

5-HT Receptor Regulation of Neurotransmitter Release

KLAUS B. FINK AND MANFRED GÖTHERT

Department of Pharmacology, Bonn University Clinic, Bonn, Germany

| | |
|--------------------------------------------------------------------------------------------------------------------------------|-----|
| Abstract | 000 |
| I. Introduction: background, definitions, and scope | 000 |
| II. Experimental approaches | 000 |
| A. In vitro studies | 000 |
| B. In vivo studies | 000 |
| C. Advantages and limitations of the techniques applied | 000 |
| III. Modulation of cholinergic neurotransmission | 000 |
| A. General aspects | 000 |
| B. 5-Hydroxytryptamine 1A receptors | 000 |
| 1. Function, location, and classification | 000 |
| 2. Concluding summary statement | 000 |
| C. 5-Hydroxytryptamine 1B and 5-hydroxytryptamine 1D receptors | 000 |
| 1. Function and location | 000 |
| 2. Classification and development of nomenclature | 000 |
| a. 5-Hydroxytryptamine 1B/1D ligands | 000 |
| b. Rat 5-hydroxytryptamine 1B receptor | 000 |
| c. Development of guinea pig and human 5-hydroxytryptamine 1B and 5-hydroxytryptamine 1D auto- and heteroreceptor nomenclature | 000 |
| d. Guinea pig 5-hydroxytryptamine 1B receptor | 000 |
| e. Human 5-hydroxytryptamine 1B receptor? | 000 |
| 3. Specific aspects | 000 |
| 4. Concluding summary statement | 000 |
| D. 5-Hydroxytryptamine 2 receptors | 000 |
| 1. Function, location, and classification | 000 |
| 2. Concluding summary statement | 000 |
| E. 5-Hydroxytryptamine 3 receptors | 000 |
| 1. Function and location | 000 |
| 2. Classification | 000 |
| 3. Specific aspects | 000 |
| 4. Concluding summary statement | 000 |
| F. 5-Hydroxytryptamine 4 receptors | 000 |
| 1. Function, location, and classification | 000 |
| 2. Concluding summary statement | 000 |
| IV. Modulation of dopaminergic neurotransmission | 000 |
| A. General aspects | 000 |
| B. Dopamine transporter-mediated dopamine release | 000 |
| 1. Function, location, and mechanism | 000 |
| 2. Concluding summary statement | 000 |
| C. Modulation via 5-hydroxytryptamine receptors putatively located on interneurons | 000 |
| 1. 5-Hydroxytryptamine 1B receptors on interneurons | 000 |
| a. Function, site of action, and classification | 000 |
| b. Concluding summary statement | 000 |

Address correspondence to: Dr. Klaus B. Fink, Department of Pharmacology, Bonn University Clinic, Reuterstr. 2b, 53113 Bonn, Germany. E-mail: finkk@uni-bonn.de

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| | | |
|-----|--------------------------------------------------------------------------------------------------------|-----|
| 2. | 5-Hydroxytryptamine 2A receptors on interneurons | 000 |
| a. | Function, location, and mechanism | 000 |
| b. | Concluding summary statement | 000 |
| 3. | 5-Hydroxytryptamine 2C receptors on interneurons | 000 |
| a. | Function, location, and classification | 000 |
| b. | Concluding summary statement | 000 |
| D. | 5-Hydroxytryptamine 1B and 5-hydroxytryptamine 2 receptors identified in vitro or in vivo | 000 |
| 1. | Presynaptic 5-hydroxytryptamine 1 and/or 5-hydroxytryptamine 2 receptor-mediated modulation? | 000 |
| 2. | Presynaptic 5-hydroxytryptamine 1B receptors | 000 |
| a. | Function, site of action, and classification | 000 |
| b. | Concluding summary statement | 000 |
| 3. | Presynaptic 5-hydroxytryptamine 2A and 5-hydroxytryptamine 2C receptors? | 000 |
| a. | Function, site of action, and classification | 000 |
| b. | Concluding summary statement | 000 |
| E. | 5-Hydroxytryptamine 3 receptors | 000 |
| 1. | Function, location, and classification | 000 |
| 2. | Concluding summary statement | 000 |
| F. | 5-Hydroxytryptamine 4 receptors | 000 |
| 1. | Function, location, and classification | 000 |
| 2. | Concluding summary statement | 000 |
| V. | Modulation of noradrenergic neurotransmission | 000 |
| A. | General aspects | 000 |
| B. | 5-Hydroxytryptamine 1A receptors | 000 |
| 1. | Function, location, and classification | 000 |
| 2. | Concluding summary statement | 000 |
| C. | 5-Hydroxytryptamine 2 receptors | 000 |
| 1. | Function and location | 000 |
| 2. | Concluding summary statement | 000 |
| D. | 5-Hydroxytryptamine 3 receptors | 000 |
| 1. | Function and location | 000 |
| 2. | Concluding summary statement | 000 |
| VI. | Modulation of GABAergic neurotransmission | 000 |
| A. | General aspects | 000 |
| B. | 5-Hydroxytryptamine 1A receptors | 000 |
| 1. | Function, location, and classification | 000 |
| 2. | Mechanism | 000 |
| 3. | Concluding summary statement | 000 |
| C. | 5-Hydroxytryptamine 1B receptors | 000 |
| 1. | Function | 000 |
| 2. | Classification | 000 |
| 3. | Location | 000 |
| 4. | Concluding summary statement | 000 |
| D. | 5-Hydroxytryptamine 2A receptors | 000 |
| 1. | Function, location, and classification | 000 |
| 2. | Concluding summary statement | 000 |
| E. | 5-Hydroxytryptamine 3 receptors | 000 |
| 1. | Function, location, and classification | 000 |
| 2. | Mechanism | 000 |
| 3. | Interaction between presynaptic 5-hydroxytryptamine 3 and 5-hydroxytryptamine 1A receptors | 000 |
| 4. | Concluding summary statement | 000 |
| F. | 5-Hydroxytryptamine 4 receptors | 000 |
| 1. | Function, location, and classification | 000 |
| 2. | Concluding summary statement | 000 |

| | |
|-----------------------|-----|
| VII. Synopsis | 000 |
| Acknowledgments | 000 |
| References | 000 |

Abstract—Serotonergic neurons in the central nervous system impinge on many other neurons and modulate their neurotransmitter release. This review focuses on 1) the function of presynaptic 5-hydroxytryptamine (5-HT) heteroreceptors on axon terminals of central cholinergic, dopaminergic, noradrenergic, or GABAergic neurons and 2) the role of GABAergic interneurons expressing 5-HT heteroreceptors in the regulation of acetylcholine, dopamine, or noradrenaline release. In vitro studies on slices or synaptosomes and in vivo microdialysis experiments have shown that 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, and/or 5-HT₄ heteroreceptors mediate this modulation. 5-HT_{1B} receptors on neocortical cholinergic, striatal dopaminergic, or hippocampal GABAergic axon terminals are examples for release-inhibiting 5-HT heteroreceptors; 5-HT₃ receptors on hippocampal GABAergic or 5-HT₄ receptors on hippocampal cholinergic axon terminals are examples for release-facilitating 5-HT heteroreceptors. GABA released from GABAergic

interneurons upon activation of facilitatory 5-HT receptors, e.g., 5-HT_{2A} or 5-HT₃ receptors, mediates inhibition of the release of other neurotransmitters such as prefrontal neocortical dopamine or neocortical acetylcholine release, respectively. Conversely, attenuated GABA release in response to activation of inhibitory 5-HT heteroreceptors, e.g., 5-HT_{1A} or 5-HT_{1B} receptors on GABAergic interneurons is involved in paradoxical facilitation of hippocampal acetylcholine and striatal dopamine release, respectively. Such 5-HT heteroreceptors are considered potential targets for appropriate 5-HT receptor ligands which, by enhancing the release of a relevant neurotransmitter, can compensate for its hypothesized deficiency in distinct brain areas. Examples for such deficiencies are the impaired release of hippocampal or neocortical acetylcholine, striatal dopamine, and hippocampal or neocortical noradrenaline in disorders such as Alzheimer's disease, Parkinson's disease, and major depression, respectively.

I. Introduction: Background, Definitions, and Scope

Serotonin [5-hydroxytryptamine (5-HT¹)] is an important neurotransmitter in the CNS that influences neu-

¹ Abbreviations: 5-HT, 5-hydroxytryptamine (unselective 5-HT receptor agonist); CNS, central nervous system; ACh, acetylcholine; NA, noradrenaline; DA, dopamine; WAY 100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexane-carboxamide maleate (5-HT_{1A} antagonist); SB 224289, 2,3,6-tetrahydro-1'-methyl-5-[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl]furo [2,3-*f*]indole-3-spiro-4'-piperidine hydrochloride (5-HT_{1B} antagonist); CP-94253, 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine (5-HT_{1B} agonist); GR 127935 *N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide (5-HT_{1B/1D} antagonist); CGS 12066B, 7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrrolo[1,2-*a*] quinoxaline 1 (5-HT_{1B/1D} agonist); BRL 15572, 1-phenyl-3-[4-(3-chlorophenyl)piperazin-1-yl]phenylpropan-2-ol dihydrochloride (5-HT_{1D} antagonist); DOI, (±)-2,5-dimethoxy-4-iodoamphetamine hydrobromide (5-HT₂ agonist); DOB, (±)-4-bromo-2,5-dimethoxyphenylisopropylamine hydrobromide (5-HT_{2A/2C} agonist); AMI-193, 8-[3-(4-fluorophenoxy)propyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one (5-HT₂ antagonist); RS 102221, 8-[5-(5-amino 2,4-dimethoxyphenyl)-5-oxopentyl]-1,3,8-triazaspiro[4,5]decan-2,4-dione (5-HT_{2C} antagonist); RO 60-0175, (*S*)-2-(6-chloro-5-fluorindol-1-yl)-1-methylethylamine (5-HT_{2C} agonist); SB 242084, 6-chloro-5-methyl-1-[6-(methylpyridin-3-yloxy)pyridin-3-yl-carbamoyl] indoline (5-HT_{2C} antagonist); mCPPB, *m*-chlorophenylbiguanide (5-HT₃ agonist); BIMU 1, 3-ethyl-2,3-dihydro-*N*-[endo-8-methyl-8-azabicyclo(3.2.1)oct-3-yl]-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride (5-HT₄ agonist); GR 113808, [1-[2(methylsulfonylamino)ethyl]-4-piperidinyl]-methyl-1-methyl-indole-3-carboxylate maleate (5-HT₄ antagonist); BIMU 8, endo-*N*-8-methyl-azabicyclo [3.2.1] oct-3-yl-2,3-dihydro-3-isopropyl-2-oxo-1*H*-benzimidazol-1-carboxamide hydrochloride (5-HT₄ agonist); GR 125487, [1-[2(methylsulfonylamino)ethyl]-4-piperidinyl]-methyl-5-fluoro-2-methoxy-1*H*-indole-3-carboxylate hydrochloride (5-HT₄ antagonist); SB 204070 (8-amino-7-chloro-*N*-butyl-4-piperidyl) methylbenzo-1,4-dioxan-5-carboxylate) hydrochloride (5-HT₄ antagonist);

ronal activity via 14 genetically, pharmacologically, and functionally distinct 5-HT receptors belonging to seven families termed 5-HT₁ through 5-HT₇ (Langer, 1980; Hoyer et al., 1994, 2002; Baumgarten and Göthert, 1997;

SDZ 205,557, 4-amino-5-chloro-2-methoxybenzoic acid 2-(diethylamino) ethyl ester hydrochloride (5-HT₄ antagonist); 8-OH-DPAT, 8-hydroxy-2-(*di-n*-propylamino)tetralin HBr (5-HT_{1A} agonist); TTX, tetrodotoxin (Na⁺-channel blocker); NAN-190, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (5-HT_{1A} partial agonist/antagonist); 5,7-DHT, 5,7-dihydroxytryptamine (serotonergic neurotoxin); CP-93129, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-*b*]pyridin-5-one (5-HT_{1B} agonist); RU 24969, 5-methoxy-3-[1,2,3,6-tetrahydropyridin-4-yl]-1*H*-indole (5-HT_{1B/1D} agonist); TFMPP, *N*-(3-trifluoromethylphenyl)piperazine hydrochloride (5-HT_{1B} agonist); MDL 72222, 3-tropanyl-3,5-dichlorobenzoate (5-HT₃ antagonist); 5-CT, 5-carboxamidotryptamine (5-HT₁ agonist); SB 216641, *N*-[3-(2-dimethylamino) ethoxy-4-methoxyphenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1'-biphenyl)-4-carboxamide (5-HT_{1B} antagonist); mCPP, *m*-chlorophenylpiperazine (5-HT_{2C/2B} agonist/partial agonist); LY-53,857, 6-methyl-1-(1-methylethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methyl-propyl ester maleate (5-HT₂ antagonist); MK-212, 6-chloro-2-(1-piperaxinyl)pyrazine (5-HT_{2B/2C} agonist); GR 38032F, 1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1-imidazol-1-yl) methyl]-4*H*-carbazol-4-one (5-HT₃ antagonist); DAU 6215, (3-α-tropanyl)1*H*-benzimidazolone-3-carboxamide chloride (5-HT_{3/4} antagonist); VC 135, 7-(4-methyl-1-piperazinyl)-6*H*-[1]benzopyranol[3,4-*c*]quinoline (5-HT₃ antagonist); MDMA, 3,4-methylenedioxyamphetamine (amphetamine derivative); RS23597, 3-[piperidin-1-yl]propyl-4-amino-5-chloro-2-methoxybenzoate hydrochloride (5-HT₄ antagonist); GBR12909, 1-(2-[bis(4-fluorophenyl) methoxy]ethyl)-4-(3-phenylpropyl)piperazine (DA uptake inhibitor); GBR12935, 1-(2-[diphenylmethoxy]ethyl)-4-[3-phenylpropyl]piperazine (DA uptake inhibitor); ICS 205,930, 3-tropanyl-indole-3-carboxylate, tropisetron (5-HT₃ antagonist); MDL 100,907 *R*-(+)-α-(2,3-dimethoxyphenyl)-1-(2-(4-fluorophenyl) ethyl)-4-piperidine-methanol (5-HT_{2A} antagonist); SB 206553

Barnes and Sharp, 1999; Sari, 2004; Bockaert et al., 2006). Seven of these 14 receptors are discussed in the context of presynaptic modulation of ACh, NA, DA, or GABA release, and of the role of GABAergic interneurons in the release modulation of these neurotransmitters. The main features, their occurrence in the brain, and their functional and therapeutic implications are summarized in Table 1.

The cell bodies and dendrites of the serotonergic neurons are located mainly in the raphe nuclei of the midbrain from where they project to almost all brain areas and the spinal cord (Törk, 1990; Baumgarten and Göthert, 1997). Exceptionally, the serotonergic axons innervating the cerebellum originate to a major part in the nucleus reticularis paragigantocellularis and nucleus pontis oralis of the brainstem (Bishop and Ho, 1985). As a result of 5-HT release from nerve terminals in virtually all regions of the CNS, the serotonergic system is involved in many important physiological functions such as the control of blood pressure, body temperature, appetite, release of prolactin and other hormones, perception of pain, and emotional behavior. Accordingly, the serotonergic system also plays a role in the pathogenesis of diseases in which these functions are disturbed, e.g., hypertension, hormonal dysfunction, depression, and anxiety. In this context, it should also be noted that the serotonergic system is influenced by clinically effective drugs such as antihypertensives, antidepressants, anxiolytics, and antipsychotics, many of which act at one or more of the 14 receptor types mentioned above. 5-HT receptors may also become targets of newly developed drugs with more favorable properties.

To define the receptors under review, a simple model of three successive neurons should be considered (Fig. 1). The

(5-methyl-1-(3-pyridylcarbonyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) hydrochloride (5-HT_{2B/2C} antagonist/inverse agonist); SB 204741, *N*-(1-methyl-5-indolyl)-*N'*-(3-methylisothiazol-5-yl)urea (5-HT_{2B} antagonist); VTA, ventral tegmental area; BW 723C86, α -methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine hydrochloride (5-HT_{2B} agonist); SB 243213, 5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxyl]-5-pyridyl]carbonyl]-6-trifluoromethyl-indone (5-HT_{2C} antagonist); SR 46349B, (*trans*-4-(3*Z*)-[2-(dimethylaminoethyl)oxyimino]-3-(2-fluorophenyl)propen-1-yl]-phenol hemifumarate (5-HT_{2A} antagonist); DRN, dorsal raphe nucleus; DAU 6285, endo-6-methoxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxylate hydrochloride (5-HT_{3/4} antagonist); SB 207710, (1-*n*-butyl-4-piperidinyl)methyl-8-amino-7-iodo-1,4-benzodioxane-5-carboxylate (5-HT₄ antagonist); MDL 73005EF, 8-[2-(1,4-benzodioxan-2-ylmethylamino)ethyl]-8-azaspiro[4.5]decane-7,9-dione (5-HT_{1A} agonist); MKC-242, 5-[3-[[[(25)-1,4-benzodioxan-2-ylmethyl]amino]propoxy]-1,3-benzodioxole HCl (5-HT_{1A} agonist); ICI 170,809, 2,2-[dimethylamino-2-methylpropylthio]-3-phenylquinoline hydrochloride (5-HT₂ antagonist); DOM, *R*(-)-1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (5-HT₂ agonist); IPSC, inhibitory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; IPSP, inhibitory postsynaptic potential; SCN, suprachiasmatic nucleus; 4-DAMP, 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (M₃ antagonist); AFDX-116, 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5-11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepine-6-one (M₂ antagonist).

first neuron is serotonergic ("neuron I") and releases 5-HT at axoaxonal synapses directly to the 5-HT receptors of the terminal boutons/varicosities of the axon of a second, nonserotonergic neuron ("neuron II," e.g., cholinergic, dopaminergic, or noradrenergic). Alternatively, 5-HT may be released from neuron I terminals into the vicinity of neuron II terminals (Beaudet and Descarries, 1978; Törk, 1990) and reaches the 5-HT receptors on the neuron II terminals by diffusion over relatively long distances. The terminals of neuron II form synapses with "neuron III." Looking back from neuron III, the 5-HT receptors on the neuron II terminals are considered presynaptic or prejunctional. The presynaptic 5-HT receptors on the neuron II terminals belong to the category of heteroreceptors, which by definition are stimulated by neurotransmitters other than those released by the neuron on which the receptor resides. Accordingly, presynaptic 5-HT heteroreceptors can be defined as release-modulating 5-HT receptors on nonserotonergic varicosities (synaptic boutons) of axon terminals (Fig. 1).

Previous reviews referred to the function of presynaptic 5-HT autoreceptors (Moret, 1985; Timmermans and Thoolen, 1987; Middlemiss, 1988; Starke et al., 1989; Göthert and Schlicker, 1997) and presynaptic heteroreceptors on serotonergic axon terminals (Göthert and Schlicker, 1991). The present article completes the review series on the cross-talk between serotonergic and nonserotonergic axon terminals by focusing on presynaptic 5-HT heteroreceptors on nonserotonergic axon terminals.

However, in many cases the exact location of the modulatory 5-HT receptors, i.e., presynaptically or somadentritically on the directly modulated neuron or on interneurons, is still an open question due to the lack of appropriate *in vitro* experiments (e.g., 5-HT₄ receptors facilitating ACh release; section III.F.). In several *in vivo* and *in vitro* investigations, modulation of release by 5-HT receptor ligands was interpreted in terms of direct operation of a 5-HT heteroreceptor on the respective neuron although the primary cell effect, depending on the receptor signal transduction, did not conform (was even opposite) to such an interpretation. Examples are 5-HT₃ receptors inhibiting ACh release (section III.E.) and 5-HT_{1A} receptors facilitating ACh release (section III.B.). These results should be reinterpreted. In both cases inhibitory GABAergic interneurons rather than the cholinergic nerve terminals may be assumed to be the site of action of 5-HT. The release-modulating 5-HT receptors involved are located, at least to a major part, on the axon terminals of GABAergic interneurons (Fig. 2). A crucial prerequisite for the indirect modulation of the release of a neurotransmitter via an inhibitory GABAergic interneuron has to be taken into account: the nerves from which this neurotransmitter is released must be endowed with GABA receptors. In fact, evidence has been presented that inhibitory GABA_A and/or GABA_B receptors are operative on cholinergic (Supavilai and Karobath, 1985; Moor et al., 1998; Ikarashi et al.,

TABLE 1
5-HT receptors discussed in this review, their cellular signaling pathways, ligands, and selected functional and therapeutic implications
For an overview over the involvement in 5-HT heteroreceptor-mediated modulation of neurotransmitter release, see Table 11.

| 5-HT Receptor | Signal Transduction | Agonists | Antagonists | Function, Occurrence, Therapeutic Implications |
|--------------------|-----------------------------------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 5-HT _{1A} | G _{i/o} AC ↓ | 8-OH-DPAT ^a , ipsapirone | Spiroxatrine, cyanopindolol, WAY 100635 ^a | Inhibition of cellular function: somatodendritic "autoreceptor" in raphe nuclei; also highly expressed in cingulate and entorhinal cortex, hippocampus, and lateral septum; involved in learning, memory, in anxiety and depression, and in ACTH release |
| 5-HT _{1B} | G _{i/o} AC ↓ | Sumatriptan, CP-94253, L-694,247, 5-nonyloxytryptamine, CGS 12066B | SB 224289 ^a , cyanopindolol, NAS-181 ^a , GR 127935 | Inhibition of cellular function; presynaptic autoreceptor; highly expressed in basal ganglia, hippocampus, and striatum; involved in anxiety and depression, drug abuse, and migraine therapy |
| 5-HT _{1D} | G _{i/o} AC ↓ | Sumatriptan, L-694,247, CGS 12066B | BRL 15572 ^a , GR 127935 | Inhibition of cellular function; present in basal ganglia, periaqueductal grey, and spinal cord; involved in migraine therapy |
| 5-HT _{2A} | G _{q/11} PLC ↑ | α-Methyl-5-HT, DOI, DOB | Ketanserin ^a , R-96544 ^a , AMI-193 | Facilitation/stimulation of cellular function and neuronal depolarization; highly expressed in neocortex, pyriform and entorhinal cortex, caudatum, claustrum, nucleus accumbens, olfactory tubercle, and hippocampus; involved in learning and therapy of schizophrenia with atypical antipsychotics |
| 5-HT _{2C} | G _{q/11} PLC ↑ | α-Methyl-5-HT, RO 60-0175 ^a , WAY 161503 | RS 102221 ^a , N-desmethyleclozapine, SB 242084 ^a | Facilitation/stimulation of cellular function; strongly expressed in choroid plexus, less in pyriform cortex, cingulate, nucleus accumbens, hippocampus, amygdala, caudatum, and substantia nigra; involved in cerebrospinal fluid secretion; therapy of schizophrenia with atypical antipsychotics |
| 5-HT ₃ | Na ⁺ /K ⁺ /Ca ²⁺ channel | 2-Methyl-5-HT ^a , SR 57227, mCPBG ^a | Ondansetron ^a , tropisetron ^a , Y-25130 ^a , zacopride | Facilitation/stimulation of cellular function; in hippocampus, striatum, and superficial neocortex layers; involved in emesis, anxiety, and reward |
| 5-HT ₄ | G _s AC ↑ | BIMU 1 ^a , BIMU 8 ^a , ML10302 ^a , RS 67506 ^a , zacopride | GR 113808 ^a , GR 125487 ^a , RS 39604 ^a , SB 204070 ^a , SDZ 205,557 ^a | Facilitation/stimulation of cellular function and excitatory function on cholinergic transmission; occurrence in nigrostriatal and mesolimbic system, and neocortex; involved in cognitive performance |

AC, adenylyl cyclase; spiroxatrine, 8-[(2,3-dihydro-1,4-benzodioxin-2-yl)methyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one (5-HT_{1A} antagonist); ACTH, adrenocorticotropic; L-694,247, 2-[5-{3-(4-methylsulfonylamino)benzyl-1,2,4-oxadiazol-5-yl}-1H-indole-3-yl]ethanamine (5-HT_{1B} agonist); α-methyl-5-HT, α-methyl-5-hydroxytryptamine (5-HT_{2A} agonist); R-96544, (2R,4R)-5-[2-[2-(2-(3-methoxyphenyl)ethyl)-1-methyl-3-pyrrolidinol hydrochloride (5-HT_{2A} antagonist); WAY 161503, 8,9-dichloro-2,3,4,4-tetrahydro-1H-pyrazolo[1,2-α]quinoxalin-5(6H)-one hydrochloride (5-HT_{2C} agonist); SR 57227, 1-(6-chloro-2-pyridinyl)-4-piperidinamine hydrochloride (5-HT₃ agonist); Y-25130, N,1-azabicyclo[2.2.2]-oct-3-yl-6-dihydro-4-methyl-3-oxo-2H-1,4-benzoxazine-8-carboxamide hydrochloride (azasetron; 5-HT₃ antagonist); ML10302, 2-(1-piperidinyl)ethyl-4-amino-5-chloro-2-methoxybenzoate (5-HT₄ agonist); RS 39604, 1-[4-amino-5-chloro-2-(3,5-dimethoxyphenyl)methyl]oxy]-3-[1-[2-methylsulphonylamino]piperidin-4-yl]propan-1-one hydrochloride (5-HT₄ antagonist); RS 67506, 1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-*n*-butyl-4-piperidinyl)-1-propanone (5-HT₄ agonist).
^a Selective ligand at the respective receptor.

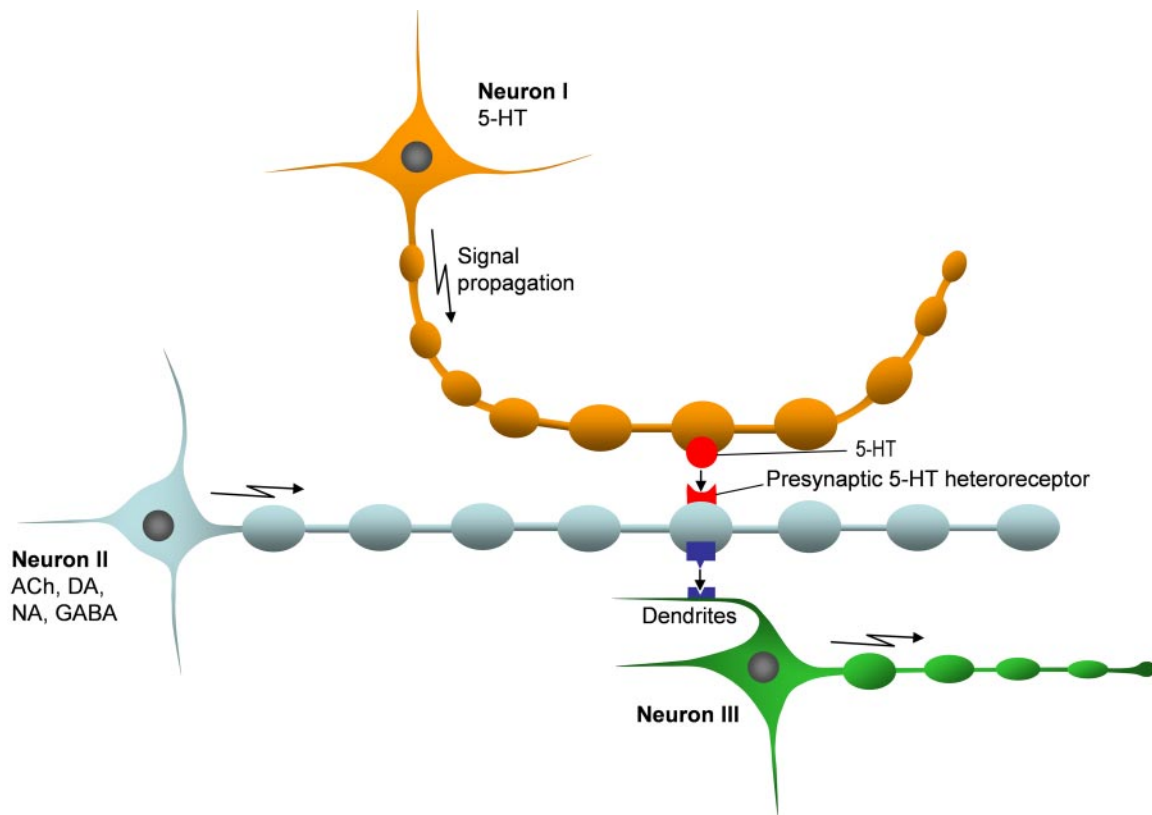


FIG. 1. Schematic model involving presynaptic 5-HT heteroreceptors (red), which are activated by 5-HT released from neuron I (orange) and which are located on the terminals (varicosities) of a nonserotonergic neuron II (blue) from which the respective neurotransmitter is released upon a third neuron (green). Modulation of neurotransmitter release from neuron II is a subject of this article, and investigations providing evidence in favor of this modulation of neurotransmitter release by presynaptic 5-HT heteroreceptors are considered. 5-HT may inhibit, facilitate, or stimulate neurotransmitter release from neuron II.

1998; Vazquez and Baghdoyan, 2003), dopaminergic (Ronken et al., 1993; Steiniger and Kretschmer, 2003), and noradrenergic neurons (Fung and Fillenz, 1983; Suzdak and Gianutsos, 1985; Tanaka et al., 2002; Sakamaki et al., 2004; Ushigome et al., 2004)

Presynaptic 5-HT heteroreceptors play mainly a physiological role in the local fine regulation of the release of the transmitter from the axon terminal on which the receptors are located. They inhibit or facilitate transmitter release in response to action potentials invading the varicosities. In contrast, somadendritic receptors modify the function of the whole neuron with all branches of its axon. Local modulation via presynaptic heteroreceptors is more pronounced as the 5-HT concentration in the biophase of the receptors increases. This concentration depends not only on the serotonergic neuronal activity but also on the activity of the transmitter transporter. In addition to these modulatory effects, presynaptic 5-HT heteroreceptors can directly stimulate neurotransmitter release; this has recently been demonstrated for, e.g., presynaptic 5-HT₃ receptors on GABAergic axon terminals (Turner et al., 2004; Dorostkar and Boehm, 2007; Dorostkar and Boehm, 2005).

This review focuses on 5-HT heteroreceptor-mediated modulation of transmitter release from cholinergic, dopaminergic, noradrenergic, and GABAergic neurons; in

particular, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, and 5-HT₄ receptors play a role in modulation of these neurotransmitters (Table 1). A previous article reviewed the literature before 1998 on presynaptic and nonsynaptic interactions between the same transmitters as are the focus of this article (Vizi and Kiss, 1998). It is complementary to this article because it focused on hippocampal transmission only, including the interaction between glutamatergic neurons, GABAergic interneurons, raphehippocampal serotonergic, coeruleohippocampal noradrenergic, and septohippocampal cholinergic projections. A more recent article reviewed the literature on the modulation of neurotransmitter release via multiple auto- and heteroreceptors in the human brain (Raiteri, 2006). Only a small part of that article is devoted to human 5-HT receptors and, thus, it overlaps only to a minor extent with this review.

II. Experimental Approaches

A. *In Vitro* Studies

Evidence for the operation of 5-HT heteroreceptors mediating modulation or stimulation of neurotransmitter release has been provided by *in vitro* experiments in which the effects of 5-HT receptor ligands on the overflow of radioactively labeled or endogenous neurotrans-

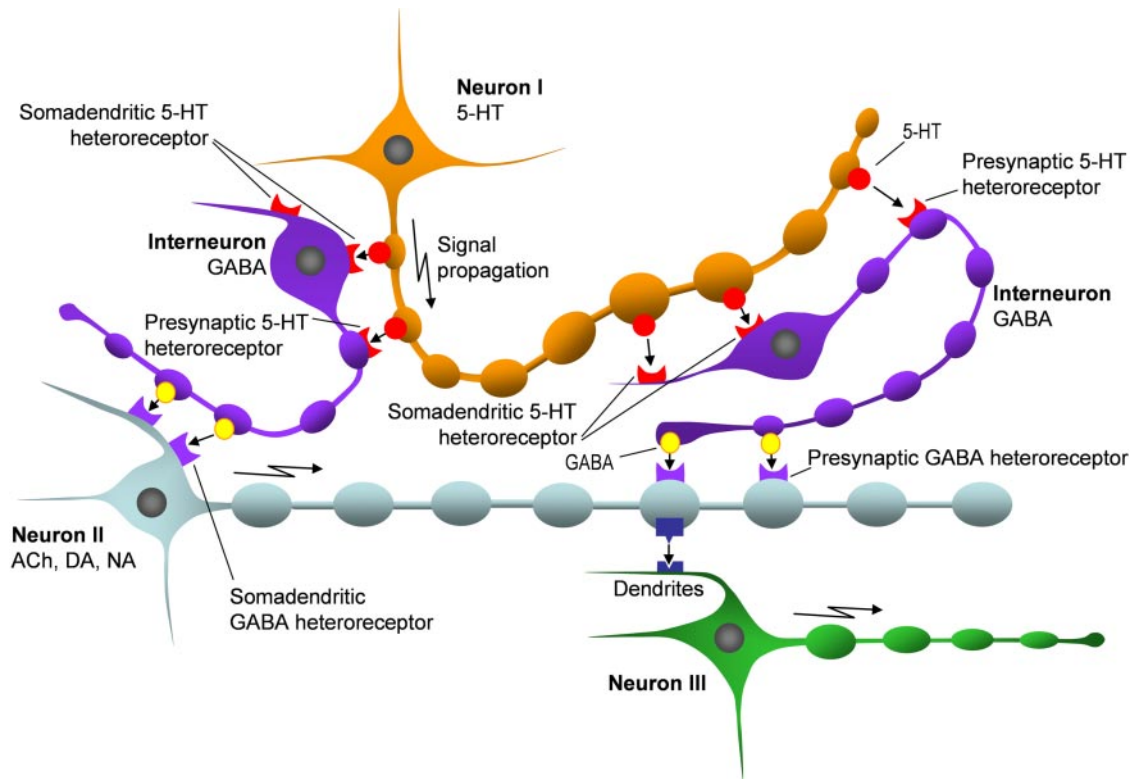


FIG. 2. Schematic model that highlights the role of inhibitory GABAergic interneurons (lilac) in the modulation of neurotransmitter release from a nonserotonergic neuron II (blue) by 5-HT released from neuron I (orange). Consistent with Fig. 1, modulation of neurotransmitter release from neuron II [which, in turn, releases its neurotransmitter upon a third neuron (green)] is a main subject of this article. 5-HT acts via somadendritic or presynaptic 5-HT receptors (red) on the GABAergic interneurons that innervate either the somadendritic or terminal region of neuron II. Investigations providing evidence in favor of this interneuron-mediated modulation of neurotransmitter release from neuron II are reviewed. 5-HT may inhibit, facilitate, or stimulate GABA release from the interneuron, thus facilitating or inhibiting neurotransmitter release from neuron II. The effect of 5-HT on neuron II is inverted by the inhibitory GABAergic interneuron, which means that stimulatory or facilitatory effects of 5-HT mediated by 5-HT₂ or 5-HT₃ receptors on the interneuron will result in an inhibition of neurotransmitter release from neuron II; conversely, inhibitory effects of 5-HT mediated by 5-HT₁ receptors on the inhibitory interneuron will result in a disinhibition, i.e., facilitation, of neurotransmitter release from neuron II.

mitter from brain or spinal cord slices or synaptosomes has been investigated (for references, see Tables 2–11). Briefly, CNS preparations were preincubated with the labeled transmitter or its precursor. Subsequently stimulation-evoked overflow of radioactivity from the superfused (or incubated in a few cases) preparations was measured. As a rule, electrical impulses, high K⁺ or, in the case of excitatory 5-HT₃ receptors, 5-HT₃ receptor agonists were used for stimulation (see Tables 2–11). Stimulation-evoked overflow of radioactivity or labeled transmitter, in particular in the presence of an inhibitor of the respective neuronal transmitter transporter, reflects the release of endogenous transmitter. Therefore, in such cases, the term transmitter release is used. Determination of depolarization-evoked transmitter release from superfused synaptosomes, i.e., torn-off and resealed varicosities, is the most appropriate method to prove the presynaptic location of the 5-HT heteroreceptor under study in such functional investigations. An alternative approach is the measurement of K⁺-evoked transmitter release from superfused slices in the presence of tetrodotoxin (TTX), which blocks the Na⁺ channel-dependent propagation of action potentials along the axon, thus excluding the involvement of somadendritic receptors.

According to the concept of transmitter modulation via presynaptic inhibitory or facilitatory 5-HT heteroreceptors, 5-HT receptor agonists should inhibit or facilitate, respectively, transmitter release in a manner susceptible to blockade by an appropriate 5-HT receptor antagonist. Experiments on slices also provide an answer to the question whether the receptor is tonically activated by 5-HT released from neuron I of Fig. 1; if so, appropriate 5-HT receptor antagonists, given alone, should produce an effect opposite to that of corresponding 5-HT agonists by blocking the effect of endogenous 5-HT released into the biophase of the 5-HT-heteroreceptor. Such information cannot be derived from experiments on superfused synaptosomes because the superfusion flow effectively removes endogenous 5-HT, released from serotonergic synaptosomes, from the biophase of 5-HT heteroreceptors on neighboring nonserotonergic synaptosomes.

Knockout mice have been particularly useful for the identification of a presynaptic auto- or heteroreceptor. If modulation of neurotransmitter release only occurs in wild-type but not in the respective receptor knockout mice, the function of a presynaptic receptor can be convincingly shown. This method has been used to prove the

function of the presynaptic 5-HT_{1B} receptor in the mouse hippocampus (Rutz et al., 2006).

5-HT heteroreceptor research experienced a renaissance with the development of more sophisticated procedures comprising cell biological and electrophysiological techniques, which were applied to GABAergic terminals (Table 10). For example, such experiments were performed on isolated single hippocampal CA1 pyramidal neurons with numerous adherent functional presynaptic terminals of GABAergic interneurons which, in turn, are endowed with presynaptic 5-HT heteroreceptors. This preparation is called the "synaptic bouton preparation" (Rhee et al., 1999; Akaike and Moorhouse, 2003). The neurons were isolated using an enzyme-free mechanical procedure. In this preparation voltage clamp recordings from the pyramidal cells were performed, and single boutons were focally stimulated. Administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and the 5-HT₃ receptor agonist, *m*-chlorophenylbiguanide, revealed that all boutons (= varicosities) contained inhibitory 5-HT_{1A} receptors and that a subset of boutons was endowed with both 5-HT_{1A} and excitatory 5-HT₃ receptors (Katsurabayashi et al., 2003). Another approach to the identification of presynaptic 5-HT₃ heteroreceptors on GABAergic axon terminals is based on the ability of rat hippocampal neurons to form autapses in single neuron microcultures; the function of this neuron can be analyzed by conventional whole-cell patch-clamp recordings from the neuronal soma (Dorostkar and Boehm, 2005, 2007). Using this preparation of hippocampal GABAergic neurons, 5-HT caused rapidly activating inward currents, which were reduced by tropisetron [3-tropanylindole-3-carboxylate hydrochloride (5-HT₃ antagonist)], leading to the conclusion that GABAergic hippocampal neurons express 5-HT₃ receptors, activation of which causes depolarization and a stimulation of GABA release. This interpretation was supported by experiments on conventional mass cultures of hippocampal neurons in which 5-HT produced a 25-fold rise in the frequency of miniature inhibitory postsynaptic currents in a manner sensitive to antagonism by tropisetron (Dorostkar and Boehm, 2005, 2007).

Attempts have been made to demonstrate the presence of certain presynaptic 5-HT heteroreceptors by modern histochemical and cytochemical techniques. In one type of these experiments, *in situ* hybridization analyses of the respective 5-HT receptor mRNA was combined with determination of the corresponding binding sites by quantitative autoradiography. When the respective 5-HT binding sites can be identified in certain brain regions but no corresponding mRNA is detectable, the 5-HT receptor under consideration may be assumed to occur as presynaptic receptor. This assumption is based on the fact that these receptors are synthesized in the somadendritic area of the neuron and transported to the terminals which, in contrast to the somadendritic area, contain at best low amounts of the respective 5-HT

receptor mRNA. Such investigations are available for only few receptors, among them 5-HT_{1B} and 5-HT_{1D} receptors (Bruinvels et al., 1994a,b). However, suggestions concerning the type of neurotransmitter whose release is modulated at the corresponding nerve terminals are largely speculative; not only heteroreceptors on different types of nerve terminals but also autoreceptors may be visualized. In another approach to identifying presynaptic receptors more directly, synaptosomes have been used. Thus, in synaptosomes prepared from corpus striatum, hippocampus, amygdala, and cerebellum of the rat (Nayak et al., 1999), 5-HT₃ receptor protein was detected by immunocytochemistry. In synaptosomes from all of these regions the 5-HT₃ receptor immunoreactivity was colocalized with the synaptic vesicle protein synaptophysin, clearly indicating a presynaptic localization of these 5-HT₃ receptors. However, the synaptosomal preparation did also not allow assignment of the presynaptic 5-HT₃ receptor to certain types of neurons. Such studies, in particular when considering the limitations mentioned, are not the subject of this review article dealing with the release of neurotransmitters.

B. *In Vivo* Studies

The most frequently applied *in vivo* technique has been intracerebral microdialysis in anesthetized or freely moving animals (for references, see Tables 2–6, 8, and 9). The respective neurotransmitter and its metabolites in the dialysates have been determined by high-pressure liquid chromatography. This technique made it possible to directly apply 5-HT receptor agonists and/or antagonists via the dialysis probe to the brain area under study and to simultaneously measure their influence on the overflow of the neurotransmitter under investigation. In many cases, the receptor ligands have been injected systemically; if not supplemented by other experimental results, such data hardly allow a conclusion as to the location within the CNS of the receptor involved (section II.C.). Two further *in vivo* methods providing similar informations on drug-induced modification of transmitter release in certain brain regions, i.e., the technique of an epidurally implanted cup on the cerebral cortex (Beani et al., 1968; Siniscalchi et al., 1991) and the push-pull technique (Guan and McBride, 1989), have also been used for the identification of presynaptic 5-HT heteroreceptors in the brain. Microdialysis experiments on receptor knockout mice will provide considerable progress also in *in vivo* receptor identification and determination of the functional role.

C. *Advantages and Limitations of the Techniques Applied*

In several conventional superfusion experiments on CNS preparations, the location of a 5-HT heteroreceptor on a certain nerve terminal (neuron II) (Fig. 1) has been unanimously proven by determination of depolarization-evoked release of the respective transmitter from slices

in the presence of TTX or from synaptosomes. In many other cases, the suggestion that a presynaptic 5-HT heteroreceptor is operative on an axon terminal (neuron II) (Fig. 1) has been derived from superfusion experiments without TTX on slices prepared from brain areas that contain axons and axon varicosities, but no cell bodies, of the respective neuron (neuron II). The involvement of a 5-HT heteroreceptor on these varicosities themselves was the simplest and most plausible interpretation, which is generally accepted. In particular, this conclusion could be drawn when identical data, obtained on slices in the presence of TTX or on synaptosomes, were available from other investigations. However, in the absence of TTX, alternatives cannot be excluded. Thus, the axon terminal of neuron II, which is subject to modification of transmitter release may be under control of an interneuron (Fig. 2) between neuron I and neuron II. In fact, the 5-HT heteroreceptor may be located on the axon terminals or the somadendritic area of such an interneuron, whose transmitter GABA finally modifies the release of neuron II transmitter. Furthermore, as an even more complex alternative, a circuit consisting of several interneurons within the brain area under study is conceivable, with the first member of the circuit being the site of action of the 5-HT receptor ligand.

In other experiments on slices in the absence of TTX or in intracerebral *in vivo* microdialysis experiments in which receptor ligands were injected systemically (section II.B.), the presence of 5-HT heteroreceptors mediating inhibition of neuron II transmitter release on an inhibitory GABAergic interneuron is by far the most reasonable explanation (Fig. 2). This is the case when agonists at receptors increasing cell function, e.g., 5-HT₃ receptor agonists, inhibit transmitter release from neuron II. Basically, 5-HT₃ receptors occur somadendritically and preferentially on the terminals of neurons (Kidd et al., 1993; Boschert et al., 1994; Sari et al., 1999). Activation of somadendritic 5-HT₃ heteroreceptors induces mainly Na⁺ and K⁺ flux through the receptor ion channel leading to strong depolarization and generation of action potentials propagating to the axon terminals, where Ca²⁺ influx occurs via voltage-gated Ca²⁺ channels (Derkach et al., 1989; Hargreaves et al., 1994). In nerve terminals this sequence of events is not valid. As shown on synaptosomes from rat striatum by means of confocal microscopy imaging, 5-HT₃ receptor channels on nerve terminals (potentially different in subunit composition from those in the perikaryon) appear to be exclusively Ca²⁺-permeable and their activation induces Ca²⁺ influx into the varicosities in a manner independent of voltage-gated Ca²⁺ channels (Rondé and Nichols, 1998). Irrespective of the somadendritic or presynaptic location of the 5-HT₃ receptor in the neuron under consideration, administration of a 5-HT₃ receptor agonist should lead to an increase, rather than an inhibition, of the release of its transmitter. Therefore, the inhibition of transmitter release from neuron II by 5-HT₃ receptor

activation can be most convincingly explained by 5-HT₃ receptors located on inhibitory GABAergic interneurons (Fig. 2). They invert the excitatory or facilitatory effect of 5-HT₃ receptor stimulation to an inhibition of transmitter release from neuron II. Typical examples for such a constellation are the 5-HT₃ agonist-induced inhibitions of acetylcholine release in the rat entorhinal cortex (Barnes et al., 1989) and in the guinea pig neocortex (Bianchi et al., 1990). Most plausibly, the 5-HT₃ receptor should not be located on the cholinergic axon terminals but rather on varicosities or cell bodies of inhibitory interneurons, probably GABAergic neurons (Fig. 2) (section III.E.), which are abundant throughout the brain. Recent investigations of the synaptic bouton preparation on mechanically isolated GABAergic interneurons (Katsurabayashi et al., 2003) and on single GABAergic neurons in microcultures (Dorostkar and Boehm, 2005, 2007) unequivocally proved the expression and operation not only of 5-HT₃ but also of 5-HT_{1A} heteroreceptors on GABAergic axon terminals.

In most of the *in vivo* investigations on presynaptic 5-HT heteroreceptors, the intracerebral microdialysis technique has been applied. In a major part of microdialysis studies, 5-HT receptor agonists and/or antagonists were directly administered via the dialysis probe to a certain brain area containing axon terminals (but no cell bodies) of the nonserotonergic neuron under investigation (neuron II) (Figs. 1 and 2). When in such experiments 5-HT receptor ligands caused a modulation of transmitter release the authors postulated, as a rule, that the 5-HT heteroreceptors involved are located presynaptically, and this suggestion represents a probable and plausible interpretation of the data (Fig. 1). Nevertheless, the same restrictions as in the *in vitro* experiments on slices in the absence of TTX are valid in such *in vivo* studies, i.e., the possibility of an involvement of a local circuit of interneuron(s) (Fig. 2) cannot be excluded. However, if the results of such *in vivo* experiments are identical to those obtained on slices in the presence of TTX or on synaptosomes, the *in vivo* operation of a presynaptic 5-HT heteroreceptor becomes likely.

Data obtained in *in vivo* microdialysis (or push-pull perfusion or cup superfusion) studies, in which systemically applied 5-HT receptor ligands modulate nonserotonin transmitter release, raise a particular problem: they can only be interpreted in terms of a presynaptic location of a 5-HT heteroreceptor if they do not substantially differ from the results of *in vitro* experiments and/or at least from data of *in vivo* microdialysis investigations in which the 5-HT receptor ligands are administered locally. In other words, suggesting a presynaptic location of a 5-HT heteroreceptor based exclusively on microdialysis investigations in which 5-HT receptor ligands are systemically administered is questionable. For a more reliable interpretation the data must be

related to those of appropriate in vitro and local microdialysis studies.

Comparison of local application and systemic injection of drugs in in vivo microdialysis studies provides hints at whether or not the drugs pass the blood-brain barrier. Furthermore, such a comparison makes it possible to get an idea about the overall importance of the presynaptic 5-HT heteroreceptor ligands applied systemically in the control of transmitter release.

III. Modulation of Cholinergic Neurotransmission

A. General Aspects

Pharmacological in vitro experiments examining the operation of 5-HT heteroreceptors on cholinergic neurons have been performed predominantly in the rat brain and to a minor extent in the guinea pig or human brain. According to the presence of serotonergic as well as cholinergic innervation in certain brain areas, investigations of serotonergic modulation of ACh release have been performed in the cortex, hippocampus, or striatum. The cerebral cholinergic system plays a major role for memory and cognition, and an increase in ACh concentration in the synaptic cleft represents a therapeutic option in dementia associated with Alzheimer's disease. The role of cholinergic-serotonergic interactions in cognition has been extensively reviewed (Cassel and Jeltsch, 1995; Steckler and Sahgal, 1995; Buhot, 1997; Feuerstein and Seeger, 1997; Ruotsalainen et al., 1998; Buhot et al., 2000). The interneuronal, somadendritic, and/or presynaptic location of any 5-HT receptor type mediating an increase in ACh release (and, hence, representing a putative target for pharmacotherapy of Alzheimer's dementia) will be discussed with particular care.

It will become evident in the following sections that a clear-cut statement concerning the location of the various 5-HT receptor types is often very difficult. For the 5-HT₁ receptor family, the discussion of location and function is particularly complex for four reasons: 1) there is a functional antagonism between the 5-HT_{1A} and 5-HT_{1B} receptors; 2) the receptor under consideration may be located both presynaptically and somadendritically; 3) it may be located on noncholinergic interneurons; and 4) the nomenclature of 5-HT_{1B/1D} receptors has been changed in the meantime. As will be outlined in some detail, presynaptic 5-HT_{1B/1D} receptors played a crucial role in the identification of the main common criterion underlying a rational and unifying classification. A possible location of 5-HT receptors modulating ACh release on noncholinergic interneurons also is involved in the context of 5-HT₂ and 5-HT₃ receptors.

B. 5-Hydroxytryptamine 1A Receptors

1. *Function, Location, and Classification.* In vivo experiments in which 5-HT_{1A} receptor ligands were ad-

ministered systemically revealed that 8-OH-DPAT and/or other 5-HT_{1A} receptor agonists such as buspirone and ipsapirone [2-[4-[4-(2-pyrimidinyl)-1-piperazinyl]-butyl]-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide (5-HT_{1A} agonist)] increase(s) ACh release in the hippocampus and cortex of guinea pigs and rats [using epidurally implanted cups (Bianchi et al., 1990; Siniscalchi et al., 1991) or microdialysis (Wilkinson et al., 1994; Ichikawa et al., 2002)]; this effect was sensitive to blockade by 5-HT_{1A} receptor antagonists. Analogous results were obtained in the spinal cord of anesthetized rats in which a microdialysis probe was placed dorsally at approximately the C5 level (Kommalage and Høglund, 2005). The selective 5-HT_{1A} receptor antagonist WAY 100635, given alone, inhibited ACh release in the rat frontal cortex, probably by counteracting the release-increasing effect of endogenous 5-HT (Consolo et al., 1996). With respect to the location of the 5-HT_{1A} receptor involved, different possibilities based on contradictory findings have been considered.

By using immunohistochemical double staining it was demonstrated (Kia et al., 1996a,b,c) that 5-HT_{1A} receptors occur on cholinergic cell bodies in the septum and project to the hippocampus and neocortical areas. Concerning the functional significance of septal 5-HT_{1A} receptors, it was shown in slices using intracellular recording techniques that 5-HT_{1A} receptors in the septum mediate hyperpolarization (Van den Hooff and Galvan, 1992). Analogously, using intracellular current clamp recording on immunohistochemically identified cholinergic nucleus basalis neurons (projecting to the neocortex) in guinea pig basal forebrain slices, 8-OH-DPAT and 5-HT caused a hyperpolarization of these neurons, suggesting that the drugs inhibit tonic firing via somadendritic 5-HT_{1A} receptors (Khateb et al., 1993). These results do not conform to the increase in ACh release found in the in vivo experiments. An explanation may be that inhibitory 5-HT_{1A} receptors are present on the cholinergic neurons of the septum and nucleus basalis, i.e., anatomically and functionally homogenous structures crucially involved in learning and memory (Buhot et al., 2000), whereas in the in vivo experiments in hippocampus and neocortex the released ACh originates from less focused neuronal territories in which 5-HT induces via 5-HT_{1A} receptors either directly or indirectly a facilitation of ACh release. The possibility of a direct facilitation of ACh release via presynaptic 5-HT_{1A} heteroreceptors on the axon terminals of cholinergic neurons was ruled out by the finding that 8-OH-DPAT did not influence stimulation-evoked [³H]ACh release from guinea pig hippocampal synaptosomes (Harel-Dupas et al., 1991), guinea pig cortical slices (Bianchi et al., 1990), and rat hippocampal slices (Maura et al., 1989); analogously, a rather preliminary in vivo study revealed that 8-OH-DPAT applied directly to the guinea pig hippocampus via the dialysis probe did not modify ACh release in this brain region (Wilkinson et al., 1994).

In the context of the facilitation of ACh release observed *in vivo*, it should be kept in mind that 5-HT_{1A} receptors are, as a rule, coupled to G_{i/o} proteins (Hamon, 1997) and mediate inhibitory effects. Coupling to other signal transduction pathways has been shown under optimized *in vitro* conditions for recombinant 5-HT_{1A} receptors in various cell lines but not in isolated tissues or *in vivo* (Raymond et al., 1999). Hence, non-G_{i/o} protein coupling is a very improbable alternative to explain the facilitatory effect of 5-HT_{1A} receptor agonists on ACh release *in vivo*. Therefore, it is likely that the 5-HT_{1A} receptor is located on a noncholinergic neuron directly or indirectly innervating the ACh neuron (Izumi et al., 1994; Lüttgen et al., 2005).

One explanation of the 5-HT_{1A} receptor-mediated facilitatory effect on ACh release reported in the literature is based on the assumption that serotonergic neurons exert an indirect inhibitory tone on cholinergic neurons (Bianchi et al., 1990). In this inhibition a 5-HT₃ receptor-mediated stimulation of inhibitory GABAergic interneurons (section VI.) is probably involved. In detail, the authors suggested that 8-OH-DPAT, by activating the inhibitory somadendritic autoreceptors on the serotonergic neurons, inhibits the activity of the serotonergic neurons; the inhibition may be assumed to lead to a decreased stimulation of the inhibitory GABAergic interneurons, which, in turn, results in a disinhibition of the cholinergic neurons (Fig. 2).

An alternative explanation of the facilitatory effect of systemically applied 5-HT_{1A} receptor agonists is founded on additional *in vivo* experiments in which 8-OH-DPAT and other 5-HT_{1A} receptor ligands were administered to the rat hippocampus via the dialysis probe (Izumi et al., 1994). This administration mimicked the effect of systemically injected 8-OH-DPAT, i.e., increased ACh release in a manner sensitive to blockade by the (partial) 5-HT_{1A} receptor antagonist NAN-190. These findings are compatible with the suggestion that the inhibitory 5-HT_{1A} receptor is located on inhibitory hippocampal interneurons, most probably GABAergic interneurons that are densely innervated by serotonergic afferents (Freund et al., 1990; Halasy et al., 1992) and whose synaptic boutons are endowed with presynaptic heteroreceptors (Katsurabayashi et al., 2003) (section VI.): the 5-HT_{1A} receptor-mediated inhibition of the release of the inhibitory transmitter GABA may be assumed to result in an increase of ACh release from GABAergically innervated hippocampal (and presumably also neocortical) cholinergic neurons. Thus, a presynaptic 5-HT heteroreceptor, albeit on a noncholinergic neuron, appears to be involved in the increase in ACh release *in vivo*.

2. Concluding Summary Statement. One or both of the suggested sites of action of systemically administered 5-HT_{1A} receptor agonists, i.e., the inhibitory somadendritic 5-HT_{1A} autoreceptor and/or the inhibitory 5-HT_{1A} receptor on GABAergic boutons, is/are probably

involved in the indirect facilitation of ACh release *in vivo* (Table 11). Morphological and electrophysiological investigations revealed that in the septum and nucleus basalis the cholinergic neurons are directly inhibited by 5-HT_{1A} receptors on their cell bodies and dendrites.

C. 5-Hydroxytryptamine 1B and 5-Hydroxytryptamine 1D Receptors

1. Function and Location. Early evidence that serotonergic input exerts an inhibition of striatal cholinergic neuronal activity was provided by a study on rat striatal slices in which ouabain-induced ACh release was measured (Vizi et al., 1981). The slices were prepared from rats whose serotonergic neurons had been destroyed either by electrolytic lesioning of raphe nuclei or chemically by pretreatment with *p*-chlorophenylalanine or 5,7-dihydroxytryptamine (5,7-DHT), and in these slices ACh release was increased. Conversely, the 5-HT releaser *d*-fenfluramine reduced ACh release in striatal slices from animals with intact serotonergic nerves, an effect that did not occur in slices from rats that had undergone chemical lesioning by *p*-chlorophenylalanine or 5,7-DHT. These findings indicate that endogenous 5-HT released by *d*-fenfluramine from intact striatal serotonergic axon terminals exerts an inhibitory effect on striatal cholinergic neurons. The data suggest that under physiological conditions striatal ACh release is tonically inhibited by 5-HT released from serotonergic raphe-striatal neurons. The authors postulate the involvement of 5-HT heteroreceptors on the cholinergic nerves and their partial location on cholinergic axon terminals.

Recent studies on hippocampal slices from rats whose serotonergic neurons were lesioned by intracerebroventricular injection of 5,7-DHT confirmed the data of Vizi et al. (1981) in that increased ACh release was found (Birtheimer et al., 2002, 2003a). This could be ascribed to the attenuated activation of the inhibitory presynaptic 5-HT heteroreceptors on cholinergic projection terminals in the hippocampus. Clear evidence for 5-HT_{1B} heteroreceptors on cholinergic terminals in the hippocampus came from experiments on 5-HT_{1B}-receptor knockout mice in which the 5-HT_{1B} agonist CP-93129 did not inhibit ACh release, whereas the 5-HT receptor agonist reduced ACh release in wild-type mice (Rutz et al., 2006).

Table 2 summarizes investigations in which inhibitory effects of 5-HT receptor agonists and/or 5-HT-releasing compounds on ACh release were found. These studies clearly allow the conclusion that the receptors involved in the inhibition are located on cholinergic axon terminals because 5-HT itself and/or 5-HT receptor agonists inhibit the K⁺-evoked [³H]ACh release from synaptosomes prepared from rat (Maura and Raiteri, 1986; Bolanos and Fillion, 1989; Bolaños-Jiménez et al., 1994) and guinea pig hippocampus (Harel-Dupas et al., 1991). Hence, these receptors on cholinergic axon terminals

TABLE 2
Studies in which the involvement of presynaptic 5-HT_{1B} or non-subclassified 5-HT heteroreceptors in inhibition of ACh release have been investigated

| Species | Brain Region | Receptor Type ^a | Drugs | Release-Inhibiting Concentration | Comment | References |
|------------|--------------------|---------------------------------|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| Rat | Striatum | 5-HT ₁ | 5-HT 5-MeOT N,N-Dimethyltryptamine Bufotenin | 10 ⁻⁷ -10 ⁻⁵ M 10 ⁻⁶ M 10 ⁻⁶ M 10 ⁻⁶ M | Superfusion of slices; K ⁺ (25 mM)-induced [³ H]ACh release; inhibitory effects attenuated by methiothepine, methysergide, and cinnanserin | Gillet et al. (1985) |
| Rat | Hippocampus | 5-HT _{1B} ^b | 5-HT RU 24969 | 10 ⁻⁸ -10 ⁻⁴ M 10 ⁻⁸ -10 ⁻⁶ M | Superfusion of synaptosomes; K ⁺ (15 mM)-induced [³ H]ACh release; 8-OH-DPAT without effect | Maura and Raiteri (1986) |
| Rat | Striatum | 5-HT | Flooxetine | 10 ⁻⁶ -10 ⁻⁵ M | Superfusion of slices; electrical field stimulation; methysergide (10 ⁻⁵ M) prevented inhibition of [³ H]ACh release by flooxetine and given alone increased [³ H]ACh release stimulated at 2 Hz | Jackson et al. (1988) |
| Rat | Hippocampus | 5-HT _{1B} | 5-HT RU 24969 <i>d</i> -Flufluramine 6-Nitroquipazine | 10 ⁻⁷ -3 × 10 ⁻⁴ M 10 ⁻⁶ -10 ⁻⁵ M 10 ⁻⁶ -10 ⁻⁴ M 10 ⁻⁶ M | Superfusion of slices; electrical stimulation (3 Hz) [³ H]ACh release; DOI (10 ⁻⁶ M) without effect; effect of 5-HT antagonized by methiothepine (10 ⁻⁶ M) but not by ketanserin (10 ⁻⁶ M); methiothepine, but not ketanserin, given alone increased [³ H]ACh release; effect of <i>d</i> -flufluramine antagonized by methiothepine (10 ⁻⁶ M) | Maura et al. (1989) |
| Rat | Hippocampus | 5-HT _{1B} | TFMPP | 10 ⁻⁵ -3 × 10 ⁻⁴ M (EC ₅₀ = 3.3 × 10 ⁻⁵ M) | Superfusion of synaptosomes; K ⁺ (15 mM)-induced [³ H]ACh release; methiothepine (10 ⁻⁵ M) abolished inhibition by TFMPP; mesulergine (10 ⁻⁵ M), spiperone (10 ⁻⁵ M), and ketanserin (10 ⁻⁵ M) without effect; MDL 72222 (10 ⁻⁵ M) partially reversed the inhibitory effect of TFMPP; minaprine (10 ⁻⁸ -3 × 10 ⁻⁷ M) shifted the TFMPP curve to the right | Bolanos and Fillion (1989) |
| Guinea pig | Caudate nucleus | 5-HT | 5-HT | 10 ⁻⁴ M | Superfusion of slices; electrically stimulated (0.2 Hz) [³ H]ACh release; methiothepine (3 × 10 ⁻⁶ M) and methysergide (10 ⁻⁶ M) prevented inhibition by 5-HT; ritanserin (3 × 10 ⁻⁶ M) and (-)-propranolol (10 ⁻⁶ M) did not prevent inhibition by 5-HT | Bianchi et al. (1989) |
| Rat | Hippocampus | 5-HT ₁ ^c | TFMPP CGS 12066B 5-CT | 5 × 10 ⁻⁶ -10 ⁻³ M 10 ⁻⁵ -10 ⁻⁴ M 10 ⁻⁵ M | Superfusion of synaptosomes; K ⁺ (10 mM)-induced [³ H]ACh release; metergoline (10 ⁻⁷ M), yohimbine (10 ⁻⁶ -10 ⁻⁵ M), and methysergide blocked the TFMPP-induced inhibition of [³ H]ACh, but methiothepine (3 × 10 ⁻⁷ -10 ⁻⁶ M), propranolol (10 ⁻⁶ M), and ketanserin (10 ⁻⁷ M) did not affect this inhibition | Harel-Dupas et al. (1991) |
| Rat | Hippocampus | 5-HT _{1B} ^b | CGS 12066B | 10 ⁻⁸ -3 × 10 ⁻⁴ M | Superfusion of synaptosomes; K ⁺ (15 mM)-induced [³ H]ACh release | Bolaños-Jiménez et al. (1994) |
| Rat | Dorsal hippocampus | 5-HT _{1B} | CGS 12066B 5-HT (after 10 μM NAN-190) Clomipramine (after 10 μM NAN-190) | 10 ⁻⁴ M 10 ⁻⁵ M 2 × 10 ⁻⁶ M | In vivo microdialysis in freely moving rats; determination of extracellular levels of endogenous ACh; drugs applied via the dialysis probe; 5-HT and clomipramine did not modify ACh release when 5-HT _{1A} receptors were not antagonized by NAN-190 (inhibition via 5-HT _{1B} receptors counterbalanced by facilitation via 5-HT _{1A} receptors) | Izumi et al. (1994) |

^a 5-MeOT, 5-methoxytryptamine hydrochloride (5-HT₄ agonist).^b Receptor type involved, as suggested by the authors.^c Classification by means of drugs that do not discriminate between the rat and guinea pig 5-HT_{1B} receptors according to the current nomenclature.^d Non-5-HT_{1A}, 5-HT_{1B}, or "5-HT_{1C}".

may be denoted as presynaptic 5-HT heteroreceptors (Fig. 1). In analogy to these results, it is probable that the same type of presynaptic 5-HT heteroreceptor on cholinergic nerves is also involved in the inhibition of electrically evoked [^3H]ACh release in slices of the rat hippocampus (Maura et al., 1989; Cassel et al., 1995; Vizi and Kiss, 1998), guinea pig caudate nucleus (Bianchi et al., 1989), and human neocortex (Feuerstein and Seeger, 1997) as well as K^+ -induced [^3H]ACh release in rat striatal slices (Gillet et al., 1985; Jackson et al., 1988). A decrease in electrically evoked [^3H]ACh release by the 5-HT receptor agonist CP-93129 (in the absence of TTX) was also observed (5–9 months after grafting) in hippocampal slices from rats that had undergone electrolytic (Cassel et al., 1995) or aspirative (Suhr et al., 1999) fimbria-fornix lesions and 2 weeks later had received intrahippocampal suspension grafts of fetal septal tissue. The authors suggested on the basis of their results that inhibitory presynaptic heteroreceptors are also operative in grafted cholinergic neurons contained in hippocampal slices. Furthermore, in hippocampal slices from aged (25–27 months) rats with various levels of memory impairment shown in a spatial reference-memory task, there was no change in CP-93129-induced inhibition of [^3H]ACh release (Birthelmer et al., 2003b); in these experiments basically conditions identical to those in slices from grafted hippocampus were applied. The results indicate that aging does not alter the function of presynaptic 5-HT heteroreceptors on cholinergic nerves. Obviously, the presynaptic 5-HT heteroreceptor mediating inhibition of ACh release in the rat hippocampus is also operative in vivo because in a microdialysis study (Izumi et al., 1994) such an effect was observed in response to CGS 12066B and, after blockade of 5-HT_{1A} receptors by NAN-190, also to 5-HT and clomipramine (Table 2). There has been some controversy concerning the 5-HT receptor type that mediates the inhibitory effects of 5-HT in the rat hippocampus because this inhibition requires functional elimination of substance P interneurons endowed with 5-HT_{2A} receptors. It has been suggested that 5-HT_{1B} heteroreceptors on cholinergic terminals reduce, whereas 5-HT₂ receptors on substance P interneurons indirectly enhance, ACh release, indicating that in addition to GABA interneurons, further interneurons may be involved (Feuerstein et al., 1996).

2. Classification and Development of Nomenclature.

a. 5-Hydroxytryptamine 1B/1D ligands. A major problem in the classification of a given 5-HT receptor as 5-HT_{1B/1D} is the shortage of selective agonists and antagonists that discriminate these receptors from the other 5-HT receptors. This problem was particularly true at the time when most of the experiments designed to classify the inhibitory presynaptic 5-HT heteroreceptors were performed, i.e., roughly in the 10 years after 1985. Among the agonists applied for this purpose at least a relative preference for 5-HT_{1B/1D} receptors can be

ascribed to CGS 12066B, RU 24969 and 5-CT. Only a few antagonists that were able to counteract the activating effect of 5-HT receptor agonists at the 5-HT_{1B} and 5-HT_{1D} receptors were available; however, drugs such as methiothepine and metergoline are nonselective in that they also block other 5-HT receptors.

The studies performed in that period revealed different pharmacological properties of the presynaptic 5-HT heteroreceptors on cholinergic nerve terminals in the rat brain on the one hand and the guinea pig and human brain on the other. The receptor expressed in the rat brain was named 5-HT_{1B} and that in the brain of guinea pig and humans was named 5-HT_{1D}. Subsequent investigations comprising molecular biological techniques revealed that 5-HT_{1B} and 5-HT_{1D} receptors are different entities coexisting in each of these species. 5-HT_{1B} receptors in the guinea pig, rabbit, and human brain, although encoded by orthologous genes, exhibit pharmacological properties that are different from those of the rat 5-HT_{1B} receptor. New selective 5-HT_{1B} and 5-HT_{1D} ligands finally made it possible and necessary to revise the nomenclature of guinea pig and human presynaptic 5-HT receptors; they also belong to the 5-HT_{1B} subclass. In this context, presynaptic autoreceptors that, in conjunction with 5-HT heteroreceptors, played an additional important role in the development of this classification are relevant.

b. Rat 5-hydroxytryptamine 1B receptor. The most comprehensive studies in the rat hippocampus (Bolanos and Fillion, 1989; Maura et al., 1989) revealed 1) that 5-HT, RU 24969, and TFMPP inhibited [^3H]ACh release, 2) that methiothepine, but not the non-5-HT_{1B} antagonists spiperone and ketanserin [3-[2-[4-(4-fluorobenzoyl)piperidino]ethyl]-2,4-(1*H*,3*H*)-quinazolidione tartrate (5-HT_{2A} antagonist)], counteracted the inhibition, and 3) that methiothepine, but not ketanserin, given alone increased [^3H]ACh release probably by preventing endogenous 5-HT from activating the presynaptic 5-HT heteroreceptor. These data were compatible with the conclusion that the latter receptor belongs to the 5-HT_{1B} type.

The inhibitory effect of 5-HT receptor agonists on [^3H]ACh release is mimicked in slices and in vivo by 5-HT uptake blockers such as 6-nitroquipazine (Maura et al., 1989), clomipramine (Izumi et al., 1994), and fluoxetine (Jackson et al., 1988) or by the 5-HT releasing drug *d*-fenfluramine (Maura et al., 1989). Both types of compounds, because of their specific effects on 5-HT inactivation in, and release from, serotonergic neurons, respectively, increase the concentration of endogenous 5-HT in the biophase of the receptor.

In view of the low number of 5-HT receptor ligands examined in most of the studies of ACh release in the rat hippocampus and striatum summarized in Table 2, each of these studies alone would not be suitable to draw a reliable conclusion concerning the 5-HT receptor type involved. However, the effects produced by each of the

few ligands applied in the investigations (Maura and Raiteri, 1986; Bolaños-Jiménez et al., 1994; Izumi et al., 1994) in the rat hippocampus conform to the functional and pharmacological properties of the inhibitory presynaptic 5-HT_{1B} heteroreceptors, thus leading the authors to suggest that their study is dealing with this receptor type, a conclusion that appears to be justified.

In two studies on rat striatal slices (Gillet et al., 1985; Jackson et al., 1988) (Table 2), the ability of methysergide and cinanserin to antagonize the inhibitory effect of endogenous 5-HT (available at increased concentration in the presence of fluoxetine) or of exogenous 5-HT receptor agonists points to the operation of another inhibitory receptor type, putatively in addition to the presynaptic 5-HT_{1B} heteroreceptor. A possible candidate would be a 5-HT₂ receptor. Because of this uncertainty, the authors concluded more cautiously that a 5-HT₁ receptor or even less specifically that a 5-HT receptor is involved.

In the context of the 5-HT_{1B} character of the inhibitory 5-HT heteroreceptor on cholinergic axon terminals in the rat hippocampus (and putatively also in the rat striatum where it may be coexpressed with a non-5-HT_{1B} receptor), it should be noted that the presynaptic 5-HT autoreceptor in the brain of this species also belongs to the 5-HT_{1B} class defined by radioligand binding in rat brain membranes (Engel et al., 1986). This observation is of particular interest because presynaptic autoreceptors played a significant role in the development of the 5-HT_{1B/1D} receptor nomenclature.

c. Development of guinea pig and human 5-hydroxytryptamine 1B and 5-hydroxytryptamine 1D auto- and heteroreceptor nomenclature. Radioligand binding experiments on guinea pig cerebral membranes (Waeber et al., 1989) analogous to those in rat brain membranes revealed (despite many similarities) clearly different pharmacological properties of the respective binding sites, leading the authors to the conclusion that a different receptor type named “5-HT_{1D}” is involved, a nomenclature that was premature according to our current knowledge. The same pharmacological characteristics as for the “5-HT_{1D}” binding sites on guinea pig cerebral membranes were established for the presynaptic autoreceptor in the guinea pig brain which, hence, was also classified as “5-HT_{1D}” (Hoyer and Middlemiss, 1989; Limberger et al., 1991). This “old” nomenclature, although revised in the meantime (see below), will be used first (but in quotation marks) to avoid discrepancies with the cited original articles in which it was applied.

Taking the identity of presynaptic 5-HT heteroreceptors on cholinergic nerves and presynaptic 5-HT autoreceptors in the rat brain into account, the questions arose 1) whether the inhibitory presynaptic 5-HT heteroreceptor on cholinergic nerves is a member of the same receptor class as the presynaptic 5-HT autoreceptor in the guinea pig also, i.e., is a “5-HT_{1D}” receptor and 2) which drugs available at that period were suitable to discrim-

inate the guinea pig “5-HT_{1D}” receptor from the rat 5-HT_{1B} receptor.

The latter question was answered by comparing the results in the radioligand binding experiments in rat and guinea pig brain membranes mentioned above. Examples for differences between rat 5-HT_{1B} and guinea pig “5-HT_{1D}” binding sites are the properties of β -adrenoceptor antagonists such as propranolol and cyanopindolol [4-(3-((1,1-dimethylethyl)amino)-2-hydroxypropoxy)-1H-indole-2-carbonitrile (5-HT_{1A/1B} antagonist)]. The affinity of these compounds was high for 5-HT_{1B} sites but only low for “5-HT_{1D}” sites. Other ligands that discriminate between both sites are the rauwolfia alkaloids yohimbine and rauwolscine, which exhibit a clear-cut preference for “5-HT_{1D}” sites. These pharmacological tools should contribute to establishing the 5-HT₁ receptor subtype identity of the guinea pig presynaptic 5-HT auto- and heteroreceptors and extend the suggestion of Hoyer and Middlemiss (1989) that 5-HT_{1B} and “5-HT_{1D}” receptors are species homologs with respect to their function and distribution. Two investigations as listed in Table 2 in which the pharmacological properties of the guinea pig presynaptic 5-HT heteroreceptor were analyzed are available.

In guinea pig caudate nucleus slices, a 5-HT concentration as high as 100 μ M was necessary to produce an 18 to 20% inhibition of the electrically evoked [³H]ACh release (Bianchi et al., 1989). This effect was inhibited by methiothepine and methysergide but not by ritanserin, a pattern of antagonist actions that would be compatible with the involvement of a receptor belonging to the 5-HT₁ family. Nevertheless, the authors did not make a suggestion concerning the receptor type involved, presumably because of the low potency of 5-HT. However, the latter may be due to an interaction of this inhibitory receptor with an additional excitatory 5-HT₂ receptor identified in the same study on the same slices. An important finding for the reinterpretation of the pharmacological properties of the inhibitory presynaptic heteroreceptors on the cholinergic nerves of the guinea pig hippocampus was the failure of (-)-propranolol to prevent the 5-HT-induced inhibition. Taken together, most of these results are compatible with a classification of the presynaptic heteroreceptors as “5-HT_{1D}.”

In guinea pig hippocampal synaptosomes, three 5-HT receptor agonists, two of which (CGS 12066B and 5-CT) are relatively selective for 5-HT_{1B} and “5-HT_{1D}” receptors, were shown to inhibit K⁺-evoked [³H]ACh release (Harel-Dupas et al., 1991). The failure of methiothepine to antagonize the inhibition of release induced by TFMP (the third 5-HT receptor agonist examined) is the only finding that does not fit to the 5-HT_{1B} or “5-HT_{1D}” character of the receptor involved. In contrast, the ability of yohimbine to counteract the inhibition caused by the agonist and the inability of propranolol to act as an antagonist strongly pointed to a “5-HT_{1D}” receptor character of the presynaptic 5-HT heteroreceptor under study. The latter was classified by the authors

as a 5-HT₁ receptor distinct from the 5-HT_{1A}, 5-HT_{1B}, "5-HT_{1C}" (5-HT_{2C} according to the present classification) subtype. Perhaps the term "5-HT_{1D}" was avoided because the existence of such a receptor type was not yet generally accepted.

In conclusion, it may be stated that the inhibitory presynaptic 5-HT heteroreceptor on cholinergic neurons of the guinea pig caudate nucleus and hippocampus could be classified as "5-HT_{1D}," i.e., the same 5-HT₁ subclass as the autoreceptor on the serotonergic axon terminals of this species (Hoyer and Middlemiss, 1989; Limberger et al., 1991).

In this context it should be noted that "5-HT_{1D}" recognition sites with binding properties virtually identical to those in the guinea pig brain (Waeber et al., 1989) were also identified in the human brain (Waeber et al., 1988). Accordingly, the guinea pig rather than the rat brain is an appropriate model for the human brain in studies of receptors belonging to the 5-HT₁ subfamily. Hence, any finding concerning this receptor family obtained in one of these species is also relevant for the other. This should be kept in mind to understand the development of the "5-HT_{1D}" subtype nomenclature.

A step forward in the classification was achieved by the isolation of two different genes from the human brain that code for receptors with binding properties virtually identical to those previously determined for guinea pig and human "5-HT_{1D}" binding sites but different from those of rat 5-HT_{1B} sites (Hartig et al., 1992). These receptors were named "5-HT_{1D α} " and "5-HT_{1D β} ." They could be distinguished by ketanserin, which has a 75-fold higher affinity for cloned 5-HT_{1D α} than for cloned 5-HT_{1D β} receptors (Zgombick et al., 1995).

Ketanserin had already been applied in a study (Harel-Dupas et al., 1991) designed to characterize the inhibitory presynaptic 5-HT heteroreceptor on the cholinergic axon terminals of the guinea pig hippocampus (Table 2) without knowledge of the capability of the drug to discriminate between 5-HT_{1D α} and 5-HT_{1D β} receptors. The failure of ketanserin at high concentration to antagonize the inhibitory effect of the 5-HT receptor agonist TFMPP suggests that the presynaptic heteroreceptor could be subclassified as 5-HT_{1D β} . In the context of the subtype identity of this receptor with the presynaptic 5-HT autoreceptor, it is of interest that the autoreceptor of the guinea pig (Bühlen et al., 1996) and human brain neocortex (Fink et al., 1995) has been subclassified as 5-HT_{1D β} .

Among the two genes identified in the human brain mentioned previously, the gene coding for the 5-HT_{1D β} receptor was highly homologous to the gene isolated from the rat brain that codes for a receptor with the pharmacological properties of the 5-HT_{1B} binding sites in rat cerebral membranes (Hartig et al., 1992) and, accordingly, also for the inhibitory presynaptic 5-HT_{1B} heteroreceptors on cholinergic nerves in the rat brain. Obviously, despite the pharmacological differences, the

presynaptic 5-HT_{1B} hetero- and autoreceptors of the rat brain on the one hand and the presynaptic 5-HT_{1D β} hetero- and autoreceptors in the human and guinea pig brain on the other are the products of orthologous genes. On the basis of these findings a rational, less confusing, and unifying nomenclature has been developed (Hartig et al., 1996), which is still valid. Nomenclature is no longer based primarily on the pharmacological, transductional, and operational receptor properties but on the primacy of the human genome. A name first established for the amino acid sequence of a receptor type deduced from a human gene is used for all receptors of various species that are the products of orthologous genes, irrespective of whether or not their pharmacological properties are identical. The species is specified by brief letter prefixes such as h for human, gp for guinea pig, and r for rat. For the sake of completeness it should be noted that a rat ortholog (Bach et al., 1993) of the human gene encoding the 5-HT_{1D α} receptor, i.e., the 5-HT_{1D} receptor of the current classification (Hartig et al., 1992) was also identified.

d. Guinea pig 5-hydroxytryptamine 1B receptor. Taking the above rules into account, the inhibitory presynaptic 5-HT heteroreceptors on the cholinergic axon terminals and the presynaptic 5-HT autoreceptors in guinea pig brain are species homologs of the rat brain 5-HT_{1B} hetero- and autoreceptor (section III.C.2.b) and, accordingly, should be denoted as guinea pig 5-HT_{1B}.

The relevance of this classification has subsequently been confirmed by the development of selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists, SB 216641 and BRL 15572, respectively (Price et al., 1997), as well as by their application in a study designed to confirm the 5-HT_{1B} character of the guinea pig presynaptic 5-HT autoreceptor (Schlicker et al., 1997). In the latter experiments on superfused guinea pig cortical slices, SB 216641, but not BRL 15572, antagonized the 5-HT-induced inhibition of electrically evoked [³H]5-HT, indicating that a guinea pig 5-HT_{1B} receptor is involved.

e. Human 5-hydroxytryptamine 1B receptor? Using neocortical synaptosomes one group has classified the inhibitory presynaptic 5-HT receptor on the cholinergic nerve terminals in the human brain as 5-HT₃ (Maura et al., 1992) (section III.C.), whereas others studying hippocampal slices claimed that it belongs to the 5-HT_{1F} subclass (Feuerstein and Seeger, 1997). In the latter study an involvement of 5-HT_{1B/1D} receptors was excluded by the low potency of 5-CT, which fits better to the pharmacological profile of, e.g., 5-HT_{1F} receptors. In any case, classification of this human receptor is premature; it was based on few rather nonselective drugs only. To clarify this issue a careful reinvestigation on slices and synaptosomes using highly selective 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F} receptor antagonists is needed. As long as such data are not available, the existence of release-inhibiting 5-HT_{1B} receptors on human cholinergic terminals cannot be excluded.

3. *Specific Aspects.* An investigation in rat hippocampal synaptosomes (not included in Table 2) was devoted to the question whether the function of the inhibitory presynaptic 5-HT_{1B} heteroreceptor on cholinergic synaptic boutons can be modified by modulators of the 5-HT transporter (Bolaños-Jiménez et al., 1993). The serotonin uptake inhibitor citalopram and another antidepressant, tianeptin, which, however, stimulates reuptake of 5-HT (Mennini et al., 1987; Fattaccini et al., 1990) were used as pharmacological tools. Both citalopram and tianeptin reduced the inhibitory effect of CGS 12066B on the K⁺-evoked [³H]ACh release, whereas the muscarinic autoreceptor-mediated inhibition of release by carbachol was not affected by tianeptin. Thus, both inhibition and stimulation of the transporter decreased the inhibitory effect of the 5-HT receptor agonist CGS 12066B on ACh release; therefore, this antagonism is obviously independent of the activity state of the transporter and its putative consequences on the concentration of endogenous 5-HT in the biophase of the presynaptic 5-HT_{1B} heteroreceptors. Concerning the latter point, it should be noted that in superfused synaptosomes changes of endogenous 5-HT in the biophase of the receptors are not involved because at sufficient superfusion speed 5-HT molecules released from serotonergic synaptosomes are effectively prevented from accumulation in the receptor biophase due to rapid removal by the superfusion stream. A plausible explanation of the interaction between modulators of the 5-HT transporter and the heteroreceptor is the existence of a direct inhibitory molecular link between a 5-HT transporter-independent recognition site for modulators of the transporter located on cholinergic varicosities and the presynaptic 5-HT_{1B} heteroreceptor on the same varicosities.

The existence and operation of such a 5-HT transporter-independent mechanism of action of antidepressants has been confirmed in an *ex vivo* study (Bolaños-Jiménez et al., 1994). In these experiments rats received saline, citalopram, or tianeptin for 14 days. Twenty-four hours after the last injection the potency of CGS 12066B in inhibiting K⁺-evoked [³H]ACh release from hippocampal synaptosomes was reduced by both citalopram and tianeptin. This finding is compatible with the suggestions that the decreased sensitivity of the presynaptic 5-HT_{1B} heteroreceptor is mediated by an interaction of the antidepressants (irrespective of whether they inhibit or stimulate 5-HT reuptake) and the heteroreceptor at the level of the cholinergic varicosity.

In an *in vivo* study (Izumi et al., 1994), the classification of the inhibitory presynaptic 5-HT heteroreceptor on hippocampal cholinergic neurons as 5-HT_{1B} was based mainly on the inhibitory effect of CGS 12066B. 5-HT and clomipramine failed to produce an inhibition of ACh release when 5-HT_{1A} receptors were operative, but an inhibitory effect was disclosed after 5-HT_{1A} receptor blockade. These findings indicate that under *in vivo*

conditions the inhibitory effect of endogenous 5-HT on ACh release mediated by 5-HT_{1B} receptors on the one hand and the 5-HT_{1A} receptor-mediated facilitatory action on the other (section III.A.) can neutralize each other, i.e., can be completely counterbalanced. Hence, to obtain an exclusive inhibition or increase of ACh release in the hippocampus, a selective 5-HT_{1B} receptor agonist or a selective 5-HT_{1A} receptor agonist, respectively, must be administered.

In contrast to the occurrence of inhibitory presynaptic 5-HT_{1B} heteroreceptors on cholinergic axon terminals of the hippocampus, caudate nucleus, and (possibly) striatum discussed so far, 5-HT heteroreceptors in the rat frontal cortex mediating stimulation of ACh release were reported (Consolo et al., 1996). In a microdialysis study in freely moving rats, the 5-HT releaser and uptake inhibitor *d*-norfenfluramine (0.63–2.5 mg/kg *i.p.* or 20 and 40 μM via the probe) increased frontal cortical ACh release. This effect was not modified by 5-HT₃ or 5-HT₄ receptor antagonists or by the selective 5-HT_{1A} antagonist WAY 100635, but it was blocked by the 5-HT_{1A/1B} receptor antagonists (–)-pindolol and (–)-propranolol. The conclusion that a 5-HT_{1B} receptor is involved was further supported by the ability of locally applied CP-93129, a selective 5-HT_{1B} receptor agonist, to mimic the *d*-norfenfluramine-induced stimulation of ACh release. The authors concluded that the 5-HT_{1B} heteroreceptor in the rat frontal cortex is located on an inhibitory interneuron; activation of the inhibitory 5-HT_{1B} heteroreceptor leads to a disinhibition, i.e., an increase of ACh release. It is an open question whether these receptors are located on the cell body and/or the axon terminals of the interneuron. Obviously, the inhibitory or facilitatory control of *in vivo* ACh release differs not only with respect to the 5-HT receptor type but also with the brain area involved.

4. *Concluding Summary Statement.* ACh release is modulated in the rat, mouse, and guinea pig hippocampus and striatum by inhibitory presynaptic heteroreceptors that in all these species belong to the 5-HT_{1B} class. This conclusion was based on the development of the current classification of 5-HT_{1B} and 5-HT_{1D} receptors to which studies on presynaptic 5-HT auto- and heteroreceptors have significantly contributed.

In the rat hippocampus it was also demonstrated that presynaptic 5-HT_{1B} receptor-mediated inhibition of ACh release induced by 5-HT and other nonselective 5-HT receptor agonists requires functional elimination of substance P interneurons by blockade of 5-HT_{2A} receptors. An inhibitory molecular link was claimed to exist on cholinergic varicosities between the presynaptic 5-HT_{1B} receptors and a 5-HT transporter-independent recognition site for antidepressants. Activation of this site by drugs such as citalopram reduces the inhibitory effect of presynaptic 5-HT_{1B} receptor stimulation. In rat hippocampus the inhibitory effect of presynaptic 5-HT_{1B} receptors on ACh release is attenuated when the ACh

release-facilitating 5-HT_{1A} receptors on GABAergic interneurons (section III.B.) are simultaneously activated by a nonselective 5-HT receptor agonist, leading to facilitation of ACh release. In rat cerebral cortex, ACh release is indirectly facilitated via inhibitory 5-HT_{1B} heteroreceptors on GABAergic interneurons.

D. 5-Hydroxytryptamine 2 Receptors

1. *Function, Location, and Classification.* The *in vitro* studies on superfused rat hippocampal (Muramatsu et al., 1988a) and neocortical slices and synaptosomes (Muramatsu et al., 1990a) summarized in Table 3 revealed that the 5-HT receptor agonists 5-HT and DOB inhibit the K⁺-evoked [³H]ACh release in a manner sensitive to blockade by selective and nonselective 5-HT₂ receptor antagonists, suggesting that the inhibitory effect of the agonists on [³H]ACh release is 5-HT₂ receptor-mediated. On the basis of the following two main lines of evidence the receptors involved have been suggested to be located as 5-HT heteroreceptors on the cholinergic axon terminals (Fig. 1): 1) the inhibitory effect of 5-HT was observed not only on synaptosomes but also on slices superfused in the presence of tetrodotoxin, thus excluding the involvement of an interneuron; and 2) electrolytic lesioning of the medial septum, i.e., the major location of cell bodies of cholinergic nerves projecting to the hippocampus, reduced the number of hippocampal [³H]ketanserin-binding sites and led to a loss of the inhibitory effect of 5-HT on the K⁺-induced [³H]ACh release in the hippocampus (Muramatsu et al., 1988a). Analogously, it had been reported previously (Quirion et al., 1985) that lesioning of the nucleus basalis magnocellularis, the area in which the cell bodies of cholinergic nerves projecting to the neocortex are located, markedly decreased [³H]ketanserin binding to lamina IV of the anterior and middle neocortex, suggesting that the 5-HT₂ receptors, at least a certain proportion, are located on cholinergic axon terminals in the neocortex.

In contrast with the inhibition of release by these 5-HT₂ heteroreceptors, the *in vivo* operation of an excitatory presynaptic 5-HT₂ receptor subtype (section III.D.2.) on cholinergic terminals in the rat frontal neocortex has been suggested (Hirano et al., 1995). It was found that systemic administration of the 5-HT releaser fenfluramine increased ACh release in a manner sensitive to blockade by i.p. applied ketanserin. The authors' conclusion concerning the presynaptic location of the receptor involved was based on the ability of locally applied fenfluramine (via the dialysis probe) to mimic the stimulatory effect of systemic application of this 5-HT releaser on ACh release. This conclusion implies that the same 5-HT receptor type was involved in the effect of locally and systemically applied fenfluramine.

The involvement of 5-HT₂ receptors or, more specifically, one of its subtypes in stimulation of ACh release was also found in more recent *in vivo* microdialysis

studies in rats in which the probes were placed in the medial prefrontal cortex or dorsal hippocampus (Nair and Gudelsky, 2004) or intraspinally at the level of C5 (Kommalage and Høglund, 2005). In the spinal cord, locally applied α -methyl-5-HT increased ACh release in a manner sensitive to antagonism by ketanserin. In the cortex DOI injected systemically or locally via the dialysis probe increased ACh release, and this effect was blocked by systemically and locally, respectively, applied LY-53,857, a 5-HT_{2A/2B/2C} antagonist. The stimulatory response to DOI was mimicked by i.p. applied MK-212, a 5-HT₂ receptor agonist. In the hippocampus only systemically applied DOI increased ACh release, an effect antagonized by LY-53,857. Thus, in the spinal cord and cortex the 5-HT receptors mediating ACh release may be assumed to be located on the cholinergic axon terminals, whereas the corresponding 5-HT receptor mechanism in the hippocampus appears to be located outside of this brain area. An effect outside of the hippocampus was also postulated (Zhelyazkova-Savova et al., 1999) in an *in vivo* microdialysis study in the hippocampus, in which *m*-chlorophenylpiperazine (mCPP), a 5-HT_{2A/2C} receptor agonist, increased ACh release when applied i.p. but not when given locally via the dialysis probe; the increase in ACh release induced by systemic mCPP was prevented by i.p. injected mesulergine, a 5-HT_{2A/2C} receptor antagonist.

In guinea pig striatal slices, a stimulatory effect of 5-HT and DOI on ACh release mediated by 5-HT₂ receptors was also found, but this effect was abolished in the presence of 0.5 μ M tetrodotoxin, suggesting that the 5-HT₂ receptors are not located on the axon terminals of the cholinergic neurons but either on their somadendritic area or upstream on an interneuron within the slice (Bianchi et al., 1989; Siniscalchi et al., 1990).

Taken together, it is obvious that the results providing evidence for 5-HT₂ receptor-mediated modulation of ACh release are inconsistent and incomplete with respect to the receptor subclassification. The members of the 5-HT₂ receptor family are similar in structure, signal transduction pathways, and most pharmacological properties. The 5-HT_{2C} receptor was originally classified as 5-HT_{1C} on the basis of some of its pharmacological properties, i.e., high affinity for [³H]5-HT, yet not for [³H]ketanserin (Pazos et al., 1984), but was renamed largely because of (among other reasons) its homology in molecular structure and identity in signal transduction with the 5-HT_{2A} receptor, which is the prototypic 5-HT₂ receptor (Göthert, 1992).

In the *in vitro* studies in which a direct inhibition of ACh release by 5-HT receptor agonists was reported (Muramatsu et al., 1988b, 1990a) (Table 3), none of the ligands applied, except DOB (which exhibits a moderately higher affinity for 5-HT_{2A} and 5-HT_{2C} than for 5-HT_{2B} receptors) and ketanserin (which binds with clearly higher affinity to 5-HT_{2A} than to the other two 5-HT₂ receptor types), had a substantial preference for

TABLE 3
Studies in which the involvement of 5-HT₂ heteroreceptors in modulation of ACh release has been investigated
 Effects of 5-HT receptor agonist or fenfluramine on ACh release in various brain regions of the rat are shown.

| Species | Brain Region | Receptor Type ^a | Drugs | Modifying Concentration | Comment | References |
|---------|----------------------|----------------------------|---------------|----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| Rat | Hippocampus | 5-HT ₂ | 5-HT | 10 ⁻⁵ –10 ⁻⁴ M inhibition | Superfusion of slices; K ⁺ (25 mM)-induced [³ H]ACh release; inhibition by 5-HT observed in the presence of tetrodotoxin (10 ⁻⁷ M); minaprine without effect by itself, but attenuated inhibition by 5-HT; minaprine displaced [³ H]ketanserin binding; electrolytic lesioning of the medial septum decreased the number of [³ H]ketanserin-binding sites | Muramatsu et al. (1988a) |
| Rat | Neocortex | 5-HT ₂ | DOB 5-HT | 10 ⁻⁵ –10 ⁻⁴ M inhibition 10 ⁻⁴ –10 ⁻³ M inhibition | Superfusion of slices; K ⁺ (25 mM)-induced [³ H]ACh release; inhibition by 5-HT observed in the presence of tetrodotoxin (10 ⁻⁷ M); minaprine (10 ⁻⁴ M), ketanserin (10 ⁻⁵ M), methysergide (10 ⁻⁵ M), or cocaine (10 ⁻⁵ M) without effect when given alone, but attenuated effect of 5-HT and DOB | Muramatsu et al. (1990b) |
| Rat | Frontal neocortex | 5-HT _{2A} | Fenfluramine | 3–10 mg/kg i.p. increase | Superfusion of synaptosomes; minaprine (10 ⁻⁴ M) given alone without effect, but attenuated inhibition by 5-HT | Hirano et al. (1995) |
| Rat | Hippocampus | 5-HT _{2C} | mCPP | 8 mg/kg i.p. increase | In vivo microdialysis, increase in ACh release; 5 mg/kg i.p. ketanserin without effect when given alone, but prevented fenfluramine-induced increase in ACh release; local application of fenfluramine via the dialysis probe also enhanced ACh release, but interaction with ketanserin not tested | Zhelyazkova-Savova et al. (1999) |
| Rat | Prefrontal neocortex | 5-HT ₂ | DOI | 1–2 mg/kg i.p. increase, 100 μM via probe | In vivo microdialysis in freely moving rats, DOI (i.p.)-induced increase in ACh release was inhibited by LY-53857 (3 mg/kg i.p.); DOI (per probe)-induced increase in ACh release was inhibited by LY-53857 (100 μM) | Nair and Gudelsky (2004) |
| Rat | Spinal cord C5 level | 5-HT _{2A} | 2-Methyl-5-HT | Increase | In vivo microdialysis in anesthetized rats, drugs applied via probe; the CH ₃ -5-HT-induced increase in ACh release was inhibited by ketanserin | Kommalage and Hoglund (2005) |

^a Receptor type involved, as suggested by the authors.

one or two of the 5-HT₂ receptors (Knight et al., 2004). However, the effective concentrations of DOB and ketanserin in the hippocampus and neocortex (Table 3) were much higher than the range (<1 μM) at which the drugs would be able to discriminate between the 5-HT₂ receptor types (Knight et al., 2004). Therefore, no conclusion concerning the involvement of a particular member of the 5-HT₂ subfamily can be drawn. Further experiments including the 5-HT₂ subtype-selective antagonists currently available (Knight et al., 2004) will be necessary for the subclassification of the inhibitory 5-HT₂ heteroreceptor presumed to be located on cholinergic axon terminals (Muramatsu et al., 1988a, 1990a).

Because all three 5-HT₂ receptor types are positively coupled to phospholipase C and thus mobilize intracellular Ca²⁺, a facilitation rather than inhibition should be expected. The issue may be resolved by considering the possibility of coupling to other signal transduction systems such as K⁺ channels, which was basically shown for 5-HT receptors (Andrade and Nicoll, 1987; Colino and Halliwell, 1987). In this context another pharmacological tool, minaprine, has been brought into play (Muramatsu et al., 1990b). In the rat cerebral cortex this drug exhibits blocking properties both at 5-HT₂ receptors and at voltage-dependent K⁺ channels on cholinergic axon terminals: [³H]ketanserin binding to cortical membranes was inhibited by minaprine, and in cortical slices and synaptosomes the K⁺-evoked [³H]ACh release was inhibited not only by minaprine but also by the voltage-dependent K⁺ channel blockers tetraethylammonium and 4-aminopyridine. These channel blockers also inhibited specific [³H]minaprine binding to brain membranes. On the basis of these results, the possibility may be considered that the 5-HT₂ receptors involved in the inhibition of ACh release are coupled via an unknown mechanism to the voltage-dependent K⁺ channels described (Muramatsu et al., 1990b). Nevertheless, doubts concerning a direct inhibition of ACh release via presynaptic inhibitory 5-HT₂ heteroreceptors on cholinergic axon terminals can hardly be ruled out, in particular, when the results of the microdialysis experiments are taken into consideration.

The microdialysis studies in the rat cerebral cortex, hippocampus (Hirano et al., 1995; Zhelyazkova-Savova et al., 1999; Nair and Gudelsky, 2004), and spinal cord (Kommalage and Høglund, 2005), although revealing the expected excitatory action in response to 5-HT₂ receptor stimulation, are also inconsistent with respect to the 5-HT₂ receptor subtype suggested to be involved. This inconsistency is related to the fact that only a few compounds of questionable selectivity were applied. Thus, on the basis of the study in the rat frontal cortex (Hirano et al., 1995), it was claimed that the 5-HT receptors mediating stimulation of ACh release in the rat frontal neocortex belong to the 5-HT_{2A} class (Table 3). Because this conclusion was exclusively based on the finding that systemic application of ketanserin at only

one rather high dose antagonizes the stimulatory effect of fenfluramine, the subclassification of the receptor as 5-HT_{2A} has to be regarded as premature (as is the putative presynaptic location). Further experiments with additional doses of ketanserin and with other subtype-selective antagonist (Knight et al., 2004) are necessary for a reliable classification. The same notes of caution are necessary with respect to the interpretation 1) of the data obtained in the rat spinal cord by Kommalage and Høglund (2005), who also proposed the involvement of 5-HT_{2A} receptors on the basis of responses to α-methyl-5-hydroxytryptamine, DOI, and ketanserin and 2) of the results obtained in the rat hippocampus by Zhelyazkova-Savova et al. (1999), who suggested the involvement of 5-HT_{2C} receptors on the basis of the application of mCPP and mesulergine only. In any case, all of these findings allow the interpretation that a release-stimulating receptor belonging to the 5-HT₂ family is involved. Such a more cautious conclusion has been drawn from data obtained in the prefrontal cortex with DOI, MK-212, and LY-53,857 (Nair and Gudelsky, 2004).

2. Concluding Summary Statement. On the basis of in vitro experiments it was suggested that the cholinergic terminals in rat neocortex and hippocampus are endowed with inhibitory presynaptic 5-HT₂ heteroreceptors. Results of in vivo experiments are, in contrast, compatible with the idea that facilitatory presynaptic 5-HT₂ receptors modify ACh release in the rat cortex and spinal cord. In the rat hippocampus a 5-HT₂ receptor agonist-induced increase in release has been suggested to be mediated by a facilitatory interneuron. In guinea pig striatal slices a stimulatory effect on ACh release via 5-HT₂ receptors (not located on cholinergic terminals) was found (Table 11).

In conclusion, the results providing evidence for 5-HT₂ receptor-mediated modulation of ACh release are inconsistent. This inconsistency is particularly true for the data obtained in the rat cerebral cortex. Although supported by experiments on slices and synaptosomes, a direct inhibition of ACh release via inhibitory presynaptic 5-HT₂ receptors is difficult to understand on the basis of what is known about the general properties of all 5-HT₂ receptor subtypes. They mediate stimulatory functions because of their positive coupling to phospholipase C via G_{Q/11} proteins (Table 1). As a first step to clarifying the issue, both the in vitro and the in vivo experiments in rat brain cortex should be repeated. The outcome will determine subsequent procedures.

E. 5-Hydroxytryptamine 3 Receptors

1. Function and Location. Modulation of ACh release by 5-HT₃ receptor activation was studied in rat, guinea pig, and human entorhinal cortex, neocortex, and hippocampus (Table 4). The conditions under which the operation of these receptors was examined differed among the various investigations and contradictory results (inhibition or increase of release) were obtained.

TABLE 4
 Studies in which the involvement of 5-HT₃ heteroreceptors in modulation of ACh release has been investigated
 Effects of 5-HT receptor agonists or 5-HT-releasing compounds on ACh release in various brain regions and species are shown.

| Species | Brain Region | Receptor Subtype ^a | Drugs | Release-Modifying Concentration | Comment | References |
|------------------------|----------------------------|----------------------------------------------|---------------------------------------------------|-------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| Rat (Lister ♀) | Entorhinal cortex | 5-HT ₃ | 2-CH ₃ -5-HT | 3 × 10 ⁻⁵ M: inhibition | Superfusion of slices; K ⁺ (20 mM)-induced [³ H]ACh release; inhibitory effect of 2-CH ₃ -5-HT only in the presence of ritanserin (10 ⁻⁶ M); 5-HT ₃ antagonists GR 38032F (3 × 10 ⁻¹⁰ -3 × 10 ⁻⁹ M) or zacopride (3 × 10 ⁻¹⁰ -10 ⁻⁹ M) abolished the effect of 2-CH ₃ -5-HT ^b | Barnes et al. (1989) |
| Guinea-pig | Cerebral neocortex | 5-HT ₃ | 2-CH ₃ -5-HT | 500 μg i.c.v.: inhibition | In vivo collection of solution from epidural cup of freely moving guinea pigs; inhibition of ACh release by 5-HT [in the presence of methiothepine (2 mg/kg s.c.) or by 2-CH ₃ -5-HT abolished by tropisetron (0.5 mg/kg s.c.) | Bianchi et al. (1990) |
| | Cerebral neocortex | | 5-HT | 500 μg/i.c.v.: inhibition | Superfusion of slices; 2-CH ₃ -5-HT (10 ⁻⁵ M) without effect on electrically evoked [³ H]ACh release | |
| | Cerebral neocortex | | 2-CH ₃ -5-HT | No effect | In vivo collection of solution from epidural cup of freely moving guinea-pigs; inhibition by 2-CH ₃ -5-HT i.c.v. or chlorimipramine s.c. prevented by MDL 72222 (1 mg/kg s.c.); chronic chlorimipramine treatment without effect (10 mg/kg daily for 14 days) | Simiscalchi et al. (1991) |
| Guinea-pig | Cerebral neocortex | 5-HT ₃ | 2-CH ₃ -5-HT | 500 μg i.c.v.: inhibition | | |
| | Cerebral neocortex | | Chlorimipramine | 10 mg/kg s.c. | | |
| Human | Neocortex synaptosomes | 5-HT ₃ | 5-HT | 10 ⁻⁸ -10 ⁻⁶ M: inhibition | Superfusion of synaptosomes; K ⁺ (15 mM)-induced [³ H]ACh release, tropisetron (10 ⁻⁸ -10 ⁻⁷ M) and ondansetron (10 ⁻⁷ M) blocked 5-HT-induced inhibition; 8-OH-DPAT, spiperone, and ketanserin without effect | Maura et al. (1992) |
| | Entorhinal cortex | | 1-PBG | 10 ⁻⁶ M: inhibition | | |
| Rat (Sprague-Dawley ♂) | Entorhinal cortex | 5-HT ₃ not confirmed ² | 2-CH ₃ -5-HT | No effect | Superfusion of slices; K ⁺ (20 mM)-induced [³ H]ACh or endogenous ACh release; 2-CH ₃ -5-HT (10 ⁻⁹ -10 ⁻⁶ M) in the presence of ritanserin (10 ⁻⁶ M) or ondansetron (10 ⁻⁹ M) without effect | Johnson et al. (1993) |
| Rat (Lister ♀) | Entorhinal cortex | | 2-CH ₃ -5-HT | No effect | Superfusion of slices; 2-CH ₃ -5-HT (2 × 10 ⁻⁶ M) without effect on K ⁺ (20 mM)-evoked [³ H]ACh release | |
| Rat (aged Wistar) | Entorhinal cortex | | 2-CH ₃ -5-HT, 5-HT | No effect | Superfusion of slices; 2-CH ₃ -5-HT (2 × 10 ⁻⁶ M) without effect on K ⁺ (20 mM)-evoked [³ H]ACh release | |
| Rat (CD ♀) | Dorsal hippocampus | 5-HT ₃ | <i>d</i> -Fenfluramine, <i>d</i> -norfenfluramine | 20 mg/kg i.p. increase, 7.5 mg/kg i.p.: increase | In vivo microdialysis in freely moving rats; release of endogenous ACh determined; tropisetron (0.5 mg/kg i.p.) or DAU 6215 (60 μg/kg i.p.) antagonized the norfenfluramine effect; | Consolo et al. (1994b) |
| | | | 2-CH ₃ -5-HT | 250 μg i.c.v. or 10 ⁻⁵ M by reverse dialysis: increase | DAU 6215 (60 μg/kg i.p.) or ondansetron (60 μg/kg i.p.) prevented the effects of 2-CH ₃ -5-HT; 5-HT ₃ receptor antagonists, given alone, had no effect | |
| Rat (Wistar ♂) | Entorhinal cortex Striatum | 5-HT ₃ | 2-CH ₃ -5-HT 1-PBG | 10 ⁻⁶ M: no effect 10 ⁻⁶ M: no effect | Superfusion of slices; K ⁺ (20 mM)-induced [³ H]ACh release; ondansetron and granisetron (10 ⁻⁸ -10 ⁻⁶ M) enhanced spontaneous and K ⁺ -evoked [³ H]ACh release; 2-CH ₃ -5-HT counteracted the release-enhancing effect of ondansetron (10 ⁻⁶ M); bicuculline (10 ⁻⁶ M) produced potentiation of [³ H]ACh release, haloperidol (10 ⁻⁶ M) and naloxone (10 ⁻⁶ M) had no effect | Ramirez et al. (1996) |
| Rat | Neocortex | 5-HT ₃ | mCPBG | 10 ⁻⁶ M: inhibition | Superfusion of synaptosomes; K ⁺ (15 mM)-induced [³ H]ACh release; VC 135 (3 × 10 ⁻⁸ M) blocked the effect of mCPBG | Crespi et al. (1997) |
| Rat (Wistar ♂) | Entorhinal cortex | 5-HT ₃ | | | Superfusion of slices; K ⁺ (20 mM)-induced [³ H]ACh release; ondansetron, granisetron, or MDL72222 (10 ⁻⁸ -10 ⁻⁶ M) enhanced K ⁺ -evoked [³ H]ACh release; bicuculline (10 ⁻⁷ M) or flumazenil (10 ⁻⁵ M) potentiated the effect of ondansetron but not of granisetron or MDL72222 | Díez-Ariza et al. (1998) |

2-CH₃-5-HT, 2-methyl-5-HT (5-HT₃ agonist); 1-PBG, 1-phenylbiguanide (5-HT₃ agonist).

^a Receptor type involved, as suggested by the authors.

^b Experiments aimed to confirm the conclusion of Barnes et al. (1989) concerning the 5-HT₃ receptor-mediated inhibition of ACh release.

It was first demonstrated on slices of the rat entorhinal cortex (Barnes et al., 1989), that 5-HT₃ receptor activation inhibits K⁺-evoked [³H]ACh release in a manner sensitive to blockade by 5-HT₃ receptor antagonists. This effect only occurred in the presence of the 5-HT₂ receptor antagonist ritanserin. These results could, however, not be confirmed (Johnson et al., 1993) in a carefully designed study on slices from the entorhinal cortex of rats belonging to three different strains.

Subsequent investigations on rat entorhinal cortex slices revealed that 5-HT₃ receptor blockade by ondansetron [1,2,3,9-tetrahydro-9-methyl-3-((2-methyl-1*H*-imidazol-1-yl)methyl)-4*H*-carbazol-4-one hydrochloride (5-HT₃ antagonist)] stimulated [³H]ACh release (Ramírez et al., 1996; Díez-Ariza et al., 1998): This effect was reversed by the 5-HT₃ receptor agonist 2-methyl-5-HT (Ramírez et al., 1996). These findings are compatible with the operation of tonically activated 5-HT₃ heteroreceptors modulating ACh release. In contrast to this suggestion, it was found in a microdialysis study in the frontoparietal cortex of freely moving rats that the 5-HT₃ receptor antagonist tropisetron altered neither basal nor K⁺-evoked ACh release, irrespective of whether the drug was applied subcutaneously or locally via the perfusion probe (Giovannini et al., 1998). On the other hand, that in vivo investigation resembled the in vitro studies in that it also disclosed a 5-HT₃ receptor-mediated effect: 5-HT (which did not modify basal ACh release), phenylbiguanide and *m*-chlorophenylbiguanide, administered via the perfusion probe, inhibited the K⁺-evoked ACh release (the inhibition by phenylbiguanide was tetrodotoxin-sensitive) and tropisetron blocked the effect of 5-HT or phenylbiguanide.

An inhibition of K⁺-evoked [³H]ACh release by a single concentration (1 μM) of the 5-HT₃ receptor agonist *m*-chlorophenylbiguanide was also observed in rat neocortical synaptosomes (Crespi et al., 1997). The *m*-chlorophenylbiguanide-induced inhibition was very weak and variable (by 18 ± 11%) and 2-methyl-5-HT, another 5-HT₃ receptor agonist, did not modify basal or K⁺-evoked [³H]ACh release. The inhibition by *m*-chlorophenylbiguanide was counteracted by VC 135, an infrequently applied 5-HT₃ receptor antagonist, but ondansetron (0.1 μM) mimicked the effect of the agonist, i.e., acted inhibitory by itself. As, furthermore, no attempt was made to explain the purported direct inhibition of ACh release by the excitatory 5-HT₃ receptor, this study left more questions open than it contributed to the clarification of the 5-HT₃ receptor-mediated modulation of ACh release. Nevertheless, it should be noted that analogously to the study by Crespi et al. (1997), an inhibitory effect of 5-HT₃ receptor stimulation on K⁺-evoked [³H]ACh release had already been found previously in human cortical synaptosomes (Maura et al., 1992). This effect was susceptible to blockade by 5-HT₃ receptor antagonists.

At first sight these results appear to strongly support the suggestion that the 5-HT₃ receptors involved are

located on cholinergic axon terminals, but a major concern about this interpretation is the fact that the 5-HT₃ receptor is a ligand-gated ion channel whose stimulation ultimately results in an increase in Ca²⁺ influx into the terminals. This increased influx, in turn, leads to an increase, rather than inhibition, of transmitter release (section II.C.) (Rondé and Nichols, 1998). Thus, the possibility that the 5-HT₃ receptor agonists do not directly act at receptors on the cholinergic axon terminals was considered by most of the authors cited above. In fact, such an indirect effect may result from inhibitory interneurons, in particular GABAergic neurons (Fig. 2) (Ramírez et al., 1996; Díez-Ariza et al., 1998). Thus, in rat entorhinal cortical slices the GABA_A receptor antagonist bicuculline and the antagonist at the benzodiazepine recognition site of the GABA_A receptor flumazenil mimicked the increasing effect of ondansetron on [³H]ACh release. In interaction experiments, the [³H]ACh-releasing effect of ondansetron was markedly potentiated by bicuculline or flumazenil (Ramírez et al., 1996; Díez-Ariza et al., 1998). Evidence has been presented that 5-HT₃ receptor-like immunoreactivity is primarily associated with GABAergic neurons in the cerebral cortex and hippocampus (Morales et al., 1996; Morales and Bloom, 1997). In addition, it was demonstrated in the rat hippocampus (Katsurabayashi et al., 2003) that 5-HT₃ receptors are also present on GABAergic varicosities (sections II.A. and VI.E.). Hence, the mechanism underlying the inhibition of ACh release may comprise the following steps: activation of somadendritic and presynaptic 5-HT₃ receptors on GABAergic interneurons results in the release of GABA which, by activating inhibitory GABA receptors on cholinergic nerve terminals, finally leads to the reduction of ACh release. On the basis of this mechanism of action, the ability of 5-HT₃ receptor agonists to inhibit ACh release from superfused human cortical synaptosomes (Maura et al., 1992) may be due to an insufficient superfusion speed related to the abundance of GABA molecules released into the vicinity of the cholinergic synaptosomes.

In contrast to the results obtained in the in vitro experiments on rat and human cerebral cortical preparations, a microdialysis study in the hippocampus of rats (Consolo et al., 1994b) (Table 4) revealed that ACh release in response to 5-HT₃ receptor activation (by systemically applied exogenous 5-HT or an increase in synaptic availability of endogenous 5-HT by *d*-fenfluramine or *d*-norfenfluramine) was facilitated in a manner susceptible to blockade by 5-HT₃ receptor antagonists. Basically the same facilitatory effect was observed when 5-HT₃ receptors were activated by 2-methyl-5-HT applied either locally via the dialysis probe or intracerebroventricularly. The authors stated that these results did not yet make it unequivocally possible to decide whether the 5-HT₃ receptor exerts its facilitatory effect on cholinergic activity directly on cholinergic nerve terminals or indirectly with one or more neurons inserted

between serotonergic and cholinergic nerve terminals. An interpretation beyond this conclusion is not possible at the present time because *in vitro* experiments on slices or synaptosomes are missing. However, in view of the depolarization of the neuronal membrane evoked by 5-HT₃ receptor activation, a presynaptic location of the 5-HT₃ receptors on cholinergic axon terminals is compatible with its stimulatory effect on ACh release. In any case, it is not yet possible to decide whether the contradictory results obtained in the experiments in the rat brain *in vitro* and *in vivo* (inhibition versus facilitation) represent differences between the brain areas (cerebral cortex versus hippocampus, respectively) or whether there are other reasons.

In the guinea pig cerebral cortex *in vivo*, the functional property of the release-modulating 5-HT₃ receptors is identical to that in rat entorhinal cortex slices. Thus, an inhibitory effect on ACh release by 5-HT₃ receptor activation with 2-methyl-5-HT, exogenous 5-HT, or chlorimipramine-induced increase in synaptic endogenous 5-HT was also observed *in vivo* in the neocortex of freely moving guinea pigs (Bianchi et al., 1990; Siniscalchi et al., 1991) (Table 4). All of these drugs were applied systemically or intracerebroventricularly, thus excluding any reliable conclusion concerning the location of the receptor involved. Taking the failure of 2-methyl-5-HT to modify the electrically evoked [³H]ACh release in neocortical slices (Bianchi et al., 1990) and the well known stimulation on the neuronal cell membrane into account, the authors interpreted the inhibition of ACh release *in vivo* as an indirect effect; the latter was suggested to be mediated by neuronal systems known to inhibit ACh release, such as GABAergic neurons (Fig. 2). Hence, the mechanism of action appears to be basically identical to that in the rat entorhinal cortex: GABA released from GABAergic neurons upon activation of their somadendritic and presynaptic 5-HT₃ receptors inhibits ACh release via specific GABA receptors on cholinergic axon terminals. However, in view of the negative result in the experiments on guinea pig slices (in contrast to that in the rat cortex) (Table 4), the location of the inhibitory 5-HT₃ receptor in the guinea pig cortex appears to differ from that of the receptor in the rat cortex: the serotonergically innervated GABA interneuron is probably located further upstream of the cholinergic neuron, i.e., outside the cortex.

2. Classification. The 5-HT receptors modulating ACh release in response to 5-HT₃ receptor ligands in the rat, guinea pig, and human cerebral cortex and in the rat hippocampus can unequivocally be classified as 5-HT₃ (Barnes et al., 1989; Bianchi et al., 1990; Siniscalchi et al., 1991; Maura et al., 1992; Consolo et al., 1994b). This statement is proven by the findings that inhibition or increase of release is induced by 5-HT₃ receptor agonists such as 2-methyl-5-HT or 1-phenylbiguanide (or by exogenous or endogenous 5-HT) and that the effect of the agonists is blocked by 5-HT₃ receptor antagonists such

as GR 38032F, zacopride, ondansetron, tropisetron, DAU 6215, or MDL 72222 (Table 4).

Five different 5-HT₃ receptor subunits named 5-HT_{3A}, 5-HT_{3B}, 5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E} have been cloned and characterized (Maricq et al., 1991; Davies et al., 1999; Dubin et al., 1999; Niesler et al., 2003, 2007). In addition, splice variants of the mouse (Hope et al., 1993) and human (Brüss et al., 2000) 5-HT_{3A} subunit exist. Only the 5-HT_{3A} subunit can form functional homopentameric 5-HT₃ receptors, whereas the other subunits and the splice variants, when coexpressed with the 5-HT_{3A} subunit, can modify the ion conductance of the receptor channel. However, no conclusion concerning the subunit composition of the 5-HT₃ receptors mediating modulation of ACh release can be drawn from the experiments quoted, because the pharmacological tools applied cannot discriminate between the homo- and heteropentameric receptors.

3. Specific Aspects. In the guinea pig and rat cerebral cortex the 5-HT₃ receptor mediating inhibition of ACh release interacts with other 5-HT receptors modulating ACh release. Thus, the increase in ACh release evoked by the 5-HT_{1A} receptor agonist 8-OH-DPAT in the guinea pig neocortex *in vivo* was reduced by the chlorimipramine-evoked increase in synaptic endogenous 5-HT, an effect that was antagonized by the 5-HT₃ receptor antagonist MDL 72222 (Siniscalchi et al., 1991). A plausible interpretation may be that both 5-HT_{1A} and 5-HT₃ receptors mediating opposite modulation of ACh release may be located on the same GABAergic interneurons, predominantly on their varicosities (Fig. 2) (Katsurabayashi et al., 2003) in the rat hippocampus (sections VI.B. and VI.E.). Thus, 5-HT₃ receptor stimulation attenuates the disinhibition of ACh release which, in turn, is probably due to activation of the inhibitory 5-HT_{1A} receptors located on the inhibitory GABAergic neurons (see sections VI.B. and VI.E.). In the rat entorhinal cortex another receptor interaction on ACh release comes into play: blockade of 5-HT₂ receptors by ritanserin was necessary to unmask the inhibitory effect of α -methyl-5-HT on K⁺-evoked [³H]ACh release (Barnes et al., 1989). However, the failure to confirm this result (Johnson et al., 1993) must be taken into account.

Interestingly, Siniscalchi et al. (1991) observed in the guinea pig cerebral cortex *in vivo* that long-term exposure of the animals to chlorimipramine did not modify the 5-HT₃ receptor-mediated inhibition of ACh release (Table 4), whereas the 5-HT_{1A} receptor-mediated increase in ACh release was reduced. (1991). Hence, the 5-HT₃ receptors, in contrast with the 5-HT_{1A} receptors, appear not to be down-regulated by long-term increases in the synaptic concentration of endogenous 5-HT.

The inhibition of ACh release by 5-HT₃ receptor agonists is of interest in the context of the role that ACh plays for memory and learning. If these receptors are tonically activated in humans, *in vivo* 5-HT₃ receptor antagonists should increase ACh release. Accordingly,

these compounds have been considered as putatively suitable for the treatment of cognitive disorders (Bentley and Barnes, 1995).

4. *Concluding Summary Statement.* Depending on the brain area, opposite effects on ACh release are mediated via 5-HT₃ receptors. In the rat entorhinal cortex as well as in the guinea pig and human neocortex 5-HT₃ receptors, probably located on inhibitory interneurons, mediate an indirect inhibition of ACh release. In the rat hippocampus ACh release is facilitated by activation of presynaptic 5-HT₃ receptors, which are assumed to be located on the cholinergic terminals (Table 11).

F. 5-Hydroxytryptamine 4 Receptors

1. *Function, Location, and Classification.* A microdialysis study in the frontal cortex of freely moving rats revealed that ACh release is modulated via facilitatory

5-HT₄ receptors (Consolo et al., 1994a) (Table 5). This suggestion concerning the function and classification was based on the ability of the selective 5-HT₄ receptor agonists BIMU 1 and BIMU 8 to increase ACh release, an effect susceptible to blockade by the 5-HT₄ receptor antagonists GR 125487 and GR 113808. Because these drugs were not applied locally via the dialysis probe but intracerebroventricularly, the experiments do not allow a conclusion as to whether the receptors are located presynaptically on the axon terminals of the cholinergic nerves. Although a direct facilitation is conceivable when the coupling of 5-HT₄ receptors to G_s proteins is considered, a location outside the neocortex on cell bodies of cholinergic neurons in other brain regions is possible.

These results obtained with the selective 5-HT₄ receptor agonists and antagonists (Consolo et al., 1994a) were ba-

TABLE 5

Studies in which the involvement of 5-HT₄ heteroreceptors in modulation of ACh release has been investigated

Effects of 5-HT receptor agonists, 5-HT-releasing compounds, or 5-HT uptake inhibitors on ACh release in various brain regions and species are shown.

| Species | Brain Region | Receptor Subtype ^a | Drugs | Release-Modifying Concentration | Comment | References |
|----------------|--------------------------|-------------------------------|------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| Rat (CD ♀) | Frontal neocortex | 5-HT ₄ | BIMU 1 BIMU 8 | 40 nmol i.c.v.: increase 30–60 nmol i.c.v.: increase | Release of ACh determined by microdialysis in freely moving rats; GR 125487 (20 nmol) or GR 113808 (15 nmol) i.c.v. prevented BIMU 1 facilitation of ACh release | Consolo et al. (1994a) |
| Rat (Wistar ♂) | Frontal neocortex | 5-HT ₄ | PCA | 1–3 mg/kg i.p.: increase | Release of ACh determined by microdialysis in freely moving rats; PCA-induced increase in ACh release inhibited by RS23597 (5 × 10 ⁻⁵ M) or GR 113803 (10 ⁻⁶ M); WAY 100135, (-)-pindolol, (-)-propranolol, ritanserin, or ondansetron had no effect | Yamaguchi et al. (1997a) |
| Rat (Wistar ♂) | Frontal neocortex | 5-HT ₄ | Indeloxazine | 10 ⁻⁵ –3 × 10 ⁻⁵ M | Release of ACh determined by microdialysis in freely moving rats; indeloxazine applied locally by reverse dialysis increased ACh release, which was inhibited by RS23597 (5 × 10 ⁻⁵ M), GR 113803 (10 ⁻⁶ M); WAY 100135, (-)-pindolol, (-)-propranolol, ritanserin, or ondansetron had no effect | Yamaguchi et al. (1997b) |
| Guinea pig | Neocortex Hippocampus | 5-HT ₄ | BIMU 8 BIMU 8 | 0.2–5 × 10 ⁻⁶ M increase 0.2–5 × 10 ⁻⁶ M: no effect | Electrically stimulated (0.5 Hz) release of [³ H]ACh from slices preincubated with [³ H]choline; slices were superfused in the presence of 10 μM hemicholinium-3; GR 125487 prevented effect of BIMU 8; hippocampal synaptosomes were depolarized with K ⁺ (20 mM) | Siniscalchi et al. (1999) |

PCA, *p*-chloramphetamine (5-HT-releasing drug); indeloxazine, indeloxazine hydrochloride (5-HT and NE uptake inhibitor).

^a Receptor type involved, as suggested by the authors.

sically confirmed by microdialysis studies in the rat frontal cortex (Yamaguchi et al., 1997a,b) and in the rat prefrontal cortex (Nair and Gudelsky, 2005). In the latter study ACh release was increased by i.p. 3,4-methylene-dioxymethamphetamine (MDMA), an effect counteracted by systemic administration of the 5-HT₄ receptor antagonist SDZ 205,557, but not the 5-HT_{2A,2B,2C} receptor antagonist LY-53,857; this finding is compatible with the suggestion that MDMA releases endogenous 5-HT which, in turn, produces the increase in ACh release by activating 5-HT₄ receptors. In the experiments of Yamaguchi et al. (1997a,b) the 5-HT₄ receptors were also stimulated by increasing the synaptic availability of endogenous 5-HT. This increase was achieved by systemic application of the 5-HT releaser *p*-chloramphetamine (Yamaguchi et al., 1997a) or by local application (via the microdialysis probe) of the 5-HT uptake inhibitor citalopram or by systemic or local application of indeloxazine. The latter drug is a 5-HT and noradrenaline uptake inhibitor which, in addition, directly increases 5-HT release (Yamaguchi et al., 1997b). The resulting increase in ACh release was counteracted by the selective 5-HT₄ receptor antagonists GR 113808 and RS23597 administered locally via the dialysis probe. The authors concluded that the release-modulating 5-HT₄ receptors are, at least in part, present in the frontal cortex [which represents one step further ahead compared with the suggestions based on the study by Consolo et al. (1994)], but that further experiments were required to determine whether 5-HT modulates ACh release directly via 5-HT₄ receptors located on cholinergic terminals or indirectly through other neuronal systems.

Such experiments were performed in superfused guinea pig brain slices and synaptosomes preincubated with [³H]choline (Siniscalchi et al., 1999). BIMU 8 did not modify basal tritium efflux but increased the electrically (0.5 Hz) evoked [³H]ACh release in slices of the cerebral cortex, hippocampus, and nucleus basalis magnocellularis in a manner sensitive to blockade by GR 125487, a selective 5-HT₄ receptor antagonist, suggesting that facilitatory 5-HT₄ receptors may be present on the cholinergic nerve terminals. However, at first sight, the failure of BIMU 8 to modify the K⁺ (20 mM)-evoked [³H]ACh release from hippocampal synaptosomes does not conform to this interpretation, but the possibility that modulation of stimulation-evoked transmitter release via presynaptic receptors may not be detectable if too strong stimuli, leading to near maximal Ca²⁺ influx, are applied should be considered. In fact, low-frequency (0.5 Hz) electrical stimulation of slices was weak, whereas a K⁺ stimulus of 20 mM applied to synaptosomes was relatively strong.

The facilitatory 5-HT₄ receptors involved are not tonically activated as indicated by the failure of 5-HT₄ receptor antagonists, given alone, to increase ACh release (Consolo et al., 1994a; Yamaguchi et al., 1997a,b; Siniscalchi et al., 1999). The ability of 5-HT₄ receptor agonists to facilitate ACh release led Consolo et al.

(1994a) and Siniscalchi et al. (1999) to suggest that such compounds offer an additional means to overcome the cholinergic deficit in memory disorders.

2. Concluding Summary Statement. In vivo analyses of ACh release in the rat cortex revealed that release was enhanced by 5-HT₄ receptor activation (Table 11), but that the receptor location (presynaptic on the terminals or on the cell bodies of the cholinergic neuron or on an interneuron) is not yet clear. Operation as a presynaptic heteroreceptor would be compatible with the coupling to G_s proteins. Experiments on slices and on synaptosomes are suitable to elucidate this issue. Such in vitro experiments in guinea pig cortex, hippocampus, and nucleus basalis magnocellularis preparations suggested direct activation of presynaptic 5-HT₄ heteroreceptors on cholinergic terminals because 5-HT₄ receptor stimulation increased ACh release.

IV. Modulation of Dopaminergic Neurotransmission

A. General Aspects

The operation of 5-HT receptors modulating DA release has been investigated in the rat and mouse brain (Tables 5–7). By far, most of the studies have been performed in the striatum; in fewer cases DA release has been examined in the nucleus accumbens and prefrontal neocortex, i.e., brain areas that are of particular interest with respect to extrapyramidal motor control, drug dependence, and the antipsychotic action of 5-HT receptor ligands. The anatomical basis underlying the modulation of DA release by endogenous 5-HT is represented by projections from serotonergic cell bodies in the raphe nuclei not only to cell bodies of dopaminergic neurons in the substantia nigra and ventral tegmental area but also to their projection areas, i.e., the striatum on the one hand and nucleus accumbens and prefrontal neocortex on the other, respectively (Bobillier et al., 1975; Fibiger and Miller, 1977; Hervé et al., 1987; Nedergaard et al., 1988).

It is evident that a 5-HT-receptor agonist-induced modification of DA release in vivo observed in microdialysis of brain regions containing cell bodies and dendrites but no axon terminals of dopaminergic neurons is not compatible with the operation of presynaptic 5-HT heteroreceptors on these terminals. This conclusion can be drawn, e.g., from a microdialysis study on freely moving rats, in which systemic injection of 8-OH-DPAT increased DA release in the ventral tegmental area (Chen and Reith, 1995); this effect was further increased by cocaine applied locally via the dialysis probe. In such experiments, DA release may be hypothesized to occur from dopaminergic dendrites. However, the 5-HT_{1A} receptors that are probably involved in the increase in release must be located elsewhere because 5-HT_{1A} receptors do not mediate a facilitation but an inhibition. It is likely that they are located as pre- and/or postsynaptic

5-HT_{1A} heteroreceptors on inhibitory nondopaminergic, probably GABAergic, interneurons that exert an inhibitory tone on the activity of the dopaminergic neurons. Activation of the inhibitory 5-HT_{1A} receptors, thus, can disinhibit, i.e., increase, somadendritic DA release and may also enhance DA release in projection areas.

In addition, the involvement of presynaptic 5-HT receptors on dopaminergic axon terminals can be ruled out when under certain *in vivo* conditions local administration of a 5-HT receptor ligand into a brain area containing cell bodies results in a change of DA release in the respective projection area, whereas local application of the ligand into the projection area is without effect. A typical example is a microdialysis study in the nucleus accumbens of freely moving rats (Imperato and Angelucci, 1989), which provided indirect evidence for an action of endogenous 5-HT on the dopaminergic cell bodies in the ventral tegmental area. Tropicsetron when injected (subcutaneously or) locally into the ventral tegmental area, but not when administered into the nucleus accumbens, antagonized the morphine (1 mg/kg s.c.)-induced stimulation of DA release in the nucleus accumbens. Obviously morphine injection is associated with an activation of serotonergic neurons leading to tonic activation of stimulatory 5-HT₃ receptors on the dopaminergic cell bodies in the ventral tegmental area. Blockade of these receptors by 5-HT₃ receptor antagonists leads to suppression of DA release in the nucleus accumbens.

In brain areas containing dopaminergic terminals, various 5-HT receptors, in particular 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, and 5-HT₄ receptors, have been shown to be involved in the modulation of DA release. These receptors are putative targets for therapeutic tools in disorders related to alterations in dopaminergic neurotransmission such as Parkinson's disease, Huntington's chorea, and schizophrenia. At first glimpse, there is a confusing diversity for and in many cases even contradictory effects that, however, can be arranged in a rational and plausible order when considering the fact that a given 5-HT receptor type can produce direct effects via presynaptic heteroreceptors and indirect effects via 5-HT receptors on interneurons. In addition to influences mediated by 5-HT receptors, 5-HT and certain 5-HT₃ receptor agonists can induce release of DA in biologically significant amounts by a DA carrier-mediated mechanism.

B. Dopamine Transporter-Mediated Dopamine Release

1. Function, Location, and Mechanism. Particular caution and care is necessary for a correct interpretation of 5-HT-, 2-methyl-5-HT-, and phenylbiguanide-evoked increases in DA efflux from the rat striatum and nucleus accumbens. 5-HT-evoked [³H]DA efflux from striatal synaptosomes was first described by De Belleruche and Bradford (1980). This finding was confirmed by others (Nurse et al., 1988), who also showed that the stimulant effect of 5-HT was abolished when the DA transporter

inhibitors nomifensine or benzotropine were included in the superfusion buffer. The latter observation already pointed to the mechanism involved. For an appropriate explanation the fact that 5-HT and phenylbiguanide, apart from being 5-HT receptor agonists with the latter acting selectively at 5-HT₃ receptors, also act via another presynaptic 5-HT- and phenylbiguanide-recognizing functional protein, namely the DA transporter (Benuck and Reith, 1992) has to be taken into account. By being transported into the dopaminergic nerve terminal by this carrier (Shaskan and Snyder, 1970; Feuerstein et al., 1986) and to a minor extent potentially also by entering the nerve terminal by diffusion (Benuck and Reith, 1992), they evoke DA efflux by reversed direction DA transport into the synaptic cleft by the DA carrier (Schmidt and Black, 1989; Yi et al., 1991; Benuck and Reith, 1992; Zazpe et al., 1994; Santiago et al., 1995; De Deurwaerdère et al., 1997). This occurs presumably in a manner analogous to the DA-releasing action of *d*-amphetamine (Parker and Cubeddu, 1986a,b). In addition, a further less important mechanism has been suggested to contribute to the increase in DA efflux, namely the release of vesicular DA into the cytoplasmic pool. There it is available for reversed direction transport by the dopamine carrier (Yi et al., 1991; Benuck and Reith, 1992). DA efflux mediated by these mechanisms is Ca²⁺-independent and blocked by inhibitors of the DA transporter, as proven by *in vitro* and *in vivo* investigations:

The study of Schmidt and Black (1989) was originally designed to confirm the results of Blandina et al. (1988) who concluded that DA release in the striatum is modulated by facilitatory 5-HT₃ receptors (section IV.E.). However, such a conclusion could not be drawn from the data of Schmidt and Black (1989), who found that the phenylbiguanide-evoked [³H]DA efflux from rat striatal slices occurred in both the presence and absence of Ca²⁺; this efflux was inhibited by nomifensine but not by tropisetron. These results clearly support the DA transporter-mediated mechanism of action, as did those of Yi et al. (1991). They observed that the 5-HT-induced increase of "basal" [³H]DA efflux from striatal synaptosomes superfused with Ca²⁺-free buffer and of Ca²⁺ evoked [³H]DA efflux were antagonized by DA transporter blockade with nomifensine or cocaine but not by 5-HT₃ receptor antagonism with MDL 72222 or GR 38032F (stimulation with Ca²⁺ was achieved by introduction of 0.6 mM CaCl₂ into the previously Ca²⁺-free superfusion fluid, yielding a final concentration of 0.6 mM). Analogous results were obtained by Benuck and Reith (1992) who studied the stimulatory actions of both phenylbiguanide and 5-HT on [³H]DA efflux from rat striatal slices. The presence of nomifensine but not of the 5-HT₃ receptor antagonists zacopride or tropisetron again prevented the increase in 5-HT- or phenylbiguanide-evoked outflow, which did not require the presence of Ca²⁺ ions and which

also occurred in preparations with depleted vesicular DA stores from reserpinized animals.

The previous results were confirmed by measuring [³H]DA efflux from superfused tissue strips from the rat striatum and were extended to a subregion of the striatum, i.e., the rat nucleus accumbens (Jacocks and Cox, 1992). The 5-HT-stimulated efflux from both brain regions was Ca²⁺-independent and abolished by GBR12909, a blocker of the DA transporter, but was not affected by tropisetron or ketanserin, a 5-HT_{2A} receptor antagonist. The authors concluded that endogenous 5-HT in the striatum and accumbens may function as a local regulator of DA release by acting via the transporter-dependent mechanism. 5-HT-, 2-methyl-5-HT- and phenylbiguanide-induced release of [³H]DA and endogenous DA was also observed in slices from the rat striatum and olfactory tubercle, a terminal field of the mesolimbic DA system (Zazpe et al., 1994). The crucial role of the DA transporter in this effect was proven by its blockade with nomifensine and cocaine, despite the inability of 5-HT₃ receptor antagonists to do so.

An investigation aimed to provide more direct evidence for the functional interaction between a representative 5-HT₃ receptor agonist and the DA transporter was conducted on synaptosomes from the nucleus accumbens and the caudate putamen with 1-(*m*-chlorophenyl)-biguanide, a more potent 5-HT₃ receptor-stimulating analog of phenylbiguanide (Campbell et al., 1995). The 5-HT agonist inhibited both the binding of [³H]GBR12935, a ligand of the DA transporter, to membranes from the two brain regions and the uptake of [³H]DA into the synaptosomes.

A microdialysis study by Santiago et al. (1995) in freely moving rats provided *in vivo* evidence for a 5-HT₃ receptor agonist-induced, DA transporter-mediated increase in DA release. They measured the influence of drugs applied locally via the dialysis probe on the efflux of endogenous DA from the striatum. The phenylbiguanide-induced DA efflux was not changed by MDL 72222 or tetrodotoxin but was inhibited by nomifensine.

2. Concluding Summary Statement. The site of action underlying the 5-HT₃ receptor-independent increase in DA efflux in the rat striatum, nucleus accumbens, and olfactory tubercle induced by 5-HT and selective 5-HT₃ receptor agonists is the dopamine transporter in the cell membrane. After being taken up into the cytoplasm of the DA terminal, these compounds evoke DA efflux by reversed direction DA transport into the synaptic cleft by the same DA transporter. According to this mechanism of action, this DA "release" can be inhibited by compounds that block the DA transporter (e.g., nomifensine) but not by 5-HT₃ receptor antagonists. It is possible that under physiological or pathological conditions associated with very pronounced stimulation of serotonergic neurons this presynaptic DA transporter-mediated action of 5-HT contributes to the induction of DA release in the above-mentioned brain

areas. Misinterpretation of studies designed to identify 5-HT₃ heteroreceptor-mediated stimulation or facilitation of DA release can most reliably be avoided if such experiments are performed in the presence of an inhibitor of the DA transporter. However, such compounds have been applied in a few investigations only.

C. Modulation via 5-Hydroxytryptamine Receptors Putatively Located on Interneurons

In vivo experiments designed to determine whether 5-HT receptors are directly or indirectly involved in modulation of DA release in the striatum and medial prefrontal cortex are summarized in Table 6. Basically the results are compatible with these possibilities, but conclusions concerning the exact location of the receptors could in most cases not be achieved although, as a rule, the drugs affecting the serotonergic system were applied locally via the dialysis probe. Nevertheless, tentative suggestions concerning the location could be made when one examined whether the signal transduction of the receptor type assumed to be involved, leading to negative or positive coupling to the effectors, does not conform to the observed effect of this receptor. Thus, if, e.g., a facilitation of transmitter release is mediated by a receptor that is negatively coupled to its effectors, a direct effect on the studied neuron is unlikely. A plausible explanation for the discrepancy is the involvement of an inhibitory interneuron endowed with the receptor. This explanation is particularly valid when the occurrence of such an interneuron that inverts the final response is supported by literature.

In most of the reports discussed in this section the classification of the receptors remained vague because of the low number and selectivity of the ligands on which the classification was based. However, the pooled information from several investigations on the pharmacological profile of the receptor involved is helpful in the attempt to characterize the receptor according to the current classification. Thus, these investigations are valuable in that they enabled an up-to-date reinterpretation of the data.

1. 5-Hydroxytryptamine 1B Receptors on Interneurons.

a. Function, site of action, and classification. In their report Benloucif and Galloway (1991) (Table 6) stated that "5-HT₁ receptor agonists" increased DA release in the rat striatum and that the 5-HT₁ receptor antagonist pindolol partially reduced the effect of 5-HT. However, the authors did not consider that the so-called "5-HT_{1C}" receptor activated by mCPP does not belong to the 5-HT₁ receptor family; this is surprising because it had already been reported (Hoyer, 1988; Hartig, 1989) that "5-HT_{1C}" receptors are members of the 5-HT₂ receptor family [for the development of the 5-HT₂ receptor classification, see also another report (Göthert, 1992)]. Taking the DA release-increasing effect not only of exogenous but also of endogenous 5-HT, released by fenfluramine, into ac-

TABLE 6
In vivo and ex vivo studies in which the involvement of 5-HT heteroreceptors in modulation of DA release has been investigated

Effects of various drugs acting at different levels of the 5-HT system on DA release determined in microdialysis studies of the rat or mouse nigrostriatal or mesocortical DA system are shown. Unless stated otherwise, the drugs were applied by the probe perfusion fluid. As a rule, the results did not allow clear-cut statements concerning the receptor type(s) involved. 5-HT receptor localization on interneurons as a rule is probable.

| Species | Brain Region | Receptor Type | Drugs | Release-Increasing Concentration | Comment | References |
|---------|---------------------------------------|---------------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| Rat | Striatum | | 5-HT mCPP TFMPP RU 24969 8-OH-DPAT Fenfluramine TFMPP | 4×10^{-11} - 4×10^{-9} M 4×10^{-10} - 4×10^{-8} M 10^{-8} - 1.6×10^{-7} M 2×10^{-9} M 2×10^{-9} M 4×10^{-10} - 4×10^{-8} M 3-30 mg/kg | In vivo microdialysis in anesthetized (with chloral hydrate) rats; pindolol (4 nmol/40 μ l fraction) inhibited the 5-HT (0.4 nmol/40 μ l fraction)-evoked increase in DA release | Benloucif and Galloway (1991) |
| Rat | Striatum | | | | Ex vivo determination of 3-MT ^w as an indirect parameter for DA release in vivo with HPLC in homogenates of striatal tissue; 3-MT, TFMPP, or ritanserin applied by s.c. injection; ritanserin without effect | Nissbrandt et al. (1992) |
| Rat | Striatum | 5-HT ₁ , 5-HT ₃ | 5-HT RU 24969 5-MeOT 8-OH-DPAT DOI | 10^{-5} - 10^{-4} M 3×10^{-5} - 3×10^{-4} M 3×10^{-4} - 3×10^{-3} M 10^{-4} - 3×10^{-3} M 3×10^{-4} - 3×10^{-3} M | In vivo microdialysis in anesthetized rats; drugs applied by probe perfusion; pindolol (4 nmol/40 μ l fraction) reduced the effect of RU 24969 (2 nmol/40 μ l fraction); ICS 205,930 (4 nmol/40 μ l fraction), and MDL 72222 (4 nmol/40 μ l fraction) reduced the facilitatory effect of 5-HT; ritanserin without effect; involvement of 5-HT ₁ and 5-HT ₃ , but not of 5-HT ₂ , receptors suggested | Benloucif et al. (1993) |
| Rat | Striatum | | 5-HT Alaproclate ^e CGS 12066B | 10^{-6} - 10^{-5} M DA, DOPAC, and HVA increased 4×10^{-4} M 10^{-6} M | In vivo microdialysis in freely moving rats; 5-HT, 5-HIAA, DA, DOPAC, and HVA determined in the dialysate; drugs; 5,7-DHT pretreatment abolished the effect of the 5-HT uptake inhibitor alaproclate on dialysate levels of DA | Yadid et al. (1994) |
| Rat | Medial prefrontal cortex | 5-HT _{2A} | MDL 100,907 | 0.1-1 mg/kg s.c. or 10^{-6} M directly i.c.v. | In vivo microdialysis in freely moving rats; ritanserin (2.5 mg/kg s.c.) without effect; 5-HT _{2A} receptors probably involved, but location on interneurons probable | Schmidt and Fadaye (1995) |
| Mouse | 5-HT _{1B} ko Dorsal striatum | | CP-93,129 | 5×10^{-5} M: 5-fold increase | In vivo microdialysis in anesthetized mice; CP-93,129 increased DA release in 5-HT _{1B} ko and in wild-type mice | De Groote et al. (2003) |

3-MT, 3-methoxytryptamine (dopamine metabolite); HPLC, high-pressure liquid chromatography; 5-MeOT, 5-methoxytryptamine (5-HT₄ agonist); DOPAC, 3,4-dihydroxyphenylacetic acid (dopamine metabolite); HVA, homovanillic acid (catecholamine metabolite); 5-HIAA, 5-hydroxyindoleacetic acid (5-HT metabolite); ko, knockout.

^a Accumulation of the DA metabolite 3-MT formed by catechol-O-methyltransferase after inhibition of monoamine oxidase by pargyline was used as an indirect measure of dopamine release and metabolism in vivo.

^b 5-HT reuptake blocker.

count, the main result of the study by Benloucif and Galloway (1991) was that the serotonergic innervation of the striatum "may exert a facilitatory influence on DA release," presumably via 5-HT₁ receptors (although the latter was not clearly stated). However, the data do not provide an answer to the question whether the facilitation of DA release is a consequence of activation of presynaptic receptors on dopaminergic nerve terminals or whether 5-HT acts via interneurons in the striatum. In fact, 5-HT, 8-OH-DPAT, and RU 24969, in contrast to mCPP, activate 5-HT_{1A} and/or 5-HT_{1B} receptors. In view of the inhibitory character of the 5-HT₁ receptors due to their coupling to G_i/G_o proteins, it is more plausible that activation of somadendritic and/or presynaptic 5-HT_{1A} and 5-HT_{1B} receptors on inhibitory interneurons (probably GABAergic ones) disinhibit DA release (Fig. 2).

More insight into the site and mechanism of action of 5-HT receptor agonists was not provided by a subsequent study of the same group (Benloucif et al., 1993) (Table 6). That investigation, in addition to confirming the facilitatory role of 5-HT_{1B} receptors, mainly extended the previous study in two aspects: 1) the 5-HT₂ receptor agonist DOI increased DA release, but surprisingly this effect was not modified by the 5-HT₂ receptor antagonist ritanserin; and 2) the 5-HT₃ receptor antagonists MDL 72222 and tropisetron weakly counteracted the facilitatory effect of 5-HT, suggesting that DA release is facilitated via 5-HT₃, but not via 5-HT₂, receptors. In contrast to the probable location of the 5-HT₁ receptors on interneurons, the 5-HT₃ receptors may represent genuine presynaptic heteroreceptors on dopaminergic axon terminals, a site of action supported by various *in vitro* and *in vivo* experiments discussed in section IV.E.

In agreement with the facilitatory effect of 5-HT_{1B} receptor activation by RU 24969 (Benloucif and Galloway, 1991; Benloucif et al., 1993), two other 5-HT_{1B} receptor agonists, TFMPP and CGS 12066B, were also shown to increase DA release in the rat striatum (Nissbrandt et al., 1992; Yadid et al., 1994) (see Table 6 for details). Furthermore, the facilitatory effect of 5-HT was mimicked by blockade of the 5-HT transporter with alaproclate (Yadid et al., 1994), an effect that is probably brought about by increasing the concentration of endogenous 5-HT at its site of action. Similarly, an *in vivo* microdialysis study in conscious and freely moving rats in which 5-HT_{1A} and 5-HT_{1B} receptor ligands such as 5-CT, anpirtoline, and isamoltan were administered locally into the striatum via the microdialysis probe (Ng et al., 1999) led to the conclusion that 5-HT₁ receptors are involved in the facilitation of DA release and that further investigations are necessary to clarify the location of these receptors in the striatum.

A microdialysis study in another brain area, i.e., the prefrontal cortex, of awake rats (Iyer and Bradberry, 1996) examined the effect of 5-HT and 5-HT receptor ligands, applied locally via the dialysis probe, on DA

release. 5-HT increased DA release in the prefrontal cortex to a greater extent than in the striatum. Whereas the 5-HT_{2A} and 5-HT₃ receptor antagonists MDL 100,907 and MDL 72222, respectively, failed to attenuate the 5-HT-induced increase of DA release in the prefrontal cortex, the effect of 5-HT was abolished by the 5-HT_{1B/1D} receptor antagonist GR 127935. The selective 5-HT_{1B} receptor agonist CP-94253 mimicked the increasing effect of 5-HT in a manner sensitive to blockade by GR 127935. These data indicate that 5-HT_{1B} receptors mediate the facilitatory effect of 5-HT and CP-94253 on DA release. As a potential mechanism of action, the authors suggested that 5-HT receptor agonists increase DA release by decreasing release of a neurotransmitter such as GABA that inhibits DA release, (Fig. 2) (Gruen et al., 1992; Murai et al., 1994). In this context they refer to a report on 5-HT_{1B}-mediated inhibition of GABA release from corresponding interneurons (Johnson et al., 1992). Whereas all of these results are compatible with the operation of an inhibitory 5-HT_{1B} receptor on inhibitory striatal and prefrontal cortical interneurons, it should be noted that *in vitro* experiments revealed that, in addition to these interneuronal 5-HT_{1B} receptors, inhibitory presynaptic 5-HT_{1B} heteroreceptors are present on the dopaminergic nerve terminals of the striatum (section IV.D.2.).

In contrast to the results summarized in Table 6 that were discussed so far, it is difficult to explain the facilitatory effect of the 5-HT_{1B} receptor agonist CP-93129 on DA release in the dorsal striatum of 5-HT_{1B} knockout mice using *in vivo* microdialysis (De Groote et al., 2003) (Table 6). This result does not conform to a role of 5-HT_{1B} heteroreceptors on inhibitory interneurons (or on dopaminergic nerve terminals) in the dorsal striatum of mice. In the context of this facilitatory effect in 5-HT_{1B} knockout mice, it should be noted that in such mice, inhibitory presynaptic 5-HT_{1B} heteroreceptors on dopaminergic terminals in the striatum could be unequivocally demonstrated (section IV.D.2.) (Sarhan et al., 2000).

b. Concluding summary statement. In the rat striatum 5-HT_{1B} heteroreceptors mediate an indirect facilitation (disinhibition) of DA release (Table 11). As concluded from several reports, these receptors, which are coupled to the effector by G_{i/o} proteins (Table 1), may be assumed to be located on inhibitory GABAergic interneurons rather than on striatal dopaminergic projection terminals [the latter are endowed with presynaptic 5-HT_{1B} heteroreceptors mediating direct inhibition of DA release (section IV.D.2.)]. Indirect facilitation of striatal DA release is due to the inhibition of the release of the inhibitory transmitter GABA in response to activation of the 5-HT receptors on GABAergic neurons. In future experiments in which TTX is applied locally to the striatum *in vitro* and *in vivo*, the facilitation of DA release should be abolished, thus providing direct evidence for this indirect mechanism.

2. 5-Hydroxytryptamine 2A Receptors on Interneurons.

a. Function, location, and mechanism. The presence of release-modulating 5-HT_{2A} receptors in the rat medial prefrontal cortex has been postulated on the basis of the ability of systemically or locally applied MDL 100,907 to increase DA release (Schmidt and Fadayel, 1995) (Table 6). The failure of ritanserin to mimic this effect was explained by the claim that the dose injected was too low and that the selectivity of ritanserin for 5-HT_{2A} receptors is lower than that of MDL 100,907. This observation is certainly in agreement with the current knowledge that ritanserin has similar affinity for all 5-HT₂ receptor subtypes (Knight et al., 2004). The increasing effect of MDL 100,907 on DA release implies tonic inhibition of DA release by prefrontal 5-HT_{2A} receptors. The drug was without effect on DA efflux in the rat striatum. This may be of importance for the use of selective 5-HT_{2A} receptor antagonists in the treatment of schizophrenia. The 5-HT_{2A} receptors in the rat medial prefrontal cortex do not appear to be located on the dopaminergic nerve terminals but rather presynaptically and/or somadendrically on inhibitory GABAergic interneurons (Fig. 2). GABA released from these neurons may inhibit dopamine release by activating GABA_B receptors in the medial prefrontal area (Santiago et al., 1993). This suggestion is compatible with the coupling of 5-HT_{2A} receptors to G_q proteins and, accordingly, their excitatory rather than inhibitory effect. In addition, the authors supported this view by quoting *in vitro* electrophysiological studies, which revealed that 5-HT_{2A} receptors are involved in the activation of GABAergic interneurons in the piriform cortex (Sheldon and Aghajanian, 1991) and that MDL 100,907 reduces the 5-HT-induced activation of these inhibitory interneurons in cortical slices (Marek and Aghajanian, 1993). A major drawback of the study in the rat medial frontal cortex with MDL 100,907 (Schmidt and Fadayel, 1995) should not be disregarded, namely, that no attempt has been made to counteract the facilitatory effect of that 5-HT₂ receptor antagonist in interaction experiments with an appropriate 5-HT₂ receptor agonist. A 5-HT₂ receptor-mediated indirect effect may not only involve GABA interneurons but has also been demonstrated in rat and human hippocampus for substance P interneurons (Feuerstein et al., 1996). A substance P interneuron, however, would not explain the 5-HT₂ receptor-mediated inhibition because substance P neurons exert a facilitatory effect via NK₁ receptors.

The same concern as raised about the study of Schmidt and Fadayel (1995), *i.e.*, lack of interaction experiments with 5-HT₂ receptor agonists, holds for a microdialysis study in medial prefrontal cortex and anterolateral caudate-putamen of freely moving rats (Pehk et al., 1993). In this study the effect of *i.p.* amperozide, a 5-HT₂ receptor antagonist less selective than MDL 100,907, on DA release was examined. In addition

to its ability to antagonize 5-HT_{2A} receptors with moderate preference over 5-HT_{2C} receptors, this drug exhibits further pharmacological properties; thus, it possesses low affinity for dopamine D₂ receptors and it blocks DA uptake. Amperozide shared the property of MDL 100,907 to increase DA release in the prefrontal cortex. However, the increase did not occur exclusively in this brain area as in the case of MDL 100,907 (Schmidt and Fadayel, 1995), yet the potency in the prefrontal cortex was clearly higher than that in the striatum.

Several mechanisms may be responsible for the *in vivo* failure, or at least less pronounced ability, of 5-HT_{2A} receptor blockade to increase DA release in the striatum. 1) One of them implies that 5-HT_{2A} receptors mediating indirect tonic inhibition of DA release by endogenous 5-HT also occur in the striatum and/or in the substantia nigra at functionally relevant density. This inhibition may be counterbalanced by a second, not yet mentioned population of 5-HT_{2A} receptors identified in a microdialysis study with striatally infused 5-HT₂ ligands (Lucas and Spampinato, 2000). These receptors, which appear to be located as facilitatory presynaptic heteroreceptors on the dopaminergic nerve terminals themselves, modulate DA release only when dopaminergic nerve activity is enhanced (for more details, see section IV.D.3.). This seems not to be the case in the striatum, a hypothesis that is compatible with previous findings of others (Ichikawa and Meltzer, 1992, 1995; Schmidt et al., 1992; Gudelsky et al., 1994) that 5-HT_{2A} receptor agonists and antagonists modified striatal DA release only when it was enhanced by amphetamine or by MDMA. 2) An alternative explanation for the failure, or less pronounced ability, of 5-HT_{2A} receptor antagonists to increase striatal DA release may be that facilitatory 5-HT_{2A} heteroreceptors on GABAergic interneurons are not expressed or are expressed at lower density in the corpus striatum. In addition, one may speculate that for anatomical or functional reasons the GABAergic input to dopaminergic neurons is much more pronounced in the prefrontal cortex than in the striatum and/or that the 5-HT_{2A} receptors on the GABAergic interneurons in the striatum are tonically activated to a much less extent than in the medial prefrontal cortex.

b. Concluding summary statement. Application of 5-HT_{2A} receptor antagonists induced an indirect increase of DA release in the rat prefrontal cortex but not or less pronounced in the striatum. The 5-HT_{2A} receptors involved are probably located as release-increasing heteroreceptors on inhibitory GABAergic interneurons. Activation of these receptors inhibits DA release indirectly (Table 11). The preferential increase in DA release in the prefrontal cortex induced by 5-HT_{2A} receptor antagonists may be of importance for the use of such compounds against negative symptoms in schizophrenia. The mechanism described deserves further experimental support by agonist-antagonist interaction experiments.

3. 5-Hydroxytryptamine 2C Receptors on Interneurons.

a. Function, location, and classification. In vivo microdialysis of the nucleus accumbens and striatum of rats anesthetized with chloral hydrate was used to determine the contribution of another 5-HT₂ receptor subtype, the 5-HT_{2C} receptor, to the control of DA release from mesolimbic and mesocortical dopaminergic neurons (Di Giovanni et al., 1999). After the i.p. injection of the 5-HT_{2C/2B} receptor antagonist/inverse agonist SB 206553, basal DA release was enhanced in both the nucleus accumbens and the striatum, whereas the 5-HT_{2A} and the 5-HT_{2A/2B/2C} receptor antagonists SR 46349B and ritanserin, respectively, also administered systemically, were ineffective in this respect. The authors suggested that 5-HT_{2C/2B} receptors mediate a tonic inhibitory control on both the mesolimbic and the nigrostriatal dopaminergic pathways; in addition they stated that, although SB 206553 does not discriminate between 5-HT_{2C} and 5-HT_{2B} receptors, results reported in the literature allow ascribing of the serotonergic inhibitory control disclosed by this drug more specifically to the 5-HT_{2C} receptor. Thus, the levels of 5-HT_{2B} receptor mRNA and protein are low in these rat brain regions (Pompeiano et al., 1994; Flanigan et al., 1995; Duxon et al., 1997), and the selective 5-HT_{2B} receptor antagonist SB 204741 failed to influence the firing activity of dopaminergic neurons of the ventral tegmental area (VTA) or the accumbal DA release (Lejeune et al., 1997).

The suggestion of an inhibitory control of DA release via 5-HT_{2c} receptors was directly supported by the ability of the selective 5-HT_{2c} receptor agonist RO 60-0175 injected i.p. to decrease DA release in the rat nucleus accumbens in a manner sensitive to blockade by the selective 5-HT_{2c} receptor antagonist SB 242084 (Di Matteo et al., 2000a,b), whereas the selective 5-HT_{2B} receptor agonist BW 723C86 was without effect (Di Matteo et al., 2000a). Analogously, i.v. SB 242084 blocked the inhibitory effects of the nonselective 5-HT_{2c} receptor agonists mCPP and MK-212, injected i.v., on DA release in the rat nucleus accumbens (Di Giovanni et al., 2000). Furthermore, a recent investigation revealed that i.p. administration of the selective 5-HT_{2C} receptor antagonist SB 243213 induced an increase in DA release in the nucleus accumbens (Berg et al., 2006).

The data with SB 206553 and SB 243213 do not make it possible to identify the location of the 5-HT_{2C} receptors involved in the control of DA release, because the drugs were administered systemically. However, hints at the location of the receptors can be derived from results published by others. Basically, 5-HT_{2C} receptors were found in all brain regions that contain the cell bodies and terminals of the mesolimbic and nigrostriatal dopaminergic neurons (Molineaux et al., 1989; Mengod et al., 1990; Abramowski et al., 1995). However, 5-HT_{2C} receptor mRNA is expressed by GABAergic nerves, but not by DA neurons, within the substantia nigra and the

VTA (Eberle-Wang et al., 1997) and 5-HT excites substantia nigra pars reticulata neurons (presumably GABAergic) by stimulating 5-HT_{2C} receptors (Rick et al., 1995). These findings point to an indirect enhancing effect of SB 206553 on DA release resulting from the removal of the 5-HT-induced tonic inhibition on GABAergic neurons by 5-HT_{2C} receptor blockade (Fig. 2) (Di Giovanni et al., 1999) [for a schematic representation, see Di Matteo et al. (2001a)]. This hypothesis of a tonic excitation by 5-HT_{2C} receptor stimulation of GABAergic neurons by endogenous 5-HT is compatible with the G_q protein coupling of this receptor type to the phospholipase C pathway. Whether 5-HT_{2C} receptors in the nucleus accumbens and striatum might participate in the effect on DA release and, if so, to which extent they might contribute remains an open question. In the context of signal transduction, it should be kept in mind that the 5-HT_{2C} receptor can also couple to other pathways such as the phospholipase A₂ signaling cascade (Leysen, 2004) or to phospholipase D via G_{α13} (McGrew et al., 2002).

The problem of the location of the 5-HT_{2C} receptors was addressed (at least with respect to the putative presence of such receptors in the striatum) in an in vivo study on awake rats (Lucas and Spampinato, 2000); microdialysis was performed in the striatum and 5-HT₂ receptor ligands were administered via the dialysis probe. In contrast to the release-enhancing effect of systemic injection of the 5-HT_{2C/2B} receptor antagonist/inverse agonist SB 206553, local administration of this drug in the striatum induced an inhibition of release, suggesting that in the striatum DA release underlies a direct tonic facilitation via 5-HT_{2C} receptors (Lucas and Spampinato, 2000) which may be located on the dopaminergic nerve terminals themselves (for further details, see section IV.D.3.). Hence, the indirect tonic inhibition of DA activity via 5-HT_{2C} receptors, which are probably located on GABAergic interneurons, seems to be restricted to the area of dopaminergic cell bodies, i.e., the substantia nigra in this case. The indirect inhibitory control appears to be more efficient than the positive modulation exerted by the striatal 5-HT_{2C} receptors putatively located on the dopaminergic nerve endings. This conclusion can be drawn because systemic injection of SB 206553 leading to blockade of the 5-HT_{2C} receptors in both brain areas resulted in an enhancement of release (Di Giovanni et al., 1999).

The importance of the 5-HT_{2C} receptors in the VTA for basal and immobilization stress-induced DA release in the rat prefrontal cortex was examined by means of in vivo microdialysis in conscious rats (Pozzi et al., 2002). The 5-HT_{2C} receptor agonist RO 60-0175, injected i.p. or locally into the VTA, abolished the stress-induced, but not basal, DA release in the prefrontal cortex, whereas the drug applied locally to the prefrontal cortex via the dialysis probe was without effect on both basal and stress-induced DA release. The selective 5-HT_{2C} recep-

tor antagonist SB 242084, injected i.p., increased basal DA release and prevented the effect of i.p. or intravenotroglmental RO 60-0175 on the stress-induced increase of DA release; however, SB 242084 was without effect when given alone to stressed rats. It was concluded 1) that endogenous 5-HT tone on 5-HT_{2C} receptors in the VTA maintains low DA release in the prefrontal cortex under basal conditions, 2) that stimulation of these receptors with an exogenous agonist preferentially inhibits stress-induced DA release in the prefrontal cortex, and 3) that the inhibitory tone exerted by 5-HT_{2C} receptors on basal DA release might be reduced during stress.

It was claimed that 5-HT_{2C} receptors do not play a relevant role in the control of the nigrostriatal dopaminergic system (Di Matteo et al., 2001a,b). The authors emphasized that preferential disinhibition of mesocorticolimbic dopaminergic function by 5-HT_{2C} receptor antagonists resulting from a minor role in the nigrostriatal might be relevant for the use of these compounds in the treatment of the negative symptoms of schizophrenia. In support of their view, the authors pointed to two investigations in which the selective and potent 5-HT_{2C} receptor antagonist SB 242084 did not affect striatal DA release significantly (Di Giovanni et al., 1999; Gobert et al., 2000), and evidence was presented for the presence of direct facilitatory 5-HT_{2C} receptors in the rat striatum (Lucas and Spampinato, 2000) (section IV.D.) whose effect would be counteracted by the 5-HT_{2C} receptor antagonist under consideration. In contrast, arguments compiled in another review article (De Deurwaerdère and Spampinato, 2001) supported a relevant role of the 5-HT_{2C} receptors in the control of the nigrostriatal system: the magnitude of the increase in DA release induced by SB 206553 was, as a rule, similar in the striatum and the nucleus accumbens; furthermore it was argued that, according to several studies, systemic administration of SB 206553 and/or SB 242084 elicits a long-lasting increase in basal or stimulated DA release in the rat striatum. It was concluded that the tonic inhibitory control exerted by the 5-HT_{2C} receptors on the nigrostriatal pathway might be less pronounced, although by no means negligible, compared with the mesocortical pathway (De Deurwaerdère and Spampinato, 2001). A significant role of striatal inhibitory 5-HT_{2C} receptors was also found in a further *in vivo* microdialysis study (Alex et al., 2005): 1) local administration of the 5-HT_{2B/2C} inverse agonist SB 206553 via the dialysis probe into the striatum increased DA release; and 2) systemic administration of the preferential 5-HT_{2C} receptor agonist mCCP not only decreased striatal DA release but also attenuated the effect of SB 206553.

It is an interesting aspect of the 5-HT_{2C} receptors mediating an indirect inhibition of DA release that they exhibit constitutive activity both in the nucleus accumbens and in the striatum *in vivo* (De Deurwaerdère et al., 2004; Berg et al., 2005). Evidence for this result was presented by a microdialysis study in both brain regions

of rats systemically injected with SB 206553 (inverse agonist at 5-HT_{2C/2B} receptors), SB 242084 (a neutral 5-HT_{2C} receptor antagonist), and/or RO 60-175 (a 5-HT_{2C} receptor agonist). SB 206553 and SB 242084 elicited increases of DA release in both brain regions, with the increase by SB 206553 being much more pronounced than that of SB 242084, whereas RO 60-175 inhibited DA release in both brain areas. In interaction experiments, SB 242084 counteracted not only the inhibitory effect of RO 60-175 but also the SB 206553-induced increase of release in excess of that caused by SB 242084 itself. An essential finding in the context of the above controversy was that the nigrostriatal and mesoaccumbal pathways did not substantially differ in their sensitivity to the administration of 5-HT_{2C} ligands.

Important therapeutic perspectives are brought up by the different behavior of the inverse agonist compared with the neutral antagonist (De Deurwaerdère et al., 2004); thus, the inverse agonistic activity of certain atypical antipsychotic drugs such as clozapine and olanzapine at constitutively active 5-HT_{2C} receptors may contribute to their clinical superiority (Herrick-Davis et al., 2000; Rauser et al., 2001). Inverse 5-HT₂ receptor agonists may exert selective therapeutic action due to their response, cell phenotype, and state-dependent activity (Berg et al., 2005). The ability of clozapine compared with haloperidol to alter the constitutive activity of 5-HT₂ receptors has recently been investigated using *in vivo* microdialysis in halothane-anesthetized rats (Navailles et al., 2006). Both antipsychotic drugs injected systemically increased DA release in the nucleus accumbens and in the striatum. The effect of clozapine was blocked by the 5-HT_{2C} receptor antagonist SB 242084 (which did not modify the response to haloperidol) but was not affected by the 5-HT_{2C} inverse agonist SB 206553 (which potentiated the haloperidol-induced increase in release). These findings are compatible with the suggestion that the atypical antipsychotic drug clozapine (but not haloperidol) behaves as a 5-HT_{2C} inverse agonist *in vivo*.

A further therapeutic perspective, namely the potential antiaddictive property of RO 60-0175 (Grottick et al., 2001), led Di Matteo et al. (2004) to test the hypothesis that activation of the 5-HT_{2C} receptor might block the stimulatory action of nicotine on accumbal DA release. In fact, they found in a microdialysis study in freely moving rats that i.p. RO 60-0175 reduced the stimulation of DA release in the nucleus accumbens in rats treated repeatedly (for 10 consecutive days) with i.p. injections of this alkaloid.

b. Concluding summary statement. 5-HT_{2C} receptors mediate an indirect tonic inhibitory control on the DA release in both the nucleus accumbens and the striatum (Table 11). As a consequence, selective and nonselective 5-HT_{2C} receptor antagonists or inverse agonists increase DA release. Evidence for the location of the 5-HT_{2C} receptors on GABAergic interneurons is much

stronger than that in the case of 5-HT_{1B} and 5-HT_{2A} receptors indirectly modulating DA release; 5-HT_{2C} mRNA is expressed in GABAergic but not dopaminergic neurons. This indirect control via interneurons seems to affect DA neurons, predominantly in the VTA and substantia nigra where the cell bodies are located.

5-HT_{2C} receptors on the interneurons in the VTA are of high importance for the control of basal and stress-induced DA release in the prefrontal cortex: the endogenous 5-HT tone on 5-HT_{2C} receptors in the VTA maintains low DA release in the prefrontal cortex under basal conditions. Stimulation of these receptors with an exogenous agonist preferentially inhibits stress-induced DA release in the prefrontal cortex.

The 5-HT_{2C} receptors mediating an indirect inhibition of DA release are constitutively active. Accordingly, inverse agonists produce a more pronounced increase in basal DA release than pure 5-HT receptor antagonists. The inverse agonistic property of certain atypical antipsychotics such as clozapine and olanzapine may contribute to their advantageous clinical effects. It is a matter of discussion whether 5-HT_{2C} receptors are less important in the control of the nigrostriatal than in that of the mesocortical dopaminergic pathway. If so, this finding would be relevant for the treatment of negative symptoms of schizophrenia. 5-HT_{2C} receptor agonists exhibit antiaddictive properties in patients with nicotine dependence.

D. 5-Hydroxytryptamine 1B and 5-Hydroxytryptamine 2 Receptors Identified in Vitro or in Vivo

1. *Presynaptic 5-Hydroxytryptamine 1 and/or 5-Hydroxytryptamine 2 Receptor-Mediated Modulation?* Table 7 summarizes details of an influential study (Ennis et al., 1981) that provided the first convincing evidence that inhibitory presynaptic 5-HT heteroreceptors are involved in the modulation of DA release. 5-HT and other 5-HT receptor agonists reduced the K⁺-evoked, Ca²⁺-dependent release of [³H]DA in superfused rat striatal slices, an effect that was counteracted by various 5-HT receptor antagonists. The inhibitory effect of 5-methoxytryptamine was not affected by the presence of tetrodotoxin in the superfusion fluid, indicating that the modulatory 5-HT receptors are located on the dopaminergic axon terminals themselves. A 5-HT-induced inhibitory effect of 5-HT on depolarization-evoked [³H]dopamine release sensitive to blockade by the 5-HT receptor antagonist methysergide was also found in rat striatal slices in which release was induced by electrical field stimulation (Westfall and Tittermary, 1982) (Table 7). In these experiments, the superfusion buffer contained the DA uptake inhibitor bentspiron to avoid carrier-mediated [³H]DA efflux (section IV.B.). The results obtained by these authors confirmed that the dopaminergic nerve terminals are endowed with presynaptic 5-HT heteroreceptors. Basically the same conclusion can be drawn from experiments on rat nucleus accumbens synaptosomes, which suggested that in this area also pre-

synaptic 5-HT heteroreceptors on dopaminergic nerve endings inhibit both DA release and synaptosomal tyrosine hydroxylase (Hetey and Drescher, 1986; Hetey, 1988), a suggestion that was based on experiments with two non-selective ligands, 5-HT and methiothepine, only. Accordingly, the receptor type involved was left open, but we may assume that it does not differ from that in the rat striatum.

Apart from the application of relatively few subtype-selective 5-HT receptor ligands, classification of release-modulating 5-HT receptors can be achieved by the determination of the potency of a relatively large number of nonselective 5-HT receptor agonists and antagonists and by relating their potency to their affinity for the various 5-HT receptor subtypes determined in radioligand binding experiments. The potency of the receptor antagonists applied by Ennis et al. (1981) in the rat striatum correlated with their potency in inhibiting [³H]5-HT binding to whole rat brain homogenates. The nonselective character of the radioligand [³H]5-HT at the various 5-HT receptors does not allow us to draw a reliable conclusion concerning the receptor type involved. However, the potencies of agonists and antagonists at the inhibitory presynaptic 5-HT heteroreceptors on the dopaminergic nerve terminals are basically compatible with the involvement of 5-HT₁ and 5-HT₂ receptors. In view of the results of the more recent experiments listed in Table 7 and discussed in the following sections, one may assume that they identified either 5-HT_{1B} or certain 5-HT₂ receptor subtypes or a mixture of them in the rat striatum and nucleus accumbens. In other words, for a clear-cut classification beyond the basic evidence for the operation of inhibitory presynaptic 5-HT heteroreceptors in the striatum reported so far, the experiments with the 5-HT subtype-selective ligands described in the following two sections were urgently needed.

2. Presynaptic 5-Hydroxytryptamine 1B Receptors.

a. Function, site of action, and classification. The operation of inhibitory presynaptic 5-HT_{1B} heteroreceptors on dopaminergic axon terminals (Fig. 1) of the rat and mouse striatum has been demonstrated in a series of investigations by the same group who investigated incubated striatal synaptosomes (Sarhan et al., 1999, 2000; Sarhan and Fillion, 1999) (Table 7). In all of these studies, the 5-HT_{1B} receptor agonist CP-93129 inhibited the K⁺-evoked [³H]DA release from rat striatal synaptosomes in a manner sensitive to blockade by the selective 5-HT_{1B} receptor antagonist SB 224289, indicating that inhibitory 5-HT_{1B} receptors mediate this effect and that the latter are probably located on the dopaminergic nerve terminals themselves. Thus, in one carefully planned type of experiment, the function, classification, and location of the receptor could be established. The only point of criticism that might be raised refers to the fact that the synaptosomes were not superfused but that sus-

TABLE 7
Studies in which the involvement of 5-HT₁ or 5-HT₂ heteroreceptors in modulation of DA release has been investigated in vitro
 Effects of 5-HT₁ or 5-HT₂ receptor ligands on DA release in the rat corpus striatum are shown.

| Species | Brain Region | Receptor Type ^a | Drugs | Release-Inhibiting Concentrations or pA ₁₀ of Antagonists | Comment | References |
|---------------------------------------|--------------|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Rat | Striatum | 5-HT | 5-HT 5-MeOT 5-MeO-N,N-dimethyltryptamine Methysergide Metergoline Methiothepine Cinanserin Cyproheptadine Mianserin 5-HT | pIC ₂₅ = 6.09 pIC ₂₅ = 6.45 pIC ₂₅ = 6.45 pA ₁₀ = 8.26 ² pA ₁₀ = 7.06 ² pA ₁₀ = 6.95 ² pA ₁₀ = 6.80 ² pA ₁₀ = 6.13 ² pA ₁₀ = 6.00 ² 10 ⁻⁶ -10 ⁻⁵ M | Superfusion of slices; K ⁺ (25 mM)-evoked [³ H]DA release; pA ₁₀ values for antagonism of 5-MeOT-induced inhibition ^b ; inhibitory effect of 5-MeOT unaffected by TTX (0.1 μM) in the superfusion buffer | Ennis et al. (1981) |
| Rat | Striatum | 5-HT | 5-HT | 3 × 10 ⁻⁷ -10 ⁻⁶ M | Superfusion of slices, electrically induced release of [³ H]DA (5 Hz); superfusion in the presence of benzotropine (10 ⁻⁶ M); reversal of 5-HT-induced inhibition by methysergide (1.7 × 10 ⁻⁵ M) | Westfall and Tittermary (1982) |
| Rat | Striatum | 5-HT ₂ | 5-HT | 10 ⁻⁷ -10 ⁻³ M 10 ⁻⁶ -10 ⁻³ M | Superfusion of slices; K ⁺ (25 mM)-evoked [³ H]DA release in vitro; inhibition (also in the presence of 0.1 μM TTX) reversed by miniprine (10 ⁻⁵ M), ketanserin (10 ⁻⁶ M), or mianserin (10 ⁻⁵ M) | Muramatsu et al. (1988b) |
| Rat | Striatum | 5-HT _{1B} | 5-HT CP-93,129 | 10 ⁻⁷ -10 ⁻³ M 10 ⁻⁶ -10 ⁻³ M | Incubation of synaptosomes; K ⁺ (20 mM)-induced [³ H]DA release inhibited by 5-HT and by the 5-HT _{1B} agonist CP-93129; sumatriptan had no effect; the selective 5-HT _{1B} receptor antagonist SB 224289 counteracted the inhibitory effects of 5-HT and CP-93129 | Sarhan and Fillion (1999) |
| Rat | Striatum | 5-HT _{1B} | CP-93,129 | 10 ⁻⁸ -10 ⁻⁴ M | Incubation of synaptosomes; K ⁺ (20 mM)-induced [³ H]DA release inhibited by 5-HT _{1B} agonist CP-93129; the selective 5-HT _{1B} receptor antagonist SB 224289 abolished inhibitory effect of CP-93129 with K _i = 1.8 × 10 ⁻⁹ M. | Sarhan et al. (1999) |
| Rat | Striatum | 5-HT _{1B} | CP-93,129 | 10 ⁻⁷ -10 ⁻³ M | Incubation of synaptosomes; K ⁺ (20 mM)-induced [³ H]DA release inhibited by 5-HT _{1B} agonist CP-93129; SB 224289 abolished inhibitory effect of CP-93129; preincubation with a specific 5-HT _{1B} receptor antibody or with 5-HT moduline (tetrapeptide LSAL) reduced the inhibitory effect of CP-93129 in a noncompetitive manner | Sarhan et al. (2000) |
| Mouse and 5-HT _{1B} ko mouse | Striatum | 5-HT _{1B} | CP-93,129 5-CT | 10 ⁻⁷ -10 ⁻⁵ M 10 ⁻⁷ -10 ⁻⁵ M | Incubation of synaptosomes; K ⁺ (20 mM)-induced [³ H]DA release studied; the selective 5-HT _{1B} agonist CP-93129 caused inhibition of [³ H]DA release in wild-type but had no effect in 5-HT _{1B} ko mouse; the 5-HT _{1B/7} agonist sumatriptan had no effect in wild-type or in 5-HT _{1B} ko mice; the selective 5-HT _{1B} antagonist SB 224289 abolished inhibitory effect of CP-93129 in wild-type mice | Sarhan et al. (2000) |

5-MeOT, 5-methoxytryptamine (5-HT₄ agonist); 5-MeO-N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine (5-HT_{1A} agonist); ko, knockout.

^a Receptor type involved, as suggested by the authors.

^b pA₁₀, negative logarithm of the molar concentration of antagonist that increased the control IC₂₅ of the agonist by a factor of 10.

pensions of the synaptosomes were used. Theoretically, this procedure may enable the individual synaptosomes to communicate with each other. Yet the location of the 5-HT_{1B} receptors on nondopaminergic synaptosomes which, in turn, influence the dopaminergic synaptosomes is unlikely because of the relatively long diffusion distance between the individual synaptosomes.

The classification of the presynaptic 5-HT heteroreceptors on dopaminergic nerve terminals as 5-HT_{1B} was supported by several lines of evidence. The inhibitory effect of CP-93129 was not mimicked by selective 5-HT_{1A}, 5-HT₂, or 5-HT₃ receptor agonists or antagonized by 5-HT₂ or 5-HT₃ receptor antagonists, but it was blocked by various selective and nonselective 5-HT_{1B} receptor antagonists (Sarhan et al., 1999) (Table 7). Furthermore, the CP-93129-induced inhibition was reduced 1) by pretreatment with a specific polyclonal 5-HT_{1B} receptor antibody directed against a region of the receptor protein involved in signal transduction and 2) by pretreatment with 5-HT moduline, a tetrapeptide with the amino acid sequence LSAL (Sarhan et al., 2000). This endogenous peptide was shown to be a selective noncompetitive 5-HT_{1B/1D} antagonist, probably acting as an allosteric modulator of the 5-HT_{1B} receptor protein (Massot et al., 1996; Rousselle et al., 1998). It is also compatible with the 5-HT_{1B} character of the presynaptic 5-HT heteroreceptor that G_i/G_o proteins appear to be involved in signal transduction, as suggested by the attenuation of the inhibitory effects of 5-HT_{1B} receptor agonists by pertussis toxin (Sarhan and Fillion, 1999).

Inhibitory presynaptic 5-HT_{1B} receptors on dopaminergic nerve terminals have also been shown in the mouse striatum by application of the same elegant approach as in the rat striatum: the K⁺-evoked [³H]DA release was inhibited by CP-93129 from mouse striatal synaptosomes and this effect was sensitive to blockade by SB 224289 (Sarhan et al., 2000). Unequivocal evidence for the involvement of the 5-HT_{1B} receptor subtype was provided by experiments on knockout mice, which revealed that the inhibitory effects of CP-93129 and 5-CT in wild-type mice were absent in synaptosomes from 5-HT_{1B} receptor knockout mice (Sarhan et al., 2000).

Comparison of the sensitivity of the 5-HT_{1B} heteroreceptors on dopaminergic nerve terminals and of the 5-HT_{1B} autoreceptors in rat striatum revealed that the potencies of 5-HT and CP93129 to inhibit the K⁺-evoked [³H]DA release were 400 and 88 times lower, respectively, than their potencies to inhibit K⁺-evoked [³H]5-HT release from synaptosomes prepared from the same brain region (Sarhan and Fillion, 1999) (Table 7). As possible explanations the authors suggested that these differences may originate from a different proportion of the receptors coupled to the G_i/G_o protein or that 5-HT_{1B} auto- and heteroreceptors may be coupled to different effectors.

b. Concluding summary statement. DA release in the striatum is directly modulated by inhibitory presynaptic 5-HT_{1B} heteroreceptors (Table 11). Thus, when one is considering the additional indirect facilitation of DA release in which presumably GABAergic interneurons are involved (section IV.C.1.), striatal DA release is under tight complementary dual 5-HT_{1B} receptor-mediated control mechanisms.

3. Presynaptic 5-Hydroxytryptamine 2A and 5-Hydroxytryptamine 2C Receptors?

a. Function, site of action, and classification. In vitro experiments on superfused slices from the rat striatum revealed that 5-HT reduces the K⁺-evoked [³H]DA release (Muramatsu et al., 1988b) (Table 7). This effect is mediated by 5-HT₂ receptors as it was abolished by ketanserin at a concentration that inhibits all 5-HT₂ receptor subtypes (Knight et al., 2004) and by the nonselective 5-HT₂ receptor antagonist mianserin. The inhibitory action was also observed in slices superfused with buffer containing tetrodotoxin, suggesting that 5-HT activates presynaptic 5-HT₂ receptors on the dopaminergic axon terminals themselves. This conclusion is supported by an in vivo microdialysis investigation in conscious and freely moving rats in which DA release was found to be modulated by inhibitory 5-HT₂ receptors, as suggested by direct administration of DOI and ketanserin into the striatum via the dialysis probe (Ng et al., 1999); because 6-hydroxydopamine lesioning of the nigrostriatal DA pathway led to a selective decrease in 5-HT₂ receptors in the striatum, the authors concluded that there are 5-HT₂ receptors on the dopaminergic nerve terminals of this brain area.

An inhibitory function mediated by 5-HT₂ receptors is an unexpected finding because, as a rule, activation of a G_q protein-coupled receptor should stimulate phospholipase C, leading to increased intraneuronal Ca²⁺ due to the formation of inositol-1,4,5-trisphosphate; this, in turn, should result in a facilitation rather than an inhibition of transmitter release. A hint at the inhibitory mechanism that may be involved in the reduction of DA release mediated by presynaptic 5-HT₂ heteroreceptors comes from experiments with minaprine. This drug also abolished the 5-HT-induced inhibition of release. It is a compound with atypical antidepressant properties, and it exhibits antagonistic characteristics at both 5-HT receptors and voltage-dependent K⁺ channels (Muramatsu et al., 1990b). In analogy to the mode of action proposed for the presynaptic 5-HT₂ heteroreceptor-mediated inhibition of ACh release, it is conceivable that the 5-HT₂ receptors involved in presynaptic inhibition of DA release are coupled via an unknown mechanism to voltage-dependent K⁺ channels [for details of the results of Muramatsu et al. (1990a) underlying this hypothesis, see section III.D.].

Whereas, thus, the in vitro results suggested the presence of inhibitory presynaptic (not subclassified) 5-HT₂ receptors on the dopaminergic nerve terminals or of

5-HT₂ receptors on an inhibitory interneuron, an *in vivo* microdialysis investigation in freely moving rats led to the identification of directly DA release-increasing 5-HT_{2A} and 5-HT_{2C} receptors in the striatum (Lucas and Spampinato, 2000). The microdialysis probe was inserted in the striatum and 5-HT₂ receptor ligands could, thus, be administered locally into this brain area via the probe. Both basal and haloperidol-stimulated (*s.c.* injection) outflow of DA and 3,4-dihydroxyphenylacetic acid were determined; a change in extracellular 3,4-dihydroxyphenylacetic acid reflects a change in intraneuronal DA synthesis (Zetterström et al., 1988). Basal DA release was not affected by the 5-HT_{2A} receptor antagonist SR 46349B, but this compound as well as the 5-HT_{2A/2B/2C} receptor antagonist ritanserin reduced the haloperidol-stimulated DA release. It was concluded that striatal 5-HT_{2A} receptors, putatively through activation of DA synthesis, positively modulate DA release only under activated conditions.

In contrast to the failure of SR 46349B to affect basal DA release, the latter was reduced by the 5-HT_{2C} receptor antagonist SR 206553 and enhanced by the 5-HT_{2A/2B/2C} receptor agonist DOI, whose effect was abolished by SB 206553 but not by SR 46349B. Haloperidol-stimulated release was not affected by SB 206553. The authors concluded that striatal 5-HT_{2C} receptors exert a tonic and phasic facilitatory control of basal DA release.

b. Concluding summary statement. DA release has been suggested to be modulated by inhibitory presynaptic 5-HT receptors belonging to the 5-HT₂ receptor family (Table 11). Only nonselective 5-HT₂ receptor ligands were applied, which excludes subclassification. The assumption of a presynaptic location of the inhibitory 5-HT₂ receptor was based mainly on the ability of 5-HT to modulate K⁺-evoked DA release in rat striatal slices in the presence of tetrodotoxin; however, a G_q-coupled receptor would, as a rule, be expected to induce a stimulation or facilitation of release instead of an inhibition. Therefore, the possibility should not be rejected that the 5-HT₂ receptors involved may be located on an inhibitory interneuron. To clarify the open questions, experiments on synaptosomes with selective 5-HT_{2A} and 5-HT_{2C} receptor agonists and antagonists should be performed.

In vivo experiments in the striatum revealed that 5-HT_{2A} and 5-HT_{2C} receptors mediate an increase in DA release, the former putatively by stimulation of DA synthesis under activated conditions of the dopaminergic nerves (Table 11). The data leading to the identification of the 5-HT_{2A} and 5-HT_{2C} receptors do not allow identification of the exact location of the receptors involved. It is tempting to postulate that they represent presynaptic facilitatory receptors on dopaminergic nerve terminals; however, they may also be located on a neighboring neuron, releasing a facilitatory transmitter which, in turn, increases DA release. Alternatively, they may also

be a component of a larger neuronal circuit. Repetition of key experiments after local application of the drugs *in vivo* and *in vitro* might help to identify the location of the receptors.

E. 5-Hydroxytryptamine 3 Receptors

1. Function, Location, and Classification. Stimulatory or facilitatory presynaptic 5-HT₃ receptors occur on dopaminergic axon terminals of the projection areas of the mesolimbic/mesocortical dopaminergic system (Fig. 1). In the striatum only one study revealed a 5-HT₃ receptor-mediated stimulation of DA release, whereas in all other investigations no such effect was observed.

The study yielding the exceptional result was performed in rat striatal slices superfused in the presence of nomifensine. 5-HT and 2-methyl-5-HT stimulated the release of endogenous DA in a manner sensitive to competitive blockade by the 5-HT₃ receptor antagonist tropisetron (Blandina et al., 1988, 1989). The major part of the effect of 5-HT persisted in the presence of tetrodotoxin (Blandina et al., 1989). These results are compatible with the operation of 5-HT₃ receptors stimulating DA release because of their membrane-depolarizing, Ca²⁺ influx-inducing property; their location on the dopaminergic axon terminals was considered as most plausible. As an alternative, the authors admit that these receptors may also occur on neighboring nerve terminals that mediate tetrodotoxin-insensitive release of another neurotransmitter such as glutamate, which in turn may stimulate DA release (Blandina et al., 1989). 5-HT and 2-methyl-5-HT also increased the K⁺-evoked [³H]DA release. More comprehensive results in favor of the operation of facilitatory 5-HT₃ receptors, were obtained in the rat olfactory tubercle, a terminal field of the mesolimbic dopaminergic system. These 5-HT₃ receptors are probably located at least in part on the dopaminergic nerve terminals (Zazpe et al., 1994). On slices of this brain region superfused without nomifensine, 5-HT, 2-methyl-5-HT, and phenylbiguanide increased the K⁺-evoked, Ca²⁺-dependent, and largely tetrodotoxin-insensitive release of [³H]DA, an effect that was blocked by 5-HT₃ receptor antagonists (Table 8).

Both in the nucleus accumbens and in the medial prefrontal cortex, *in vivo* administration of a 5-HT₃ receptor agonist either intracerebroventricularly or via the dialysis probe increased DA release in a manner sensitive to blockade by 5-HT₃ receptor antagonists (Jiang et al., 1990; Chen et al., 1992) (Table 8), whereas 5-HT_{1A} and 5-HT₂ receptor agonists were ineffective. Both groups of authors assumed that these effects were mediated by presynaptic 5-HT₃ receptors on the dopaminergic nerve terminals, although the conditions in both studies are not appropriate to determine the site of action of the drugs. However, on the basis of the *in vitro* experiments, the assumption of the presence of the 5-HT₃ receptors on dopaminergic nerve terminals

TABLE 8
Studies in which the involvement of 5-HT₃ or 5-HT₄ heteroreceptors in modulation of DA release has been investigated
 Effects of 5-HT₃ or 5-HT₄ receptor ligands on DA release in various brain regions of rat and mouse are shown.

| Species | Brain Region | Receptor Type ^a | Drugs | Release-Modifying Concentration | Comment | References |
|---------|--------------------------|----------------------------|---------------------------------|-----------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| Rat | Striatum | 5-HT ₃ | 2-CH ₃ -5-HT 5-HT | 3 × 10 ⁻⁶ -10 ⁻⁵ M: stimulation 10 ⁻⁶ -6 × 10 ⁻⁶ M: stimulation | Superfusion of slices in the presence of tyrosine (5 × 10 ⁻⁵ M), nomifensine (10 ⁻⁵ M), and pargyline (10 ⁻⁵ M); spontaneous endogenous DA release determined; ICS 205,930 (4-9 × 10 ⁻⁹ M) reduced the stimulatory effect of 5-HT | Blandina et al. (1988) |
| Rat | Striatum | 5-HT ₃ | 2-CH ₃ -5-HT | 3 × 10 ⁻⁶ -5 × 10 ⁻⁵ M: stimulation | Superfusion of slices in the presence of nomifensine (10 ⁻⁵ M) and pargyline (10 ⁻⁵ M); spontaneous endogenous DA release determined; concentration-response curves for 5-HT and 2-CH ₃ -5-HT shifted to the right by ICS 205,930 (4 × 10 ⁻¹⁰ M); 5-HT-stimulated DA release was Ca ²⁺ -dependent and to a major extent TTX-insensitive; DOI or 5-CONH ₂ -T had no stimulatory effect; methysergide and methiothepine did not reduce the stimulatory effect of 5-HT K ⁺ (20 mM)-evoked DA release | Blandina et al. (1989) |
| Rat | Nucleus accumbens | 5-HT ₃ | 2-CH ₃ -5-HT i.c.v. | 10 ⁻⁷ -10 ⁻⁵ M: stimulation 5 × 10 ⁻⁵ M: increase 10 ⁻⁵ M: increase | In vivo chronocoulometric ^b recording in chloral hydrate-anesthetized rats; drugs applied i.c.v.; facilitatory effect of 2-CH ₃ -5-HT (1.5 × 10 ⁻⁹ M) blocked by granisetron (10 ⁻⁹ M), but not by metergoline (10 ⁻⁹ M); 8-OH-DPAT and DOI were ineffective; 2-CH ₃ -5-HT effect was abolished after 6-OHDA injection into medial forebrain bundle | Jiang et al. (1990) |
| Rat | Medial prefrontal cortex | 5-HT ₃ | PBG | 10 ⁻³ M: increase | In vivo microdialysis in freely moving rats; endogenous DA level determined; zacopride (10 ⁻³ M) in the perfusion solution abolished the effect of PBG | Chen et al. (1992) |
| Rat | Olfactory tubercle | 5-HT ₃ | 5-HT 2-CH ₃ -5-HT | 10 ⁻⁶ -10 ⁻⁴ M: increase 10 ⁻⁶ -10 ⁻⁵ M: increase | Superfusion of slices preincubated with [³ H]DA; increase in K ⁺ (20 mM) evoked [³ H]DA release determined; effects were almost completely TTX-insensitive; ondansetron (5 × 10 ⁻⁶ -2 × 10 ⁻⁶ M), granisetron, or tropisetron (2 × 10 ⁻⁷ M) blocked 5-HT ₃ receptor agonist effects | Zazpe et al. (1994) |
| Rat | Nucleus accumbens | 5-HT ₃ | PBG | 10 ⁻⁶ -10 ⁻⁴ M: increase | In vivo microdialysis in halothane-anesthetized rats; endogenous DA levels determined; ondansetron and (S)-zacopride reduced DA release induced by electrical stimulation of the dorsal raphe nucleus in the nucleus accumbens but not in the striatum, | Deurwaerdère et al. (1998) |
| Rat | Nucleus accumbens | 5-HT ₃ | Ondansetron (S)-Zacopride | 0.1 mg/kg s.c.: decrease 0.3 mg/kg s.c.: decrease | In vivo microdialysis in freely moving rats; endogenous DA efflux induced by morphine (1-10 mg/kg s.c.) determined | Deurwaerdère et al. (2005) |
| Rat | Striatum | 5-HT ₄ | MDL 72222 5-HT | 10 ⁻⁶ -5 × 10 ⁻⁶ M: stimulation | In vivo microdialysis in halothane-anesthetized rats; drugs applied via the dialysis probe; spontaneous DA release determined; DAU 6285 inhibited 5-HT ₄ - and BIMU 8-induced DA release; ondansetron without effect | Bonhomme et al. (1995) |
| Rat | Striatum | 5-HT ₄ | BIMU 8 5-HT 5-MeOT | 10 ⁻⁴ M: stimulation 10 ⁻⁵ M: increase 10 ⁻⁵ M: increase | Superfusion of slices, determination of endogenous DA release by HPLC; DA release induced by 5-HT ₄ receptor agonists; the selective 5-HT ₄ receptor antagonist GR 113808 (10 ⁻⁷ M) abolished the stimulatory effect of the 5-HT ₄ receptor agonist renzapride; in vivo microdialysis in freely moving rats GR 113808 (10 ⁻⁶ M) abolished the stimulatory effect of the 5-HT ₄ receptor agonist renzapride (10 ⁻⁴ M) or 5-MeOT (10 ⁻⁵ M); drugs were administered through the dialysis probe | Steward et al. (1996) |
| Rat | Striatum | 5-HT ₄ | Renzapride (S)-Zacopride | 10 ⁻⁵ M: increase 10 ⁻⁵ M: increase | Superfusion of synaptosomes in the presence of L-[3,5- ³ H]tyrosine; drug-induced release of newly synthesized [³ H]DA studied | Deurwaerdère et al. (1997) |
| Rat | Striatum | 5-HT ₄ | 5-HT 5-MeOT 5-HT | 10 ⁻⁶ -10 ⁻⁵ M: increase 10 ⁻⁵ M: increase 10 ⁻⁶ M: increase | Superfusion of synaptosomes preincubated with [³ H]DA; drug-induced release of [³ H]DA studied | |

2-CH₃-5-HT, 2-methyl-5-hydroxytryptamine (5-HT₃ agonist); 5-CONH₂-T, 5-carboxamidoxytryptamine (5-HT₁ agonist), i.c.v., intracerebroventricular; 6-OHDA, 6-hydroxydopamine (dopaminergic neurotoxin); PBG, phenylbiguanide; HPLC, high-pressure liquid chromatography; 5-MeOT, 5-methoxytryptamine (5-HT₄ agonist).

^a Receptor type involved, as suggested by the authors.

^b Chronocoulometry is a modification of chronoamperometry in which the charge is determined instead of the oxidative current of DA using a Nafion-coated carbon fiber microelectrode implanted in the nucleus accumbens vs. a reference electrode placed on the surface of the frontal cortex.

(among putatively additional locations) (Fig. 1) appears to be justified.

An investigation (De Deurwaerdère et al., 1998) (Table 8) was devoted to the question whether endogenous 5-HT via 5-HT₃ receptors exerts a facilitatory control selectively in either the mesoaccumbal or the nigrostriatal dopaminergic pathway. Microdialysis in the nucleus accumbens and striatum of halothane-anesthetized rats was combined with dorsal raphe nucleus (DRN) electrical stimulation. This stimulation induced an increase of DA release in the nucleus accumbens, but a reduction of DA release in the striatum. Subcutaneous injection of the 5-HT₃ receptor antagonists ondansetron and (S)-zacopride reduced the increase in accumbal DA release evoked by DRN stimulation but did not modify the stimulation-induced inhibition of striatal DA release.

In this context, the authors also mentioned that, with the exception of two studies (Blandina et al., 1988, 1989) as mentioned earlier. This statement conforms to our own attempt to reproduce the data of Blandina et al. in experiments on superfused striatal slices preincubated with [³H]DA: we failed to obtain a stimulatory effect of 5-HT₃ receptor agonists on [³H]DA release (K. B. Fink). In view of the multiple futile attempts to reproduce the results by Blandina et al., it is tempting to postulate that they measured DA release in striatal slices which, to a major extent, were composed of the nucleus accumbens.

An even closer determination of the conditions underlying the operation of 5-HT₃ receptors in the nucleus accumbens, using in vivo microdialysis of this brain area (De Deurwaerdère et al., 2005), was based on the hypothesis that the control of accumbal DA release by these receptors is not only dependent on increased tone of the serotonergic neurons but also requires an additional specific permissive factor to operate. In freely moving rats, pretreatment with a 5-HT₃ receptor antagonist (ondansetron or MDL 72222 s.c.) reduced accumbal DA release evoked by 1 or 10 mg/kg morphine (s.c.). Administration of ondansetron into the nucleus accumbens via the dialysis probe of freely moving rats decreased DA release only when it was strongly stimulated by s.c. injection of 10 mg/kg morphine but not when a weaker stimulus, i.e., injection of 1 mg/kg morphine, was applied. In halothane-anesthetized rats, intra-accumbal administration of ondansetron reduced the DA release evoked by DRN electrical stimulation. The authors suggested that 5-HT₃ receptors in the nucleus accumbens play a role in DA release only when central dopaminergic and serotonergic tones are concomitantly increased. Concerning the location of the 5-HT₃ receptors, the data obtained in the experiments with intra-accumbal ondansetron only allow the conclusion that they occur in the nucleus accumbens itself.

2. Concluding Summary Statement. Activation of 5-HT₃ receptors increases DA release in the nucleus accumbens and the medial prefrontal cortex. Because of

their direct excitatory property as ligand-gated ion channels, a plausible interpretation of in vitro and in vivo results is that these receptors are expressed as presynaptic 5-HT₃ heteroreceptors on the dopaminergic nerve terminals in the brain regions mentioned (Table 11). A transsynaptic effect involving an interneuron releasing an excitatory transmitter such as glutamate has been suggested as an alternative possibility. It is probable that both mechanisms are responsible for the facilitation of DA release. According to elegant, more advanced studies, endogenous 5-HT exerts its facilitatory control of DA release selectively in the mesoaccumbal and probably mesocortical dopaminergic pathway, leaving the nigrostriatal pathway unaffected. Evidence was presented that, as a prerequisite for a relevant modulation of DA release in vivo, dopaminergic and serotonergic tones have to be concomitantly increased.

F. 5-Hydroxytryptamine 4 Receptors

1. Function, Location, and Classification. An early hint at the operation of DA release-increasing 5-HT₄ receptors in the rat striatum was provided by Benloucif et al. (1993) in their microdialysis study on chloral hydrate-anesthetized rats. They found that 5-methoxytryptamine, a nonselective 5-HT₄ receptor agonist, enhanced dopamine release, an effect that was attenuated by tropisetron at a 5-HT₄ receptor-antagonizing high concentration. More clear-cut evidence for the operation of release-enhancing 5-HT₄ receptors came from a microdialysis study in the striatum of halothane-anesthetized rats (Bonhomme et al., 1995) (Table 8). Locally applied 5-HT or BIMU 8, a selective 5-HT₄ receptor agonist, enhanced spontaneous DA release. This stimulatory effect was antagonized by the 5-HT_{3/4} receptor antagonist DAU 6285. Because a role of 5-HT₃ receptors was ruled out by the failure of ondansetron to counteract the increasing effect of 5-HT, the pattern of effects of the drugs mentioned so far conformed best to the involvement of 5-HT₄ heteroreceptors. 5-HT₁ and 5-HT₂ receptors also were not additional sites of action because the 5-HT₁ receptor antagonist (-)-pindolol and the 5-HT₂ receptor antagonists ketanserin and cinanserin did not block the effect of 5-HT.

Two comprehensive investigations, in which both in vitro and in vivo experiments were performed (Steward et al., 1996; De Deurwaerdère et al., 1997) (Table 8), not only confirmed the 5-HT₄ character and the release-enhancing property of the receptors in response to their stimulation but also were designed to provide an answer to the question of the location of the receptors: the authors claimed that the 5-HT₄ receptors, which are positively coupled to adenylate cyclase via G_s proteins, are present at least to a major part on neighboring nerves within the striatum, although the simplest model would be their location on the dopaminergic nerve terminals themselves.

In the first of these investigations, part of the experiments was performed in continuously superfused rat striatal slices (Steward et al., 1996) (Table 8). The spontaneous release of endogenous DA was enhanced not only by 5-HT but also by the nonselective and selective 5-HT₄ receptor agonists 5-methoxytryptamine and renzapride, respectively, as well as by the mixed 5-HT₃ receptor antagonist/5-HT₄ receptor agonist (*S*)-zacopride. The effect of the latter drug was blocked by the 5-HT₃/5-HT₄ receptor antagonist SDZ 205,557. Similarly, in incubated striatal slices ("a static release preparation"), renzapride increased the basal DA release in a manner sensitive to blockade by the selective 5-HT₄ receptor antagonist GR 113808. These findings were supplemented by experiments on freely moving rats using the microdialysis technique, which also revealed that 5-methoxytryptamine and renzapride increased DA release and that this effect was antagonized by GR 113808. Because tetrodotoxin abolished the increase in striatal DA release induced by 5-HT₄ receptor activation in the *in vitro* experiments, the authors suggested that the 5-HT₄ receptors are, at least to a major extent, not located on the dopaminergic axon terminals but rather on neighboring nerve terminals releasing a facilitatory transmitter (potentially, e.g., glutamate). This conclusion was supported by the results of an autoradiography study with the radiolabeled 5-HT₄ receptor antagonist [¹²⁵I]SB 207710, which revealed that destruction of the dopaminergic neurons by the neurotoxin 6-hydroxydopamine did not decrease the number of labeled 5-HT₄ receptors, whereas the destruction of neurons with cell bodies in the striatum by kainic acid led to a marked decrease in 5-HT₄ receptor density (Patel et al., 1995). Analogous results were obtained in a study in which the density of 5-HT₄ receptors in the post mortem brains of patients with Parkinson's disease and Huntington's disease were determined (Reynolds et al., 1995): in Parkinson's disease associated with a loss of dopaminergic neurons in the nigrostriatal system, no change in density of 5-HT₄ receptors in basal ganglia was observed, whereas in Huntington's disease associated with a profound loss of nondopaminergic (e.g., GABAergic, glutamatergic) neurons in basal ganglia, a decrease in 5-HT₄ receptor density in the putamen occurred. These findings conform to the putative involvement of a facilitatory neurotransmitter released in the vicinity of the dopaminergic nerve terminals. This transmitter substance, by activating specific receptors, may increase the release of DA in response to action potentials invading the chain of dopaminergic varicosities.

In addition to these considerations of the authors, it should be noted that the failure of 6-hydroxydopamine to decrease the number of radiolabeled 5-HT₄ receptors in the striatum does not exclude the possibility that these receptors are located on the dopaminergic nerve terminals. The number of such presynaptic heteroreceptors may be so small compared with the number of 5-HT₄

receptors on other anatomical structures in the striatum that they cannot be detected in lesion experiments with conventional radioligand binding or autoradiography. The authors themselves (Steward et al., 1996) pointed to the limited resolution of autoradiography studies and suggested that a minor proportion of the 5-HT₄ receptors mediating the release-increasing effect may be located as facilitatory presynaptic receptors on the dopaminergic nerve terminals. As they also stated, the positive coupling of the 5-HT₄ receptors to adenylate cyclase would conform to this possibility.

In the second combined *in vitro* and *in vivo* study (De Deurwaerdère et al., 1997) (Table 8), *in vivo* microdialysis of the striatum of halothane-nitrous oxide-anesthetized rats was supplemented by *in vitro* experiments on superfused synaptosomes. In the *in vivo* experiments, 5-HT enhanced DA release in a manner sensitive to partial blockade by the 5-HT₄ receptor antagonist GR 125,487 and the 5-HT_{3/4} antagonist DAU 6285, but not by methiothepine. The DA release-increasing effect of 5-HT was mimicked by (*S*)-zacopride. In the presence of tetrodotoxin, 1) the effect of 5-HT was reduced and was no longer antagonized by GR 125,487 or DAU 6285, and 2) (*S*)-zacopride also no longer increased DA release. However, it should be considered that in the presence of tetrodotoxin, the absolute basal DA release was lowered to approximately 10% of the level in the absence of this drug; such a considerable difference in basal conditions is a major obstacle against the possibility of a reliable comparison of the effect of DA receptor ligands in the presence and absence of tetrodotoxin. In striatal synaptosomes, 5-HT increased the release of newly synthesized [³H]DA whereas BIMU 8 and (*S*)-zacopride failed to do so. The 5-HT-induced enhancement of release was not antagonized by DAU 6285 but was reduced by the DA uptake inhibitor nomifensine, irrespective of whether or not DAU 6285 was present simultaneously; the latter findings indicate that the 5-HT-induced [³H]DA release (probably also part of the 5-HT-evoked release *in vivo*) is mediated by the DA transporter (section IV.B.). In agreement with the findings with newly synthesized [³H]DA, 5-HT but not (*S*)-zacopride also increased [³H]DA release from synaptosomes prelabeled with this tritiated neurotransmitter.

The *in vivo* data prove that the so-called basal DA release is enhanced by activation of facilitatory 5-HT₄ receptors in the corpus striatum. Based on the lack of effect of 5-HT₄ receptor ligands on synaptosomes and the failure of such compounds to modulate DA release *in vivo* in the presence of tetrodotoxin, the authors further concluded that these receptors are not located on dopaminergic nerve terminals but "postsynaptically," i.e., that the 5-HT₄ receptors are located on cell bodies in the striatum and that the effects of 5-HT₄ receptor ligands may, thus, involve neuronal circuitry.

Yet, an alternative interpretation is at least as plausible. It is evident that under control conditions without

tetrodotoxin DA release in vivo was not really basal: obviously, it was evoked by depolarization of the nerve terminals by invading action potentials because after blockade of the Na⁺ channels with tetrodotoxin basal DA release was strongly inhibited, a condition under which 5-HT₄ receptors no longer influenced DA release. Receptors on nerve terminals are well known to decrease or increase the release of its neurotransmitter by, e.g., reducing or enhancing the depolarization-induced Ca²⁺ influx into the respective terminals (Langer, 1977; Starke, 1977; Westfall, 1977; Göthert, 1982; Starke et al., 1989; Göthert and Schlicker, 1997). Such a presynaptic site and mechanism of action may also underlie the enhancing effect of 5-HT₄ receptors on DA release in vivo, which was only observed in the absence of tetrodotoxin. The failure of 5-HT₄ receptor ligands to modulate basal [³H]DA release in synaptosomes does not argue against this possibility because the effect of the ligands was not examined in the presence of a depolarizing stimulus, i.e., high K⁺ concentration. The operation of 5-HT₄ receptors modulating depolarization-evoked release could only have been excluded if experiments on synaptosomes would have revealed a failure of 5-HT₄ receptor ligands to modulate K⁺-evoked DA release.

Taken together, the available data are not sufficient to decide reliably whether the DA release-modulating 5-HT₄ receptors are located on the dopaminergic nerve terminals or on other nerves in the striatum releasing an excitatory neurotransmitter or whether both sites of action are involved. Thus, the exact mechanisms by which 5-HT₄ receptor ligands regulate DA release remained unexplained (Bockaert et al., 1997, 1998).

More recently, the role of the functional state of the dopaminergic system in the 5-HT₄ receptor-mediated control of DA release has been investigated (Lucas et al., 2001). In a microdialysis study on freely moving rats, the 5-HT₄ receptor antagonist GR 125487 injected i.p. did not affect basal DA release, but decreased the enhanced release in the striatum induced by s.c. haloperidol [for mechanism underlying haloperidol-induced enhancement, see (Lidsky and Banerjee, 1993)], whereas systemic administration of GR 125487 modified neither basal nor haloperidol-stimulated DA release in the nucleus accumbens. In agreement with the influence of systemically injected GR 125487, intrastriatal applications of this drug also selectively attenuated the haloperidol-stimulated DA release in this brain area, leaving the basal DA release unaffected. In single unit electrophysiological recordings, GR 125487 reduced the haloperidol-stimulated impulse flow in nigrostriatal but not in mesoaccumbal dopaminergic neurons. The authors concluded that 5-HT₄ receptors exert a facilitatory control on striatal DA release and nigrostriatal dopaminergic impulse flow only when the nigrostriatal dopaminergic system is activated. In addition, one may postulate that the release-increasing 5-HT₄ receptors are located both somadendritically on the do-

paminergic cell bodies of the substantia nigra and on nonspecified neurons in the corpus striatum. Furthermore, the authors conclude that the control by 5-HT₄ receptors occurs selectively in the nigrostriatal dopaminergic system, because 5-HT₄ receptors are without influence on mesoaccumbal dopaminergic activity. Finally, they also suggest that 5-HT₄ receptor ligands, permitting an independent modulation of the nigrostriatal dopaminergic system, may have potential for improved treatment of Parkinson's disease.

A selective 5-HT₄ receptor-mediated regulation of stimulation-dependent DA release in the corpus striatum was also observed in a microdialysis study in rats with morphine. The morphine (s.c.)-induced increase in striatal DA release was reduced by the selective 5-HT₄ receptor antagonists GR 125487 or SB 204070 and potentiated by the 5-HT₄ receptor agonist prucalopride (each of the latter drugs injected i.p.). Neither of these compounds affected morphine-stimulated DA release in the nucleus accumbens (Porrás et al., 2002). These findings support the idea of a putative application of 5-HT₄ receptor agonists in Parkinson's disease.

2. Concluding Summary Statement. Activation of 5-HT₄ receptors resembles 5-HT₃ receptor stimulation in that it increases DA release. It is not yet clear to what extent this increase is induced directly via 5-HT₄ receptors on DA neurons compared with an indirect modulation via an interneuron (Table 11). However, the two receptor types differ from each other in that they selectively modulate "reciprocal" dopaminergic systems: for 5-HT₄ receptors the release in the striatum but not in the nucleus accumbens is modified, whereas for 5-HT₃ receptors it is the opposite.

Preferential expression of 5-HT₄ receptors on striatal nerve terminals releasing a facilitatory neurotransmitter such as glutamate rather than on dopaminergic terminals was proven by combined lesion and radioligand binding studies and by measurement of 5-HT₄ receptor density in the post-mortem brains of patients with Parkinson's or Huntington's disease. In Parkinson's disease characterized by a loss of dopaminergic neurons in the nigrostriatal system, no change in 5-HT₄ receptors was observed in basal ganglia. In contrast, in Huntington's disease associated with a loss of, e.g., GABAergic and glutamatergic neurons in basal ganglia, 5-HT₄ receptor density in the putamen was decreased.

Furthermore, it was shown that 5-HT₄ receptors facilitate nigrostriatal dopaminergic impulse flow and striatal DA release only when this dopaminergic system is activated. Because 5-HT₄ receptors are without influence on mesoaccumbal dopaminergic activity, permitting independent modulation of the nigrostriatal dopaminergic system, the development of 5-HT₄ receptor agonists for improved treatment of Parkinson's disease is conceivable.

V. Modulation of Noradrenergic Neurotransmission

A. General Aspects

Modulation of NA release has been studied mainly in the rat CNS; in only one investigation listed in Table 9 were rabbit hippocampal slices used. The hippocampus has been the most thoroughly brain region examined both *in vitro* and *in vivo*; in addition, 5-HT heteroreceptor-mediated control of NA release has been studied in the hypothalamus, frontal cortex, ventral tegmental area, and spinal cord. The preferential investigation of the hippocampus is due to the generally accepted hypothesis that an increase in availability of NA in the biophase of adrenoceptors in this brain region is involved in the mechanism of action of antidepressants and, accordingly, 5-HT receptor ligands enhancing NA release may be expected to positively influence this mood disorder.

The pattern of presynaptic heteroreceptors on noradrenergic nerves in the CNS is identical in several aspects to that in the peripheral postganglionic sympathetic nerves (see previous reviews, Starke, 1977; Westfall, 1977; Langer, 1980) and original articles (Schlicker et al., 1989; Molderings et al., 1992). In particular, inhibitory presynaptic 5-HT_{1B/1D} receptors have been identified on the noradrenergic axon terminals of the cardiovascular system of various species both *in vitro* and *in vivo* (Charlton et al., 1986; Göthert et al., 1986; Molderings et al., 1987, 1990, 1996; Medhurst et al., 1997; Morán et al., 1998; Harris et al., 2002). Therefore, one could expect that the noradrenergic nerve terminals in the CNS might also be endowed with inhibitory presynaptic 5-HT_{1B/1D} receptors. However, attempts to identify such receptors on the noradrenergic nerves of the brain failed (Taube et al., 1977; Schlicker et al., 1983).

Basically, 5-HT_{1A}, 5-HT₂, and 5-HT₃ receptors have been shown to mediate changes in NA release, but in most cases the location of these receptors remained uncertain. Furthermore, for 5-HT₃ receptors, contradictory results have been obtained with respect to their function and location.

B. 5-Hydroxytryptamine 1A Receptors

1. Function, Location, and Classification. Basic evidence for the involvement of 5-HT_{1A} receptors in the modulation of NA release has been provided by microdialysis studies in awake rats in which 5-HT_{1A} ligands have been injected subcutaneously. The 5-HT_{1A} receptor agonist 8-OH-DPAT increased NA release in the hippocampus (Done and Sharp, 1994; Hajós-Korcsok and Sharp, 1996), frontal cortex (Hajós-Korcsok and Sharp, 1996), hypothalamus (Suzuki et al., 1995), and ventral tegmental area (Chen and Reith, 1995). NA release was also increased by other partial or full 5-HT_{1A} receptor agonists, in particular buspirone, gepirone (investigated in anesthetized rats) ipsapirone, MDL 73005EF, NAN-

190, and MKC-242. The increasing effect of MDL 73005EF, MKC-242, and/or 8-OH-DPAT was counteracted by the 5-HT_{1A} receptor antagonists WAY 100135 (Suzuki et al., 1995) and WAY 100635 (Hajós-Korcsok and Sharp, 1996; Hajós-Korcsok et al., 1999). The 5-HT_{1A} receptors are not stimulated tonically because WAY 100635 given alone did not modify NA release (Hajós-Korcsok and Sharp, 1996). As an immunocytochemical marker of noradrenergic neuronal activation, the 5-HT_{1A} receptor agonists ipsapirone and tandospirone (Hamamura et al., 1997) as well as 8-OH-DPAT and MDL 73005EF (Hajós-Korcsok et al., 1999) were shown to increase the expression of the immediate early gene *c-fos* in the locus coeruleus, an effect sensitive to antagonism by WAY 100135 (Hamamura et al., 1997) or WAY 100635 (Hajós-Korcsok and Sharp, 1999).

The increasing effect of 5-HT_{1A} receptor agonists on NA release was suggested to be due to activation of the somadendritic 5-HT_{1A} receptors on the serotonergic neurons themselves (Done and Sharp, 1994). This suggestion was based on the observations that MDL 73005EF and NAN-190, which have low efficacy at postsynaptic 5-HT_{1A} receptors but are almost full agonists of the inhibitory somadendritic 5-HT_{1A} autoreceptors (Gartside et al., 1990; Hjorth and Sharp, 1990), mimicked the effect of the full somadendritic autoreceptor agonist 8-OH-DPAT. Furthermore, 5-HT₂ receptor antagonists also increased NA release, and this analogy of action led the authors to suggest that 5-HT₂ receptors may be involved in the 5-HT_{1A} receptor-mediated increase in NA release. According to their hypothesis, the 5-HT₂ receptors are not located in the locus coeruleus, i.e., on the cell bodies and dendrites of the noradrenergic neurons, but rather on inhibitory GABAergic neurons interposed between the serotonergic and noradrenergic neurons (Gorea et al., 1991; Chiang and Aston-Jones, 1993). Inhibition of serotonergic neuronal activity by 5-HT_{1A} autoreceptor stimulation would result in decreased 5-HT₂ receptor-mediated activation of inhibitory GABAergic neurons innervating the soma and dendrites of noradrenergic neurons. Thus, their activity is disinhibited, i.e., increased with the consequence of increased NA release (Done and Sharp, 1994). However, the possibility of a primary site of action of the 5-HT_{1A} receptor ligands on the 5-HT neuron itself was virtually excluded by the findings that pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (Chen and Reith, 1995; Hajós-Korcsok and Sharp, 1996) or destruction of 5-HT neurons by intracerebroventricular injection of the neurotoxin 5,7-dihydroxytryptamine (Suzuki et al., 1995) failed to modify the 5-HT_{1A} receptor agonist-induced increase in NA release suggesting that the 5-HT_{1A} receptor must be located postsynaptically, i.e., behind the 5-HT neuron.

Because local administration of 8-OH-DPAT into the hypothalamus via the dialysis probe had no effect on NA release (Suzuki et al., 1995), the 5-HT_{1A} receptor in-

TABLE 9
Studies in which the involvement of 5-HT₂ and/or 5-HT₃ heteroreceptors in modulation of NA release has been investigated
Effects of various 5-HT receptor ligands or 5-HT-releasing drugs on NA release or drugs modulating monoamine inactivation in various brain regions of rabbit and humans are shown.

| Species | Brain Region | Receptor Type ^a | Drugs | Release-Modifying Concentration or Dose | Comment | References |
|---------|-----------------------------------------------|----------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Rat | Spinal cord | 5-HT ₂ | DOI | 3 × 10 ⁻⁷ M: inhibition | Superfusion of slices; electrically (5 Hz) stimulated [³ H]NA release; ketanserin (10 ⁻⁷ M) prevented the effect of DOI | Celuch et al. (1992) |
| Rat | Hippocampus | 5-HT ₂ | DOI | 0.2-0.5 mg/kg: inhibition | In vivo microdialysis in anesthetized rats; drug application by s.c. injection; determination of noradrenaline release; ritanserin (0.4 mg/kg s.c.) and spiperone (0.2-1 mg/kg s.c.) prevented the inhibitory effects of 5-HT _{2c} agonists; sulpiride without effect; 5-HT re-uptake localization on inhibitory afferents; the locus coeruleus probable | Done and Sharp (1992) |
| Rabbit | Hippocampus | 5-HT ₃ | DOB p-Chloramphetamine 5-HT 2-CH ₃ -5-HT 5-CONH ₂ -T | 1 mg/kg: inhibition 2 mg/kg: inhibition 10 ⁻⁶ -10 ⁻⁵ M: increase 10 ⁻⁵ -3.2 × 10 ⁻⁵ M: increase 10 ⁻⁶ -3.2 × 10 ⁻⁵ M: increase | Superfusion of slices; electrically (3 Hz)-evoked [³ H]NA release; MDL 72222 (10 ⁻⁵ M) or tropisetron (10 ⁻⁶ M) antagonized the facilitatory effect of 5-HT; ketanserin (10 ⁻⁷ M) without effect | Feuerstein and Hertting (1986) |
| Rat | Hippocampus | 5-HT ₃ | 6-Nitroquipazine 2-CH ₃ -5-HT 5-HT Paroxetine | 10 ⁻⁶ M: increase 10 ⁻⁵ -10 ⁻⁴ M: increase 3 × 10 ⁻⁵ M: increase 10 ⁻⁶ M: increase | Superfusion of slices; electrically (3 Hz) stimulated [³ H]NA release; desipramine (10 mg/kg/day for 21 days) decreases the magnitude of the enhancing effect of 2-CH ₃ -5-HT on evoked [³ H]NA release; fluoxetine, trimipramine, maprotiline, and moclobemide without effect | Mongeau et al. (1994b) |
| Rat | Hypothalamus | 5-HT ₃ | 5-HT 2-CH ₃ -5-HT | 10 ⁻⁷ -10 ⁻⁵ M: inhibition 7 × 10 ⁻⁸ -10 ⁻⁵ M: inhibition | Superfusion of slices in the presence of nomifensine (10 ⁻⁵ M) and tyrosine (5 × 10 ⁻⁵ M); determination of L-Glu (10 ⁻⁶ -10 ⁻³ M)-induced release of endogenous noradrenaline; ICS 205,930 (10 ⁻⁹ M) inhibited the effects of 5-HT and 2-CH ₃ -5-HT; ritanserin (10 ⁻⁶ M) or methysergide (10 ⁻⁶ M), given alone, without effect; ritanserin (10 ⁻⁶ M) or methysergide (10 ⁻⁶ M) potentiated inhibition by 5-HT; α-CH ₃ -5-HT (10 ⁻⁶ M) reversed effect of 2-CH ₃ -5-HT (10 ⁻⁶ M); presynaptic localization of 5-HT receptors questionable | Goldfarb et al. (1993) |
| Rat | Hippocampus Hypothalamus Frontal cortex | 5-HT ₃ | 2-CH ₃ -5-HT | 10 ⁻⁵ -10 ⁻⁴ M: increase 10 ⁻⁵ -10 ⁻⁴ M: increase 10 ⁻⁴ M: increase | Superfusion of slices; electrically (3 Hz) stimulated [³ H]NA release; release-increasing effect of 2-CH ₃ -5-HT antagonized by ondansetron, that of paroxetine blocked by ondansetron, tropisetron, and (S)-zacopride; 5-carboxamidotryptamine without effect; lesioning of 5-HT fibers with 5,7-DHT without influence on 2-CH ₃ -5-HT-induced increase of release | Mongeau et al. (1994a) |
| Rat | Hippocampus | 5-HT ₃ | 5-HT 2-CH ₃ -5-HT Fluoxetine | 10 ⁻⁸ -10 ⁻⁵ M: inhibition 10 ⁻⁸ -10 ⁻⁵ M: inhibition 10 ⁻⁵ M: inhibition | In vivo microdialysis in freely moving rats; determination of NA release stimulated by K ⁺ (120 mM) through the microdialysis probe; drugs applied through microdialysis probe; ondansetron (10 ⁻⁶ -10 ⁻⁵ M) blocked inhibitory effect of 5-HT and 2-CH ₃ -5-HT; 5-HT receptor localization on inhibitory interneurons probable | Matsumoto et al. (1995) |

2-CH₃-5-HT, 2-methyl-5-hydroxytryptamine (5-HT₃ agonist); 5-CONH₂-T, 5-methoxytryptamine (5-HT₁ agonist); L-GLU, L-glutamate (unselective glutamate receptor agonist); α-CH₃-5-HT, α-methyl-5-hydroxytryptamine (5-HT_{2A/2B} agonist).

^a Receptor type involved, as suggested by the authors.

^b Denoted as "5-HT_M" in the article (nomenclature valid at that time).

volved in the elevated NA release evoked by systemic administration of 5-HT_{1A} receptor agonists cannot be located on the noradrenergic nerve terminals of this brain area; this possibility was also improbable in view of the inhibitory character of the 5-HT_{1A} receptors. The 5-HT_{1A} receptors mediating the increase in NA release can also hardly be located in the somadendritic area of noradrenergic neurons when one considers that 5-HT_{1A} receptors preferentially couple to G_{i/o} proteins and that the locus coeruleus is not among the brain regions with a substantial density of 5-HT_{1A} receptors (Gozlan et al., 1988; Pompeiano et al., 1992; Kung et al., 1995). Rather, locus coeruleus afferent neurons releasing an inhibitory neurotransmitter, e.g., GABA, to the cell bodies and dendrites of the noradrenergic nerves seem to be the primary sites of action of the 5-HT_{1A} ligands (Fig. 2) (Hajós-Korcsok and Sharp, 1999). However, the exact location of the 5-HT_{1A} receptors still is an open question (Hajós-Korcsok et al., 1999).

2. *Concluding Summary Statement.* NA release in the hippocampus, frontal cortex, hypothalamus, and ventral tegmental area is increased by activation of 5-HT_{1A} receptors. These receptors are not located in the somadendritic or terminal area of the noradrenergic neurons. Because the somadendritic 5-HT_{1A} autoreceptor could also be excluded as the site of action by demonstrating the persistence of the effect after destruction of serotonergic neurons, the most probable location appears to be a GABAergic interneuron (Table 11). Inhibition of its activity by activation of the G_{i/o}-coupled 5-HT_{1A} receptor presumably located as the presynaptic heteroreceptor on synaptic boutons of GABAergic neurons (Katsurabayashi et al., 2003) leads to less release of GABA and as a consequence to disinhibition of NA release. This sequence, if proven by further experiments, would be analogous to that suggested as an explanation for the 5-HT_{1A} receptor-mediated increase in ACh release. It should be kept in mind that 5-HT_{1A} receptor agonists enhancing NA release such as ipsapirone positively influence depression.

C. 5-Hydroxytryptamine 2 Receptors

1. *Function and Location.* In vitro and in vivo data suggest that 5-HT₂ receptors mediate modulation of NA release in the rat CNS (Table 9). Thus, in slices of the spinal cord, the nonselective 5-HT₂ receptor agonist DOI inhibited the electrically evoked [³H]NA release in a manner sensitive to blockade by ketanserin at a concentration that does not discriminate between 5-HT₂ receptor subtypes (Celuch et al., 1992). However, in view of the facilitatory character of the 5-HT₂ receptors (as a rule, coupled to G_q proteins), a location of the 5-HT₂ receptors on the noradrenergic nerve terminals is improbable; such facilitatory receptor systems, stimulation of which ultimately results in an inhibitory function, probably occur on a neuron releasing an inhibitory neurotransmitter onto the noradrenergic axon terminal.

For the 5-HT₂ receptor modulating NA release in the rat hippocampus, this effect is indirect as well and the location of the receptor is also by no means clear (Done and Sharp, 1992). These restrictions are valid because the respective microdialysis study in anesthetized rats was almost exclusively based on subcutaneous injection of nonselective 5-HT₂ receptor ligands. Thus, the experiments revealed an inhibition of NA release 1) by 5-HT₂ receptor agonists such as DOB and DOI, 2) by the nonselective 5-HT receptor agonist quipazine, and 3) by endogenous 5-HT released by *p*-chloramphetamine. The DOI-induced inhibition was antagonized by ritanserin and spiperone, a mixed 5-HT₂/dopamine D₂ receptor antagonist. In awake rats, the 5-HT₂ receptor antagonists ritanserin and ICI 170,809 given alone increased NA release, suggesting tonic activation of the 5-HT₂ receptors (Done and Sharp, 1994). The inhibition of NA release in the hippocampus (Done and Sharp, 1992) was suggested to be directly related to a decrease in the firing rate of noradrenergic neurons in the locus coeruleus in response to systemic administration of DOB, DOM, and quipazine, an effect sensitive to blockade by 5-HT₂ receptor antagonists (Rasmussen and Aghajanian, 1986; Rasmussen et al., 1986; Gorea and Adrien, 1988). A direct inhibitory action of the 5-HT₂ receptor ligands on the noradrenergic nerve terminals of the hippocampus was excluded in preliminary experiments by the failure of DOI, applied locally via the dialysis probe, to modify NA release. In addition, a direct inhibitory action of 5-HT₂ receptor agonists on the somadendritic area of NA neurons in the locus coeruleus is unlikely in view of the predominantly facilitatory character of the 5-HT₂ receptor system. The most plausible possibility is that the 5-HT₂ receptors are located on inhibitory afferents to the locus coeruleus, such as putatively GABAergic nerves originating from the hypoglossus nucleus in the medulla oblongata (Fig. 2) (Gorea et al., 1991; Chiang and Aston-Jones, 1993). It is a shortcoming that no attempt was made to determine the 5-HT₂ receptor subtype.

2. *Concluding Summary Statement.* Activation of not yet subclassified 5-HT₂ receptors mediates an indirect inhibition of NA release in the hippocampus and spinal cord. These receptors are presumably located on inhibitory, probably GABAergic, interneurons that invert the stimulative character of the 5-HT₂ receptors to an inhibition of noradrenaline release (Table 11). Experiments with subtype-selective ligands have to be performed to determine the 5-HT₂ receptor subtype involved.

D. 5-Hydroxytryptamine 3 Receptors

1. *Function and Location.* Both facilitatory and inhibitory modulations of NA release via 5-HT₃ receptors have been found in the rat and rabbit brain (Table 9). A facilitation of electrically evoked [³H]NA release by 5-HT₃ receptor activation, observed in experiments on

rabbit hippocampal slices with 5-HT, 5-carboxamidotryptamine, and the 5-HT₃ receptor agonist 2-methyl-5-HT, was in agreement with the expectation (Feuerstein and Hertting, 1986): stimulation of this ligand-gated ion channel should result in an increase in Ca²⁺ influx into the axon terminal on which the 5-HT₃ receptor channel is located (in the present case on the noradrenergic nerve terminal) (Rondé and Nichols, 1998), leading to a facilitation of depolarization-evoked noradrenaline release. The 5-HT₃, but not 5-HT₂, character of the receptor involved was unequivocally established by the finding that the facilitatory effect of 5-HT was antagonized by MDL 72222 or tropisetron (formerly ICS 205,930) but not by ketanserin. A facilitation of NA release could also be brought about by increasing the concentration of endogenous 5-HT in the biophase of the presynaptic facilitatory 5-HT₃ receptors on the noradrenergic nerve terminals (Fig. 1) by administration of the selective inhibitor of the neuronal 5-HT transporter, 6-nitroquipazine, an effect that was sensitive to antagonism by tropisetron (Feuerstein and Hertting, 1986). Finally, the authors stated that, thus, the central noradrenergic nerve terminals of the rabbit resemble the peripheral noradrenergic nerve terminals of this species [(rabbit cardiac sympathetic nerves (Fozard and Mwaluko, 1976)) (Göthert and Duhrsen, 1979) in that they are endowed with presynaptic facilitatory and stimulatory receptors, respectively.

Largely identical experimental procedures (electrical stimulation of [³H]NA-prelabeled brain slices) and pharmacological tools as in the study on the rabbit hippocampus were applied to investigate whether 5-HT₃ receptors mediate facilitation of [³H]NA release in the hippocampus, hypothalamus, and frontal cortex of the rat (Mongeau et al., 1994a) (Table 9). In fact, the existence of such facilitatory 5-HT₃ receptors was proven. In the hippocampus it was also confirmed that stimulation of these receptors can be achieved by increasing the level of endogenous 5-HT by selective 5-HT reuptake blockade, in this case with paroxetine. In contrast to the rabbit hippocampus, 5-carboxamidotryptamine did not modify the electrically evoked [³H]NA release in this brain area of the rat, which is not surprising in view of the selectivity of action of this drug on 5-HT₁ receptors. The question of desensitization of the 5-HT₃ receptor after prolonged agonist stimulation was also addressed. There was no decrement in the effectiveness of 2-methyl-5-HT in increasing [³H]NA release after 20 min compared with 8 min of exposure, indicating that, surprisingly, no desensitization occurred within this time period. In the context of whether or not the 5-HT₃ receptors involved are located on noradrenergic nerve terminals themselves a combined radioligand binding/denervation study was conducted (Kidd et al., 1993). Binding of [³H](S)-zacopride to 5-HT₃ receptors was not decreased by noradrenergic denervation. At first sight this finding argues against the presence of presynaptic

5-HT₃ receptors on noradrenergic nerve endings. However, the number of 5-HT₃ receptors on the noradrenergic nerve fibers compared with their number in the innervated brain structures may be too small as to be detectable by such studies. Hence, a final decision can only be made on the basis of experiments using synaptosomes.

A follow-up study on rat hippocampal slices (Mongeau et al., 1994b) (Table 9) investigated the effects of long-term treatment with various classes of antidepressant drugs on the modulation of electrically evoked [³H]NA release by 2-methyl-5-HT. Desipramine treatment diminished the enhancing effect of 2-methyl-5-HT on evoked [³H]NA release, whereas fluoxetine, trimipramine, maprotiline, and moclobemide did not. The desipramine-induced desensitization of the NA release-enhancing 5-HT₃ receptors was suggested to be due to an increased intraneuronal Ca²⁺ concentration resulting from the desipramine treatment.

In contrast to these reports, other *in vitro* and *in vivo* investigations revealed that 5-HT₃ receptor agonists inhibit depolarization-evoked NA release (for details of some of the studies, see Table 9). Blockade of 5-HT₂ receptors by ritanserin or methysergide was a prerequisite for the disclosure of such an inhibitory effect of 5-HT on the K⁺ (20 mM)-evoked release of endogenous NA from hypothalamic slices; this effect was blocked by tropisetron (Blandina et al., 1991). In a subsequent study (Table 9) on rat hypothalamic slices superfused with Mg²⁺-free buffer containing nomifensine and tyrosin, endogenous NA release was stimulated with glutamate (Goldfarb et al., 1993). 5-HT or 2-methyl-5-HT inhibited glutamate-evoked NA release in a manner sensitive to blockade by tropisetron. Ritanserin or methysergide potentiated the 5-HT-induced inhibition of glutamate-evoked NA release, whereas the 5-HT₂ receptor agonist α -methyl-5-HT abolished this effect. These results suggested that 5-HT₃ receptors are involved in the inhibition of glutamate-evoked NA release. 5-HT₂ and 5-HT₃ receptors mediate opposite effects, thus explaining the fact that blockade of 5-HT₂ receptors was a prerequisite for the disclosure of the inhibitory effect of 5-HT₃ receptor stimulation.

It is tempting to relate the discrepancies between the various *in vitro* experiments (Table 9) to two methodological differences: 1) electrical pulses versus high K⁺ concentrations or glutamate, respectively, were used for stimulation; and 2) preloaded [³H]NA versus endogenous NA, respectively, were measured. However, these two factors may account for quantitative differences in the amplitude of modulation, whereas the qualitative differences remain unexplained.

A 5-HT₃ receptor-mediated (yet 5-HT₂ receptor-independent) inhibition of K⁺ (120 mM)-evoked NA release was also found in an *in vivo* microdialysis study in the hippocampus of freely moving rats (Matsumoto et al., 1995). The pharmacological tools were administered via

the dialysis probe. Exogenous 5-HT and 2-methyl-5-HT as well as an increase in endogenous 5-HT in the bio-phase of the 5-HT₃ receptors by fluoxetine acted inhibitory; this effect was blocked by ondansetron.

The paradox that the membrane depolarization by 5-HT₃ receptor activation inhibits K⁺-evoked release of endogenous NA both in vitro and in vivo was discussed by both groups of authors. As a possible explanation they suggested that the inhibition may be indirect, i.e., mediated by the release of another transmitter which, in turn, inhibits NA release (Blandina et al., 1991; Matsumoto et al., 1995). GABAergic (among other) neurons may play a role in the 5-HT₃ receptor-mediated inhibition of release, involving presynaptic GABA heteroreceptors on noradrenergic nerve terminals (Fig. 2) (Matsumoto et al., 1995). It was suggested that this functional interaction may be involved in the mechanisms underlying the pathogenesis of anxiety and depression (Matsumoto et al., 1995).

2. Concluding Summary Statement. Activation of 5-HT₃ receptors in the rabbit and rat brain mediates facilitation of NA release. In analogy to the role of 5-HT₃ receptors on peripheral noradrenergic nerves and in agreement with the depolarizing function, they are located as facilitatory presynaptic receptors on the noradrenergic nerve terminals of the rabbit hippocampus. In the rat hippocampus, cortex, and hypothalamus this location is also probable but evidence is not yet unequivocal (Table 11). Additional experiments on synaptosomes should clarify this issue.

In the rat hypothalamus and hippocampus activation of 5-HT₃ receptors can also induce an (in this case indirect) inhibition of NA release, thus allowing a very differentiated fine tuning of release. This is presumably brought about by stimulation of inhibitory GABAergic interneurons, on which they are expressed. These 5-HT₃ receptors also interact in a complex manner with glutamatergic nerve systems and with 5-HT_{2A} receptors.

VI. Modulation of GABAergic Neurotransmission

A. General Aspects

As outlined in previous sections, 5-HT receptor-mediated modulation of GABA release from inhibitory GABAergic interneurons plays an important role in the regulation of the release of various nonserotonin, non-GABA neurotransmitters in the CNS (Fig. 2): inhibition of GABA release from the axon terminals of such interneurons has been considered as an explanation of paradoxical facilitatory effects mediated by inhibitory 5-HT receptors: examples are the 5-HT_{1A} receptor-mediated stimulation of ACh and NA release (sections III.B. and V.B., respectively) and the 5-HT_{1B} receptor-mediated stimulation of DA release (section IV.C.1.). Conversely, facilitation of GABA release from inhibitory GABAergic interneurons has been suggested as a mechanism underlying the inhibition of transmitter release induced by

activation of facilitatory 5-HT receptors, e.g., the 5-HT₃ receptor-mediated inhibition of ACh release (section III.E.), the 5-HT_{2A} and 5-HT_{2C} receptor-mediated inhibition of DA release (sections IV.C.2. and IV.C.3., respectively), and the 5-HT₂ and 5-HT₃ receptor-mediated inhibition of NA release (sections V.C. and V.D., respectively).

Whereas in previous sections the role of GABAergic neurons as inhibitory interneurons (Fig. 2) has been considered, this section is devoted to these GABAergic neurons as primary targets of 5-HT (neuron II in Fig. 1) released from serotonergic axon terminals present in virtually all regions of the CNS, as are GABAergic neurons. Modulation of GABA release has been investigated in vitro in the hippocampus, septum, basolateral amygdala, periaqueductal gray, suprachiasmatic nucleus, caudate/putamen, globus pallidus, ventral tegmental area, and neocortex from rats, mice, guinea pigs, and humans (Table 10 and text). Inhibitory 5-HT_{1A} and 5-HT_{1B/D} receptors as well as stimulatory or facilitatory 5-HT_{2A}, 5-HT₃, and 5-HT₄ receptors have been identified. Interestingly, these receptors have been proven to be located, at least to a major extent, as presynaptic heteroreceptors on the GABAergic varicosities (synaptic boutons) themselves (Fig. 1).

In view of the importance of GABAergic neurons for the 5-HT heteroreceptor-mediated modulation of various neurotransmitters, it should be noted that, in turn, the serotonergic neurons are under the control of GABAergic nerves. This control has been investigated in midbrain slices containing the dorsal and medial raphe nuclei. [³H]GABA release and [³H]5-HT release were determined. The reciprocal interaction may serve as a local tuning in the neural connection between, e.g., neocortex and midbrain raphe nuclei (Bagdy et al., 2000).

B. 5-Hydroxytryptamine 1A Receptors

1. Function, Location, and Classification. The nystatin perforated patch or the conventional whole-cell patch-clamp technique was used on mechanically dissociated neurons from various brain areas of the rat for voltage clamp recording of pharmacologically isolated miniature inhibitory postsynaptic current (mIPSC) arising from attached GABAergic nerve terminals. The preparation is called the synaptic bouton preparation (Fig. 2): axonal varicosities of GABAergic interneurons attached to the cell body of neuron II (Rhee et al., 1999; Akaike and Moorhouse, 2003). In basolateral amygdala neurons (Koyama et al., 1999, 2002), periaqueductal gray neurons (Kishimoto et al., 2001), and hippocampal CA1 pyramidal neurons (Katsurabayashi et al., 2003), 5-HT reversibly reduced the GABAergic mIPSC frequency without affecting the mean amplitude. This effect was mimicked by the 5-HT_{1A} receptor agonist 8-OH-DPAT, and the inhibitory effect of the agonists was blocked by partial and by nonselective 5-HT_{1A} receptor antagonists such as NAN-190 and spiperone, respec-

TABLE 10
Studies in which the involvement of presynaptic 5-HT heteroreceptors in direct modulation of GABA release has been investigated
 Effects of various 5-HT receptor ligands and *p*-chloramphetamine on GABA release in various brain regions of rodent and human brain are shown.

| Species | Brain Region | Receptor Type ^a | Drugs | Release-Modifying Concentration or Dose | Comment | References |
|---------------------|------------------------------|----------------------------------------------|----------------------------|--------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| Rat, Wistar | Basolateral amygdala | 5-HT _{1A} | 8-OH-DPAT | 10 ⁻⁶ M: inhibition | Whole-cell voltage-clamp recording from acutely mechanically dissociated neurons with adherent GABAergic synaptic boutons (synaptic bouton preparation); spontaneous GABAergic mIPSPs determined | Koyama et al. (1999) |
| Rat, Wistar | Basolateral amygdala | 5-HT _{1A} | 8-OH-DPAT | 10 ⁻⁶ M: inhibition | Whole-cell voltage-clamp recording from acutely mechanically dissociated neurons; synaptic bouton preparation; spontaneous GABAergic mIPSPs | Koyama et al. (2002) |
| Rat, Wistar | Hippocampus CA1 | 5-HT _{1A} | 8-OH-DPAT | 10 ⁻⁶ M: inhibition | Whole-cell voltage-clamp recording from acutely mechanically dissociated CA1 pyramidal neurons; synaptic bouton preparation; electrical stimulation of single boutons | Katsurabayashi et al. (2003) |
| Human | Neocortex | ^a 5-HT _{1D} ^b | Sumatriptan | 10 ⁻⁷ -10 ⁻⁵ M: inhibition | Superfusion of slices; methiothepine (10 ⁻⁶ M) prevented inhibition by sumatriptan | Feuerstein et al. (1996) |
| Rat | Ventral tegmental area | 5-HT _{1B} | CP-93129 | 1.5-4.5 × 10 ⁻⁷ M: inhibition | Superfusion of slices in the presence of nipecotic acid and aminooxyacetic acid; SB 216641 prevented inhibition; 8-OH-DPAT and WAY100635 without effect | Yan and Yan (2001) |
| Rat mouse | Suprachiasmatic nucleus | 5-HT _{1B} | CP-93129 | 1.5-4.5 × 10 ⁻⁷ M: inhibition | | |
| Rat mouse | Suprachiasmatic nucleus | 5-HT _{1B} | CP-93129 | 10 ⁻⁶ M: inhibition | Frequency, but not amplitude, of mIPSPs recorded in suprachiasmatic nucleus neurons decreased in wild-type mice, yet no effect of CP-93129 in 5-HT _{1B} receptor knockout mice | Bramley et al. (2005) |
| Rat ♂ | Neocortex | 5-HT _{2A} | DOI | 10 ⁻⁶ M: increase | Superfusion of synaptosomes, GABA release determined; trazodone (10 ⁻¹⁰ -10 ⁻⁶ M) antagonized effect of DOI | Luparini et al. (2004) |
| Rat | Caudate putamen | 5-HT ₃ | <i>p</i> -Chloramphetamine | 10 ⁻⁷ M: facilitation | Superfusion of slices; determination of the enhancing effect of <i>p</i> -chloramphetamine on K ⁺ (20 mM)-evoked [³ H]GABA release; effect of <i>p</i> -chloramphetamine blocked by ICS 205,930 (5 × 10 ⁻⁸ M) or MDL 72222 (10 ⁻⁷ M) | Meyer et al. (1991) |
| Rat, Wistar | Basolateral amygdala | 5-HT ₃ | mCPBG | 10 ⁻⁶ M: facilitation | Whole-cell voltage-clamp recording from acutely mechanically dissociated basolateral amygdala neurons; synaptic bouton preparation; spontaneous GABAergic mIPSPs | Koyama et al. (2000) |
| Rat, Wistar | Basolateral amygdala | 5-HT ₃ | mCPBG | 10 ⁻⁶ M: facilitation | Whole-cell voltage-clamp recording from acutely mechanically dissociated basolateral amygdala neurons; synaptic bouton preparation; synaptic bouton preparation; spontaneous GABAergic mIPSPs | Koyama et al. (2002) |
| Rat, Wistar | Hippocampus | 5-HT ₃ | mCPBG | 1-3 × 10 ⁻⁶ M: facilitation | Whole-cell voltage-clamp recording from acutely mechanically dissociated CA1 pyramidal neurons; synaptic bouton preparation; electrical stimulation of single boutons | Katsurabayashi et al. (2003) |
| Rat, Sprague-Dawley | Hippocampus | 5-HT ₃ | 5-HT | 5 × 10 ⁻⁵ -10 ⁻⁴ M: facilitation | Whole-cell voltage-clamp recording from CA1 pyramidal neurons in hippocampal slices; 5-HT elicited GABAergic mIPSPs blocked by ICS 205,930 (2 × 10 ⁻⁹ M) but not by TTX | Turner et al. (2004) |
| Mouse | Hippocampus | 5-HT ₃ | 5-HT | 10 ⁻⁴ M: facilitation | Superfusion of synaptosomes prelabelled with [³ H]GABA; osmotically by 0.5 M sucrose evoked release of [³ H]GABA; 5-HT effect blocked by ICS 205,930 (10 ⁻⁶ M) | Turner et al. (2004) |
| Rat | Cultured hippocampal neurons | 5-HT ₃ | 5-HT | 10 ⁻⁵ M: facilitation | Whole-cell patch-clamp recordings from single neuron microcultures that form autapses; ICS 205,930 3 × 10 ⁻⁷ M abolished the effect of 5-HT | Dorostkar and Boehm (2005, 2007) |

^a Receptor type involved, as suggested by the authors.

^b According to the old nomenclature; current nomenclature human 5-HT_{1B}.

tively. These results indicate that GABA release in the brain regions investigated and (and probably also in other brain areas) is controlled by inhibitory presynaptic 5-HT_{1A} heteroreceptors on the attached GABAergic nerve terminals.

Further evidence for the location of 5-HT_{1A} receptors on the GABAergic varicosities was achieved by focal electrical stimulation of single GABAergic boutons in the synaptic bouton preparation of single hippocampal CA1 pyramidal neurons (Katsurabayashi et al., 2003). In all boutons tested, 8-OH-DPAT decreased the amplitude of evoked IPSCs, whereas in only part of the boutons, mCPBG increased the amplitude of evoked IPSCs. The authors concluded that all GABAergic boutons are endowed with inhibitory 5-HT_{1A} receptors, whereas only a subset of the boutons contain both inhibitory 5-HT_{1A} and excitatory 5-HT₃ receptors (for the latter, see section VI.E.). The interaction between the 5-HT_{1A} and 5-HT₃ receptors on the GABAergic boutons as well as its functional significance will be discussed below (section VI.E.).

2. *Mechanism.* The mechanism underlying the 5-HT_{1A} receptor-mediated inhibition of GABA release from GABAergic boutons was investigated in basolateral amygdala neurons with attached GABAergic nerve terminals. The 5-HT-induced inhibition of the mIPSC frequency was not affected in either K⁺- or Ca²⁺-free external solution (Koyama et al., 1999) but was abolished by *N*-ethylmaleimide, a GTP-binding protein inhibitor (Koyama et al., 1999, 2002). The frequency of mIPSC was increased by stimulation with high K⁺, and this increase was inhibited by 8-OH-DPAT; activation of adenylyl cyclase by forskolin also increased synaptic mIPSC, and forskolin pretreatment prevented the 5-HT-induced inhibition of mIPSC frequency (Koyama et al., 1999). The authors concluded that the inhibition of GABAergic mIPSC frequency, i.e., inhibition of GABA release from synaptic boutons, caused by stimulation of the G protein-coupled presynaptic 5-HT_{1A} receptors is due to the inactivation of the adenylyl cyclase/cAMP signal transduction pathway and that this intracellular pathway directly acts on the GABA release process independent of K⁺ and Ca²⁺ channels.

3. *Concluding Summary Statement.* In several areas of the rat brain GABA release is controlled by inhibitory presynaptic 5-HT_{1A} heteroreceptors on GABAergic nerve terminals (Table 11). This release-decreasing control is caused by the inhibition of the cAMP signal transduction pathway, thus negatively acting directly on GABA release.

C. 5-Hydroxytryptamine 1B Receptors

1. *Function.* Electrophysiological investigations, which provided the first steps in the identification of a role of 5-HT_{1B} receptors in modulation of GABA release, were performed in rat septal (Joëls and Gallagher, 1988) and hippocampal slices (Segal, 1990). In cells contained

in these tissues (in the case of hippocampus, CA1 cells), 5-HT blocked GABA_B synaptic potentials, leaving the synaptic potentials mediated by GABA_A receptors unaffected or enhanced. Because these results did not allow identification of the 5-HT receptor type involved, this question was addressed in a study on rat midbrain slices in which intracellular recordings from dopaminergic neurons were made (Johnson et al., 1992). These studies confirmed that 5-HT reduced the amplitude of GABA_B synaptic potentials and did not reduce the amplitude of synaptic potentials mediated by GABA_A receptors. Several steps farther ahead toward the identification of presynaptic 5-HT_{1B} receptors involved, the 5-HT_{1B} receptor agonist TFMP was shown to mimic the inhibitory action of 5-HT. The reduction of GABA_B potentials was counteracted by the 5-HT_{1B/1A} receptor antagonist cyanopindolol but not by the 5-HT_{1A}/DA receptor antagonist spiperone. Using the same technique and tissue preparations, it was reported that cocaine inhibited the GABA_B-mediated inhibitory potentials (Cameron and Williams, 1995). This action of cocaine was blocked by pretreatment with the 5-HT-depleting agent *p*-chloramphetamine and was mimicked not only by 5-HT and the 5-HT-releasing agent fenfluramine but also by sumatriptan [1-[3-(2-dimethylaminoethyl)-1*H*-indol-5-yl]-*N*-methyl-methanesulfonamide succinate], a 5-HT_{1B/1D} receptor agonist. However, pretreatment with *p*-chloramphetamine failed to affect the actions of sumatriptan (Cameron and Williams, 1994, 1995). These results suggested that 5-HT receptor agonists and compounds increasing the concentration of endogenous 5-HT in the biophase of the 5-HT receptors, such as cocaine and fenfluramine, inhibit midbrain GABA transmission through 5-HT_{1B} receptors.

Based on these findings Morikawa et al. (2000) performed a study on ventral midbrain slices of wild-type and 5-HT_{1B} receptor knockout mice in which IPSPs from dopaminergic neurons were recorded. In neurons from wild-type animals, 5-HT markedly reduced the IPSP amplitude, but failed to do so in 5-HT_{1B} receptor knockout mice. An increase of endogenous 5-HT in the biophase of the 5-HT receptor by cocaine or fenfluramine, inhibited the IPSP amplitude in wild-type mice but only marginally affected this parameter in knockout mice. Brains from 5-HT_{1B} receptor knockout mice and wild-type mice, in addition to rat brains, were also used to record (in the whole cell configuration) mIPSCs arising from nonretinal GABAergic terminals in suprachiasmatic nucleus (SCN) neurons in hypothalamic slices (Bramley et al., 2005). It is worthwhile to mention that GABAergic neurons form an extensive network in the SCN (Strecker et al., 1997; Castel and Morris, 2000). The 5-HT_{1B} receptor agonist CP-93129 decreased the frequency of miniature IPSPs in SNC neurons of both rat and wild-type mouse hypothalamic slices with no clear effect on mIPSC amplitude. In 5-HT_B knockout

mice, CP-93129 had no clear effect on the frequency and amplitude of mIPSCs.

5-HT_{1B} receptor knockout mice have also been used to study the functional importance of the 5-HT receptors in the SCN. The lack of functional 5-HT_{1B} receptors results in an attenuated response to light (Sollars et al., 2003). This may be due to increased GABAergic neural activity in the SCN as a consequence of the lack of effect of 5-HT on 5-HT_{1B} receptors. Drugs with antagonistic properties at 5-HT_{1B} receptors may also cause an attenuation of the response to light.

Results analogous to those in the electrophysiological studies were obtained in biochemical experiments on superfused brain slices. Thus, it was found in human neocortical slices that the 5-HT_{1B/1D} receptor agonist sumatriptan inhibits the electrically evoked release of endogenously formed [³H]GABA in a manner sensitive to blockade by the nonselective 5-HT receptor antagonist methiothepine (Feuerstein et al., 1996). In rabbit neocortical slices no such effect was found, pointing to a significant species difference and to the limitations of using receptor-mediated modulation of transmitter release in animals as a model for modulation of release in humans. Similar to the results in the human neocortical slices (Feuerstein et al., 1996), 5-HT_{1B} receptor activation by CP-93129 inhibited high K⁺-evoked [³H]GABA release in globus pallidus slices (Chadha et al., 2000). In the rat ventral tegmental area slices preincubated with [³H]GABA, CP-93129 and RU 24969, but not 8-OH-DPAT, inhibited the K⁺-evoked [³H]GABA release (Yan and Yan, 2001). The RU 24969-induced inhibition of release was antagonized by the selective 5-HT_{1B} receptor antagonist SB 216641 (Price et al., 1997; Schlicker et al., 1997) and the 5-HT_{1B/1A} receptor antagonist cyanopindolol, but not by the selective 5-HT_{1A} receptor antagonist WAY 100635.

2. Classification. It may be suggested that in all tissues of rat, mouse, and humans dealt with in this section, the same receptor type at the same subcellular location of GABAergic neurons is involved in modulation of GABA release. The failure of 5-HT receptor agonists to inhibit GABA release in 5-HT_{1B} receptor knockout mice in contrast with the ability of these drugs to cause an inhibition in wild-type mice and the ability of the selective 5-HT_{1B} receptor antagonist SB 216641 to counteract the agonist-induced inhibition of GABA release in the rat prove the involvement of inhibitory 5-HT_{1B} heteroreceptors in the modulation of GABA release. The release-inhibitory 5-HT receptors in the human neocortex had been classified as “5-HT_{1D}” according to the old nomenclature (Feuerstein et al., 1996) (section III.C.2.), assuming that the human 5-HT_{1D} receptor is the species analog of the rodent 5-HT_{1B} receptor. However, according to current knowledge, 1) both 5-HT_{1B} and 5-HT_{1D} receptors are expressed as different entities in all species considered here, 2) 5-HT_{1B} and 5-HT_{1D} receptors exhibit similar pharmacological properties but differ

from each other, at least in part, in their distribution and function (section III.C.2.), 3) sumatriptan, in particular, cannot discriminate between 5-HT_{1B} and 5-HT_{1D} receptors, and 4) in a comparison of the 5-HT_{1B} receptors of the various species with each other it is evident that they mediate the same effects (section III.C.2.). Therefore, it may be postulated that the human neocortex human 5-HT_{1B} receptors, when activated, also mediate inhibition of GABA release.

3. Location. Rat and mouse 5-HT_{1B} receptors are present on the axon terminals of the GABAergic neurons (Morikawa et al., 2000; Yan and Yan, 2001; Bramley et al., 2005). This conclusion is based largely on data supporting the idea that 5-HT_{1B} receptors are expressed in various species predominantly as presynaptic 5-HT auto- and heteroreceptor on nerve terminals of serotonergic and nonserotonergic neurons (Kidd et al., 1993; Boschert et al., 1994; Sari et al., 1999). This line of evidence supports the contention that in the human neocortex inhibitory presynaptic 5-HT_{1B} heteroreceptors also modulate GABA release. However, irrespective of the probably predominant location of the 5-HT_{1B} receptors on the chain of GABAergic varicosities in the brain areas of the species considered, at least some 5-HT_{1B} receptors may be assumed to also be located on the cell bodies of GABAergic neurons.

4. Concluding Summary Statement. In multiple brain areas of rat, mouse, and human (Table 11) activation of 5-HT_{1B} receptors induces an inhibition of GABA release. This has been proven by electrophysiological techniques, by transmitter release studies, and by use of 5-HT_{1B} receptor-deficient mice. Because of the availability of selective 5-HT_{1B} antagonists the 5-HT_{1B} character of the receptors could be unequivocally established. They are predominantly located as presynaptic heteroreceptors on GABAergic nerve terminals.

D. 5-Hydroxytryptamine 2A Receptors

1. Function, Location, and Classification. The first evidence for the operation of GABA release-increasing 5-HT_{2A} receptors came from a study, in which preferential 5-HT_{2A} receptor antagonists such as ketanserin and MDL 100907 inhibited the K⁺-evoked GABA release in cortical slices (Cozzi and Nichols, 1996). This inhibition was presumably brought about by counteracting a facilitatory tone exerted by endogenous 5-HT. In agreement with this suggestion, a microdialysis study revealed that local infusion of the nonselective 5-HT₂ receptor agonist DOI increased cortical GABA release (Abi-Saab et al., 1999).

A very comprehensive investigation comprising in vitro and in vivo studies has been performed to prove convincingly that presynaptic 5-HT_{2A} receptors modulate GABA release in the rat cerebral cortex (Table 10) (Luparini et al., 2004). In superfused cortical synaptosomes the 5-HT₂ receptor agonist DOI increased the spontaneous release of endogenous GABA, and

this effect was antagonized by the 5-HT₂ receptor antagonist/5-HT reuptake inhibitor trazodone (a compound used as an antidepressant drug) at high potency (pIC₅₀ 8.31). In the same preparation trazodone inhibited [³H]5-HT accumulation at low potency (pIC₅₀ 5.9). In superfused neocortical slices, trazodone exerted a biphasic effect on GABA release: at concentrations of 0.1 to 100 nM it acted inhibitory, and at 1 to 100 μM it increased GABA release. The inhibitory effect of trazodone in the low concentration range was mimicked by the 5-HT_{2A} receptor antagonist AMI-193. In contrast, the 5-HT₂ receptor agonist DOI increased GABA release in the slice preparation.

The data obtained with DOI and trazodone in synaptosomes unequivocally allow the conclusion that the GABAergic nerve terminals are endowed with facilitatory presynaptic 5-HT₂ receptors. Because the selective 5-HT_{2A} receptor antagonist AMI-193 mimicked the effect of trazodone, the receptors can be subclassified as 5-HT_{2A}. The inhibitory effect of trazodone and AMI-193 on GABA release in the slice preparation is due to their antagonistic action on the facilitatory tone exerted by endogenous 5-HT via these presynaptic heteroreceptors. In the higher concentration range of trazodone, the biphasic concentration of endogenous 5-HT at the presynaptic 5-HT_{2A} heteroreceptors on the GABAergic varicosities appears to be increased so dramatically by the trazodone-induced 5-HT reuptake inhibition that it outweighs the trazodone antagonism at the presynaptic 5-HT_{2A} heteroreceptors, resulting in an increase in GABA release. The biphasic effect of trazodone, i.e., the inhibition of GABA release at a low subcutaneous dose and an increase of GABA release at a higher dose, was also found in vivo in the microdialysis experiments on the cerebral cortex.

In the context of the potential clinical significance of this finding, the authors also investigated the influence of the modification of GABA release on 5-HT release (Luparini et al., 2004). Trazodone increased 5-HT release through a dual mechanism: at a low concentration this is primarily due to blockade of the facilitatory 5-HT_{2A} receptors on the GABAergic nerve terminals, thus attenuating an inhibitory GABAergic tone regulating 5-HT release in the cerebral cortex and at a high concentration it is caused by the inhibition of the 5-HT transporter. The increase in 5-HT release, resulting from this double mechanism, may be responsible for the antidepressant properties of trazodone, possibly through 5-HT_{1A} receptor down-regulation (Subhash et al., 2002). As outlined above, the increase in 5-HT release is accompanied by the rise in GABA release which may explain the sedative and anxiolytic actions that accompany the antidepressant activity of trazodone.

2. Concluding Summary Statement. Activation of 5-HT_{2A} receptors in rat neocortex increases GABA release. These receptors are located presynaptically on the GABAergic terminals (Table 11). They are tonically ac-

tivated by endogenous 5-HT. As a consequence, nonselective and selective 5-HT_{2A} receptor antagonists inhibit GABA release, leading to disinhibition, i.e., increase of transmitter release from neurons (including the serotonergic ones) innervated by GABAergic neurons. This mechanism is of clinical importance for the antidepressant effect of trazodone, a mixed 5-HT₂ receptor antagonist/5-HT transporter inhibitor. The effect of the trazodone-induced modification of GABA on 5-HT release explains at least in part, the antidepressant and anxiolytic actions of the drug.

E. 5-Hydroxytryptamine 3 Receptors

1. Function, Location, and Classification. Various in vitro investigations using different techniques unanimously revealed that the 5-HT₃ receptor agonist mCPBG, exogenous 5-HT, or endogenous 5-HT released by *p*-chloramphetamine facilitates or stimulates GABA release in a manner sensitive to blockade by 5-HT₃ receptor antagonists such as tropisetron (ICS 205,930) and MDL 72222 (Table 10). This finding has been proven 1) by measurement of the enhancing effect of *p*-chloramphetamine on the K⁺-evoked [³H]GABA release in superfused slices of the rat caudate-putamen (Meyer et al., 1991), 2) by recording GABAergic IPSCs in the synaptic bouton preparation, in particular in mechanically dissociated rat basolateral amygdala (Koyama et al., 2000, 2002) and hippocampal CA 1 pyramidal neurons (Katsurabayashi et al., 2003) with adherent synaptic GABAergic boutons (for details, see section VI.A.), 3) by recording of GABAergic mIPSC in CA1 pyramidal neurons in rat hippocampal slices, 4) by estimating the osmotically (0.5 M sucrose) evoked [³H]GABA release in superfused mouse hippocampal synaptosomes (Turner et al., 2004), and 5) by whole-cell patch-clamp recordings in microcultures from single hippocampal neurons that form autapses (Dorostkar and Boehm, 2005, 2007). These data unequivocally allow the conclusion that GABA release is modified by presynaptic facilitatory or stimulatory 5-HT₃ receptors in all of the brain regions investigated.

In the rat basolateral amygdala (Table 10), these receptors are unequivocally located on GABAergic varicosities because the synaptic bouton preparation contains only torn-off GABAergic axonal varicosities but no cell bodies of such neurons (Koyama et al., 2000, 2002; Katsurabayashi et al., 2003) (Fig. 2: axonal varicosities of GABAergic interneurons attached to the cell body of neuron II). The same holds true for rat and mouse hippocampal synaptosomes (Turner et al., 2004). Although in caudate putamen (Meyer et al., 1991), the effect of endogenous 5-HT and of 5-HT₃ receptor antagonists on K⁺-evoked release was investigated in the absence of tetrodotoxin only (allowing no clear-cut conclusion concerning the subcellular location), the release-facilitating 5-HT₃ receptors in these brain areas may be assumed to also be located, at least in part, on GABAergic axon

terminals. In addition, it is tempting to postulate that GABAergic neurons in other brain areas, such as the cerebral cortex, and of other species are also endowed with facilitatory 5-HT₃ receptors on their axon terminals. Consistent with this hypothesis, it was suggested on the basis of a radioligand binding study that 5-HT₃ receptors in the central nervous system are located on nerve terminals (Kidd et al., 1993).

2. *Mechanism.* The mechanism underlying the 5-HT₃ receptor-mediated facilitation of GABA release was studied in synaptic bouton preparations of basolateral amygdala neurons (Koyama et al., 2000). 5-HT₃ receptor activation by mCPBG increased the mIPSC frequency in buffer containing Ca²⁺ and Cd²⁺, the latter to block all voltage-dependent Ca²⁺ channels. However, the 5-HT₃ receptor agonist-induced increase was absent in Ca²⁺-free external solution. These findings suggest that Ca²⁺ influx through 5-HT₃ receptor channels is crucial for the facilitation of synaptic GABA release, whereas influx through voltage-dependent Ca²⁺ channels plays a negligible role. The same conclusion was drawn from results obtained in hippocampal slices, in which Cd²⁺ at a concentration blocking all voltage-dependent Ca²⁺ channels and omission of Ca²⁺ produced effects identical to those in the synaptic bouton preparation of amygdala neurons

3. *Interaction between Presynaptic 5-Hydroxytryptamine 3 and 5-Hydroxytryptamine 1A Receptors.* GABA release in the brain is modulated not only via facilitatory 5-HT₃ receptors but also via inhibitory 5-HT_{1A} receptors (section VI.B.). Part of the GABAergic synaptic boutons in the rat hippocampus are endowed with both receptor types; i.e., a ligand-gated ion channel and a G protein-coupled receptor, respectively (Katsurabayashi et al., 2003). Studies in other neuronal systems had suggested that activation of G protein-coupled receptors inhibits ionotropic receptor channel-mediated function (Huidobro-Toro et al., 1996; Liu et al., 2000). Therefore, the rat synaptic bouton preparation of basolateral amygdala neurons was used to investigate whether presynaptic 5-HT_{1A} and 5-HT₃ receptors on varicosities of GABAergic axon terminals interact with each other (Koyama et al., 2002). Application of 8-OH-DPAT and its subsequent removal led to an inhibition of mIPSC frequency, which disappeared after washout. However, long after the removal of 8-OH-DPAT the mCPBG-induced facilitatory responses were still suppressed. This suppression was abolished by pretreatment with *N*-ethylmaleimide, a pertussis toxin-sensitive GTP-binding protein inhibitor. Accordingly, in the absence of *N*-ethylmaleimide, intracellular signaling induced by activation of 5-HT_{1A} receptors is responsible for the attenuation of the 5-HT₃ receptor-mediated facilitation of GABA release. In particular, phosphorylation of the 5-HT₃ receptor at its intracellular phosphorylation sites may be modified, thus inducing a conformational change that may lead to a long-lasting reduction of 5-HT₃ receptor channel func-

tion. This, in turn, would be associated with a decreased Ca²⁺ influx via the 5-HT₃ receptor channel and, as a consequence, with a decrease in 5-HT₃ receptor-mediated facilitation of GABA release. It should be kept in mind that stimulation of 5-HT₃ receptors on varicosities leads to Ca²⁺, but not Na⁺, influx through these channels (Rondé and Nichols, 1998).

In the basolateral amygdala the presence of both 5-HT₃ and 5-HT_{1A} receptors on the same GABAergic axon terminals and their interaction may have functional consequences in vivo in the neurons that are GABAergically innervated (neuron II in Fig. 2) (Koyama et al., 2002): an initial rapid action of 5-HT on such neurons consists of an augmented GABAergic inhibition as a result of the dominance of fast 5-HT₃ receptors. However, the long-term action of 5-HT is characterized by attenuated GABAergic inhibition of the postsynaptic neurons because the 5-HT_{1A} receptor-mediated inhibition of GABA release becomes dominant over the effect of 5-HT₃ receptors. In addition, the function of the latter receptors is attenuated by their desensitization. It was speculated that such time-dependent differences in serotonergic modulation of GABAergic inhibition in the amygdala might contribute to the variability of emotional responses (Koyama et al., 2002). Basically, the same time-dependent modulation comes into play in the hippocampus. This has been shown in the synaptic bouton preparation of hippocampal CA1 neurons in which single synaptic boutons were focally stimulated (Katsurabayashi et al., 2003).

4. *Concluding Summary Statement.* 5-HT₃ receptor activation in the rat and mouse hippocampus, amygdala, and caudate putamen facilitate or stimulate GABA release. The 5-HT₃ receptors are located as presynaptic heteroreceptors on the GABAergic axon terminals (Table 11). Ca²⁺ influx via 5-HT₃ receptor channels plays a crucial role for the facilitation of GABA release, whereas influx through voltage-dependent Ca²⁺ channels is negligible. Part of the GABAergic synaptic boutons are endowed not only with 5-HT₃ receptors but also with 5-HT_{1A} receptors that interact with each other: 5-HT_{1A} receptor activation attenuates 5-HT₃ receptor channel function. This is probably due to modified phosphorylation of the 5-HT₃ receptor at the intracellular phosphorylation sites. As a functional consequence of the operation of the two receptors at different speed of signal transduction, the fast facilitatory 5-HT₃ receptor dominates in the initial phase, whereas the attenuation of this response by the slow 5-HT_{1A} receptor dominates the subsequent long-term effect.

F. 5-Hydroxytryptamine 4 Receptors

1. *Function, Location, and Classification.* Superfused guinea pig hippocampal slices were used to study the effects of 5-HT₄ receptor ligands on basal efflux and electrically evoked release of endogenous GABA (Bianchi et al., 2002). The 5-HT₄ receptor agonist BIMU 8 did

not modify basal GABA efflux but modulated the electrically evoked GABA release in a complex manner: at low concentrations (0.2–0.4 μM) it increased and at higher concentrations (0.7–2.0 μM) it inhibited GABA release. Both of these responses were counteracted by GR 125487, a selective 5-HT₄ receptor antagonist, indicating that 5-HT₄ receptors are involved in modulation of GABA release.

Because BIMU 8 increased ACh release in rat and guinea pig hippocampal slices (section III.F.) (Siniscalchi et al., 1999), the hypothesis of whether the cholinergic system is involved in the biphasic effect of BIMU 8 was examined (Bianchi et al., 2002). Such an indirect effect of 5-HT₄ receptor stimulation was proven by the ability of the nonselective muscarinic receptor antagonist atropine to abolish the dual action of BIMU 8. To determine the muscarinic receptor subtype involved it was shown that the muscarinic M₁ and M₃ receptor antagonist 4-DAMP prevented the BIMU 8-evoked increase in GABA release, whereas the M₂ receptor antagonist AFDX-116 did not. Conversely, AFDX-116 extinguished the inhibitory effect of BIMU 8 on GABA release, which, in turn, was left unaffected by 4-DAMP (Bianchi et al., 2002). In conclusion, by stimulating 5-HT₄ receptors 5-HT induces release of ACh which, in turn, bidirectionally modulates GABA release: at relatively low concentrations it increases GABA release via M₁ and/or M₃ receptors and at higher concentrations it inhibits GABA release via M₂ receptors. Two results achieved with different methods conform to the involvement of 5-HT₄ receptors in these modulatory processes: 1) 5-HT₄ receptors are widely expressed in the hippocampus (Eglen et al., 1995) where they appear to be involved in cognitive processes (Lamirault and Simon, 2001) and 2) M₁, M₂, M₃, and M₄ muscarinic receptors also occur in the hippocampus where each subtype can play a specific role in the cholinergic modulation of excitatory and inhibitory circuits (Levey et al., 1995; Rouse and Levey, 1996).

2. Concluding Summary Statement. In guinea pig hippocampus stimulation of 5-HT₄ receptors (probably located on cholinergic neurons) increases ACh release which, in turn, bidirectionally modulates GABA release. At high potency activation of 5-HT₄ receptors GABA release is increased via M₁ and/or M₃ receptors; at low potency activation it is inhibited via M₂ receptors (Table 11). Thus, the 5-HT₄ receptor-mediated modulation of GABA release, which comprises a cholinergic neuron additionally inserted between the serotonergic and GABAergic neurons, is an example of a more complex regulatory neuronal circuit than that illustrated in Figs. 1 or 2.

VII. Synopsis

The present article is focused on the 5-HT heteroreceptor-mediated local modulation of the release of the

neurotransmitters ACh, DA, NA, and GABA in the CNS. As demonstrated in synaptosomes and slices in vitro and in microdialysis experiments in vivo (these techniques are the backbone of the methodology), 5-HT heteroreceptor-mediated modulation occurs mainly via two mechanisms (Table 11): 1) a direct modulation is accomplished via presynaptic 5-HT receptors on the axon terminals from which the respective transmitter is released; and 2) indirectly, release is controlled via interneurons, in particular GABAergic interneurons. The latter are endowed with 5-HT heteroreceptors inhibiting or facilitating the release of GABA which, in turn, influences the release of the transmitter under consideration. It is remarkable that most types of 5-HT heteroreceptors modulating GABA release are located on the axon terminals of GABAergic (inter)neurons (Table 11), indicating that not only in the case of direct modulation of the release of a given neurotransmitter but also in the case of local involvement of GABAergic interneurons presynaptic signal transduction plays a relevant role.

Table 11 shows for cholinergic, dopaminergic, noradrenergic, and GABAergic neurotransmission that modulation of the release of each of these neurotransmitters via 5-HT heteroreceptors is very complex. This complexity comprises several important aspects that deserve consideration.

Thus, the release of each of the neurotransmitters investigated is modulated by several (up to five) 5-HT receptor types which, because of their main signal transduction systems, mediate inhibitory or facilitatory/stimulatory actions, or in other words, partly synergistic and partly opposite effects (Table 11). In detail, the patterns of 5-HT receptors modulating the release of the four neurotransmitters comprise the following receptor types (the G protein coupled in response to receptor activation or to a receptor activation-induced increase in open probability of the cation channel is shown in parentheses): 5-HT_{1A}, 5-HT_{1B} (G_{i/o}); 5-HT_{2A}, 5-HT_{2C} (G_q); 5-HT₄ (G_s); and 5-HT₃ (Na⁺- and Ca²⁺-conducting ligand-gated ion channel; see section II.C.). In this context it should be pointed out that details of signal transduction mechanisms in individual axon terminals are largely obscure, implying all this more that this refers to receptor-mediated modification of these processes. The reasons are that most of these terminals are too small for patch-clamp recording and that results obtained in direct measurements of biochemical parameters in the synaptosomal fraction allow at best limited conclusions concerning individual types of axon terminals because this fraction represents a mixture of synaptosomes originating from different neurons and containing different neurotransmitters. Furthermore, 5-HT receptors, including presynaptic receptors, may be assumed to be engaged in complexes of many newly discovered proteins interacting with G protein-coupled receptors (Bockaert et al., 2006); investigating the functions of these proteins has just started.

TABLE 11
Synopsis of 5-HT heteroreceptors in this article

The 5-HT heteroreceptors modifying the release of ACh, DA, NA, and GABA, i.e., the neurotransmitters that are the focus of the present article, in the CNS, their location on the axon terminals of the corresponding neuron and/or an interneuron, the CNS region(s) and species in which they have been identified, the method(s) and preparation(s) used for this purpose and the sections of this article in which details are given.

| Neuron | Receptor | Function ^a | Location ^b | | CNS Region ^c | | | Species ^d | | Method; Preparation ^e | | Section |
|--------|----------------------------------------|-----------------------|-----------------------|----------------------|-------------------------|--------|----------------------|----------------------|--------------|----------------------------------|----|---------|
| | | | Presyn. | Intern. ^f | Hippocampus | Cortex | Striatum | Others ^g | In vitro | In vivo | | |
| ACh | 5-HT _{1A} | + | | X | X | X | | r, gp | | | md | III.B. |
| | 5-HT _{1B} | - | X | X | X | X | CN | r, gp, m | sl, syn | | md | III.C. |
| DA | "5-HT ₂ " not subclassified | + | X? | X? | X | X | SC | r, gp | sl, syn | | md | III.D. |
| | 5-HT ₃ | - | X? | X | X | X | EC | r, h, gp | sl | | md | III.D. |
| | 5-HT ₄ | + | X? | X? | X | X | NBM | r | sl, syn | | md | III.E. |
| | 5-HT _{1B} | + | X? | X? | X | X | | r, gp | | | md | III.F. |
| NA | "5-HT ₂ " not subclassified | - | X | X? | X | X | | r, m | syn | | md | IV.D.2. |
| | 5-HT _{2A} | - | X? | X? | X ^h | X | | r | sl | | md | IV.D.3. |
| | 5-HT _{2C} | + | X? | X ⁱ | X | X | NAC | r | | | md | IV.C.2. |
| | 5-HT ₃ | + | X? | X ⁱ | X | X | | r | | | md | IV.D.3. |
| GABA | 5-HT _{1A} | + | | X? | X | X | NAC, OT | r | sl | | md | IV.D.3. |
| | 5-HT _{1B} | - | X | X | X | X | | r | sl, syn | | md | IV.E. |
| | 5-HT _{2A} | + | X? | X | X | X | HY, VTA | r, rb | sl | | md | V.B. |
| | 5-HT ₃ | + | X? | X | X | X | SC | r, rb | sl | | md | V.C. |
| GABA | 5-HT _{1A} | + | X? | X? | X | X | HY | r | sl | | md | V.D. |
| | 5-HT _{1B} | - | X | X | X | X | AM, PG | r | sl | SBP | md | V.D. |
| | 5-HT _{2A} | + | X ^h | X ^h | X | X | GLP, SE, SN, VM, VTA | r, h, m | sl | | md | VIC. |
| | 5-HT ₃ | + | X | X | X | X | AM, CP | r, m | SBP, sl, syn | | md | VID. |
| GABA | 5-HT ₃ | + | X | X ^{l,m} | X | X | | gp | sl | | md | VI.E. |
| | 5-HT ₄ | - | X | X ^{l,n} | X | X | | gp | sl | | md | VI.F. |

^a +, stimulation or facilitation; -, inhibition.
^b X, location (at least probable) of 5-HT heteroreceptors on axon terminals ("Presyn.", presynaptic) of the neuron under study and/or on an interneuron ("Intern.", X?), location suggested, but uncertain; in some cases, both alternatives are marked because they have been suggested with equally good arguments.
^c X, CNS regions investigated.
^d r, rat; gp, guinea pig; m, mouse; h, human; rb, rabbit.
^e md, microanalysis; sl, slice; syn, synaptosomes; SBP, synaptic bouton preparation, electrophysiological recording from dissociated cells.
^f GABAergic interneuron, unless stated otherwise in specific footnote.
^g CN, caudate nucleus; SC, spinal cord; EC, entorhinal cortex; NEM, nucleus basalis magnocellularis; NAC, nucleus accumbens; OT, olfactory tubercle; HY, hypothalamus; PG, periaqueductal gray; GLP, globus pallidus; SE, septum; SN, substantia nigra; VM, ventral midbrain; VTA, ventral tegmental area; AM, amygdala; CP, caudate putamen.
^h Prefrontal cortex.
ⁱ Neighboring neuron releasing a facilitatory transmitter, acting on dopaminergic nerve terminals.
^j Result of Blandina et al. (1988), but all other investigations agreed in that 5-HT₃ receptors are not involved in the control of DA release. Striatal slices used by Blandina's group may have been composed of a major portion of nucleus accumbens.
^k Cholinergic and/or somatodendritic on GABAergic neuron.
^l Presynaptic "interneuron."
^m Via M₁ and M₃ receptors at low 5-HT₄ receptor agonist concentration.
ⁿ Via M₂ receptors at high 5-HT₄ receptor agonist concentration.

According to the signal transduction mechanisms initiated by 5-HT receptor coupling to the respective G proteins or to the conformational change of the 5-HT₃ receptor channel leading to the increase in its cation permeability, activation of a presynaptic 5-HT_{1A} or 5-HT_{1B} heteroreceptor should result in inhibition of transmitter release, whereas activation of presynaptic 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, or 5-HT₄ heteroreceptors should lead to stimulation or facilitation of release. In agreement with these predictions, examples for such release-modulating heteroreceptors are inhibitory 5-HT_{1A} receptors on GABAergic nerve terminals and inhibitory 5-HT_{1B} receptors on cortical cholinergic, striatal dopaminergic, and hippocampal GABAergic nerve terminals as well as facilitatory 5-HT₃ receptors on hippocampal GABAergic and, presumably, 5-HT₄ receptors on hippocampal cholinergic nerve terminals (Table 11).

However, the complexity arising from the heterogeneity of release-modulating 5-HT receptors is even more pronounced when we consider that in many cases activation of a receptor does not induce the expected but rather the opposite effect (Table 11). Besides alternative coupling of the receptor to other signal transduction pathways, the involvement of the above-mentioned inhibitory GABAergic interneurons endowed with the modulatory 5-HT receptors comes into play, leading to the inversion of the expected effect. Thus, GABA released from GABAergic interneurons upon activation of facilitatory 5-HT heteroreceptors, e.g., 5-HT_{2A} and 5-HT₃ receptors, mediates inhibition of the release of other neurotransmitters such as prefrontal cortical dopamine and cortical acetylcholine release, respectively. Conversely, attenuated GABA release in response to activation of inhibitory 5-HT heteroreceptors, e.g., 5-HT_{1A} and 5-HT_{1B} receptors, on GABAergic interneurons is involved in "paradoxical" facilitation of hippocampal acetylcholine and striatal dopamine release, respectively (Table 11).

Another complex finding was that release of certain transmitters in the rat brain is inhibited and facilitated by the same type of 5-HT receptor. This has been demonstrated, e.g., for 5-HT₃ receptors modulating ACh and NA release and for both 5-HT_{2A} and 5-HT_{2C} receptors modulating DA release (Table 11). One explanation that has to be considered is that they are expressed in different brain regions or, if within the same brain region, on axon terminals originating from different neurons releasing the transmitter under study. Another possibility is that one effect is mediated by receptors present on the axon terminals releasing the transmitter under study, whereas the opposite effect is mediated by receptors expressed on GABAergic interneurons. However, all such attempts for an explanation are speculative.

As a rule, the function (inhibition or facilitation/stimulation of release) mediated by a certain presynaptic 5-HT receptor type does not differ among species or neurons on which it occurs. An example is the presyn-

aptic 5-HT_{1B} receptor (Table 11) encoded by orthologous species-specific genes, which inhibits ACh, DA, and GABA release in the CNS of rat, mouse, guinea pig, and human. Involvement of the same receptor type does not exclude the possibility that its pharmacological properties differ to a certain extent between the species (section III.C.2.).

Identification of 5-HT heteroreceptors modulating neurotransmitter release in the CNS is of particular importance because they are potential targets for new therapeutic compounds and they may already play a role in the mechanism of action of currently available drugs. Inhibition or facilitation of release can be accomplished with appropriate receptor agonists, antagonists, and, if relevant, inverse agonists at the 5-HT heteroreceptors listed in Table 11. However, it is a drawback that none of the 5-HT receptor types modulating the release of ACh, DA, and NA exclusively controls the release of just one of these neurotransmitters (Table 11), thus rendering it difficult to develop ligands with sufficient selectivity of action, i.e., drugs with relatively few side effects. Nevertheless, it is conceivable that a preference of modulation of the release of a certain transmitter can be achieved if, e.g., the receptor under consideration might exhibit constitutive activity. Evidence for a functional role of constitutive activity in certain brain regions has been obtained for, e.g., 5-HT_{2C} receptors (section IV.C.3.).

Table 11 also contains a list of the brain regions in which the various 5-HT receptor types have been found to modulate the release of the transmitters under study. These patterns of regions do not indicate that the receptors exclusively occur in the brain areas listed, but they reflect the selection of brain areas that are relevant for specific CNS disorders and that may become the targets of novel 5-HT receptor ligands as therapeutic drugs for these disorders. Such drugs may compensate for the shortage of a specific neurotransmitter in certain brain areas by increasing its release. Examples for such disorders are the deficiency in hippocampal and cortical acetylcholine, striatal dopamine, and hippocampal noradrenaline release in Alzheimer's disease, Parkinson's disease, and major depression, respectively. Accordingly, 5-HT receptor-mediated modulation of acetylcholine release has most frequently been studied in the hippocampus and cortex, modulation of DA release in the striatum, and modulation of NA release in the hippocampus (Table 11). As a perspective it is justified to state that 5-HT receptor ligands modifying transmitter release have a considerable potential not only for these three diseases but also, as mentioned in various sections of this article, in other central nervous system disorders such as schizophrenia, drug dependence, and anxiety.

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