}\)), which generates the intracellular second messenger inositol-1,4,5-trisphosphate (IP\(_3\)), accumulation of the downstream IP\(_3\) metabolite inositol monophosphate (IP\(_1\)), and DAG. IP\(_3\) interacts with the IP\(_3\) receptor, leading to increased i Ca\textsuperscript{2+} into the cytoplasm; Ca\textsuperscript{2+} mobilization, measured with calcium-binding fluorescent dyes, and IP\(_1\) levels, assessed with \(^{3}H\)-inositol, are well characterized to be elevated following activation of the 5-HT\textsubscript{2C} receptor [for reviews, see Raymond et al. (2001) and Millan et al. (2008)]. Utilizing cell-permeable small peptide disruptors mimicking the C terminal of Ga\textsubscript{q11} Sanders-Bush and colleagues demonstrated that a Ga\textsubscript{q1} but not a Ga\textsubscript{q} disruptor was able to block 5-HT\textsubscript{2C} receptor–mediated phosphoinositide hydrolysis in choroid plexus endothelial cells (Chang et al., 2000). Furthermore, a PLC\(_{\beta1}\), but not a PLC\(_{\beta2}\), peptide blocked 5-HT\textsubscript{2C} receptor activation, suggesting that 5-HT\textsubscript{2C} receptor–evoked phosphoinositide hydrolysis...
is mediated through a $\alpha_q$- and PLC$_{\beta1}$-dependent mechanism (Chang et al., 2000).

Hydrolysis of phosphoinositides generates the signaling lipid DAG, leading to activation of PKC and downstream stimulation of the MAPK cascade, resulting in phosphorylation of ERK$_{1/2}$ (Werry et al., 2005). In fact, 5-HT$_{2C}$ receptor–transfected CHO cells were shown to couple ERK$_{1/2}$ via a PLD- and PKC-dependent pathway likely through $\alpha_{12/13}$ proteins (Werry et al., 2005). The PLD and PKC involvement in ERK$_{1/2}$ phosphorylation evoked by 5-HT$_{2C}$ receptor stimulation was recently validated in a hypothalamic cell line (mHypA-2/10) derived from the periventricular nucleus of an adult male mouse (Lauffer et al., 2016). These further analyses indicated that the native 5-HT$_{2C}$ receptor activates the cellular transcription factor CREB via PKC-induced ERK$_{1/2}$ activation in this cellular model (Lauffer et al., 2016).

Desensitization and resensitization processes regulate the functional activity of 5-HT$_{2C}$ receptor. Agonist-dependent desensitization is associated with 5-HT$_{2C}$ receptor phosphorylation involving G protein–coupled receptor kinase (GRK) 2 (Berg et al., 2001), binding of $\beta$-arrestins, and uncoupling of the receptor from the G protein to result in receptor internalization into endosomes; resensitization and recycling to the plasma membrane occurs with dephosphorylation (Marion et al., 2004; Schlag et al., 2004). The interaction of the 5-HT$_{2C}$ receptor with the C-terminal domain of PSD-95/Disc large/Zonula occludens (PDZ) domain–containing proteins (Bécamel et al., 2002, 2004; Anastasio et al., 2010; Anastasio et al., 2014b) is known to play an important role in 5-HT$_{2C}$ receptor desensitization/resensitization processes and trafficking (Gavarrini et al., 2006). Intracellular Ca$^{2+}$ release in mouse cortical neurons in primary culture is regulated by the PDZ proteins postsynaptic density 95/PDZ large/Zonula occludens (PDZ) domain–containing proteins (Bécamel et al., 2002, 2004; Anastasio et al., 2010; Anastasio et al., 2014b) is known to play an important role in 5-HT$_{2C}$ receptor desensitization/resensitization processes and trafficking (Gavarrini et al., 2006). Intracellular Ca$^{2+}$ release in mouse cortical neurons in primary culture is regulated by the PDZ proteins postsynaptic density 95 (PSD-95) and MAGUK p55 subfamily member 3 (MPP3) (Gavarrini et al., 2006; Möller et al., 2013). Although PSD-95 and MPP3 do not modify the efficacy of 5-HT$_{2C}$ receptor signaling triggered by a single 5-HT exposure, PSD-95 increases signal desensitization and trafficking upon repeated agonist exposure, an effect that is blocked by a peptidyl mimetic of the 5-HT$_{2C}$ receptor C-terminus, which disrupts the interaction between the 5-HT$_{2C}$ receptor and PSD-95 (Gavarrini et al., 2006). On the other hand, MPP3 stabilizes the 5-HT$_{2C}$ receptor at the plasma membrane and prevents desensitization of the 5-HT$_{2C}$ receptor–mediated Ca$^{2+}$ release (Gavarrini et al., 2006). This regulation correlates with surface expression of the receptor and indicates that 5-HT$_{2C}$ receptor signaling is highly regulated by PDZ proteins.

2. Phospholipase D. The 5-HT$_{2C}$ receptor also activates PLD, an enzyme that catalyzes the conversion of phosphatidylcholine to choline and phosphatidic acid; phosphatidic acid transduces most of PLD-activated activity, whereas soluble choline diffuses into the cytosol, but has little second messenger activity [for reviews, see Frohman (2015) and Nelson and Frohman (2015)]. The activation of PLD can occur through the canonical receptor/G protein/effector signal transduction cascade via $\alpha_{12/13}$. In rat choroid plexus epithelial cells, 5-HT–evoked PLD activation occurs at levels similar to PLC activation; however, PLD activation is not downstream to G protein–linked PLC activation (McGrew et al., 2002). The 5-HT$_{2C}$ receptor antagonist SB206553 and a peptide targeting the $\alpha_{13}$, but not the $\alpha_q$, subunit blocked 5-HT–evoked PLD activation (McGrew et al., 2002). Inactivation of RhoA GTPase in NIH3T3 cells stably expressing the 5-HT$_{2C}$ receptor by the C3 exoenzyme from Clostridia botulinum blocks 5-HT$_{2C}$ receptor–evoked PLD, but not PLC, signaling (McGrew et al., 2002). Also, in a NIH3T3 cell line derived from $\alpha_q$-deficient mice, 5-HT$_{2C}$ receptor–dependent stress fiber formation is dependent on $\alpha_{13}$ and Rho signaling (Gohla et al., 1999). The PLD signaling was not seen in cells transfected with the 5-HT$_{2C}$-VGV receptor; this highly edited isoform has a diminished ability to couple to $\alpha_{13}$ and is unable to promote Rho GTPase activity (McGrew et al., 2004). Together, these studies suggest that 5-HT$_{2C}$ receptor–mediated PLD signaling is dependent on $\alpha_{13}$ activation of Rho, a property of this receptor that is affected by pre-RNA editing.

3. Phospholipase A$_2$. Stimulation of the 5-HT$_{2C}$ receptor is thought to activate cytosolic PLA$_2$, which hydrolyzes arachidonic acid–containing phospholipids to produce free arachidonic acid and a host of its metabolites (for reviews, see Burke and Dennis, 2009a,b). In an early study, 5-HT was demonstrated to stimulate the release of arachidonic acid through activation of PLA$_2$, but not phosphoinositide turnover, in hippocampal neurons cocultured with glial cells, but not in glial cultures alone; the studies supported the involvement of the 5-HT$_2$ receptor subtype in the PLA$_2$ activation (Felder et al., 1990). In 5-HT$_{2C}$ receptor–transfected CHO cells, 5-HT increases the release of arachidonic acid, an effect that is blocked by the PLA$_2$ inhibitor mepacrine, which had no effect on 5-HT$_{2C}$ receptor–mediated phosphoinositide hydrolysis (Berg et al., 1996). The G protein effector is sensitive to pertussis toxin inactivation, but the exact G proteins involved are as of yet unknown (Felder et al., 1990). This research was extended to demonstrate that the relative efficacy of agonists is distinct for the PLA$_2$–arachidonic acid versus PLC-phosphoinositide pathways. For example, DOI acts as a full agonist to stimulate arachidonic acid release (equivalent to 5-HT), whereas 3-trifluoromethylphenylpiperazine and d-LSD preferentially activate the PLC-phosphoinositide and PLA$_2$–arachidonic acid pathways, respectively (Berg et al., 1998b). In addition, arachidonic acid release, though sensitive to pretreatment with 5-HT, is not as sensitive
as phosphoinositide hydrolysis, suggesting that arachidonic acid release may be more difficult to demonstrate in vivo as a 5-HT<sub>2C</sub> receptor–mediated output when compared with phosphoinositide hydrolysis (Berg et al., 1998b). The involvement of arachidonic acid stimulation in 5-HT<sub>2</sub> receptor signaling in vivo is supported by the observation that systemic administration of DOI resulted in increased incorporation of labeled arachidonic acid into brain membranes (Qu et al., 2005). The increases were seen in brain regions with the highest densities of 5-HT<sub>2A</sub> receptor (e.g., cerebral cortex) but not in the choroid plexus, which expresses the highest 5-HT<sub>2C</sub> receptor density (choroid plexus). Future studies are necessary to clarify the relative roles of the 5-HT<sub>2A</sub> receptor and 5-HT<sub>2C</sub> receptor in the control of arachidonic acid incorporation in vivo (Basselin et al., 2012).

4. Cyclic Nucleotides. Signaling through cyclic nucleotides, such as cAMP and cGMP, are also reported to be engaged by the 5-HT<sub>2C</sub> receptor. Activation of the 5-HT<sub>2C</sub> receptor inhibits forskolin-stimulated cAMP production in AV12 fibroblast cells that stably express the receptor at high density (~12 pmol/mg membrane protein); at low 5-HT<sub>2C</sub> receptor density (~150 fmol/mg of membrane protein), 5-HT couples to the PLC-IP pathway but evokes a stimulation rather than an inhibition of cAMP production (Lucaites et al., 1996). Upon pertussis toxin treatment, a modest 5-HT<sub>2C</sub> receptor stimulatory effect on cAMP accumulation as well as its potential dependence on the G<sub>i/o</sub> family of G proteins was observed (Lucaites et al., 1996). In Xenopus oocytes transfected with the 5-HT<sub>2C</sub> receptor and G proteins, the 5-HT<sub>2C</sub> receptor was shown to couple to G<sub>i</sub> in addition to G<sub>q</sub> (Quick et al., 1994). Because there is limited evidence that the 5-HT<sub>2C</sub> receptor links to cAMP in cells natively expressing the receptor (porcine choroid plexus) (Palacios et al., 1986), further analyses as to its biologic significance in vitro and in vivo are required.

The antimaligrae medication dihydroergotamine, which inhibits [3H]-mesulergine binding to the 5-HT<sub>2C</sub> receptor and is a full 5-HT<sub>2C</sub> receptor agonist in porcine choroid plexus (Brown et al., 1991), elevates cGMP levels in LMTK<sup>−</sup> fibroblasts stably expressing the 5-HT<sub>2C</sub> receptor and is a full 5-HT<sub>2C</sub> receptor agonist in porcine choroid plexus (Brown et al., 1991), elevates cGMP levels in LMTK<sup>−</sup> fibroblasts stably expressing the 5-HT<sub>2C</sub> receptor (Schaerlinger et al., 2003). 5-HT rapidly elevates cGMP production in the 5-HT<sub>2C</sub> receptor–enriched porcine choroid plexus tissue slices (Kaufman et al., 1995) with an efficacy similar to phosphatidylinositol turnover (Conn et al., 1986); the potencies of antagonists that suppress 5-HT–mediated cGMP formation align with their affinity for the 5-HT<sub>2C</sub> receptor (Kaufman et al., 1995). The pertussis toxin–insensitive 5-HT<sub>2C</sub> receptor–mediated cGMP formation is dependent on calcium and PLA<sub>2</sub>-arachidonate release as well as lipoxigenase (Kaufman et al., 1995). Thus, the 5-HT<sub>2C</sub> receptor appears to exhibit efficacy to evoke cGMP formation in a native tissue in a PLA<sub>2</sub>-dependent manner (Kaufman et al., 1995) as well as regulate NMDA-mediated production of nitric oxide and elevation of cGMP (Marcoli et al., 1997).

5. Extracellular Signal-Regulated Kinases. The activation of the 5-HT<sub>2C</sub> receptor can diverge to influence several G protein–dependent downstream cascades and may also converge on others, such as members of the MAPK class, P42 and 44 (p44/p42-MAPK), also known as ERK<sub>1/2</sub>. In fact, there is evidence to suggest that phosphorylation of ERK<sub>1/2</sub> is an important integrator of the multiple upstream signaling events for the 5-HT<sub>2C</sub> receptor. In CHO cells stably expressing the 5-HT<sub>2C</sub> receptor, 5-HT stimulated phosphorylation of ERK<sub>1/2</sub>, which is inhibited by the 5-HT<sub>2C</sub> receptor antagonist mianserin (Werry et al., 2005). Though PKC and PLD inhibitors suppress 5-HT–mediated phosphorylation of ERK<sub>1/2</sub>, PLC and PLA<sub>2</sub> inhibitors are ineffective, suggesting the 5-HT<sub>2C</sub> receptor–mediated pERK<sub>1/2</sub> is dependent on PKC and PLD signaling (Werry et al., 2005). Activation of ERK<sub>1/2</sub> by 5-HT<sub>2C</sub> receptor ligands is not solely mediated by coupling of the 5-HT<sub>2C</sub> receptor to G proteins and can, in fact, be mediated by coupling to other protein transducers. The C terminus of the 5-HT<sub>2C</sub> receptor contains a calmodulin domain that is critical for β-arrestin recruitment and ERK<sub>1/2</sub> signaling (Labasque et al., 2008). A calmodulin mutant prevented phosphorylation of ERK<sub>1/2</sub> in cortical neurons and choroid plexus epithelial cells, suggesting that the calmodulin interaction is critical for G protein–independent signaling (Labasque et al., 2008). In HEK293 cells transiently expressing the 5-HT<sub>2C</sub> receptor, pERK<sub>1/2</sub> levels were increased over nontransfected cells, suggesting the expression of constitutive activity of the 5-HT<sub>2C</sub> receptor (Labasque et al., 2008). This elevated basal pERK<sub>1/2</sub> was inhibited by a 5-HT<sub>2C</sub> receptor inverse agonist toward PLC (Labasque et al., 2008), again suggesting that pERK<sub>1/2</sub> activity is PLC-mediated. Interestingly, this activity was unaffected by depletion of G<sub>α</sub><sub>12</sub> or G<sub>α</sub><sub>13</sub> proteins, whereas in cells lacking β-arrestin or calmodulin, basal pERK<sub>1/2</sub> levels were decreased, providing the first evidence of constitutive activity of a G protein–coupled receptor toward a G protein–independent, β-arrestin–dependent signaling mechanism (Labasque et al., 2008).

6. Ion Channels. The impact of 5-HT<sub>2C</sub> receptor on the function of ion channels has been addressed extensively in the choroid plexus, which controls the composition and secretion of cerebrospinal fluid, a function in which chloride (Cl<sup>−</sup>) and potassium (K<sup>+</sup>) channels play a key role (Millar et al., 2007). 5-HT acting through the 5-HT<sub>2C</sub> receptor has been noted to activate Cl<sup>−</sup> and inhibit K<sup>+</sup> channels in mouse and rat choroid plexus epithelium (Hung et al., 1993; Speake et al., 2004), and the 5-HT<sub>2C</sub> receptor has been shown to inhibit K<sup>+</sup> channels through a PKC-dependent pathway in rat choroid plexus epithelial cells (Speake et al., 2004). Activation of the 5-HT<sub>2C</sub> receptor leads to G protein–dependent PLC activation, IP<sub>3</sub> and DAG production, and
that the 5-HT2C receptor inhibits K\(^+\) (Panicker et al., 1991), which contrasts the observation that the 5-HT2C receptor as a 5-HT2C receptor antagonist blocks foci in Xenopus oocytes, 5-HT suppresses K\(^+\) conductance through a calcium/calmodulin-activated phosphatase thought to dephosphorylate the K\(^+\) channel in a kinase-dependent manner, evoking its closure (Hoger et al., 1991).

The 5-HT\(_{2C}\) receptor also modulates the Kv1.5 channel through a PLC-dependent pathway in Xenopus oocytes (Panicker et al., 1991), which contrasts the observation that the 5-HT\(_{2C}\) receptor inhibits K\(^+\) channels through a PKC-dependent pathway in rat choroid plexus epithelial cells (Speake et al., 2004). The 5-HT\(_{2C}\) receptor also inhibits the GABA\(_{A}\) receptor in Xenopus oocytes through a calcium-dependent, phosphorylation-independent mechanism (Huidobro-Toro et al., 1996b) and by suppressing an inwardly rectifying K\(^+\) current in striatal cholinergic interneurons and rat brain slices (Blomeley and Bracci, 2005; Blomeley and Bracci, 2009). The 5-HT\(_{2C}\) receptor was GABA\(_{A}\)-coupled to PLC activation and phosphoinositide hydrolysis to directly inhibit GIRK channels in POMC neurons of the arcuate (Qiu et al., 2007) as well as in lateral (but not basolateral) amygdala neurons (Yamamoto et al., 2014), which are known to stabilize resting membrane potential and therefore polarization of these neurons (Delmas and Brown, 2005). Hence, though stimulation of 5-HT\(_{2C}\) receptor significantly modulates ion channel function, the involvement of specific signal transduction molecules in the processes is variable and underexplored at present.

F. The 5-HT\(_{2C}\) Receptor as an Oncogene

5-HT is known to act as a mitogen to regulate the proliferation and differentiation of a variety of cells through its binding to several 5-HT receptors and control of downstream signaling (for review, see Fanburg and Lee, 1997). The NIH3T3 mouse fibroblast cells are often used to identify oncogenes (genes that can transform a cell to a tumor cell) because they display several well-characterized phenotypes that are indicative of cellular transformation, including the formation of foci (regions of dense cell growth within an otherwise confluent monolayer) (Land et al., 1983). Stimulation of NIH3T3 cells transfected with the 5-HT\(_{2C}\) receptor leads to the generation of transformed foci, maintenance of which requires the activation of the 5-HT\(_{2C}\) receptor as a 5-HT\(_{2C}\) receptor antagonist blocks foci formation (Julius et al., 1988; Julius et al., 1989). Upon injection of these cells into nude mice, tumors form, a finding that led to the initial conclusion that the 5-HT\(_{2C}\) receptor is a proto-oncogene (Julius et al., 1989).

The constitutive activity of the 5-HT\(_{2C}\) receptor also stimulated cell division in the transfected NIH3T3 cells, and these data further suggested that multiple G proteins and signaling pathways were engaged in the cell division generated by agonist-evoked and constitutively active receptor signaling (Westphal and Sanders-Bush, 1996).

G. Clinical Relevance of the 5-HT\(_{2C}\) Receptor

The 5-HT\(_{2C}\) receptor is widely distributed throughout the basal ganglia, limbic system, and prefrontal cortex (Hoyer et al., 1986; Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990) and is well poised to mediate 5-HT-dependent appetite, cognition, mood, movement, and sleep, whereas dysfunctional 5-HT\(_{2C}\) receptor signaling has been implicated in neuropsychiatric (e.g., addiction, anxiety, and depression) and neuropathological conditions (e.g., schizophrenia) as well as obesity and metabolic disorders (Berg et al., 2008a; Di Giovanni et al., 2010; Fig. 14). Therefore, the 5-HT\(_{2C}\) receptor is a therapeutic target of great interest (for other reviews, see Cunningham and Anastasi, 2014; Howell and Cunningham, 2015; Sullivan et al., 2015; Di Giovanni and De Deurwaerder, 2016; Fig. 12). The behavioral pharmacology of 5-HT\(_{2C}\) receptor ligands as well as the clinical relevance of a dysfunctional 5-HT\(_{2C}\) receptor system has recently been reviewed in detail (Cunningham and Anastasio, 2014; Howell and Cunningham, 2015; Sullivan et al., 2015; Di Giovanni and De Deurwaerder, 2016), and an overview of clinical implications of the 5-HT\(_{2C}\) receptor system is provided here.

1. Substance Use Disorders (Addiction)

The pharmacological and molecular mechanisms underlying the effects of abused drugs have been the subject of extensive study. As early work unfolded, a central concept emerged that the dopamine pathway projection from the VTA to the nucleus accumbens plays a mechanistic role in the rewarding and incentive-salience value of abused drugs (for reviews, see Koob and Volkow, 2010; Volkow et al., 2010). As the research progressed, the field began to recognize that the transition to problematic drug abuse and substance use disorder (addiction and dependence) involves an “expanding cycle of dysfunction” (Koob and Volkow, 2010), engaging multiple neurotransmitter substrates within limbic-corticostratal circuitry, including 5-HT (for reviews, see Kalivas and Volkow, 2005; Koob and Volkow, 2010; Volkow et al., 2010; Cunningham and Anastasio, 2014; Muller and Homberg, 2015; Di Giovanni and De Deurwaerdere, 2016; Wolf, 2016). Much of the research has focused on abused drugs as rewarding substances and the evoked, long-lasting dysregulation of the dopamine mesoaccumbens pathway (for reviews, see Koob and Volkow, 2010; Volkow et al., 2010). However, a second focus has evolved toward identifying genotypic and phenotypic drivers of individual differences in.
vulnerability to substance use disorders and relapse, as drug abuse culminates in addiction in only a subset of users (SAMHSA, 2015). Impulsivity, a predisposition toward rapid unplanned reactions to stimuli without regard to the negative consequences, is one such phenotype that contributes to initial drug use and is perpetuated by continued use of the abused drug (for reviews, see Moeller et al., 2001a,b; Cunningham and Anastasio, 2014). The impact of impulsivity in psychostimulant addiction is best described with roles for 5-HT and dopamine prominently identified (for reviews, see Moeller et al., 2001a; Dalley and Roiser, 2012; Bari and Robbins, 2013; Cunningham and Anastasio, 2014; Logue and Gould, 2014). Cue reactivity, the sensitivity to cues previously linked with the drug-taking experience, is a second such phenotype that plays a prominent role in craving and relapse in humans (Carter and Tiffany, 1999; O’Brien et al., 1998; Drummond, 2001). The extended limbic-cortico-striatal circuitry underlies both impulsivity and cue reactivity with multiple neurotransmitters involved (Childress et al., 1999; Bechara, 2005; Goldstein et al., 2007; Liu et al., 2012). Thus, addiction involves the generation of drug use within a background of vulnerability (e.g., propensity for impulsive behavior) and progresses with repeated drug exposure, neuronal plasticity, and the entrainment of addictive behaviors. In particular, the role of the 5-HT<sub>2C</sub> receptor in various aspects of these addictive processes has been well studied for the class of psychostimulants; several studies have reported the efficacy of 5-HT<sub>2C</sub> receptor agonists to suppress nicotine intake and nicotine seeking (Grottick et al., 2001; Levin et al., 2011; Higgins et al., 2012). Relevant to this are the extensive studies of the role of the 5-HT<sub>2C</sub> receptor in the rewarding and incentive-saliency value of cocaine as well as factors involved in vulnerability to addiction and relapse, especially impulsivity and cue reactivity.

Cocaine is a psychomotor stimulant that inhibits 5-HT reuptake (Koe, 1976). Employing the self-administration assay, the preclinical model with the best validity for human drug taking, studies demonstrated that voluntary cocaine administration elevates 5-HT efflux in the nucleus accumbens (Parsons and Justice, 1993; Parsons et al., 1996; Howes et al., 2000). Depletion of forebrain 5-HT induces compulsive cocaine seeking, which is reversed by a 5-HT<sub>2C</sub> receptor antagonist (Pelloux et al., 2012). Constitutive knockout of the 5-HT<sub>2C</sub> receptor increases the motivation to take cocaine and enhances cocaine-induced elevation in dopamine in the nucleus accumbens (but not the dorsal striatum) of mice (Rocha et al., 2002). Pretreatment with a 5-HT<sub>2C</sub> receptor agonist, systemically or into the VTA, enhances, whereas systemic administration of a 5-HT<sub>2C</sub> receptor antagonist inhibits, the elevated dopamine efflux in the NAc evoked by nonresponse contingent cocaine administration (Navailles et al., 2004, 2008; Cathala et al., 2015). The 5-HT<sub>2C</sub> receptor control over dopamine function is thought to mediate, in large part, the efficacy of a selective 5-HT<sub>2C</sub> receptor agonist (e.g., RO60-0175 or WAY163909) to suppress the voluntary intake of cocaine (Grottick et al., 2000; Fletcher et al., 2002a; Fletcher et al., 2004; Neisewander and Acosta, 2007; Cunningham et al., 2011).

Stimulation of the 5-HT<sub>2C</sub> receptor also dose-dependently suppresses reinstatement induced by cocaine and cocaine-associated cues as measures of cue reactivity (Grottick et al., 2000; Neisewander and Acosta, 2007; Burbassi and Cervo, 2008; Fletcher et al., 2008; Cunningham et al., 2011; Swinford-Jackson et al., 2016). Conversely, systemic administration of 5-HT<sub>2C</sub>
receptor antagonists have been shown to exert effects opposite to those following agonist administration, thus enhancing cocaine self-administration (Fletcher et al., 2002a) and cue reactivity (Fletcher et al., 2002a; Pelloux et al., 2012). In nonhuman primates, a 5-HT$_{2C}$ receptor agonist attenuated the stimulant, reinforcing, and reinstatement effects of cocaine, effects reversed by the selective 5-HT$_{2C}$ receptor antagonist SB242084 (Manvich et al., 2012a,b; Ruedi-Bettschen et al., 2015). Interestingly, SB242084 induced modest stimulant effects and exhibited reinforcing effects in primates (Manvich et al., 2012a,b) but had contrasting results (Ruedi-Bettschen et al., 2015).

The efficacy of 5-HT$_{2C}$ receptor agonists to suppress cue reactivity upon systemic administration is mirrored following intracranial microinjection of a 5-HT$_{2C}$ receptor agonist into the mPFC, which attenuates both cocaine- and cue-induced reinstatement (Pentkowski et al., 2010). These data highlight the importance of the 5-HT$_{2C}$ receptor in regulation of cortical substrates underlying cocaine-associated cue reactivity specifically, as 5-HT$_{2C}$ receptor agonist microinfusions did not alter cocaine intake (Pentkowski et al., 2010). Thus, the 5-HT$_{2C}$ receptor provides inhibitory tone over cocaine reward and cue reactivity as well as the neurochemical effects of cocaine (for reviews, see Cunningham and Anastasio, 2014; Howell and Cunningham, 2015; Di Giovanni and De Deurwaerdere, 2016), and mPFC-localized 5-HT$_{2C}$ receptors underlie, in part, the generation of cue reactivity.

Cocaine administered nonresponse contingently has been reported to result in 5-HT$_{2C}$ receptor neuroadaptations (Zayara et al., 2011; Craigie et al., 2015) as well as regulation of the brain-specific snoRNA MBII-52, which is involved in the regulation of the 5-HT$_{2C}$ receptor pre-mRNA (Chen et al., 2014). Abstinent cocaine users exhibit lower sensitivity to the effects of a 5-HT$_{2C}$ receptor agonist (Lee and Meltzer, 1994; Buydens-Branchez et al., 1997; Patkar et al., 2006), whereas the highest cue reactivity was observed in those cocaine-dependent subjects carrying the C23S SNP in the 5-HT$_{2C}$ receptor gene (Anastasio et al., 2014a), which may be associated with diminished 5-HT$_{2C}$ receptor signal transduction (Lappalainen et al., 1995; Okada et al., 2004; Piva et al., 2011; Walstab et al., 2011). Further evidence that the functional status of the 5-HT$_{2C}$ receptor in the mPFC (Lopez-Gimenez et al., 2001; Liu et al., 2007; Nocjar et al., 2015) influences the incentive-motivational effects of cocaine and cocaine-associated cues has accumulated in rats (Anastasio et al., 2014a,b; Swinford-Jackson et al., 2016). Cunningham and colleagues found that the highest levels of cocaine cue reactivity correlated with the lowest levels of mPFC 5-HT$_{2C}$ receptor protein and a blunted sensitivity to the suppressive effects of the selective 5-HT$_{2C}$ receptor agonist WAY163909 (Anastasio et al., 2014a; Swinford-Jackson et al., 2016). The efficacy of WAY163909 to suppress high levels of cue reactivity associated with extended forced abstinence (“incubation”) was also reduced at a time point (30 days) at which lower synaptosomal expression of 5-HT$_{2C}$ receptor protein was observed in the mPFC (Swinford-Jackson et al., 2016), a key site involved in incubation phenomena (Koya et al., 2009; Whitfield et al., 2011; Ma et al., 2014). Furthermore, a greater proportion of the expressed 5-HT$_{2C}$ receptor protein was sequestered in the cytoplasmic (vs. membrane) compartment of the mPFC at prolonged versus early forced abstinence, and there was an inverse correlation of the membrane to cytoplasmic 5-HT$_{2C}$ receptor ratio in the mPFC with levels of cocaine cue reactivity. Collectively, these outcomes indicate that the functional status of the 5-HT$_{2C}$ receptor system in the mPFC is a key contributor to cocaine cue reactivity and its incubation.

Cocaine-dependent subjects who express high cocaine cue reactivity express high impulsivity (Liu et al., 2011b), and a similar relationship has been observed in cigarette smokers (Doran et al., 2007, 2008). In outbred rats, lower mPFC 5-HT$_{2C}$ receptor membrane protein levels and an increase in edited 5-HT$_{2C}$ receptor mRNA variants with reduced 5-HT$_{2C}$ receptor signaling capacity distinguish high impulsive rats from low impulsive rats (Anastasio et al., 2014b) as well high and low responders to novelty, another model of addiction vulnerability (Dracheva et al., 2009). The virally mediated knockdown of the 5-HT$_{2C}$ receptor localized to the mPFC also results in elevated impulsivity and cue reactivity relative to controls (Anastasio et al., 2014b), suggesting that reduced 5-HT$_{2C}$ receptor tone in the mPFC confers vulnerability to these interlocked behaviors (Anastasio et al., 2014b). The status of 5-HT$_{2C}$ receptor function in the orbitofrontal cortex may also be a contributor to the vulnerability of impulsive rats to cocaine reward and cue reactivity (Besson et al., 2013). Together, these data suggest that the functional status of the cortical 5-HT$_{2C}$ receptor system may be a mechanistic driver in the generation of cocaine use disorder and relapse phenomena.

The body of knowledge in support of a role of the 5-HT$_{2C}$ receptor in regulating the rewarding properties and voluntary intake of other abused substances is less well developed than that of cocaine and nicotine. However, stimulation of the 5-HT$_{2C}$ receptor has been noted to suppress ethanol self-administration (Maurel et al., 1999; Tomkins et al., 2002; Kasper et al., 2013; Rezvani et al., 2014) and reinstatement in rodents (Kasper et al., 2013). Exposure to ethanol vapor for several days is associated with increased expression of the 5-HT$_{2C}$ receptor transcript in several corticostriatal and hypothalamic nodes (Yoshimoto et al., 2012), higher levels of the 5-HT$_{2C}$ receptor protein in the NAc (Yoshimoto et al., 2012), and enhanced pre-mRNA editing of the 5-HT$_{2C}$ receptor (Watanabe et al., 2014), suggesting that neuroadaptations in the 5-HT$_{2C}$
5-HT receptors are mechanistically involved in ethanol preference behavior.

Pretreatment with 5-HT1C receptor ligands have been shown to impact various behavioral sequelae associated with d-amphetamine (O’Neill et al., 1999; Ripberger et al., 2015; Wohr et al., 2015), MDMA (Banksen and Cunningham, 2002; Fletcher et al., 2002b), methamphetamine (Steed et al., 2011; Graves and Napier, 2012), and the marijuana alkaloid Δ9-THC (Ji et al., 2006), suggesting that rich prospects to explore the potential therapeutic value of selective 5-HT1C receptor agonists in addictive processes engaged by these abused drugs remain.

5-HT is involved in the pharmacology of opioid abused drugs (including heroin and prescription opioids) (Tao and Auerbach, 1994; Tao et al., 1998; Singh et al., 2003), and systemic administration of a 5-HT releaser (e.g., dexfenfluramine) was shown to suppress heroin self-administration in rats (Wang et al., 1995). Pretreatment with a selective 5-HT1C receptor agonist also reduced opioid-induced behavioral sensitization (Wu et al., 2015a; Zhang et al., 2016). Recent studies have further demonstrated that lorcaserin significantly decreases the reinforcing effects of oxycodone and shifts the oxycodone dose-effect curve downward at doses that do not alter motor activity (Neelakantan et al., 2017). Thus, 5-HT1C receptor agonists may prove therapeutically useful to promote recovery and extend abstinence from several classes of abused drugs.

2. Appetite, Satiety, and Obesity. Hunger is the physiologic need for food. Appetite (the desire for food), satiation (the end of the desire for food during a meal), and satiety (the feeling of “fullness” that prevents further eating before the return of hunger) include both internal (e.g., glucose homeostasis) and conditioned factors (e.g., hedonics) (Blundell, 1999). 5-HT in the CNS has long been implicated in the control of these processes involved in satiation and satiety (Lucki, 1998; Halford and Blundell, 2000; Saper et al., 2002; Voigt and Fink, 2015). The 5-HT releaser d-fenfluramine, employed clinically for weight loss (until withdrawn in 1997), its metabolite d-norfenfluramine, and preferential 5-HT1C receptor agonists (e.g., mCPP) evoke hypophagia in rodents, which is associated with increased satiety, an effect blocked by 5-HT1C receptor antagonists or constitutive knockdown of the 5-HT1C receptor (Kennett and Curzon, 1988; Tecott et al., 1995; Halford et al., 1997; Vickers et al., 1999; Dalton et al., 2006; Nonogaki et al., 2008). Selective 5-HT1C receptor agonists (e.g., lorcaserin, RO60-0175) have been consistently demonstrated to suppress food intake (Clifton et al., 2000; Somerville et al., 2007; Thomsen et al., 2008; Grottick et al., 2015; Higgs et al., 2015; for review, see Higgins et al., 2020). In fact, the selective 5-HT1C receptor agonist WAY163909 dose-dependently decreases food intake in normal Sprague-Dawley rats, obese Zucker rats, and mice with diet-induced obesity (Dunlop et al., 2005) without the anxiogenic profile of mCPP (Dunlop et al., 2006). Tecott et al. (1995) reported that the constitutive 5-HT1C receptor knockout mouse exhibited hypophagia and increased body mass in the context of both insulin resistance and late-onset obesity (Nonogaki et al., 2008), whereas weight gain as well as a greater relative risk of metabolic dysfunction and diabetes develops with the chronic treatment of atypical antipsychotics with 5-HT1C receptor antagonist properties (e.g., olanzapine) in humans and animals (Wirshing et al., 1999; Kirk et al., 2009). Interestingly, a selective 5-HT1C receptor antagonist has been reported to variably increase (Bonhaus et al., 1997) or decrease food intake depending on the preclinical model employed (Kennett et al., 1997; Murotani et al., 2011).

The behavioral satiety sequence (BSS) describes the orderly process through which eating transitions to other behaviors (e.g., grooming and resting) and is a well validated model for analyzing satiation (meal termination) and satiety (postigestive inhibition of food intake) in rodents and humans (for review, see Rodgers et al., 2010). Consistent with the proposed role of 5-HT to promote satiety, d-fenfluramine, its metabolite analogs, and preferential and selective 5-HT1C receptor agonists (Halford et al., 1998; Clifton et al., 2000; Hewitt et al., 2002; Dalton et al., 2006; Somerville et al., 2007) accelerate the BSS without disruption of its integrity (for review, see Rodgers et al., 2010). For example, d-fenfluramine and RO60-0175 reduced the rate of feeding and meal size as well as increased the latency to feed consistent with enhanced satiety (Clifton et al., 2000). The effects of d-fenfluramine on the BSS was markedly reduced in mice constitutively lacking the 5-HT1C receptor (Vickers et al., 1999). In contrast, appetite-enhancing drugs (e.g., cyproheptadine) (Chinuck et al., 2007) disrupt the satiety sequence (Bergen, 1964; Ishii et al., 2003). A recent microstructural analysis of ingestive behavior found that lorcaserin reduced the number of bouts of licking behavior (Higgs et al., 2016) indicative of the promotion of satiety (Davis et al., 2001). Thus, the 5-HT1C receptor is an important mediator of food intake through the control of satiety mechanisms (for reviews, see Lucki, 1998; Halford and Blundell, 2000; Voigt and Fink, 2015).

Investigations of 5-HT involvement in the mechanisms underlying satiety have focused predominantly on neural loci in the hypothalamus and midbrain/hindbrain circuits, which synchronize energy balance and glucose homeostasis in concert with peripheral systems (for reviews, see Saper et al., 2002; Gautron et al., 2015; Voigt and Fink, 2015). The 5-HT neurons in the dorsal and median raphe innervate multiple hypothalamic subnuclei (van de Kar and Lorenz, 1979; Peyron et al., 1998), which richly express 5-HT1C receptor mRNA and protein (Hoffman and Mezey, 1989; Molineaux et al., 1989; Mengod et al., 1990). A subpopulation of arcuate POMC neurons
express the 5-HT2C receptor and are activated by d-fenfluramine and mCPP (Heisler et al., 2002; Lam et al., 2008). Elmquist and colleagues elegantly demonstrated that the activation of the 5-HT2C receptor localized to POMC neurons stimulates POMC synthesis and its cleavage into α-melanocyte-stimulating hormone, which acts on melanocortin 4 receptors in the paraventricular nucleus of the hypothalamus to promote satiety, weight loss, and glucose regulation (Heisler et al., 2007a; Zhou et al., 2007; Xu et al., 2008; Berglund et al., 2013). Stimulation of the 5-HT2C receptor following application of mCPP depolarized a subpopulation of POMC neurons potentially via PLC-dependent activation of transient receptor potential channels, independent of GIRK channel activity (Sohn et al., 2011). In mice that selectively lack the 5-HT2C receptor in POMC neurons, body weight was normal; however, these mice were insensitive to d-fenfluramine or mCPP-evoked hypophagia and developed metabolic dysfunction, including hyperinsulinemia, hyperglycagomenia, hyperglycemia, and insulin resistance (Berglund et al., 2013). Rescue of 5-HT2C receptor in POMC neurons of 5-HT2C receptor–null mice normalized food intake, adiposity, and body weight as well as the anorexigenic effects of d-fenfluramine and mCPP (Xu et al., 2008). Interestingly, POMC expression within 5-HT2C receptor–expressing neurons in the arcuate regulates whole body energy balance, body weight, and adiposity in male, but not female, mice; these authors proposed that this molecular mechanism may explain, in part, sex differences in the prevalence of obesity (Burke et al., 2016). This landmark discovery marshaled in an era of advanced understanding of the CNS circuits involved in obesity [for review, see Friedman (2014)]. It was not long before genetic deletion of the leptin receptor in neurons was shown to activate the same POMC neurons activated by leptin (Qiu et al., 2010). Lastly, coadministration of the preferential 5-HT2C receptor agonist mCPP plus leptin was recently shown to have an additive effect on reducing body weight in diet-induced obese mice (Yan et al., 2015). The growing knowledge of the interface between leptin and 5-HT regulatory systems prompted Halford and Blundell to propose that these systems function independently, but coordinate within the hypothalamus, to control satiety and energy reserves (Halford and Blundell, 2000).

The drive to eat palatable foods, composed essentially of high fat and/or sugars, involves hedonic mechanisms, which are also important for maintaining the homeostatic nutritional requirements for energy balance [for reviews, see Saper et al. (2002) and Volkow et al. (2012)]. These foods activate limbic-corticostratial systems, which mediate reward and motivation [for reviews, see Gautron et al. (2015) and Voigt and Fink (2015)], and hedonic eating has been identified as a contributor to the obesity epidemic (Saper et al., 2002; Volkow et al., 2012). Preclinical analysis of the rewarding (reinforcing) and motivational effects of food self-administration can be evaluated on a fixed ratio and a progressive ratio (PR) schedule, respectively, in either freely fed or food-restricted animals. Performance under PR schedules is thought to reflect the motivational “efficacy” of the food given that deprivation level as well as reinforcer magnitude can increase breakpoints on the PR schedule (Hodos and Kalman, 1963). The preferential (mCPP, MK212) and selective 5-HT2C receptor agonists (R0 60-0175) decrease the breakpoint for a grain reinforcer on a PR schedule in food-restricted pigeons (Wolff and Leander, 2000). WAY163909 dose-dependently reduces self-administration of sucrose on a fixed ratio schedule in freely fed rats, an effect blocked by pretreatment with SB242084 (Cunningham et al., 2011), whereas mCPP decreases the breakpoint for Ensure in freely fed mice (Ward et al., 2008). Lorcaserin efficaciously suppresses the rewarding effects of food in food-restricted rats based on its 5-HT2C receptor agonist actions [Higgins et al., 2012; for review, see Higgins et al. (2020)]. Interestingly, though 5-HT2C receptor agonists effectively suppress cocaine seeking [for reviews, see Cunningham and Anastasio (2014) and Howell and Cunningham (2015)], WAY163909 failed to affect sucrose seeking (Cunningham et al., 2011), suggesting that the 5-HT2C receptor differentially regulates the incentive-salience value of cocaine- versus sucrose-associated cues. Thus, there is evidence that the 5-HT2C receptor system controls the hedonic, rewarding aspects of palatable food; the brain locus of action for the 5-HT2C receptor to control these behaviors requires further evaluation (Pratt et al., 2009; Pratt et al., 2012; Clissold et al., 2013).

Multiple lines of investigation suggest a relationship between 5-HT2C receptor SNPs and obesity,
antipsychotic-induced weight gain, and transcriptional activity of the HTR2C gene. Eight HTR2C gene polymorphisms have been reported in the literature: three polymorphisms, as well as a GT nucleotide repeat variation, have been identified in the promoter (Xie et al., 1996); three polymorphisms have been reported within intronic regions (Gibson et al., 2004); one polymorphism has been reported in the coding region, resulting in the replacement of cysteine with serine at amino acid 23 (C23S) in the amino-terminus of the receptor (Lappalainen et al., 1995); and there is one polymorphism in the 3’ untranslated region (Song et al., 1999).

The C23S SNP occurs with a frequency of approximately 10%–15% in the human population (Lappalainen et al., 1995). However, there is no evidence for an association of this SNP with obesity (Lentes et al., 1997; Gibson et al., 2004). The promoter haplotype −995A/−759T/−697C has been reported to be associated with obesity in a Japanese population (Yuan et al., 2000) and the −759C allele to be more common in obese than nonobese Caucasian women (Pooley et al., 2004). Several studies suggest that the −759T allele may be associated with less weight gain following antipsychotic drug treatment (Reynolds et al., 2003; Miller, 2005; Templeman et al., 2005). Although additional research has reported no association between antipsychotic-induced weight gain (AIWG) and HTR2C promoter polymorphisms (Basile et al., 2002; Tsai et al., 2002; Theisen et al., 2004; Templeman et al., 2005), others have reported an association of the −759C allele with AIWG (Wallace et al., 2011). It is interesting to note that greater promoter activity, resulting in increased HTR2C transcription, has been reported to be associated with the −759T allele (Yuan et al., 2000). Also, in a luciferase-based reporter assay, HTR2C promoter haplotypes containing the −759C allele showed lower transcriptional activity than those containing the −759T haplotype (Buckland et al., 2005). Although these studies suggest that the −759C/T polymorphism may regulate gene transcription in vitro, a subsequent study reported that 5-HT2C receptor mRNA levels in the frontal cortex of 43 subjects are unaffected by −759C/T status (Pooley et al., 2004).

3. Schizophrenia. Both selective 5-HT2C receptor agonists and 5-HT2C receptor antagonists have been suggested for the treatment of schizophrenia. Antipsychotic medications with a profile as a 5-HT2C receptor antagonist seem to be effective to suppress positive symptoms, whereas 5-HT2C receptor agonists appear useful for inhibition of the negative symptoms and cognitive impairments in schizophrenia (Wood et al., 2001; Rosenzweig-Lipson et al., 2007a, 2012) with fewer motor side effects (Di Giovanni et al., 2006; Di Giovanni and De Deurwaerdere, 2016). The therapeutic potential of a specific antipsychotic would depend indirectly on the opposite modulation that these receptors exert on dopaminergic systems and the preferential 5-HT2C receptor modulation of the mesocortical and limbic system versus the nigrostriatal system (Di Giovanni et al., 2006; Di Giovanni and De Deurwaerdere, 2016). Moreover, the analysis of the pharmacological profile of some atypical antipsychotics led to 5-HT2C receptor blockade as a valuable strategy to improve the efficacy of dopamine antagonists in long-term treatments (Meltzer, 1999). Vabicaserin is a novel antipsychotic and anorectic agent with high agonist efficacy at the 5-HT2C receptor (Dunlop et al., 2011) and has been shown to be effective in treating schizophrenia, improving positive symptoms (Shen et al., 2014). Unfortunately, the clinical development of vabicaserin by Pfizer was terminated because the drug failed to meet the primary efficacy end point in clinical trials (https://clinicaltrials.gov/ct2/show/results/NCT00563706?term=vabicaserin&rank=2).

Sertindole, a newer antipsychotic (Jurueña et al., 2011), alternatively exerts a potent inverse agonist activity at the 5-HT2C receptor (Herrick-Davis et al., 2000), along with dopamine D2, α1-adrenergic receptor and 5-HT2A receptor blockade (Hietala et al., 2001). Sertindole is effective in reducing anxiety and improving cognition/memory and brain plasticity, most probably by reducing 5-HT2C receptor tonic activation (Hietala et al., 2001).

Several lines of evidence have identified the 5-HT2C receptor in the origin of some side effects, both motor and metabolic, associated with the chronic use of antipsychotic drugs. For instance, 5-HT2C receptor blockade seems to contribute to AIWG, one of the most common and debilitating side effects induced by chronic treatment with these medications (Reynolds et al., 2005; Shams and Muller, 2014). Therefore, 5-HT2C receptor agonists may have an antipsychotic activity without inducing AIWG and alterations of glucose homeostasis caused by atypical antipsychotics. However, agomelatine as a 5-HT2C receptor antagonist has a more favorable profile and does not influence body weight in depressed patients (Pompili et al., 2013).

Other important side effects induced by the chronic use of antipsychotics that may be related to the activation of the 5-HT2C receptor are the movement disorders, such as dystonia, acute Parkinsonism, and tardive dyskinesia, referred to globally as antipsychotic-induced extrapyramidal side effects (EPS) (Tarsy and Baldessarini, 1984; Tarsy et al., 2002; Janno et al., 2004). The inverse agonism at the 5-HT2C receptor evoked by some atypical antipsychotics might indeed explain their fewer EPS effects (Herrick-Davis et al., 2000). Consistently, ritanserin, a nonselective 5-HT2 antagonist, limits the occurrence of EPS in patients treated with typical neuroleptic drugs (Bersani et al., 1990). Moreover, the affinity of typical antipsychotics toward the 5-HT2C receptor inversely correlates to the EPS severity (Richtand et al., 2007; Richtand et al., 2008). Nevertheless, experimental evidence suggests that concurrent 5-HT2C receptor agonism might
increase the efficacy of typical and atypical antipsychotics, allowing dose-sparing with a reduction of side effects (Grauer et al., 2009).

4. Mood Disorders and Anxiety. Depression and anxiety are complex illnesses that have in common an altered central 5-HT tone. Compelling evidence accumulating over more than four decades has indicated the 5-HT$_{2C}$ receptor is critically involved in the serotonergic regulation of these pathologic states. Indeed, mCPP and MK-212 induce anxiogenic-like behaviors in animals (Kennett et al., 1991; Sevy et al., 1994; Southwick administered in humans (Lowy and Meltzer, 1988; Kahn and Wetzler, 1991; Sevy et al., 1994; Southwick et al., 1997; Gatch, 2003). These anxiogenic effects are likely due to the activation of the 5-HT$_{2C}$ receptor. In agreement, Tecott and colleagues showed that 5-HT$_{2C}$ receptor knockout mice exhibit an anxiolytic-like phenotype not attributable to locomotor alterations (Heisler et al., 2007b). Moreover, desensitization of the 5-HT$_{2C}$ receptor in SERT knockout mice has been shown to contribute to the moderation of the anxiety phenotype (Martin et al., 2014b) and to the antidepressant effects (Prisco and Esposito, 1995; Di Giovanni et al., 2006). On the other hand, mCPP induced anxiolytic effects in mice (Nic Dhonnchadha et al., 2003) but showed antidepressant-like properties in the anhedonia model in rats (Moreau et al., 1996) and was recently observed to be anorexigenic without inducing anxiety/depression in humans (Thomas et al., 2014). Moreover, RO60-0175 presented an antidepressant profile or an anxiolytic/anticompulsive profile in some tests (Cryan and Lucki, 2000; Nic Dhonnchadha et al., 2003). Interestingly, anxiogenic responses induced by RO60-0175 (Martin et al., 2013, 2014a) are sometimes related to its sedative properties (Kennett et al., 2000). CP809101 is ineffective in some models (Siuciak et al., 2007) and anxiogenic in others (Strong et al., 2009, 2011; Christianson et al., 2010).

Consistent with proposed anxiogenic effects of 5-HT$_{2C}$ receptor activation, 5-HT$_{2C}$ receptor antagonists display anxiolytic/antidepressant properties in numerous tests (Kennett et al., 1994, 1996, 1997; Wood et al., 2001; Millan, 2005; Harada et al., 2006). Interestingly, there are models in which both 5-HT$_{2C}$ receptor agonists and antagonists display anxiolytic/antidepressant properties, including the chronic mild stress–induced anhedonia model and the activity consequent to olfactory bulbectomy. The tendency is that selective 5-HT$_{2C}$ receptor agonists would be more appropriate in the treatment of depression, obsessive-compulsive disorder (OCD), or panic attacks, whereas the antagonists would be better suited for generalized anxiety and obsessive-compulsive disorder (Jenck et al., 1998; Millan, 2003, 2005). On the contrary, the atypical antidepressants mirtazapine and mianserin (Hayasaka et al., 2015) and a recently developed new antidepressant agomelatine (Millan et al., 2003, 2011; Millan, 2005) have clear antagonistic 5-HT$_{2C}$ receptor profiles. The lack of selectivity of the pharmacological 5-HT$_{2C}$ receptor tools employed to date as well as the limited appreciation of 5-HT$_{2C}$ receptor function and its unique molecular mechanisms (e.g., edited and spliced 5-HT$_{2C}$ receptor isoforms, constitutive activity, and biased signaling) complicate final conclusions at present.

The paradoxical efficacy of both 5-HT$_{2C}$ receptor agonists and antagonists is likely due to the complex neurobiological basis of depression/anxiety and the fact that different behavioral responses involve different areas expressing the 5-HT$_{2C}$ receptor, of which activation produces opposite effects (Millan et al., 2005). Indeed, local activation of the 5-HT$_{2C}$ receptor in the basolateral part of the amygdala induces anxiety (Campbell and Merchant, 2003), whereas the activation of those in the dorsal periaqueductal gray triggers anxiolytic responses (Yamashita et al., 2011). Thus, the long-term antidepressant effect of compounds such as SSRIs may be related to a region-dependent desensitization of the 5-HT$_{2C}$ receptor in determined regions (Prisco and Esposito, 1995; Di Giovanni et al., 2006; Martin et al., 2014a).

In summary, selective 5-HT$_{2C}$ receptor antagonists may present promising molecules for developing new antidepressant/anxiolytic drugs. These could be considered as monotherapy or for augmentation strategies to improve other antidepressant responses (Cremer et al., 2004, 2007) with the ability to reverse several SSRI-induced side effects.

5. Epilepsy. Early evidence implicated a 5-HT involvement in the pathophysiology of epilepsy (Bonnycastle et al., 1957). Since then, evidence has accumulated clearly showing that there is a direct link between 5-HT levels and epilepsy. Increasing 5-HT levels in the CNS is generally antiepileptic, whereas a decrease favors epileptogenesis and seizure generation (for reviews, see Bagdy et al. (2007) and Giardi and Di Giovanni (2015)). Moreover, a common 5-HT dysfunction might underlie both epilepsy and comorbid depression seen in patients with epilepsy (Kanner et al., 2012; Giardi and Di Giovanni, 2015).

The 5-HT$_{2C}$ receptor is thought to be involved in seizure generation and cell excitability (Jakus et al., 2003; Isaac, 2005). Tecott and colleagues showed that 5-HT$_{2C}$ receptor knockout mice display spontaneous convulsive generalized seizures, which cause their high mortality rate (Tecott et al., 1995) and a reduced threshold for various convulsing stimuli (Applegate and Tecott, 1998; Heisler et al., 1998). Conversely, 5-HT$_{2C}$ receptor activation increased the threshold of general convulsion induced by pentylentetrazole and electroshock in mice (Upton et al., 1998). The 5-HT$_{2C}$ receptor also negatively controls nonconvulsive generalized seizures. Di Giovanni and colleagues showed that, in the polygenic animal model of absence epilepsy, the Genetic Absence Epilepsy
Rat from Strasbourg (Danobe et al., 1998), RO60-0175 (unpublished data), lorcaserin, and CP809101 were capable of blocking spike and wave discharges (Venzi et al., 2016). Interestingly, as expected, SB242084 blocked the effect of lorcaserin and CP809101 but also showed some antiabsence effects. One possible mechanism by which 5-HT2C receptor activation exerts antiepileptic effects is via the normalization of the aberrant GABA_A receptor tonic inhibition in the ventrobasal thalamus seen in different animal models of absence epilepsy (Cope et al., 2009; Cruenelli and Di Giovanni, 2014, 2015). Findings with the Genetic Absence Epilepsy Rat from Strasbourg animal model of absence epilepsy are in agreement with those obtained in another model of absence epilepsy, the Wistar Albino Glaxo/Rij-rat, in which mCPP decreases the cumulative duration of spike and wave discharges via the activation of the 5-HT2C receptor (Jakus et al., 2003; Jakus and Bagdy, 2011).

The 5-HT2C receptor system seems devoid of any modulatory role in partial seizures or, paradoxically, has a proepileptic role in this type of epilepsy. Indeed, observations from Di Giovanni’s group show that mCPP and lorcaserin, but not RO60-0175, were able to halt hippocampal after discharges in a rat model of temporal lobe epilepsy, an effect potentiated and insensitive to SB242084 pretreatment (Orban et al., 2014). These data indicate that other 5-HT receptors are involved in the antiepileptic effect of mCPP and lorcaserin, probably the 5-HT1A/7 receptor (Orban et al., 2013) or unknown targets, confirming previous findings (Damjanoska et al., 2003; Navailles et al., 2013a; Orban et al., 2014).

In summary, 5-HT2C receptor agonists may have new therapeutic utility in epilepsy. In particular, the FDA-approved lorcaserin may be useful for the treatment of human generalized convulsive and nonconvulsive epilepsy, which is very important in consideration of the fact that the epileptic drug pipeline is limited. Moreover, activation of the 5-HT2C receptor may also be useful for treating comorbid neuropsychiatric comorbidity commonly seen in patients with epilepsy (Di Giovanni and De Deurwaerdere, 2016; Venzi et al., 2016).

6. Sleep. A role for 5-HT in the sleep-wake cycle is well documented, as 5-HT has been shown to promote wakefulness and reduce REM sleep [for review, see Monti (2011)]. In general, nonselective 5-HT2A/2C receptor agonists and selective 5-HT2C receptor agonists increase wakefulness and decrease SW and/or REM sleep following systemic administration in rats, whereas nonselective 5-HT2A/2C receptor antagonists and selective 5-HT2C receptor antagonists tend to decrease wakefulness and promote SW with reduced REM sleep [for review, see Monti (2011)]. In contrast to what may be anticipated, 5-HT2C receptor knockout mice display increased wakefulness with reduced SW and non-REM sleep (Frank et al., 2002). The increased wakefulness and reduced SW sleep have been attributed to compensatory mechanisms, possibly including the enhanced dopaminergic and adrenergic neurotransmission that occurs as a result of the constitutive knockout of the 5-HT2C receptor (Frank et al., 2002).

The nonselective 5-HT2C2A receptor antagonists ritalserin and ketanserin enhanced SW sleep in a dose-dependent manner in human subjects with normal sleep patterns (Sharpley et al., 1990; Idzikowski et al., 1991). Similar results were observed following treatment with seganserin, ICI169369, and SR46349B (Dijk et al., 1989; Landolt et al., 1999). In addition, ritanserin has been reported to improve SW sleep in subjects with insomnia (Adam and Oswald, 1989), major depression (Staner et al., 1992), and generalized anxiety disorder (da Roza Davis et al., 1992). In patients with schizophrenia, typical and atypical antipsychotic drugs with nonselective 5-HT2C2A receptor antagonist properties tend to increase total sleep time and efficiency but reduce the latency and duration of REM sleep (Taylor et al., 1991; Nofzinger et al., 1993; Wetter et al., 1996; Sharpley et al., 2000; Muller et al., 2004). Taken together, these results indicate an involvement of the 5-HT2C receptor in the maintenance of normal sleep architecture and suggest possible avenues for future research and medication development for the treatment of insomnia.

7. Clinical Impact of RNA Editing of the 5-HT2C Receptor. Many studies have attempted to analyze 5-HT2C receptor RNA editing profiles in postmortem samples from patients with psychiatric disorders. The majority of these reported that 5-HT2C receptor RNA editing in postmortem prefrontal cortex samples from patients with schizophrenia or bipolar disorder is not altered (Niswender et al., 2001; Dracheva et al., 2003, 2008b; Iwamoto and Kato, 2003; Zhu et al., 2012). In contrast, mixed results were obtained from postmortem prefrontal cortex samples from patients with major depression, with some studies reporting no change (Niswender et al., 2001; Zhu et al., 2012; Lyndon et al., 2013) and other studies reporting a decrease (Gurevich et al., 2002b) or increase in 5-HT2C receptor RNA editing (Iwamoto and Kato, 2003). Several studies have reported that psychiatric patients who committed suicide had increased 5-HT2C receptor RNA editing with increased levels of the less-active 5-HT2C receptor isoforms (Niswender et al., 2001; Gurevich et al., 2002b; Iwamoto and Kato, 2003; Dracheva et al., 2008b; Lyndon et al., 2013; Di Narzio et al., 2014) as well as increased expression of ADAR1 (Simmons et al., 2010). However, there were notable differences in the patterns of 5-HT2C receptor RNA editing that were reported in these studies. In a similar fashion, there is great variation in the reported effects of antipsychotic and antidepressant medications on RNA editing (Sodhi et al., 2005; Abbas et al., 2010; Iwamoto et al., 2011; Martin et al., 2014a) and the effect of altered synaptic 5-HT levels on 5-HT2C receptor RNA editing (Gurevich
et al., 2002a; Abbas et al., 2010; Lyddon et al., 2010; Moya et al., 2011). These variations in results highlight the requirement of a large sample size and the need to analyze hundreds of sequences per sample to obtain an accurate profile of all possible RNA editing events. In some of the more recent studies, the statistical power of the results has been increased by employing high-throughput sequencing methods that allow larger numbers of samples to be processed, in a more timely and less expensive manner, along with the ability to analyze hundreds or even thousands of sequences from a single sample (Lanfranco et al., 2009; Abbas et al., 2010; Morabito et al., 2010b; O’Neil and Emeson, 2012; Zhu et al., 2012; Lyddon et al., 2013; Anastasio et al., 2014b; Di Narzo et al., 2014). Although these studies have reported changes in 5-HT_{2C} receptor RNA editing patterns following chronic drug treatment in rodent models or changes in editing in patients with psychiatric disorders who committed suicide, the magnitude of the observed change is small, typically less than 10%. This raises several important questions. Could such a small change in 5-HT_{2C} receptor editing be physiologically relevant? Could there be larger changes in 5-HT_{2C} receptor RNA editing occurring within discrete neuronal populations, and could these changes be underestimated or masked when sampling an entire brain region?

A few of the studies described in the preceding paragraph examined RNA editing in both cortical and subcortical regions, whereas many studies examined RNA editing only in the prefrontal cortex of patients with psychiatric illnesses. Because the 5-HT_{2C} receptor is located within mesolimbic and mesostriatal regions that have been shown to regulate the activity of ascending dopamine, it still remains possible that changes in RNA editing occurring within discrete populations of neurons in cortical and/or subcortical regions could play a role in the etiology of psychiatric disorders. Future studies capable of examining RNA editing profiles of individual neuronal populations within localized subregions will provide a more accurate picture of the relationship between 5-HT_{2C} receptor RNA editing, 5-HT_{2C} receptor isoform expression, and the functional regulation of 5-HT and dopamine neurotransmission in psychiatric disorders.

X. 5-HT_{3} Receptor

A. Introduction

The 5-HT_{3} receptor is a Cys-loop ligand-gated ion channel, which is structurally and functionally distinct from the other six classes of 5-HT receptors whose metabotropic actions are mediated via G proteins. 5-HT_{3} receptors are pentameric assemblies of five identical or nonidentical subunits that pseudosymmetrically surround the ion pore (Boess et al., 1992; Green et al., 1995). Each subunit has a large extracellular domain (ECD), four transmembrane domains (TMD I–IV), and an intracellular domain (ICD) between M3 and M4.

The ECD contains the agonist binding site, which is located at the interface of two adjacent subunits and is formed by three loops (A–C) from one (the principal) subunit and three β-strands (referred to as loops D–F) from the adjacent or complementary subunit; key residues that contribute to the binding pocket in these loops have been identified from structural data (Hassaine et al., 2014), and these are supported by a range of functional studies [for reviews, see Barnes et al. (2009) and Thompson et al. (2010)]. The binding site contains the aromatic box, which is found in all Cys-loop receptors, and in the homomeric 5-HT_{3}A receptor, is constituted of W183 (loop B), W90 (loop D), Y153 (loop E), F226 (loop C), and Y234 (loop C).

The transmembrane domain of each 5-HT_{3} receptor subunit is composed of four (M1–M4) transmembrane α-helices, with short loops between M1 and M2 (intracellular) and M2 and M3 (extracellular). The M2 α-helices line the ion pore, and pore-facing residues contribute to ion flux and selectivity. M1, M3, and M4 all protect M2 from the surrounding membrane lipids and play a role in receptor function. The conserved proline in M1, for example, is essential for activation, and the receptor is expressed but cannot function when this proline is replaced by alanine, glycine, or leucine (Dang et al., 2000). However, substitution with noncanonical amino acids that lack hydrogen bond donor activity yields active channels similar to wild-type receptors. These suggest flexibility in secondary structure in this region of M1 is a key element in channel gating.

The ICD is formed primarily by the large M3–M4 intracellular loop; this region is responsible for receptor modulation and also plays a role in trafficking. Deletion studies reveal the ICD is not essential, as the mouse 5-HT_{3}A receptor subunit ICD can be replaced by the heptapeptide M3–M4 linker of GLIC without loss of function (Jansen et al., 2008). Further evidence that the ICD can function as a separate domain comes from studies in which it was added to the GLIC linker peptide, resulting in modification of function by the intracellular protein RIC-3 (Goyal et al., 2011).

ICD structural details are sparse, but each subunit is known to possess an α-helix, which contributes to openings, known as portals, just below the level of the membrane. The residues that line these portals are important for ion conductance; when the 5-HT_{3}A subunit residues are replaced with those found in the 5-HT_{3}B subunit, the single channel conductance, which is very low in the homomeric 5-HT_{3}A receptor, is increased to that of the heteromeric 5-HT_{3}AB receptor (Kelley et al., 2003b).

Only 5-HT_{3}A subunits can form functional homomeric 5-HT_{3} receptors. These subunits have been cloned from a range of species, including human hippocampus,
amygdala, and colon (Beelli et al., 1995; Miyake et al., 1995); guinea pig small intestine (Lankiewicz et al., 1998); ferret colon (Mochizuki et al., 2000); and dog brain (Jensen et al., 2006); however, homologs are absent from invertebrates (although there is a related 5-HT–gated anion selective receptor). Multiple isoform of 5-HT3A subunits are known. Alternative splicing of the transcript encoding guinea pig, mouse, and rat, (but not dog, ferret, or human) 5-HT3A subunits results in “long” [5-HT3A(a)] and short [5-HT3A(b)] isoforms, where the 5-HT3A(b) isoform lacks five or six amino acid residues within the M3–M4 intracellular loop, which results in some subtle differences in receptor properties (Hope et al., 1993; Lankiewicz et al., 1998). In the human 5-HT3A subunit, three different splice variants have been described. Two of these (5-HT3AL and HT3Rext) would result in larger proteins, whereas the other (5-HT3AT) codes for a truncated subunit containing only a single transmembrane domain. 5-HT3Rext has not yet been functionally evaluated, but 5-HT3AT and 5-HT3AL, while not functional when expressed alone, form receptors with modified functional properties when coexpressed with canonical 5-HT3A subunits (Brüss et al., 2000).

A range of other 5-HT3 receptor subunits have been identified (B–E), of which only the 5-HT3B subunit has been extensively investigated (Davies et al., 1999; Dubin et al., 1999; Niesler et al., 2003). Coexpression of this subunit with the 5-HT3A subunit to yield heteromeric 5-HT3AB receptors resulted in functional receptors with properties that more closely represented those found in some native neuronal receptors, including relatively high single-channel conductance and relatively low Ca2+ permeability compared with homomeric 5-HT3A receptors.

The stoichiometry of heteromeric receptors is still not clear, although the presence of at least one 5-HT3A subunit appears to be obligatory in heteromeric receptors (Niesler et al., 2008; Holbrook et al., 2009). 5-HT3AB receptors were originally suggested to possess a 3B:2A subunit ratio, and using atomic force microscopy with tagged subunits indicated a BABBBA arrangement (Barrera et al., 2005). The physiologic relevance of this, however, has been questioned in more recent studies, in which the presence of an AA interface was clearly demonstrated (Lochner and Lummis, 2010; Thompson et al., 2011b), and fluorescently tagged subunits indicate a 3A:2B arrangement at the plasma membrane (Miles et al., 2013). This is also consistent with the near identical orthosteric binding site pharmacology when comparing h5-HT3A and h5-HT3AB receptors (Brady et al., 2001). The arrangement and number of 5-HT3C, 5-HT3D, and 5-HT3E subunits in functional receptors has not yet been determined, although there are many potential 5-HT3 receptor isoforms arising from the utilization of multiple subunits in different combinations.

For more information of the structure of the 5-HT3 receptor, see XVI. B. 5-HT Ligand-Gated Ion Channels.

B. Expression

5-HT3 receptors are located in many brain areas, including the hippocampus (Fig. 15), entorhinal cortex, frontal cortex, cingulated cortex, dorsal horn ganglia, amygadala, nucleus accumbens, substantia nigra, and ventral tegmental area (Parker et al., 1996a; Barnes et al., 2009). The dorsal vagal complex in the brainstem, which is key to the vomiting reflex and contains the area postrema and nucleus tractus solitarius, has the highest levels, consistent with the potent antiemetic properties of 5-HT3 receptor antagonists (Pratt et al., 1990; Parker et al., 1996; Fig. 16).

The functional studies are supported by expression studies, with 5-HT3A receptor mRNA and protein being observed in regions of the CNS known to have 5-HT3 receptors. Such studies also indicate 5-HT3 receptor expression in a wide range of other tissues, including peripheral and sensory ganglia and the gastrointestinal tract (e.g., Michel et al., 2005; Barnes et al., 2009; Fig. 17). In addition, expression of the 5-HT3A subunit has been reported in immune cells such as monocytes, chondrocytes, T cells, synovial tissue, and platelets (Fiebich et al., 2004; Stratz et al., 2008).

There was initial controversy as to the presence of 5-HT3B subunits in brain, but later studies show it is expressed here (e.g., Brady et al., 2007), with a preference for distinct “brain-type” isoforms. The longer canonical 5-HT3B subunit is broadly expressed in many tissues, including kidney, liver, and the gastrointestinal tract, with relatively high levels in spleen, colon, small intestine, and kidney (Tzvetkov et al., 2007; Holbrook et al., 2009).

5-HT3C, 5-HT3D, and 5-HT3E receptor subunits were first identified in humans, and genes for these proteins have now been shown to exist in a range of species, although not in rodents (Niesler et al., 2008; Holbrook et al., 2009). Initial studies suggested that the 5-HT3D and 5-HT3E subunits had a very restricted expression in the GI tract, but more recent data suggests all these subunits have a relatively widespread distribution. Studies examining protein levels have lagged behind the genetic work, but expression of 5-HT3C, 5-HT3D, and 5-HT3E subunits at the protein level in the GI tract has recently been confirmed (Kapeller et al., 2011).

C. Post-translational Modifications

The human 5-HT3A subunit has four consensus sequence N-glycosylation sites in the N-terminal ECD domain and all can be N-glycosylated (Monk et al., 2004). N-glycosylation is essential for export from the ER, cell surface expression, and radioligand binding, although it is not necessary to preserve a ligand binding site once the receptor has matured (Green
et al., 1995; Boyd et al., 2003; Quirk et al., 2004). Three of the four $N$-glycosylation sites are conserved between a range of species (N104, N170, and N186) and appear to be critical, whereas the $N$-glycosylation site at residue 28 is less important and indeed absent in rodents (Monk et al., 2004).

The other subunits have been less well studied, but it has been shown that 5-HT3B receptor subunits, when expressed alone, fail to exit the ER, providing a possible explanation as to why these subunits cannot form functional homomeric receptors. ER retention is due, at least in part, to a CRAR retention motif, which forms part of the M1–M2 intracellular loop (Boyd et al., 2003). Coexpression with the 5-HT3A subunit may shield this ER retention motif, allowing heteromeric 5-HT$_3$AB receptors to reach the cell surface. There is some evidence that the 5-HT3B subunit forces a preference for expression of the heteromeric 5-HT$_3$ receptor, as coexpression of the 5-HT3A and 5-HT3B subunits in tsA-201 cells did not indicate the presence of homomeric 5-HT$_3$A receptors (Barrera et al., 2005), although the physiologic relevance of this finding is not yet clear.

BiP, calnexin, and RIC-3 have been identified as ER chaperone proteins that associate with the 5-HT$_3$ receptor and are likely to promote correct folding, oligomerisation, post-translational modification, and/or export from the ER (Boyd et al., 2003). RIC-3 has been the most widely studied but has different effects depending on the subunits, the species they originated from, and the expression system (Castillo et al., 2005; Cheng et al., 2005, 2007). Expression of human homomeric 5-HT$_3$A receptors in transfected mammalian cells, for example, is enhanced by RIC-3, but it causes inhibition of heteromeric (5-HT$_3$AB) receptor expression, and mouse 5-HT$_3$A receptor expression in oocytes is completely abolished (Cheng et al., 2005). This apparent discrepancy may be due to other proteins that could influence 5-HT$_3$ receptor expression. Cyclophilin A, for example, promotes 5-HT$_3$A receptor expression in the cell membrane via an integral peptidyl prolyl isomerase activity (Helekar and Patrick, 1997), and there may be a range of other proteins yet to be identified that can modify 5-HT$_3$ receptor expression.

Similar to the human 5-HT3A subunit, the human 5-HT3B subunit has a number of consensus sequences for $N$-glycosylation sites in the N-terminal ECD domain (five; Massoura et al., 2011), and all can be $N$-glycosylated (Massoura et al., 2011). $N$-glycosylation of the human 5-HT3B subunit at each of the five consensus sites facilitates export from the ER and efficient cell surface expression when the 5-HT3B subunit is coexpressed with the human 5-HT3A subunit to generate hetermeric 5-HT$_3$AB receptors (Massoura et al., 2011).

**D. Pharmacology**

There are many selective and potent compounds that act at 5-HT$_3$ receptors. Many 5-HT$_3$ receptor agonists have in common a basic amine, an aromatic ring, a hydrophobic group, and two hydrogen bond acceptors; potent agonists include 2-methyl-5-HT, phenylbiguanide, and m-chlorophenylbiguanide (Kilpatrick et al., 1990; Cockcroft et al., 1995). Data from AChBP suggest that agonists tend to be relatively small compounds, as they need to allow the C loop to close over the binding site, initiating the gating process; the structure of 5-HT binding protein (5-HTBP),
a modified version of AChBP, which binds 5-HT and granisetron, supports a similar mechanism of action for the 5-HT₃ receptor (Tsetlin and Hucho, 2009; Kesters et al., 2013).

A range of highly selective 5-HT₃ receptor partial agonists with a range of intrinsic activities are available (e.g., Manning et al., 2011, 2014; Roberts et al., 2020) that have been proposed as therapeutics to treat the symptoms of, for example, irritable bowel syndrome (IBS) with diarrhea (IBS-D) and carcinoid syndrome (Roberts et al., 2020).

5-HT₃ receptor competitive antagonists, which bind at the orthosteric (agonist) binding site, are usually larger than agonists; they require an aromatic part, a basic moiety, and an intervening hydrogen bond acceptor. For most antagonists, these are a rigid aromatic or heteroaromatic ring system, a basic amine, and a carbonyl group (or isosteric equivalent) that is co-planar to the aromatic system (Evans et al., 1991), and there are slightly longer distances between the aromatic and amine group when compared with the agonist pharmacophore. Only small substituents, such as a methyl group, can be accommodated on the charged amine (Schmidt and Peroutka, 1989). Many potent antagonists of 5-HT₃ receptors have 6.5 heterocyclic rings, and the most potent compounds contain an aromatic six-membered ring. Morphine and cocaine (Gaddum and Picarelli, 1957) were the first antagonists used to characterize the 5-HT₃ receptor, with more selective 5-HT₃ receptor antagonists being developed in the 1980s, including MDL72222 or bemestron (Fozard, 1984) and ICS 205-930 or tropisetron (Donatsch et al., 1985). Other compounds that were developed include ondansetron, granisetron, and zacopride, which act at nanomolar concentrations, and there is now a wide range of similarly potent compounds, with many containing bicyclic heteroaromatic structures, that is, quinoxalines, quinazolines, or quinolines (Verheij et al., 2012). Data indicate that these compounds, and therefore possibly all ligands that bind to the orthosteric site, are stabilized in the binding pocket by interaction with water molecules.
Some 5-HT₃ receptor inhibitors act by blocking the channel; picrotoxin, which was originally considered to be relatively selective for the GABA<A receptor, also blocks the 5-HT₃ receptor channel; the binding of picrotoxinin (the active component of picrotoxin) has been localized to the 6’ position of M2 (Das and Dillon, 2003; Thompson et al., 2011a). Compounds structurally similar to picrotoxin, such as the gingkolides and bilobalide, act similarly (Thompson et al., 2011a). Diltiazem, which blocks voltage-gated calcium channels, is also known to block the 5-HT₃ receptor pore and acts close to the 7’ and/or 12’ residues in homomeric receptors (Gunthorpe and Lummis, 1999; Thompson et al., 2011a). Morphine and its analog methadone as well as the antimalarial compounds quinine and mefloquine may also exert their inhibitory effects via binding to the pore, highlighting the common mechanisms that many of these drugs share and also the promiscuity that many of these compounds display (Brady et al., 2001; Deeb et al., 2009; Baptista-Hon et al., 2012).

There are a number of allosteric modulators that effect 5-HT₃ receptor function, including n-alcohols, anesthetics, antidepressants, cannabinoids, opioids, steroids, and natural compounds; these can inhibit or enhance receptor activity, and many also modulate other Cys-loop receptors, although not always in the same direction [see reviews by Parker et al. (1996), Bentley and Barnes (1998) for impact on native 5-HT₃ receptors], although alcohols and inhaled anesthetics have been shown to have reduced sensitivity at 5-HT₃AB receptors, whereas the effects of etomidate, propofol, and pentobarbital are similar at 5-HT₃A and 5-HT₃AB receptors (Solt et al., 2005; Stevens et al., 2005; Rusch et al., 2007).

A further pharmacological phenomenon termed cryptic orthosteric modulation was identified using the human 5-HT₃A receptor as a model (Powell et al., 2016); this pharmacological mechanism may convey therapeutic benefits when targeting ligand-gated ion channels (Powell et al., 2016).

**E. Function**

The 5-HT₃ receptor pore is a relatively nonselective cation channel, constructed of five pseudosymmetrically arranged M2 α-helices (one from each of the five subunits). The residues that line the ion-accessible face of M2 are predominantly nonpolar and are the major controlling influence on ion flux (Reeves et al., 2001; Panicker et al., 2002; McKinnon et al., 2011). Currents are primarily carried by Na⁺ and K⁺ ions, although divalent and small organic cations are also permeable (Derkach et al., 1989; Yang, 1990; Maricq et al., 1991).

Ionic selectivity is predominantly mediated via residues in M2. A triple mutant receptor with a proline insertion at −1’ and the substitutions E−1’A and V13’T resulted in an anion-permeable mouse 5-HT₃A receptor (Gunthorpe and Lummis, 2001), although subsequent studies showed that the replacement of only two residues (E−1’ and S19’R) was needed to invert ion selectivity (Thompson and Lummis, 2003).
5-HT$_3$A receptors are almost equally permeable to monovalent and divalent cations (P$_{Ca}$/P$_{Cs}$ = 1.0–1.4) (Brown et al., 1998; Davies et al., 1999; Livesey et al., 2011). However, human 5-HT$_3$A receptors have lower Ca$^{2+}$ permeability [P$_{Ca}$/P$_{Cs}$ = 0.6 (Davies et al., 1999)], possibly the consequence of the 20’ residue being neutral (Asn and not Asp) in human 5-HT$_3$B subunits. Consistent with this, a D20’A substitution in 5-HT3A subunits reduces Ca$^{2+}$ permeability [P$_{Ca}$/P$_{Cs}$ = 0.4 (Livesey et al., 2008)]. Recent studies suggest that the ICD may also play a role in Ca$^{2+}$ permeability, as substitutions of charged residues in this region can have a major effect on Ca$^{2+}$ permeability (Livesey et al., 2011).

The single-channel conductance of the homomeric receptor is low; values of 0.4–0.76 pS have been reported (Davies et al., 1999; Gunthorpe et al., 2000; Kelley et al., 2003). The presence of Lys in the M2 regions was originally considered a possible explanation, but substitutions here revealed this was not the case (Gunthorpe et al., 2000). Subsequent work revealed that the low conductance was due to Arg residues located in the amphipathic helix of the M3–M4 loop, which forms holes, known as portals, through which ions can cross between the intracellular vestibule of the receptor and the cell interior (Kelley et al., 2003).

Heteromeric 5-HT$_3$AB receptors show a range of distinct biophysical characteristics when compared with 5-HT3A receptors: their desensitization is more rapid, concentration-response curves reveal lower EC$_{50}$ values and Hill slopes, their voltage dependence is linear, and their divalent cation permeability is much reduced; most noticeable, however, is the large single-channel conductance (13–16 pS) (Peters et al., 2005). This has been shown to be due to the substitution of Arg residues in the M3–M4 helical region of the 5-HT3A subunit by neutral or negative-charged residues in the 5-HT3B subunit (Kelley et al., 2003).

Other heteromeric receptors have not yet been extensively investigated, but studies to date indicate that they are more similar to homomeric 5-HT$_3$A receptors. The gate of the 5-HT$_3$A receptor channel is located centrally within M2 (Panicker et al., 2002), consistent with the “hydrophobic girdle” model of channel gating. The hydrophobic girdle is a region in the center of M2 that is less than 3.5 Å diameter over a distance of ~8 Å; the residues that face the pore here are hydrophobic (13’Val and 9’Leu), making it effectively impermeable to ions in the closed conformation (Miyazawa et al., 2003). Data consistent with this hypothesis are substitution of Val13’ residues in the 5-HT$_3$A receptor by threonine or serine, which caused an increase in agonist potency (Dang et al., 2000) or spontaneous channel openings (Bhattacharya et al., 2004a), and substitutions of Leu9’ by a range of amino acids, which affected agonist potency and desensitization rates (Yakel et al., 1993).

F. Clinical Relevance

Studies implicate the malfunction of 5-HT$_3$ receptors in a range of neurologic and gastrointestinal disorders (Walstab et al., 2010; Niesler, 2011). Selective 5-HT$_3$ receptor antagonists (e.g., first-generation selective antagonists such as ondansetron and granisetron and second-generation antagonists such as palonosetron) have revolutionized the control of emesis, particularly in patients receiving high aggressive cytotoxic anticancer chemotherapy (e.g., cisplatin) and radiotherapy. Thus, the release of substantial amounts of 5-HT from enterochromaffin cell stores in the gastrointestinal tract as a consequence of antimitotic anticancer therapy may activate local (vagal) and central (chemoreceptor trigger zone) 5-HT$_3$ receptors to evoke nausea and vomiting. Simple antagonism of these 5-HT$_3$ receptors offers substantial relief to patients. See XX. 5-HT Receptors and the Gastrointestinal Tract for further discussion of the 5-HT$_3$ receptor and emesis.

The identification of 5-HT$_3$ receptors in immune cells also suggests a possible role of 5-HT$_3$ receptors in immunologic processes and inflammation and suggests that they may plausibly be involved in diseases such as atherosclerosis, tendomyopathies, and fibromyalgia (Fiebich et al., 2004; Stratz et al., 2008).

Some SNP's have been identified in patients with bipolar affective disorder (BPAD) or schizophrenia, disorders that segregate with cytogenetic abnormalities involving a region on chromosome 11 that harbors the HTR3A gene (Weiss et al., 1995). Further studies are needed with two SNPs found in schizophrenic patients (R344H and P391R) to determine if they contribute to disease in these patients, but a significant association was found in BPAD with a P16S mutation in the 5-HT3A subunit, with reporter constructs indicating this mutant could modulate expression levels. (Niesler et al., 2001; Thompson et al., 2006; Krzywkowski et al., 2007). Additional SNPs in the HTR3A gene result in the 5-HT3A(A33T) and 5-HT3A(M257I) subunit variants, both of which are associated with reduced levels of cell surface expression, and the 5-HT3A(S253N) variant, which does not appear to compromise plasma membrane expression (Krzywkowski et al., 2007). The significance of these is yet to be determined.

In the 5-HT3B subunit, there has been an extensive investigation into a very common SNP, Y129S, which is linked both to BPAD and major depression in women (Krzywkowski, 2006; Hammer et al., 2012). Unusually, the Y129S variant is a gain of function mutation, as 5-HT3AB(Y129S) receptors have an increased maximal response to 5-HT, decreased desensitization and
deactivation kinetics, and a sevenfold increase in mean channel open time in comparison with heteromeric receptors containing the wild-type 5-HT3B subunit (Krzywkowski et al., 2008). An intermediate effect is apparent for receptors assembled from a mixture of wild-type 5-HT3A, wild-type 5-HT3B, and 5-HT3B(Y129S) subunits, suggesting that signaling via the 5-HT3AB receptor in heterozygous as well as homozygous individuals is altered by this SNP (Krzywkowski et al., 2008).

Studies in the more recently discovered HTR3C, HTR3D, and HTR3E genes also indicate possible involvement in disease. An SNP in the 5-HT3C gene (N163K) has been correlated with IBS, and expression studies suggest it causes an increase in receptor density (Kapeller et al., 2011). Increased expression has also been associated with an SNP in the 3’ untranslated region of the HTR3E gene, also associated with IBS, which inhibits the binding of a microRNA, as described above (Kapeller et al., 2008).

IBS-D is another disorder in which 5-HT3 receptor antagonists can be effective therapeutic agents, although side effects that may be associated with a high level of 5-HT3 receptor inhibition supports the development of 5-HT3 receptor partial agonists (e.g., Roberts et al., 2020). See XX. 5-HT Receptors and the Gastrointestinal Tract for discussion of the role of the 5-HT3 receptor and IBS.

Additional studies suggest that a wide range of other diseases have the potential to be treated with 5-HT3 receptor–selective drugs, including addiction, pruritis, migraine, chronic heart pain, bulimia, and neurologic phenomena such as anxiety, psychosis, nociception, and cognitive function (Thompson and Lummis, 2007; Walstaw et al., 2010).

See XVIII. 5-HT Receptors and the Brain, XIX. 5-HT Receptors and the Cardiovascular System, XX. 5-HT Receptors and the Gastrointestinal Tract, and XXI. 5-HT Receptors and the Immune System for further discussion of the clinical relevance of the 5-HT3 receptor.

XI. 5-HT4 Receptors

A. Introduction

An atypical 5-HT receptor was initially identified to be positively coupled to adenylyl cyclase in colliculi neurons (Dumuis et al., 1988), guinea pig hippocampus (Bockaert et al., 1990), and human and porcine cardiac atria (Kaumann, 1990; Kaumann et al., 1990; Villalon et al., 1990). This receptor was insensitive to 5-HT1 receptor and 5-HT2 receptor antagonists and was antagonized (but with a relatively low affinity) by tropisetron (at the time thought to be a relatively selective 5-HT3 receptor antagonist) but not by another 5-HT3 receptor antagonist, MDL 72222. This new 5-HT receptor was named the “5-HT4” receptor.

B. Gene and Primary Structure

The cloning of the rat cDNA by Gerald et al. (1995) revealed the first primary sequence of 5-HT4 receptor. It is a classic GPCR with seven transmembrane domains (TMD). Two splice variant sequences were found initially [5-HT4S and 5-HT4L, later renamed 5-HT4(a) and 5-HT4(b)] coding for proteins of 387 and 388 amino acids, respectively. The splicing occurs within the C-terminal domain following the splicing site coding for the leucine 358 (L358). This splicing site at position L358 was subsequently found in the majority of the numerous splice variant transcripts sequenced in rodents, pig, and human (Blondel et al., 1997, 1998; Claeysen et al., 1999; Bach et al., 2001; Brattelid et al., 2004a; Bockaert et al., 2006; Coupar et al., 2007). Subsequently in human, additional splice variants differing in their C-terminal sequences following L358 have been identified [a, b, c (a shorter nonrelevant form of the c variant has been reported that is due to a sequencing error), d, e, f, g, i, and n; Bockaert et al., 2006; Coupar et al., 2007; Fig. 18]. For a complete analysis of intron/exon junctions of the human gene, see Bockaert et al. (2004a). Another splice variant (b) occurs in the second extracellular loop (Bender et al., 2000). In pig, at least nine other different splice variants, not described in humans, have been reported, including a functional homofusion variant (De Maeyer et al., 2008a). The coding sequence of the 5-HT4 receptor gene (HTR4) extends over more than 200 kb (Genes, Ensembl release 82) and is localized on chromosome 5 (5q31-q33) (Claeysen et al., 1997). It contains at least 14 exons. Each of the C-terminal variant sequences contains one of the exons 8–13 (Bockaert et al., 2006). The variant h is the only form that results from expression of exon 6. The presence or not of exon 1 in the 5-HT4a transcript is debated (Hodge et al., 2013), as the ATG starting site is localized in exon 2. Recently, a novel transcript has been found in the human brain in which exon 1 and 2 are substituted for a novel exon (N) containing an expected ATG starting site, the C-terminal sequence being identical to the 5-HT4b receptor splice variant (Hodge et al., 2013). The predicted protein sequence for this 5-HT4 receptor variant should have a different N terminus sequence, as it is longer and the N-linked glycosylation site is at N2 instead of N7 (Hodge et al., 2013). Similar additional N-terminal exons have been identified in mouse brain and heart (Azim et al., 2012). However, further studies to clone the complete cDNA sequences and express and functionally characterize them are required before concluding on the physiologic importance of this splice variant. As some exons (such as h or i) do not present any in-frame stop codons, many additional combinations probably remain to be discovered (Brattelid et al., 2004a). SNPs within the noncoding region of HTR4 gene have been associated with chronic obstructive pulmonary disease and asthma/airways obstruction (Repapi et al., 2010; Hodge et al., 2013). The locus presents features consistent with a regulatory region and involves SNPs that significantly alter the binding motifs of a transcription factor regulating lung development (Foxp1)
(Hodge et al., 2013). Consistent with this finding, mice devoid of the 5-HT4 receptor have an altered baseline lung function (House et al., 2015).

C. Expression Profile

1. Central Nervous System. Brain distribution of 5-HT4 receptors has been studied using radiolabeled antagonists [3H]GR 113808, [125I]SB 207710, and [3H]R116712 in many species, including mouse, rat, guinea pig, monkey, and human (Eglen et al., 1995b; Bockaert et al., 1997; Bonaventure et al., 2000; Fig. 19). Generally, a heterogeneous and comparable distribution is found in the adult brain of these various species with some interspecies differences in the globus pallidus, the substantia nigra, and the interpeduncular nucleus. The highest receptor densities are found in several regions of the limbic system (islands of Calleja, olfactory bulbs, striatum, ventral pallidum, septum, hippocampus, and amygdala) or in areas belonging to several pathways such as hippocampo-habenulo-interpeduncular and striato-nigro-tectal pathways (Waeb er et al., 1993, 1994, 1996; Jakeman et al., 1994). Abundant localization of 5-HT4 receptor mRNA in the rat brain was reported in the olfactory system, striatum, medial habenula, and the hippocampus (Ullmer et al., 1996). In the rat striatum, lesion studies showed the presence of 5-HT4 receptor on the cell bodies of neurons that project to the substantia nigra and/or globus pallidus (Compan et al., 1996), whereas in situ hybridization experiments performed in guinea pig reported a localization of 5-HT4 receptors on the terminals of the striatopallidal and striatonigral projections (Mengod et al., 1996). In the rat brain, comparison of mRNA distribution with [125I]SB 207710 binding sites confirmed that 5-HT4 receptors are localized both somatodendritically (caudate putamen) and on axon terminals (substantia nigra and globus pallidus) (Vilaró et al., 1996). Bonaventure et al. (2000) performed a mapping

![Fig. 18. Primary structure of human 5-HT4 receptors. The amino acid sequences of the identified splice variants are depicted. 5-HT4 receptors have identical sequences up to L1358 and differ by the length and composition of their C-terminal domain. The 5-HT4(hb) variant, which presents an insertion of 14 residues in the second extracellular loop, has been isolated in combination with the b isoform, and it is called 5-HT4(hb). N-glycosylation sites on N17 and N180 are indicated. Palmitoylation sites on C328, C329 (common to all splice variants), and C386 (in the a isoform) are schematized. This figure has been established in accordance with the last human genome assembly (Genes, Ensembl release 82) and modified from Padayatti et al. (2013).](image_url)
analysis of both 5-HT4 receptor mRNA and binding sites in postmortem human brains and reported combined receptor detection in caudate nucleus, putamen, nucleus accumbens, and hippocampus with moderate to low densities in the cortex. Mismatches between absence of 5-HT4 receptor mRNA with high densities of receptor-binding sites in the globus pallidus and the substantia nigra suggested that the receptors may be localized on axonal projections originating from the striatum (Bonaventure et al., 2000).

In the cortex, hippocampus, and amygdala, 5-HT4 receptors were described on cholinergic neurons, where they stimulate the release of acetylcholine, as well as on the glutamatergic neurons (King et al., 2008). In the striatum and nucleus accumbens, the receptors have been found on the intrinsic GABA neurons (spiny neurons) but also on glutamatergic neurons (King et al., 2008). Work using dual-label in situ hybridization found precise localization of 5-HT4 receptors in relation with the cholinergic system. In the basal forebrain, 5-HT4 receptor mRNA was not detected in the cholinergic cell population but in parvalbumin synthesizing and glutamatergic cells (Penas-Cazorla and Vilaro, 2015). Thus, noncholinergic cell populations within the basal forebrain, hippocampal and cortical areas that express 5-HT4 receptors (likely glutamatergic cells) would mediate the 5-HT4 receptor–mediated enhancement of acetylcholine (Penas-Cazorla and Vilaro, 2015) and other neurotransmitters, such as the increase in 5-HT release in the hippocampus (Ge and Barnes, 1996).

![Fig. 19. 5-HT4 receptor expression in the human brain. In situ hybridization detection of 5-HT4 receptor mRNA (B–D) and 5-HT4 receptor autoradiography of radioligand binding sites ([3H]R116712 total binding (G and H) and nonspecific binding (J and K). Acc, nucleus accumbens; CA1-3, hippocampal fields; Cd, caudate nucleus; DG, dentate gyrus; Ent, Cx entorhinal cortex; Ic, internal capsule; Pu, putamen; S, subiculum; SN, no mRNA evident, substantia nigra; TCd, tail of caudate nucleus. Adapted from Bonaventure et al. (2000) (with permission).]
Localization of 5-HT₄ receptor splice variants in rodents appears to be similar within brain areas and concordant to the binding studies, with a low level of 5-HT₄ receptor mRNA in the cerebellum, where no receptor-binding site is detected (Claeysen et al., 1999; Vilaro et al., 1996). A complex pattern of human 5-HT₄ receptor isoforms, which depends not only on the region studied but also on each individual brain, requires analysis of a larger cohort to be conclusive (Bender et al., 2000). However, the variations in C-terminal domain of the 5-HT₄ receptor influence its constitutive activity (Claeysen et al., 1999), internalization properties (Mnie-Filali et al., 2010), and subcellular localization via interacting partners (Joubert et al., 2004) (see 2.). The distribution of 5-HT₄(a) receptors in the juvenile rat brain and spinal cord recently achieved using a polyclonal antibody have produced global data in accordance with the literature. However, striking staining of cerebellum cells raises a doubt on the specificity of the antibody used, which has not been tested on animals devoid of 5-HT₄ receptor (KO) (Suwa et al., 2014).

5-HT₄ receptors are also located on the respiratory Pre-Boetzinger complex in the ventrolateral medulla of the brainstem, where they modulate spontaneous respiratory activity (Manzke et al., 2003; Richter et al., 2003).

2. Periphery.

a. Heart. In porcine and human heart, 5-HT₄ receptors are mainly expressed in the atrial myocytes, and their expression was long believed to be atria-selective (Kaumann et al., 1991; Ouadid et al., 1992; Kaumann and Levy, 2006). However, expression was later also found in human and porcine ventricle (Bach et al., 2001; Brattelid et al., 2004a,b), and during heart failure and cardiac hypertrophy, their mRNA level and expression increased in the porcine, rat, and human ventricles (Brattelid et al., 2004b; Qvigstad et al., 2005a,b,c), possibly reflecting reactivation of a fetal gene expression pattern (Brattelid et al., 2012).

b. Gastrointestinal tract. In rodents, 5-HT₄ receptors are expressed in the smooth muscle of the esophagus. In many species, they are present on the intrinsic afferent neurons, motor neurons, and enterocytes of the gastrointestinal tract (Hegde and Eglen, 1996). The expression of 5-HT₄ receptors on excitatory motor neurons in the gut facilitates acetylcholine release, which stimulates gastrointestinal motility (Liu et al., 2005a; Gershon and Tack, 2007; Ren et al., 2008). In the human colon, 5-HT₄ receptors are also expressed on circular smooth muscle cells, where they induce relaxation (McLean et al., 1995). The 5-HT₄(b) receptor variants appear to be predominantly expressed in porcine GI mucosa, suggesting their contribution to the 5-HT₄ receptor–mediated mucosal effects of 5-HT (Prem et al., 2013). In human colon, RT-PCR analysis shows that 5-HT₄(d) and 5-HT₄(g) receptor splice variants are significantly less likely to be detected in mucosa and longitudinal muscle compared with the 5-HT₄(a), 4(b), 4(c), 4(n) splice variants (Yaakob et al., 2015).

c. Adrenal gland. 5-HT₄ receptors are present in glomerulosa and zona fasciculata of the adrenal cortex (Cartier et al., 2005). Moreover, a different splicing mechanism seems to occur in these tumors, where the 5-HT₄(a) and 5-HT₄(b) receptor splice variants, usually present in normal adrenal cortex, are absent, and the 5-HT₄(d), a rare isoform, is over-represented (Cartier et al., 2005).

d. Salivary glands. In the rat submandibular gland, expression of 5-HT₄(b) but not 5-HT₄(a) receptors was demonstrated and linked to cyclic AMP formation and regulation of volume and protein content of saliva, together with 5-HT₇ receptors (Turner et al., 1996; Bourdon et al., 2000).

e. Urinary bladder. In the bladder, 5-HT₄ receptors are present on cholinergic/purinergic neurons that innervate the smooth muscle (detrusor) (Candura et al., 1996). Their stimulation with cisapride enhances human bladder contraction without affecting urethral contractions or relaxations (Kullmann et al., 2013).

f. Lung. 5-HT₄ receptor mRNA has been detected at low density in human alveolar epithelial cells type II, airway epithelial cell lines, and human airway smooth muscle (Bayer et al., 2007; Einstein et al., 2008; Hodge et al., 2013).

D. Post-translational Modifications

Post-translational modifications of 5-HT₄ receptors and their related functional consequences have been studied on receptors expressed in heterologous cells rather than native tissues.

1. N-Glycosylation. There are two putative N-linked glycosylation sites in the extracellular side of 5-HT₄ receptors that conform to the consensus sequence N-X-S/T, where X can be any amino acid except a Pro residue (N-terminal N7 and second extracellular loop N180; Fig. 18). The pattern of glycosylation is dependent on the nature of the cell in which 5-HT₄ receptors are expressed. Salom et al. (2012) report that the glycosylation pattern of 5-HT₄ receptors ectopically expressed in mouse rod cells in vivo is more homogeneous than the glycosylation pattern of 5-HT₄ receptors expressed artificially in HEK293 cells. The h5-HT₄(b) receptors expressed in mouse rod cell display high-mannose–type and complex-type sugars at both N7 and N180 sites. Most of the oligosaccharides had the core structure of three mannoses (Man) and two N-acetylgalactosamine
intracellular loop (I-4) with rhodopsin reveals that after the TM7, there is a fourth
phosphates attached, respectively (Barthet et al., 2009). Loss of phosphate on fragmentation indicated
the turnover rate for receptor-bound palmitate. They identified C328/C329 as potential acylation sites in the
5-HT4(a) receptor, but, in contrast to most other palmitoylated GPCRs, an additional cysteine residue C386,
located in the very distal portion of the C-terminal domain, was also identified as a palmitoylation site.
Therefore, “complete” palmitoylation of the 5-HT4(a) receptor peptide was found to be phosphorylated in
rod cells. Palmitoylation of those cysteines are associated with the wide spectrum of GPCRs biologic activities, from coupling to G proteins and regulated endocytosis to receptor phosphorylation and desensitization. Ponimaskin et al. (2002a) were the first to show that, in insect Sf9 cells, palmitoylation of the 5-HT4(d) is a reversible process and that agonist increases the turnover rate for receptor-bound palmitate. They identified C328/C329 as potential acylation sites in the 5-HT4(d) receptor.

2. Palmitoylation. The first crystal structure of rhodopsin reveals that after the TM7, there is a fourth intracellular loop (I-4) with α-helical structure. Thus, this I-4 loop is also called helix 8 and terminates with two palmitoylated cysteines that are well conserved in many GPCRs as in the 5-HT4 receptors (C328 and C329).

3. Phosphorylation. The 5-HT4 receptor C-terminal sequence, common to all splice variants (upstream of L358), contains a remarkable cluster of serine and threonine residues (S347TTTSGTHVL358), which are potential phosphorylated sites for GRK. This cluster is absolutely required for β-arrestin/dynamin–dependent receptor endocytosis but not for receptor uncoupling (Barthet et al., 2005). This cluster is also required for β-arrestin binding to the receptor (Barthet et al., 2005). Like many GPCRs, 5-HT4 receptors stimulate ERK. However, this stimulation is Gs/Gβ/γ/Gα/cAMP/PKA-independent as well as β-arrestin–independent. In contrast, this stimulation requires activation of Src tyrosine kinase (Barthet et al., 2007). This Gα-independent Src-mediated ERK activation is inhibited by GRK5, which is physically associated with the proximal region of the C terminus (upstream of the S/T cluster) (Barthet et al., 2009). Tandem mass spectra of the stimulated 5-HT4 receptors in HEK293 cells expressing GRK5 revealed a peptide (R336-P359) found under a nonphosphorylated and three phosphorylated forms with one, two, and three phosphates attached, respectively (Barthet et al., 2009). Loss of phosphate on fragmentation indicated phosphorylation of S354 in the monophosphorylated peptide and the presence of an additional phosphorylated residue within the S347TTT350 motif. The triphosphorylated peptide incorporated an additional phosphate within the S347TTT350 motif. This indicates that GRK5 sequentially phosphorylates the peptide, first on S354 and then in the S347TTT350 motif. The same 5-HT4 receptor peptide was found to be phosphorylated in receptors expressed in mouse rods (0–5 phosphorylated residues) (Salom et al., 2012). In the same model, another peptide was found to be phosphorylated within the I-3 loop (A232 to R250). S235, S236, S238, and S242 residues were the targets for phosphorylation as well as S247 or T248 (Salom et al., 2012).

E. Pharmacology

1. Agonists. Very early, benzamides substituted by 2-methoxy-4-amino-5-chloro such as metoclopramide, renzapride, cisapride, or zacopride were identified as potent agonists of the 5-HT4 receptors (for reviews, see Bockeaert et al. (1997, 2004a, 2008) and Langlois and Fischmeister (2003)). This was an important step in the pharmacological characterization of 5-HT4 receptors (Clarke et al., 1989) and prompted a number of pharmaceutical companies to develop selective ligands for this receptor.

The first class of 5-HT4 receptor to be discussed includes indole derivatives such as tryptamines (including 5-HT) and indole carbazimidamines derivatives. Tegaserod, prescribed to treat IBS in women suffering from constipation, belongs to this class. This compound was withdrawn from the market in 2007 because of cardiovascular side effects but has made a recent return to the market, albeit with limitations on use.

The second class includes benzamide derivatives such as metoclopramide, which was commercialized as an antiemetic (action via dopamine D2 receptors and 5-HT3 receptors at higher doses) and stimulating gastrointestinal transit (action via 5-HT4 receptors). Mosapride, which also acts as a 5-HT4 receptor agonist, is used to treat gastrointestinal disorders, as it accelerates gastric emptying (Curran and Robinson, 2008; Tack et al., 2012), but a main metabolite is pharmacologically active and antagonizes the 5-HT3 receptor.

The benzofurane carboxamide prucalopride is selective for the 5-HT4 receptor (Briejer et al., 1999) and has been approved for the treatment of chronic constipation, in case of laxative resistance, in Europe (2009) and in Canada (2011) (Tack et al., 2013). Lead of the benzoates class is ML10302, which presents a high affinity for 5-HT4 receptor and weak affinity for 5-HT3 receptors (Yang et al., 1997). ML10302 was described as the first potent and selective 5-HT4 receptor agonist, but further investigation in vivo was limited by its susceptibility to hydrolysis.
Benzodioxane class is represented by only one product, the SL65.0155, particularly proactive as a mnemonic in several species. This compound has reached Phase IIb for the treatment of Alzheimer disease (Moser et al., 2002). It was abandoned for undisclosed reasons that may be related to its low efficacy and very partial agonist profile on 5-HT4 receptors.

The sixth class includes aryl ketones, which were developed to overcome the metabolic lability related to the related 5-HT4 receptor ester ligands. These compounds have a high bioavailability and easily pass the blood-brain barrier. RS 67333 (Eglen et al., 1995a), containing a large alkyl group, was widely used in vivo in rodent and particularly for behavioral studies demonstrating the procognitive effects of 5-HT4 receptor agonists (Fontana et al., 1997; Marchetti et al., 2000; Lamirault and Simon, 2001; Lelong et al., 2001; Kulla and Manahan-Vaughan, 2002).

The seventh class is represented by a carboxamide pyridine, PRX-03140 (also known in the literature as VRX-03011), another partial 5-HT4 receptor agonist and promnemonic substance that reached Phase IIb for the treatment of Alzheimer disease (Mohler et al., 2007) in 2008, although development of the drug was then stopped.

Benzimidazolone derivatives constitute the eighth class with BIMU1 and BIMU8 as examples, which are potent and efficacious 5-HT4 receptor agonists with good brain-penetrating properties (Dumuis et al., 1991; Rizzi et al., 1992). However, their affinity for 5-HT3 and σ2 receptors limits their utility (Bonhaus et al., 1993).

The last two classes include naphthalimide derivatives (Eglen et al., 1994) (RS 56532 and RS 66332) and the quinoline derivative velusetrag (Beattie et al., 2008). Velusetrag displays promising efficacy in patients with constipation (e.g., Nelson et al., 2017) and may also be useful in Alzheimer disease.

Other 5-HT4 receptor agonists are listed in Table 16, indicating key pharmacological parameters.

2. Antagonists. GR 113808, belonging to the indole class, was the first selective 5-HT4 receptor antagonist presenting low affinity for 5-HT3 receptors (Gale et al., 1994) and the first tritiated radioligand commercially available. This molecule enabled the accurate definition of pharmacological characteristics and localization of 5-HT4 receptors, especially in the brain (Grossman et al., 1993). Other antagonists are benzoate derivatives (such as SDZ 205557) or benzoate dioxane derivatives such as SB 204070, which by substitution of the chlorine by a radioactive iodine, lead to [125I]SB 207710, another excellent radioligand (Kaumann et al., 1995). Other antagonist classes include benzimidazolones, imidazolpyridines, and aryl ketones.

5-HT4 receptor antagonists have been mainly developed for the purpose of binding studies and in situ labeling using radioligands. Recent work includes the development of imaging probes for positron emission tomography (PET) (Dubost et al., 2012; Buitier et al., 2013; Tavares et al., 2014). However, because of the presence of 5-HT4 in the failing heart, some antagonists have been designed for cardioprotective purposes, trying to avoid CNS penetration and improving oral administration route (Brudeli et al., 2010, 2013a,b, 2014).

Some 5-HT4 receptor antagonists are listed in Table 16, indicating key pharmacological parameters.

3. Inverse Agonists. Inverse agonists of the 5-HT4 receptor (Claeysen et al., 2000; Joubert et al., 2002) have

**TABLE 16**

Affinities and efficacies of several 5-HT4 receptor ligands in radioligand binding (pKi) and functional (pEC50 or pIC50) studies

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Affinity</th>
<th>Efficacy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pK_i</td>
<td>pEC50 or pIC50</td>
<td>E_max or I_max</td>
</tr>
<tr>
<td>Agonists</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF-00885706</td>
<td>8.4</td>
<td>8.2–8.4</td>
<td>78%–84%</td>
</tr>
<tr>
<td>PF-01354082</td>
<td>8.7</td>
<td>8.1–8.5</td>
<td>74%–66%</td>
</tr>
<tr>
<td>Compound 26</td>
<td>7.9</td>
<td>9.4</td>
<td>63%</td>
</tr>
<tr>
<td>ATI-75025 (Naronapride)</td>
<td>8.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 2d</td>
<td>9.3–9.8</td>
<td>9.2–9.6</td>
<td>7%–33%</td>
</tr>
<tr>
<td>Compound 3</td>
<td>8.1–8.6</td>
<td>8.7–9.1</td>
<td>21%–63%</td>
</tr>
<tr>
<td>TD-2749</td>
<td>8.0</td>
<td>8.6</td>
<td>85%</td>
</tr>
<tr>
<td>PF-4895274 (TBPT)</td>
<td>9.5</td>
<td>9.0</td>
<td>19%</td>
</tr>
<tr>
<td>SSP-002392</td>
<td>9.1–9.2</td>
<td>10.4–10.8</td>
<td>–100%</td>
</tr>
<tr>
<td>TD-8954</td>
<td>9.4</td>
<td>8.6</td>
<td>55%</td>
</tr>
<tr>
<td>Dual ligands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT4 agonist/5-HT3 antagonist Cpd 17</td>
<td>6.7 (5-HT4)</td>
<td>7.4 (H3R)</td>
<td>7.3 (H3R)</td>
</tr>
<tr>
<td>Donecopride 5-HT4 agonist/AChE inhibitor</td>
<td>8.1 (5-HT4)</td>
<td>9.0 (5-HT4)</td>
<td>7.8 (pIC50 AChE)</td>
</tr>
<tr>
<td>Antagonists</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 34d</td>
<td>8.8</td>
<td>9.2</td>
<td>80%–100%</td>
</tr>
<tr>
<td>Compound 30</td>
<td>8.8</td>
<td>7.9 (pKb)</td>
<td>100%</td>
</tr>
<tr>
<td>Compound 12g</td>
<td>8.7</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>Compound 20</td>
<td>11.9</td>
<td>10.6 (pKb)</td>
<td></td>
</tr>
<tr>
<td>Compound 9</td>
<td>9.9</td>
<td>8.6 (pKb)</td>
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</tr>
<tr>
<td>Compound 25</td>
<td>10.1</td>
<td>9.1 (pKb)</td>
<td></td>
</tr>
</tbody>
</table>
been generated by Roche in collaboration with Bockaert’s group (RO 116-0086, RO 116-1148, and RO 116-2617; Fig. 20). SB 207266, which is a carboxylate indole derivative, is also an inverse agonist at the 5-HT₄ receptor (Fig. 20).

4. Multifunctional Ligand. Dallemagne and colleagues reported the synthesis and characterization of donecopride, a multitarget directed molecule combining 5-HT₄ receptor agonist properties with acetylcholinesterase (AChE) inhibition (Lecoutey et al., 2014); the drug may display disease-modifying actions by increasing soluble amyloid precursor protein (sAPPα) release as well as procognitive effects (Lecoutey et al., 2014; Rochais et al., 2015).

5. 5-HT₄ Receptor Radioligands. The first tritiated or iodinated 5-HT₄ receptor compounds, mainly antagonists, were particularly useful to determine brain regional distribution of the receptor (Grossman et al., 1993; Waeber et al., 1993, 1994, 1996), [³H]GR 113808 and [¹²⁵I]SB 207710 being the lead compounds (Kaumann et al., 1995). Then, several [¹¹C]-labeled compounds have been developed for use in PET (Gee et al., 2008; Xu et al., 2010; Buiter et al., 2013), enabling live imaging studies in animals (Kornum et al., 2009) and in humans (Marner et al., 2009; Madsen et al., 2011). New [¹²⁵I]-labeled antagonists have also been developed for use as PET tracers (Dubost et al., 2012). More recently, promising fluoride radioligands have been described: [¹⁸F]MNI-698 and [¹⁸F]MNI-699 (Caille et al., 2013; Tavares et al., 2014).

F. Function

1. Physiologic Function. The 5-HT₄ receptors have many physiologic functions. They are implicated in learning and memory and in the stimulation of the nonamyloidogenic cleavage of APP (Bockaert et al., 2008, 2011; Claeyssen et al., 2015). Thus, they are potential targets to treat Alzheimer disease.

A role of 5-HT₄ receptors in feeding behavior is now clear. Agonists and antagonists have hypo- and hyperphagic properties, respectively (Jean et al., 2007; Bockaert et al., 2011).

5-HT₄ receptor stimulation has rapid antidepressant action and may contribute to the actions of SSRI (Lucas, 2009; Samuels et al., 2016).

In the periphery, the role of 5-HT₄ receptors in gastrointestinal tract is well established. 5-HT₄ receptors evoke gastric emptying, peristaltic reflex, intestinal motility, inhibition of hypersensibility, short free fatty acid–stimulated HCO₃⁻ secretion, and, importantly, enteric nervous system development and adult neurogenesis of enteric plexus (Matsuyoshi et al., 2010; Gershon, 2011, 2013; Hoffman et al., 2012; Akiha et al., 2015). For further discussion, see XX. 5-HT Receptors and the Gastrointestinal Tract.

Although it was initially believed that 5-HT₄ receptors were confined to the atria of porcine and human heart (Kaumann and Levy, 2006), functional 5-HT₄ receptors mediating increased cardiac contractility (inotropic effect), as well as hastened relaxation (lusitropic effect), were later found in porcine and human ventricle (Brattelid et al., 2004b). In human, and also rat ventricle, 5-HT₄ receptor mRNA expression and function is increased or even induced in pathologies such as heart failure and hypertrophy, and following cardiac infarction (Brattelid et al., 2004b; Qvigstad et al., 2005a,c), maybe reflecting reactivation of foetal gene expression (Brattelid et al., 2012). 5-HT₄ receptor antagonists may be useful in those cardiac pathologies (Birkeland et al., 2007a; Kjekshus et al., 2009).

Fig. 20. Structure and activities of 5-HT₄ receptor inverse agonists. The inverse agonist activity was studied according to the ability of the compounds to inhibit the constitutive activity (activity in the absence of agonists). 5-HT₄ (agonist) receptors were expressed in COS-7 cells (1500 ± 130 fmol/mg). The constitutive cAMP production in presence of the receptor is equal to 720% ± 50% of the activity obtained in the absence of receptor. From Joubert et al. (2002).
Although 5-HT₄ receptors are weakly expressed in whole lung, airways epithelial cells, and airways smooth muscle cells, meta-analyses of genome-wide association studies indicate that intronic SNPs in HTR4 gene are associated with some pulmonary diseases (Hancock et al., 2010; Repapi et al., 2010; Soler Artigas et al., 2011; Hodge et al., 2013).

5-HT₄ receptors are present in corticoadrenal gland where they stimulate aldosterone production, in salivary glands where they modify the volume and protein content of saliva (Turner et al., 1996; Bourdon et al., 2000), and in urinary bladder (Hegde and Eglen, 1996; Lefebvre et al., 2015).

2. Cellular Function.

a. G protein coupling of 5-HT₄ receptors and primary signaling events. 5-HT₄ receptors stimulate adenyl cyclase in colliculi neurons as well as in hippocampus (Dumuis et al., 1988). This coupling has also been found in nucleus accumbens (Jean et al., 2007) and in heart atrium (Kaumann et al., 1990; Ouadid et al., 1992) and ventricle (Afzal et al., 2008). Thus, in native tissues, the 5-HT₄ receptor–mediated Gₐ-cAMP/PKA signaling pathway is well established. In primary cortical neurons and HEK293 cells, cAMP produced by 5-HT₄ receptors also activates the exchange factor Epac. Epac, via an Epac/Rap1/Ras pathway, activates an α-secretase and the release of sAPPα (Lezoualc’h and Robert, 2003; Cochet et al., 2013).

In transfected cell lines, several signaling pathways have been found, including coupling to G₁₅, G₉₁, and G₁₃ (Ponimaskin et al., 2002b; Bockaert et al., 2006; Woehler and Ponimaskin, 2009). Expression of different 5-HT₄ receptor splices variants in heterologous cells (COS and HEK293 cells, rodent cardiac myocytes) have identified putative differences in their signaling coupling. One clear difference is their constitutive activities measured on Gₛ coupling. Generally, the shorter the C terminus, the higher the constitutive activity (Claeysen et al., 1999). In addition, it has been reported that, in HEK293 cells and adult cardiac myocytes, 5-HT₄ₐ₅ₐ but not 5-HT₄ₐ₄ or 5-HT₄ₐ₆ are coupled to both Gₛ and G₁₅ (Pindon et al., 2002). In similar heterologous systems, activation of transiently expressed 5-HT₄ₐ₅ₐ receptors by 5-HT and other agonists induce both cAMP and inositol phosphate accumulation (Chang et al., 2007; Gaven et al., 2013).

Following PKA activation, a series of ionic currents are modulated in colliculi and CA1 hippocampal neurons (Andrade and Chaput, 1991; Ansanay et al., 1995). These include a long-lasting inhibition of K⁺ currents, including Ca²⁺-activated K⁺ channels (mediated by a PKA-dependent inhibition of phosphatases), which generates neuronal excitability and a decrease in spike accommodation (Ansanay et al., 1995) as well as activation of the hyperpolarization-activated current known to adjust repetitive discharge behavior in CA1 neurons (Andrade and Chaput, 1991). In prefrontal cortex pyramidal neurons, GABAergic transmission are either stimulated or inhibited by 5-HT₄ receptors. A role of PKA and A kinase anchoring proteins has been reported (Cai et al., 2002).

5-HT₄ receptors also activate L-type Ca²⁺ channels in human and porcine atrial myocytes via a cAMP/PKA pathway (Ouadid et al., 1992; Kaumann and Levy, 2006).

The receptor-Gₛ uncoupling phase of 5-HT₄ receptor desensitization is very rapid and efficient in colliculi neurons (Ansanay et al., 1992) and rat esophagus (Ronde et al., 1995) and is specifically dependent on the presence of a high expression of GRK2 but not of its catalytic activity (Barthet et al., 2005). In contrast, the β-arrestin/dynamin–dependent endocytosis of 5-HT₄ receptors in HEK293 cells does not require a high expression of GRK2 and is dependent of the S/T cluster upstream of L²⁵⁸ (Barthet et al., 2005).

5-HT₄ receptor–operated Src/ERK pathway, but not the Gₛ pathway, was negatively regulated by GRK5 physically preassociated with the proximal C-terminal region of the receptor in both human HEK293 cells and mouse colliculi neurons (Barthet et al., 2009). This desensitization requires two sequences of events: the association of β-arrestin1 to the phosphorylated ST cluster already described and the phosphorylation, by GRK5, of β-arrestin1 (at S⁴¹⁵) bound to the receptor. Phosphorylated β-arrestin1, in turn, prevented the activation of Src constitutively bound to 5-HT₄ receptors (Barthet et al., 2009).

3. Gastrointestinal Tract. 5-HT₄ receptor localization and functions in GI tract have been extensively studied in many species, including guinea pig, pig, dog, mice, rat, and human (Craig and Clarke, 1990; McLean et al., 1995; Hegde and Eglen, 1996; Poole et al., 2006; Gershon and Tack, 2007; Sanger, 2008; Priem et al., 2013).

Activation of 5-HT₄ receptors is prokinetic on GI and stimulate guinea pig ileum contractions is concomitant to discovery of 5-HT₄ receptors (Craig and Clarke, 1990). Subsequently, 5-HT₄ receptor facilitation of the peristaltic reflex has been identified (Hegde and Eglen, 1996; Grider et al., 1998). The mechanism by which 5-HT₄ receptors stimulate peristaltic reflex is debated. Two proposals have been provided: 1) Within circular muscles of colon (human), 5-HT₄ receptors stimulate the release the inhibitory (descending relaxation) and excitatory (ascending contractions) neurotransmitters
(nitric oxide and acetylcholine respectively) (Tonini et al., 1989; Liu et al., 2005; Cellek et al., 2006; Ren et al., 2008). 2) 5-HT4 receptors localized on the luminal surface of epithelial cells, including enterochromaffin cells and goblet cells, stimulate the peristaltic effect via secreted mucus, 5-HT, and fluid secretion (Hegde and Eglen, 1996). Luminal HCO$_3^-$ secretion is an important component of normal colonic fluid and electrolyte movement and is a major fraction of the fluid secreted in several diarrheal diseases. It has been recently found that short-chain fatty acids (SCFAs) stimulate HCO$_3^-$ secretion. This effect involves several steps, including the following ones. SCFA via the stimulation of a GPCR receptor localized on enterochromaffin cells (free fatty acid 2) increases 5-HT release, and 5-HT4 receptors localized on epithelial cells and afferent neurons stimulate HCO$_3^-$ secretion (Akiba et al., 2015).

4. Visceral Pain. Tegaserod, a 5-HT4 receptor agonist, administered orally in human reduces abdominal pain and discomfort (De Maeyer et al., 2008b; Sanger, 2008). Because tegaserod is also a 5-HT2B receptor antagonist, its exact mechanism of action is not clear. Hoffman et al. (2012) found that on a colorectal distension model of visceral pain, a more selective 5-HT4 receptor agonist (naronapride) is also active and that both tegaserod and naronapride effects were blocked by a 5-HT4 receptor antagonist. The mechanism of this antivisceral pain action of 5-HT4 receptor agonists is unknown.

5. Enteric Nervous System Development and Enteric Nervous System Adult Neurogenesis. 5-HT4 receptors are important in the early development of enteric neurons, whose number increases through 4 months of age (Gershon, 2013). In mice lacking 5-HT4 receptors (KO), the early increase fails, and the later decline is more severe. 5-HT4 receptors specifically protect cultured enteric neurons from apoptosis and also activate CREB (Liu et al., 2009). In adults, 5-HT4 receptors are also able to stimulate neurogenesis in mice (Gershon, 2011). Adult neurogenesis appears to only start when ENS is injured, either following rectal transection and end-to-end anastomosis or chemical ablation with, for example, benzalkonium chloride (Matsuyoshi et al., 2010; Laranjeira et al., 2011). In the former model, mosapride, a 5-HT4 receptor agonist, promoted the regeneration of the neural circuit in the impaired myenteric plexus and the recovery of the defeation reflex in the distal gut (Matsuyoshi et al., 2010; Kawahara et al., 2012).

6. Central Nervous System.

a. Learning and memory. One of the first roles of 5-HT4 receptors in vivo was their promnesic effect in rat, mouse, monkey, and human (Eglen et al., 1995b; Bockaert et al., 2008, 2011; Haahr et al., 2013; Meneses, 2015). Procognitive effects of 5-HT4 receptor agonists have been described, both on short-term memory (such as social olfactory memory) and on long-term olfactory memory (such as olfactory associative memory). Acute treatments with 5-HT4 receptor agonists induced an increase in memory acquisition in autoshaping task, object and social recognition, Morris water-maze, task with long intertrial intervals (2 hours), delayed matching performance, and impeded spontaneous alteration scores (King et al., 2008; Bockaert et al., 2011). Chronic treatments also improve memory performance (Quiedeville et al., 2015) and increase working memory. Old rats have poor memory relative to adult rats that can be upgraded with 5-HT4 receptor agonists, which are also reported to antagonize atropine- or scopolamine-induced learning and memory deficits in a variety of tasks, such as spatial navigation in Morris water maze, spontaneous alternation, and olfaction (Lamirault and Simon, 2001; Lelong et al., 2003; King et al., 2008; Marchetti et al., 2011). In 5-HT4 receptor KO mice, the loss of learning and memory appears to be circumvented by adaptive changes in cholinergic systems (Buhot et al., 2003).

Hippocampal 5-HT4 receptor expression correlates inversely with human memory (Haahr et al., 2013). The cellular basis of such learning and memory effects may be an increase in acetylcholine release found in frontal cortex and hippocampus, a complex modulation of synaptic plasticity (long-term potentiation and LTD) and a potentiation of learning-induced spine growth in the hippocampus [see Kemp and Manahan-Vaughan (2004), King et al. (2008), and Bockaert et al. (2011)]. Interestingly, the 5-HT4 receptor agonist SL65.0155 enhances simultaneous olfactory discrimination performance and potentiates learning-induced dendritic spine growth in the mouse hippocampus (Restivo et al., 2008). Finally, an association between 5-HT4 receptor mRNA and protein expression in cortical areas, hippocampus, olfactory tubercles on one hand, and memory consolidation on the other hand has been reported (Manuel-Apolinar et al., 2005).

b. Control of mood and role of 5-HT4 receptor agonists. SSRIs are common drugs to treat depression that eventually increase tonic 5-HT release in relevant limbic structures such as the hippocampus. It has been established that stimulation of 5-HT4 receptors localized on pyramidal mPFC stimulate the activity of a fraction of DRN 5-HT neurons (responder neurons with high-frequency discharges), an effect expected to result in a positive effect on mood behavior (Lucas and Debonnel, 2002; Lucas, 2009). Thus, 5-HT4 receptor agonists may have relatively fast antidepressant actions or may be used in combination to reduce the delay of action of SSRIs when given alone (Samuels et al., 2016).

5-HT4 receptors interact with p11, a protein that is downregulated in brain of depressed patients. This interaction is particularly clear in brain regions important for anxiety/depression and cognition such as the hippocampus (Warner-Schmidt et al., 2009; Egeland et al., 2011). The antidepressant-like activity of RS 67333, a 5-HT4 receptor agonist, was abolished in p11-KO mice, which are known to have a depressive-like phenotype (Warner-Schmidt et al., 2009).
Adult hippocampal neurogenesis in the subgranular zone has gained considerable attention in relation to mood and depression. The hypothesis is that a decrease in newborn dentate granule cell production leads to depression, whereas enhanced neurogenesis (proliferation, survival, and maturation) is required for treatment of depression (Duman and Monteggia, 2006; Samuels et al., 2016). Chronic antidepressant treatments, including SSRIs, stimulate adult hippocampal neurogenesis (proliferation of newborn cells as well as the survival and maturation of the young neurons). 5-HT_4 receptor agonists can also induce a rapid neurogenesis in the hippocampus in adult rodents (Duman and Monteggia, 2006; Pascual-Brazo et al., 2012; Ishizuka et al., 2014) and the synthesis of BDNF, a key growth factor implicated in antidepressant actions and neurogenesis. Interestingly, the 5-HT_4 receptor antagonist GR 125487 partially blocks the neurogenic effects of chronic fluoxetine treatment and its antidepressant and anxiolytic effects (Mendez-David et al., 2014), which may relate to a reduced hippocampal 5-HT release evident with 5-HT_4 receptor antagonists (Ge and Barnes, 1996). In addition to neurogenesis, SSRIs can reverse neuronal maturation in the adult hippocampal granular cell neurons via 5-HT_4 receptor-mediated signaling (Koyabashi et al., 2010).

c. Feeding behavior. In 5-HT_4 receptor KO mice, the stress-induced hypophagia and novelty-induced exploratory activity were reduced (Compan et al., 2004). This suggests that 5-HT_4 receptors may be involved in stress-induced anorexia. Anorexia and bulimia are motivation disorders, which may simulate a reward structure such as nucleus accumbens that displays relatively high expression of 5-HT_4 receptors. Direct stimulation of 5-HT_4 receptors in the nucleus accumbens reduced the physiologic drive to eat and increased the mRNA of the anorectic peptide cocaine- and amphetamine-regulated transcript in WT but not KO mice (Jahn et al., 2007, 2012). 5-HT_4 receptors control cocaine- and amphetamine-regulated transcript mRNA expression via a cAMP/PKA signaling pathway. Finally, intra-accumbal injection of 5-HT_4 receptor antagonists or siRNA-mediated 5-HT_4 receptor KO decrease satiety (Jahn et al., 2007).

d. Cardiac system. 5-HT via 5-HT_4 receptors induces cardioexcitation in atrial but not in ventricles of healthy humans and pig (Kaumann, 1990; Kaumann et al., 1991; Jahnel et al., 1992). However, an inotropic effect can also be found in human and porcine cardiac ventricle under phosphodiesterase inhibition (Brattelid et al., 2004b). In the atrium, 5-HT_4 receptors activate and phosphorylate L-type Ca^{2+} channels via a cAMP/PKA signaling pathway (Ouali et al., 1992; Kaumann and Levy, 2006). It has been proposed that atrial fibrillation, mediated by 5-HT_4 receptors, may occur in case of altered circulation during, for example, ageing (Kaumann and Levy, 2006). 5-HT_4 receptors expressed in sinoatrial node in piglets, and possibly in humans, may be responsible for some 5-HT-mediated arrhythmias (Sanders and Kaumann, 1992). In addition, in failing human heart (Brattelid et al., 2004b), there is an upregulation of 5-HT_4 receptor mRNA level in ventricles accompanied by a 5-HT_4 receptor-mediated positive inotropic response to 5-HT. Similar observations have been reported in infarcted, failing, and hypertrophic rat hearts (Qvigstad et al., 2005a,c; Brattelid et al., 2007a). Note that 5-HT_4 receptors are not expressed in healthy rat ventricles. Because in those pathologies there is an increase in plasma 5-HT concentration, likely released from platelets, this may result in cardiotoxic effects mediated via 5-HT_4 receptors.

Cardiac remodeling in heart failure is characterized by activation of a fetal gene program. Indeed, Brattelid et al. (2012) found, in rat, that 5-HT_4 receptor mRNA expression and 5-HT_4 receptor–mediated inotropic response are augmented not only in heart failure but also in ventricles during late fetal development. In rodent cardiomyocytes, pralclopride responses, characterized by a high propensity to trigger diastolic Ca^{2+} events, absent in controls, can be achieved following BDNF and imipramine treatments (Meschin et al., 2015). Because both treatments increase expression of p11, the authors are logically proposing that it is p11 that upregulates 5-HT_4 receptor responses as seen in the brain. This is likely but not formally demonstrated in the report. The difference in p11 expression in human and rodent cardiomyocytes could explain their different responsiveness to 5-HT_4 receptor agonists. This remains to be demonstrated.

For further discussion, see XIX. 5-HT Receptors and the Cardiovascular System.

e. Respiratory system. At the level of the brainstem central respiratory center, the Pre-Boeztinger complex, 5-HT_4(a) receptors via a cAMP signaling pathway, control respiration (Manzke et al., 2003). They stimulate phrenic nerve activity and respiratory minute volume. Thus, 5-HT_4 receptors are able to avert opioid-induced breathing depression, likely by having an opposing effect on the cAMP decrease induced by those drugs in the respiratory center (Manzke et al., 2003). Although noncoding variant SNPs in HTR4 are associated with pulmonary diseases in human genome-wide association studies (Hancock et al., 2010; Repapi et al., 2010; Soler Artigas et al., 2011; Hodge et al., 2013), the relatively low expression of 5-HT_4 receptors in lungs cast some doubt on the functional impact of 5-HT_4 receptors in pulmonary functions (Hodge et al., 2013). However, 5-HT_4 receptor KO mice display an altered baseline lung function (lung resistance, tissue resistance, and tissue elastance) and an increase in methacholine and 5-HT–induced airway hyper-responsiveness (House et al., 2015).

f. Adrenal gland. In the human adrenal gland, 5-HT_4 receptors are mainly localized on zona glomerulosa (Lefebvre et al., 2015), which is consistent with 5-HT stimulating preferentially aldosterone secretion.
rather than cortisol in vitro. Similarly, 5-HT$_4$ receptor agonists administered to healthy individuals increases plasma aldosterone levels without any change in plasma cortisol concentrations (Lefebvre et al., 2015).

g. Urinary bladder. Neuromuscular cholinergic transmission in human isolated detrusor muscle is facilitated by neural 5-HT$_4$ receptors (Tonini et al., 1994). In contrast, in urinary bladder strips from rhesus and Cynomolgus monkeys, experiments using direct electrical stimulation of bladder smooth muscle indicate that the 5-HT$_4$ receptors are located post-junctionally (Waikar et al., 1994).

**XII. 5-HT$_{5A}$ Receptors**

A. Introduction

In the early 1990s, the genes encoding the human and rodent 5-HT$_{5A}$ receptor genes were cloned and localized. Subsequent work in the mid-1990s revealed the primary protein structure, expression pattern, and cellular function of the receptor using in vitro preparations (Plassat et al., 1992; Erlander et al., 1993; Matthes et al., 1993; Pasqualetti et al., 1998a; Grailhe et al., 2001). Yet, the function of the receptor in vivo remained elusive until the early 2000s. By generating the Htr5A receptor knockout mouse, Grailhe et al. (1999, 2001) were the first to provide evidence of 5-HT$_{5A}$ receptor function in vivo. Subsequently, an ex vivo approach was used to characterize the electrophysiological effects of 5-HT$_{5A}$ receptors in native mouse and rat cortical brain tissue and the consequences of its deletion (Goodfellow et al., 2012), which led to the Receptor Nomenclature Committee promoting to receptor status (i.e., 5-HT$_{5A}$ to 5-HT$_{5A}$ receptor). Though further investigations have been pursued in vivo (Curtin et al., 2013; Muñoz-Islas et al., 2014; Yamazaki et al., 2014, 2015), clinical examination has been severely limited by the lack of selective ligands.

B. Gene and Primary Structure

The human HTR5A is located on chromosome 7 at position 7q36 (Matthes et al., 1993). Partial sequencing revealed an intron-exon boundary located in the middle of the third cytoplasmic loop at exactly the same position as in the mouse and rat 5-HT$_{5A}$ genes (Rees et al., 1994; Grailhe et al., 2001). The homologous mouse Htr5A is located on chromosome 5 at position 5B (Matthes et al., 1993). Partial sequence analysis revealed that the 5-HT$_{5A}$ receptor gene contains an intron in the third cytoplasmic loop (Matthes et al., 1993), approximately 8 kb in length (Matthes et al., 1993). The human 5-HT$_{5A}$ receptor, composed of a 1071-bp open reading frame, displays approximately 82% nucleotide homology with the mouse 5-HT$_{5A}$ gene (Rees et al., 1994; Grailhe et al., 2001).

Polymorphisms in the 5-HT$_{5A}$ receptor have been reported, including two nucleotide substitutions in the 5’ untranslated region and two synonymous substitutions in the coding region as well as a Pro15Ser amino acid substitution near the N terminus (Shimron-Abarbanell et al., 1997; Iwata et al., 1998; Birkett et al., 2000; Iwata et al., 2001; Arias et al., 2001; Dubertret et al., 2004). The latter polymorphism is proximal to a phosphorylation site and is located in a region likely involved in agonist-induced downregulation (Iwata et al., 1998; Thomas, 2006). Additional polymorphisms in the promoter region of the 5-HT$_{5A}$ receptor have been identified (Zhang et al., 2010).

Hydropathy analysis revealed that the 5-HT$_{5A}$ receptor contains seven hydrophobic domains (numbered I–VII), a classic feature of GPCRs (Plassat et al., 1992; Erlander et al., 1993; Matthes et al., 1993; Rees et al., 1994; Grailhe et al., 2001; Thomas et al., 2004). Sequencing analysis of the 5-HT$_{5A}$ receptor protein uncovered a single long open reading frame and a poly A tail (Plassat et al., 1992; Matthes et al., 1993; Hurley et al., 1998; Grailhe et al., 2001; Thomas et al., 2004). The length of the 5-HT$_{5A}$ receptor protein is 357 amino acids for the human, mouse, and rat proteins (Plassat et al., 1992; Erlander et al., 1993; Matthes et al., 1993; Rees et al., 1994; Hurley et al., 1998; Grailhe et al., 2001) but 356 amino acids in length for the guinea pig receptor (Thomas et al., 2004). In all species, the 5-HT$_{5A}$ receptors display N-linked glycosylation sites on the amino terminals as well as consensus phosphorylation sites for protein kinase C and protein kinase A on the presumed cytoplasmic domains (Plassat et al., 1992; Erlander et al., 1993; Grailhe et al., 2001).

C. Expression Profile

The 5-HT$_{5A}$ receptor is expressed widely in the central nervous system (Plassat et al., 1992; Rees et al., 1994; Pasqualetti et al., 1998a; Grailhe et al., 2001; Kinsey et al., 2001; Tanaka et al., 2012; Fig. 21) but seemingly not in peripheral organs (Plassat et al., 1992; Rees et al., 1994; Grailhe et al., 2001). There is some controversy over its expression in the peripheral nervous system (Pierce et al., 1996; Chen et al., 1998a; Wang et al., 2000; Nicholson et al., 2003; Avila-Rojas et al., 2015).

In the human brain, the 5-HT$_{5A}$ receptor mRNA expression has been reported in many regions of the cerebral cortex, including prefrontal, parietal, temporal, occipital, and entorhinal cortices (Pasqualetti et al., 1998b; Grailhe et al., 2001; Lambe et al., 2011; Fig. 22). The 5-HT$_{5A}$ mRNA was also detected in the hippocampus, the amygdala, the cerebellum, the striatum, the caudate nucleus, and the substantia nigra (Rees et al., 1994; Pasqualetti et al., 1998a; Grailhe et al., 2001; Fig. 22). Similar expression patterns have been reported in the mouse (Plassat et al., 1992; Tanaka et al., 2012) and the rat (Erlander et al., 1993; Kinsey et al., 2001; García-Alcocer et al., 2010; Lopez-Esparza et al., 2015). Expression was also noted in the suprachiasmatic nucleus of the hypothalamus, raphe nuclei, the ventral tegmental area,
and the locus coeruleus (Duncan et al., 2000; Oliver et al., 2000). Because the 5-HT$_{5A}$ receptor mRNA has not been detected in astrocytes (Hirst et al., 1998), expression of this receptor in the central nervous system is likely restricted to neuronal cells, consistent with the morphol-

genesis of cells expressing 5-HT$_{5A}$ receptor immunoreactiv-

ey (Oliver et al., 2000).

D. Post-translational Modifications and Impact

Treatment with the N-glycosylation inhibitor tunicamycin fails to alter [3H]5-HT binding to the 5-HT$_{5A}$ receptor yet results in redistribution of the 5-HT$_{5A}$ receptor from the cell membrane to the intracellular compartment, suggesting that the N-glycosylation sites may play a role in targeting the 5-HT$_{5A}$ receptor to the cell membrane (Dutton et al., 2008). Although both the N6 and N21 residues appear N-glycosylated within the mature human receptor protein, amino acid mutagenesis demonstrates that N-glycosylation of only the N6 residue results in protein insertion into the cell membrane (Dutton et al., 2008), a presumed prerequisite for receptor function.

E. Pharmacology

The psychedelic hallucinogen LSD binds to and stimulates the 5-HT$_{5A}$ receptor with high affinity, as does another nonselective 5-HT receptor agonist, 5-CT. However, the quest to characterize the functions of the 5-HT$_{5A}$ receptor in native tissue has been limited by a lack of selective agonists. Such characterization has relied on nonselective agonists in the presence of antag-

ons for other 5-HT receptors (Goodfellow et al., 2012).

Among selective antagonists for 5-HT$_{5A}$ receptors, SB699551 (Corbett et al., 2005) has been most widely used in the literature (Table 17). It is important to note, however, that although SB-699551 possesses high affinity for the human and guinea pig 5-HT$_{5A}$ receptor ($\sim pK_i$ 8.3), it displays a 100-fold lower affinity for the rat and mouse 5-HT$_{5A}$ receptor ($pK_i$, 6.3; Thomas et al., 2006), limiting its utility in preclinical studies.

Other 5-HT$_{5A}$ receptor antagonists have been pro-

posed, including A-842377 (Garcia-Ladona et al., 2006) and 2-amino diarylquinazolines (Peters et al., 2008). However, only SB699551 and a series of compounds, ASP5736 (Yamazaki et al., 2014, 2015), AS2030680, and AS2674723 (Yamazaki et al., 2015) have extensive published pharmacological validation. Nonselective antagonists for the 5-HT$_{5A}$ receptor include methiothe-

pin and ritanserin (Thomas et al., 2004).

Based on rank order of affinity alone, the following in vitro pharmacological profile can be suggested for the human and mouse 5-HT$_{5A}$ receptors: LSD (human $pK_i$ 8.40–8.70; mouse $pK_i$ 9.10) > 5-CT (human $pK_i$ 7.6–8.28; mouse $pK_i$ 7.8) > 5-HT (human $pK_i$ 6.7–7.40; mouse $pK_i$ 6.6–7.12) > 8-OH-DPAT (human $pK_i$ 5.6–6.07; mouse $pK_i$ 6.13–5.9) (Plasat et al., 1992; Rees et al., 1994; Francken et al., 1998; Grailhe et al., 2001; Thomas et al., 2004). By contrast, the human and mouse 5-HT$_{5A}$ receptors display low affinity for ketanserin, norepi-

nephrine, dopamine, or spiperone ($pK_i$ < 5) (Plasat et al., 1992; Rees et al., 1994; Francken et al., 1998).

F. Function

1. Cellular Function. Functional effects of 5-HT$_{5A}$ receptors have been examined in cell systems, in native tissue, and in vivo. Broadly, these data suggest that the 5-HT$_{5A}$ receptor can activate pertussis toxin–sensitive G proteins (Francken et al., 1998; Hurley et al., 1998; Thomas et al., 2004) and suppress adenyl cyclase and production of cAMP (Francken et al., 1998; Hurley et al., 1998; Thomas et al., 2000; Noda et al., 2003). Electrophysiological examination in native rodent cerebral cortex is consistent with coupling to Go$\alpha$$_{i/o}$ proteins that activate Kir3 channels (Goodfellow et al., 2012). Studies in vivo indicate 5-HT$_{5A}$ receptors may influence cognitive function and memory (Gonzalez et al., 2013; Yamazaki et al., 2014, 2015; Nikiforuk et al., 2016) and participate in acoustic startle circuitry (Curtin et al., 2013) and potentially antinociception (Muñoz-Islas et al., 2014).

2. G Protein Coupling of the 5-HT$_{5A}$ Receptor In Vitro. To elaborate on the functional work in cell systems, treatment of cell lines transfected with the 5-HT$_{5A}$ receptor with either 5-HT or 5-CT increases labeled GTP$_\gamma$S binding (Francken et al., 1998; Hurley et al., 1998; Thomas et al., 2004), an effect that could be abolished by pretreatment with pertussis toxin (Francken et al., 1998; Thomas et al., 2004). Conversely, pretreat-

ment of the nonhydrolysable GTP analog, Gpp(NH)$_p$,
reduced the high affinity binding of [3H]5-CT in transfected HEK293 cells (Francken et al., 1998). Moreover, stimulation of the 5-HT5A receptor with 5-HT can reduce the basal activity of adenylyl cyclase (Noda et al., 2003) and inhibit forskolin-induced formation of cAMP in both C6 glioma and HEK293 cell lines (Francken et al., 1998;  

Fig. 22. In situ hybridization detection of 5-HT5A receptor mRNA expression in human brain. (i and ii) Dark-field autoradiographs of coronal sections of human hippocampus and surrounding regions. CA1, CA3 fields and the dentate gyrus (DG) of hippocampus, entorhinal cortex (EC), and subiculum (S). Scale bars, 0.2 cm. (A) Dark-field autoradiograph of a coronal section of the cerebellar cortex: the Purkinje cells are heavily labeled [high magnification of Purkinje cells in bright-field (B) and dark-field (C)]. Scale bars, 600 μm (A), 500 μm (B), and 900 μm (C). Adapted from Pasqualetti et al. (1998b) (with permission).  

TABLE 17  
Affinities of selective antagonists at 5-HT5A and other 5-HT receptors  
Adapted from data reported in Corbett et al. (2005) (for SB699551; compound 11a), Thomas (2006) (for SB699551), and Yamazaki et al. (2015) (for ASP5736, AS2030680, and AS2674723). Values are binding IC50 or Ki (where indicated). Note Ki values are given for the human, mouse, and rat cloned 5-HT5A receptors, whereas the other values have been reported mainly for the human receptor (also see Thomas et al., 2004 for guinea pig values).  

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Species</th>
<th>SB699551</th>
<th>ASP5736</th>
<th>AS2030680</th>
<th>AS2674723</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT1A</td>
<td>Human</td>
<td>Ki = 501 nM</td>
<td>&gt;1000 nM</td>
<td>K1 = 21 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Ki = 84 nM</td>
</tr>
<tr>
<td>5-HT1B</td>
<td>Human</td>
<td>Ki = 316 nM</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>5-HT1D</td>
<td>Human</td>
<td>Ki = 398 nM</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>5-HT3</td>
<td>Human</td>
<td>Ki &gt; 1000 nM</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>5-HT5A</td>
<td>Human</td>
<td>Ki = 794 nM</td>
<td>&gt;1000 nM</td>
<td>&gt;300 nM</td>
<td>&gt;300 nM</td>
</tr>
<tr>
<td>5-HT7</td>
<td>Human</td>
<td>Ki = 1000 nM</td>
<td>&gt;1000 nM</td>
<td>K1 = 22 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;300 nM</td>
</tr>
<tr>
<td>5-HT12</td>
<td>Human</td>
<td>Ki = 398 nM</td>
<td>K1 = 287 nM</td>
<td>&gt;300 nM</td>
<td>&gt;300 nM</td>
</tr>
<tr>
<td>5-HT14</td>
<td>Human</td>
<td>NR</td>
<td>&gt;1000 nM</td>
<td>&gt;100 nM</td>
<td>&gt;300 nM</td>
</tr>
<tr>
<td>5-HT16</td>
<td>Guinea pig</td>
<td>NR</td>
<td>&gt;1000 nM</td>
<td>&gt;100 nM</td>
<td>&gt;300 nM</td>
</tr>
<tr>
<td>5-HT2A</td>
<td>Human</td>
<td>K1 = 3 - 6 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>K1 = 4 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>K1 = 1 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>K1 = 1 nM&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5-HT2B</td>
<td>Rat</td>
<td>Ki = 501 nM</td>
<td>K1 = 2 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>K1 = 1 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>K1 = 1 nM&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5-HT2C</td>
<td>Mouse</td>
<td>Ki = 501 nM</td>
<td>K1 = 4 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>K1 = 3 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>K1 = 2 nM&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5-HT3</td>
<td>Human</td>
<td>Ki &gt; 1000 nM</td>
<td>&gt;1000 nM</td>
<td>K1 = 39 nM</td>
<td>&gt;300 nM</td>
</tr>
<tr>
<td>5-HT5</td>
<td>Human</td>
<td>Ki &gt; 1000 nM</td>
<td>K1 = 125 nM</td>
<td>K1 = 10 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>K1 = 7 nM&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NR, not reported.  
<sup>a</sup>High affinity binding at 5-HT5A receptors or medium- to high-affinity nonselective binding.
et al., 2004). Moreover, pretreatment with pertussis toxin prevented this 5-HT-induced inhibition of basal adenyl cyclase activity (Noda et al., 2003). These findings are also consistent with the 5-HT5A receptor coupling to a Gαi/o protein pathway. However, this inhibitory effect of the 5-HT5A receptor on cAMP could not be replicated in transfected HeLa or COS-M6 cells (Erlander et al., 1993). Similarly, in both HEK and NIH-3T3 cell lines, stimulation of the transfected 5-HT5A receptors had no detectable effect in basal cAMP levels, forskolin-induced cAMP levels, or accumulation of inositol phosphates (Grailhe et al., 2001). In C6 glioma cells, 5-HT5A receptor has been shown to inhibit ADP-ribosyl cyclase activity in response to 5-HT, an effect that was abolished following pretreatment with pertussis toxin (Noda et al., 2003).

3. G Protein Coupling of the 5-HT5A Receptor Ex Vivo. Research in native tissue has shown that cerebral cortical 5-HT5A receptors couple to G proteins and has assessed the electrophysiological consequences of such coupling. In the presence of clozapine and spiperone, cortical slices from wild-type mice display a rightward shift of the [3H]5-CT binding following pretreatment with Gpp(NH)p, suggesting that in the native system, the 5-HT5A receptor is coupled to G proteins (Waeger et al., 1998). The 5-HT5A receptor–activated ion current has been characterized in native prefrontal cortical tissue of both rats and mice (Goodfellow et al., 2012). The native 5-HT5A receptor produces a small outward current that has a potent inhibitory effect on excitability of layer V pyramidal neurons of the prefrontal cortex. 5-HT has submicromolar potency at the native 5-HT5A receptor and activates inwardly rectifying potassium channels (Goodfellow et al., 2012).

4. 5-HT5A Receptor Function in the Spinal Cord. Spinal 5-HT5A receptors are upregulated by painful stimuli (Muñoz-Islas et al., 2014) and have been suggested to participate in antinoiception and pain (Doly et al., 2004; Muñoz-Islas et al., 2014; Avila-Rojas et al., 2015). Their spinal localization suggests possible motor, somatosensory, and autonomie functions (Doly et al., 2004).

5. Function In Vivo. Grailhe et al. (1999) generated 5-HT5A receptor knockout mice. In terms of its biologic phenotype, the central nervous system of the 5-HT5A receptor knockout mice appeared normal. There were no major differences in the cytoarchitectonic divisions, neuronal morphology, or distribution of glia (Grailhe et al., 1999). Moreover, 5-HT5A receptor knockout mice display no differences in the distribution of neuronal markers for the monoaminergic system, calcium binding protein, neuropeptides, nitric oxide synthase, or amino acid receptor subunits (Grailhe et al., 1999).

There appears to be the potential for significant cross talk between 5-HT5A receptors and 5-HT1A receptors in cerebral cortex given their electrophysiological function (Goodfellow et al., 2012, 2014). In mice constitutively deleted for Htr5A, however, there were striking, suprcompensatory changes in the magnitude of the prefrontal 5-HT1A receptor currents, with 5-HT5A receptor knockout mice displaying nearly a doubling of the 5-HT1A receptor outward current in layer V neurons from the prefrontal cortex (Goodfellow et al., 2012). The mechanism underlying this homeostatic plasticity is unknown. Further electrophysiological examination of 5-HT5A receptor knockout mice revealed no difference in GABAergic Receptor–mediated outward current, 5-HT1A receptor protein levels, or cell intrinsic properties in the adult prefrontal cortical neurons (Goodfellow et al., 2012).

Mice deleted for Htr5A display an increase of explorative-like behaviors, rather than anxiety-like behaviors, on the open field, the elevated plus maze, and the marble burying task (Grailhe et al., 1999). Moreover, 5-HT5A receptor knockout mice display greater increase in the number of entries into the central region after the introduction of the novel object into the center of the open field, suggesting an increase in “inspective” explorative-like behaviors (Grailhe et al., 1999). In addition, 5-HT5A receptor knockout mice displayed a reduction on LSD-elicited increase in locomotion in the open field (Grailhe et al., 1999). These changes occurred in the absence of any difference in motor activity (Grailhe et al., 1999). Given the evident considerable homeostatic plasticity detected in the cerebral cortex of the 5-HT5A receptor knockout mice (strong upregulation of 5-HT1A receptor–mediated effects; Goodfellow et al., 2012), the behavioral characterization of the 5-HT5A receptor knockout may underestimate its behavioral effects.

G. Clinical Relevance

The 5-HT5A receptors are positioned neuroanatomically to play a role in emotional regulation, cognition, antinoiception, and control of circadian rhythms and metabolism (Plassat et al., 1992; Pasqualetti et al., 1998a,b; Oliver et al., 2000; Duncan et al., 2000; Grailhe et al., 2001; Doly et al., 2004; Lambe et al., 2011; Tanaka et al., 2012). Unfortunately, clinical examination of 5-HT5A receptors has been restricted by the lack of selective ligands.

1. Psychosis and Depression. A nonsynonomous SNP (Pro15Ser) in the HTR5A gene has been linked to schizophrenia (Iwata et al., 2001; Dubertret et al., 2004) and depression (Birkett et al., 2000), and polymorphisms in the promoter region of the 5-HT5A receptor link to elevated triglyceride levels (Zhang et al., 2010). The high affinity of the psychedelic hallucinogen LSD for the 5-HT5A receptor also underscores a potential link to psychosis (Thomas, 2006), keeping in mind that LSD, like many other ergolines, has affinity for multiple 5-HT receptors, of which only the 5-HT2A receptor has been shown to play a significant role in hallucinations or psychosis. Yet in additional relevance to schizophrenia,
5-HT$_{5A}$ receptors have been suggested to participate in prepulse inhibition of the acoustic startle response (Curtin et al., 2013). This effect was demonstrated in goldfish with SB-699551 and with A-843277. The latter compound has been reported to have antipsychotic and antidepressant properties in rodent models, in line with the distribution of the receptor in higher cortical and limbic regions (Garcia-Ladona et al., 2006; Jongen-Relo et al., 2006; but see Kassai et al., 2012). Recent work suggests that blocking 5-HT$_{5A}$ receptors may ameliorate positive symptoms in a rodent model of schizophrenia and will potentially enhance cognition and social behavior in such models (Yamazaki et al., 2014; Nikiforuk et al., 2016). It may be relevant that the atypical antipsychotic drug asenapine has high affinity for h5-HT$_{5A}$ receptors (along with a number of other receptors; Shahid et al., 2009).

2. Memory and Circadian Rhythm. Yamazaki et al. (2014, 2015) demonstrated the 5-HT$_{5A}$ receptor antagonists (ASP5736, AS2030680, and AS2674723; Table 17) benefit memory, supporting a role for 5-HT$_{5A}$ receptors in memory consolidation (Gonzalez et al., 2013). Additional preclinical work focuses on the localization of 5-HT$_{5A}$ receptors in the hypothalamus, where it is densely expressed in the suprachiasmatic nucleus (Oliver et al., 2000; Duncan et al., 2000), suggesting potential roles in circadian rhythm and metabolism.

XIII. 5-HT$_{5B}$ Receptors

A. Introduction

The genes encoding the human and rodent 5-HT$_{5B}$ receptor were identified in 1993. The human 5-HT$_{5B}$ receptor gene HTR5B is located on chromosome 2 at position 1q11–13 (Matthes et al., 1993). However, this gene contains stop codons in exon I, resulting in a transcriptional product likely yielding a presumed nonfunctional truncated protein (Grailhe et al., 2001). The mouse 5-HT$_{5B}$ receptor gene Htr5B is located on chromosome 1 at position 1F (Matthes et al., 1993) and forms a functional protein upon heterologous expression. Partial sequence analysis revealed that the 5-HT$_{5B}$ receptor gene contained an intron in the third cytoplasmic loop (Matthes et al., 1993; Grailhe et al., 2001).

B. Receptor Structure

Using hydropathy analysis, the predicted 5-HT$_{5B}$ receptor protein has been shown to contain seven hydrophobic domains (numbered I–VII), a classic feature of GPCRs (Plassat et al., 1992, 1992; Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993; Rees et al., 1994; Grailhe et al., 2001). Sequencing analysis of the 5-HT$_{5B}$ receptor protein revealed a single long open reading frame and a poly A tail (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993). The length of the 5-HT$_{5B}$ receptor is 370 amino acids in both the rat and mouse protein (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993).

C. Expression Profile

The 5-HT$_{5B}$ receptor has a restricted expression profile within the central nervous system. In the rodent, expression of the 5-HT$_{5B}$ receptor is exclusively found in the CA1 field of the hippocampus, the habenula, the inferior olivary nucleus, and the raphe nuclei (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993; Kinsey et al., 2001; Serrats et al., 2004, 2004; Tanaka et al., 2012; Fig. 23). Interestingly, the 5-HT$_{5B}$ receptor mRNA was strongly expressed in the medial portion of the raphe nuclei and was found to have high coexpression with 5-HT transporter, suggesting that this receptor may be localized on 5-HT–producing neurons (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993; Serrats et al., 2004; Fig. 24). Expression of the 5-HT$_{5B}$ receptor has not been detected in any peripheral organs, including the heart, kidney, lung, liver, or intestine (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993). Evidence for the 5-HT$_{5B}$ receptor being expressed primarily in endosomes rather than the cell membrane raises potentially novel mechanisms of the functional relevance of this protein (Niebert et al., 2017) by impacting cell membrane expression of the 5-HT$_{1A}$ receptor via intracellular direct protein-protein interaction.

D. Regulatory Mechanisms and Post-translational Modifications

The 5-HT$_{5B}$ gene expression is regulated by the transcription factor ATF-7, which can directly bind to the 5-HT$_{5B}$ promoter region, resulting in histone methylation and, ultimately, silencing of the receptor gene transcription (Maekawa et al., 2010). The 5-HT$_{5B}$ receptor displays N-linked glycosylation sites on the amino-terminal end as well as consensus phosphorylation sites for protein kinase C and protein kinase A on the presumed cytoplasmic domains (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993).

E. Pharmacology

To date, selective ligands for the 5-HT$_{5B}$ receptor have not been reported. Nonselective agonists include LSD and 5-CT, and a nonselective antagonist is methiothepin. Based on affinity alone, the following in vitro pharmacological profile can be suggested for rodent 5-HT$_{5B}$ receptors: LSD (rat pKi 7.49), 5-CT (mouse pKi 7.4; rat pKi 6.26–8.88), and methiothepin (mouse pKi 7.8; rat pKi 7.35–8.87) but low affinity for ketanserin, dopamine, and norepinephrine (Matthes et al., 1993).

F. Function at Cellular, Tissue, and In Vivo Levels

The transduction pathway of the 5-HT$_{5B}$ receptor has not been well examined. Pretreatment with Gpp(NH)p reduced $[^{3}$H]5-CT binding in 5-HT$_{5B}$–transfected COS1 cells, suggesting that the 5-HT$_{5B}$ receptor readily couples...
to G protein (Wisden et al., 1993). However, this receptor had no effect on basal cAMP levels in either HeLa or COS-M6 cell lines (Erlander et al., 1993), although high levels of 5-htr5b expression in mouse brain are associated with low cAMP levels (Vogelgesang et al., 2017).

It is speculated that the 5-htr5b receptor acts as an inhibitory autoreceptor on 5-HT neurons in the raphe in rodents. However, neither an electrophysiological nor pharmacological profile has been described for the 5-htr5b receptor in native tissue.

In a mouse model of Rett syndrome, there is a relatively high level of 5-htr5b receptor expression in the brainstem because of a failure to downregulate receptor expression (Vogelgesang et al., 2017), with further studies suggesting a role for the 5-htr5b receptor in the modulation of the complex breathing phenotype of the mouse model of Rett syndrome (Vogelgesang et al., 2018) as well a potential link to the native receptor inhibiting generation of cAMP.

G. Clinical Relevance

Functional 5-htr5b receptors are likely not expressed in humans (Grailhe et al., 2001), which limits interest in this receptor particularly from the pharmaceutical industry.

XIV. 5-HT6 Receptors

A. Introduction

The 5-HT6 receptor has moderately high affinity for 5-HT, with an apparent binding affinity of approximately 30 nM. The gene for the 5-HT6 receptor was
originally cloned from rat in the early 1990s (Monsma et al., 1993; Ruat et al., 1993a) and later from human (Kohen et al., 1996) and mouse (Kohen et al., 2001) cDNA libraries; the human gene was located on chromosome 1 (Kohen et al., 1996). For each known species, the complete gene contains three exons and has a total length of around 14 kb with a coding sequence of approximately 1.4 kb. The receptor protein is composed of 440 amino acids in humans and mice and 436 amino acids in rats; a sequencing error in the originally published rat sequence was later corrected (Kohen et al., 1996). In humans, there is one described polymorphism (a C267T variant; Masellis et al., 2001) as well as a truncated, nonfunctional splice variant (Olsen et al., 1999). No replicated polymorphisms in the human gene have been associated with human diseases, including schizophrenia, methamphetamine-associated psychosis, tardive dyskinesia, Alzheimer disease, obesity, or antidepressant drug responsiveness.

The protein structure of the 5-HT_6 receptor conforms to the typical motif of GPCRs with the typical seven transmembrane domain configuration. There have been no detailed investigations of whether this receptor exists in dimers or higher-order oligomers.

B. Expression

5-HT_6 receptor mRNA is expressed primarily in the brain (Fig. 25), being detectable in rat brain by day 12 of embryogenesis (Grimaldi et al., 1998). Originally, Northern blot analyses detected RNA transcripts in brain tissue with the following regional density profile: hypothalamus > hippocampus > mesencephalon > cerebral cortex = olfactory bulb > olfactory tubercle (Monsma et al., 1993). Ruat et al. (1993a) also reported
a very similar distribution, noting highest expression in
dorsal and ventral striatum, olfactory tubercle, and
hippocampus, with lower expression in stomach and
very low to no expression detected in a variety of
other peripheral tissues. Radioligand binding and
immunohistochemical localization supported a similar
distribution in rat and human brain (Gerard et al., 1997;
Marazziti et al., 2013b). Using RT-PCR, Hirst et al.
(2003) detected a similar distribution of 5-HT₆ receptor
mRNA in various regions of rat and human brain,
whereas mouse brain had much lower apparent levels
of 5-HT₆ receptor mRNA and, unlike in other species, no
enrichment in the basal ganglia. Specific binding of
[^125I]-SB258585, a highly selective 5-HT₆ receptor
radioligand, displayed the same patterns of regional
localization, with substantially higher levels of 5-HT₆
receptors in striatum than in other regions in rats and
humans but fairly homogenous levels of expression in
all of the mouse brain regions that expressed 5-HT₆
receptors. Differences in 5-HT₆ receptor pharmacology
between these species were evident but did not explain
the disparate distributions of receptor expression,
highlighting important species differences in 5-HT₆
receptor distribution (and pharmacology).

The cellular profile of expression has been exam-
ined in some detail in several brain regions. In the
hippocampus and cortex, 5-HT₆ receptor mRNA is
present in glutamatergic neurons containing vGluT1
mRNA as well as a subset of GABAergic interneurons
that coexpress the 5-HT₃A receptor; 5-HT₆ receptor
mRNA is also detected in other classes of interneurons
at a lower frequency (Helboe et al., 2015). 5-HT₆
receptor immunoreactive glial cells have also been
reported in human cortex (Lorke et al., 2006;
Marazziti et al., 2013b). In striatum, 5-HT₆ receptor
mRNA is expressed in both direct and indirect pathway
medium spiny neurons (Ward and Dorsa, 1996; Helboe
et al., 2015) and occasionally in cholinergic interneur-
os (Bonsi et al., 2007; Helboe et al., 2015).

5-HT₆ receptors are not presynaptic autoreceptors
on 5-HT neurons, as 5-HT₆ receptor mRNA in rat
is not altered by ablation of the DRN with 5,7-
dihydroxytryptamine (Gérard et al., 1996) and is not
colocalized with SERT mRNA (Helboe et al., 2015).
Nonetheless, their abundant expression on forebrain
cortical neurons thought to project to DRN 5-HT
neurons may explain how 5-HT₆ receptor ligands can
modify 5-HT neuronal firing (see Electrophysiology
below).

The localization of 5-HT₆ receptors within neurons is
still somewhat controversial, perhaps because of diffe-
rences in antibody specificity. Initial studies described

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**Fig. 25.** In situ hybridization detection of 5-HT₆ receptor mRNA expression in rat brain. Autoradiographic visualization of 5-HT₆ receptor mRNA in
rat brain (A–D) and relative absence of signal when adjacent sections probed with the sense control (c and d). ANA, anterior nucleus accumbens; CgCx,
cingulate cortex; Cx, cortex; DG, dentate gyrus; Hb, habenula; Hp, hippocampus; OT, olfactory tubercle; PFCx, prefrontal cortex; PyCx, pyriform cortex.
Adapted from Ward et al. (1995) (with permission).
5-HT₆ receptors in the neuropil and electron microscopy suggested that immunolabeling was predominantly associated with dendrites (Gerard et al., 1997). A subsequent electron microscopy study revealed that 5-HT₆ receptor immunolabeling is not only associated with dendrites but also with primary neuronal cilia (Hamon et al., 1999; Brailov et al., 2000). This is a surprising result because there are few GPCRs that localize to primary neuronal cilia (e.g., somatostatin sst3 receptor), and the 5-HT₆ receptor is the only 5-HT receptor that may do so (Berbari et al., 2008). The ciliary localization may require the presence of key amino acids in the third intracellular loop of the receptor, which is the case for 5-HT₆ receptors in mice, rats, and humans (Berbari et al., 2008). This subcellular distribution may have implications for interactions with signaling proteins, such as adenylyl cyclase 3, which is localized exclusively in primary cilia (Baker et al., 1998), and resultant functional consequences of 5-HT₆ receptor signaling. However, the distribution and function of 5-HT₆ receptors that are heterologously expressed may be strongly dependent on a number of factors, including the differentiation state of the neuron and the amount of receptor expressed. As an example, exogenous overexpression of transfected 5-HT₆ plasmid DNA has a strong effect on cilia localization, with heavy expression leading to increased rates of non-ciliary localization.

C. Pharmacology

There are important species differences between mammalian 5-HT₆ receptors. The mouse 5-HT₆ receptor has similar sequence homology to the homologous receptor of human, pig, or rat, but the pharmacological profile is much closer between human and rat than mouse (Setola and Roth, 2003). Hirst et al. (2003) detected marked differences in the affinity of a number of ligands for the 5-HT₆ receptor in these three species. For example, the antagonist compound Ro 04-6790 binds to recombinant rat or human 5-HT₆ receptors with similar affinity (pKᵢ ≈ 7.4) but has relatively very low affinity for mouse 5-HT₆ receptor (pKᵢ < 4); several other antagonists (including SB-258585 and mianserin) have lower affinities (5- and 12-fold, respectively) at mouse compared with rat or human receptors (Hirst et al., 2003); these differences are explained to some degree by variances in the binding pocket as demonstrated by site-directed mutagenesis. On the other hand, several highly selective agonists have similar affinities for mouse, human, and rat 5-HT₆ receptors. There are relatively far lower levels of 5-HT₆ receptor radioligand binding sites in mouse brain, with lesser density variation across regions, than is evident in rat and human brain. Importantly, this reduced level of receptor binding and rather homogenous density across the mouse brain is method-independent, as it is equally observed using a variety of techniques to localize the receptors. Differences in residues 188 in TM5 and 290 in TM6 contribute to these species differences.

A null mutation (knockout) for the mouse 5-HT₆ receptor results in only subtle changes to the physiological and behavioral phenotype (Bonasaera et al., 2006), consistent with a limited contribution of the 5-HT₆ receptor to baseline mouse behavior. Thus, rats may be a more useful model for studying the pharmacological and (patho)physiological roles of 5-HT₆ receptors in relation to humans, whereas the availability of a knockout strain makes mice very useful for studying other aspects of the biology of this receptor.

Several strategies for radiolabeling 5-HT₆ receptor have been described. [³²P]-SB258585 (Hirst et al., 2006) is the most selective, commercially available option; other radioligands require masking of non–5-HT₆ receptor sites and include [³H]-LSD (Sleight et al., 1998), [³H]-clozapine (Glatt et al., 1995), and [¹¹C]-GSK215083 (Parker et al., 2012, 2015).

Several selective 5-HT₆ receptor agonists are available. 2-Ethyl-5-methoxy-N,N-dimethyltryptamine was the first moderately selective agonist developed (Glennon et al., 2000); it is brain penetrant and has >10-fold selectivity for 5-HT₆ compared with other 5-HT receptors. More selective agonists include WAY181187, WAY208466 (Schechter et al., 2008), and ST1936 (Borsini et al., 2015). Some of these ligands display partial agonism under certain circumstances, such as EMD386088 (Jastrzebska-Wiesek et al., 2013) and E6801 (Romero et al., 2006); many nonselective 5-HT₆ receptor agonists are either full or partial agonists at 5-HT₆ receptors, which can be useful tools in specific cases, but care must be given to rule out actions at other 5-HT receptors. For instance, EMD386088 has moderate affinity (IC₅₀ = 34 nM) for the 5-HT₃ receptor (Mattson et al., 2005), and ST1936 has moderate affinity for 5-HT₇, 5-HT₂B, and α₂-adrenoceptors (Kᵢ = 168, 245, and 300 nM, respectively; Riccioni et al., 2011). However, the contribution of partial agonism to the behavioral or physiologic effects of 5-HT₆ receptor ligands has not been thoroughly characterized.

A number of clinically effective drugs have affinity for 5-HT₆ receptors but also for other receptor targets. Several antidepressants and antipsychotics have high affinity for 5-HT₆ receptors (Monsma et al., 1993; Ruat et al., 1993a), but none of these are selective for the receptors. After the discovery of the 5-HT₆ receptor, a number of modestly selective antagonists were reported based on high-throughput screening of chemical libraries, although these often shared affinity for other 5-HT or dopamine receptors (Upton et al., 2008). The first CNS penetrant selective 5-HT₆ receptor antagonists were produced by Roche (e.g., Ro 04-6790; Sleight et al., 1998) and GlaxoSmithKline (e.g., SB-271046; Bromidge et al., 1999). Subsequently, more highly selective 5-HT₆ receptor antagonists were developed, which are useful for both in vitro and in vivo...
experimentation. Among these are SB258585, SB399885 (Hirst et al., 2003), and Ro4368554 (Lieben et al., 2005).

In heterologous systems expressing wild-type or constitutively active 5-HT_{6} receptors, a number of the selective agonists may show partial agonist activity, whereas numerous antagonists display inverse agonist properties (Purohit et al., 2003; Romero et al., 2006).

The first 5-HT_{6} receptor somewhat selective PET ligand, [^{11}C]GSK215083 (with approximately fivefold-lower affinity for the 5HT_{2A} receptor), demonstrates 5-HT_{6} receptor occupancy in the human striatum (Parker et al., 2012, 2015) and will no doubt help to investigate the role of this receptor in human pathology and in therapeutic response/target engagement of existing or new therapeutics or drugs.

D. Post-translational Modifications and Protein Interactions

There is a predicted glycosylation site in the amino terminal of the 5-HT_{6} receptor and a number of predicted phosphorylation sites (Monsma et al., 1993; Ruat et al., 1993a; Kohen et al., 1996, 2001), although none of these have been examined systematically. However, phosphorylation of serine 350 appears to be mediated by cyclin-dependent kinase 5 (Cdk5); treatment of NG108 cells with a 5-HT_{6} receptor–selective agonist leads to coimmunoprecipitation of 5-HT_{6} receptor and Cdk5, whereas the 5-HT_{6} receptor–selective antagonist SB258585 reduces the receptor phosphorylation and coimmunoprecipitation with Cdk5 (Duhr et al., 2014). Furthermore, bioluminescence energy transfer experiments confirm the direct interaction of this receptor and Cdk5 in transfected NG108 cells, which is also inhibited by the 5-HT_{6} receptor antagonist SB258585. Duhr et al. (2014) demonstrated that Cdk5-dependent phosphorylation of the 5-HT_{6} receptor leads to activation of Cdc42 (a Rho GTPase), which can in turn activate further downstream mechanisms. The role of serine 350 phosphorylation was confirmed by site-directed mutagenesis, which may result in constitutive activity of the receptor in the absence of agonist and inhibition of phosphorylation and downstream events by the 5-HT_{6} receptor–selective antagonist SB258585. These signaling events may enhance NG108 cell differentiation and neurite outgrowth both in this cell line and in striatal and cultured hippocampal and striatal neurons.

Using HEK293 cells transfected with epitope-tagged 5-HT_{6} receptors, Marin’s group also detected direct interactions of this receptor with mTOR and several related proteins in the mTOR complex 1 (Meffre et al., 2012). 5-HT_{6} receptor–selective agonists induce mTOR activation, social cognition impairments in mice, and object recognition impairment in rats that could be blocked with the mTOR antagonist rapamycin. In summary, these data suggest that direct protein-protein interactions involving the 5-HT_{6} receptor may lead to activation of multiple signaling cascades in addition to the canonical G_{s}-mediated activation of adenyl cyclase (see XVII. C. 5-HT_{6} Receptor Receptosome: Toward New Signaling Mechanisms Underlying Its Control of Cognition and Neurodevelopmental Processes for further discussion of protein-protein interactions with the 5-HT_{6} receptor).

Though not examined in detail, dimerization of 5-HT_{6} receptors can be inferred from the immunoprecipitation and Western blot analysis (Meffre et al., 2012; Duhr et al., 2014).

E. Signaling Pathways

There is growing appreciation for the complexity of the pharmacological and signaling properties of the 5-HT_{6} receptor. The ability of the 5-HT_{6} receptor to activate adenyl cyclase in a G_{s}-dependent manner was first described when the receptor was originally cloned and expressed heterologously as well as in native tissues (Monsma et al., 1993; Ruat et al., 1993a; Sebben et al., 1994; Unsworth and Molinoff, 1994; Choi et al., 2007; Kim et al., 2014b). 5-HT_{6} receptors can activate several adenyl cyclase isoforms, including AC3, AC5, and AC8, but not AC1 or AC8 (Baker et al., 1998). Because 5-HT_{6} receptors activate adenyl cyclase, thereby increasing cAMP and therefore protein kinase A activity, a number of additional downstream signaling consequences can be presumed, although these have not been systematically examined. Svenningsson et al. (2002) examined the potential synergistic interactions between 5-HT_{6} and D_{1} dopamine receptors; 5-HT_{6} receptor activation in striatum regulates DARPP_{32}, an enzyme previously associated mainly with dopaminergic neurotransmission. Selective 5-HT_{6} receptor agonists modulate the DARPP_{32} phosphorylation state in a manner consistent with increasing this enzyme’s activity; phosphorylation of DARPP_{32} is blocked by selective 5-HT_{6} receptor antagonists; 5-HT_{6}–mediated c-Fos activation and motor activity is blunted in DARPP_{32} knockout mice (Svenningsson et al., 2002). However, 5-HT regulation of DARPP_{32} is unlikely to involve exclusively 5-HT_{6} receptors, as 5-HT_{4} and 5-HT_{7} receptors can also activate DARPP_{32}. This pathway is an interesting regulatory node that potentially integrates 5-HT and dopamine signaling in neurons that coexpress 5-HT_{6} and D_{1} receptors, such as striatonigral (direct pathway) medium spiny neurons.

The 5-HT_{6} receptor induces fyn kinase activation. (Yun et al., 2007; Riccioni et al., 2011). Fyn is an src tyrosine kinase family member, present in both cilia and neuronal soma. Fyn is expressed in the same brain regions as 5-HT_{6} receptor. Phosphorylated-Fyn is thought to activate Erk1/Erk2 kinases via the Ras-Raf-MEK pathway. Direct protein interactions with Cdk5 also predict that 5-HT_{6} receptors signal through this kinase pathway, which was shown to regulate neurite outgrowth in NG108 cells and primary neurons (Duhr et al., 2014).
This pathway regulates neuronal migration in both slice cultures and in vivo (Jacobshagen et al., 2014). 5-HT₆ receptor activation phosphorylates doublecortin and focal adhesion kinase, two mediators of neuronal migration, and is Cdk5-dependent, although additional or alternative mediators may be at play. Similarly, mTOR interactions were predicted based on studies of protein interactions, and 5-HT₆ receptor agonists induce phosphorylation of mTOR into the active form, which is blocked by selective 5-HT₆ receptor antagonists both in vitro and in vivo (Meffre et al., 2012).

The constitutive activity of 5-HT₆ receptors represents an intriguing issue. Constitutive activity implies that the receptor couples to and activates downstream effectors in the absence of the agonist; the clearest evidence for this with 5-HT₆ receptors comes from heterologous expression systems and with activation of Cdk5, Fyn, and mTOR signaling events (Meffre et al., 2012; Duhr et al., 2014; Jacobshagen et al., 2014; see XVII. C. 5-HT₆ Receptor Receptosome: Toward New Signaling Mechanisms Underlying Its Control of Cognition and Neurodevelopmental Processes). However, it has been more difficult to confirm this in systems that are dependent on activation of 5-HT₆ receptors expressed in native cells or tissues. For example, Sebben et al. (1994) described 5-HT₆ receptor–mediated cAMP accumulation in primary cultured mouse striatal neurons, and several full agonists, such as 5-HT and LSD, caused cAMP production, but no constitutive activity was apparent. Because the cell type, signal transduction pathways, and level of receptor expression may all affect the extent of constitutive activity, its contribution to 5-HT₆ function likely varies a great deal depending on the cellular context; hence, it still remains to be demonstrated with natural expression levels in vivo. However, the 5-HT₆ receptor clearly has a propensity to display constitutive activity, and many of the available antagonists are in fact inverse agonists, meaning they will reduce 5-HT₆ signaling if constitutive activity is present (Purohit et al., 2003).

F. Function

Prior to the availability of selective 5-HT₆ receptor antagonists, antisense oligonucleotides were used to reduce expression/function of the 5-HT₆ receptor; yawning and stretching behavior (Bourson et al., 1995), weight loss, and reduced retention of spatial learning in the Morris water maze were reported following receptor knockdown (Woolley et al., 2001), later replicated by the use of selective antagonists. Interestingly, the extent of 5-HT₆ receptor knockdown was limited (20%–30% using [³H]LSD binding), and it is only more recently that knockout mice have been available. Tecott and Brennan (2000) produced the first 5-HT₆ receptor knockout mouse and reported weight loss in a patent, which triggered the exploration of 5-HT₆ receptor antagonists as appetite suppressants (Heal et al., 2008; Higgs et al., 2016). More recently, germ-line knockout of 5-HT₆ receptors produced no changes in viability, health, weight, or longevity of the null mutant mice (Bonasera et al., 2006) and no deficits in fertility or maternal behavior. Furthermore, no overt changes in circadian activity, emotional behavior, sensorimotor gating, or cognition were detected. The only behavioral change evident was a reduced sensitivity to the sedative and ataxic effects of acute ethanol administration. However, mouse 5-HT₆ receptor KO may underestimate the potential roles of 5-HT₆ receptors in any of these effects, as mice have much lower 5-HT₆ receptor expression than many other mammalian species (Hirst et al., 2003), as discussed above.

Though 5-HT₆ null mutant mice seem to develop normally, there are very interesting effects of acute modulation of 5-HT₆ receptors during cortical development. Using both in vivo and explant culture models, Dayer and colleagues have shown that modulating 5-HT₆ receptor signaling produces deficits in neuronal migration (Jacobshagen et al., 2014). The 5-HT₆ receptor partial agonist EMD386088 delays the rate of pyramidal neuron and interneuron migration (Riccio et al., 2009, 2001), which can be blocked by the antagonist SB258585, although the concentrations of drugs used are rather high, questioning receptor classification.

The effect of activation versus inhibition of 5-HT₆ receptor activity is complex, though, as short hairpin RNA knockdown of endogenous 5-HT₆ receptors produces deficits in neuronal speed and extent of neuronal migration (Jacobshagen et al., 2014). This deficit is partially rescued by coexpressing 5-HT₆ receptors or overexpressing Cdk5 but not by coexpressing 5-HT₆ receptor mutants that disrupt Gs-coupling and thus adenyl cyclase activation. Furthermore, 5-HT₆ receptor–mediated Cdk5 signaling facilitates dendritic outgrowth. There may be additional mechanisms of 5-HT₆ receptor–mediated developmental effects, as the selective agonist WAY181187 activates mTOR in mouse cortex, and this is blocked by SB258585 (Meffre et al., 2012). The psychomimetic drug phencyclidine, which models aspects of the development and symptoms of schizophrenia, induces mTOR activation in neonatal rat cortex and impairs social cognition in the adult mouse, both of which are blocked by the mTOR inhibitor rapamycin or the 5-HT₆ receptor–selective antagonist SB258585. Interestingly, both drugs also reverse visual recognition impairments associated with isolation rearing in rats (Marsden et al., 2011; Meffre et al., 2012). Though adenylyl cyclase, mTOR, Cdk5, and fyn kinase signaling are complex and impacted by many parallel pathways, the potential that multiple signaling pathways emanating from 5-HT₆ receptors play a crucial role in establishing normal cortical circuitry has far-reaching implications given that drugs that modulate 5-HT dynamics (such as SSRIs and other antidepressants) or that directly interact with 5-HT₆ receptors (whether exclusively so or not) may
produce alterations in cortical development. These issues need to be examined more fully to understand the consequences of 5-HT drug treatments during gestation or in adolescence.

It is reasonable to conclude that 5-HT\(_6\) receptors may increase the excitability of neurons, as this is the expected result following activation of adenylyl cyclase. The 5-HT\(_6\) receptor agonist WAY181187 increases fos-like immunoreactivity in cortex (Burnham et al., 2010), and the 5-HT\(_6\) receptor antagonist Ro4368554 reduces scopolamine-induced increase of fos-like immunoreactivity in lateral amygdala (Mitchell et al., 2009b); however, both of these manipulations produce complex behavioral and pharmacological changes that make it difficult to conclude that the effects are directly related to 5-HT\(_6\) receptors or are more indirect consequences of circuitry level events.

5-HT\(_6\) receptors modulate many different neurotransmitter systems (Mitchell and Neumaier, 2005; Dawson, 2011). The impacts on extracellular levels of acetylcholine, glutamate, GABA, dopamine, and noradrenaline have been reported using microdialysis. Selective 5-HT\(_6\) receptor ligands alter neuronal firing in a variety of brain regions, but it is difficult to conclude whether these effects are direct or indirect. In any event, it appears that 5-HT\(_6\) receptor ligands modulate numerous brain regions and neurotransmitter systems.

There is a rich literature addressing the potential cognitive effects of 5-HT\(_6\) receptor modulation (Woolley et al., 2004; Mitchell and Neumaier, 2005; King et al., 2008; Codony et al., 2011; Marsden et al., 2011; Meneses, 2015). In most cases, 5-HT\(_6\) receptor agonists display procognitive effects in a variety of rodent memory tasks, such as spatial learning, novel object recognition, social discrimination, and autoshaping (Mitchell and Neumaier, 2005). Generally, these experiments used parenteral drug administration either just before or just after a memory “training” session that was then assessed after a short interval. The procognitive effects of 5-HT\(_6\) receptor agonists have often been shown to be associated with changes in other neurotransmitter systems, especially acetylcholine, glutamate, or GABA release. However, it is difficult to reach mechanistic conclusions based on the correlative nature of these observations. At this time, it is not possible to attribute the memory-enhancing effects of 5-HT\(_6\) receptor agonists to a single brain region or neurotransmitter system, and it seems likely that multiple mechanisms are responsible.

Although there are numerous reports that inhibiting 5-HT\(_6\) receptors promotes (or restores) memory, there is some apparent inconsistency in the cognitive role of 5-HT\(_6\) receptor agonists. For example, WAY117187 (an agonist) impaired social recognition in an antagonist-sensitive manner whether given systemically or locally into frontal cortex (Loiseau et al., 2008); EMD386088 or WAY466 impaired memory in the autoshaping or social recognition tasks, respectively (Meneses et al., 2008; Schechtler et al., 2008). However, other reports have observed procognitive effects of 5-HT\(_6\) receptor agonists (Kendall et al., 2011; Woods et al., 2012; Pereira et al., 2015). There are several potential explanations for these discrepancies. One explanation is that 5-HT\(_6\) receptor activation in some brain regions is procognitive, whereas inhibition of 5-HT\(_6\) receptors in other brain regions is also procognitive. Another possible explanation is that the effect of increased or decreased 5-HT\(_6\) receptor activity is entirely dependent on the specific cognitive domain being tested. A third possibility is that either high or low levels of 5-HT\(_6\) receptor activity enhance cognition in specific regions, albeit by different neurochemical mechanisms (e.g., facilitating glutamatergic function in states with a relative deficit or facilitating GABAergic function in states of excessive glutamatergic activity). Even when the same cognitive task has been used, each laboratory likely performs these tests in at least slightly different ways—with or without amnestic maneuvers (such as using scopolamine to disrupt memory function), in young or old animals, or with short- versus long-interval testing; thus, it seems unlikely that agonists or antagonists will “win” this battle, but instead there may be a role for both 5-HT\(_6\) receptor agonists and antagonists for different cognitive problems in different pathologic states.

The effects of striatal 5-HT\(_6\) receptors on autoshaping and related instrumental learning tasks have been investigated based on the early observations that 5-HT\(_6\) receptor antagonists facilitate cholinergic function (Mitchell and Neumaier, 2005); it was thought that these drugs facilitate consolidation of memory by cortical or hippocampal mechanisms. However, because 5-HT\(_6\) receptors are most heavily expressed in medium spiny neurons in striatum, these neurons might also be an important site of 5-HT\(_6\) receptor action in cognition. Because the autoshaping task used extensively by Meneses’ group was clearly sensitive to 5-HT\(_6\) receptor antagonism, this task was studied in mice with striatum-specific increased 5-HT\(_6\) receptor expression (Meneses 2015; Mitchell et al., 2007). High 5-HT\(_6\) receptor density in dorsomedial striatum impaired the acquisition but not the expression of previously learned instrumental learning. This effect is unlikely to involve memory consolidation, as the deficit is only reversed by giving a 5-HT\(_6\) receptor antagonist before but not after the training session. The operant conditioning parameters were altered to allow single-session acquisition; increased 5-HT\(_6\) receptor expression interfered with learning under these conditions (Eskenazi and Neumaier, 2011b). Furthermore, the type of learning affected by increasing the local expression of 5-HT\(_6\) receptors depended entirely on the subregion of striatum targeted (Mitchell et al., 2007; Ferguson et al., 2008; Eskenazi and Neumaier, 2011a,b). These studies do not exclude a role of 5-HT\(_6\) receptors in cholinergic interneurons in striatum,
as the focus was on the direct and indirect striatal output pathways, which have generally opposing effects on behavior (Yager et al., 2015). Increased 5-HT$_6$ receptor expression in indirect pathway medium spiny neurons is sufficient to disrupt instrumental learning in dorsomedial striatum or to facilitate learning of new behavior in overtrained animals when the dorsolateral striatum was targeted (Eskenazi et al., 2015). Indeed, 5-HT$_6$ receptors tend to oppose the effects of dopamine on striatal-based behaviors including learning, as they are expressed in both output pathways, whereas D$_1$ and D$_2$ dopamine receptors are differentially expressed in these pathways (Gerfen and Surmeier, 2011). These subtleties illustrate how 5-HT$_6$ receptors can have diverse effects on behavior depending on the cells that express these receptors. Thus, a full understanding of how 5-HT$_6$ receptors modulate learning and memory in a specific cognitive disorder may depend, at least in part, on how that disorder changes 5-HT receptor signaling.

In addition to effects on cognition, 5-HT$_6$ receptors have been proposed to promote satiety and reduce feeding and body weight (Woolley et al., 2001; Fisas et al., 2006; Voigt and Fink, 2015; Higgs et al., 2016); however, not all studies report effects of chronic 5-HT$_6$ receptor antagonism on body weight in rats or humans (Mitchell et al., 2009; Wilkinson et al., 2014; Quiedeville et al., 2015). Most of these studies used animals with normal body weight, whereas the 5-HT$_6$ receptor antagonist idalopirdine reduced feeding, peritoneal fat, and body weight when rats were fed a high-fat diet (Dudek et al., 2015). This metabolic profile might be advantageous clinically, as reducing feeding only in obese individuals would mitigate the risks of the drug when used for treating individuals with dementia. Furthermore, as several atypical antipsychotics that have high affinity for 5-HT$_6$ receptors (along with many other sites) are associated with increased appetite, weight gain, and the development of the metabolic syndrome, the preclinical and limited clinical data available suggest that 5-HT$_6$ receptor antagonism is unlikely to be responsible for these adverse effects, which are more likely due, at least in part, to 5-HT$_2C$ receptor antagonism.

The 5-HT$_6$ receptor has also been examined in relation to drug dependence given their high affinity for 5-HT$_6$ receptors (along with many other sites) are associated with increased appetite, weight gain, and the development of the metabolic syndrome, the preclinical and limited clinical data available suggest that 5-HT$_6$ receptor antagonism is unlikely to be responsible for these adverse effects, which are more likely due, at least in part, to 5-HT$_2C$ receptor antagonism. Moderate levels of 5-HT$_6$ receptors are also expressed in the DRN, and local injection of the agonist WAY-208466 decreases REM sleep and increases wakefulness (Monti et al., 2013). Single-unit recording in the anesthetized rat shows that systemic application of the full agonist WAY-181187 increases and the antagonist SB-399885 decreases firing of putative DRN 5-HT neurons; further studies are needed to establish if this involves cortical feedback loops, interneurons, and/or a direct effect on 5-HT neurons as proposed by the authors (Brouard et al., 2015). Consistent with this idea of indirect modulation of 5-HT release in the raphe, the 5-HT$_6$ receptor antagonist SB399885 inhibits spontaneous firing of GABAergic interneurons (expressing 5-HT$_6$ receptor mRNA) in the DRN in mouse brain slices (Asaoka et al., 2015). This mechanism may be
relevant to the action of antipsychotic drugs, as the effect is mirrored by olanzapine.

G. Clinical Relevance

Treatment of cognitive impairment is a leading potential application of 5-HT₆ receptor ligands in humans. There is ample evidence that manipulation of 5-HT₆ receptors can improve cognitive function based on preclinical animal models. Though schizophrenia is associated with positive and negative symptoms that can respond to available antipsychotic drugs, cognitive impairment is an often disabling and persistent problem that typically does not respond well to antipsychotic medications. Although there are some preliminary clinical trials in schizophrenia, these trials had technical limitations because the 5-HT₆ receptor ligands investigated were as add-ons to treatments already underway, and in some cases, the ongoing treatments were atypical antipsychotic drugs that possess potent antagonist properties at 5-HT₆ receptors, making it unlikely that additional benefits from 5-HT₆ receptor blockade could be readily detected. Early clinical studies with 5-HT₆ receptor ligands are reviewed elsewhere (Heal et al., 2008; Codony et al., 2011). A double-blind, placebo-controlled phase II trial for treatment of dementia associated with Alzheimer Disease (Wilkinson et al., 2014) investigated whether the addition of Lundbeck’s idalopirdine, a selective 5-HT₆ receptor antagonist, to an established treatment (donepezil, a cholinesterase inhibitor) delays the progression of dementia. This study was successful in two important ways: the addition of idalopirdine was well tolerated and led to improved cognitive function as compared with addition of placebo. However, disappointingly, idalopirdine suffered a late-stage failure in a pivotal phase III trial. Along the same lines, intepirdine, previously known as RVT-101, a 5-HT₆ receptor antagonist originally developed by GSK, displayed positive effects in patients with AD in a phase II clinical trial. A subsequent phase III trial of the drug after it was acquired by Axovant failed to achieve any of its main efficacy targets in mild to moderate AD patients. Intepirdine did not improve symptoms compared with placebo on two widely used AD symptom measures—the ADAS-Cog (the Alzheimer’s Disease Assessment Scale—Cognitive Subscale) and ADCSADL (Alzheimer’s Disease Cooperative Study Activities of Daily Living Scale) scales. Subsequently, intepirdine was evaluated in a clinical trial of patients with dementia with Lewy bodies also without success, leading to a halt in the development of this drug. Hence, although the availability of new effective treatments for dementia is a crucial health imperative, it would appear that 5-HT₆ receptor antagonism may not transform the preclinical promises into clinical practice; understanding the mechanisms that underlie this apparent failure is important and may allow opportunity for patient stratification to better identify patients that may respond favorably to 5-HT₆ receptor ligands.

Several other pathologies might also be amenable to treatment with 5-HT₆ receptor ligands, as preclinical data suggests that other progressive dementing disorders, depression, obesity, and epilepsy have potential to become indications for 5-HT₆ receptor ligands. As now appreciated, the multiple signal transduction pathways engaged by 5-HT₆ receptors and various 5-HT₆ receptor ligands with differing degrees of intrinsic efficacy; the role of agonism, partial agonism, antagonism, and inverse agonism; and biased signaling need to be considered in future development of 5-HT₆ receptor therapeutic agents. Such pharmacological complexity may also account for the apparent opposing actions of 5-HT₆ receptor ligands in preclinical behavioral models as discussed above.

XV. 5-HT₇ Receptors

A. Introduction

The 5-HT₇ receptor was the last 5-HT receptor to be discovered. In 1993, several research groups, almost at the same time, identified the 5-HT₇ receptor from the screening of cDNA libraries from various species, including humans. 5-HT₇ receptor mRNA is localized in discrete areas of the mammalian brain, including thalamus, hippocampus, and cortex, and matched with the expression of 5-HT₇ receptor protein. Based on its distribution in the CNS, the 5-HT₇ receptor is proposed to be involved in thermoregulation, circadian rhythm, learning and memory, hippocampal signaling, and sleep. Various drugs (clozapine, cyproheptadine, and amitryptiline) and pharmacological tools (5-CT and 8-OH-DPAT) binding to 5-HT₇ receptor poses the question of whether some of the effects of these compounds are mediated, at least in part, by 5-HT₇ receptor. Initially, the lack of selective 5-HT₇ receptor agonists and antagonists slowed down the elucidation of the (patho)physiologic role of the receptor, especially because of the high sequence homology in the transmembrane domains of 5-HT₇ and 5-HT₁₅ receptors. Moreover, the two receptors are distributed in the same areas of the CNS and, at the cellular level, they may have opposite effects: the 5-HT₇ receptor is positively coupled to adenyl cyclase whereas the 5-HT₁₅ receptor is negatively coupled. After 2000, various selective antagonists (SB-258719 and SB-269970) and agonists (AS-19, LP-211, and E-55888) became available, providing the scientific community with powerful pharmacological tools to get deeper insights on the role of 5-HT₇ receptors in health and disease.

B. Cloning of the Gene

The 5-HT₇ receptor is a class A GPCR, cloned by screening of cDNA libraries from mouse (Plasmat et al., 1993), rat (Lovenberg et al., 1993a, Meyerhof et al., 1993, Rua et al., 1993b; Shen et al., 1993), human (Bard et al., 1993), guinea pig (Tsou et al., 1994), Xenopus laevis (Nelson et al., 1995), pig (Bhalla et al., 2002a),...
Caenorhabditis elegans (Hobson et al., 2003), and honeybee (Schlenstedt et al., 2006).

The open reading frame of the human cDNA codes for a protein of 445 amino acids with 57% sequence identity within the transmembrane regions in comparison with the Drosophila melanogaster 5-HT_dro1 receptor and 39%–53% homology with human 5-HT₁₅, 5-HT₂₅, 5-HT₅, and 5-HT₆ receptors (Bard et al., 1993). The gene encoding the human 5-HT₇ receptor is located on chromosome 10 (q21-q24) (Gelernter et al., 1995) and contains several introns in the coding region (Ruat et al., 1993b, Erdmann et al., 1996; Heidmann et al., 1997). The guinea pig 5-HT₇ receptor has 466 amino acids, with an amino acid homology within the transmembrane regions with other 5-HT receptors of 34% and 48% (Tsou et al., 1994). The mouse brain 5-HT₇ receptor cDNA has one long open reading frame for 448 amino acids. The homology with other 5-HT receptors is low, with the best score with the 5-HT₇dro1 receptor (42%) and the next closest homology being with 5-HT₁₅, 5-HT₁₆, 5-HT₅, and 5-HT₆ receptors (Plassat et al., 1993). The 5-HT₇ receptor was cloned from rat by four open reading frames, which reported different amino acid lengths ranging from 404 to 448. Shen et al. (1993) reported the sequencing of a full-length clone isolated from a rat hippocampal cDNA library, revealing a 404-amino-acid protein with seven hydrophobic regions. Within these regions, rat 5-HT₇ receptor is 44%–50% identical with members of the 5-HT₁₅, 5-HT₅, and 5-HT₆ subfamilies, with lower homology to the 5-HT₂ receptor subtypes (37%–40%). Lovenberg et al. (1993a) isolated and determined the nucleotide sequence of a clone showing an open reading frame that encoded a 435-amino-acid protein. Within the conserved transmembrane domains of known 5-HT receptors, the rat 5-HT₇ receptor exhibited the greatest identity with the 5-HT₇dro1 receptor (54%). Instead, the entire coding sequence showed low identity (33%–39%) with 5-HT₇dro1, 5-HT₁₅, 5-HT₁₆, 5-HT₅, 5-HT₆ receptor subtypes. Ruat et al. (1993b) reported the characterization of a nucleotide sequence containing an open reading frame encoding a 448-amino-acid protein. This rat 5-HT₇ receptor showed the highest sequence homology in the hydrophobic regions with the 5-HT₇dro1 receptor (60%). In the transmembrane domains, the homologies with other 5-HT receptors were as follows: 5-HT₁₅A, 51%; 5-HT₁₅B, 55%; 5-HT₁₅D, 52%; 5-HT₁₆, 53%; 5-HT₂₅, 52%; 5-HT₂₆, 43%; 5-HT₂₇, 40%; 5-HT₂₈, 42%; 5-HT₅, 48%; and 5-HT₆, 45%. The rat 5-HT₇ receptor reported by Meyerhof et al. (1993) has an open reading frame encoding a 448-amino-acid protein, showing the highest sequence homology with 5-HT₇dro1 receptor (36% identity). The 5-HT₇ receptor from pig (Bhalla et al., 2002a) encoded an open reading frame of a 447-amino-acid protein that showed high homology (92%–96%) with the 5-HT₇ receptor protein cloned from the other species.

The presence of introns in the 5-HT₇ receptor gene results in a number of functional splice variants. Although no alternative splicing has been reported for the first intron, located in the sequence encoding the second intracellular loop of the receptor, alternative splicing at the second intron, which is located in the sequence encoding the C-terminal end, generates a number of splice variants, namely 5-HT₇(a), (b), (c), (e) receptors in rat and 5-HT₇(a), (b), (d) receptors in man (Heidmann et al., 1998; Krobert et al., 2001; Liu et al., 2001). Splice variants in dog, marmoset, and zebrafish are to be found in the www.ensembl.org database, but no related reports are available in the literature. In the mouse, two additional splice variants have been described, named 5-HT₇(b) and 5-HT₇(e) in analogy with the rat (Gellynck et al., 2008). In guinea pig and pig, the 5-HT₇ receptor is homologous to the human 5-HT₇(a) variant. Although the splice variants differ in the lengths of their carboxy terminal ends, they do not show major differences in their membrane localization nor significant differences in their respective pharmacology and signal transduction properties or functional coupling to Gₛ protein (see below).

A transcribed human 5-HT₇ receptor pseudogene has been identified by a degenerate PCR approach. The original clone (S771) has homology greater than 90% to the 5-HT₇ receptor sequence; expression of the pseudogene transcript is detected throughout the brain and peripheral tissues, in general agreement with 5-HT₇ receptor mRNA localization. However, the transcript was also detected in tissues not known to express the 5-HT₇ receptor (i.e., liver and kidney) (Olsen et al., 1999).

There is as yet no crystal structure of the 5-HT₇ receptor, but molecular modeling and site-directed mutagenesis has identified essential residues for ligand binding and activation of the human receptor (Impellizzeri et al., 2015).

C. Expression

1. mRNA. The distribution of mRNA encoding the 5-HT₇ receptor protein has been studied in several species using various techniques, as summarized in Tables 18 and 19. In all species, high levels of 5-HT₇ receptor mRNA are expressed in the CNS (hypothalamus, thalamus, and hippocampus; Fig. 26). In peripheral tissues, 5-HT₇ receptor mRNA is present in the ileum, spleen, endocrine glands, and arteries. In blood vessels and the gastrointestinal tract, 5-HT₇ receptor mRNA expression is generally present in smooth muscle cells.

The relative abundance of the three human 5-HT₇ isoforms 5-HT₇(a), (b), and (d) within brain (fetal brain, caudate, hippocampus) and peripheral tissues (uterus, trachea, small intestine, stomach, saphenous vein) has been examined (Krobert et al., 2001; Guthrie et al., 2005). These tissues expressed all three isoforms. Although the 5-HT₇(b) isoform is most prevalent, the relative amounts of 5-HT₇(a) and 5-HT₇(d) differed by...
tissue type, with the 5-HT\textsubscript{7(d)} isoform being most abundant in smooth muscle and least common in brain tissues (Krobert et al., 2001; Guthrie et al., 2005). In the rat, the 5-HT\textsubscript{7(a)} receptor isoform is most prevalent in both the CNS (cerebellum, cortex, hippocampus, hindbrain, thalamus) and the periphery (heart, kidney, spleen) (Heidmann et al., 1997, 1998).

2. Radioligand Binding. Radioligand binding assays have been used to study the distribution of 5-HT\textsubscript{7} receptor protein in native tissues. A first study to define 5-HT\textsubscript{7} receptor binding sites in rat hypothalamic membranes was performed using \(^{3}H\)5-HT in the presence of 100 nM pindolol that blocks the binding of the radioligand to 5-HT\textsubscript{7A} and 5-HT\textsubscript{7B} receptors. The pharmacology of the identified binding sites correlated well with that of rat recombinant 5-HT\textsubscript{7} receptors (Sleight et al., 1995). A subsequent study showed that 100 nM of pindolol does not completely mask 5-HT\textsubscript{7A} and 5-HT\textsubscript{7B} receptors and that the population of pindolol-insensitive receptors labeled by \(^{3}H\)5-HT in rat hypothalamus appeared to be heterogeneous (Gobbi et al., 1996). Also, \(^{3}H\)SB-269970 failed to define a homogeneous population of 5-HT\textsubscript{7} binding sites in rat hypothalamus homogenates even in the presence of various masking agents (pindolol, sumatriptan, DOI) (Stowe and Barnes, 1998). \(^{3}H\)5-CT has been used to label 5-HT\textsubscript{7} receptors in guinea pig brain cortex membranes in the presence of sumatriptan and cyanopindolol (To et al., 1995).

The pharmacology of these binding sites was well correlated to that of the guinea pig cloned 5-HT\textsubscript{7} receptor. Because affinity values of reference compounds were consistently lower in the binding assay performed in native tissue, the authors hypothesized that sumatriptan and cyanopindolol were likely to occupy, at least in part, the 5-HT\textsubscript{7} receptors. \(^{3}H\) Mesulergine labels 5-HT\textsubscript{7} receptors in guinea pig ileal longitudinal muscle in the presence of several masking agents (cinanserin, prazosin, raclopride, RS 102221, and yohimbine). However, under these same conditions, no binding was detected in the rat jejunum (Hemedah et al., 1999).

\[^{3}H\]SB-269970 is the first selective radioligand to label with high affinity 5-HT\textsubscript{7} receptors in rat, mouse, guinea pig, pig, marmoset, and human brain homogenates. Guinea pig brain homogenate displays markedly higher 5-HT\textsubscript{7} receptor expression in comparison with
receotor double-knockout mice by using $[3H]5$-CT ligands. $[3H]5$-CT has been used in the presence of 2002).

In guinea pig and rat brain, the distribution of various masking agents (To et al., 1995; Waeber and Moskowitz, 1995a; Gustafson et al., 1996; Mengod et al., 1996). In guinea pig and rat brain, the distribution of the 5-HT7 receptor binding sites was largely consistent with that reported for 5-HT7 receptor mRNA. The affinity for 5-HT7 receptor sufficient to compete with $[3H]5$-CT to some degree for the 5-HT7 receptor. In a subsequent study, the localization of 5-HT7 receptor was studied in 5-HT1A receptor knockout and 5-HT1A/B receptor double-knockout mice by using $[3H]5$-CT (5-HT1A, 5-HT1B, and 5-HT7 agonist) and $[3H]8$-OH-DPAT (5-HT1A and 5-HT2 agonist) (Bonaventure et al., 2002b). $[3H]8$-OH-DPAT was better than $[3H]5$-CT for measuring 5-HT7 receptor binding sites, even if it displays lower 5-HT7 receptor affinity than $[3H]5$-CT in tissue homogenates. The anatomic distribution of the $[3H]8$-OH-DPAT–labeled sites observed in these knock-out mice was in agreement with the distribution of 5-HT7 receptor mRNA and immunoreactivity reported previously. Within the hippocampal formation, strong labeling was found in the CA3 region, whereas low binding was found in CA1 region. 5-HT7 receptors were also found within the dorsal raphe and the hypothalamus, including the suprachiasmatic nucleus.

The selective high-affinity radioligand $[3H]$SB-269970 was used to localize the 5-HT7 receptors in human brain (Varnäs et al., 2004; Fig. 27). The distribution of the 5-HT7 receptors was largely similar to that shown by the autoradiographic studies in rat (Gustafson et al., 1996), guinea pig (To et al., 1995), and mouse (Martin-Cora and Pazos, 2004). High receptor density was detected in thalamus, hypothalamus, and hippocampus. However, unlike in rodents, human brain 5-HT7 receptors were also found in high levels in caudate nucleus, putamen, and substantia nigra (Varnäs et al., 2004). Eventually, using $[3H]$SB-269970, Horisawa et al. (2013) reported that 5-HT7 receptor distribution in rat brain was similar

<table>
<thead>
<tr>
<th>Species</th>
<th>Technique</th>
<th>Localization (Relative Abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>In situ hybridization</td>
<td>Hippocampus (+ + +), periventricular thalamus (+ + +), superficial cortex (+ + +), cerebellar granule cells (+ + +)</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>In situ hybridization</td>
<td>Medial thalamic nucleus (+ + +), hippocampal formation (+ + +), superficial layer cortex (+ + +), medial geniculate nucleus (+ + +), amygdala (+ + +), hypothalamus (+ + +), midbrain (+ + +), hindbrain (+ + +)</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Northern Blot</td>
<td>Parietal cortex (+ + +), hippocampus (+ + +), frontal cortex (+ + +), cerebellum (+ + +), ileum (+ + +), spleen (+ + +)</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Northern Blot</td>
<td>Thalamus (+ + +), brainstem (+ + +), hypothalamus (+ + +), substantia nigra (+ + +), olfactory bulb (+ + +), olfactory tubercle (+ + +) (not detected in peripheral organs)</td>
</tr>
<tr>
<td>Hamster</td>
<td>In situ hybridization</td>
<td>Artery smooth cells (+ + +), pulmonary artery smooth cells (+ + +) (not detected in coronary artery, pulmonary artery, aortic endothelial)</td>
</tr>
<tr>
<td>Human</td>
<td>RT-PCR</td>
<td>Brain (+ + +), kidney (+ + +), liver (+ + +), pancreas (+ + +), spleen (+ + +), coronary artery (+ + +), stomach (+ + +), descending colon (+ + +), ileum (+ + +)</td>
</tr>
<tr>
<td>Human</td>
<td>RT-PCR</td>
<td>Trigeminal ganglia</td>
</tr>
<tr>
<td>Human</td>
<td>RT-PCR</td>
<td>Colonic circular muscle</td>
</tr>
<tr>
<td>Mouse</td>
<td>Northern Blot</td>
<td>Forebrain (+ + +), brain stem (+ + +), cerebellum (+ + +), colliculi (+ + +), intestine (+ + +), heart (+ + +), not detected in spleen, kidney, lung, liver</td>
</tr>
<tr>
<td>Mouse</td>
<td>RT-PCR</td>
<td>Myometrium</td>
</tr>
<tr>
<td>Pig</td>
<td>RT-PCR</td>
<td>Pulmonary artery (+ + +), coronary artery (+ + +), cerebral artery (+ + +), cerebral vein (+ + +)</td>
</tr>
<tr>
<td>Pig</td>
<td>RT-PCR</td>
<td>Brain cortex, trigeminal ganglion, cerebellum, pulmonary artery, coronary artery, superior vena cava, saphenous vein, not detected in heart</td>
</tr>
</tbody>
</table>

$^a$Tsou et al. (1994).
$^b$To et al. (1995).
$^c$Mengod et al. (1996).
$^d$Ruat et al. (1995).
$^e$Duncan and Franklin (2007).
$^f$Ullmer et al. (1995).
$^g$Pierce et al. (1997).
$^h$Graveleau et al. (2000).
$^i$Bard et al. (1993).
$^j$Terrin et al. (2001).
$^k$Irving et al. (2007).
$^l$Plassat et al. (1993).
$^m$Yang et al. (2014).
$^n$Kitazawa et al. (2001).
$^o$Bhalla et al. (2002).
to that in human (Varnäs et al., 2004), with small differences in regions with an intermediate to low density of 5-HT7 receptors.

3. Immunoreactivity. Rabbit polyclonal antibodies raised against amino acid sequence 8–23 of the rat 5-HT7 receptor are commercially available. Immunocytochemistry has been used to localize the distribution of 5-HT7 receptors in rat forebrain (Neumaier et al., 2001), which were detected in the cortex, hippocampal formation, tenia tecta, thalamus, and hypothalamus, in agreement with the localization reported for the 5-HT7 receptor mRNA. In particular, in the suprachiasmatic nucleus, both cell bodies and proximal fibers were strongly stained, suggesting a somatodendritic subcellular distribution of the receptor. The presence of 5-HT7 receptors was also reported in both pre- and postsynaptic GABA, vasoactive intestinal polypeptide, and vasopressin processes in the suprachiasmatic nucleus in mouse (Belenky and Pickard, 2001). 5-HT7 receptors are detected in Purkinje cells of rat cerebellum but not in the cerebellar cortex or in deep nuclei (Geurts et al., 2002).

5-HT7 receptor immunolabeling was detected mainly in the two superficial laminae of the dorsal horn and in small- and medium-sized dorsal root ganglion cells in the rat spinal cord (Doly et al., 2005), consistent with a predominant role of 5-HT7 receptor in nociception. In addition, moderate labeling was found in the lumbar dorsolateral nucleus (Onuf’s nucleus), suggesting an involvement of the receptor in the control of pelvic floor muscles (Doly et al., 2005). Electron microscopic examination of the dorsal horn shows three main localizations of the receptor: 1) a postsynaptic localization on peptidergic cell bodies in laminae I–III and in numerous dendrites, 2) a presynaptic localization on unmyelinated and thin myelinated peptidergic fibers,
and 3) in lamina I and II in astrocytes (Doly et al., 2005). 5-HT7 receptors have been localized to numerous myenteric neurons, some submucosal neurons, and a few smooth muscle cells of guinea pig ileum (Tonini et al., 2005) and in human corneal epithelium and endothelium (Grueb et al., 2006). An upregulated expression of 5-HT7 receptors was detected during maturation of bone marrow–derived dendritic cells, suggesting a critical role of 5-HT7 receptors in the regulation of immune cell polarization and, thus, in the peripheral inflammatory processes (Holst et al., 2015).

D. Pharmacology

1. Agonists. Various selective 5-HT7 receptor agonists have been identified (Table 20). The aminotetraline derivative AS-19 has a $K_d$ of 0.6 nM at human cloned 5-HT7 receptors, with high selectivity over all the other 5-HT receptor subtypes (>100-fold) except 5-HT1D receptors (11-fold). However, AS-19 behaves as a potent partial 5-HT7 receptor agonist ($EC_{50} = 9$ nM) with a maximal effect reaching 77% of that of 5-HT in a functional assay measuring cAMP stimulation in HEK293F cells overexpressing human 5-HT7 receptors (Brenchat et al., 2009), which can complicate interpretation of arising data using tissue and in vivo preparations.

E-55888 is another potent and selective 5-HT7 receptor agonist with high efficacy, displaying high affinity for 5-HT7 receptors ($K_d = 2.5$ nM), low affinity for 5-HT1A ($K_d = 700$ nM), and no significant affinity for the other 5-HT receptors. E-55888 behaves as a full agonist ($EC_{50} = 16$ nM) and increases cAMP levels in HEK293F-expressing human 5-HT7 receptors (Brenchat et al., 2009). Another selective 5-HT7 receptor agonist is LP-211, with high but different affinities for rat and human recombinant 5-HT7 receptors ($K_d = 0.58$ and 15.0 nM, respectively) and moderate to low affinity for other 5-HT receptors, including 5-HT1A (Leopoldo et al., 2008; Hedlund et al., 2010). LP-211 induces 5-HT7–mediated relaxation of substance P-stimulated guinea pig ileum contracture (82% of the maximal effect elicited by 5-CT). However, in HEK293 cells stably expressing human 5-HT7 receptor (Atanes et al., 2013), LP-211 displayed insurmountable antagonism of 5-CT–stimulated cAMP signaling. These results were unexpected, as LP-211 have been extensively characterized as a 5-HT7 receptor agonist in several ex vivo and in vivo studies [for review, see Di Pilato et al. (2014)]. The authors suggested that the inhibitory effects of LP-211 are due to an irreversible stabilization of an inactive conformational state of the receptor that is tightly associated with Gs protein, independent of agonist binding (Bruheim et al., 2003; Andressen et al., 2006).

2. Antagonists. Various selective 5-HT7 receptor antagonists have been identified (Table 20). The first was SB-258719, which displays useful 5-HT7 receptor affinity ($K_i = 31.6$ nM) with 100-fold selectivity against all the other 5-HT receptors (Forbes et al., 1998). In HEK293 cells stably expressing the human 5-HT7 receptor, SB-258719 did not stimulate basal adenyl cyclase activity, suggesting a lack of agonist activity, but produced a surmountable antagonism of the 5-CT response with a $pK_B$ of 7.0. Further chemical optimization of SB-258719 led to SB-269970, which is the
SB-269970 displays high affinity at both human cloned 5-HT7 receptors (pK$_i$ 5.89) and in guinea pig cortex (pK$_i$ 5.83). The compound is at least 100-fold selective against a wide range of receptors, except for the human 5-HT5A receptor (about 50-fold). SB-269970 displays potent antagonism in both HEK293 cells stably expressing the 5-HT7 receptor (pA$_2$ 5.85) and in the guinea pig hippocampal membranes (pK$_B$ 5.83). SB-269970 also produces a small inhibition of basal adenylyl cyclase activity in the absence of added 5-CT, consistent with inverse agonism (Hagan et al., 2000). Subsequent studies led to the identification of the antagonist SB-656104, which is as potent as SB-269970 (K$_i$ 2 nM; pA$_2$ 5.84) but displays only 10-fold selectivity over the 5-HT1D receptor. Both SB-269970 and SB-656104 are brain-penetrant, and the latter is more stable metabolically (Thomas et al., 2003).

### 3. Allosteric Modulators

Oleamide, an amidated fatty acid, is an allosteric modulator of the 5-HT7 receptor (Thomas et al., 1997a, 1999b; Hedlund et al., 1999; Alberts et al., 2001). In cells expressing the 5-HT7 receptor, oleamide increases cAMP accumulation in a concentration-dependent manner but with a lower efficacy than 5-HT. In the presence of 5-HT, oleamide displayed the opposite effect on cAMP, causing insurmountable antagonism of 5-HT, suggesting that oleamide acts at an allosteric site on the 5-HT7 receptor. In receptor-binding studies, oleamide modulated the binding properties of 5-HT7 receptors expressed in vitro (Hedlund et al., 1999). In addition, the binding of both agonists and antagonists at human recombinant 5-HT7 receptors stably expressed in HEK293 cells was allosterically inhibited by zinc (Satała et al., 2018).

### E. Signaling

The 5-HT7 receptor is positively coupled to adenylyl cyclase through activation of Gs, resulting in intracellular increase in cAMP (Bard et al., 1993; Lovenberg et al., 1993a; Ruat et al., 1993b). The human, rat, and mouse splice variants all activate adenylyl cyclase (Heidmann et al., 1998; Krobert et al., 2001; Krobert and Levy, 2002) and do not show evident differences in their respective pharmacology and signal transduction properties or functional coupling to Gs protein (Heidmann et al., 1998; Krobert et al., 2001). The only reported functional difference is that the human 5-HT7(d) receptor displays a differential pattern of receptor internalization (Guthrie et al., 2005). Furthermore, variation in the length of the C-terminal tails and in the number of consensus sites for phosphorylation by PKA and PKC raises the possibility that the splice variants can display different desensitization or trafficking properties (Heidmann et al., 1997). Moreover, the human 5-HT7(b) and the rat 5-HT7(b) and 5-HT7(c) receptor splice variants contain recognition sites for PDZ domain-containing proteins (Hamblin et al., 1998), suggesting that these splice variants may be targeted to active zones within cells and may couple to alternative signaling pathways.

Stimulation of the human 5-HT7(a) receptor in HEK293 cells coexpressed with various adenylyl cyclase isoforms revealed that this receptor not only activates the Gs-sensitive AC5 isoform but also the Gs-insensitive...
and Ca\(^{2+}\)/calmodulin-stimulated AC1 and AC8 isoforms (Nielsen et al., 1996; Xia and Storm, 1997; Baker et al., 1998).

5-HT\(_7\) receptor expression density does not appear to influence agonist or partial agonist potency (or efficacy), suggesting the absence of receptor reserve effects (Bruheim et al., 2003; Andressen et al., 2006) and implying independence from receptor-Gs stoichiometry. This may also suggest the 5-HT\(_7\) receptor is preassociated with Gs (Adham et al., 1998; Alberts et al., 2001; Krobert et al., 2001), which was supported by a more recent study investigating receptor-immobilized fluorescence recovery after photobleaching and fluorescence resonance energy transfer (Andressen et al., 2018) to compare the Gs coupling of the 5-HT\(_7\) and 5-HT\(_4\) receptors.

Prolonged stimulation by 5-HT induces both homodimer and heterologous desensitization of Gs signaling in HEK293 cells. A similar effect is seen on prolonged incubation with the inverse agonists SB-269970 and methiothepin, implying that desensitization is independent of Gs activation (Krobert et al., 2006).

Clozapine and olanzapine, which are 5-HT\(_7\) receptor inverse agonists [see Krobert and Levy (2002)], display functional selectivity at the human 5-HT\(_7\) receptor (i.e., different ligands at the same receptor elicit different functional effects) (Andressen et al., 2015). Thus, clozapine and olanzapine downregulate 5-HT\(_7\) receptors expressed in HEK293 cells, an effect normally evident for agonists but not for antagonists or inverse agonists. This inverse agonist–mediated receptor downregulation may be mediated by interaction with the G protein–associated sorting protein GASP-1 (Manfra et al., 2015).

In rat hippocampal neurons, 5-HT stimulation leads to the activation of serine/threonine kinases ERK1 and ERK2, and most of this activity is mediated by 5-HT\(_7\) receptors (Errico et al., 2001). A PKA-dependent pathway for 5-HT\(_7\) receptor–mediated ERK1 and ERK2 activation has been proposed (Norum et al., 2003, 2005). 5-HT\(_7\) receptor activation increases intracellular cAMP levels to activate PKA, which phosphorylates the guanine nucleotide exchange factor (GEF) Ras-GRF1 that controls the activity of Ras, an upstream activator of c-Raf (or Raf-1; a member of the Raf kinase family; Norum et al., 2003). Consequently, c-Raf phosphorylates and activates various kinases, including MEK1 and, further downstream, ERK1/2. An alternative pathway could involve the activation of GEF proteins Epac1 and Epac2 by cAMP, which in turn activate the Rap1 proteins through GDP-GTP exchange. GTP-bound Rap1 proteins can bind and activate different members of the Raf kinase family (i.e., B-Raf, A-Raf, and c-Raf), leading to ERK1/2 activation (Norum et al., 2005). The contribution of these GEFs in the ERK1/2 activation pathway mediated by 5-HT\(_7\) receptor has been demonstrated in the pheochromocytoma PC12 cells stably overexpressing 5-HT\(_7\) receptor and in rat hippocampal neurons (Lin et al., 2003; Johnson-Farley et al., 2005). Moreover, ERK1/2 seems to be required for normal neuronal function, such as neurotrophin-stimulated neuronal differentiation and neuroprotection as well as regulation of neurite outgrowth and formation of neuronal networks (Hetman et al., 1999; Speranza et al., 2013, 2015).

Stimulation of 5-HT\(_7\) receptor expressed in HEK293 cells increases intracellular Ca\(^{2+}\) levels (Baker et al., 1998). However, the exact mechanism for this increase is not known. In rat adrenal glomerulosa cells, this 5-HT\(_7\) receptor–mediated increase of intracellular Ca\(^{2+}\) levels is mediated by T-type Ca\(^{2+}\) channels in a cAMP-PKA-dependent manner (Lenglet et al., 2002a,b), which is likely to contribute to ERK1/2 activation (Lin et al., 2003; Norum et al., 2003; Johnson-Farley et al., 2005).

The 5-HT\(_7\) receptor interacts with the G\(_{12}\) member of the G protein family (Kvachnina et al., 2005; Kobe et al., 2012), which can activate multiple signaling pathways. The prominent downstream effectors are members of the Rho family of small GTPases (Rho, Rac, and Cdc42). 5-HT\(_7\) receptor–mediated stimulation of G\(_{12}\) protein results in Rho-dependent activation of the transcription factor serum response factor, which binds to the serum response element (SRE). The stimulation of 5-HT\(_7\) receptor increases SRE-driven gene expression even in the presence of a PKA inhibitor or pertussis toxin, suggesting that the receptor-mediated SRE activation is not PKA-dependent (Kvachnina et al., 2005). Stimulation of 5-HT\(_7\) receptor/G\(_{12}\) signaling pathway selectively activates both RhoA and Cdc42 (Kvachnina et al., 2005), suggesting a cross talk between Cdc42 and RhoA pathways. The 5-HT\(_7\) receptor/G\(_{12}\) signaling pathway in cultured hippocampal neurons promotes formation of dendritic spines and accelerates synaptogenesis, leading to enhanced spontaneous synaptic activity (Kobe et al., 2012). A morphogenic action of the 5-HT\(_7\) receptor is confirmed in neuronal primary cultures from the cortex, hippocampus, and striatal complex of embryonic rat or mouse brain (Speranza et al., 2013, 2015) and postnatal cortical and striatal neurons (Speranza et al., 2017). The involvement of mTOR, Cdc42, Cdk5, and ERK in this process suggests these proteins to be downstream targets of G\(_{12}\) (Speranza et al., 2013, 2015). In the hippocampus, the 5-HT\(_7\) receptor/G\(_{12}\) signaling pathway undergoes strong developmental regulation. In organotypic hippocampal cultures from juvenile mice, G\(_{12}\) signaling potentiates formation of dendritic spines, increases the basal neuronal excitability, and modulates synaptic plasticity. In contrast, in preparations from older mice, 5-HT\(_7\) receptor stimulation had no effect on neuronal morphology, synaptogenesis, and synaptic plasticity (Kobe et al., 2012). Consistently, the expression level of both 5-HT\(_7\) receptor and G\(_{12}\) proteins in the hippocampus...
progressively decreases during postnatal development (Kobe et al., 2012).

F. Post-translational Modifications

1. Regulatory Mechanisms. The 5-HT\textsubscript{7} receptor appears to undergo N-glycosylation and palmitoylation. The receptor is N-glycosylated at the asparagine residues N5 and N66 (Gellynck et al., 2012), but this does not appear to influence agonist binding, potency, or efficacy. Furthermore, immunocytochemical studies revealed the presence of the N-glycosylation mutants at the cell surface.

The mouse 5-HT\textsubscript{7} receptor expressed in Sf9 insect cells undergoes dynamic palmitoylation in an agonist-dependent manner (Kvachnina et al., 2009). Mutation analysis shows that cysteines located in the C-terminal receptor domain at positions 404, 438, and 441 represent the main potential palmitoylation sites. Palmitoylation-deficient mutants reveal that agonist-induced activation of Gs and G12 proteins is unaffected. Instead, mutation of the Cys404 alone or in combination with Cys438/Cys441 increases G\textsubscript{12}-mediated constitutive activity of the 5-HT\textsubscript{7} receptor (agonist-independent), whereas the activation of G12 protein is not affected. Thus, palmitoylation of 5-HT\textsubscript{7} receptors might be directly involved in the isomerization of the receptor from the inactive to the active form in the absence of agonists. Considering that the 5-HT\textsubscript{7} receptor is coupled to both Gs and G12 proteins, dynamic palmitoylation may represent a molecular mechanism responsible for selective Gs- or G12-mediated signaling.

2. Interacting Proteins. Apart from G proteins, only a few 5-HT\textsubscript{7} receptor–interacting proteins have been described. Interacting proteins mostly act as adaptor or scaffolding proteins and help in trafficking of the receptor, not only to the plasma membrane but also in receptor internalization, recycling, and degradation. Among the many regulatory proteins that are involved in GPCR desensitization and downregulation, GPCR-associated sorting proteins (GASPs) participate in the sorting of several receptors toward the degradation pathway (Bornert et al., 2013). GASP-1 and GASP-2 interact with several GPCRs, including 5-HT\textsubscript{7} receptors, through interaction of C-terminal tails containing two critical amino acid residues within the sequence that corresponds to helix 8 in the three-dimensional structure of rhodopsin (Simonin et al., 2004).

PLAC-24 (protein that localizes at cell-cell contacts), also known as the eukaryotic initiation of translation factor 3, subunit k, is another intracellular interaction partner for all three splice variants of the human 5-HT\textsubscript{7} receptor (De Martelaere et al., 2007). PLAC-24 might be involved in the transport of newly synthesized 5-HT\textsubscript{7} receptors toward the plasma membrane, as this transport is hampered by the overexpression of certain domain constructs of PLAC-24. PLAC-24 has been proposed to be additionally involved in the stabilization of the receptor at the cell surface by anchoring it at the actin cytoskeleton, as PLAC-24 might be part of a multishubunit complex that links the actin and microtubule cytoskeleton and causes an increase in the expression of the receptor.

Periplakin (an actin- and intermediate filament–binding protein) and the neurite-outgrowth promoting protein, neurochondrin, strongly interact with the C-terminal tail of 5-HT\textsubscript{7} receptor. The functional consequences of these specific interactions are not yet known (Ward et al., 2009).

RhoBTB3, a member of the Ras superfamily of small GTPases, is expressed in several brain regions where the 5-HT\textsubscript{7} receptor is also localized, including hippocampus and nucleus accumbens. The physical interaction between the 5-HT\textsubscript{7\textalpha} receptor and RhoBTB3 has been demonstrated through yeast two hybrid, GST pull-down, and coimmunoprecipitation assays (Matthys et al., 2012). Not only the C-terminal tail but also the third intracellular loop of the 5-HT\textsubscript{7\textalpha} receptor seem to be involved in the interaction. The 5-HT\textsubscript{7\textalpha} receptor may be targeted for proteasomal degradation following endocytosis at the plasma membrane, and RhoBTB3 appears to strongly inhibit proteasomal degradation of the receptor.

S100B is a Ca\textsuperscript{2+}-regulatory protein that controls Ca\textsuperscript{2+} homeostasis in various cell types, including astrocytes. S100B also regulates the activity of adenyl cyclase in preparations from rat brain and skeletal muscle (Donato et al., 2009). S100B physically interacts with 5-HT\textsubscript{7} receptors; it negatively regulates cAMP accumulation in 5-HT\textsubscript{7}–transfected HeLa cells and mouse cortical astrocytes. Overexpression of S100B causes brain region–specific dysregulation of cAMP pathways in vivo that may relate to depressive-like behavior, which can be normalized by 5-HT\textsubscript{7} receptor blockade by SB-269970 (Stroth and Svenningsson, 2015).

For further discussion of interacting proteins, see XVII. B. A Survey of 5-HT Receptor GIPs.

3. Homo- and Heteromeric Receptor Associations. GPCRs can form oligomers, and it is now widely accepted that homo- and heterodimerization provides an additional mechanism for regulating cellular processes through the fine tuning of receptor-mediated signaling (Devi, 2001; Bulenger et al., 2005). The 5-HT\textsubscript{7} receptor forms homodimers in both intact HEK293 cells and neuroblastoma N1E-115 cells transfected with 5-HT\textsubscript{7} receptor and rat cortical astrocytes (Teitler et al., 2010; Smith et al., 2011; Renner et al., 2012; Teitler and Klein, 2012).

Heterodimerization between 5-HT\textsubscript{1\textalpha} and 5-HT\textsubscript{7} receptors has been demonstrated by coimmunoprecipitation and by fluorescence resonance energy transfer approaches (Renner et al., 2012); the coimmunoprecipitation studies in mouse brain provide direct evidence that 5-HT\textsubscript{1\textalpha} and 5-HT\textsubscript{7} receptors can form heterodimers in vivo. Such heterodimerization alters the signaling properties of the 5-HT\textsubscript{1\textalpha} receptor by attenuating the ability of 5-HT\textsubscript{1\textalpha}...
receptor to activate G protein, in contrast to 5-HT receptor-mediated activation of G protein, which is not affected. In addition, heterodimerization reduces the ability of 5-HT1A receptors to activate GIRK channels, an effect mediated through the Gβγ subunits of G proteins (Reuveny et al., 1994; Kofuji et al., 1995). The inhibitory effect of heterodimerization on GIRK currents is also evident in mouse hippocampal neurons, suggesting a physiologic relevance in vivo.

Heterodimerization between the 5-HT1A and 5-HT7 receptors appears to promote agonist-mediated internalization of the 5-HT1A receptor (5-HT1A receptors expressed alone are relatively resistant to the agonist-induced internalization). The pharmacological blockade of 5-HT7 receptors, but not of 5-HT1A receptors, abolishes internalization of both 5-HT7 homo- and heterodimers, suggesting that 5-HT7 receptor-mediated signaling is an initial step responsible for 5-HT1A receptor cotranslocation. Once internalized, 5-HT1A receptors can activate G protein–independent signaling pathways such as a β-arrestin–mediated coupling to MAPK. Thus, depending on the relative amount of 5-HT1A receptors participating in dimers, stimulation by 5-HT can activate distinct ERK-mediated pathways (i.e., G protein–dependent or β-arrestin–dependent), further implicating the physiologic relevance of heterodimerization.

G. Function

Hedlund et al. (2003) first reported on the generation of 5-HT7 KO mice by targeted disruption within exon II of the 5-HT7 receptor gene, thus inactivating all known splice variants of the receptor. 5-HT7 KO mice (Roberts et al., 2004a; Guscott et al., 2005) grow and reproduce normally, suggesting that the receptor does not play an essential role during development. These mice also have normal body weight and basal rectal temperature and appear to be in good health (Guscott et al., 2005). No differences are detected between 5-HT7 KO mice and the wild-type littermates in general motor ability, visual acuity, pain sensitivity, anxiety-like behavior, or the capacity to show freezing behavior in the habituation, conditioning, or cued components of the cued and contextual conditioning procedure (Roberts et al., 2004a). Also, in the prepulse inhibition paradigm, no difference is observed in the 5-HT7 receptor KO mice (Guscott et al., 2005; Semenova et al., 2008).

1. Thermoregulation. 5-HT7 agonists induce a considerable hypothermic response in vivo in various species (Yamada et al., 1988; Won and Lin, 1988; Sugimoto et al., 1991; Guscott et al., 2003; Hedlund et al., 2003, 2004; Naumenko et al., 2011). Initially, the 5-HT1A receptor was thought responsible, largely on the basis of data obtained using the 5-HT1A receptor agonist 8-OH-DPAT (Hjorth, 1985) before this drug was also recognized as a 5-HT7 receptor agonist (Guscott et al., 2003; Hedlund et al., 2004). It appears the 5-HT7 receptor is more important at lower 5-HT concentrations, playing a role in the fine tuning of temperature homeostasis, whereas the 5-HT1A receptor is activated at higher 5-HT concentrations, possibly as a defense against hyperthermia (Hedlund et al., 2004), such that both 5-HT1A and 5-HT7 receptors are important (Brenchat et al., 2012a).

2. Learning and Memory. 5-HT7 receptor KO mice under certain types of examination can display specific impairments in contextual learning. Two forms of place learning, spatial (Barnes maze) and contextual (fear conditioning), in addition to three hippocampus-dependent learning tests have been studied (motor, cued conditioning, and operant conditioning) to demonstrate effects of 5-HT7 receptor KO mice in contextual fear conditioning, whereas there was no evident effect in the other learning tests. Of potential relevance, electrophysiological studies on hippocampal slices from 5-HT7 receptor KO mice demonstrate deficits in long-term potentiation (Roberts et al., 2004).

Further behavioral studies demonstrate hippocampus-associated spatial memory deficits in 5-HT7 receptor KO mice (impairments in memory compilation required for resolving spatial tasks), which result in impaired hippocampus-dependent allocentric memory (Sarkisyan and Hedlund, 2009), whereas no effect was evident in the novel object recognition test. Egocentric spatial memory, which is striatum-dependent, remains intact. On the other hand, 5-HT7 receptor KO mice do not exhibit learning impairments and/or dysfunctions in short-term memory if the environment remains static, such as in the Barnes maze test. In the same test, 5-HT7 receptor KO mice have no impairment in long-term memory or memory consolidation.

3. Antipsychotic Potential. 5-HT7 receptor antagonists have been evaluated in animal models used to test antipsychotic-like activity. The selective 5-HT7 receptor antagonist SB-258741 reverses hyperactivity induced by PCP in rats (Pouzet et al., 2002). SB-269970 partially but significantly blocks hyperactivity induced by ketamine in mice (Galici et al., 2008). Furthermore, 5-HT7 receptor KO mice display less pronounced deficits in the PCP-induced prepulse inhibition compared with WT mice (Semenova et al., 2008), although this effect was not replicated by SB-269970 treatment (Semenova et al., 2008). Collectively, the available data suggest that the antipsychotic-like activity elicited by selective 5-HT7 receptor blockade is weaker than that obtained with clinically proven antipsychotic drugs (Thomas and Hagan, 2004).

Current antipsychotic drugs are not very effective to reverse the cognitive deficits associated with schizophrenia. Cognitive impairments induced by subchronic PCP administration in rats are believed to mimic cognitive deficits in schizophrenia (Javitt and Zukin, 1991; Jentsch and Roth, 1999). PCP selectively impairs performance in reversal learning test (Abdul-Monim et al., 2006, 2007; McLean et al., 2009a), attentional set-shifting test (McLean et al., 2008), and novel object
recognition test (a paradigm for studying visual episodic memory) (Grayson et al., 2007). The acute administration of SB-269970 reverses subchronic PCP-induced deficits in a reversal learning task in rats (McLean et al., 2009b) and in the novel object recognition test in rats (Horiguchi et al., 2011). Pretreatment with SB-269970 or lurasidone reverses the subchronic PCP-induced deficit in reversal learning in mice (Rajagopal et al., 2016). This effect was not elicited by the agonist AS-19, confirming that antagonism, but not agonism, at 5-HT7 receptors restores function in principal cortical neurons impaired by NMDA receptor blockade.

Cognitive deficits in mice induced by dizocilpine are also used to model impaired working memory in schizophrenia. SB-269970 reverses dizocilpine-induced cognitive deficits in a translational behavioral model of working memory, the delayed nonmatching to position task. At a neurochemical level, SB-269970 normalizes the dizocilpine-induced glutamate efflux but not dizocilpine-induced dopamine extracellular levels in the cortex of freely moving rats (Bonaventure et al., 2011). SB-269970 also reverses dizocilpine-induced memory deficits in an autoshaping Pavlovian instrumental learning task in rats (Meneses, 2004). Moreover, 5-HT7 receptor blockade by another antagonist, SB-656104, reverses dizocilpine-induced learning and memory impairments in the passive avoidance and Morris water maze tests in rats (Horisawa et al., 2011).

Ketamine-based animal models represent a valuable tool in preclinical research because ketamine is commonly used in the clinic to model the transient neurocognitive impairments in healthy volunteers (Krystal et al., 1994). Acute administration of SB-269970 in rats ameliorates ketamine-induced cognitive deficits in the attentional set-shifting task (a measure of cognitive flexibility) and the novel object recognition test (Nikiforuk et al., 2013). SB-269970 reverses memory deficits in an autoshaping Pavlovian instrumental learning task in rats after an intraprefrontal infusion of ketamine (Liy-Salmeron and Meneses, 2008).

Experimental evidences suggest a role of 5-HT7 receptor blockade in the procognitive actions of the atypical antipsychotics amisulpride and lurasidone. Both drugs ameliorate the PCP-induced deficits in the novel object recognition task in rats (Horiguchi et al., 2011). This effect is reversed by coadministration of the selective 5-HT7 agonist AS-19. Lurasidone attenuates the dizocilpine-induced deficits in the passive avoidance test in rats. Also, in this case, AS-19 abolishes the effect of lurasidone (Horisawa et al., 2013).

The pharmacological blockade of 5-HT7 receptors may also have therapeutic implications for the treatment of negative symptoms in schizophrenia because SB-269970 ameliorates ketamine-induced social withdrawal in rats (Nikiforuk and Popik, 2013). Consistently, acute administration of amisulpride reverses ketamine-induced social withdrawal. The prosocial efficacy of amisulpride is abolished by the agonist AS-19. Finally, coadministration of subactive doses of SB-269970 and amisulpride results in prosocial effects in rats (Holuj et al., 2015).

4. Antidepressant-Like Behavior. 5-HT7 receptor KO mice have an antidepressant-like behavior, with reduced immobility in commonly used preclinical animal models of depression, such as the tail suspension test (TST) and the forced swim test (Guscott et al., 2005; Hedlund et al., 2005; Sarkisyan et al., 2010). The decreased immobility in both models is most likely not due to a general increase in motor activity, as no genotype difference in locomotor activity or motor learning were evident between 5-HT7 receptor KO and WT mice (Roberts et al., 2004).

Because 5-HT7 receptor KO mice display an antidepressant-like behavior, the potential role of the 5-HT7 receptor was also investigated in OCD models: it is recognized OCD involves the 5-HT system and patients may benefit from antidepressant therapy. Thus, 5-HT7 receptor KO mice have been tested in three models believed to mimic some of the stereotypic aspects of OCD (Hedlund and Sutcliffe, 2007). Inactivation of the 5-HT7 receptor leads to decreased marble burying, a model linked to OCD and anxiety. However, there is no difference between 5-HT7 receptor KO and WT mice in the two other models (i.e., head dipping and plastic-mesh screen chewing models). Thus, a possible role of the receptor in OCD remains an open question.

5. Sleep. Overall, a normal sleep pattern is observed in 5-HT7 receptor KO mice, although during rest, 5-HT7 receptor KO mice spend less time in REM sleep (with less frequent but longer REM episodes) compared with WT mice (Hedlund et al., 2005). There is no difference between the genotypes in time spent awake or in slow wave sleep, and the frequency of slow wave sleep episodes is not altered. These sleep patterns of 5-HT7 receptor KO mice are in agreement with the antidepressant-like profile observed in this genotype.

A variety of circadian parameters in 5-HT7 receptor KO mice, including rate of entrainment and photic responsiveness, have been investigated. There are no evident differences in the average number of days that 5-HT7 receptor KO mice need to reach entrainment to an advance of 6 hours in the light/dark cycle compared with WT mice (Gardani and Biello, 2008). Both groups of mice display minimal effects to light stimulation during the subjective day, whereas, during the early night, light induces phase delays and, later in the subjective night, results in advances of the circadian phase.

Similarly, administration of the 5-HT7 receptor antagonists SB-269970 and SB-656104 to rats reduces the total amount of REM sleep, whereas wake and slow wave sleep are not affected (Hagan et al., 2000; Thomas et al., 2003; Monti and Jantos, 2006). Furthermore, both
genetic inactivation and pharmacological blockade of 5-HT7 receptor augmented the effects of SSRIs on REM sleep suppression (Bonaventure et al., 2007; Shelton et al., 2009).

As for the effect of 5-HT7 receptor agonists on sleep, systemic administration of LP-211 during the light phase increases wakefulness in rats and reduces REM sleep duration and periods. To assess the potential neural sites that mediate the changes in REM and wake in the rat, LP-211 was microinjected into the brain regions involved in sleep-wake regulation. Local administration of LP-211 into the DRN, locus coeruleus, lateral basal forebrain, and laterodorsal tegmental nucleus suppressed REM sleep, and microinjection of LP-211 into the basal forebrain augmented wake (Monti et al., 2014). The authors proposed that activation of 5-HT7 receptors expressed by GABAergic interneurons decreases the activity of REM sleep–promoting cholinergic neurons in the laterodorsal and pedunculopontine tegmental nuclei and reduces REM sleep. Suppression of REM sleep was observed also when LP-44, another 5-HT7 receptor agonist, was microinjected into the DRN (Monti et al., 2008).

A possible explanation for these apparently contradictory findings is that 5-HT7 receptors can stimulate GABAergic neurons only in the absence and/or at low concentrations of 5-HT, whereas at high 5-HT concentrations, 5-HT7 receptors inhibit these GABAergic neurons. Therefore, a concentration-dependent switch in 5-HT7 receptor signaling could explain why inhibition of 5-HT7 receptor activity, elevated 5-HT concentrations, or administration of 5-HT7 receptor agonist at high concentrations prevent 5-HT7 receptor–mediated stimulation of the REM stimulatory GABAergic neurons.

5-HT7 receptors have also been implicated in the regulation of the mammalian circadian clock located in the suprachiasmatic nucleus. Studies have demonstrated that 8-OD-PAT induces nonphotic phase resetting through activation of 5-HT7 receptors in vitro and in vivo. This effect is reversed by genetic inactivation or pharmacological blockade by the antagonists SB-269970, DR-4004, and JNJ-18038683 (Sprouse et al., 2004; Guscott et al., 2005; Shelton et al., 2015). Consistently, the agonist LP-211 induced a phase advancement of the circadian rhythm in mice (Adriani et al., 2012). Moreover, activation of 5-HT7 receptors by the partial agonist AS19 shortens the period length of oscillation of clock gene period circadian protein homolog 2 expression in the suprachiasmatic nucleus. Period circadian protein homolog 2 expression is used to monitor changes in circadian period length and amplitude (Westrich et al., 2013).

6. Autism Spectrum Disorders. Clinical studies suggest a deficient brain 5-HT system as a causal mechanism in autism spectrum disorders (McDougle et al., 1996; Boccuto et al., 2013). Moreover, the lack of 5-HT during early stages of development may contribute to disrupt the wiring architecture of the brain (Azmitia et al., 2011).

5-HT7 receptor activation corrects molecular, electrophysiological, and behavioral manifestations in mice models of Fragile X syndrome (FXS) and Rett syndrome (RTT), both genetic forms of intellectual disabilities associated with autistic behavior (Costa et al., 2012, 2015; De Filippis et al., 2014, 2015). FXS is the most common inherited intellectual disability; it is caused by silencing of the Fmr1 gene coding for the Fragile X Mental Retardation Protein (Pieretti et al., 1991). Activation of 5-HT7 receptors by the agonists 8-OD-PAT and LP-211 rescues mGluR-LTD in Fmr1 knockout mice and restores LTD levels to those of WT mice (Costa et al., 2012, 2015). This might have important functional consequences, as long-term synaptic plasticity plays a fundamental role in shaping the structure and function of brain circuits. LTD is crucially involved in learning and memory, in novelty detection, and in the extinction of previously acquired memories, and it is believed to underlie behavioral flexibility (Collingridge et al., 2010). On that basis, Costa et al. (2012, 2015) suggested that selective activation of 5-HT7 receptors, by restoring mGluR-mediated synaptic plasticity to normal levels, might also rescue cognitive functions and behavioral flexibility in the mouse model of FXS.

De Filippis et al. (2014) have demonstrated that the selective 5-HT7 agonist LP-211 rescues the behavioral impairments in MeCP2-308 male mice, a mouse model of RTT. RTT is a rare neurodevelopmental disorder characterized by severe behavioral symptoms, including autistic-like behaviors, anxiety, motor disturbances, stereotypic hand movements, and severe cognitive dysfunction (Hagberg, 2002; Ricceri et al., 2013). Mutations in the methyl-CpG–binding protein 2 (MeCP2) gene have been identified as the main genetic cause of RTT. MeCP2 encodes a multifunctional protein that binds to methylated DNA and mainly acts as a key transcriptional regulator. Systemic treatment with LP-211 rescues RTT-related defective performance: anxiety-related profiles in a light/dark test, motor abilities in a dowel test, the exploratory behavior in the marble burying test, and memory in the novelty preference task. At a molecular level, LP-211 administration in MeCP2-308 mice restores levels of the Rho GTPases effector molecules p21 activated kinases and coflin, which both are key regulators of actin cytoskeleton dynamics and, thus, crucially involved in neuronal plasticity. A follow-up study reported similar effects in MeCP2-308 heterozygous female mice; the genetic and hormonal milieus of these mice more closely resemble those of RTT patients (De Filippis et al., 2015). In addition, targeting 5-HT7 receptors can rescue brain mitochondrial dysfunction in heterozygous female MeCP2-308 and MeCP2-Bird mice (a more severely affected model). Moreover, LP-211 treatment
completely restores the radical species overproduction by brain mitochondria in the MeCP2-308 model and partially recovers the oxidative imbalance in MeCP2-Bird mice (Valenti et al., 2017).

A core symptom of autism spectrum disorder is repetitive and stereotypic behavior. The dual 5-HT$_{1A}$/5-HT$_{7}$ partial agonist (+)-5-(2’-fluorophenyl)-N,N-dimethyl-1,2,3,4-tetrahydronaphthalen-2-amine reduces or eliminates stereotypes in three different mouse models of stereotypy: idiopathic jumping, repetitive body rotations after treatment with the NMDA antagonist dizocilpine, and repetitive head-twitching after treatment with the 5-HT$_{2}$ receptor agonist DOI (Canal et al., 2015) without altering locomotor behavior.

Finally, it has been proposed that the reduced behavioral inflexibility elicited by 5-HT$_{7}$ receptor antagonists (Nikiforuk, 2012; Nikiforuk and Popik, 2013) might be of relevance in autism spectrum disorder, as reduced behavioral flexibility (i.e., a reduced ability to replace a previously acquired rule with a new one in adaptation to a new environmental context) is a typical feature of this pathology (Ciranna and Catania, 2014).

There is a suggestive link between a HTR7 genetic abnormality, which encodes the 5-HT$_{7}$ receptor, and neurodevelopmental disorders (Helsmoortel et al., 2016). The whole-genome sequencing of a severely affected dizygotic twin with an autism spectrum disorder and intellectual disability revealed a compound heterozygous mutation in the HTR7 gene as the only variation.

7. Epilepsy. The involvement of 5-HT$_{7}$ receptors in epilepsy has been actively investigated. The 5-HT$_{7}$ receptor antagonist SB-258719 reduces spontaneous epileptic activity in the WAG/Rij rat model of absence epilepsy (Graf et al., 2004). In pilocarpine-induced rat models of temporal lobe epilepsy, SB-269970 also reduces the number of seizures (Yang et al., 2012). Interestingly, genomic studies in humans suggest a link between variants in the gene encoding the 5-HT$_{7}$ receptor and alcoholism (Zlojutro et al., 2011; Kim et al., 2014). In this respect, mice exposed to alcohol vapors present increased expression of 5-HT$_{7}$ receptors in brain areas specifically involved in dependence (Yoshimoto et al., 2012). However, blockade of 5-HT$_{7}$ receptors with SB-258719 does not alter alcohol drinking behavior in the mice exposed to alcohol vapor (Yoshimoto et al., 2012).

9. Pain. Much effort has been made to investigate the potential (patho)physiologic role of 5-HT$_{7}$ receptors in nociception and chronic pain. Early studies suggested a peripheral pronociceptive action of 5-HT through 5-HT$_{7}$ receptor activation (Meuser et al., 2002). The pain-promoting effect of 5-HT or 5-CT injection into a hindpaw on formalin-induced local nociceptive responses is blocked by SB-269970 (Rocha-González et al., 2005).

In rat models of neuropathic pain (i.e., chronic constriction injury to the sciatic nerve or spinal nerve ligation) systemic administration of SB-269970 reduces hyperalgesia and tactile allodynia (Amaya-Castellanos et al., 2011; Viguier et al., 2012). Intrathecal administration of SB-269970 also reduces tactile allodynia in spinal nerve-ligated rats, suggesting the involvement of 5-HT$_{7}$ receptors in pronociceptive mechanisms at the spinal level (Amaya-Castellanos et al., 2011). A pronociceptive effect of 5-HT$_{7}$ receptor stimulation has been reported at the trigeminal level. SB-656104 significantly decreases the c-Fos immunostaining in the spinal nucleus of the trigeminal nerve in response to intracisternal injection of capsaicin (Martínez-García et al., 2011).

However, such findings appear in contradiction with the data from Brenchat et al. (2009, 2010), in which systemic administration of SB-269970 or SB-258719 enhance mechanical hypersensitivity associated with capsaicin-induced hyperalgesia or nerve injury in mice. Interestingly, local injection of SB-269970 or SB-258719 in control mice does not promote hypersensitivity, suggesting that 5-HT$_{7}$ receptors might be involved in some pronociceptive modulatory mechanisms only under neuronal sensitization conditions.

In addition to possible species differences, the selected model of neuropathic pain might also have relevance with respect to the aforementioned contradictions. The antinociceptive potential of 5-HT$_{7}$ receptor antagonists would suggest a pronociceptive effect of 5-HT$_{7}$ receptor agonists for which there is some support (Brenchat et al., 2010; Martínez-García et al., 2011). However, other studies report 5-HT$_{7}$ receptors mediate antinociceptive effects. Thus, blockade of spinal 5-HT$_{7}$ receptors by intrathecal injection of SB-269970 prevents the antinociceptive effects of systemic administration of morphine, tramadol, or cannabinoids in the tail flick test (Dogru and Seyrek, 2006; Dogru et al., 2009; Yanarates...
et al., 2010), and intrathecal administration of E-57431 and E-55888 inhibit mechanical hypersensitivity caused by capsaicin injection or nerve injury–induced mechanical hypersensitivity in both mice and rats (Brenchat et al., 2011; Viguier et al., 2012). Furthermore, systemic treatment with 5-HT7 receptor agonists produce marked reductions in mechanical and thermal hypersensitivity in various chronic pain models with central and/or peripheral sensitization (Brenchat et al., 2009, 2010, 2012a,b; Ulugol et al., 2012; Viguier et al., 2012, 2013).

H. Clinical Relevance

1. Depression. Multiple experimental approaches tend to support the hypothesis that 5-HT7 receptor blockade or genetic inactivation displays an antidepressant-like activity (Guscott et al., 2005; Hedlund et al., 2005; Sarkisyan et al., 2010). In agreement with mouse KO data, the pharmacological blockade of 5-HT7 receptors results in antidepressant-like effects in the TST (Hedlund et al., 2005; Wesolowska et al., 2006a; Bonaventure et al., 2007) and in the FST in both mice (Hedlund et al., 2005; Wesolowska et al., 2006b) and rats (Wesolowska and Kowalska et al., 2008; Mnie-Filali et al., 2011).

The 5-HT7 receptor antagonist SB-269970 was assessed in the olfactory bulbectomy paradigm, which is considered as a “chronic” behavioral model of depression, in which classic antidepressants require the administration for 2 to 3 weeks before any antidepressant-like effects can be observed (Song and Leonard, 2005). SB-269970 induced a faster antidepressant-like response when compared with 1 week of treatment with the SSRI fluoxetine (Mnie-Filali et al., 2011). 5-HT7 receptor blockade may also augment the effects of antidepressant drugs; thus, the combination of an ineffective dose of SB-269970 with an ineffective dose of one of several antidepressants, results in a synergistic reduction in immobility in both FST and TST (Wesolowska et al., 2006b; Bonaventure et al., 2007; Wesolowska and Kowalska, 2008; Sarkisyan et al., 2010) that correlates with increases in 5-HT release in the prefrontal cortex (Bonaventure et al., 2007; Wesolowska and Kowalska, 2008). Besides the prefrontal cortex, the hippocampus has also been implicated in the effects of SB-269970 and imipramine, as intrahippocampal SB-269970 administration reduces immobility in the rat FST (Wesolowska et al., 2006b).

Preclinical tests in mice suggest that the clinically established antidepressant effect of the atypical antipsychotic drugs amisulpride, aripiprazole, or lurasidone may be due to 5-HT7 receptor blockade. Thus, amisulpride, aripiprazole, and lurasidone are potent but nonselective 5-HT7 receptor antagonists that have clear antidepressant actions (Lecrubier et al., 1997; Smeraldi, 1998; Lawler et al., 1999; Shapiro et al., 2003; Nakamura et al., 2009; Ishibashi et al., 2010; Citrome, 2011) that reduce immobility in the TST and the FST in WT but not in 5-HT7 KO mice (Abbas et al., 2009a; Sarkisyan et al., 2010; Cates et al., 2013), strongly suggesting a role for the 5-HT7 receptor.

The 5-HT7 receptor antagonist JNJ-18038683 (Bonaventure et al., 2012) reduces immobility in mice in the TST. Coadministration of subeffective doses of citalopram and JNJ-18038683 elicits an antidepressant effect. However, JNJ-18038683, when tested in patients with major depressive disorder, produced no statistically significant improvement over placebo on the Montgomery-Åsberg Depression Rating Scale, although escitalopram in that same study was also inactive, complicating the interpretation.

2. Sleep. The potent and selective 5-HT7 receptor antagonist JNJ-18038683 prolonged REM latency and decreased REM sleep duration in healthy volunteers (Bonaventure et al., 2012). Furthermore, JNJ-18038683 appeared to enhance REM sleep suppression induced by citalopram.

XVI. High-Resolution Structure of 5-HT Receptors

A. 5-HT GPCRs

Since the initial cloning of a 5-HT receptor in 1988 (Julius et al., 1988), it has been appreciated that the G protein–coupled 5-HT receptors would have a topology similar to other members of the GPCR superfamily (Kroeze et al., 2003). These features included a predicted seven-transmembrane helical arrangement with an orthosteric binding pocket near the upper one-third of the helical array (Choudhary et al., 1992, 1993, 1995). Indeed, early molecular models—bolstered by site-directed mutagenesis studies—predicted that 5-HT and serotonergic drugs such as ergolines and ergopeptines would be anchored by a highly conserved aspartic acid in helix III and aromatic residues in helix VI (Choudhary et al., 1993, 1995; Wang et al., 1993; Sealon et al., 1995).

These predictions were confirmed in 2013 with the publication of the first high-resolution structures of the human 5-HT1B (Wang et al., 2013) and 5-HT2B (Wacker et al., 2013) receptors—both in complex with the ergopeptine ergotamine (Fig. 28). Additionally, the 5-HT1B receptor was also solved in complex with dihydroergotamine (Wang et al., 2013), revealing an essentially identical ligand orientation. Perhaps not surprisingly, and as predicted many years ago (Choudhary et al., 1995), ergotamine was anchored by the highly conserved TMIII aspartic acid residue and stabilized by hydrophobic and edge-on-face interactions with aromatic residues in helix VI (Wacker et al., 2013; Wang et al., 2013).

Given the large differences between 5-HT1B and 5-HT2B receptors in terms of pharmacology, signal transduction, and amino acid sequence, the high-resolution structures of both receptors allowed the investigators to
identify both important structural commonalities and dissimilarities between these two distinct 5-HT receptor families (Wacker et al., 2013; Wang et al., 2013); the orthosteric binding pockets of both receptors are nearly identical, with only two amino acids in the conserved orthosteric binding site differing (Wang et al., 2013). This high degree of conservation readily explains why many serotonergic drugs are promiscuous. Indeed, LSD was found to be a potent agonist at every G protein–coupled 5-HT receptor except the 5-HT7 (Wacker et al., 2013).

Subtle differences in the general region of the binding pocket were able to explain heretofore puzzling aspects of 5-HT receptor pharmacology. For instance, the apparent preference of rodent versions of the 5-HT1B, 5-HT1D, 5-HT1E, and 5-HT1F receptors for β-adrenergic antagonists (Hoyer and Middlemiss, 1989; Adham et al., 1994) was now apparent based on structural, mutagenesis, and modeling studies (Wang et al., 2013). Thus, a Thr7.39→Asn7.39 to mutation, changing the human to a rodent version, led to the formation of a polar interaction network now favoring the binding of the propanolamine moiety of adrenergic antagonists. Additionally, the high affinity of norfenfluramine for 5-HT2B receptors, which may lead to valvular heart disease (Rothman et al., 2000), was explained by the orientation of a nonconserved methionine residue (M7.39) that was oriented in the binding pocket in the 5-HT2B receptor and absent in all other 5-HT receptors (Wang et al., 2013). Finally, distinctly different patterns of signaling by ergotamine at 5-HT1B receptors, where it is unbiased, and 5-HT2B receptors, where it shows arrestin bias, led to explication of the structural features responsible for functional selectivity (Wacker et al., 2013).

Thus, the apparent arrestin-biased signaling by ergotamine at 5-HT2B receptors is due to a preferential stabilization of inactive states of particular G protein conformational microswitches (which are essential for mediating canonical signaling) and the preferential allowance of conformations responsible for arrestin-dependent signaling (Wacker et al., 2013).

More recent studies have clarified the mechanisms responsible for the differential activation of G protein versus arrestin signaling (Wacker et al., 2017a) at 5-HT2A (Wacker et al., 2017b) and 5-HT2B receptors (Wacker et al., 2017b; McCorvy et al., 2018). In particular, a detailed study of several 5-HT2B receptor structures (Wacker et al., 2017b; McCorvy et al., 2018) disclosed that key interactions with A5.46 and P3.37 are essential for canonical G protein signaling, whereas interactions with extracellular loop residue L7.35 are essential for arrestin-ergic signaling. Additionally, other structures of an intermediate-active state of the 5-HT2C receptor (Peng et al., 2018; Fig. 28) and the G protein heterotrimer-stabilized active state of the
5-HT<sub>1B</sub> receptor (Garcia-Nafria et al., 2018) implicated a key “trigger motif” P-I-F as being essential for the active-inactive switch and biased signaling (Wacker et al., 2017a; McCorvy et al., 2018).

B. 5-HT Ligand-Gated Ion Channels

5-HT<sub>3</sub> receptors are ion channels of the Cys-loop receptor family (Thompson et al., 2010; Corringer et al., 2012). Thus, they share a common architecture with other members, such as nicotinic acetylcholine, ionotropic γ-aminobutyric, and glycine receptors. All of them are composed of five subunits, symmetrically disposed around a central ionic pore axis, forming an urn-like architecture (Fig. 29). Each subunit has three functional domains (Fig. 29): a large extracellular domain harboring the neurotransmitter binding pocket, a four-helix transmembrane domain establishing the ion channel, and an intracellular domain that mediates the receptor interaction with intracellular proteins.

Recent years have seen the emergence of a solid structural framework to help interpret functional and pharmacological studies. First, 5-HT, granisetron, and palonosetron have been crystallized in complex with a soluble model protein (termed 5-HTBP; Kesters et al., 2013; Price et al., 2016), providing possible orientations of ligands and adding up to the huge variety of nicotinic receptor ligands cocrystallized with the same type of model protein. Second, the initial mouse 5-HT<sub>3A</sub> receptor X-ray structure (Hassaïne et al., 2014) has provided an almost complete picture (~60 unstructured residues missing in the intracellular domain) of a 5-HT<sub>3</sub> receptor but with empty neurotransmitter sites capped by stabilizing llama antibodies instrumental to crystallization. Third, a series of distinct conformations of the full-length murine receptor imaged by Cryo-Electron Microscopy (Basak et al., 2018a,b; Polovinkin et al., 2018) has shed light on its gating mechanism and revealed how 5-HT and antiemetic drugs such as tropisetron bind in the neurotransmitter site. Specialized reviews discuss Cys-loop receptor structure at length (daCosta and Baenziger, 2013; Nys et al., 2013; Sauguet et al., 2013; Wu et al., 2015; Nemecz et al., 2016).

The five equivalent neurotransmitter binding pockets of the homopentameric 5-HT<sub>3A</sub> receptor are located in electronegative clefts at interfaces between two adjacent subunits (Fig. 30). Three loops of the principal subunit and four portions of beta strands of the complementary subunit contribute to the pocket (Fig. 30). Therefore, the shape of the binding pocket arises from the combination of the quaternary arrangement (how one subunit is oriented relative to its neighbor) and of the local conformation of loops and side chains. During activation of Cys-loop receptors and gating of the channel, both global subunit/subunit orientation and more local conformation changes take place (Sauguet et al., 2013; Du et al., 2015; Basak et al., 2018a; Polovinkin et al., 2018). The 5-HTBP and other model proteins present a stiff quaternary structure in which the subunit/subunit orientation is fixed, and in that respect, they are imperfect models for ligand binding.

The binding cleft of the 5-HT<sub>3A</sub> receptor is surrounded by aromatic residues forming a 10-Å wide box, including W156 (loop B), W63 (loop D), Y126 (loop E), F199, and Y207 (loop C). W156 (W145 in 5-HTBP) lies at the bottom of the cleft and has cation-Pi interaction with 5-HT.

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**Fig. 29.** Architecture and ligand binding sites of 5-HT<sub>3</sub> receptors. (A) Cartoon representation of a single subunit viewed parallel to the plane of the membrane. (B) Cartoon representation of the entire pentameric receptor, in the same orientation [the subunit of (A) is equivalent to the yellow subunit]. One out of the five stabilizing VHH15 single-chain llama antibodies is shown in pale green and labeled VHH15. (C) Surface representation of the receptor highlighting binding clefts in blue (neurotransmitter site), yellow (anesthetics intrasubunit site), orange (PU-02 and anesthetics intersubunit site), purple (extracellular allosteric pocket), and olive (pore blockers site). The subunit equivalent to the one of (A) appears in darker gray.
Beene et al., 2002). This part of the site looks rigid with many intra- and intersubunit interactions. In contrast, the mouth of the cleft seems prone to reorganization. The C loop adopts different conformations in the apo, the antagonist-bound, and 5-HT-bound (Fig. 30). Other important residues lining the site include N101 (loop A), T152 and T154 (loop B), R65 (loop D), D177, S179, I180 (loop F), D42, and I44 (loop G).

A picture of the molecular mechanism of 5-HT\textsubscript{3} receptors starts to emerge from the accumulated structural data. The closed apo conformation resembles those of the receptor inhibited by small molecules (antiemetics such as tropisetron) or by the inhibitory VHH15. Upon 5-HT binding, the receptors undergo a transition to the open state characterized by 1) a compaction of the neurotransmitter pocket because of quaternary reorganization of the extracellular domain and 2) an opening of the transmembrane pore linked to the pivot of each subunit transmembrane domain. Two independent cryo-EM studies have captured a second 5-HT\textsubscript{3}–bound state featuring a closed pore similar to that of the resting state (Basak et al., 2018a; Polovinkin et al., 2018). Based on functional experiments, this state was tentatively described as preactive rather than desensitized (Polovinkin et al., 2018).

There is no direct structural data on allosteric modulation sites of 5-HT\textsubscript{3} receptors. Nevertheless, allosteric sites identified on homologous Cys-loop receptors are relevant to the 5-HT\textsubscript{3} pharmacology. For instance, PU-02, a selective inhibitor of the 5-HT\textsubscript{3} receptor acting in the micromolar range, binds to a transmembrane intersubunit site (orange in Fig. 29).
that correlates to the ivermectin site of the *C. elegans* glutamate-gated chloride channels receptor (Hibbs and Gouaux, 2011) and to the ethanol and bromoform sites of the model bacterial GLIC receptor (Sauguet et al., 2013). In the 5-HT₃A receptor structure, a deep cleft between subunits is indeed accessible from the lipid bilayer and lined by residues determining PU-02 activity [16]: L266, S270, G282, and V288 on the (+) side and S226, I268, and T272 on the (−) side (Fig. 29). A second membrane-accessible site, this time located within a single subunit (Fig. 29), has been identified as the site of action of the positive allosteric modulator TMPPAA (Gasiorek et al., 2016; Polovinkin et al., 2018) and might be involved in anesthetics inhibition (Lopreato et al., 2003). Some compounds acting as physical blockers of the ion flux bind directly in the channel lumen. A variety of sites have been identified at different depths in the pore, spanning almost the whole transmembrane part, from divalent cations binding close to the intracellular mouth to TEA and lidocaine in the middle (Hilf et al., 2010) and memantine that binds close to the extracellular mouth (between 13′ and 16′) (Rammes et al., 2001; Ulens et al., 2014).

The 5-HT₃ receptor structures also shed light on the neglected intracellular domain. Only part of it is seen in the structures: a short helical stretch after the M3 transmembrane helix and a long helix MA continuous with the M4 transmembrane helix (Figs. 29 and 31). At their N-terminal side, MA helices form a tight pentameric bundle stabilized by hydrophobic interactions (Fig. 31). Further up, and closer to the mouth of the transmembrane pore, they carry charged residues whose mutations were shown to profoundly affect the channel conductance. At this level, the spacing between MA helices results in lateral fenestrations, plugged in the closed-pore structures by the loop linking M3 and MA (Fig. 31). Recent cryo-EM structures have shown that this intracellular region is drastically reorganized in the open-pore state, with wide portals, and displays flexibility. The ion exit pathway and the way in which the ion flux depends on the flexibility and the local electrostatic environment remain to be fully described (Di Maio et al., 2015).

Molecular dynamic simulations established the existence of a dewetted zone in the transmembrane pore in the closed-pore conformations and its conversion to a fully hydrated pore permeant to cations in the open conformations (Yuan et al., 2016; Polovinkin et al., 2018).

**XVII. 5-HT GPCRs and Their Interacting Proteins**

**A. Introduction**

The history of cell signaling started more than 60 years ago with the seminal discovery of cAMP by Earl Sutherland (Nobel prize in 1971). The mechanisms by which some receptors activate the production of second messengers has been revealed by Martin Rodbell (Nobel Prize 1994) in the 1970s when he showed that...
the receptors do not directly stimulate second messenger synthesizing enzymes but indirectly, via an allosteric activation of G proteins. The GPCR ternary complex consisting of a receptor, a G protein, and an effector, enzyme, or channel was born. Surprisingly, five decades later, the nature and functions of the huge protein complexes comprising GPCRs and GPCR-interacting proteins (GIPs) still remain poorly characterized. During their cellular journey, GPCRs are assisted by GIPs for their proper folding, targeting proper subcellular compartments, trafficking to and out of the plasma membrane, and select several alternative signaling pathways, including G protein–independent pathways. In addition, GIPs are able to allosterically modify the pharmacology of associated GPCR and even activate some GPCRs in the absence of ligands. This section of the review is dedicated to GIPs of 5-HT receptors, which are among the GPCRs for which those proteins are the most extensively characterized, mainly thanks to proteomics screens. Given the large number of 5-HT receptors identified, it provides a wide overview of their role in GPCR physiology. Recent advances in the role of GIPs in fine-tuning 5-HT6 receptor signaling and associated physiologic functions, including neurodevelopment and cognition, are particularly highlighted.

The evolution of multicellular organisms has been closely linked to their capacity to communicate with their environment and to develop sophisticated communications between their own cells. Most of these communications involve chemical messengers (e.g., hormones, neurotransmitters, growth factors) that interact with one or several transmembrane receptors. Among those receptors, GPCRs are the most numerous ones (Bockaert and Pin, 1999). Around 10,000 genes encode such receptors in the human genome (representing 3% to 4% of the genome), of which ~300 are nonolfactory receptors (Fredriksson et al., 2003). 5-HT receptors are particularly well represented in this important receptor family. The diversity of 5-HT GPCRs is certainly due to molecular tinkering from one ancestral gene and has been instrumental for the implication of 5-HT in a large number of physiologic and pathophysiological functions both in the CNS and peripheral tissues. Because of their structural diversity, 5-HT receptors are able to trigger a large panel of signaling events (Marin et al., 2012). Many of them are transduced by different G proteins. In addition, signaling events elicited by 5-HT receptor activation as well as by many GPCRs are triggered or finely modulated through their interaction with multiple intracellular proteins, “GIPs,” and are assembled into functional complexes designated as “receptosomes” (Bockaert et al., 2003, 2004b, 2010b; Maurice et al., 2011; Marin et al., 2012). GIPs are not only involved in GPCR signaling but also in their targeting to subcellular compartments, including axonal and dendritic compartments, in their trafficking in and out of the plasma membrane through the endoplasmic reticulum, Golgi apparatus, endosomal, and lysosomal compartments (Bockaert et al., 2003, 2004b, 2010; Maurice et al., 2011; Marin et al., 2012). Several reviews have been published on GIPs, including two dedicated to 5-HT receptor GIPs (Allen et al., 2008; Marin et al., 2012). Accordingly, only a brief description of the nature, cellular, and physiologic functions of 5-HT receptor GIPs will be provided here (see Table 21), focusing on intracellular GIPs and not considering 5-HT receptor association with other GPCRs (homodimer/heteromer formation). A more detailed account of proteins interacting with the 5-HT6 receptor is presented to highlight the interest of a comprehensive description of GIPs for a given receptor to unravel novel mechanisms underlying GPCR activation and physiologic functions controlled by those receptors.

B. A Survey of 5-HT Receptor GIPs

1. 5-HT1A Receptor. The 5-HT1A receptor recruits and activates both G13 and G5 (Bockaert et al., 2006; Marin et al., 2012). Coupling to G13 is predominant in the soma of 5-HT raphe neurons and mainly leads to adenylyl cyclase (AC) inhibition, whereas coupling to G5 prevails in hippocampus and leads to inhibition of GIRK channels (Mannoury la Cour et al., 2006); see also Marin et al. (2012) for a detailed review of G5/G13-mediated signaling events elicited by 5-HT1A receptor and their physiologic impact. The molecular basis of the difference in G protein coupling is unknown. Only two GIPs are known to interact with 5-HT1A receptors, namely calmodulin (Della Rocca et al., 1999; Turner et al., 2004) and Yif1B (Carrel et al., 2008; Al Awabdh et al., 2012; Alterio et al., 2015). The Ca2+-calmodulin complex binds to two distinct sites located in the third intracellular loop (i3) of the receptor (Turner et al., 2004), whereas Yif1B (Yip1 interacting factor homolog B) associates with its short C-terminal domain (Ct). Ca2+-calmodulin is implicated in receptor internalization, Erk1/2 activation, and modulation of PKC-dependent receptor phosphorylation (Della Rocca et al., 1999; Turner et al., 2004). In addition, Ca2+-calmodulin contributes to receptor-operated Janus kinase 2 (Jak2) and type 1 sodium-proton exchanger (NHE-1) activation (Turner et al., 2007). 5-HT1A receptor activation leads to the assembly of a ternary signaling complex that includes activated Jak2, Ca2+-calmodulin, and NHE-1, in which calmodulin is activated by tyrosine phosphorylation instead of cellular Ca2+ elevation. This results in tighter interaction of calmodulin with NHE-1, NHE-1 conformational change, and increased NHE-1 transport activity (Turner et al., 2007). Yif1B is a protein implicated in the traffic of proteins from the endoplasmic reticulum (ER) to the Golgi (Alterio et al., 2015) that plays a key role in the selective targeting of 5-HT1A receptors to dendrites (Carrel et al., 2008). It has been proposed that Yif1B serves as a scaffold allowing the recruitment of 5-HT1A receptor to a complex comprising...
TABLE 21

5-HT receptor–interacting proteins and their cellular and physiologic functions

For each receptor, the nature of identified GIPs, their site of interaction, the function of their interaction with the receptor (if known) at the cellular (receptor targeting, trafficking, and signal transduction) and physiologic levels as well as their role in pathologies are indicated. The question marks indicate that the site of interaction of the GIP in the receptor sequence or the physiologic/pathologic functions of its interaction with the receptor remain unknown.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>GIP</th>
<th>Site of Interaction</th>
<th>Cellular Functions</th>
<th>Physiologic/Pathologic Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT1A</td>
<td>Calmodulin</td>
<td>i3/Ct</td>
<td>Endocytosis</td>
<td>?</td>
<td>Della Rocca et al., 1999; Turner et al., 2004, 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PKC–dependent receptor activation</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>NHE-1 activation</td>
<td></td>
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<tr>
<td></td>
<td>Yif1B</td>
<td>Ct</td>
<td>Anterograde trafficking</td>
<td>?</td>
<td>Al Awabdh et al., 2012; Alterio et al., 2015</td>
</tr>
<tr>
<td>5-HT1D</td>
<td>p11</td>
<td>i3</td>
<td>Cell surface density and signaling</td>
<td>Depression in rodent models</td>
<td>Svenningsson et al., 2006; Eriksson et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Emotional memory in rodents</td>
<td></td>
</tr>
<tr>
<td>5-HT2A</td>
<td>β-arrestin 2</td>
<td>?</td>
<td>Delayed recycling (human receptor)</td>
<td>Hallucinogenic effects induced by high 5-HT concentration</td>
<td>Schmid et al., 2008; Bhattacharya et al., 2010; Schmid and Bohn, 2010; Allen et al., 2008</td>
</tr>
<tr>
<td></td>
<td>MAP1A</td>
<td>?</td>
<td>Trafficking in apical dendrites</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arf1-6</td>
<td>i3/Ct</td>
<td>Coupling to phospholipase D</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jak/STAT3</td>
<td>?</td>
<td>Receptor phosphorylation and desensitization</td>
<td>Putative implication in psychiatric disorders (Coffin-Lowry syndrome)</td>
<td>Guillet-Deniaux et al., 1997; Shefler et al., 2006; Allen et al., 2008; Strachan et al., 2009</td>
</tr>
<tr>
<td></td>
<td>RSK2</td>
<td>i3</td>
<td></td>
<td></td>
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<tr>
<td>5-HT2A</td>
<td>Calmodulin Caveolins</td>
<td>i3/Ct</td>
<td>Gq coupling</td>
<td></td>
<td>Turner and Raymond, 2005 Bhatnagar et al., 2004</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Targeting to lipid rafts</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>PSD-95</td>
<td>Ct (PDZ ligand-SCV)</td>
<td>Dendritic targeting</td>
<td>Hallucinogenic effects in mice Antipsychotic drug effects Inflammatory and neuropathic pain</td>
<td>Xia et al., 2003; Becamel et al., 2004; Abbas et al., 2009; Pichon et al., 2010; Vogrigr et al., 2013; Wattiez et al., 2013</td>
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<tr>
<td></td>
<td>SAP97</td>
<td>Ct (PDZ ligand-SCV)</td>
<td>Postsynaptic localization</td>
<td>Drug dependence</td>
<td>Allen et al., 2008</td>
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<td></td>
<td>MUPP1</td>
<td>Ct (PDZ ligand-SCV)</td>
<td>Postsynaptic localization</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAGI2</td>
<td>Ct (PDZ ligand-SCV)</td>
<td>Postsynaptic localization</td>
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<td>Allen et al., 2004</td>
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<td>CIPP</td>
<td>Ct (PDZ ligand-SCV)</td>
<td>Postsynaptic localization</td>
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<td>Becamel et al., 2004</td>
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<td>NHERF3</td>
<td>?</td>
<td>Gq signaling</td>
<td></td>
<td>Walther et al., 2015</td>
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<td>5-HT2B</td>
<td>MUPP1</td>
<td>Ct (PDZ ligand-SSV)</td>
<td>Postsynaptic localization</td>
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<td>Becamel et al., 2001</td>
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<tr>
<td></td>
<td>PDZ protein</td>
<td>Ct (PDZ ligand-SSV)</td>
<td>NO synthesis</td>
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<td>Manivet et al., 2000</td>
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<td>5-HT3C</td>
<td>Calmodulin</td>
<td>Ct</td>
<td>Erk1/2 signaling</td>
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<td>Becamel et al., 2002; Labasque et al., 2008 Labasque et al., 2008; Labasque et al., 2010; Marion et al., 2004</td>
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<tr>
<td></td>
<td>β-arrestins</td>
<td>i3?</td>
<td>Constitutive and agonist-dependent endocytosis</td>
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<tr>
<td></td>
<td>MUPP1</td>
<td>Ct (PDZ ligand-SSV)</td>
<td>?</td>
<td></td>
<td>Becamel et al., 2001; Parker et al., 2003</td>
</tr>
<tr>
<td></td>
<td>PSD-95</td>
<td>Ct (PDZ ligand-SSV)</td>
<td>?</td>
<td></td>
<td>Becamel et al., 2002; Gavarini et al., 2006</td>
</tr>
<tr>
<td></td>
<td>MPP3</td>
<td>Ct (PDZ ligand-SSV)</td>
<td>?</td>
<td></td>
<td>Becamel et al., 2002; Gavarini et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Velici3/CASK/MintMAGI2, SAP102, PSD93</td>
<td>Ct (PDZ ligand-SSV)</td>
<td>?</td>
<td></td>
<td>Becamel et al., 2002; Gavarini et al., 2006</td>
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<tr>
<td>5-HT2C</td>
<td>PTEN</td>
<td>Ct</td>
<td>?</td>
<td>Rewarding effects of THC and nicotine</td>
<td>Bécamel et al., 2002; Ji et al., 2006</td>
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<td></td>
<td>Picot</td>
<td>i3</td>
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<td>5-HT4</td>
<td>Src</td>
<td>?</td>
<td>Erk1/2 signaling</td>
<td>?</td>
<td>Gill et al., 2005; Barthet et al., 2007; Barthet et al., 2009; Warmer-Schmidt et al., 2009</td>
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<tr>
<td></td>
<td>p11</td>
<td>i3</td>
<td>Cell surface localization</td>
<td>Depression in rodent models</td>
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<td></td>
<td>ADAM10</td>
<td>?</td>
<td>?</td>
<td>β-amyloid plaques</td>
<td>Cochet et al., 2013</td>
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<td></td>
<td>GRK5</td>
<td>Ct</td>
<td>Src activation</td>
<td></td>
<td>Barthet et al., 2009</td>
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<td></td>
<td>SNX27a</td>
<td>?</td>
<td>Erk1/2 signaling</td>
<td></td>
<td>Joubert et al., 2004</td>
</tr>
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</table>

(continued)
Rab6, kinesin family member 5B, and dynein that coordinates its anterograde traffic to terminal dendrites (Al Awabdh et al., 2012).

2. **5-HT<sub>1B</sub> Receptor.** In addition to its coupling to G<sub>i</sub>/G<sub>o</sub> (Bockaert et al., 2006; Marin et al., 2012), the 5-HT<sub>1B</sub> receptor interacts via its i3 loop with a particularly interesting GIP, named p11 (Svenningsson et al., 2006; Svenningsson and Greengard, 2007). p11 (also known as S100A10) is a member of the S100 EF-hand protein family. S100 proteins are small acidic proteins (10–12 kDa) characterized by two calcium-binding sites that have helix-loop-helix (“EF-hand type”). p11 increases cell surface density of 5-HT<sub>1B</sub> receptor and, thus, receptor-operated signaling (Svenningsson et al., 2006). Reduction in p11 level has been found in postmortem human tissue from depressed individuals and suicide victims as well as in a rodent model of depression, whereas its level in rodent brain is increased by antidepressants or electroconvulsive therapy (Svenningsson et al., 2006; Svenningsson and Greengard, 2007). Furthermore, invalidation of the gene encoding p11 induces a depression-like phenotype, reduces response to antidepressants and emotional memory, and enhances dependence to cocaine (Svenningsson and Greengard, 2007; Eriksson et al., 2013; Svenningsson et al., 2014).

3. **5-HT<sub>2A</sub> Receptor.** The 5-HT<sub>2A</sub> receptor is mainly coupled to G<sub>q</sub>/G<sub>11</sub> proteins. 5-HT<sub>2A</sub> receptors also engage G<sub>q</sub>/G<sub>11</sub>-mediated signaling upon stimulation by hallucinogenic agonists, including LSD and synthetic agonists such as DOI (Bockaert et al., 2006; Gonzalez-Maeso et al., 2007; Marin et al., 2012; Karaki et al., 2014). Like many GPCRs, the 5-HT<sub>2A</sub> receptor also recruits β-arrestins following agonist stimulation (Bhattacharya et al., 2010). Intriguingly, it has been found that β-arrestin 2 is specifically involved in the hallucinogenic-like effects elicited by high concentrations of 5-HT (or its metabolites) but not in those induced by DOI (Schmid et al., 2008). These hallucinogenic-like effects are mediated by the activation, via β-arrestin 2, of a PI3K/Src/Akt signaling cascade (Schmid and Bohn, 2010). Likewise, β-arrestins are required for Erk1/2 activation by 5-HT but not by DOI (Schmid et al., 2008).

The 5-HT<sub>2A</sub> receptor (like several other 5-HT receptors, including 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors; see below) expresses a canonical recognition motif for PDZ domain-containing proteins at its C-terminal extremity. PDZ domain–containing proteins (or PDZ proteins) are scaffolding proteins containing one or several PDZ domains, in addition to other protein-protein interaction domains. PDZ domains have been classified according to their specificity for PDZ motifs (or PDZ ligands):

### TABLE 21—Continued

<table>
<thead>
<tr>
<th>Receptor</th>
<th>GIP</th>
<th>Site of Interaction</th>
<th>Cellular Functions</th>
<th>Physiologic/Pathologic Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NHERF</strong></td>
<td>Ct (PDZ ligand-SCF)</td>
<td>Receptor recycling?</td>
<td>?</td>
<td>Joubert et al., 2004</td>
<td></td>
</tr>
<tr>
<td><strong>CRMp2</strong></td>
<td>Ct (PDZ ligand-SCF)</td>
<td>Targeting to microvilli</td>
<td>?</td>
<td>Joubert et al., 2004</td>
<td></td>
</tr>
<tr>
<td><strong>5-HT&lt;sub&gt;4a&lt;/sub&gt;</strong></td>
<td>Ct</td>
<td>Neuronal architecture</td>
<td>?</td>
<td>Weninger et al., 2014</td>
<td></td>
</tr>
<tr>
<td><strong>5-HT&lt;sub&gt;4b&lt;/sub&gt;</strong></td>
<td>Ct (PDZ ligand-VPV)</td>
<td>Regulation of cAMP signaling</td>
<td>?</td>
<td>Joubert et al., 2004</td>
<td></td>
</tr>
<tr>
<td><strong>5-HT&lt;sub&gt;6&lt;/sub&gt;</strong></td>
<td>Ct (PXHXPR)</td>
<td>Cell surface expression</td>
<td>?</td>
<td>Yun et al., 2007</td>
<td></td>
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<tr>
<td><strong>Fyn</strong></td>
<td>i3/Ct</td>
<td>Cell surface expression</td>
<td>?</td>
<td>Kim et al., 2014</td>
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<tr>
<td><strong>Jab1</strong></td>
<td></td>
<td>-Jun phosphorylation</td>
<td>?</td>
<td>Ha et al., 2015</td>
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</tr>
<tr>
<td><strong>Map1B-LC1</strong></td>
<td>Ct</td>
<td>Endocytosis</td>
<td>?</td>
<td>Dayer et al., 2015</td>
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</tr>
<tr>
<td><strong>SNX14</strong></td>
<td>i3</td>
<td>Gs signaling</td>
<td>?</td>
<td>Ward et al., 2009</td>
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<tr>
<td><strong>mTORC1</strong></td>
<td>Ct/unknown</td>
<td>mTOR signaling</td>
<td>Cognitive impairment in neurodevelopmental models of schizophrenia</td>
<td>Meffre et al., 2012</td>
<td></td>
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<tr>
<td><strong>Cdk5, p35</strong></td>
<td>Ct</td>
<td>Receptor phosphorylation</td>
<td>Neurite growth migration of pyramidal neurons</td>
<td>Dayer et al., 2014; Jacobshagen et al., 2014; Dayer et al., 2015</td>
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<tr>
<td><strong>5-HT&lt;sub&gt;7&lt;/sub&gt;</strong></td>
<td>Neurochondrin</td>
<td></td>
<td>?</td>
<td>Ward et al., 2009</td>
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<td></td>
<td>Periplakin</td>
<td></td>
<td>?</td>
<td>Stroth and Svenningsson, 2015</td>
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<tr>
<td></td>
<td>S100B</td>
<td>i3</td>
<td>cAMP</td>
<td>Behavioral despair</td>
<td>Stroth and Svenningsson, 2015</td>
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</tbody>
</table>

PICO, protein kinase C interacting cousin of thioredoxin.
class I PDZ domains preferentially bind to -S/Txϕ, class II to -ϕxϕ, and class III to -E/Dxϕ motifs (where ϕ represents a hydrophobic residue and X any residue), respectively. The 5-HT$_{2A}$ receptor PDZ ligand (-SCV) clearly belongs to class I. Several PDZ proteins have been found to interact with the 5-HT$_{2A}$ receptor. The majority of them have been identified thanks to affinity purification coupled to mass spectrometry (AP-MS) proteomic strategies or two-hybrid screens. These include Multi-PDZ protein 1 (MUPP1), PSD-95, membrane-associated guanylate kinase with inverted domain structure 2 (MAGI2), synapse-associated protein (SAP)97, channel interacting PDZ protein (CIPP), and MAGUK p55 subfamily member 2 (MPP2) and 3 (MPP3) (Becamel et al., 2001; Bécamel et al., 2004; Pichon et al., 2010). Among the 5-HT$_{2A}$ receptor GIPs identified, PSD-95 is certainly the most extensively studied with respect to the functional impact of its association with the receptor. PSD-95, which is located in postsynaptic spine membranes together with other receptor PDZ partners, is required for dendritic targeting of the receptor (Xia et al., 2003; Allen et al., 2008). In addition, microtubule-associated protein (MAP)1A may scaffold the receptor to the microtubule cytoskeleton and facilitate its trafficking to apical dendrites (Allen et al., 2008). PSD-95, like SAP97 and MUPP1, increases receptor cell surface density likely by inhibiting receptor endocytosis (Allen et al., 2008; Jones et al., 2009). The impact of the 5-HT$_{2A}$ receptor/PSD-95 complex upon receptor-operated signaling is less clear. On the one hand, 5-HT$_{2A}$ receptor-mediated downstream signaling is impaired in PSD-95–deficient mice (Abbas et al., 2009), suggesting that the interaction promotes receptor-operated signal transduction. On the other hand, acute disruption of 5-HT$_{2A}$ receptor/PSD-95 association with an interfering peptide increases receptor-mediated cytosolic Ca$^{2+}$ increase and its antihyperalgesic effects in models of neuropathic and inflammatory pain in the rat (Pichon et al., 2010; Wattiez et al., 2013), suggesting that this interaction inhibits receptor-operated signaling. These opposite effects of sustained (PSD-95 knockout) and acute (interfering peptide) disruption of the 5-HT$_{2A}$ receptor/PSD-95 association with an interfering peptide increases receptor-mediated cytosolic Ca$^{2+}$ increase and its antihyperalgesic effects in models of neuropathic and inflammatory pain in the rat (Pichon et al., 2010; Wattiez et al., 2013), suggesting that this interaction inhibits receptor-operated signaling. These opposite effects of sustained (PSD-95 knockout) and acute (interfering peptide) disruption of the 5-HT$_{2A}$ receptor/PSD-95 association with an interfering peptide disrupts the 5-HT$_{2A}$ receptor/PSD-95 interaction may reflect long-term adaptive changes in PSD-95 knockout mice. In line with the antihyperalgesic effects produced by disruption of 5-HT$_{2A}$ receptor/PSD-95 interaction with a peptide, docking simulation studies identified a series of substituted indoles as potential small-molecule inhibitors of this interaction. One of them efficiently inhibited mechanical hyperalgesia in an experimental model of traumatic neuropathic pain in the rat (Vogrig et al., 2013). Collectively, these studies identify the 5-HT$_{2A}$ receptor/PSD-95 complex as a new therapeutic target for the treatment of neuropathic pain, which remains poorly controlled by currently available analgesics. Consistent with the inhibition of 5-HT$_{2A}$ receptor–operated signaling in PSD-95 deficient mice, the hallucinogenic-like effects of drugs are impaired in these mice (Abbas et al., 2009). Likewise, deletion of PSD-95 renders ineffective atypical antipsychotics such as clozapine, which act as 5-HT$_{2A}$ receptor inverse agonists, against PCP-induced disruption of prepulse inhibition, a widely used pharmacological model of schizophrenia (Abbas et al., 2009). Na$^{+}$/H$^{+}$ exchanger regulatory factor (NHERF) 3 is another PDZ protein that interacts with the 5-HT$_{2A}$ receptor but, surprisingly, in a PDZ-independent manner (Walther et al., 2015). NHERF3 negatively regulates 5-HT$_{2A}$ receptor endocytosis and positively influences 5-HT$_{2A}$ receptor–stimulated inositol phosphate formation (Walther et al., 2015).

Several 5-HT$_{2A}$ receptor–interacting proteins have been identified in addition to PDZ proteins. Targeting of 5-HT$_{2A}$ receptor to caveolae microdomains is ensured by its interaction with the multifunctional proteins caveolins (Allen et al., 2008). Moreover, association of the receptor with caveolins has a profound impact on receptor-operated signal transduction and functions; interaction of 5-HT$_{2A}$ receptor with caveolin-1 within lipid raft/caveolae membranes enhances receptor-mediated signal transduction by facilitating its coupling to G$_{q}$ protein in C6 glioma cells (Bhatnagar et al., 2004), whereas its interaction with caveolin-3, the predominant caveolin isoform in heart, upon 5-HT stimulation, negatively regulates receptor-mediated hypertrophy in cardiomyoblasts and neonatal cardiomyocytes (Mialet-Perez et al., 2012).

ARF1 and, to a lesser extent, ARF6, associate with the i$_{3}$ loop and Ct of the 5-HT$_{2A}$ receptor (Robertson et al., 2003). This association is enhanced by GTP loading and is essential for 5-HT$_{2A}$ receptor–mediated PLD activation, which seems to be independent of G$_{q11}$ (Robertson et al., 2003). 5-HT$_{2A}$ receptors also bind to the tyrosine kinase Jak2 in fetal myoblasts (Guillet-Deniau et al., 1997). This is accompanied by Jak2 autophosphorylation, the recruitment of the transcription factor signal transducer and activator of transcription (STAT)3, and its translocation to the nucleus (Guillet-Deniau et al., 1997). P90 ribosomal S6 kinase 2 (RSK2) interacts with 5-HT$_{2A}$ receptor i$_{3}$ loop and exerts a tonic break on receptor-operated signaling (Sheffler et al., 2006; Allen et al., 2008). RSK2 phosphorylates 5-HT$_{2A}$ receptor at Ser$^{314}$ located in the i$_{3}$ loop. Ser$^{314}$ phosphorylation underlies heterologous desensitization of the receptor elicited by growth factors (Allen et al., 2008; Strachan et al., 2009). A role of 5-HT$_{2A}$ receptors in symptoms associated with Coffin-Lowry syndrome (because of null mutations in RSK2 gene), such as moderate to severe mental retardation, movement disorders, and schizophrenia-like psychosis, in heterozygote females has been proposed (Allen et al., 2008). Calmodulin interacts in a Ca$^{2+}$–dependent manner with consensus binding motifs located in 5-HT$_{2A}$ receptor i$_{2}$ loop and Ct. This association inhibits receptor coupling to G$_{q}$ (Turner and Raymond, 2005).

4. 5-HT$_{2B}$ Receptor. The 5-HT$_{2B}$ receptor is mainly coupled to G$_{q}$ (see also Bockaert et al., 2006), Marin
et al. (2012) for detailed review of Gq/Gi-mediated signaling events elicited by this receptor.

A number of recognized signaling proteins interact with the 5-HT2B receptor, including constitutive and inducible NOS and the G proteins Gaq, Ga11, and Ga13. The 5-HT2B receptor also binds to the multivalent PDZ scaffold protein, MUPP1; this is in common with the other 5-HT receptors 5-HT2A and 5-HT2C. 5-HT2A and 5-HT2B receptors also bind MUPP1-PDZ domains in vitro and share the C-terminal -E-X-V/I-S-X-V sequence (Becamel et al., 2001). This PDZ motif is also required for the recruitment of the constitutive NO synthase cNOS (Manivet et al., 2000).

Ubiquitin E3 ligases (E3s) confer specificity to ubiquitination by recognizing target substrates. The ligand of numb protein X (LNX) family of E3s is a group of PDZ domain–containing RING-type E3 ubiquitin ligases. The substrate recognition mechanism of LNX E3s involves their specific PDZ domains by binding to the C termini of the target proteins. The human C-terminal LNX1 PDZ3-binding motifs of the 5-HT2B receptor promotes ubiquitination by LNX1ΔPDZ4 (Guo et al., 2012).

5. 5-HT2C Receptor. The 5-HT2C receptor activates phospholipase C (PLC) via Gq/11, PLD via G13, phospholipase A2 (PLA2), and Akt/glycogen synthase kinase (GSK)3β via G12 (Bockaert et al., 2006). 5-HT2C receptors also activate Erk1/2 signaling via a mechanism entirely independent of G proteins, but dependent of their interaction with both Ca2+/calmodulin and β-arrestin 2 (Labasque et al., 2008; Marin et al., 2012). As already mentioned, the receptor expresses a canonical class I PDZ ligand at its C-terminal extremity. MUPP1 was the first PDZ protein identified as a partner of the 5-HT2C receptor in 1998, using the yeast two hybrid system (Ullmer et al., 1998). MUPP1 contains 13 PDZ domains (it is the PDZ protein that contains the largest number of PDZ domains identified) and selectively interacts with the receptor via its 10th PDZ domain (Becamel et al., 2001). This interaction is dynamically regulated by agonist-dependent receptor phosphorylation at Ser458 located in the PDZ binding motif (Parker et al., 2003). The gene encoding MUPP1 (Mpdz) has been identified as a quantitative trait underlying physical depressive states (Svenningsson and Greengard, 2007) and postsynaptic localizations such as the ternary complex Vel13/CASK/Mint1, consistent with their different distribution at the synapse (Bécamel et al., 2004). 5-HT2C receptor PDZ partners differentially regulate receptor endocytosis and desensitization: PSD-95 increases both constitutive and agonist-induced receptor endocytosis and desensitization, whereas MPP3 stabilizes the receptor at the plasma membrane and prevents its desensitization (Gavarini et al., 2006). Note that PSD-95 has an opposite effect on 5-HT2A and 5-HT2C receptor endocytosis (Xia et al., 2003; Gavarini et al., 2006).

5-HT2C receptors also interact via their i3 loop with PTEN, a phosphatase that negatively controls the Akt/PKB signaling pathway and thus is a tumor suppressor (Ji et al., 2006). The 5-HT2C receptor/PTEN interaction was established in the VTA, the site of inhibition of the rewarding effects of many drugs of abuse by 5-HT2C receptor agonists. This inhibition is linked to the ability of receptors to prevent the increase in the firing rate of VTA dopaminergic neurons projecting to the nucleus accumbens elicited by drugs. Disrupting the 5-HT2C receptor-PTEN interaction with a cell-penetrating interfering peptide reduced the firing rate of VTA dopaminergic neurons and prevented rewarding effects of drugs of abuse such as THC and nicotine, suggesting that this interaction might be a relevant target for the treatment of drug addiction (Ji et al., 2006). 5-HT2C receptors also interact with protein kinase C interacting cousin of thioredoxin, a PKC theta-interacting protein with a thioredoxin homology domain involved in the regulation of the thioredoxin system (Bécamel et al., 2002). The functional consequence of this interaction remains to be established.

6. 5-HT1 Receptors. 5-HT1 receptors engage signaling pathways through multiple G proteins, especially G5 and in some cell lines G13, Gq, and Gi (for reviews, see Coupar et al. (2007), Bockaert et al. (2008)). In addition, 5-HT1 receptors stimulate Erk1/2 in a G protein– and β-arrestin–independent mechanism that requires activation of the tyrosine kinase Src constitutively associated with 5-HT1 receptor (Gill et al., 2005; Barthelet et al., 2007; Bockaert et al., 2011). This pathway is also implicated in the activation of PLCγ1-mediated inhibition of NHE-1 in intestinal epithelial cells (Gill et al., 2005). Furthermore, GRK5 interacts with the proximal Ct of 5-HT1 receptors and phosphorylates β-arrestin 1. This prevents Src activation and underlies desensitization of Src-dependent Erk1/2 activation by 5-HT1 receptors (Bockaert et al., 2008; Barthelet et al., 2009).

Like 5-HT1B receptors, 5-HT1 receptors interact with p11 via their i3 loop (Warner-Schmidt et al., 2009). p11 enhances receptor cell surface localization and signal transduction (Warner-Schmidt et al., 2009). Given the extensive data showing that p11 is downregulated in depressive states (Svenningsson and Greengard, 2007) and that activation of 5-HT1 receptors has antidepressant effects (Ge and Barnes, 1996; Bockaert et al., 2011),
this interaction might be of particular interest for the treatment of depression.

The 5-HT₄ receptor interacts, directly or indirectly, with the α-secretase ADAM10. This interaction might be an important step in the constitutive (agonist-independent) activation of nonamyloidogenic cleavage of amyloid precursor protein (APP) and release of soluble APPα (sAPPα) fragment elicited by the receptor (Cochet et al., 2013). sAPPα has neurotrophic and neuroprotective properties, and its release on constitutive (and agonist-dependent) receptor activation might contribute to receptor-mediated reduction of amyloid pathology observed in several mouse models of Alzheimer disease (Cochet et al., 2013; Tesseur et al., 2013; Pimenova et al., 2014; Claeyssen et al., 2015).

The 5-HT₄ receptor is one of the GPCRs for which alternative mRNA splicing generates the most variants that differ in their Ct, with some variants (a, e, and f) expressing canonical PDZ ligands, suggesting that these receptors may recruit specific GIPs, including different sets of PDZ partners. Ten proteins have been shown to interact with the Ct of the 5-HT₄(a) Receptor. Most of them are PDZ proteins that associate with its canonical class I PDZ motif (-SCF) (Joubert et al., 2004). These include SNX27a, a member of the sorting nexin family, which targets 5-HT₄(a) receptors to early endosomes in cell lines (Joubert et al., 2004). A more recent study showed that SNX27 associates with β₂-adrenergic receptor PDZ ligand and that this interaction is essential for PDZ-directed receptor recycling (Temkin et al., 2011). It also showed that SNX27 serves as an essential adaptor protein linking PDZ motif containing cargo to the retromer. SNX27 also directly interacts with endosomes through its lipid-binding phox homology (PX) domain. A similar role of SNX27a in endocytic 5-HT₄ receptor trafficking is likely. The interaction of the 5-HT₄(a) receptor with the PDZ protein NHERF promotes its recruitment to microvilli, where it localizes with activated ezrin, consistent with a role of 5-HT₄ receptors in cytoskeleton remodeling (Joubert et al., 2004). The Ct of the 5-HT₄(a) receptor also recruits CRMP2, a member of the collapsing response mediator protein (CRMP) family through a PDZ-independent mechanism (Joubert et al., 2004). 5-HT₄ receptors cause G13- and RhoA-dependent neurite retraction and cell rounding in neuroblastoma cells (Ponimaskin et al., 2002b). Their association with CRMP2 might also contribute to regulation of neuronal architecture.

Three GIPs associate with the class II PDZ ligand (-VPV) of the 5-HT₄(a) receptor. These include two PDZ partners, namely CIPP and nNOS, the only NOS isoform expressing a PDZ domain. The third protein that binds to the Ct of 5-HT₄(c) receptor is Sec23A, a protein of the COPII complex, which is likely involved in its trafficking from the ER to the Golgi (Joubert et al., 2004). The 5-HT₄(b) receptor interacts with the phosphodiesterases PDE3A1 and PDE4D3, which may thereby regulate receptor-operated cAMP signaling (Weninger et al., 2014).

7. 5-HT₇ Receptor. The 5-HT₇ receptor is coupled to Gs and G₁₂ (Bard et al., 1993; Ruat et al., 1993b). 5-HT₇ receptor stimulation promotes neurite outgrowth and SRE-mediated gene transcription through G₁₂-dependent activation of RhoA and Cdc42 GTPases (Kvachnina et al., 2005; Guseva et al., 2014b). The 5-HT₇ receptor Ct interacts with neurochondrin, a protein expressed in bone and brain that promotes neurite growth. Neurochondrin interacts with several GPCRs, including melanin-concentrating hormone receptor 1, orexin-1 and thromboxane A2 receptors, and the mGlut family metabotropic glutamate receptor (Ward et al., 2009; Marin et al., 2012). Generally, neurochondrin inhibits GPCR coupling to G proteins. The 5-HT₇ receptor Ct also recruits the intermediate filament perilplakin, which, like neurochondrin, inhibits receptor-operated Ca²⁺ signaling (Ward et al., 2009). The role of neurochondrin in neurite outgrowth elicited by 5-HT₇ receptor stimulation remains unexplored.

5-HT₇ receptors, via their i₃ loop, interact with S100B, a member of the S100 family, and S100B negatively regulates 5-HT₇ receptor-induced cAMP production (Stroth and Svenningsson, 2015). S100B seems to play a role in the antidepressant effects of fluoxetine (Baudry et al., 2010), and its serum concentration may serve as a biomarker predicting response to antidepressant treatment (Arölt et al., 2003). Notably, 5-HT₇ receptor antagonists have been reported to display rapid antidepressant action, making the receptor a promising target for antidepressants with improved onset of clinical efficacy compared with most of the currently available treatments (Mnie-Filali et al., 2011). Suggestive of a possible influence of 5-HT₇ receptor/S100B interaction in the pathogenesis of mood disorders and their treatment, transgenic female mice overexpressing S100B show depressive-like behavior that is normalized by administration of a 5-HT₇ receptor antagonist (Stroth and Svenningsson, 2015).

The human and rat genes coding for the 5-HT₇ receptors generate different splice variants (Heidmann et al., 1997). One of them (5-HT₇(b) receptor) is common to rat and human and expresses at its extreme Ct a canonical class II PDZ ligand (-FVL). To date, no PDZ partner of 5-HT₇(b) receptor has been identified.

C. 5-HT₆ Receptor Receptosome: Toward New Signaling Mechanisms Underlying Its Control of Cognition and Neurodevelopmental Processes

1. Introduction. The 5-HT₆ receptor has early been considered as a promising target for psychiatric diseases in line with its near exclusive expression in the central nervous system (Hirst et al., 2003). Although initial studies suggested that 5-HT₆ receptors are almost exclusively localized on GABA interneurons, more recent studies, including some in human brain,
indicate that they are also present on pyramidal glutamatergic neurons in prefrontal cortex and hippocampus (Woolley et al., 2004; Marazziti et al., 2013b). A more detailed study of 5-HT_6 receptor mRNA distribution showed highest expression in both D_1 and D_2 receptor–containing medium-size spiny neurons of caudate putamen and nucleus accumbens and confirmed consistent expression in glutamatergic neurons of hippocampus and cerebral cortex expressing vGluT1 (Helboe et al., 2015). It also showed the presence of 5-HT_6 receptor mRNA in a minor fraction of GABAergic cortical neurons, including mainly neurons coexpressing 5-HT_3A receptor and a subset of calbindin- and calretinin-positive neurons (Helboe et al., 2015). 5-HT_6 receptors thus display an ideal distribution to regulate the balance between excitatory and inhibitory signaling in brain regions implicated in cognition, which is altered in schizophrenia (Uhlhaas and Singer, 2010). The pharmacology of 5-HT_6 receptors includes a great number of ligands exhibiting agonist or antagonist activities. Some of them have shown putative interest for treating cognitive deficits, depression, anxiety, sleep, and feeding disorders as well as pain (Mitchell and Neumaier, 2005; Svenningsson et al., 2007; Fone, 2008; Ly et al., 2013; Wilkinson et al., 2014; Claeyssen et al., 2015; Karila et al., 2015; Pereira et al., 2015). Unfortunately, phase III clinical trial results for treating cognitive impairment have been disappointing. An increase in our understanding of how the cellular and molecular events associated with the 5-HT_6 receptor translate to behavioral responses may inform and support stratified medicine approaches.

The 5-HT_6 receptor is positively coupled to G_s and, like many GPCRs, activates Erk1/2 (Ruat et al., 1993a; Sebben et al., 1994) (Fig. 32). As G_s/cAMP and Erk1/2 pathways can have a positive influence on cognition, it was unlikely that their inhibition would mediate the procognitive effects of 5-HT_6 receptor antagonists. This suggested that alternative coupling mechanisms might be involved. Likewise, the cellular mechanisms controlling 5-HT_6 receptor functional activity remained largely unexplored until the use of unbiased strategies to identify 5-HT_6 receptor–interacting proteins. These included two-hybrid screens (Yun et al., 2007, 2010; Kim et al., 2014) and AP-MS proteomic strategies based on different methods to purify receptor partners, such as coimmunoprecipitation (Meffre et al., 2012) or pull-down assays using particular receptor sequences as baits (Duhr et al., 2014; Ha et al., 2015).

2. Fine-Tuning of 5-HT_6 Receptor Trafficking and Signal Transduction by GIPs. The nonreceptor tyrosine kinase Fyn was the first GIP discovered for 5-HT_6 receptor, thanks to a two-hybrid screen using the receptor Ct as bait (Fig. 32). Fyn is a member of the Src family of tyrosine kinases that are highly expressed in brain and involved in synaptic plasticity. Several lines of evidence suggest that Fyn might be implicated in Alzheimer disease pathogenesis: Fyn phosphorylates Tau at Tyr^18 and controls its association with microtubules (Lee et al., 2004); it has also been involved in Aβ-induced synaptic deficits and neurotoxicity (Yang et al., 2011). In addition, Fyn plays a key role in neurodevelopmental processes: in combination with Src, Fyn controls cortical lamination, the formation of...
the Purkinje cell plate, and, thus, neuronal migration (Kuo et al., 2005). It is involved in netrin-1–mediated axon attraction of cortical neurons via the phosphorylation of Trio, a Rho/Rac-GEF (DeGeer et al., 2013), and in the maturation of GABAergic synapses elicited by neural cell adhesion molecule (Chattoerpadhyaya et al., 2013). Fyn binds to 5-HT6 receptor Ct (likely to the conserved polyproline sequence PXhPXR, where X represents any amino acid and h any hydrophobic amino acid) via its SH3 domain (Yun et al., 2007). Functional studies showed that Fyn increases 5-HT6 receptor cell surface localization and, consequently, receptor-operated G protein signaling (Yun et al., 2007). Conversely, 5-HT6 receptor activation triggers Fyn phosphorylation, as assessed by an increase in Tyr^{Tyr} phosphorylation, an event contributing to 5-HT6 receptor–induced activation of the Ras/Erk1/2 pathway (Fig. 32) (Yun et al., 2007). Whether 5-HT6 receptor–mediated Fyn activation is involved in neuronal migration, attraction, and maturation of GABAergic synapses remains to be elucidated.

Further two-hybrid screens identified two other 5-HT6 receptor–interacting proteins that control receptor trafficking. The first one is Jun activation domain–binding protein (Jab1), a protein initially isolated as a c-Jun and Jun D binding partner that stabilizes their binding to activator protein 1 (AP-1) sites and potentiates them as transcription factors (Claret et al., 1996; Yun et al., 2010). Jab1 interacts with both i3 loop and Ct of the receptor (Yun et al., 2010). Jab1 stabilizes surface expression of 5-HT6 receptor and is necessary to maintain activity of endogenous receptors. In turn, 5-HT6 receptor stimulation increases nuclear translocation of Jab1, suggesting that they may play a role in the regulation of gene expression via Jab1 (Fig. 32) (Yun et al., 2010). Furthermore, 5-HT6 receptors and Jab1 are upregulated following middle cerebral artery occlusion–induced focal cerebral ischemia in rats. Likewise, exposure of cultured cells to hypoxic insults increased expression of both protein partners, which in turn reduce cell death induced by hypoxia (Fig. 32) (Yun et al., 2010). The second protein identified is light chain 1 (LC1) subunit of MAP1B protein (MAP1B-LC1), a microtubule-associated protein highly expressed in the brain (Kim et al., 2014). MAP1B-LC1 interacts with the Ct of the receptor and reduces receptor endocytosis, thereby increasing its cell surface expression and signal transduction activity (Gα coupling and Erk1/2 activation) (Kim et al., 2014).

The 5-HT6 receptor i3 loop recruits several proteins of the endocytic machinery, such as dynamin, AP-2, amphiphysin, and epsin as well as SNX14 (Ha et al., 2015). Like SNX27, SNX14 belongs to the sorting nexin family, predicted to have a role in protein sorting and vesicular trafficking (Carlton et al., 2005). It contains a putative RGS domain and a phox homology (PX) domain, an N-terminal hydrophobic region, and a PX-associated domain of unknown function. SNX14 is expressed at high levels in the nervous system (Carroll et al., 2001) and plays a critical role in both excitatory and inhibitory transmissions (Huang et al., 2014). SNX14 was found to inhibit 5-HT6 receptor–dependent signaling via two different mechanisms: 1) it specifically binds to and sequesters Gαs, thus inhibiting the Gs-cAMP pathway; and 2) it promotes 5-HT6 receptor endocytosis and degradation (Fig. 32) (Ha et al., 2015). Furthermore, PKA phosphorylates SNX14 at two serine residues located in the RGS domain. SNX14 phosphorylation strongly reduces its affinity for Gαs and thereby prevents Gαs sequestration. Accordingly, phosphorylated SNX14 preferentially associates with 5-HT6 receptor and can thereby enhance receptor internalization (Ha et al., 2015). Therefore, SNX14 has been considered as a dual, sequential, negative regulator of 5-HT6 receptor–operated signaling, first by sequestering Gαs and second by inducing receptor endocytosis (Ha et al., 2015). Collectively, these findings indicate that 5-HT6 receptor functional activity is finely modulated by its association with GβγPs that exert contrasting effects on receptor trafficking and receptor-mediated signal transduction.

3. Recruitment of mTOR Complex 1 by 5-HT6 Receptor: Potential Role in Cognitive Deficits Associated with Schizophrenia. Using a proteomics strategy combining coimmunoprecipitation of full-length receptor and tandem mass spectrometry, Meffre et al. (2012) identified 28 proteins that specifically associate with the 5-HT6 receptor. This “receptosome” showed a remarkable enrichment in proteins of the mTOR pathway (Meffre et al., 2012). These include mTOR itself; Raptor (regulatory associated protein of TOR), an activator of mTOR that together with mTOR and proline-rich Akt substrate of 40 kDa constitutes the mTOR complex 1 (mTORC1); the small GTPase Rheb (Ras homolog enriched in brain), which directly activates mTOR when bound to GTP; the Tti1/Tel2 complex, required for assembly and stability of mTOR complexes; and the Ras GTPase activating protein neurofibromin, an upstream negative regulator of the pathway leading to mTOR activation (Fig. 33) (Meffre et al., 2012). This “receptosome” also includes Vps34, a protein of the class III PI3K family implicated in autophagosome formation (Bockaert and Marin, 2015). In line with this remarkable enrichment of the 5-HT6 receptor complex in proteins of the mTOR pathway, agonist stimulation of the receptor results in the activation of the mTOR pathway both in a transfected cell line and in rodent brain, particularly in prefrontal cortex and striatum (Meffre et al., 2012). Notably, 5-HT6 receptor–elicited mTOR activation depends on both its physical association with mTOR and the canonical PI3K/Akt/TSC/Rheb pathway involved in activation of mTORC1 by tyrosine kinase receptors (Fig. 33) (Meffre et al., 2012). An overactivation of mTOR is observed in many genetic diseases in which mental retardation or
Cognitive deficits are observed [for a review, see Bockaert and Marin (2015)]. One classic example is tuberous sclerosis (TSC) caused by mutations in the \textit{TSC1} or \textit{TSC2} genes, which encode proteins of the TSC complex, namely, hamartin and tuberin, respectively. The TSC complex is the main GTPase activating protein for Rheb. Mutations in \textit{TSC1} or \textit{TSC2} result in inactivation of TSC, a process leading to nonphysiologic mTOR activation. About 50% of TSC patients show intellectual disabilities as well as deficits in memory, attention, and executive functions, and 20%–60% display ASD (Bockaert and Marin, 2015). Moreover, the mTORC1 inhibitor rapamycin rescues cognitive deficits in a mouse model of TSC (\textit{Tsc2}^{1/2} mice). Likewise, systemic administration of rapamycin prevents deficits in social cognition and novel object recognition induced by a 5-HT_{6} receptor agonist in rats treated with pilocarpine, a model of temporal lobe epilepsy (Wang et al., 2014). mTOR is also overactivated in the pilocarpine model, and a 5-HT_{6} receptor antagonist prevents mTOR activation, increases latency of seizures, and reduces their severity (Wang et al., 2014). This suggests that blocking the 5-HT_{6}/mTOR pathway might be a valuable therapeutic strategy for epilepsy treatment.

4. Recruitment of Cyclin-Dependent Kinase 5 by 5-HT_{6} Receptor: An Essential Step in Its Control of Neuronal Migration and Differentiation. The 5-HT_{6} receptor is implicated in early steps of brain development, including neurulation and neuronal migration within the cortex (Jacobshagen et al., 2014; Dayer et al., 2015). Although the cAMP pathway contributes to the control of neuronal migration by 5-HT_{6} receptors, a PKA inhibitor only partially reversed the effect of its stimulation upon neuronal migration (Riccio et al., 2009),

![Diagram showing engagement of Cdk5 and mTOR signaling pathways by 5-HT_{6} receptor and their role in neurodevelopmental processes and cognition.](image-url)
suggesting that other signaling pathways might be involved. Cyclin-dependent kinase (Cdk)5, which has been identified as a 5-HT<sub>6</sub> receptor partner by different AP-MS proteomics strategies (Fig. 33; Meffre et al., 2012; Duhr et al., 2014), was an obvious candidate. Indeed, Cdk5 is known to control actin cytoskeleton dynamics and various neurodevelopmental processes, such as neuronal migration (including migration of cortical pyramidal neurons), neurite growth, and synapse morphogenesis (Jessberger et al., 2009; Lalioti et al., 2010). Moreover, the 5-HT<sub>6</sub> receptor recruits, via its Ct, not only Cdk5 and its activator p35 but also a network of proteins functionally connected with Cdk5, including Wiskott-Aldrich syndrome protein–family verprolin homologous protein 1 (WAVE-1) and G protein–inducer of neurite growth 1, two Cdk5 substrates; phosphatase 2A, which dephosphorylates and activates WAVE-1; and the Arp2/3 complex, which is also known to be activated by WAVE-1 (Duhr et al., 2014). Several lines of evidence indicate a role of Cdk5, under the control of the 5-HT<sub>6</sub> receptor, in the migration of pyramidal neurons: in utero electroporation of a 5-HT<sub>6</sub> receptor short hairpin RNA at E14.5 induces a mispositioning of these neurons, which can be rescued by electroporation of plasmids encoding wild-type 5-HT<sub>6</sub> receptor or 5-HT<sub>6</sub> receptors unable to couple to G<sub>s</sub> or to bind to 5-HT (Jacobshagen et al., 2014). This indicates that the effect of 5-HT<sub>6</sub> receptors on pyramidal neuron migration is agonist-independent and is not mediated by the G<sub>s</sub>-adenylyl cyclase pathway. Defect in migration elicited by silencing 5-HT<sub>6</sub> receptor expression is rescued by electroporation of plasmids expressing Cdk5 and its activator p35 (Jacobshagen et al., 2014). Moreover, 5-HT<sub>6</sub> receptor knockdown significantly reduces phosphorylation of doublecortin (at Ser<sup>297</sup>) and focal adhesion kinase (at Ser<sup>735</sup>) in primary cortical neurons, two Cdk5 substrates known to control migration of pyramidal neurons (Xie et al., 2003; Tanaka et al., 2004). Collectively, these findings indicate that 5-HT<sub>6</sub> receptors control migration of cortical pyramidal neurons through an agonist-independent, Cdk5-dependent mechanism (Jacobshagen et al., 2014; Dayer et al., 2015).

Beyond neuronal migration, the 5-HT<sub>6</sub>/Cdk5 complex also controls neurite outgrowth and neuronal differentiation (Fig. 33). Its role in neurite growth was not only established in neuroblastoma-glioma NG108-15 cells, a commonly used cellular mode for investigating mechanisms underlying neuronal development, but also in primary neurons and brain explants (Duhr et al., 2014; Seo and Tsai, 2014). Reminiscent of its control of neuronal migration, the growth-promoting effects of the receptor is G<sub>i</sub>– and agonist-independent. They are reversed by the selective 5-HT<sub>6</sub> receptor antagonist SB258585, which thus behaves as an inverse agonist in this model (Duhr et al., 2014). Likewise, SB258585 inhibits Cdk5 association with the 5-HT<sub>6</sub> receptor, indicating a specific recruitment of Cdk5 by a constitutively active receptor conformation. 5-HT<sub>6</sub> receptor–elicited neurite growth also depends on receptor phosphorylation at Ser<sup>350</sup> by associated Cdk5. This suggests a reciprocal interplay between 5-HT<sub>6</sub> receptor and Cdk5, whereby the receptor stimulates Cdk5 activity and is itself a Cdk5 substrate. The signaling events downstream of the 5-HT<sub>6</sub>/Cdk5 complex contributing to neurite growth have been partially characterized and involve Cdk5-dependent activation of the Rho GTPase cell division cycle (Cdc42), a key regulator of actin cytoskeleton dynamics (Fig. 33) (Duhr et al., 2014). The precise mechanisms that control 5-HT<sub>6</sub> receptor/Cdk5 interaction, including the potential influence of other receptor partners, and the cellular events downstream of the 5-HT<sub>6</sub>/Cdk5/Cdc42 pathway remain to be explored. As already mentioned, the regulatory role of Cdk5 during brain development extends to synapse formation. Whether 5-HT<sub>6</sub> receptor–dependent activation of Cdk5 controls synaptogenesis in addition to neuron migration and shaping also remains to be investigated.

**XVIII. 5-HT Receptors and the Brain**

**A. Introduction**

The diversity of 5-HT receptor signaling pathways and associated functional complexity in the brain is greater than any other tissue or organ. For historical perspectives on the contribution of neurophysiology, pharmacology, and molecular biology to the discovery of 5-HT receptor subtypes in the brain, see reviews in Bradley et al. (1986), Barnes and Sharp (1999), and Bockaert et al. (2010).

**B. 5-HT Receptor Signaling in Neurons**

All 5-HT receptor subtypes found in the brain are also found in the periphery except 5-HT<sub>1e</sub>, 5-HT<sub>2C</sub>, and 5-HT<sub>6</sub> receptors, for which there is little evidence for functional expression outside the CNS. The signaling properties of 5-HT receptors in the brain are identical to those in the periphery. Thus, the metabotropic 5-HT receptors couple to the three canonical signaling pathways, namely G<sub>i</sub>, G<sub>q</sub>, and G<sub>v/11</sub>, which elicit the expected second messenger cascades, and this is proven to occur in neurons in most cases.

The G<sub>i</sub>-coupled 5-HT receptors that inhibit adenylyl cyclase and cause a fall in cAMP encompass the 5-HT<sub>1</sub> receptor family, comprising 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub>, and 5-HT<sub>1F</sub> subtypes. Evidence suggests that the 5-HT<sub>5</sub> receptor family, 5-HT<sub>5A</sub> and 5-HT<sub>5B</sub>, is also G<sub>i</sub>-coupled, although, to date, this has only been demonstrated in cultured cells and not native neurons. Excellent reviews on the signaling (Hannon and Hoyer, 2008; Bockaert et al., 2010; McCorry and Roth, 2015) and consequent electrophysiological (Lamb and Aghajanian, 2006; Andrade and Beck, 2010; Marek,
properties of 5-HT₁ receptors can be found elsewhere.

Independent of the involvement of a decrease in cAMP, 5-HT₁ receptors also open GIRKs to hyperpolarize neurons and inhibit the opening of voltage-gated calcium channels; this is best understood for 5-HT₁ₐ receptors (e.g., Montalbano et al., 2015). 5-HT₁ receptors are also likely to elicit other (noncanonical) G protein–dependent signals, including via ERK and Akt kinase, again as exemplified by studies of the 5-HT₁ₐ receptor. Adding further complexity, it is well established that 5-HT₁ₐ receptors have different properties depending on their pre- or postsynaptic localization. In particular, agonists tend to have greater efficacy at presynaptic 5-HT₁ₐ receptors, and the latter have greater tendency to desensitize than postsynaptic 5-HT₁ₐ receptors. The reasons for these differences are uncertain but, as discussed elsewhere (Clarke et al., 1996; Barnes and Sharp, 1999; Mannoury la Cour et al., 2006; Garcia-Garcia et al., 2014), could relate to presynaptic versus postsynaptic differences in 5-HT₁ₐ receptor reserve, efficiency of G protein coupling, non–G protein–dependent signaling, or even biased agonism—that is, the generation of signals that are ligand-dependent. Regarding the latter, emerging data strongly support the view that selective 5-HT₁ₐ receptor agonists can preferentially direct receptor signaling to particular intracellular pathways in a brain region–specific manner (Becker et al., 2016).

The 5-HT₂ receptor family, 5-HT₂ₐ, 5-HT₂₅, and 5-HT₂₇, comprise Gq₁₁-coupled receptors that activate phospholipase C, leading to increased formation of inositol trisphosphate and diacylglycerol and then mobilization of intracellular calcium and PKC activation. 5-HT₂ receptor stimulation also results in neuronal excitation in a variety of brain regions, likely involving the closure of potassium channels. A 5-HT₂ receptor–mediated increase in excitatory postsynaptic currents is also often observed, which is thought to be mediated either directly or indirectly through increased release of glutamate (Lambe and Aghajanian, 2006; Marek, 2010).

It is now recognized that 5-HT₂ receptor signaling goes well beyond activation of the phospholipase C/PKC pathway. For instance, noncanonical signaling pathways associated with 5-HT₂ₐ receptor activation include phospholipase A₂, the ERK pathway, and signaling via small G proteins (e.g., Rho A and Rab4), and there are similar such findings for 5-HT₂₅ and 5-HT₂₇ receptors (for review, see Bockaert et al. (2010), Halberstadt (2015), McCorry and Roth (2015), Maroteaux et al. (2017)). Interesting findings are emerging regarding noncanonical signaling by 5-HT₂ receptors. Specifically, the 5-HT₂₇ receptor is able to activate ERK via a G protein–independent mechanism through recruitment of calmodulin and β-arrestin (Labasque et al., 2008). Furthermore, recent studies on noncanonical signaling by the 5-HT₂₅ receptor found evidence of ligand-dependent signals and then used the crystal structure of the receptor to identify high-resolution, structural basis for the biased signaling (Wacker et al., 2013, 2017a,b). This evidence of ligand-dependent signaling through 5-HT₂ receptors has relevance to the psychotropic effects of 5-HT₂ₐ receptor agonists, some of which are hallucinogenic but not all (Gonzalez-Maeso et al., 2007), and may also explain the long-lasting effects of LSD that are difficult to explain on the basis of pharmacokinetics alone (Wacker et al., 2017). However, the signaling pathways mediating the characteristic effects of hallucinogens have not been identified conclusively. Biased agonism at 5-HT₂ receptors offers intriguing possibilities for therapeutic potential (e.g., avoidance of hallucinogenic properties), although this is yet to be explored, keeping in mind that 5-HT₂ₐ receptor agonists have profound effects on vascular and other smooth muscle in addition to their psychotropic effects. Also, findings of the existence of constitutive activity at 5-HT₂ₐ and 5-HT₂₇ receptors, and 5-HT₂ receptor ligands with inverse agonist properties, provides further avenues for 5-HT₂ receptor drug development (e.g., Aloyo et al., 2009).

The remaining metabotropic 5-HT receptors to be considered, specifically 5-HT₄, 5-HT₆, and 5-HT₇ receptors, are Gₛ₄ coupled and thus activate adenyl cyclase to increase cAMP. At the electrophysiological level, 5-HT₄ receptor activation on neurons is associated with a slow membrane depolarization mediated by a reduction of a slow after-hyperpolarization potential and facilitation of L-type calcium channels through protein kinase A activation (Andrade and Chaput, 1991; Birnstriel and Beck, 1995). Similarly, the neuronal 5-HT₇ receptor is also frequently associated with a direct depolarizing or excitatory effect, also likely mediated by actions on slow after-hyperpolarization and L-type calcium channels (Bacon and Beck, 2000; Andrade, 2006).

As with the other metabotropic 5-HT receptors, the 5-HT₄ receptor generates a diversity of signals independent of the second messenger. In addition, both the 5-HT₄ and 5-HT₆ receptors provide further examples of metabotropic 5-HT receptors that are capable of noncanonical signaling. For example, 5-HT₄ receptor stimulation activated ERK in cultured cells and neurons in a manner that was G protein–independent (Gₛ₄, Gₛ₆, Gₒ) but dependent on Src tyrosine kinase (Barthet et al., 2007; Bockaert et al., 2010), and there are analogous observations for 5-HT₆ receptors (Yun et al., 2007).

As a complement to the slow signaling elicited by metabotropic 5-HT receptors, the ionotropic 5-HT₃ receptors mediate rapid synaptic transmission. All five human 5-HT₃ receptors (5-HT₃A–5-HT₃E) are homologous with other members of the Cys-Cys loop ligand-gated channel superfamilies (e.g., nicotinic, GABAₐ, glutamate, or glycine receptors) (Barnes et al., 2009). Although there is uncertainty regarding the composition of the native 5-HT₃ receptors in the brain, pentameric coassemblies of 5-HT₃A and 5-HT₃B subunits is
a likely common occurrence [for reviews, see Niesler (2011) and Thompson and Lummis (2013)]. Allosteric modulators of the 5-HT3 receptor are emerging to add to the possibilities for 5-HT3 receptor manipulation for therapeutic benefit (e.g., Trattning et al., 2012 and Newman et al., 2013).

Overall, the complexity of neuronal signaling events elicited by 5-HT receptors is yet to be fully revealed. The metabotropic 5-HT receptors are clearly capable of both G protein–dependent and G protein–independent signaling, and it is also clear that the divergent signals generated by these receptors can be biased according to the agonist used. Moreover, as covered elsewhere, further complexity arises from the large number of protein partners influencing cellular localization and trafficking as well as signal tuning of 5-HT receptors, their homo- or heterodimerization with other GPCRs, and the alternative RNA splicing and editing of certain 5-HT receptors (especially 5-HT2C, 5-HT4, and 5-HT7 receptors) to generate variability in function (Hannon and Hoyer, 2008; Bockaert et al., 2010; McCorvy and Roth, 2015).

C. Expression of 5-HT Receptors in the Brain

Application of a combination of techniques such as receptor autoradiography, in situ hybridization, and immunocytochemistry has revealed maps of 5-HT receptor subtype distribution in the brain that are largely similar across a range of vertebrate species (although of note, the 5-ht1a receptor is not expressed in rodents, forebrain 5-HT3 receptors are differentially expressed across species, and the full-length 5-ht5b receptor is not expressed in man). The use of BAC transgenic mice engineered to express a fluorescent or colored reporter genes under the control of specific 5-HT receptor promoters, such as in the case of 5-HT2A receptor (Weber and Andrade, 2010), reveals further anatomic detail of 5-HT receptor distribution. Although the 5-HT receptor maps are largely based on data collected from the rodent brain, a high-resolution PET atlas of four 5-HT receptors (5-HT1A, 5-HT1B, 5-HT2A, and 5-HT4 receptors) and the 5-HT transporter was recently described for the human brain (Beliveau et al., 2017). It is now clear from these mapping studies that each 5-HT receptor subtype has a unique distribution pattern in the brain but one that often overlaps with that of other 5-HT receptor subtypes. These differential distribution patterns suggest that different 5-HT receptor subtypes are likely associated with distinct CNS functions amenable to manipulation using 5-HT receptor subtype selective pharmacological agents.

D. Presynaptic 5-HT Receptors

Two of the 14 5-HT receptors are highly expressed presynaptically (i.e., expressed by 5-HT neurons themselves). Thus, 5-HT1A receptors are located on the soma and dendrites of 5-HT neurons, whereas 5-HT1B receptors are expressed at the soma but then trafficked down the axons to the terminals (Riad et al., 2000). It is firmly established from both in vitro and in vivo models that 5-HT1A and 5-HT1B receptors function as autoreceptors and exert direct inhibitory control over the firing of 5-HT neurons and terminal 5-HT release, respectively. Thus, studies over 40 years ago finding that 5-HT itself, or nonselective 5-HT agonists such as LSD, inhibited the efflux of preloaded radiolabeled 5-HT from rodent brain slice preparations were followed up by extensive pharmacological analysis that identified that this effect was mediated by 5-HT1B receptors located on 5-HT nerve terminals (Gothert and Weinheimer, 1979; Mounsey et al., 1982; Engel et al., 1986; Fink and Gothert, 2007). Similarly detailed pharmacological studies using electrophysiological recordings of 5-HT neurons and in vivo microdialysis measurements of 5-HT release characterized the autoreceptor function of somatodendritic 5-HT1A receptors (e.g., Vandermaelen and Aghajanian, 1983; Sharp et al., 1989; and Adell and Artigas, 1998).

There are more recent reports of the presence of other 5-HT receptor subtypes in the midbrain raphe nuclei, particularly 5-HT1D, 5-HT1F, 5-HT2B, 5-HT2C, and 5-HT6A. However, with the exception of 5-HT2C (located on raphe GABAergic interneurons and not 5-HT neurons), expression levels of these receptors in the midbrain raphe are low, and their functional significance in terms of 5-HT neuron control is not certain. Nevertheless, recent findings demonstrate that feedback control of 5-HT neurons is not likely limited to 5-HT1 autoreceptors; instead, it includes 5-HT receptors located on postsynaptic targets that have the physiologic effects of 5-HT autoreceptors but use additional 5-HT receptor subtypes and operate via neural inputs to 5-HT neurons. For example, evidence supports a role for postsynaptic 5-HT1A, 5-HT2A, and 5-HT2C receptors in the inhibitory control of 5-HT neurons, whereas 5-HT4 and 5-HT6 receptors are excitatory in this regard (Ge and Barnes, 1996; Sharp et al., 2007; Sharp, 2010; Brouard et al., 2015). Many of these feedback pathways appear to be localized on cortical pathways projecting back to the midbrain raphe nuclei (5-HT1A, 5-HT2A, 5-HT4, and 5-HT6 receptors), although contributions from non-5-HT neurons such as habenula inputs to the raphe (5-HT2C receptors) further emphasize the complexity of 5-HT neuron control.

E. Postsynaptic 5-HT Receptors

5-HT receptor mapping studies demonstrate that the majority of 5-HT receptor subtypes are located postsynaptically (i.e., expressed by non-5-HT neurons, sometimes referred to as heteroreceptors, including the receptor subtypes involved in feedback control of 5-HT neurons) and that each 5-HT receptor subtype has an expression pattern that is distinct but often overlaps with that of other 5-HT receptors. Even 5-HT receptors...
from the same family have different CNS distributions (e.g., 5-HT2A vs. 5-HT2C and 5-HT1A vs. 5-HT1B). Some 5-HT receptors are expressed at higher levels than others; in particular, 5-HT1A and 5-HT2A receptors are among the most abundant, whereas in comparison, levels of 5-HT1D, 5-HT3, 5-HT1F, 5-HT2B, and 5-HT5A receptors are much less abundant. Detailed maps of 5-HT receptor binding sites and protein and mRNA distribution in the brain are reported in a number of review articles (Mengod et al., 2006, 2010; Palacios, 2016).

At the macrolevel, and in keeping with the widespread 5-HT innervation of the brain, 5-HT receptors of different types are expressed in many brain regions that play key roles in numerous CNS functions. For example, regions rich in 5-HT receptors include cortical and limbic areas (5-HT1A, 5-HT2A/2C, 5-HT3, 5-HT4, and 5-HT6 receptors), the basal ganglia (5-HT1B, 5-HT4, and 5-HT6 receptors), mesolimbic pathways (5-HT1B, 5-HT2A/2C, and 5-HT4 receptors), hypothalamus (e.g., paraventricular and arcuate nuclei; 5-HT2C receptor), suprachiasmatic nucleus (5-HT7 receptor), trigeminal nucleus (5-HT1B/1D receptors), dorsal vagal complex (encompassing the area postrema and nucleus tractus solitarius; 5-HT3 receptor), and the spinal cord (dorsal root ganglia; 5-HT2B/2C and 5-HT3 receptors). This list depicting 5-HT receptor distribution is of course not exclusive and does not take into account the relative density of receptors in the different regions [e.g., in many regions, 5-HT2C receptors are present at much lower densities than 5-HT2A receptors (with the remarkable exception of the choroid plexus), and this is also the case for 5-HT1D compared with 5-HT1B receptors].

F. Cellular Localization of Central Nervous System 5-HT Receptors

The principal cellular location of CNS 5-HT receptors is neurons; indeed, evidence for the expression of 5-HT receptors by adult native nonneural cells such as glial cells is, at best, inconsistent. For example, reports of glial cell expression of 5-HT1A and 5-HT2A receptors appear to depend on the antibody used. However, emerging evidence suggests that activated microglia may express 5-HT2B and other 5-HT receptors (Krabbe et al., 2012; Kolodziejczak et al., 2015). Nevertheless, 5-HT receptors are undoubtedly present in cells of the cerebrovasculature, as most evident for 5-HT1B receptors (Riad et al., 1998).

On the whole, the expression of specific 5-HT receptors is not restricted to particular neuron types. For example, among the complex microcircuitry of the cerebral cortex, hippocampus, and amygdala, 5-HT1A and 5-HT2A receptors are expressed both on pyramidal neurons (glutamatergic) and certain classes of GABAergic interneurons (particularly those expressing parvalbumin). Elsewhere in the brain, 5-HT1A receptors are localized on 5-HT neurons in the raphe nuclei (see above) and cholinergic neurons in the septum, whereas 5-HT2A receptors are expressed by midbrain dopamine neurons. On the other hand, 5-HT3 receptors in rodent cortex and hippocampus mark a specific population of GABA interneurons with distinct chemical, morphologic, and anatomic properties (Lee et al., 2010), and these receptors are also present in pyramidal neurons, particularly in humans (Brady et al., 2007). In comparison, cortical 5-HT6 receptors are preferentially (although not exclusively) expressed by pyramidal cells (Helboe et al., 2015).

Not surprisingly, there are multiple interactions between 5-HT and other neurotransmitter systems, and particular attention has been paid to 5-HT interactions with the other monoamines, noradrenaline and dopamine. These interactions are highly complex, involving multiple 5-HT receptor subtypes located at presynaptic and postsynaptic sites within complicated, overlapping circuitry, such that prediction of the overall effect of 5-HT is often not possible. However, at the level of individual receptors, the rules can be more straightforward. For example, the distribution of 5-HT2A and 5-HT7 receptors within the mesolimbic dopamine system has allowed the general consensus that 5-HT2A receptors stimulate and 5-HT7 receptors inhibit dopamine transmission (Howell and Cunningham, 2015).

Given the differential expression patterns of the 5-HT receptors, it is highly unlikely that all 14 5-HT receptors are expressed by a single neuron. Evidence from earlier double- and triple-labeling in situ hybridization studies support the idea that a single postsynaptic synapse may express a combination of two to three 5-HT receptors (Mengod et al., 2010). Studies combining whole-cell patch clamp recordings with single-cell reverse transcriptase polymerase chain reaction (RT-PCR) to characterize 5-HT receptor expression are in keeping with this idea. Thus, analysis of the 5-HT receptor mRNA content of neurons of the bed nucleus of the stria terminalis revealed that individual neurons could be subdivided according to the prominent expression of two to three distinct 5-HT receptor transcripts in largely excitatory/inhibitory combinations (e.g., 5-HT1A/5-HT7, 5-HT3/5-HT7, 5-HT1B/5-HT4, 5-HT1A/5-HT1B/5-HT2A, and 5-HT1A/5-HT2A receptors) that matched electrophysiological observations (Hazra et al., 2012). A similar analysis of the 5-HT receptor mRNA content of single neurons of the preoptic nucleus revealed two types of neurons that predominately expressed a combination of inhibitory (5-HT1A) and excitatory (5-HT2A) 5-HT receptors, whereas others expressed excitatory 5-HT receptors alone, again in keeping with electrophysiological findings (Sangare et al., 2016). This evidence of 5-HT receptor expression conferring both excitatory and inhibitory signaling effects of 5-HT at the single-cell level is born out more generally in electrophysiological experiments recording the effects of either bath application of 5-HT or electrical and optogenetic
activation of 5-HT neurons (Andrade, 2006; Andrade and Beck, 2010; Sengupta et al., 2017).

Generally speaking, there is a very good match between 5-HT receptor mRNA and protein (e.g., Mengod et al., 2010), suggesting that the majority of 5-HT receptor proteins are not trafficked significantly along axons and reside largely at the levels of the neuronal soma and dendrites. Notable exceptions to this rule are 5-HT_{1B} and 5-HT_{3} receptors. The former are trafficked either to nerve terminals of 5-HT neurons where they function as autoreceptors or to nerve terminals of other neurons and especially GABA neurons where they modulate GABA release. On the other hand, it is estimated that about 70%–80% of the 5-HT_{3} receptors are located on nerve endings, where their role is also to modulate neurotransmitter release (e.g., dopamine, cholecystokinin, glutamate, acetylcholine, and GABA) (Hannon and Hoyer, 2008; Walstab et al., 2010). A final point of note is that, unusually, 5-HT_{6} receptors are expressed on neuronal primary cilia (Hamon et al., 1999), the functions of which remain obscure.

At the ultrastructural level, electron microscopy studies show that all metabotropic 5-HT receptors visualized thus far are located extrasynaptically, which has reinforced the idea that 5-HT principally signals via volume transmission (Descarries et al., 2006). However, synaptic localization of 5-HT receptors cannot be excluded, as these are challenging studies that are often limited by the properties and specificity of available antibodies. Moreover, it is beyond doubt that 5-HT is present in vesicles that are synaptic as well as nonsynaptic. An additional complexity in signaling at 5-HT synapses is cotransmission, with coreleased glutamate emerging as a key player (e.g., Sengupta et al., 2017).

G. Behavioral Roles of 5-HT Receptors in the Brain

1. Introduction. Knowledge of the regional and cellular localization of 5-HT receptors in the brain has been immensely helpful in understanding a vast literature on the behavioral effects of pharmacological and genetic manipulation of 5-HT receptors. Collectively, these developments are casting light on the likely behavioral functions of 5-HT receptors. So far, no behavioral response can be confidently ascribed to activation of the CNS 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, or 5-HT_{5} receptors.

2. 5-HT_{1} Receptor Family.
   a. 5-HT_{1A} receptors. The behavioral functions of 5-HT_{1A} receptors have been the focus of much research given the widespread and high CNS expression of these receptors, combined with the availability of a large number of selective 5-HT_{1A} receptor ligands. It has long been known that in rodents, administration of 8-OH-DPAT and other 5-HT_{1A} receptor agonists causes a wide range of behavioral and physiologic effects, including changes in motor function (especially induction of the 5-HT behavioral syndrome), hyperphagia, hypothermia, altered sexual behavior, and changes in pain threshold. Also, there is a large literature demonstrating that 5-HT_{1A} receptor agonists have antidepressant and anxiolytic activity and influence a range of cognitive domains relevant to symptoms of schizophrenia (for reviews, see Traber and Glaser, 1987; Handley, 1995; and Newman-Tancredi, 2010). These findings accord with preclinical and clinical evidence implicating changes in 5-HT_{1A} receptors in the pathophysiology of a variety of psychiatric illnesses, including depression, anxiety and other stress-related disorders, and schizophrenia. Appropriate 5-HT_{1A} receptor function also appears critical for antidepressant drug efficacy (Richardson-Jones et al., 2010; Samuels et al., 2016). The diverse behavioral effects of 5-HT_{1A} receptor agonists are likely to involve an action at 5-HT_{1A} receptors in multiple forebrain and midbrain sites; the contribution of presynaptic and postsynaptic 5-HT_{1A} receptors to specific behavioral effects of 5-HT_{1A} receptor agonists is not always certain, and indirect effects on other transmitter systems, particularly noradrenaline and dopamine, may often be involved.

Despite these complexities, a significant body of evidence links the decrease in 5-HT transmission evoked by 5-HT_{1A} autoreceptor activation to anxiolytic effects, whereas an increase in 5-HT transmission evoked by activation of postsynaptic 5-HT_{1A} receptors is associated with antidepressant effects [see Barnes and Sharp (1999) and II. 5-HT_{1A} Receptor in the present review]. Much of the evidence for this has come from psychopharmacological studies in animals and humans, and it is being increasingly reinforced by studies using advanced genetic mouse models that have the power to make targeted manipulation of pre- versus postsynaptic 5-HT_{1A} receptors.

In particular, genetic mouse constructs with selective knockdown of 5-HT_{1A} autoreceptor expression generate an increase in anxiety phenotypes (Richardson-Jones et al., 2011). In contrast, selective knockdown of postsynaptic 5-HT_{1A} receptors is associated with depressive-like phenotype, suggesting a differential impact of pre- versus postsynaptic 5-HT_{1A} receptors on anxiety and depression mechanisms. Paradoxically, although these phenotypes associated with genetic manipulation can be reproduced by pharmacological means, they are only observed when 5-HT_{1A} receptor suppression is initiated in early life and not during adulthood. Thus, these data point to a developmental mechanism, that is, altered pre- and postsynaptic 5-HT_{1A} receptor signaling during development, causing an adult behavioral phenotype through impacting the normal formation of anxiety/depression circuitry (Richardson-Jones et al., 2011; Garcia-Garcia et al., 2014, 2016). An analogous theory has been posited to explain the behavioral phenotype of 5-HT transporter mutant mice (Ansorge et al., 2004). Consideration of developmental mechanisms may therefore be appropriate when interpreting the behavioral phenotype of other mouse models with
altered 5-HT receptor expression (Gingrich et al., 2003; Berger and Tecott, 2006; O’Leary and Cryan, 2010).

Aside from the interesting picture emerging through genetic models, there is increasing evidence that behavioral effects of 5-HT\textsubscript{1A} agonist administration are dependent on the agonist used. Divergent effects of certain 5-HT\textsubscript{1A} agonists have been reported across a range of models of cognition and emotional behavior as well as in relevant neurochemical and neurophysiological paradigms. These findings raise the possibility that different behaviors are being evoked by different signals because of biased agonism, and differential sensitivity of pre- and postsynaptic 5-HT\textsubscript{1B} receptors to the agonists may be a contributing factor. Thus, functionally and anatomically distinct subpopulations of 5-HT\textsubscript{1A} receptors, combined with an emerging diversity of biased 5-HT\textsubscript{1A} receptor agonists, forecasts new categories of 5-HT\textsubscript{1A} drugs for selective behavioral manipulation and thereby potential multiple therapeutic applications.

b. 5-HT\textsubscript{1B} receptors. Preclinical studies on the behavioral effects of the 5-HT\textsubscript{1B} receptor ligands have been hampered by the lack of drug tools with sufficient selectivity or brain penetration, and this continues to be somewhat problematic in the case of 5-HT\textsubscript{1B} receptor agonists. Early studies on the behavioral effects of 5-HT\textsubscript{1B} receptor agonists and antagonists in rodents have been extensively reviewed (Lucki, 1992; Middlemiss and Tricklebank, 1992; Barnes and Sharp, 1999). These earlier findings, combined with more recent preclinical and clinical studies, emphasize a role for 5-HT\textsubscript{1B} receptors in depression and anxiety behaviors on the one hand (for review, see Ruf and Bhagwagar (2009) and Fakhoury (2016)) and aggression and impulse control on the other (for review, see Nautiyal et al. (2015)). Moreover, the role of pre- versus postsynaptic 5-HT\textsubscript{1B} receptors in these behaviors is beginning to be addressed using tissue-specific and time-dependent conditional 5-HT\textsubscript{1B} receptor mutant mice.

Evidence from earlier neuropharmacological studies suggests on the whole that 5-HT\textsubscript{1B} receptor agonists have antidepressant effects in animal models, whereas 5-HT\textsubscript{1B} receptor antagonists are anxiolytic (Fakhoury, 2016). The findings with 5-HT\textsubscript{1B} receptor antagonists are to some extent consistent with evidence that mice with a global 5-HT\textsubscript{1B} receptor knockout demonstrate anxiolytic and antidepressant-like phenotypes (Mayorga et al., 2001; Jones and Lucki, 2005; Bechtholt et al., 2008). Moreover, it is reported these phenotypic effects can be recapitulated in genetic mice with selective loss of 5-HT\textsubscript{1B} autoreceptors (Nautiyal et al., 2015). The latter group argue that relative to 5-HT\textsubscript{1B} autoreceptors, postsynaptic 5-HT\textsubscript{1B} receptors may have an opposing effect on anxiety and depressive behaviors, thereby potentially accounting for the divergent actions of 5-HT\textsubscript{1B} receptor agonists and antagonists noted above. Importantly, an anxiolytic and antidepressant-like phenotype was generated by knockout of 5-HT\textsubscript{1B} autoreceptors in adulthood and not during early postnatal life, thereby making a developmental mechanism unlikely (Nautiyal et al., 2015). Collectively, these data suggest that drugs that selectively block 5-HT\textsubscript{1B} autoreceptors may be useful for the treatment of anxiety and depression. Currently, there are no drugs with this property, although developments with biased 5-HT\textsubscript{1A} receptor agonists (see above) offer the potential for such agents in the future.

Another interesting phenotype of mice with a global 5-HT\textsubscript{1B} receptor knockout is increased aggression and impulsivity. This finding fits in with earlier evidence that certain 5-HT\textsubscript{1B} receptor agonists (“serenics”) have antiaggressive properties (Olivier et al., 1995). Moreover, these data fit with a consistent line of evidence associating reduced brain 5-HT transmission with high levels of impulsivity and aggression. The recently available conditional 5-HT\textsubscript{1B} receptor knockout mouse has allowed for further examination of the role of pre- versus postsynaptic 5-HT\textsubscript{1B} receptors in aggression and impulsivity (Nautiyal et al., 2015). It was found that heightened aggression and impulsivity was linked to loss postsynaptic 5-HT\textsubscript{1B} receptors and not 5-HT\textsubscript{1B} autoreceptors. However, although the increase in aggression involved a development mechanism (i.e., recapitulated by early life knockout), the increase in impulsivity was separable and was linked to loss of postsynaptic 5-HT\textsubscript{1B} receptors in adulthood. These data raise the possibility that pharmacologic agents targeting 5-HT\textsubscript{1B} receptors may be therapeutically effective in disorders associated with loss of impulse control. At least in the case of 5-HT\textsubscript{1B} receptor agonists, these agents have been widely used in the clinic since the first development of sumatriptan for the acute treatment of migraine (Humphrey, 2008). It may be argued at length whether the effects of triptans in migraine involve a neuronal component versus vascular/inflammatory mechanisms (Humphrey and Goadsby, 1994) and whether triptans are considered safe in terms of CNS adverse effects.

3. 5-HT\textsubscript{2} Receptor Family. The behavioral effects of 5-HT\textsubscript{2} receptor agonists in rodents are many, ranging from changes in both unconditioned (e.g., head-twitches, increased motor activity, hypophagia, and hyperthermia) and conditioned responses (e.g., punished responding and drug discrimination) [for review, see Koek et al. (1992), Barnes and Sharp (1999), and Halberstadt (2015)]. It has become possible to identify the role of the different 5-HT\textsubscript{2} receptor subtypes in these behaviors with some degree of confidence through the availability of highly selective antagonists (for each of 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B}, and 5-HT\textsubscript{2C} receptors) and agonists (especially 5-HT\textsubscript{2C} receptor agonists) as well as mutant mice with the different receptors selectively knocked out (for review, see Berger and Tecott (2006), O’Leary and Cryan (2010), Halberstadt (2015), and Di Giovanni and De Deurwaerdere (2016)].
a. 5-HT$_{2A}$ receptors. It is clear from detailed pharmacological analysis that the 5-HT$_{2A}$ receptor mediates the effects of serotonergic hallucinogens such as LSD and psilocybin in various behavioral models in animals, including drug discrimination, the head-twitch response, and locomotion (Halberstadt, 2015). The evidence is equally strong that the 5-HT$_{2A}$ receptor mediates the hallucinogenic effects of these drugs in humans. Prior to their prohibition in the late 1960s, psychedelic drugs such as LSD and psilocybin were used extensively in the treatment of major depression as well as anxiety-related disorders and addictions, and results were generally encouraging. Despite the restrictions that remain in place around these agents, there has been renewed interest in their use for experimental medicine studies and therapeutic purposes. Psilocybin in particular has been subject to investigation in a range of human psychopharmacological and brain imaging studies, which have contributed proof-of-principle and dose/safety data for clinical trials (Nutt, 2016). Moreover, recent clinical data demonstrate that acute administration of psilocybin (combined with psychologic support) causes a sustained lowering of depression and anxiety ratings in cancer patients (Griffiths et al., 2016; Ross et al., 2016) and treatment resistant-depression (Carhart-Harris et al., 2016), which is likely to encourage further studies in these and other patient groups.

The potent 5-HT$_{2A}$ receptor antagonist properties of many second-generation antipsychotics has been linked to the reported superior antipsychotic efficacy and reduced side-effect profile of these agents compared with early drugs (Meltzer and Massey, 2011). Although selective 5-HT$_{2A}$ receptor blockade is unlikely to be sufficient to generate a useful antipsychotic effect per se, evidence that the 5-HT$_{2A}$ receptor inverse agonist pimavanserin has antipsychotic actions in relevant animal models (Vanover et al., 2006) and in certain patient populations (Fox, 2014) suggests a novel alternative way to treat psychosis.

Recent reports that 5-HT$_{2A}$ receptor antagonists augment the effect of 5-HT uptake inhibitors in preclinical models (Marek et al., 2003, 2005; Boothman et al., 2006) may link to evidence that drugs with 5-HT$_{2A}$ receptor antagonist properties are helpful as augmenting agents in treatment-resistant depression (Marek et al., 2003). Although the mechanism behind this effect of 5-HT$_{2A}$ receptor blockade is not certain, it may link to evidence of an inhibitory 5-HT$_{2A}$ receptor-mediated feedback on 5-HT neurons (Sharp et al., 2007). 5-HT$_{2A}$ receptor blockade has also been associated with impulse control; thus, selective 5-HT$_{2A}$ receptor antagonists decrease impulsivity in animal models (Winstanley et al., 2004; Winstanley, 2011), and there is support for this action from psychopharmacological studies in humans (Rock et al., 2013). Interestingly, both lithium and the organoselenium compound, ebselen, which like lithium blocks signaling through phosphoinositide

b. 5-HT$_{2B}$ receptors. The combination of low 5-HT$_{2B}$ receptor expression in the brain and the lack of selective 5-HT$_{2B}$ receptor ligands has hampered progress in establishing whether these receptors are able to elicit robust behavioral effects. This situation has moved forward with the development of 5-HT$_{2B}$ knockout mice, which have striking phenotypes across a range of modalities, including deficits in sensorimotor gating, social interaction, attention, learning, and memory as well as elevated impulsivity and altered sleep patterns (Pitychoutis et al., 2015). Furthermore, some of these effects (sleep and sensorimotor gating) can be phenocopied by administration of a selective 5-HT$_{2B}$ receptor antagonist. However, 5-HT$_{2B}$ knockout is also associated with severe cardiac abnormalities and embryonic and postnatal lethality, which might lead to neurodevelopment issues in surviving animals that could confound the interpretation of these adult CNS phenotypes (as noted above). Although studies involving conditional 5-HT$_{2B}$ knockout strategies and further pharmacological phenocopying are awaited, these data raise interesting possibilities regarding the functional role of CNS 5-HT$_{2B}$ receptors. As noted elsewhere (Hutcheson et al., 2011), 5-HT$_{2B}$ receptor agonism as a therapeutic approach is fraught by clinical evidence that such agents may induce valvulopathies, pulmonary hypertension that can have lethal consequences; thus, many therapeutic agents with 5-HT$_{2B}$ receptor agonist properties (e.g., fenfluramine, pergolide, and cabergoline) have now been withdrawn from the market (Hutcheson et al., 2011). Nevertheless, recent data showing biased agonism at the 5-HT$_{2B}$ receptor (Wacker et al., 2013) offers the potential for future 5-HT$_{2B}$ receptor agonists that may be devoid of these systemic adverse effects.

c. 5-HT$_{2C}$ receptors. Early studies recognized that several behavioral responses were likely associated with activation of central 5-HT$_{2C}$ receptors, including hypolocomotion, hypophagia, anxiety, penile erection, and hyperthermia (for review, see Barnes and Sharp (1999)). Initially, these associations were largely based on observations using nonselective 5-HT$_{2C}$ receptor ligands. However, with increased availability of 5-HT$_{2C}$ receptor–selective compounds and the creation of 5-HT$_{2C}$ knockout mice, evidence for the involvement of the 5-HT$_{2C}$ receptor in many of these responses is now compelling (for review, see Di Giovanni and De Deurwaerdere (2016)). Moreover, these developments expanded the CNS processes likely to be under 5-HT$_{2C}$ receptor control to include various behaviors and cognitions linked to compulsive drug- and food-seeking as well as the central control of energy homeostasis,
oral dyskinesia, wakefulness, and even control seizure threshold.

In the last decade or so, there has been considerable research interest in the link between 5-HT_{2C} receptors and addictive behaviors associated with food and psychostimulant drugs (Higgins and Fletcher, 2015; Howell and Cunningham, 2015). It is now clear that 5-HT_{2C} receptor agonists reduce palatable food consumption and other effects associated with obesity (increased body mass and fat content; Heisler et al., 2006, 2007a,b; Lam et al., 2008; Higgs et al., 2011, 2016). These agents can also disrupt various steps in the sequence of events leading up to compulsive use of addictive drugs such as cocaine and nicotine (including stimulant action, positive reinforcement, behavioral sensitization, and reinstatement). The mechanism is, in part, likely to involve a 5-HT_{2C} receptor–mediated decrease in mesolimbic dopamine function, but interactions with other transmitter systems (especially cortical glutamate) are likely to play a role. In addition, the lowering of food intake and metabolism by 5-HT_{2C} receptor agonists is likely to involve an action on hypothalamic nuclei that promotes satiety and regulates energy balance pathways (Heisler et al., 2006, 2007; Lam et al., 2008).

Interestingly, separate studies have revealed a link between 5-HT_{2C} receptors and impulse control; thus, 5-HT_{2C} receptor agonists reduce premature responses, whereas 5-HT_{2C} receptor antagonists have the opposite effect (Higgins et al., 2003; Winstanley et al., 2004; Fletcher et al., 2007). Given that impulsivity may influence many aspects of addictive behavior, 5-HT_{2C} receptors may modulate addiction indirectly via its effects on impulsive behavior.

Many of these experiments detect opposing interactions between 5-HT_{3C} receptors and 5-HT_{2A} receptors (e.g., 5-HT_{2A} receptor antagonists also lower impulsivity) that were detected in earlier neuropharmacological studies (Berendsen and Broekkamp, 1990). This opposing interaction between 5-HT_{2C} and 5-HT_{2A} receptors could explain the poor efficacy in drug addiction models of drugs that elevate 5-HT itself (SSRIs, fenfluramine). Consequently, it is proposed that an optimal way to control addictive behavior (and also avoid potential adverse effects associated with 5-HT_{2C} receptor agonists that have off-target effects at 5-HT_{2A} and 5-HT_{2B} receptors) may be a drug that combines in the same molecule agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor (Anastasio et al., 2015; Higgins and Fletcher, 2015). Additional approaches for future investigation could include 5-HT_{2C} receptor agonists with biased agonist properties or positive allosteric modulators of the 5-HT_{2C} receptor.

4. 5-HT_{3} Receptors. Outside of the well established role of central (as well as peripheral) 5-HT_{3} receptors in the control of emesis, 5-HT_{3} receptors have been linked to multiple behavioral effects, ranging from changes in anxiety and cognition to altered pain processing and sensitivity to addictive drugs. Most of the original evidence for this comes from reports of the behavioral effects of 5-HT_{3} receptor ligands (mostly antagonists) in animal models; these and more recent findings are extensively reviewed elsewhere (Costall and Naylor, 1992; Bentley and Barnes, 1995; Barnes and Sharp, 1999; Walstab et al., 2010; Gupta et al., 2016). However, many of the behavioral effects observed in preclinical investigations are not confirmed by studies of selective 5-HT_{3} receptor antagonists in clinical populations (Bentley and Barnes, 1995; Walstab et al., 2010). The apparent failure for the translation of these preclinical findings could, in part, be explained by suboptimal clinical dosing, as bell-shaped dose-response curves are often observed for 5-HT_{3} receptor antagonists, particularly in relation to CNS effects. Nevertheless, the involvement of 5-HT_{3} receptors in some of these behaviors receives support from more recent studies of mutant mice with altered 5-HT_{3A} receptor expression. In particular, support for a role for 5-HT_{3} receptors in depression/anxiety-related behaviors, learning and memory, and pain processing comes from the phenotypic analysis of transgenic mice with 5-HT_{3A} receptor knockout or overexpression (Harrell and Allan, 2003; Kelley et al., 2003; Berger and Tecott, 2006). However, interpretation of the phenotypes of these mice is complicated by findings that 1) they are not always phenocopied by pharmacological agents, 2) they are dependent on the mouse background strain, and 3) overexpression and deletion of the 5-HT_{3A} receptor sometimes produced similar effects (for review, see Berger and Tecott (2006) and O'Leary and Cryan (2010)).

More recent evidence associating 5-HT_{3} receptors with emotional behaviors and cognition comes from findings with vortioxetine, which has a complex pharmacology leading to potent inhibition of 5-HT_{3} receptors, although the drug is also a 5-HT_{1A} and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist, and 5-HT transporter inhibitor. Vortioxetine has significant antidepressant and procognitive activity in both rodent models and clinical trials (Mørk et al., 2012; Sanchez et al., 2015) and is currently marketed as an antidepressant with cognition-enhancing properties. Despite vortioxetine's polymodal pharmacology, inhibition of 5-HT_{3} receptors is thought to play a prominent role in its mechanism of action. Thus, in rodents, vortioxetine preferentially occupies 5HT_{3} receptors and the 5-HT transporter at low doses, and either 5-HT_{3} receptor blockade alone produces similar effects to vortioxetine or effects of vortioxetine can be replicated by coadministration of an SSRI and a 5-HT_{3} receptor antagonist (Mørk et al., 2012; Sanchez et al., 2015). One current mechanistic explanation of these findings is that blockade of 5-HT_{3} receptors on specific populations of
GABA interneurons in the cerebral cortex contributes to vortioxetine’s action (Riga et al., 2016).

5. 5-HT4 Receptors. Early preclinical studies detected positive effects of 5-HT4 receptor agonists on cognitive performance as well as a reduction in anxiety-related behaviors [for review, see Barnes and Sharp (1999)], and these observations have proven to be consistent in later work as recently reviewed by others (Bockaert et al., 2011; Claeysen et al., 2015; Hagena and Manahan-Vaughan, 2017). Stemming in part from studies of 5-HT4 knockout mice (Compan et al., 2004), a role of 5-HT4 receptors in feeding behavior also seems clear, with 5-HT4 receptor agonists and antagonists having hypo- and hyperphagic properties, respectively (Jean et al., 2007; Bockaert et al., 2011).

Procognitive effects of 5-HT4 receptor agonists have been described across a range of species and in a variety of experimental paradigms that model different aspects of short- and long-term memory, many of them dependent on the hippocampus where 5-HT4 receptors are reasonably abundant (Hagena and Manahan-Vaughan, 2017). These agents also reverse the cognitive deficits induced by factors such as ageing, pharmacological interventions (e.g., muscarinic antagonists), and Alzheimer disease–like pathology. The mechanisms underlying the procognitive effects of 5-HT4 receptor agonists are not certain but may be mediated by one or more of increased release of acetylcholine, induction of synaptic plasticity, increased synaptic spine formation, and altered hippocampal network properties (see Boddeke and Kalkman (1990), Claeysen et al. (2015), and Hagena and Manahan-Vaughan (2017)). Administration of 5-HT4 receptor agonists is also associated with increased amyloid precursor protein cleavage, which has led to speculation that these agents may be useful in the management of Alzheimer disease (Claeysen et al., 2015). A contribution from 5-HT4 receptors in hippocampus seems likely as noted above, but a role for other circuits such as corticostriatal connectivity, which is abundant in 5-HT4 receptors, seems likely.

Administration of 5-HT4 receptor agonists is also associated with rapid antidepressant effects in animal models (Lucas et al., 2007), and there is preclinical evidence that 5-HT4 receptor activation plays an important role in the action of SSRIs (Mendez-David et al., 2014). Thus, 5-HT4 receptor agonists induce a number of responses in common with repeated antidepressant treatment (e.g., efficacy in models of depression or increased expression of neural plasticity markers) but with a rapid onset of action (Vidal et al., 2014b; Samuels et al., 2016). Moreover, antidepressant administration causes adaptive changes in 5-HT4 receptor expression and function (Licht et al., 2010a). Part of the mechanism involved in the antidepressant effects of 5-HT4 receptor agonists may be the activation of a positive feedback control of midbrain 5-HT neurons via 5-HT4 receptor located in the frontal cortex (Lucas et al., 2005; Licht et al., 2010b).

The full interpretation of this literature on the behavioral effects of 5-HT4 receptor ligands is somewhat hampered by the current lack of reports concerning the effect of these agents on mood or cognitive performance in humans, although there is early evidence that a 5-HT4 receptor agonist improved the cognitive performance in nonhuman primates (Terry et al., 1998). Concerns about the potential for 5-HT4 receptor agonists to elicit adverse gastrointestinal and cardiac effects are likely to have held back the transition to clinical studies, but these do not appear to be effects common to all 5-HT4 receptor agonists (Claeysen et al., 2015), which is encouraging for future clinical studies.

6. 5-HT5 Receptors. To date, there are few studies on the CNS effects of 5-HT5 receptor ligands, and the main indications regarding the functions of this receptor currently come from studies on the phenotype of 5-HT5A receptor knockout mice (Grailhe et al., 1999). It is reported that these mice display increased exploratory activity in the open field and various other tests without evidence of altered anxiety levels. Without data from phenocopying experiments using 5-HT5 receptor antagonists or other controls for developmental origins of the phenotype, it remains uncertain whether these behaviors are driven by the absence of 5-HT5 receptor in the adult brain. Intriguingly, in 5-HT5A receptor knockout mice the locomotor activation induced by LSD was blunted, suggesting that 5-HT5A receptor activation might contribute to the psychotropic effects of psychedelic agents.

7. 5-HT6 Receptors. Despite the identification of the 5-HT6 receptor over 20 years ago, the functions of this receptor have been somewhat obscure until recently. Largely through studies on the effects of 5-HT6 receptor ligands (agonists and antagonists) in animal behavioral models, it has been discovered that the receptor likely plays an important role in cognition.

The evidence for procognitive effects of 5-HT6 receptor ligands is in keeping with the predominant localization of 5-HT6 receptors in corticostriatal circuitry and is reviewed elsewhere (King et al., 2008; Codony et al., 2011; Meneses, 2015; see also XIV. 5-HT6 Receptors). There are consistent findings that 5-HT6 receptor antagonists enhance learning and memory mechanisms in a variety of preclinical models ranging from novel object and social recognition to spatial memory tasks. These effects are observed in naive animals and in those with memory deficits induced by, for example, pharmacological means such as cholinergic antagonists.

The mechanism underlying this effect of 5-HT6 receptor blockade is uncertain. An interaction with the cholinergic system was proposed in early studies, and this is supported by more recent data. In particular, treatment with a 5-HT6 receptor antagonist augments
the effects of an acetylcholinesterase inhibitor on cognitive measures in both animal models and patients with Alzheimer disease (Wilkinson et al., 2014; Kucinski et al., 2017; although 5-HT6 receptor antagonists have failed in larger phase III clinical trial of patients with pathologic cognitive deficits), and there is neurophysiological and neurochemical evidence that this drug combination has a synergistic effect on cholinergic function (Herrik et al., 2016). However, recent data show that 5-HT6 receptors are not expressed by cholinergic neurons (Helboe et al., 2015), which would invoke an indirect mechanism. A mechanism involving increased information processing through corticostrial circuits was recently proposed (Kucinski et al., 2017).

A further complication in understanding the preclinical effects of 5-HT6 receptor antagonists is the paradoxical observation that 5-HT6 receptor agonists are also reported to have preognitive effects in some, albeit not all, studies (e.g., Schechter et al., 2008; Burnham et al., 2010; Kendall et al., 2011; Meneses et al., 2011). It is difficult to see how this paradox can be reconciled by inferring subtleties in subpopulations of 5-HT6 receptor at the neural circuit level. Part of the explanation could lie in the cognitive domain being measured, and to date, few studies have systematically compared 5-HT6 receptor agonists and antagonists in the same model. There also remains the intriguing possibility that the full answer lies in the pharmacology of 5-HT6 receptor ligands and that their categorization in terms of partial agonist, inverse agonist, and even biased agonist is not yet complete (e.g., Romero et al., 2006).

8. 5-HT7 Receptors. As with the 5-HT6 receptor, the functions of the 5-HT7 receptor are only now beginning to be revealed. This has largely come about through developments in 5-HT7 receptor pharmacology and genetic mouse models. Preclinical data link the 5-HT7 receptor to a variety of CNS processes, including regulation of circadian rhythms, body temperature, mood, cognitions, seizure threshold, and pain processing as well as mechanisms of addiction, and this is extensively reviewed elsewhere and will only be briefly discussed here (Hedlund, 2009; Hauser et al., 2015; see also XV. 5-HT7 Receptors).

The link between the 5-HT7 receptor and circadian rhythms was established in early studies, which recognized that the circadian phase shift of neurons of the suprachiasmatic nucleus evoked by the 5-HT7 receptor agonist 8-OH-DPAT was mediated by 5-HT7 and not 5-HT1A receptors (Lovenberg et al., 1993). Indeed, in this light, re-evaluation of the hypothermic effect of 8-OH-DPAT using 5-HT7 receptor knockout mice and selective antagonists revealed the involvement of 5-HT7 receptors, although 5-HT1A receptors appear to mediate the hypothermic effect of higher doses of the drug (Hedlund et al., 2004). This hypothermic effect of 5-HT7 receptor activation is sufficiently robust to make it a useful test of 5-HT7 receptor agonist activity in vivo (Di Pilato et al., 2014).

The discovery that 5-HT7 receptor knockout mice have an antidepressant-like phenotype contributed to evidence that 5-HT7 receptors have a role in emotional control. This feature of the mice is phenocopied by selective 5-HT7 receptor antagonists that were shown to have antidepressant effects in a range of models in rats and mice. These agents also augment the effect of SSRI and other antidepressants in behavioral and neurochemical models. These findings have translated into clinical trials of the 5-HT7 receptor antagonist JNJ-18038683 (Bonaventure et al., 2012), although a clear conclusion regarding efficacy of this agent has not yet been reached. It is interesting that in animal models, the antidepressant efficacy of amisulpiride is 5-HT7 receptor–dependent. Many other antidepressant and antipsychotic drugs (tricyclics, lurasidone, aripiprazole, etc.) in clinical use have 5-HT7 receptor antagonist properties, though the contribution of this receptor to their clinical effects is not known.

As a final point of interest, selective brain penetrant 5-HT7 receptor agonists are becoming available as pharmacological tools (Di Pilato et al., 2014) to help further define the function of the 5-HT7 receptor and open new therapeutic avenues.

XIX. 5-HT Receptors and the Cardiovascular System

A. Introduction

Cardiovascular effects of 5-HT are complex: when infused into the rat circulation, 5-HT produces a triphasic response with a short-lasting hypotensive phase, followed by a hypertensive phase and then a long-lasting hypotensive phase mediated by, respectively, 5-HT3, 5-HT2, and 5-HT1 receptors (Kalkman et al., 1984). The cardiovascular effects of 5-HT have been known since the middle ages when ergots produced devastating peripheral vasoconstriction, which resulted in gangrene and limb loss because of consumption of rye bread polluted with fungi that produced ergots. For a long time, the general view was that 5-HT2 receptors are largely responsible for vasoconstrictor effects, which would be blocked by 5-HT2 receptor antagonists, such as ketanserin. However, the situation was much more complex, and it was known that depending on vessel and species, compounds such as the ergolines (ergotamine, DHE, and metergoline) would produce very complex responses: often vasoconstriction, but not infrequently vasodilatation, could be observed as well. Even before the full repertoire of 5-HT receptors were known, it was suspected that “5-HT1-like” receptors would at least mediate some of these effects. One of the most important tools for probing “5-HT1-like receptor”–mediated effects was 5-carboxamidotryptamine
(5-CT), which became available in the early 1980s. 5-CT 1) potently contracted the dog saphenous vein (Feniuk et al., 1981); 2) inhibited the release of noradrenaline and 5-HT from sympathetic and central 5-HT neurons, respectively (Feniuk et al., 1981; Engel et al., 1983); and 3) displayed nanomolar affinity for the 5-HT1 receptor recognition sites (Engel et al., 1983). Furthermore, several 5-HT-induced responses (associated with “5-HT1-like” receptor recognition sites) were blocked by methiothepin and/or methysergide, but not by ketanserin, including relaxation of smooth muscle, contraction of dog saphenous vein, long-lasting hypotension in the rat, and tachycardia in the cat [see Kalkman et al. (1984), Bradley et al. (1986), Saxena and Villalón (1990)]. The migraine program led by Pat Humphrey at Glaxo used 5-CT as a template to study such effects with the idea to develop a new migraine medication that would be selective for the craniovascular bed (Humphrey et al., 1991). This research culminated with the discovery and development of sumatriptan (and other “triptans” that followed). It was then realized that sumatriptan had high affinity and potency at 5-HT1D receptor sites before the actual receptors were cloned (Peroutka et al., 1989; Schoeffter and Hoyer, 1989a,b; Hoyer et al., 1990). The high affinity of sumatriptan for 5-HT1D receptor recognition sites suggested that the sumatriptan-induced vasoconstriction was mediated by 5-HT1-like receptors resembling the 5-HT1D receptor subtype (Martin, 1994). However, the concept of 5-HT1-like receptor remained alive for some time (Hoyer et al., 1994). One of the main reasons for this uncertainty was that metergoline, a potent 5-HT1D receptor ligand, was less active as an antagonist both in vitro (e.g., canine, human, and rabbit saphenous vein and rabbit renal and cerebral arteries; Deckert et al., 1994; Hoyer et al., 1994) and in vivo (porcine and canine carotid vascular beds; Den Boer et al., 1992; Villalón et al., 1995) than methiothepin, as may have been anticipated from metergoline’s affinity at the then defined 5-HT1D receptor recognition sites. However, the experimental conditions used to detect 5-HT1D receptor binding (Waebber et al., 1988) allowed binding to, at the time unknown, 5-HT1A, 5-HT1B, and 5-HT7 receptors, which clearly complicated interpretation. The cloning of 5-HT1Dn and 5-HT1Df receptors combined with their relatively high affinity for sumatriptan, and the eventual distinction between 5-HT1B and 5-HT1D receptors, including the species differences, allowed some order to the complexity (Weinshank et al., 1992) along with an appreciation of a need for subtype-selective 5-HT1 receptor agonists and antagonists. Eventually it was realized that the loosely defined “5-HT1-like receptor” covered at least three structurally distinct receptors, namely, the 5-HT1B, 5-HT1D, and 5-HT7 receptors. Here, basic and more recent knowledge as to the involvement of each 5-HT receptor in the cardiovascular system in normal physiological and pathophysiological conditions will be discussed (the cardiovascular system to include the heart, blood, vasculature, kidneys, and adrenals as well as the peripheral and central nervous systems involved in cardiovascular system regulation).

B. 5-HT in Cardiovascular Tissues

The majority of 5-HT in the body is made by 1) the gastrointestinal system and 2) the neurons of the raphe complexes in the brainstem. 5-HT makes its way to all body organs by storage in blood platelets following SERT uptake and through free 5-HT being released from the enterochromaffin cells of the intestine. Free 5-HT—external to the platelet—is measurable in the plasma of all species examined (Watts et al., 2012). 5-HT has been detected in all rat tissues examined (Linder et al., 2009) and cannot be accounted for by the presence of platelets given that electron microscopic images show no platelet adhesion to the tissues used for measuring 5-HT. Moreover, 5-hydroxyindole acetic acid (5-HIAA) can be detected in these same tissues. 5-HIAA is a metabolite of 5-HT, the product of monoamine oxidase, a mitochondrial enzyme (i.e., 5-HT has to be inside the cell to be converted to 5-HIAA). Interestingly, many cardiovascular tissues express SERT, and, at least in the rat, SERT-dependent uptake of 5-HT takes place in peripheral tissues that include arteries, heart, and adrenals (Linder et al., 2009). The SERT KO rat, created by Edwin Cuppen (Homberg et al., 2007), has been particularly useful in these studies. Moreover, certain tissues of the cardiovascular system synthesize 5-HT, independent of the gastrointestinal system and brain, using tryptophan hydroxylase (TPH), of which two forms exist, TPH1 and TPH2, the latter being neuron-specific. This includes vascular smooth muscle (Ni et al., 2004, 2008), endothelial cells (Morecroft et al., 2007), kidney (Hafli et al., 1996; Sole et al., 1986; Stier and Itskovitz, 1985; Stier et al, 1985), cardiomyocytes (Ikeda et al., 2005; Pönicke et al., 2012) and potentially adrenal glands (Brownfield et al., 1985; Csaba and Sudar, 1978; Delarue et al., 1992; Holzwarth and Brownfield, 1985; Holzwarth et al., 1984; Pönicke et al., 2012; Verhofstad and Jonsson, 1983).

C. Cardiovascular Effects Mediated by 5-HT Receptors

1. 5-HT1A Receptors. One of the best-known peripheral actions of 5-HT1A receptor stimulation in rodents is inhibition of stress-evoked cardiovascular responses, reducing the tachycardia and renal sympathoexcitation that accompany stress (Horiuichi et al., 2005, 2011; Naïlivaiko and Sgoifo, 2009). Stress models investigated include restraint stress, fear-conditioned stress, cold exposure, and elevated plus maze (anxiety-producing). Interestingly, 5-HT1A receptor agonists can be given systemically to produce their anxiolytic effects independent of sympathoinhibition (Vianna and Carrive, 2009). 5-HT1A receptors also mediate increased vagal drive (Ramage, 1990). 5-HT1A receptor agonists lower blood pressure in the normal (nonhemorrhaged, nonstressed) rat. 8-OH-DPAT given in the ventral medulla of the normal rat causes hypotension and bradycardia (Helke et al.,
carotid circulation (De Vries et al., 1998). Similarly, 8-OH-DPAT, flesinoxan, and other 5-HT_{1A} receptor agonists reduce blood pressure in both the spontaneously hypertensive rat and Wistar Kyoto rat (see, e.g., Doeds et al., 1998). The 5-HT_{1A} receptor agonist flesinoxan has been suggested to inhibit sympathetic nerve activity, at least in part, through renal nerves and to lower blood pressure in rats (Chamienia and Johns, 1994) and in cats (Wouters et al., 1988; Ramage et al., 1992). Collectively, these studies point to the central role of 5-HT_{1A} receptors modifying autonomic responses as one of the greatest contributions made by this receptor subtype. Interestingly, peripheral 5-HT_{1A} receptors can also mediate inhibition of the sympathetic vasopressor outflow in pithed rats (Villalón et al., 1998; Morán et al., 1998).

In contrast to the decrease in blood pressure caused in a normal rat, 8-OH-DPAT increases whole-body venous tone to protect against hemorrhagic shock (Tiniakov and Scroggin, 2009), described as a sympathetic mediated venoconstriction. 5-HT_{1A} receptors are not highly expressed in the vasculature, and direct effects within the vasculature are uncommon (Villalón and Centurión, 2007; Ramage and Villalón, 2008). Flesinoxan and other 5-HT_{1A} receptor agonists have been developed as blood pressure–lowering agents, but no clinical success has been reported, and these programs were abandoned.

2. 5-HT_{1B} Receptors. 5-HT_{1B} receptors are found on cerebral arteries and other vascular tissues mediating direct vasoconstriction [see Villalón et al. (2003) and Villalón and Centurión (2007)]. Furthermore, it seems that the receptor may be silent and may become responsive in conditions such as atherosclerosis (Geerts et al., 2000; Ishida et al., 2001). Peripheral effects in rats have been described, such as 1) inhibition of noradrenaline release from sympathetic nerves in vena cava (Göthert et al., 1986) and systemic vasculature (Villalón et al., 1998) and 2) inhibition of plasma extravasation produced by trigeminal ganglion stimulation (Buzzi and Moskowitz, 1991). 5-HT_{1B} receptors also mediate vasoconstriction in the rat caudal arteries (Craig and Martin, 1993) and the canine external carotid circulation (De Vries et al., 1998).

5-HT_{1B} receptors are expressed in vascular smooth muscle of several different arteries, including rat aorta (Banes and Watts, 2003), aortic vasa vasorum (Cohen et al., 2002), tail artery (Craig and Martin, 1993), and middle cerebral artery (Kovács et al., 2012); human arteries and veins, including central, pulmonary, and coronary arteries (Verheggen et al., 1998, 2004; Morecroft et al., 1999; Nilsson et al., 1999a,b; van den Broek et al., 2002; Tanaka et al., 2008b) as well as coronary endothelial cells (Ishida et al., 1998); canine internal (Centurión et al., 2001) and external (Centurión et al., 2001, Valdivia et al., 2004) carotid artery beds; rabbit saphenous vein, basilar artery (Bhattacharya et al., 2004), and renal artery (Hill et al., 2000); porcine coronary artery (Schoeffter and Hoyer, 1989, 1990); bovine pulmonary artery (McKenzie et al., 2010); and guinea pig isolated iliac artery (Sahin Erdemli et al., 1991; Jahnichen et al., 2004). Next to the 5-HT_{2A} receptor, the 5-HT_{1B} receptor is the best studied and most frequently reported in the vasculature. Expression in cerebral arteries has been a significant focus given the potential involvement of these receptors in the pathophysiology of migraine (e.g., Villalón et al., 2003; Villalón and Centurión, 2007).

The 5-HT_{1B} receptor mediates vasoconstriction in a variety of blood vessels, although some 5-HT_{1B} receptors have been described as “silent,” meaning that a vessel needs to be primed with a depolarizing stimulus such as high K\(^+\) before a functional receptor is observed (“unmasking”) (Movahedi and Purdy, 1998; Froldi et al., 2008). By contrast, functional 5-HT_{1B} receptors, coupled to nitric oxide synthase, have been reported in cultured bovine aortic endothelial cells (McDuffie et al., 1999). There is thus the potential for 5-HT_{1B} receptors to serve opposing actions in the blood vessel: contraction through smooth muscle and relaxation through the endothelium (Schoeffter and Hoyer, 1989, 1990; Sahin Erdemli et al., 1991).

Functional 5-HT_{1B} receptors are also found in the trigeminocervical complex of cats and dogs, where activation of the receptor inhibits 1) the “nociceptive traffic” within this complex in the former (Goadsby and Classey, 2003) and 2) capsaicin-sensitive trigeminal sensory nerves innervating the external carotid bed in the latter (Muñoz-Islas et al., 2006, 2009). 5-HT_{1B} receptor agonists inhibit the release of sensory neuropeptides (particularly calcitonin gene–related peptide) in migraine, providing the basis for an effective treatment in addition to the craniovascular vasocostricter effects (Ma et al., 2001; Villalón and Olesen, 2009; Gupta and Villalón, 2010). Finally, prejunctional 5-HT_{1B} receptors inhibit (by peripheral mechanisms) 1) norepinephrine release in blood vessels (Molderings et al., 1990, 1996; Villalón et al., 2001), 2) the vasopressor (Villalón et al., 1998) and cardioaccelerator (Sánchez-López et al., 2004) sympathetic outflow, and 3) the vasodepressor sensory CGRPergic outflow in rats (González-Hernández et al., 2011). Thus, the expression and function of 5-HT_{1B} receptors is complex and at multiple levels, even within just the vasculature.

In the vasculature, the 5-HT_{1B} receptor is best known for its upregulation and elevated function in pulmonary hypertension (Keegan et al., 2001). The 5-HT_{1B} receptor contractile function is also enhanced in rabbit atherosclerotic coronary arteries (Ishida et al., 2001) and rabbit carotid arteries from animals subjected to a carotid collar (Geerts et al., 2000). Also well established is the use of 5-HT_{1B/1D/1F} receptor agonists (the triptans) for the abortive treatment of migraine attacks (e.g., Villalón et al., 2003). Though useful in causing a cerebral vasoconstriction that is thought to be associated with improved symptoms, the triptans can also cause a coronary artery constriction that limits their use in the treatment of migraine in patients with a cardiac
condition; however, long-term surveillance shows a remarkably low incidence of serious side effects (Chan et al., 2011). Exploration for antimigraine mechanisms have expanded beyond the 5-HT1B receptor (there are a multitude of triptans available) with interest on the role of the 5-HT1F receptor playing a potentially non-vascular role (Chan et al., 2011; Rizzoli, 2014) with compounds such as lasmiditan.

3. 5-HT1D Receptors. The external carotid vasoconstrictor responses to 5-HT and sumatriptan are antagonized by the selective 5-HT1D receptor antagonist SB224289 but not the selective 5-HT1D receptor antagonist BRL15572 (De Vries et al., 1998), indicating that 5-HT1B receptors mediate this vasoconstrictor response. It must be noted that sumatriptan and second-generation triptans do not distinguish between 5-HT1B and 5-HT1D receptors (Villalón et al., 2003; Villalón and Centurión, 2007).

A series of isochroman-6-carboxamide derivatives, including PNU-109291 and PNU-142633, have been described as highly selective 5-HT1D receptor agonists (Ennis et al., 1998; McCall et al., 2002). These 5-HT1D receptor agonists do not produce vasoconstriction in vivo (Centurión et al., 2001) or in vitro preparations (cerebral arteries; Bouchelet et al., 2000).

At the peripheral level, the presence of 5-HT1D receptors seems to be rather limited to autonomic and trigeminal nerve terminals/ganglia (Jones et al., 1995; Molderings et al., 1996; Shepheard et al., 1997; Villalón et al., 1999).

There were attempts to develop 5-HT1D receptor agonists for migraine therapy (for references, see Villalón et al. (2003) and Chan et al. (2011)); these compounds are active in the trigeminal inflammation/plasma extravasation models yet would presumably be less prone to side effects, as the cardiovascular effects are largely negligible (Villalón et al., 2003; Chan et al., 2011). However, when tested in the clinic to treat migraine, there was little success, and development has been abandoned.

4. 5-h1e Receptors. The receptor has been immunohistochemically localized to the cerebral vasculature of humans, but mice and rats do not express a functional 5-h1e receptor (Klein and Teitler, 2012). There is no evidence for a physiologic role for 5-h1e receptors (Hoyer et al., 1994; Hoyer and Martin, 1997; Villalón and Centurión, 2007; Ramage and Villalón, 2008).

5. 5-HT1F Receptors. 5-HT1F receptor mRNA and the corresponding protein is preferentially expressed in neuronal tissues rather than vascular smooth muscle (Ullmer et al., 1995; Bouchelet et al., 1996). Accordingly, 5-HT1F receptor agonists appear devoid of direct vasoconstrictor properties (Johnson et al., 1998; Cohen et al., 1999; Villalón et al., 1999).

Most recently, lasmiditan was developed as a 5-HT1F receptor agonist (Rizzoli, 2014); it did not contract rabbit saphenous vein, which is frequently used as a surrogate for the human coronary artery for which crossover effects of migraine drugs (triptans) can be identified (Nelson et al., 2010). This is consistent with a lack of contractile response via the 5-HT1F receptor generally in this preparation (Cohen and Schenck, 2000) as well as with human cerebral and meningeal arteries (Razzaque et al., 1999; Bouchelet et al., 2000).

6. 5-HT2A Receptors. The 5-HT2A receptor is the original "D" receptor of Gaddum and Picarelli (1957). It is expressed in vascular smooth muscle, cardiac muscle, and platelets in multiple species, including humans (Ulmer et al., 1995; Derangeon et al., 2010; Watts et al., 2012).

The 5-HT2A receptor mediates contraction in arteries and veins from most species (Villalón and Centurión, 2007), including rat (Sung et al., 2013), both directly and indirectly through regulation of sympathetic nerves (Blessing and Seaman, 2003). It promotes platelet aggregation and gap junctional coupling in heart myocytes (Derangeon et al., 2010), induces the activation of cardiac fibroblasts (Yabangolu et al., 2009), and, within the nucleus tractus solitarius, stimulates a depressor and bradycardic response (Comet et al., 2007). Moreover, the direct component of 5-HT-induced tachycardia in reserpinized pithed rats is mediated by activation of 5-HT2A receptors (Centurión et al., 2002). The receptor mediates increases in contractility in rat cardiac atrium (Läer et al., 1998) and ventricle but the latter only in heart failure and cardiac hypertrophy (Läer et al., 1998; Qvigstad et al., 2005c; Birkeland et al., 2007b; Brattelid et al., 2007a,b).

In most vascular diseases, isolated blood vessels are hyper-responsive to 5-HT via the 5-HT2A receptor. Similar to the 5-HT1B receptor, upregulation of the 5-HT2A receptor has been implicated in elevated vascular tone in pulmonary hypertension (Delaney et al., 2013). The 5-HT2A receptor antagonist sarpogrelate blocked the development of pulmonary hypertension induced by monocrotaline and increased survival rate for this highly fatal disease (Hironaka et al., 2003).

The 5-HT2A receptor has been targeted for the treatment of hypertension; thus, ketanserin had been developed for this indication, but further research revealed that the antihypertensive effects of ketanserin were actually mediated by a1-adrenoceptor blockade. Interestingly, relatively selective 5-HT2A receptor antagonists (e.g., ritanserin) are devoid of antihypertensive potential in humans.

7. 5-HT2B Receptors. The 5-HT2B receptor, originally described in the stomach fundus, is expressed throughout the cardiovascular system, including vascular smooth muscle (many beds), endothelial cells, cardiac myocytes, fibroblast, and valves (Choi and Maroteaux, 1996; Jaffré et al., 2009).

In systemic arteries from normotensive rats, the 5-HT2B receptor is expressed but is apparently not functional (Banes and Watts, 2002, 2003). The endothelial 5-HT2B receptor mediates a nitric oxide–mediated relaxation in normal vessels (Ellis et al., 1995; Glusa and...
Pertz, 2000; Jahnichen et al., 2005). Strong support for the interaction of the 5-HT2B receptor with NOS was provided by Manivet et al., (2000). In endothelial cells isolated from human coronary artery, 5-HT elevates nitric oxide production through the 5-HT2B receptor (Ishida et al., 1998). The receptor also mediates calcium release in human pulmonary arterial endothelial cells (Ullmer et al., 1996). The 5-HT2B Receptor would appear necessary for cardiomyocyte survival (Nebigil et al., 2000a,b); receptor ablation leads to a cardiomyopathy without hypertrophy (Nebigil et al., 2000, 2001).

The 5-HT2B receptor is critical to development of hypoxia-induced pulmonary hypertension (Launay et al., 2002; Esteve et al., 2007) and monocrotaline-induced pulmonary hypertension (Zopf et al., 2011) and becomes functional in a number of models of experimental hypertension (Watts et al., 1995, 1996; Watts and Fink, 1999; Banes and Watts, 2002, 2003; Russell et al., 2002). Inhibition of the 5-HT2B receptor appears to block valvular myofibroblast differentiation, a process involved in calcification of the aortic valves (Hutcheson et al., 2012). The question remains whether this role of the 5-HT2B receptor also applies to pulmonary hypertension in humans, in which the 5-HT1B receptor seems to have a central role (Maclean and Dempsic, 2010). However, the use of the anorectic combination fenfluramine-phentermine precipitated pulmonary hypertension and valvular heart disease, with evidence of mediation via the 5-HT2B receptor (Fitzgerald et al., 2000; Rothman et al., 2000). Valvulopathy is also observed with other 5-HT2B receptor agonists (e.g., norfenfluramine, benfluorex, pergolide, cabergoline, and ergotamine, most of which have been withdrawn from a majority of markets because of concerns around these adverse side-effects). As a consequence of these findings, preclinical safety screens for 5-HT2B receptor agonism have become mandatory in an attempt to avoid the potential risk of valvulopathy (Hutcheson et al., 2011; Reid et al., 2013). Valvulopathy induced by serotonergic compounds did not begin with fenfluramine-phentermine; it has been observed with ergots over the centuries in central and eastern Europe and even in ancient Egypt (Hauck et al., 1990; Eadie, 2003) as well as in patients with carcinoid tumors (Druce et al., 2009). Finally, 5-HT2B receptors appear essential for isoproterenol-induced cardiac hypertrophy in the mouse (Jaffre et al., 2004), and overexpression of the 5-HT2B Receptor led to cardiac hypertrophy in the mouse (Nebigil et al., 2003b). It should also be kept in mind that MDMA (ecstasy) and its metabolite MDA, as well as a number of MDMA analogs, act as 5-HT2B receptor agonists and thus carry the risk of valvulopathies if consumed (Setola et al., 2003).

8. 5-HT3C Receptors. This receptor has not been localized to peripheral tissues with confidence, though a few reports suggest this may be so. The 5-HT3C receptor is, however, expressed in cardiovascular-controlling areas of the central nervous system (e.g., nucleus tractus solitarius) and may elevate blood pressure (Ferreira et al., 2005; Austgen et al., 2012).

9. 5-HT3A and 5-HT3AB Receptors. An important action of 5-HT3 receptors in the cardiovascular system is the ability to elicit the neuronally mediated transient von Bezold-Jarisch reflex (for references, see Kalkman et al. (1984), Saxena and Villalón (1990), and Villalón and Centurión (2007]).

Alternatively, activation of some peripheral 5-HT3 receptors evokes tachycardiac responses that may involve 1) noradrenaline release from postganglionic cardiac sympathetic neurons (Saxena and Villalón, 1990), 2) a direct action on the cardiac pacemaker (Wilson et al., 1990), and 3) CGRP release from cardiac sensory nerves (Nishio et al., 2002).

5-HT3 receptors in sympathetic ganglia may sustain chronic stress–induced hypertension in the rat (Nalivaiko and Sgoifo, 2009).

10. 5-HT4 Receptors. Cardiac expression is well established for the 5-HT4 receptor, with earliest papers of 5-HT4-like receptors in human atra dating back to 1989 (Kaumann and Levy, 2006). The 5-HT4 receptor is functional in fetal hearts (Brattelid et al., 2012), normally expressed in human and porcine atrium and ventricle (Bach et al., 2001; Brattelid et al., 2004a,b; Weninger et al., 2012), and splice variants of the receptor exist in the human heart (Bach et al., 2001; Brattelid et al., 2004a; Krobert et al., 2005; Kaumann and Levy, 2006). Endothelial cells also express 5-HT4 receptors (Nishikawa et al., 2010; Machida et al., 2013), as does the adrenal gland (Vilaró et al., 2002).

5-HT4 receptors mediate positive inotropic, chronotropic, and lusitropic effects in human and porcine atrium (Kaumann, 1990; Kaumann et al., 1990, 1993; Villalón et al., 1990, 1991; Krobert et al., 2005; De Maeyer et al., 2006; Kaumann and Levy, 2006; Gergs et al., 2009; Chai et al., 2012; Weninger et al., 2012) and positive inotropic and lusitropic effects in human and porcine ventricular myocardium (Brattelid et al., 2004b; Afzal et al., 2008).

5-HT4 receptors also mediate arrhythmogenesis in human atria (Kaumann, 1994; Pino et al., 1998), modulation of angiogenesis in cultured human umbilical vein endothelial cells (Nishikawa et al., 2010; Profirovic et al., 2013), and aldosterone secretion from the adrenal gland (Lefebvre et al., 1998, 2000). Interestingly, a model of cardiac overexpression of the human 5-HT4 receptor in the mouse heart has been developed to test for arrhythmogenesis as a cardiac side effect (Gergs et al., 2010).

As pointed out earlier, only 5-HT2A receptors mediate 5-HT-induced cardiostimulation in healthy rats (Centurión et al., 2002), but both 5-HT4 and 5-HT2A receptors mediate this effect after development of congestive heart failure (Qvigstad et al., 2005a,c). Accordingly, 5-HT4 receptors 1) become functional in ventricles during heart failure (Brattelid et al., 2004b, 2007a, 2012; Qvigstad et al., 2005a,c; Birkeland et al., 2007b) and 2) may contribute to human atrial
fibrillation (Kaumann et al., 1994; Lezoualch’ et al., 2007). Thus, 5-HT₄ receptor antagonists may have potential therapeutic usefulness for improvement of cardiac function. In line with a proposed arrhythmogenic effect of stimulation of atrial 5-HT₄ receptors (Kaumann, 1994), arrhythmogenic effects of a 5-HT₄ antagonist were demonstrated in a porcine model of atrial fibrillation (Rahme et al., 1999). However, although atrial 5-HT₄ expression levels increase in human chronic atrial fibrillation (Lezoualch’ et al., 2007), the arrhythmogenic potential of 5-HT₄ receptors may be reduced in established human atrial fibrillation (Christ et al., 2014).

By analogy with the use of betablockers to improve prognosis in heart failure (Lohse et al., 2003), the use of 5-HT₄ receptor antagonists was proposed (Qvigstad et al., 2005b; Levy et al., 2008) and delivered improvement in a rat model (Birkeland et al., 2007a). 5-HT₄ receptors are functional in human failing ventricle (Brattelid et al., 2004b; Afzal et al., 2008), and apparent clinical benefits of 5-HT₄ receptor antagonism have been detected (Kjekshus et al., 2009).

11. 5-HT₅ Receptors. The knowledge about cardiovascular responses mediated by 5-HT₅ receptors is very limited [see Ramage and Villalón (2008)]. It is suggested that putative 5-HT₅ receptors can mediate 1) the 5-HT–induced cardiac sympathoinhibition (together with 5-HT₁B/1D) receptors in pithed rats (Sánchez-López et al., 2003) and 2) the GR-127935–sensitive mechanism mediating hypotension in anesthetized rats (Sánchez-Maldonado et al., 2015).

12. 5-HT₆ Receptors. These receptors have not been localized or found to be functionally relevant within the cardiovascular system (Villalón and Centurión, 2007; Ramage and Villalón, 2008).

13. 5-HT₇ Receptors. The 5-HT₇ receptor most probably is encompassed with the originally designated “5-HT₇-like” receptor that mediates direct (endothelium-independent) vasorelaxation, the late vasodepressor response (i.e., the tertiary component of the triphasic response) following intravenous administration of 5-HT (Kalkman et al., 1984; Saxena and Villalón, 1990, 1991).

5-HT₇ receptors are primarily located in vascular smooth muscle of most species (Ullmer et al., 1995; Villalón and Centurión, 2007; Ramage and Villalón, 2008). The 5-HT₇ receptor mediates direct vasorelaxation in multiple vascular beds (e.g., Cushing et al., 1996; Terrón, 1996; Villalón et al., 1997a, 2001; Jahni–chen et al., 2005; Seto et al., 2009; Watts et al., 2015).

14. Receptor-Independent Actions of 5-HT. It is recognized that 5-HT can exert a (patho)physiologic role that is independent of cell surface 5-HT receptors.

For instance, various groups have reported that animals lacking SERT, or those in which SERT was pharmacologically inhibited, are protected from pulmonary hypertension (Fanburg and Lee, 2000; Marcos et al., 2003; Guignabert et al., 2005; Elangbam et al., 2008; Wang et al., 2012). Similarly, rodents overexpressing SERT develop pulmonary hypertension (Guignabert et al., 2006). It is known that intracellular 5-HT is able to modify proteins, a phenomenon known as “serotonylation” of proteins (Lin et al., 2014). Such protein modification may mediate mitogenic and profibrotic effects of 5-HT independent of receptor activation (Guilloy et al., 2009; Liu et al., 2011; Wei et al., 2012; Penumatsa and Fanburg, 2014). This covalent modification of target proteins by 5-HT is mediated by the enzyme transglutaminase, of which the tissue transglutaminase isofom (TG II, tTG) is abundant in vascular tissue. Targets for serotonylation include Akt (Penumatsa et al., 2014); small GTPases such as Ras, Rab 4, and Rho (Ahmed et al., 2008; Mercado et al., 2011; Walther et al., 2003b, 2011; Lin et al., 2013); fibronectin (Liu et al., 2011; Hummerich et al., 2012); and smooth muscle α-actin (Watts et al., 2009).

XX. 5-HT Receptors and the Gastrointestinal Tract

A. Introduction

The GI tracts of mammals contain a huge store of 5-HT (Gershon and Tack, 2007), melatonin (a derivative of 5-HT; Stone and Darlington, 2002), and kynurenic derivatives of tryptophan, which can interact with 5-HT receptors on GI muscle (Pomfret et al., 1987). This 5-HT is released to act within the GI tract (all known 5-HT receptors are expressed within the GI tract; see below) and externally into the blood, playing various roles in metabolism, osteogenesis, immunity, neurogenesis, and neuroprotection (Gershon, 2012, 2013). Indeed, about 95% of the 5-HT in the human body is GI tract, with 90% being in enterochromaffin (EC) cells in the mucosal epithelium and 5% in the neural structures intrinsic to the bowel wall; in common with the rest of the epithelium, EC cells are continually shed and replaced. Large amounts of 5-HT are also present in mast cells of rats and mice, but human intestinal mast cells usually contain no 5-HT (Buhner and Schemann, 2012); a subset of mast cells in patients with colon carcinoma and ulcerative colitis has been reported to contain 5-HT (Stoyanova and Gulubova, 2002). In the human GI tract, the highest amounts of 5-HT are present in the duodenum and rectum, whereas the lowest is found in the esophagus and ileum (Spiller, 2008a). In rats, the greatest amount of 5-HT is in the cecum (Hansen and Witte, 2008). 5-HT is also present in a limited (2% to 3%) number of myenteric descending interneurons (with variation among species; Gershon, 2003; Gershon and Tack, 2007), potentially regulating secretion and circular muscle movements in guinea pig jejunum (Neal and Bornstein, 2007), and in enteric sensory and motor neurons of mouse colon (Okamoto et al., 2014).

There are two GI 5-HT pools, each dependent on a different isofom of TPH (Li et al., 2011b). The larger pool is present in EC cells and is TPH1-dependent. The smaller pool, present in neurons, is TPH2-dependent, as is CNS 5-HT. These two pools can thus be selectively
depleted. Thus, TPH1 KO depletes EC cells, whereas TPH2 KO depletes neuronal 5-HT.

5-HT is released from EC cells by mechanical and chemical stimuli (e.g., pH, bile acids, and nutrients such as glucose and short-chain fatty acids) applied to the luminal surface of the bowel. 5-HT release is modulated by neuronal and hormonal inputs acting at numerous receptors (including 5-HT receptors; see below) expressed by EC cells (Gershon, 2003; Hansen and Witte, 2008). The junctions between EC cells and intrinsic and extrinsic sensory nerve terminals in the mucosa are not morphologically like traditional synapses (Wade and Westfall, 1985). Indeed, 5-HT secreted by EC cells acts locally in a paracrine fashion, although the nerves it activates may be situated far from the EC cells (Wade and Westfall, 1985). In addition, EC cells are in constant motion from the crypts to the surface; they turn over and are replaced by stem cells both in the small and large intestines. As a result of the movement and transient nature of EC cells, traditional synapses are not found (nerves are not good at innervating moving targets) and EC cells do not focus the 5-HT released as precisely as do neurons (see Gershon and Tack (2007)). Similarly, EC cells are located in relatively close proximity to mucosal lymphocytes (Yang and Lackner, 2004).

The 5-HT released into the mucosa and/or lumen does not normally penetrate to the muscle of the GI tract because of two reasons: first, 5-HT has poor ability to diffuse through the muscle; second, 5-HT is readily taken up by neurons and enterocytes via either SERT or organic cation transporters (Wade et al., 1996; Chen et al., 1998). 5-HT is also removed by the vasculature where it is taken up into blood platelets, which lack TPH and thus cannot synthesize 5-HT. Mucosal 5-HT thus does not gain access to the muscle layers of the bowel, except in pathologic conditions in which high plasma concentrations are reached (Sanger, 2008).

The released 5-HT can potentially act at multiple 5-HT receptors [see Costa et al. (2003), Beattie and Smith (2008), Hansen and Witte (2008), Sanger (2008), Chetty et al. (2009), Li et al. (2011b), Hoffman et al. (2012), and Alexander et al. (2015a,c)], which are expressed by several elements of the GI tract:

- Isolated enteric crest-derived cells (mRNA for all known 5-HT receptors)
- EC cells (5-HT2C, 5-HT3, 5-HT4)
- Goblet cells (5-HT4)
- Enterocytes (5-HT1A, 5-HT2A, 5-HT3, 5-HT4)
- Muscle (5-HT1B/1D, 5-HT2A, 5-HT2B, 5-HT4, 5-HT7)
- Interstitial cells of Cajal (5-HT3, 5-HT4)
- Motor (5-HT1A, 5-HT1B/1D, 5-HT2A, 5-HT2B, 5-HT3, 5-HT4) and sensory (5-HT3, 5-HT4) neurons of the myenteric and submucosal plexus
- Terminals of GI extrinsic sympathetic and/or parasympathetic nerves (5-HT1A, 5-HT3, 5-HT4 in rodents)

The genes encoding the 5-HT3A and 5-HT3B subunits of the 5-HT3 receptor are widely expressed by different mammalian tissues. In humans, expression of 5-HT3C and 5-HT3E mRNA is greatest within the GI tract (5-HT3C is also present elsewhere), whereas 5-HT3D mRNA is largely restricted to the kidney, colon, and liver (Niesler et al., 2003; Holbrook et al., 2009; Yaakob et al., 2015). Notably, genes encoding 5-HT3C, 5-HT3D, and 5-HT3E are found in humans and other mammals but not in rodents (Holbrook et al., 2009).

5-HT3A mRNA has been found in the mucosa and circular and longitudinal (taenia) muscle of human colon, whereas 5-HT3B mRNA may be more common in muscle and 5-HT3E mRNA in the mucosa (Chetty et al., 2009; Yaakob et al., 2015). Importantly, 5-HT3A and 5-HT3B mRNA and immunoreactivity are coexpressed with neuronal cell markers in the submucosal plexus of human intestine and therefore potentially form heteromeric 5-HT3AR receptors in these nerves (Michel et al., 2005). Others report that in cell bodies of human colon myenteric neurons, 5-HT3C, 5-HT3D, and 5-HT3E mRNA coexpress with 5-HT3A, whereas mRNA for 5-HT3A and 5-HT3D coexpress in the submucosal plexus (Kapeller et al., 2011). The physiologic significance of these different expression patterns is not clear. However, the absence of genes encoding 5-HT3C, 5-HT3D, and 5-HT3E in rodents has been linked to the specific divergence of rodents away from the primate evolutionary line and the peculiar lack of an emetic reflex in rodents (a function strongly linked in humans with 5-HT3 receptors; see below and Holbrook et al., 2009). Here it may be of interest to note that selective 5-HT3 receptor antagonists do not necessarily affect GI functions in the same way (e.g., Banner and Sanger, 1995), but whether such anomalies are explained by an ability to distinguish between different 5-HT3 receptor subunits [see Thompson and Lummis (2013) for examples] is still unknown.

Among the 5-HT GPCRs, splice variants exist in human colon for 5-HT2B, 5-HT7, and 5-HT4 receptors (Coupur et al., 2007; Chetty et al., 2009; Yaakob et al., 2015), although their roles are poorly understood. It has been speculated that the efficiency of 5-HT4 receptor intracellular coupling and/or the desensitization liability and affinity of this receptor for particular ligands depends on which COOH-terminal splice variant is expressed by a particular cell (Coupur et al., 2007; Beattie and Smith, 2008; Sanger, 2009). This would explain why 5-HT4 receptor agonists facilitate GI cholinergic functions with high intrinsic activity (leading to increased GI motility), whereas in cardiac muscle and other tissues, the intrinsic activity of the same agonist is low (De Maeyer et al., 2006). Notably, the 5-HT4(d) isoform is specifically expressed within human intestine (in addition to 5-HT4(b) and 5-HT4(a)), which are widely expressed, and the GI prokinetic renzapride (a nonselective 5-HT4 receptor agonist; Sanger, 1987a)
acts as a full agonist at this isoform but only as a partial agonist at the 5-HT_{4g} isoform (Mialet et al., 2000).

Certain 5-HT GPCRs may modulate ion channel functions within the GI tract. Sugiuara et al. (2004) showed that in mouse colon, the functions of transient receptor potential cation channel subfamily V member 1 channels were facilitated by both 5-HT_{2A} and 5-HT_{4} receptor activation. In rat colon, a synergistic link between 5-HT_{4} and 5-HT_{3} receptors has been suggested (Smith et al., 1999). A proposed intracellular “cross talk” between 5-HT_{3} and NK_{1} receptors may also provide a pathway via which certain selective 5-HT_{3} receptor antagonists can influence the receptor in an allosteric manner (e.g., palonsetron) to inhibit substance P-mediated responses and thereby exert greater antiemetic activity (Rojas et al., 2014; see later).

Finally, it should be noted that 5-hydroxyindalpine, an agonist mimicking certain atypical 5-HT–mediated responses [said to be mediated by a putative 5-HT_{1P} receptor, as yet undefined as a molecular entity despite considerable research; see Galligan (2007)], facilitated peristalsis in mouse colon but had no meaningful affinity for human 5-HT_{1A}, 1B, 1D, 2A, 2B, 2C, 3, 4, 6, and 7 receptors or for other monoamine (adrenoceptor, dopamine, and histamine) receptors (Mitchell et al., 2009). It has been speculated that at least some “atypical 5-HT responses” may be the result of ligands acting at allosteric binding sites and/or at GPCR heterodimers, such as a 5-HT_{1B/1D} and dopamine D_{2} receptor heterodimer (Galligan, 2007).

B. Functions of 5-HT

Of the many proposed roles for endogenous 5-HT in GI physiology, the most widely studied relate to its involvement in GI movements, secretion, reflex functions, and sensations. Several of these functions are mediated via 5-HT_{3} and 5-HT_{4} receptors; other receptors may also have important roles, although the available data are often limited. It is important to appreciate that GI functions can differ markedly between species, especially between rodents and humans (Sanger et al., 2011b, 2013b), and this can greatly change the functions of 5-HT and thereby complicate the translational value of certain animal models.

Additional developmental roles for endogenous 5-HT have also been reported, acting as an enteric neurotrophic or neuroprotective agent via 5-HT_{2B} and 5-HT_{4} receptors or when released from enteric neurons, to promote epithelial growth via 5-HT_{2A} receptors on submucosal cholinergic neurons (Fiorica-Howells et al., 2000; Liu et al., 2009; Li et al., 2011b; Gross et al., 2012; Gershon, 2013; Maue and Hoffman, 2013; Takaki et al., 2014). The absence of neuronal 5-HT during development in mice lacking TPH2, for example, is associated with a profound ENS hypoplasia and slow GI transit (Li et al., 2011b). Neurons that are born (become postmitotic) after enteric serotonergic neurons, in the sequence of ENS neurogenesis, are 5-HT–dependent and are particularly deficient in these animals. Thus, 5-HT is a growth factor that is required for enteric neuronal development. The mucosa was also defective in TPH2KO mice, suggesting that neuronal 5-HT promotes division of transit-amplifying cells in intestinal crypts (Gross et al., 2012). Furthermore, the normal postnatal accretion of enteric neurons and growth of mice during the first 4 months of life also does not occur in animals in which 5-HT_{4} receptors have been deleted (Liu et al., 2009).

One possibility is that neuroprotective functions of neuronal 5-HT might be integrated with a proinflammatory role of mucosal 5-HT. For example, mucosal 5-HT enhances and triggers inflammation (Bischoff et al., 2009; Ghia et al., 2009; Haub et al., 2010; Li et al., 2011a; Margolis et al., 2014), probably by stimulating dendritic cells (Li et al., 2011a). Inflammation is potentially toxic to enteric neurons (Gulbransen et al., 2012), but it is also important in protecting the bowel from microbial invasion. Mucosal 5-HT may therefore enhance the strength of the innate immune response to danger, while at the same time, neuronal 5-HT may protect enteric neurons from being damaged by the response; 5-HT can thus serve both as a sword and a shield of the gut (Gershon, 2012).

1. Movements of the Hungry Stomach. During hunger, the release of 5-HT from the upper GI tract, together with the hormone motilin, has a potential role in initiating a repeating pattern of upper GI movements known as the migrating motor complex, also associated with changes in blood flow, gall-bladder emptying, and gastric and pancreatic secretions.

The migrating motor complex, which in humans repeats every 80–120 minutes, is characterized by a relatively long period of quiescence (phase I), irregular nonpropulsive movements (phase II), and then a short burst (5–8 minutes) of high-amplitude propulsive contractions (phase III) initiated by the vagus nerve in stomach, duodenum, and jejunum but rapidly recovering (phase IV) while migrating to the terminal ileum, where the movement is terminated. Phase III removes undigested material and prevents bacterial overgrowth; it can be disrupted by disease and may help develop feelings of hunger (Sanger and Lee, 2008; Sanger et al., 2011a; Deloose et al., 2012; Tack et al., 2014). In dogs (Nakajima et al., 2010), a gradual release of 5-HT from duodenal enterochromaffin cells during phase I eventually activates 5-HT_{4} receptors within the myenteric plexus to increase GI motility (phase II), releasing more 5-HT from enterochromaffin cells to initiate phase III via 5-HT_{3} receptors in the stomach (also Morita et al., 2013) and in humans (Wilmer et al., 1993; Luiking et al., 2002) and 5-HT_{4} receptors in the intestine (dogs: Davidson et al., 1990; Nakajima et al., 2010). The vagus also increases motilin release from human mucosal
Enteroneuroendocrine cells (Wilmer et al., 1993) to re-enforce gastric phase III activity by greatly facilitating cholinergic motor nerve activity in a short-lasting manner (Brod et al., 2012).

2. Movements and Sensations of the Stomach and Duodenum after Meals. In healthy volunteers, little or no influence of endogenous 5-HT has been detected on gastric accommodation, compliance, sensation and motility, or on the rate of gastric emptying after ingestion of a meal, at least by acting at 5-HT3 (e.g., Gore et al., 1990; Kuo et al., 2002; Netzer et al., 2002; Janssen et al., 2011b; Kusakabe et al., 2014) or 5-HT4 receptors (e.g., Bharucha et al., 2000). In contrast, 5-HT3 receptor antagonism can increase gastric emptying in rodents (e.g., Costall et al., 1987; Miyata et al., 1995), although an ability of ondansetron, a racemate, to facilitate cholinergic activity in guinea pig ileum via an unknown, non–5-HT3-mediated mechanism should also be noted (Miyata et al., 1995; González and Puig, 1997). However, in the mouse, elimination of neuronal 5-HT as a result of the knockout of TPH2 accelerates gastric emptying (Li et al., 2011b), and 5-HT deletion or antagonism impairs vagal relaxation of the guinea pig stomach (Bülbring and Gershon, 1967).

If a meal is rich in glucose, amino acids, and/or lipids, high concentrations of these nutrients and/or gastric acid within the lumen of the duodenum can release 5-HT from enterochromaffin cells (and other mediators such as cholecystokinin, potentially acting synergistically together; Hayes and Covasa, 2005) to activate 5-HT3 receptors upon abdominal vagal nerve terminals to induce satiety (Feinle and Read, 1996; Savastano and Covasa, 2007) and, if necessary, nausea (see below). In rodents, a clear reduction in gastric emptying has also been observed, but in humans, intraduodenal infusion of glucose or a high-fat meal caused only a small 5-HT3 receptor-mediated reduction in gastric antrum movements and gastric emptying (Stacher et al., 1990; Raybould et al., 2003; Savoye et al., 2007). In rats, 5-HT4 receptors on intrinsic cholinergic neurons may be activated to increase duodenal bicarbonate secretion for a neutralizing action against gastric acid (Akiba et al., 2015).

3. Movements of the Small Intestine. It has long been known that 5-HT is released into the intestinal lumen by mechanical stimuli to the mucosa, leading to facilitation of the peristaltic reflex (e.g., Bülbring and Lin, 1958; Foxx-Orenstein et al., 1996; Bertrand et al., 2000, 2008; Pan and Gershon, 2000; Patel et al., 2007). However, it seems unlikely that this mechanism has a major physiologic influence on human small-intestinal movements, at least via 5-HT3 (Gore et al., 1990; Houghton et al., 2000) and 5-HT4 receptors (Bharucha et al., 2000). Nevertheless, this does not rule out the possibility that under certain nonphysiologic conditions (e.g., prevention of 5-HT reuptake by use of an SSRI), small-intestinal movements can be stimulated by the released 5-HT (Grover and Camilleri, 2013; Bundeff and Woodis, 2014; see XX. C. 5-HT in GI Pathology for discussion). Furthermore, it should be noted that 5-HT is probably released from EC cells into the lamina propria underlying these cells. Nerve fibers from intrinsic primary afferent neurons are found in this location, as are sensory nerves derived from the vagus and dorsal root ganglia. Because the entire enteroneuroendocrine system, including EC cells, secretes basolaterally, not apically, the luminal appearance of 5-HT is likely to result from spillover (5-HT is a relatively small molecule so it can diffuse into the lumen after its release into the lamina propria). It might thus be expected that endogenous 5-HT release in the small intestine would not affect motility.

4. Movements of the Colon. Endogenous 5-HT acting at 5-HT3 receptors plays a physiologic role in controlling normal movements of the colon via extrinsic and/or intrinsic nerve pathways, depending on the species of mammal. Additionally, and in certain species, 5-HT2B and 5-HT7 receptors may play similar roles (see below). However, the exact role of 5-HT on movements of the colon remain uncertain due, in part, to the high complexity of both the 5-HT system and the movements of colon in different species. This uncertainty has been discussed in a series of “cross talk” articles (Heredia et al., 2013; Smith and Gershon, 2015a,b; Spencer et al., 2015a,b).

a. 5-HT3 receptor knockout mice, from which 5-HT can no longer cause contraction of the isolated colon, display apparently normal GI transit and colorectal motility patterns (Fiorica-Howells et al., 2002).

b. 5-HT2B receptors. Propulsive movements of the colon and fecal output in vivo are reduced by 5-HT2B receptor antagonism in rodents (Bassil et al., 2009) but not in dogs (Morita et al., 2013). The receptor has been implicated in enteric nerve and in ICC development (Fiorica-Howells et al., 2000; Tharayil et al., 2010). Although exogenously applied 5-HT can cause contraction of human colon via 5-HT2B receptor activation (Borman et al., 2002), a role for endogenous 5-HT acting at the 5-HT2B receptor to affect human colonic functions has yet to be established.

c. 5-HT3 receptor antagonists. 5-HT3 receptor antagonists slow colonic motility and induce mild constipation in several species, but the mechanisms of action may vary. In humans, the ability to slow colonic motility (e.g., Gore et al., 1990; Talley et al., 1990; Houghton et al., 2000) is at least partly due to inhibition of the gastrocolic reflex, thought to be a vagus nerve–mediated colonic contractile response associated with eating but specifically evoked by distension of the gastric antrum or inclusion of lipids within the lumen of the duodenum (Prior and Read, 1993; von der Ohe et al., 1994; Björnsson et al., 1998, 2002). In these studies, the ascending and descending components of the peristaltic reflex were unaffected by 5-HT3 receptor antagonism. The latter is
consistent with an inability to detect 5-HT–mediated fast synaptic potentials in the myenteric plexus of human colon (Brookes et al., 1987) but seems at variance with the ability of local application of 5-HT to excite submucosal neurons (but not chloride ion secretion) in human small and large intestine via 5-HT3 receptors (where 5-HT3A and 5-HT3B receptor subunits are expressed; Michel et al., 2005).

In rodents, 5-HT3 receptors appear to influence colonic movements via more “local” mechanisms. Thus, the ability of 5-HT3 receptor antagonists (granisetron and tropisetron but not ondansetron) to dose-dependently inhibit fecal pellet excretion by guinea pigs could be at least partly mimicked in guinea pig mid-to-distal isolated colon, in which granisetron and tropisetron prevented movement and expulsion of endogenous fecal pellets (recovered by application of naloxone; Sanger and Wardle, 1994). Similar data were reported by Jin et al. (1999) using artificial fecal pellets and approximately the same region of colon after application of different 5-HT3 and 5-HT4 receptor antagonists. However, in guinea pig distal colon, movements of artificially inserted fecal pellets were unaffected or only transiently reduced when 5-HT4 or 5-HT3 receptor antagonists (including granisetron and ondansetron) were applied individually but were inhibited, albeit for only a short period of time, when the antagonists were given together (Kadowaki et al., 1996). In rats, proximal colon transit (measured via an indwelling cannula) and 5-HT–induced diarrhea in mice were unaffected by 5-HT4 or 5-HT3 receptor antagonists (ondansetron) applied separately but were inhibited when applied together (Nagakura et al., 1997). In contrast, Yu et al. (2015) demonstrated an ability of 5-HT3 receptor antagonism to abolish propulsive movements of rat isolated colon, again supporting a local role for 5-HT in the control of rodent colonic movements.

More recent studies confirmed a local release of 5-HT from mouse colon in response to a maintained presence of a fecal pellet, an ability of 5-HT3 receptor activation to promote pacemaker activity generated in mouse ileum by the interstitial cells of Cajal (Liu et al., 2011), and an ability of 5-HT3 receptor antagonism to prevent colonic migrating motor contractions (e.g., Bush et al., 2001; Heredia et al., 2009, 2013; Dickson et al., 2010). The question of whether the 5-HT comes from interneurons or from enterochromaffin cells to evoke these movements—or even if endogenous 5-HT is required at all—is the subject of debate, raising interesting questions about methods, the role of mucosal versus stretch-induced reflexes, putative constitutive expression of receptors (Smith et al., 2010, 2014; Heredia et al., 2013; Sia et al., 2013), and mouse strain differences (Neal et al., 2009). Despite the controversy surrounding the function of colonic 5-HT, myenteric 5-HT neurons project so extensively in the colon that these cells have been called “the central processing unit in the colon” (Okamoto et al., 2014).

d. 5-HT4 receptors. 5-HT4 receptor antagonists have little (Morita et al., 2013) or no ability to inhibit colon movements in dogs (Nagakura et al., 1996) or rodents (Kadowaki et al., 1996; Nagakura et al., 1997; Sanger et al., 1998, 2000), although reduced GI activity was observed in 5-HT4 receptor knockout mice, arguably related to loss of 5-HT4 receptor–mediated promotion of survival of enteric neurons (Gershon and Liu, 2007). In human volunteers, there were no changes in colonic transit (a trend toward delayed transit was not statistically significant), fasting or postprandial motor activity, compliance, or sensations evoked by transverse and sigmoid colon distension after 10–12 days administration with pharmacologically effective doses of a 5-HT4 receptor antagonist (Bharucha et al., 2000).

e. 5-HT7 receptors. Activation of 5-HT7 receptors expressed by human intestinal muscle causes muscle relaxation (Prins et al., 1999; Coupar et al., 2007; Irving et al., 2007). In guinea pig ileum, 5-HT7 receptors are localized both to muscle and to myenteric and submucosal neurons (Tonini et al., 2005). In this tissue, the release of 5-HT from enterochromaffin cells is thought to activate 5-HT7 receptors on intrinsic sensory neurons (defined as Dogiel type II neurons) to evoke slow depolarization (Monro et al., 2005) and perhaps also to facilitate a descending inhibitory motor pathway to relax the muscle, increasing its ability to accommodate and thereby reducing the likelihood of inducing peristalsis by intraluminal distension (Tuladhar et al., 2003; Tonini et al., 2005). By a similar process, endogenous 5-HT may activate 5-HT7 receptors in mouse colon to promote descending inhibitory interneurons and contribute to the generation of spontaneous colonic migrating motor complex (Dickson et al., 2010).

C. 5-HT in Gastrointestinal Pathology

The large amount of 5-HT in the GI tract and the expression of all 5-HT receptors on several, functionally different types of GI cells (see above) creates the interesting situation of being able to use selective 5-HT receptor antagonists to treat disease caused by release of endogenous 5-HT and also use 5-HT receptor agonists (“exogenous 5-HT”) to treat other diseases. This section discusses the involvement of endogenous 5-HT in the mechanisms of disease.

Increased release or availability of 5-HT from EC cells is associated with various GI disorders, including diarrhea (e.g., induced by cholera and other bacterial toxins and also by carcinoid tumors), inflammatory bowel disease, and functional disorders such as IBS [Gershon and Tack, 2007; Spiller, 2008b, 2011; Bertrand and Bertrand, 2010; for discussion on expression of 5-HT receptors by human dendritic and immune cells, see Idzko et al. (2004) and Shajib and Khan (2015)]. More recently, the release of 5-HT from enterochromaffin cells of the rat duodenum has been shown to increase following exposure to short-chain fatty acids (Akiba et al., 2015),
potentially caused by increased TPH1 transcription (Reigstad et al., 2015). 5-HT release is also increased by cytotoxic anticancer treatments following generation of free radicals in enterochromaffin cells (Minami et al., 2003) and acute stress via agents such as corticotropin-releasing factor (Sanger et al., 2000; Von Mentzer et al., 2007). Increases in 5-HT availability in 5-HT transporter knockout mice can change the level of expression and sensitivity of enteric 5-HT3 receptors (Gershon, 2003).

In GI disease, an increased availability of 5-HT has marked effects on certain autonomic functions (e.g., emesis), GI movements (e.g., diarrhea), and, perhaps, conscious perceptions of discomfort and/or pain. Several studies have investigated the role of 5-HT release into the circulation, 5-HT in tissue (typically by immunohistochemistry), and mRNA expression of 5-HTTLPR gene (which determines SERT levels) in rectal mucosa and platelets and DNA polymorphisms in 5-HTTLPR gene. In summary, the most consistent findings are elevated sensitivity of enteric 5-HT3 receptors (Gershon, 2003).

In patients at the end of life, perhaps with far-advanced cancer for which chemo- or radiotherapy is no longer provided, emesis can remain a severe problem for reasons associated with the use of drugs; cranial, electrolytic, or metabolic causes; and bowel obstruction, uremia, and/or sepsis. In these patients, 5-HT3 receptor antagonists have often provided effective control of emesis (e.g., Currow et al., 1997; Mystakidou et al., 1998; Buchanan and Muirhead, 2007). Exactly where the 5-HT comes from is not always clear, and 5-HT3 receptors expressed both peripherally (vagus nerve terminals) and centrally within the brainstem are likely to be involved (Sanger and Andrews, 2006).

SSRIs can induce nausea and vomiting that is reduced by 5-HT3 receptor antagonism and associated with polymorphisms of the HTR3B gene but not with genes encoding the 5-HT transporter, the 5-HT2A receptor, or the 5-HT3A receptor subunit (Sugai et al., 2006; Tanaka et al., 2008). Such drugs also stimulate small but not large bowel motility and have

1. Emesis. There are no “universal” antiemetic drugs, and multiple stimuli evoke emesis via different pathways, not all of which involve the release of 5-HT. The stimuli that involve the release of 5-HT and that have been studied most often are discussed below. In this discussion, the term “emesis” is taken to represent the combined act of vomiting (and dry retching) as well as nausea. It is, however, increasingly appreciated that the sensation of nausea is not fully explained by the pathways that induce vomiting. For example, 5-HT3 and NK1 receptor antagonists are both more effective against vomiting than they are against nausea, implying that different mechanisms are involved (Andrews and Sanger, 2014) and, hence, different approaches to treatment are required (Sanger et al., 2013).

Since the pioneering studies with animals (Costall et al., 1986; Miner and Sanger, 1986; Miner et al., 1987), the use of selective 5-HT3 receptor antagonists as antiemetic drugs has revolutionized treatment of cancer by making chemo- and radiotherapeutic treatments more tolerable (emesis is now viewed as something that can be treated rather than needs to be tolerated, and anticancer drugs can now be given in family-oriented outpatient clinics) by enabling the delivery of more aggressive treatments and by actually reducing health care costs (Currow et al., 1997; Warr and DeAngelis, 2009). Following identification of this role for the 5-HT3 receptor, it rapidly became standard practice to coadminister a 5-HT3 receptor antagonist with the corticosteroid dexamethasone to achieve even better relief from emesis evoked by moderate to severe emetogenic treatments. Later, the “triple-therapy” of 5-HT3 receptor antagonism, dexamethasone, and NK1 receptor antagonism achieved a further benefit in patients receiving treatments classified as “highly emetogenic,” not only controlling the appearance of “acute” emesis (during the first 24 hours after initiation of treatment) but also, even more importantly, now controlling the “delayed emesis,” which in these patients can occur 24–48 hours after the start of treatment (Warr, 2012).

5-HT3 receptor antagonists prevent cytotoxic-associated vomiting by blocking the ability of released 5-HT (Barnes et al., 1990; Cubeddu et al., 1990), likely from EC cells, to activate 5-HT3 receptors on abdominal vagal nerve terminals and thereby effectively “desensitize” the vagus to the proemetic stimulatory actions of other substances (e.g., prostanoids) released during the cytotoxic treatment (see Sanger and Andrews, 2006). Most recently, evidence is emerging that a long-lasting 5-HT3 receptor antagonist (palonosetron) may provide further improvements in emesis control by a mechanism not yet clearly understood but argued to involve inhibition of substance P–mediated responses via a unique interaction with the 5-HT3 receptor (Rojas et al., 2014).

Depending partly on the population studied, a minority of patients treated with moderate to highly emetogenic chemotherapy do not respond well to treatment with a 5-HT3 receptor antagonist. This may be due to mutations in a P-glycoprotein efflux transporter in intestinal epithelia and capillaries of the blood-brain barrier, affecting the availability and target engagement of these and other drugs (Perwitasari et al., 2011; Tsuji et al., 2013; He et al., 2014; Zoto et al., 2015), and/or differences in rates of metabolism of ondansetron and tropisetron associated with polymorphisms of the gene encoding CYP2D6 (Kaiser et al., 2002). Genetic variants of the 5-HT3B, but not the 5-HT3A subunit, are also reported to alter antiemetic efficacy in a small number of patients (Tremblay et al., 2003; Kaiser et al., 2004; de Wit et al., 2005).

In patients at the end of life, perhaps with far-advanced cancer for which chemo- or radiotherapy is no longer provided, emesis can remain a severe problem for reasons associated with the use of drugs; cranial, electrolytic, or metabolic causes; and bowel obstruction, uremia, and/or sepsis. In these patients, 5-HT3 receptor antagonists have often provided effective control of emesis (e.g., Currow et al., 1997; Mystakidou et al., 1998; Buchanan and Muirhead, 2007). Exactly where the 5-HT comes from is not always clear, and 5-HT3 receptors expressed both peripherally (vagus nerve terminals) and centrally within the brainstem are likely to be involved (Sanger and Andrews, 2006).
been evaluated as potential treatments of IBS, improving general well-being (Grover and Camilleri, 2013; Bundeff and Woodis, 2014).

Notably, there are many drugs and experimental tools other than the SSRIs that increase (e.g., 5-hydroxytryptophan, nonselective SSRIs, and monoamine oxidase inhibitors) or decrease 5-HT availability (e.g., fenfluramine; depletion following the initial increase), sometimes with additional abilities to antagonize certain 5-HT receptors, and these can induce or reduce emesis. The receptors include 5-HT1A, 5-HT2A, and 5-HT3, but which receptors are involved is not always clear, and studies with more selective ligands are required to understand mechanisms of action (Johnston et al., 2014).

Postoperative vomiting (POV) is caused by multiple stimuli but can be reduced by 5-HT3 receptor antagonism (e.g., Chun et al., 2014). The exact mechanism is not clear (Horn et al., 2014). Genetic variations in the HTR3A and HTR3B genes may be associated with the risk of developing POV, but given the multifactorial causes of POV, larger studies are required to determine the true clinical significance of these observations (Rueffert et al., 2009; Ma et al., 2013).

2. Eating Disorders. The 5-HT3 receptor antagonist ondansetron reduces binge-eating, vomiting, and depressive symptoms in patients with severe bulimia nervosa, leading to a return of normal eating, possibly by modulating cyclic increases in vagal nerve activity (Faris et al., 2006). Arguably, an ability of 5-HT3 receptor antagonism to increase the threshold before satiation is reached (Janssen et al., 2011b) could also play a role. Variants of the HTR3B gene have been associated with the restrictive subtype of anorexia nervosa (Hammer et al., 2009). It may also be possible to achieve long-term regulation of body weight by modulating different aspects of gastric motility (Janssen et al., 2011), perhaps with drugs that act at different 5-HT receptors (see below).

3. Carcinoid Diarrhea. Carcinoid tumors in the colon can generate high levels of circulating 5-HT, causing diarrhea by stimulating colonic motor functions (von der Ohe et al., 1993) via 5-HT3 receptors (see above) and chloride ion secretion in human colon via 5-HT2A and 5-HT4 (ascending colon) and 5-HT2A (descending colon) receptors (Borman and Burleigh, 1996). This is primarily treated with loperamide or a somatostatin analog, such as octreotide, but 5-HT3 receptor antagonists have helped, at least when given acutely, as have 5-HT2A (ketanserin) receptor antagonists and methysergide, a nonselective 5-HT1/5-HT2 receptor agonist/antagonist (Camilleri and von der Ohe, 1994; Schwörer et al., 1995; Spiller, 2008a). Given that TPH1 is responsible for 5-HT production, perhaps its inhibition may represent a new treatment paradigm in carcinoid tumors (see Camilleri, 2011).

4. Functional Gastrointestinal Disorders. These are a grouping of GI disorders that cannot be explained by structural or tissue abnormalities and as such are defined by symptoms. They include functional dyspepsia, IBS, and several others (Longstreth et al., 2006).

5. Functional Dyspepsia. The rationale for treatment of functional dyspepsia with a 5-HT receptor antagonist has not been established. Single doses of a 5-HT3 receptor antagonist did not affect symptoms (Van Lelyveld et al., 2006), although an ability of 5-HT4 receptor antagonism to prevent HCO3 release in rat duodenum by SCFAs has suggested a role when high levels of SCFAs occur in the upper GI tract during bacterial overgrowth (Akiba et al., 2015). More promisingly, a pilot study with patients dosed for 12 weeks with a 5-HT3 receptor antagonist showed some improvement in “adequate relief of pain or discomfort” (Talley et al., 2001), and an association between symptoms and 5-HT3A receptor gene polymorphism has been suggested (Mujakovic et al., 2011). Arguably, these data find some consistency with an ability of 5-HT3 receptor antagonism to prevent the experience of nausea in healthy volunteers induced by intraduodenal infusion of lipids [see Gershon and Tack (2007)], as early satiety and nausea are common symptoms in this group of patients (Vanheel et al., 2013) but are at variance with the lack of ability of 5-HT3 receptor antagonism to change sensitivity to gastric distension [see Gershon and Tack (2007)].

6. Irritable Bowel Syndrome. In some pilot studies, 5-HT3 receptor antagonism reduced sensations caused by bowel distension in patients with IBS [e.g., Prior and Read (1993) and Goldberg et al. (1996)], perhaps as a consequence of increased compliance to distension (Delvaux et al., 1998). These studies, together with the known ability to reduce colonic movements, prompted the evaluation and initial success of the 5-HT3 receptor antagonist alosetron as a treatment for patients with IBS with symptoms of diarrhea as well as abdominal discomfort and/or pain. The subsequent occurrence of ischemic colitis in a small number of treated patients restricted use of alosetron and almost entirely stopped research into this area. It has since been concluded that ischemic colitis is two- to four-times more likely to occur in patients with IBS regardless of treatment (Lewis, 2011), suggesting that any future treatments of this form of IBS should not induce severe constipation to potentially exacerbate such a liability. Furthermore, it should be noted that an ability of 5-HT3 receptor antagonists to reduce sensations evoked by bowel distension has not been consistently observed [e.g., Hammer et al. (1993) and Zigelboim et al. (1995) in patients without “psychologic disorders”), perhaps arguing for greater emphasis of future research on colonic movement disorders. Any association between IBS and HTR3 subunit gene mutations remains uncertain.
(Niesler, 2011). Nevertheless, in patients with diarrhea-predominant IBS, the ability of alosetron to reduce colonic transit may be associated with long (LL) polymorphisms of the 5-HT transporter gene 5-HTTLPR, which is associated with increased synthesis of SERT (SLC6A4) and inactivation of endogenous 5-HT (Camilleri et al., 2002). Also, in this group of patients with IBS, symptoms were improved in a pilot study using the 5-HT3 receptor antagonist ramosetron, an activity correlating with increased expression of TPH1 and with TPH1 gene polymorphism (Shiotani et al., 2015).

The 5-HT4 receptor antagonist SB-207266 reduced stress-induced defection in mice (Sanger et al., 2000), and in a pilot study with diarrhea-predominant IBS patients, it tended to reduce rectal sensitivity and reduced small-intestinal transit (Houghton et al., 1999). However, lack of significant efficacy in larger studies stopped further development for this indication (De Ponti, 2004).

In some countries, the 5-HT4 receptor agonist tegaserod was registered for treatment of IBS and chronic constipation (see below for discussion on the potential use of 5-HT4 receptor agonists in the treatment of IBS) but was then withdrawn because of potential cardiovascular liability and poor overall efficacy (Schiller and Johnson, 2008). Tegaserod has since been shown to act as a potent 5-HT2B receptor antagonist (Beattie and Johnson, 2008). Tegaserod has since been shown to act as a potent 5-HT2B receptor antagonist (Beattie et al., 2004), reducing colonic motility (in rodents, not dogs; see above) and exerting a visceral antinociceptive activity in rodents (Ohashi-Doi et al., 2010; O'Mahony et al., 2010). The extent to which each of these different activities translates to humans and/or patients with IBS is not clear. In parallel with the evidence that LL polymorphisms of the 5-HT transporter gene 5-HTTLPR result in greater changes in colonic transit with alosetron, a clinical trial has shown that LL polymorphism is associated with reduced clinical efficacy of tegaserod in patients with constipation (Li et al., 2007).

LX1031, a locally acting TPH inhibitor, that does not cross the blood-brain barrier, has been found to be safe and well tolerated in an exploratory 4-week phase 2 study in patients with symptomatic, nonconstipating IBS (Brown et al., 2011). Thus, reduction of mucosa-derived 5-HT may positively influence symptoms common to nonconstipating IBS. A relationship was observed between symptom improvement and a reduction in 24-hour urinary 5-HIAA; thus, 5-HIAA can serve as a biomarker to estimate the rate of 5-HT synthesis and target engagement by the TPH inhibitor LX1031. However, data from any further clinical development of this drug is lacking, and the company Lexicon have confirmed development has been terminated.

Any involvement of the 5-HT7 receptor in the mechanisms of visceral pain, following a proposed role in somatic pain (Andrews and O’Neill, 2011), is as yet unknown. Similarly, any involvement of 5-HT in the mechanisms of mucosal inflammation (see earlier) in patients with IBS has yet to be demonstrated.

7. Other Gastrointestinal Disorders. In colon from patients with diverticular disease, 5-HT4 mRNA expression was reduced in the muscle but increased in the mucosa; expression of 5-HT2B and 5-HT3A mRNA was unchanged. The authors speculate that these changes could influence the intestinal motor disturbances associated with this disease (Böttner et al., 2013).

D. Therapeutic Benefits of 5-HT Receptor Agonists and Antagonists

This section discusses the use of 5-HT receptor agonists (“exogenous 5-HT”) to treat diseases where any involvement of endogenous 5-HT in the mechanism of the disease is absent or unclear. The class of 5-HT3 receptor antagonists (e.g., alosetron or ondansetron) for IBS-D and 5-HT4 receptor agonists for chronic constipation or IBS-C (e.g., prucalopride) are extensively used in clinical practice and constitute first- or second-line therapeutic agents for these conditions. Efficacy and safety are documented by systematic reviews and meta-analyses (Andresen et al., 2008; Ford et al., 2009; Shin et al., 2014). The risk of ischemic colitis with alosetron (not observed with ondansetron) is estimated at about 1 in 1000 patients; however, there is also evidence that IBS itself is a risk factor for the development of ischemic colitis (Huerta et al., 2011).

1. 5-HT4 Receptor Agonists. At present, 5-HT4 receptors appear to have little or no major roles in disorders of GI hypomotility; for example, in idiopathic gastroparesis, there were no overall changes in 5-HT4 receptor expression apart from a reduced expression of the 5-HT4(c) splice variant (van Lelyveld et al., 2008), yet 5-HT3 receptor agonists have long been used to treat such disorders. This began with metoclopramide, a derivative of procarbazine found to have surprising antiemetic and gastric prokinetic properties. Understanding the mechanisms of action of metoclopramide directly led to the discovery of the antiemetic role of the 5-HT3 receptor (Miner and Sanger, 1986) and in the description of a novel “5-HT-like” receptor affecting GI motility (Sanger, 1987b), later named as the 5-HT4 receptor by Bockaert and colleagues who used similar ligands in CNS studies (Dumuis et al., 1988). Subsequently, a number of different 5-HT4 receptor agonists were launched as prokinetic agents, but none of the early examples are selective in their action, leading to cardiovascular complications (Sanger, 2009). Nevertheless, the selective 5-HT4 receptor agonist prucalopride is now marketed as a treatment of chronic idiopathic constipation; others are in development, and the potential use of such agents in the treatment of upper GI disorders associated with gastric and/or esophageal hypomotility is still being explored (Sanger, 2009; Broad et al., 2014a,b; Kessing et al., 2014). Interestingly, a low dose of the 5-HT4 receptor agonist...
mosapride has been shown to increase human gastric accommodation in healthy volunteers (Amano et al., 2015), perhaps reflecting the ability of 5-HT_{4} receptor agonists to increase nitricergic as well as cholinergic activity (Cellek et al., 2006), the former potentially increasing gastric fundus accommodation and the latter, gastric antrum motility and emptying. Because impaired gastric accommodation may contribute to the etiology of symptoms in patients with postprandial functional dyspepsia (e.g., early satiety and/or nausea; Talley, 2015), these data support the argument that such symptoms can be relieved by selective 5-HT_{4} receptor agonists (Janssen et al., 2011a; Sanger et al., 2013).

In human colon, 5-HT_{4} receptor activation is an effective prokinetic principle, as it facilitates excitatory cholinergic and inhibitory nitricergic motor nerve activities (representing the ascending excitatory and descending inhibitory components of a peristaltic reflex), decreases muscle tension, and increases chloride secretion from the mucosa into the lumen (Borman and Burleigh, 1996; Prins et al., 2000; Cellek et al., 2006; Broad et al., 2013). Evidence for facilitation of enteric sensory nerve activity remains uncertain (Gershon and Tack, 2007). More recent attention has been drawn to the possibility that 5-HT_{4} receptor activation might stimulate bicarbonate secretion in rat proximal colon (Kaji et al., 2015) and might also reduce nociception in rats exposed to colorectal distension (Hoffman et al., 2012; Gilet et al., 2014). The latter activity could depend on a synergistic interaction with 5-HT_{3} receptors in the mechanisms of allodynia (Smith et al., 1999) but also contrasts with the findings of Sugiuara et al. (2004), who showed that the nociceptive functions of transient receptor potential cation channel subfamily V member 1 channels were facilitated by 5-HT_{4} receptor activation in mouse colon. Similar responses have not yet been confirmed to occur in human colon, but if successfully translated, 5-HT_{4} agonists may be useful as suppositories or enemas for treating IBS (Kale-Pradhan and Talley, 2015), perhaps reflecting the ability of 5-HT_{4} receptor activation might promote neurogenesis in adults has received support from studies using mice (increased bromodeoxyuridine incorporation into neurons, neural precursors, or stem cells; Liu et al., 2011), guinea pigs, and rats (regeneration of neural circuitry or recovery of reflex activity after rectal transaction and anastomosis, accompanied by increased neurofilament and neural stem cell markers; Takaki et al., 2014), suggesting potential use of 5-HT_{4} receptor agonists in treatments of disorders associated with intestinal aganglia.

2. Other 5-HT Receptor Agonists. Different 5-HT_{1A} receptor agonists, including buspirone, may reduce emesis induced by different stimuli in animals, including cisplatin and motion, although species differences in actions complicates the interpretation of data (Johnston et al., 2014). In patients with functional dyspepsia, repeat-dosing with the 5-HT_{1A} receptor agonist R137696 did not reduce dyspeptic symptoms or gastric accommodation (Tack et al., 2009).

When given acutely after meals to patients with functional dyspepsia, the 5-HT_{1B/1D} receptor agonist sumatriptan delayed gastric emptying, improved gastric accommodation, and reduced the perception of gastric distension and early satiety (Tack et al., 2004). The suggestion that this receptor may be involved in the mechanisms of vomiting has not yet been resolved (Johnston et al., 2014), especially as sumatriptan may act on the yet to be defined 5-HT_{1P} receptor.

Partial 5-HT_{3} receptor agonists (e.g., pumosetrag; CSTI-300) have been identified for treatment of patients with diarrhea-predominant IBS (e.g., Moore et al., 2013; Roberts et al., 2020). CSTI-300 displays comparable efficacy to alosetron in a rat model of colon distension (Roberts et al., 2020). Pumosetrag has been evaluated in patients with gastroesophageal reflux disease (Choug et al., 2014), reducing the rate of acid reflux events but not symptoms.

A summary of some 5-HT drugs in development for GI therapeutics is summarized in Table 22 and reviewed elsewhere (Valentin et al., 2015).

XXI. 5-HT Receptors and the Immune System

A. Introduction

The defense against pathogens is mediated by innate and adaptive immune mechanisms that act in the periphery and the CNS. 5-HT regulates inflammation and immunity by acting on 5-HT receptors that are differentially expressed on immune cells, both in rodents and humans. 5-HT acts as a potent chemoattractant, recruiting innate immune cells to sites of inflammation. 5-HT also alters the production and release of cytokines and cell activation/proliferation. Some immune cells, including mast cells and T lymphocytes, have the capacity to synthesize and release 5-HT, expanding the range of tissues for 5-HT signaling.

B. How Do Immune Cells Encounter 5-HT?

Although 5-HT is largely studied as a neurotransmitter, enterochromaffin cells of the gut produce most of the body’s 5-HT that functions as a local hormone. These cells express tryptophan hydroxylase TPH1, a rate-limiting enzyme for 5-HT production (Walther et al., 2003). A second TPH isofrom, TPH2, synthesizes 5-HT
in the CNS and gut enteric nerves (Walther et al., 2003). 5-HT concentrations in blood and tissues are normally kept relatively low. Immune cells, however, may encounter 5-HT released in the gut mucosa or from platelets that sequestered 5-HT via the 5-HT transporter SERT (SLC6A4). In turn, platelets can release accumulated 5-HT at sites of injury and inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013). Inflammation. Platelet-derived 5-HT is important for attracting innate immune cells such as neutrophils to inflamed tissue (Duerschmied et al., 2013).

**D. 5-HT and the Immune Tolerance**

One well documented way to control immunity and tolerance is through the regulation of nutrients in the microenvironment of immune cells. Best described is tryptophan deficiency mediated by the catabolic enzyme indoleamine 2,3-dioxygenase (IDO), which locally depletes tryptophan and liberates immunoregulatory metabolites known as kynurenines. T-cell activation is exquisitely sensitive to local tryptophan catabolism, and thus this enzyme exerts profound protective effects in allo-fetal rejection, autoimmunity, and inflammation (Munn and Mellor, 2013). Although IDO is thought to be the major tryptophan-catabolizing enzyme outside of the liver, TPH1 shares a similar $K_m$ to IDO ($\sim 20 \mu M$) (Mckinney et al., 2005) and can also

C. 5-HT and Hematopoiesis

It has been proposed that 5-HT acts at hematopoietic stem cell progenitors directly or via modulation of the bone marrow microenvironment (Yang et al., 2007). Mice deficient in peripheral 5-HT ($Tph1^{-/-}$) display morphologic and cellular features reminiscent of ineffective erythropoiesis (Amireault et al., 2011). Other data show that the bone marrow composition of $Htr2b^{-/-}$ mice displays a significant increase in Cd11b+/Gr+ cells that represents granulocyte precursors. This is associated with a significant reduction in Cd11b+/CD31+ population that corresponds to immature endothelial progenitor cells in 5-HT2B+ mice (Launay et al., 2012). These observations support the hypothesis that 5-HT signaling controls the differentiation of myeloid precursor cells, particularly in the monocyte/macrophage lineages.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Example</th>
<th>Putative Action in Humans</th>
<th>Pharmacodynamic in Humans</th>
<th>Clinical Efficacy: Phase IIB or III</th>
<th>Safety Issues, Approval, Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPH1 blocker</td>
<td>LX-1031</td>
<td>Inhibits synthesis of 5-HT by blocking TPH1</td>
<td>Inhibition of urine 5-HIAA excretion; no studies of PD efficacy</td>
<td>Phase IIB trial in non–C-IBS: 1000-mg dose, improved adequate relief, stool consistency</td>
<td>Reported to be discontinued</td>
</tr>
<tr>
<td>5-HT3 receptor antagonist</td>
<td>Ramosetron</td>
<td>Inhibits secretion, motility, nociception</td>
<td>ND</td>
<td>Phase IIB 5- and 10-μg dose studies in IBS-D: benefit on global relief and bowel function</td>
<td>Approved in Asia, under investigation elsewhere; ischemic colitis with same drug class</td>
</tr>
<tr>
<td>5-HT4 receptor agonist</td>
<td>Prucalopride</td>
<td>Selective 5-HT4 receptor agonist; stimulates colonic motility</td>
<td>Accelerated CT in health and CC</td>
<td>Multiple phase II/III trials completed; open label experience of ~1000 cumulative patient-years; efficacy in CC (males and females in phase III and IV clinical trials), with consistent benefits shown by meta-analyses</td>
<td>No clinical cardiac AEs in clinical trials of &gt;4000 humans; approved in virtually all countries except United States</td>
</tr>
<tr>
<td>5-HT4 receptor agonist</td>
<td>Velusetrag</td>
<td>Selective 5-HT4 receptor agonist; stimulates colonic motility</td>
<td>Accelerated CT in health in dose-related fashion</td>
<td>Phase IIB efficacy; no effect on QT in health or 400 patients with constipation</td>
<td>Under investigation (although last report 2017)</td>
</tr>
<tr>
<td>5-HT4 receptor agonist; 5-HT3 receptor antagonist</td>
<td>Naronapride</td>
<td>Selective 5-HT4 receptor agonist; stimulates colonic motility</td>
<td>Accelerated CT in health</td>
<td>Under investigation (although last report 2018)</td>
<td></td>
</tr>
<tr>
<td>5-HT4 receptor agonist</td>
<td>Relefactron</td>
<td>Selective 5-HT4 receptor agonist; stimulates colonic motility</td>
<td>Accelerated CT in functional constipation</td>
<td>Phase II studies ongoing in IBS-C patients (ClinicalTrials.gov trial NCT02082457)</td>
<td>May be discontinued (last active report 2015)</td>
</tr>
</tbody>
</table>

*AEs, adverse events; CC, chronic constipation; CT, colonic transit; ND, not determined.*
potentially exhaust tryptophan to regulate immune tolerance. Indeed, in models of skin allograft tolerance, tumor growth, and experimental autoimmune encephalomyelitis (multiple sclerosis), Tph1 deficiency was shown to break allograft tolerance, to induce tumor remission, and to intensify neuroinflammation independent of the downstream product 5-HT (Nowak et al., 2012).

E. 5-HT and the Innate Immune Response

Innate immune system function involves monocytes, macrophages, dendritic cells, neutrophils, eosinophils, mast cells, and natural killer cells that act immediately in the area of infection, leading to the destruction of pathogens. Innate immunity is primarily responsible for recognizing and eradicating “nonself” molecules presented by pathogens and is therefore confined to recognizing extracellular pathogens (bacteria vs. viruses). This response is nonspecific with respect to particular invaders but provides immediate host defense against pathogens via pattern recognition by toll-like receptors (TLRs). Pathogen-associated molecular patterns (e.g., peptidoglycans, bacterial LPS, and double-stranded viral RNAs) bind TLRs on antigen-presenting cells, namely, dendritic cells and macrophages. Antigen-presenting cells then phagocytose pathogens and display pathogen-derived peptides via the major histocompatibility complex on their cell surface for recognition by leukocytes of the “adaptive” immune system. Antigen-presenting cells also secrete proinflammatory cytokines (e.g., IL1β, IL-6, and TNFα), prostaglandins, and histamine, which further activate physiologic responses, alerting the body to infection/invasion. In addition to cellular protective mechanisms, innate immunity also includes the complement system, activated by foreign substances, antigen-antibody complexes (classic pathway), and Gram-negative bacteria (alternative pathway). This system leads to cell lysis, increased vascular permeability (allowing antibodies, innate immune cells, and fluid to enter tissues), and chemotaxis. The complement system also helps to activate antigen-presenting cells, namely, dendritic cells and B cells, during specific immune responses. Innate immunity also functions to communicate the presence of pathogens to cells involved in adaptive immune responses (Baganz and Blakely, 2013). The local environment and the presence of stimulatory signals determine whether monocytes acquire dendritic cell or macrophage characteristics and functions. 5-HT receptors are expressed by a broad range of inflammatory cell types, including monocytes, macrophages, and dendritic cells.

Neutrophils are the most abundant white blood cell and serve an essential role in innate immunity, particularly against bacteria. Duerschmied et al. (2013) reported that Tph1−/− mice show mild leukocytosis (e.g., elevated white blood cells) numbers compared with WT mice, primarily driven by an elevated neutrophil count. Despite this, 50% fewer leukocytes rolled on unstimulated mesenteric venous endothelium of Tph1−/− mice. Diminished rolling in Tph1−/− mice resulted in reduced firm adhesion of leukocytes after LPS treatment, and neutrophil extravasation into lung, peritoneum, and skin wounds was reduced in Tph1−/− mice. 5-HT alone did not induce neutrophil migration in vitro, suggesting that endothelial adhesion was the primary deficit. Consequently, survival from LPS-induced endotoxic shock was improved in Tph1−/− mice. In conclusion, platelet 5-HT promotes the recruitment of neutrophils in acute inflammation (Duerschmied et al., 2013); however, the nature of the 5-HT receptors underlying these effects is unknown.

In human CD14 monocytes, mRNA expression of 5-HT1E, 5-HT2A, 5-HT3, 5-HT4, and 5-HT7 receptors has been revealed (Dürk et al., 2005). 5-HT modulates the release of IL-1β, IL-6, IL-8/CXCL8, IL-12p40, and TNF-α, whereas it has no effect on the production of IL-18 and IFN-γ in LPS-stimulated human blood monocytes. Moreover, 5-HT modulates mRNA levels of IL-6 and IL-8/CXCL8 but not of IL-1β and TNF-α. Pharmacologic experiments suggested that signaling through the 5-HT3 receptor upregulates the LPS-induced production of IL-1β, IL-6, and IL-8/CXCL8 but not that of TNF-α and IL-12p40. Furthermore, activation of the Gs-coupled 5-HT4 and 5-HT7 receptors increases secretion of IL-1β, IL-6, IL-12p40, and IL-8/CXCL8, but in contrast, it inhibits LPS-induced TNF-α release. Interestingly, 5-HT1E and 5-HT2A receptor agonists do not modulate the LPS-induced cytokine production in human monocytes (Dürk et al., 2005). Instead, 5-HT modulates cytokine production via activation of 5-HT3, 5-HT4, and 5-HT7 receptors.

5-HT has been shown to upregulate the activity of peritoneal macrophages and to increase the in vitro activity of phagocytosis in a concentration-dependent manner via 5-HT1A/7 receptors and NF-κB (Freire-Garabal et al., 2003). Gene expression profiling of proinflammatory M1 (granulate-macrophage colony-stimulating factor) and anti-inflammatory M2 (macrophage colony-stimulating factor) macrophages revealed that 5-HT2B and 5-HT7 receptor mRNAs are preferentially expressed by M2 macrophages, whereas the 5-HT7 receptor is the only 5-HT receptor expressed in M1 macrophages (de Las Casas-Engel et al., 2013). The 5-HT7 receptor is preferentially expressed by anti-inflammatory M2 macrophages and is also detected in vivo in liver Kupffer cells and in tumor-associated macrophages. Expression of 5-HT2C receptors was also reported in alveolar macrophages, where 5-HT induces a rise in intracellular Ca2+ concentration and an increased expression of CCL2 (monocyte chemoattractant protein-1) mRNA (Mikulski et al., 2010). LPS, the archetypal macrophage-activating stimulus that
signals via TLR4, was shown to regulate the expression of 5-HT_{2B} receptors in mouse macrophages. 5-HT_{2B} receptor mRNA is increased 20-fold in murine thioglycollate-elicited peritoneal macrophages (Lattin et al., 2008). 5-HT was shown to inhibit the LPS-induced release of proinflammatory cytokines, to upregulate expression of macrophage M2 polarization–associated genes, and to reduce the expression of M1-associated genes. Only 5-HT_7 receptors mediate the inhibitory action of 5-HT on the release of proinflammatory cytokines. Both 5-HT_{2B} and 5-HT_7 receptors mediate the pro-M2 skewing effect of 5-HT. Blockade of both receptors during in vitro monocyte-to-macrophage differentiation preferentially modulates the acquisition of M2 polarization markers (de Las Casas-Engel et al., 2013).

In mice, it has been established that 5-HT is an important regulator of microglia, the brain resident macrophages, which derive from yolk sac hematopoietic stem cell precursors. In the presence of 5-HT, the microglial processes moved more rapidly toward a lesion, which is considered a chemotactic response. Similarly, the chemotactic response of cultured microglia to ATP is enhanced by 5-HT. Phagocytic activity determined by the uptake of microspheres reveals that 5-HT application decreases phagocytic activity of amoeboid microglia. The presence of microglial 5-HT_{2B}, 5-HT_{5A}, and 5-HT_7 receptors was confirmed by patch-clamp experiments in culture and amoeboid microglia and by qPCR analysis of RNA isolated from primary cultured and acutely isolated adult microglia (Krabbe et al., 2012). This was recently confirmed by two-photon microscopy, showing that microglial processes moved rapidly toward the source of 5-HT via activation of the 5-HT_{2B} receptor (Kolodziejczak et al., 2015). Modulation of microglial functions such as phagocytosis and migration is fundamental for the CNS, as microglia can influence the balance of synaptogenesis and neuronal death during development and in pathology.

Platelet activation was reported in patients with various allergic disorders. Platelet-derived factors may influence monocyte differentiation into dendritic cells. Indeed, 5-HT alters differentiation of monocytes into dendritic cells (triggered by granulocyte-macrophage colony-stimulating factor and IL-4), leading to dendritic cells with reduced expression of costimulatory molecules and CD1a and higher expression of CD14. These 5-HT–triggered dendritic cells exhibit significantly reduced stimulatory activity toward allogeneic T cells. However, they show enhanced cytokine-producing capacity, including for IL-10 but not IL-12. 5-HT–induced alteration of the dendritic cells phenotype and the reduction in antigen-presenting capacity are mediated via 5-HT_{1B}/5-HT_7 receptors (Katoh et al., 2006).

Immature dendritic cells preferentially express mRNA for 5-HT_{1B}, 5-HT1E, and 5-HT_{2B} receptors, whereas mature dendritic cells mostly express 5-HT_4 and 5-HT_7 receptors. The mRNA expression level of the ligand-gated cation channel 5-HT_4 and the GPCR 5-HT_{2A} receptors are not modified during maturation. 5-HT stimulates 5-HT_3–dependent Ca^{2+} influx in both immature and mature dendritic cells. The 5-HT_{1B/1E} and 5-HT_{2A/2B} receptor stimulation induces intracellular Ca^{2+} mobilization via Gi/Gq proteins in immature, but not mature, dendritic cells. Activation of 5-HT_4 receptors induces cAMP elevation in mature dendritic cells. Functional studies indicate that activation of 5-HT_4 and 5-HT_7 receptors enhances the release of the cytokines IL-1β and IL-8 while reducing the secretion of IL-12 and TNF-α in mature dendritic cells (Idzko et al., 2004).

5-HT is able to induce oriented migration in immature but not in LPS-matured dendritic cells via activation of 5-HT_{1B/1E} and 5-HT_{2A/2B} receptors. Accordingly, 5-HT also increases migration of pulmonary dendritic cells to draining lymph nodes in vivo. By binding to 5-HT_3, 5-HT_4, and 5-HT_7 receptors, 5-HT upregulates the production of the proinflammatory cytokine IL-6. Additionally, 5-HT influenced chemokine release by human monocyte–derived dendritic cells: production of the potent T-helper cells Th1 chemoattractant IP-10/CXCL10 was inhibited in mature dendritic cells, whereas CCL2/ macrophage-derived chemokine secretion was upregulated in both immature and mature dendritic cells. Furthermore, dendritic cells matured in the presence of 5-HT switched to a high IL-10– and low IL-12p70–secreting phenotype. Consistently, 5-HT favored the outcome of a Th2 immune response both in vitro and in vivo (Müller et al., 2009). A recent study using Htr7^{−/−} mice confirmed 5-HT_7 receptor expression in CD103^{+}CD11c^{+} dendritic cells found in colon (and spleen) and its importance in immune activation and gut inflammation (Kim et al., 2013).

Interestingly, like platelets, dendritic cells can take up 5-HT from the microenvironment, and the antidepressant fluoxetine inhibits this uptake. Expression of 5-HT transporters (SERTs) is regulated by dendritic cell maturation, exposure to microbial stimuli, and physical interactions with T cells. Significantly, 5-HT sequestered by dendritic cells is stored within LAMP-1+ vesicles and subsequently released via Ca^{2+}–dependent exocytosis, as confirmed by amperometric recordings (O’Connell et al., 2006).

5-HT is chemotactic for eosinophils. Notably, allergic asthma is characterized by infiltration of eosinophils, and plasma levels of 5-HT are elevated in symptomatic asthma patients. There is solid evidence that 5-HT contributes to this eosinophil recruitment. Indeed, 5-HT alone can stimulate in vitro migration of murine and human eosinophils (Boehme et al., 2004; Kang et al., 2013). Although several 5-HT receptor subtypes are expressed, 5-HT_{2A} is the most prominent, and 5-HT_{2A} receptor antagonists inhibit 5-HT–induced but not eotaxin-induced migration. Furthermore, eosinophils roll in response to 5-HT in venules under conditions of
physiologic shear stress (Boehme et al., 2004; Kang et al., 2013). Signaling via 5-HT_{2A} receptors is associated with changes in cell shape/morphology via activation of specific intracellular signaling molecules (ROCK, MAPK, PI3K, and the PKC-caldesmon pathway) (Kang et al., 2013).

Mast cells have the capacity to synthesize and accumulate 5-HT (Kushnir-Sukhov et al., 2007). In turn, this stored 5-HT can be released upon IgE cross-linking. Furthermore, mast cells express mRNA for multiple 5-HT receptors, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{6}, and 5-HT_{7} receptors (Kushnir-Sukhov et al., 2006). 5-HT can induce mast cell adherence to fibronectin and stimulate cell migration. However, there is no evidence that 5-HT degranulates mast cells or modulates their activation by IgE. Mast cells from the 5-HT_{1A} receptor knockout mouse (Htr1a^{-/-}) do not respond to 5-HT, indicating a principal role for this receptor. Importantly, 5-HT attracts mast cells to sites of inflammation; injection of 5-HT into the skin enhances the accumulation of mast cells in wild-type but not in 5-HT_{1A} receptor–null mice.

Natural killer cells are large lymphocytes with innate killing capacity. Addition of 5-HT to mixtures of target cells and CD56− natural killer–enriched human mononuclear cells strongly augmented natural killer cell cytotoxicity via 5-HT_{1A} receptors. This effect was indirect and involved 5-HT signaling at accessory monocytes. The cytotoxicity-enhancing effect of 5-HT was additive to that induced by IFN-α, IFN-γ, or IL-2 but not to histamine (Hellstrand and Hermodsson, 1987).

**F. 5-HT and Adaptive Immunity**

The response of a second immune system division, termed the adaptive, or specific, immune system, occurs within hours of an infection and involves antigen-specific recognition and destruction of pathogens by T and B lymphocytes. The two components of the adaptive immune system involve cell-mediated and humoral immunity. Cell-mediated immunity is carried out by T cells located in the thymus, lymph nodes, and circulation. Antigen-presenting cells that migrate to lymph nodes will prime and educate T cells as to the nature of the pathogen. T cells then proliferate and differentiate into, for example, CD4+ T-helper inflammatory cells (Th1) that activate macrophages, CD4+ Th2 cells that aid antibody responses, or CD8+ cytotoxic cells that target cells infected with intracellular microbes. The second component of adaptive immunity involves the contributions of B cells, located in lymph tissue, spleen, and in the circulation. Upon stimulation, B cells become plasma cells (with or without the help of Th2) that produce and secrete antibodies (immunglobulins). Memory T and B cells recognize specific antigens and respond quickly. Thus, the adaptive immune system is distinguished from the innate immune system by its ability to identify, remember, and eliminate pathogens that have been designated as nonself. Adaptive immunity is triggered at the immune synapse, where peptide major histocompatibility complexes and costimulatory molecules expressed by dendritic cells are physically presented to T cells (Baganz and Blakely, 2013).

The mRNA expression of 5-HT receptors in lymphoid tissues of the rat, ex vivo isolated spleen, thymus, and peripheral blood lymphocytes include 5-HT_{1B}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{6}, and 5-HT_{7} receptor mRNAs. Mitogen-stimulated spleen cells additionally expressed mRNA corresponding to the 5-HT_{3} receptor (Stefulj et al., 2000). In the rhesus macaque, SERT-positive cells were found among CD4+, CD3+, and CD3+CD4+ lymphocytes, respectively (Yang et al., 2007). Fluoxetine significantly increases the number of lymphocytes expressing SERT and stimulates an enrichment of CD8+ T cells, decreasing the CD4+/CD8+ ratio. Fluoxetine administration elevates the levels of IL-4 at 1, 2, and 3 weeks and of IL-2 at 2 and 3 weeks. The IL-4/IL-2 ratio is significantly increased in fluoxetine group compared with the controls and is similar during the 3 weeks of treatment (Fazzino et al., 2009).

There is long-standing evidence that 5-HT can influence T-cell activation. Notably, mice treated with a selective, irreversible inhibitor of TPH1, parachlorophenylalanine, exhibit a reduction in the number of CD25-positive T cells (Young et al., 1993; León-Ponte et al., 2007), suggesting that 5-HT contributes physiologically to T-cell activation. A screen for 5-HT receptor subtypes in murine T cells revealed expression of three subtypes; naïve T cells selectively express 5-HT_{7} receptors, whereas following T-cell activation, there is a strong upregulation of 5-HT_{1B} and 5-HT_{2A} receptors (León-Ponte et al. 2007). Significantly, exogenous 5-HT induces rapid phosphorylation of ERK1/2 and IκBα in naïve T cells that is inhibited by preincubation with a selective 5-HT_{7} receptor antagonist. Thus, 5-HT signaling via the 5-HT_{7} receptor may contribute to early T-cell activation. Yin et al. (2006) showed that 5-HT_{1B} receptor antagonists impaired the proliferation of helper CD4+ T cells in mouse and human. Inoue et al. (2011) showed that a 5-HT_{2A} receptor agonist enhanced Concavalin-A–induced activation of murine CD4+ and CD8+ T cells, whereas a 5-HT_{2A} receptor antagonist blocked T-cell receptor–mediated IL-2 and interferon-γ production. Consistent with these data, Akiyoshi et al. (2006) showed that treatment with a 5-HT_{2A} receptor antagonist enhanced the survival of cardiac allograft in mice. Thus, these mouse data strongly support involvement of 5-HT receptors (5-HT_{7}, 5-HT_{1B}, and 5-HT_{2A}) during early- and late-stage T-cell activation.

Interestingly, although not detected in mouse, the 5-HT_{2B} receptor is found in human T cells. Gene expression profiles during human CD4+ T-cell differentiation identified the 5-HT_{2B} receptor with 10-fold greater expression in CD3^{high}CD4^{+}CD8^{−}
SP4 thymocytes over intrathymic T progenitor cells, CD3-CD4+CD8+ “double positive” thymocytes, CD3+CD4-CD8-CD45RA+CD62L+ “naive” T cells from cord blood, and CD3+CD4+CD8-CD45RA-CD62L+ “naive” T cells from adult blood (Lee et al., 2004). Furthermore, 5-HT2B receptors are differentially expressed among Th subsets. In human umbilical cord blood, Th cells cultured in the presence of cytokines promoting Th2 differentiation were found to increase 5-HT2B Receptor expression along with 50 Th2 differentially expressed genes (Aijö et al., 2012).

5-HT may also modulate migration of human T cells. Human but not mouse T cells express functional 5-HT3 receptors. 5-HT3 receptor agonists selectively decrease T-cell migration toward gradients of the chemokine CXCL12 but not to other chemokines such as CCL2 and CCL5. Interestingly, CXCL12 is highly expressed on vascular endothelium and inhibits T-cell migration across endothelium and extravasation. In transmigration experiments, 5-HT3 receptor stimulation reverses this effect of endothelial-bound CXCL12 on T-cell migration (Magrini et al., 2011). These data suggest that 5-HT can stimulate trafficking of T cells from blood to tissues.

T cells have the capacity to synthesize 5-HT, and levels of TPH1 expression increase following T-cell activation (O’Connell et al., 2006; León-Ponte et al., 2007; Urbina et al., 2014; Chen et al., 2015). The precise signaling role for T-cell–produced 5-HT is uncertain. Conceivably, TPH1 activity in T cells could act to exhaust tryptophan, as has been proposed for mast cells (Nowak et al., 2012). On the other hand, 5-HT produced by T cells might act in an autocrine or paracrine manner. Indeed, T cells express the type 1 vesicular monoamine transporter responsible for vesicular storage of 5-HT, and type 1 vesicular monoamine transporter expression increases following T-cell activation concomitant with TPH1. Furthermore, Ca2+ elevations in T cells can trigger secretion of 5-HT. Interestingly, levels of TPH1 and monoamine oxidase A, the principal catabolic enzyme for 5-HT, are greater in CD8+ compared with CD4+ T cells, suggesting a specific biologic role for 5-HT synthesis in this T-cell subset (Chen et al., 2015). B lymphocytes have the capacity to sense and sequester 5-HT via SERT. 5-HT increases mitogen-stimulated CD19+ B lymphocyte proliferation in a concentration- and time-dependent manner. These effects are reproduced by a 5-HT1A receptor agonist. 5-HT–induced increases in proliferation are blocked by 5-HT1A receptor antagonists. Moreover, LPS-activated mouse spleen cells express specific binding sites for 5-HT1A receptor, suggesting that 5-HT upregulates mitogen-stimulated B lymphocyte proliferation through 5-HT1A receptors (Iken et al., 1995). Furthermore, mitogen-activated B lymphocytes express higher levels of 5-HT1A receptor mRNA and protein than resting cells. This upregulation is seemingly dependent on NF-κB transcription factors, as selective inhibitors of this pathway prevent the increase in mRNA expression for the 5-HT1A receptor (Abdouh et al., 2001).

B lymphocytes express SERT, and uptake of 5-HT leads to apoptosis of Burkitt lymphoma cells (Serafeim et al., 2002). 5-HT may induce apoptosis via the intracellular serotonylation signaling pathway. Furthermore, long-term treatment with SSRIs in humans leads to enhanced (~30%) numbers of B lymphocytes (Hernandez et al., 2010). Interestingly, higher doses of SSRIs directly promote apoptosis of Burkitt lymphoma cells by inhibiting DNA synthesis, whereas normal peripheral and tonsilar B cells are relatively resistant to SSRI-induced apoptosis (Serafeim et al., 2003). SERT has been detected in a variety of B-cell lines (Meredith et al., 2005), revealing SERT as a potential target for a broad range of B-cell malignancies.

XXII. General Summary and Conclusions

The first official IUPHAR review on 5-HT receptors (Hoyer et al., 1994) was a landmark for the then rather complex 5-HT receptor field: it has come of age and has been cited well over 3600 times (Google Scholar). It followed a number of initiatives and meetings in the late 1980s, when the 5-HT receptor nomenclature committee was established (by our esteemed colleague and friend Paul Vanhoutte in 1987, who sadly died in 2019). The committee was constituted and met formally for the first time in 1990 at the occasion of the 5-HT meeting in Basel (a satellite to the 1990 IUPHAR main meeting); a number of recommendations were made adapting to the new findings in transduction mechanisms and molecular biology of the receptors over the subsequent decade (Humphrey et al., 1993a; Hoyer et al., 1994, 2002; Hartig et al., 1996; Hoyer and Martin, 1997; Martin et al., 1998). It is remarkable that the recommendations made at the time have been largely accepted by a community that was used to very different nomenclatures or even definitions of receptors and that very little needed to be changed or added to these recommendations (Alexander et al., 2015a,b,c 2019). In the 1994 review, it was noted that the authors had a cumulated 100 years of active 5-HT research to share. A number of our colleagues have in the meantime retired from active research or have moved to other professional priorities. There is a lot of new “blood” now on board to reflect the growing diversity of the research, which is currently performed in many different academic and SME (small or medium enterprise) pharmaceutical centers; the combined years in 5-HT research accumulated by the authorship has increased because of the considerably greater number of authors on the present paper to address a more diverse range of complex issues.

What has clearly changed, though, is the relative representation from the industry, which compared with the 1994 version, is very much reduced. In 1994, there were six out of the eight authors who worked in “Big Pharma,” whereas there are none for the present
review. This is explained by the lesser interest for exploratory 5-HT research in the industry on the one hand, with less emphasis on 5-HT translational research and a shift of research toward small or medium enterprise/Biotech; on the other hand, there is an increased interest in very basic aspects of 5-HT research, such as structural biology or the more recent advances in the immunologic aspects, which were hardly addressed in the previous review of 1994. The shift in emphasis and thus authorship was very much needed, as the 5-HT receptor field has become more complex and possibly less the subject of “classical” pharmacologists as it was in the latter decades of the last century.

The apparent good news is that no new receptors have been identified, except for some additional 5-HT₃ receptor subunits whose function still remain to be defined clearly. On the other hand, there have been major advances with respect to 5-HT₁B/₁D receptors; as far as can be told, all triptans act as 5-HT₁B/₁D receptor agonists, and in the meantime, many different triptans have reached the market primarily for the acute treatment of migraine. Some of them, such as sumatriptan, may also act as 5-HT₁F receptor agonists. Thus, the 5-HT₁B receptor is most probably the main and sole target for triptans in the treatment of migraine, whereas the 5-HT₁D receptor, which is comparatively less abundant, may only play a minor role. Indeed, a study in migraine with a selective 5-HT₁D receptor agonist (PNU142633) was not conclusive; it can be argued that the compound was only a partial agonist and that target engagement may not have been optimal. Thus, the jury is still out to define a role for the 5-HT₁D receptor in physiology and disease. Because of the triptans, of which many starting with sumatriptan) that this constitutes another target for the treatment of migraine. This is especially true for patients who want to avoid vascular side effects, as in contrast to the 5-HT₁B receptor, the 5-HT₁F receptor is not expressed in vascular tissues. The 5-HT₁A receptor is still actively investigated, especially because newer highly selective 5-HT₁A receptor ligands show very different patterns of pathway selectivity and thus may have clinical application in diseases as different as chronic pain, Parkinson disease, or Rett Syndrome and other forms of autistic disorders/mental retardation, as illustrated by the relatively recent FDA approval for compounds such as fibanserin, cariprazine, vortioxetine for the treatment of female sexual desire deficit, schizophrenia, and depression, respectively. Although these compounds have other activities, they all share 5-HT₁A receptor agonism with possible differences in receptor engagement in different brain regions. An interesting aspect with some of the newer 5-HT₁A receptor ligands is their variety of activities at different transduction systems (i.e., these compounds have various degrees of biased agonism or signaling). This is not unique to 5-HT₁A receptors, as probably most 5-HT GPCR agonists show different levels of biased signaling depending on their preferential activation of one or the other multiple combinations of receptor/G protein and accessory proteins. This has become even more evident now that crystal structures exist for both 5-HT₁B and 5-HT₂B receptors, which show that various ligands can occupy different conformation in the same orthosteric pocket, which may explain why certain 5-HT₂A receptor agonists produce hallucinations when others do not. In addition, it has become evident that the orthosteric binding pocket is not the unique target for ligands, since a number of ergolines (e.g., ergotamine or DHE) may bind an accessory (allosteric site?), which has been well described for some metabotropic GPCRs (e.g., mGlu or GABAB receptors), opening the possibility for allosteric 5-HT receptor modulators (some of which have already been proposed for, for example, 5-HT₁B/₁D or 5-HT₃ receptors).

The aspect of species differences is a recurrent theme, as illustrated by the amply documented and well accepted differences in the pharmacology of 5-HT₁B receptors (explained by a single amino acid change in the core structure of the ligand-binding site) or the multiple differences observed with splice variants of the 5-HT₁A or 5-HT₇ receptors. A potential function for the 5-HT₄e receptor in native tissue or cells is still to be identified; absence from rodents hinders the research and has made 5-HT₄e receptor drug discovery relatively unattractive in the absence of a link to disease. A similar point can be made about the 5-HT₅ receptors, in which both a relative lack of tools and the absence of a link to disease also hinder progress, although recent advances in knowledge concerning the 5-HT₅A receptor may promote further research. However, as the 5-HT₅B receptor appears to be nonfunctional in humans, this likely prevents interest as a therapeutic target.

Significant progress has been made in the 5-HT₂ receptor family. The potential role played by 5-HT₂B receptors in valvulopathies led to many of the drugs acting as agonists at these receptors being withdrawn from the market since the early 2000s (fenfluramine, norfenfluramine, benfluorex, and pergolide), keeping in mind that MDMA (“ecstacy”) and its active metabolites (explained by a single amino acid change in the core structure of the ligand-binding site) or the multiple differences observed with splice variants of the 5-HT₂A or 5-HT₇ receptors. A potential function for the 5-HT₄e receptor in native tissue or cells is still to be identified; absence from rodents hinders the research and has made 5-HT₄e receptor drug discovery relatively unattractive in the absence of a link to disease. A similar point can be made about the 5-HT₅ receptors, in which both a relative lack of tools and the absence of a link to disease also hinder progress, although recent advances in knowledge concerning the 5-HT₅A receptor may promote further research. However, as the 5-HT₅B receptor appears to be nonfunctional in humans, this likely prevents interest as a therapeutic target.

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compared with the nonedited isomorph that can couple to multiple G proteins. In addition, both agonists (lorcaserin) and antagonists (agomelatine) have been developed to treat obesity/schizophrenia/addiction and depression/generalized anxiety disorder, respectively. Interestingly, in spinal cord injury, the 5-HT2C receptor is less edited and expression is increased, resulting in constitutive activity and muscle spasms in the absence of any endogenous 5-HT, which can be reversed by inverse 5-HT2C receptor antagonists. The 5-HT2A receptor may be less investigated, as early claims that linked 5-HT2A to hypertension or sleep did not result in clinical success. However, it should be kept in mind that all three, 5-HT2A, 5-HT2B, and 5-HT1B, receptors play a role in pulmonary hypertension, a disease with unmet need for treatment, as neither endothelin antagonists nor PDE inhibitors are highly effective, although commonly prescribed. On another note, 5-HT2B receptors appear to play a significant role in fibrosis, be it in the lung, liver, and especially the heart. 5-HT2A receptor antagonism is a major component of second-generation antipsychotics (in combination with dopamine D2 receptor antagonism and often many other pharmacological activities, e.g., blonanserin), and there is evidence that a 5-HT2A receptor antagonist may show efficacy in certain subpopulations of schizophrenic patients. It may also be relevant that the 5-HT–glutamate link in schizophrenia may be related to the existence of heterodimers between 5-HT2A and mGlur2 receptors, although in vivo proof of concept remains to be established. Along these lines, there is evidence that 5-HT2A receptors are able to form homodimers, similarly to 5-HT1B or 5-HT1D receptors; however, the latter two may also form heterodimers, which may explain the codistribution that has been consistently observed for these two receptors. The 5-HT2C receptor is also capable of dimerization in vitro; it may even form tetra or octamers, and it may form heteromers with the ghrelin or the melatonin 2 receptor. Clearly, receptor homo- and heterodimerization is an ongoing subject of interest with respect to 5-HT receptors. In addition to information incorporated within relevant sections of this review, the reader is also directed to specialized papers on this subject (e.g., Lee et al., 2000; Herrick-Davis, 2013; Moreno et al., 2016; Moutkine et al., 2017; Maroteaux et al., 2019).

In the 5-HT3 receptor field, similarly to what has been long known and accepted for other members of the Cys-loop ligand-gated ion channel superfamily (e.g., nicotinic acetylcholine receptors), homomeric and heteromeric 5-HT3 receptors are evident, and new receptor molecular isomers are still being investigated. Clinically, alosetron has been developed for the treatment of IBS with diarrhea, in contrast to 5-HT4 receptor agonists, for which the primary indication remains IBS with constipation or primary constipation in spite of the withdrawal of tegaserod from most markets. Obviously, the antiemetic effects of 5-HT3 receptor antagonists, whether in the short term or in the longer term following chemotherapy or possibly surgically induced, are still a very salient feature of these drugs, even if, in the meantime, combination therapy (with, e.g., NK1 receptor antagonists) becomes the accepted treatment. There has been a lot of research dealing with 5-HT6 receptor antagonists in memory/dementia (e.g., idalopirdine), although the phase III clinical data has been disappointing, whereas the 5-HT7 receptor is somewhat less actively investigated as a clinical target. The mystery of the putative 5-HT1P receptor in the gastrointestinal tract remains, although heterodimerization remains a favored potential explanation, albeit without clear direct evidence.

The more troubling news is that whereas receptors used to be defined based on structural, operational, and transductional features, it becomes clear that some refinements are needed:

1) In terms of structural knowledge, progress has been relatively slow. X-ray diffraction data from crystals exist for the 5-HT3 receptor and also, for example, the 5-HT1B and 5-HT2B receptors. One of the limitations of this approach for GPCRs is that the conformation of the ligand-receptor complex is very much dependent on the G protein heterotrimer associated with the receptor. In more recent years, we have learned that many receptors are able to couple to various pathways and even are able to signal in the absence of G proteins. In addition, there are multiple GPCR-interacting proteins that may affect both signal transduction and receptor-ligand conformation, thus the pharmacological signature.

2) The operational definition of a receptor (pharmacological profile based on rank orders of affinity of agonists and antagonists), which was the primary feature of receptors up to the 1990s, is now recognized as increasingly complex because of the dependence on the G protein associated with the receptor under study and other GPCR-interacting proteins. Indeed, a number of drugs will have different affinities and potencies depending on the transduction system studied. Such variations may seem artificial and linked to the expression of receptors and transduction components in engineered cells, but we know that an endogenously expressed receptor can react very differently to a given ligand in a cell- and system-dependent manner (e.g., 5-HT4 receptors in the GIT). Thus, the concept of functional selectivity or biased agonism/antagonism is a reality (see so-called β-blockers, which have rather different clinical features depending on their signaling characteristics, e.g., carvedilol compared with propranolol, pindolol, and nadolol). In contrast to other receptors, such as
muscarnic or GABA receptors, there is little research addressing allosteric modulation for 5-HT receptors, with the possible exception of the 5-HT₃ receptor. However, it becomes clear from X-ray structural studies performed with some ergolines that in addition to the classic orthosteric binding pocket, some 5-HT receptor have an extended binding “site,” which is very reminiscent of that described for muscarinic allosteric ligands. Such molecular targets may offer attractive strategies for novel therapeutics.

The relatively recently recognized importance of the microbiome continues to grow (Gilbert et al., 2018), and one aspect of this relevant to the present paper is the regulation of 5-HT receptors by bacteria-derived metabolites (e.g., Yano et al., 2015; Bhattarai et al., 2017; Cohen et al., 2017); understanding the “physiologic” and “pathologic” consequences of such modulation will no doubt provide impetus for developing therapeutic strategies harnessing these mechanisms.

In conclusion, it is clear a understatement to say so has a lot has been learned since 1994, but still some old questions remain unanswered, and many new questions will keep 5-HT researchers busy for years to come. Although 5-HT/serotonin/enteramine was only discovered and characterized a little more than 70-80 years ago (Viali and Ersparmar, 1933; Rapport et al., 1947, 1948; Ersparmer and Asero, 1952; Twarog and Page, 1953), 5-HT, its receptors, enzymes, transporters and multiple accessory proteins, constitute one of the oldest transmitter systems, estimated to have originated about 800 million years ago; hence it had time to develop a certain level of complexity. The appreciated complexity of the 5-HT system relates less to the number of 5-HT receptor classes, families, and subtypes, which was, per se, a conceptual challenge for some researchers in the 1980s and 1990s, than to the actual complex nature of the ligand/receptor/G protein/antinoin and/or interacting protein complexes that vary according to species, organ, cells, gender, and disease state. This intricacy renders the pharmacological definition of a given receptor, or a ligand, challenging, as it may all depend on the nature and environment of the supramolecular complex, which is being specifically addressed.

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Wrote or contributed to the writing of the manuscript: Barnes, Ahern, Becamel, Bockert, Camilleri, Chaumont-Dubel, Cleysena, Cunningham, Fone, Gershon, Di Giovanni, Goodfellow, Halberstadt, Hartley, Hassaine, Herrick-Davis, Hovius, Lacivita, Lambe, Leopoldo, Levy, Lummis, Marin, Maroteaux, McCreasy, Nelson, Neuamaior, Neumann-Tacreli, Nury, Robert, Roth, Roumier, Sanger, Teitel, Sharp, Villalon, Vogel, Watts, Hoyer.

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1. Introduction

Serotonin (5-HT) is a neurotransmitter that plays a crucial role in various brain functions, including mood regulation, memory, appetite, and pain perception. Its deregulation is associated with several neuropsychiatric disorders, such as depression, anxiety, and addiction. Understanding the mechanisms underlying serotonin function is essential for developing effective treatments.

2. Serotonin Receptors

Several 5-HT receptors have been identified, each with distinct pharmacological profiles and functional roles. The expression of these receptors is highly region-specific, which is important in understanding their role in different brain regions.

3. Serotonin Receptor Modulation

Pharmacological modulation of 5-HT receptors is a promising approach for the treatment of diseases. Selective agonists and antagonists have been developed to target specific receptor subtypes, offering potential therapeutic benefits.

4. Future Directions

Further research is needed to elucidate the complex interactions between serotonin systems and their roles in various brain functions. Understanding these interactions will be crucial in developing targeted therapies for the treatment of neuropsychiatric disorders.

5. Conclusion

The studies presented in this article provide valuable insights into the role of serotonin in the brain, highlighting the importance of understanding the mechanisms underlying its function for the development of effective treatments.

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