Therapeutic Targeting of \( \alpha_7 \) Nicotinic Acetylcholine Receptors

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Abstract—The \( \alpha_7 \)-type nicotinic acetylcholine receptor is one of the most unique and interesting of all the members of the cys-loop superfamily of ligand-gated ion channels. Since it was first identified initially as a binding site for \( \alpha \)-bungarotoxin in mammalian brain and later as a functional homomeric receptor with relatively high calcium permeability, it has been pursued as a potential therapeutic target for numerous indications, from Alzheimer disease to asthma. In this review, we discuss the history and state of the art for targeting \( \alpha_7 \) receptors, beginning with subtype-selective agonists and the basic pharmacophore for the selective activation of \( \alpha_7 \) receptors. A key feature of \( \alpha_7 \) receptors is their rapid desensitization by standard “orthosteric” agonist, and we discuss insights into the conformational landscape of \( \alpha_7 \) receptors that has been gained by the development of ligands binding to allosteric sites. Some of these sites are targeted by positive allosteric modulators that have a wide range of effects on the activation profile of the receptors. Other sites are targeted by direct allosteric agonist or antagonists. We include a perspective on the potential importance of \( \alpha_7 \) receptors for metabotropic as well as ionotropic signaling. We outline the challenges that exist for future development of drugs to target this...
important receptor and approaches that may be considered to address those challenges.

**Significance Statement**——The z7-type nicotinic acetylcholine receptor (nAChR) is acknowledged as a potentially important therapeutic target with functional properties associated with both ionotropic and metabotropic signaling. The functional properties of z7 nAChR can be regulated in diverse ways with the variety of orthosteric and allosteric ligands described in this review.

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**I. Introduction**

“To move is all mankind can do and for such, the sole executant is muscle, whether in whispering a syllable or felling a forest.” These words by Charles Sherrington (Sherrington, 1947) draw attention to the most accessible and critically important synapses of the body: neuromuscular junctions. These synapses provide the starting point for all our studies of synaptic physiology and pharmacology. The nicotinic acetylcholine receptors (nAChRs) of the neuromuscular junction are the key mediators of this fundamental connection between the integrated output of the brain and our ability to manifest the desired output of our brain. These receptors were the first ligand-gated channels to be cloned and studied at the level of their single-channel current (reviewed in [Papke 2014]). An appreciation that nicotine was one of the most widely used and subtle but psychologically compelling drugs to which we are exposed motivated great interest in looking for homologous receptors in the brain.

**II. Diversity of Nicotinic Acetylcholine Receptor**

Early studies that probed the brain with radioligands identified two distinct and largely nonoverlapping populations of candidate receptors, with one population binding nicotine and acetylcholine (ACh) with high affinity and the other binding the snake toxin z-bungarotoxin (z-BTX) (Clarke et al., 1985). The biochemical isolation of the high-affinity nicotine-binding proteins of brain (Whiting and Lindstrom, 1986) was achieved at about the same time as the subunits for these receptors were cloned and heterologously expressed in *Xenopus* oocytes (Boulter et al., 1987). Despite having distinct pharmacological properties from the nAChRs of the neuromuscular junction, the high-affinity nicotine receptors of the brain were in many ways similar nAChRs. Functional receptors form as complexes of five subunits, which are heteromeric, requiring at least two different types of subunits (Cooper et al., 1991). One type, designated z subunits, contains essential primary elements of the ACh binding site, whereas other subunits contain complementary elements of the binding sites, which form at subunit interfaces. Each subunit in the nAChR pentameric complex has an extracellular domain followed by three transmembrane helices, a variable hydrophilic intracellular loop (Stokes et al., 2015), and a fourth hydrophobic transmembrane span. Eight different genes (CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRN2, CHRN3, and CHRN4) have been cloned from mammalian brain coding for the nAChR subunit proteins of these heteromeric neuronal receptors: z2, z3, z4, z5, z6, /2, /3, and /4 (Wang et al., 1996; Forsayeth and Kobrin, 1997; Gerzanich et al., 1997). Notably, z9 and z10 subunits have also been cloned (CHRNA9, and CHRNA10), and although z9 forms functional receptors when expressed alone, together these subunits can also form heteromeric receptors in unique locations outside the brain (Elgoyhen et al., 1994, 2001). Functional heteromeric neuronal-type receptors containing z2, z3, z4, or z6 must also contain a / subunit (/2 or /4) (Wang et al., 1996; Gerzanich et al., 1997, 1998; Dowell et al., 2003).

Although a relatively minor subtype of nAChR in the brain, receptors containing z3 subunits are of primary importance in autonomic ganglia, where they mediate the synaptic transmission through the ganglia (Wang et al., 2002). In the brain though, most high-affinity heteromeric receptors contain z4 subunits usually in combination with /2. Although these high-affinity nAChRs of the brain certainly have high structural and sequence homology to the receptors of the neuromuscular junction, they are not easily amenable to study in situ (Heinemann et al., 1990) due to fact that they are primarily located at presynaptic terminals (Wonnacott, 1997; Dani, 2001). The functional analogs of nAChRs in the brain that mediate the majority of fast excitatory transmission are structurally unrelated receptors activated by glutamate (Traynelis et al., 2010).

Although we began to gain some understanding about the high-affinity receptors in the brain facilitated by the use of heterologous expression systems (Deneris et al.,

**ABBREVIATIONS:** ACh, acetylcholine; AChBP, acetylcholine binding protein; z-BTX, z-bungarotoxin; CAP, cholinergic anti-inflammatory pathway; dEPP, 1,1-diethyl-4-phenylpiperazinium; DPP, dipicolyaminoprymidine; EVP-6124, (R)-7-chloro-N-quinalidin-3-ylbenzo[b]thiophene-2-carboxamide; FLIPR, Fluorescent Imaging Plate Reader; FRM-17874, (R)-7-fluoro-N-quinalidin-3-ylbenzo[b]thiophene-2-carboxamide; GATI07, 4-(4-bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopental[c]quinoline-8-sulfonamide; IL, interleukin; KC-1, 5'-phenylanabaseine, 6'-phe

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**Table 1: List of Abbreviations**

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ACh</td>
<td>Acetylcholine</td>
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<td>AChBP</td>
<td>Acetylcholine binding protein</td>
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<td>z-BTX</td>
<td>z-Bungarotoxin</td>
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<td>CAP</td>
<td>Cholinergic anti-inflammatory pathway</td>
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<tr>
<td>dEPP</td>
<td>1,1-diethyl-4-phenylpiperazinium</td>
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<tr>
<td>DPP</td>
<td>Dipicolyaminoprymidine</td>
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<td>EVP-6124</td>
<td>(R)-7-chloro-N-quinalidin-3-ylbenzo[b]thiophene-2-carboxamide</td>
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<td>FLIPR</td>
<td>Fluorescent Imaging Plate Reader</td>
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<td>FRM-17874</td>
<td>(R)-7-fluoro-N-quinalidin-3-ylbenzo[b]thiophene-2-carboxamide</td>
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<td>GATI07</td>
<td>4-(4-bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopental[c]quinoline-8-sulfonamide</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<td>KC-1</td>
<td>5'-Phenylanabaseine, 6'-Phenyl-3,4,5,6-tetrahydro-2,2'-bipyrindine</td>
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<tr>
<td>MALA</td>
<td>Methylylpyridinone</td>
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<td>nAChR</td>
<td>Nicotinic acetylcholine receptor</td>
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<tr>
<td>2-NDEP</td>
<td>1,1-diethyl-4-naphthalenyl-2-ylpiperazin-1-ium iodide</td>
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<tr>
<td>NOR</td>
<td>Novel object recognition</td>
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<td>OA</td>
<td>Orthosteric activation</td>
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<td>PAM</td>
<td>Positive allosteric modulator</td>
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<td>PHA-543,613,N(-[3R]-1-azabicyclo[2.2.2]oct-3-yl)furo[2,3-c]pyridine-5-carboxamide</td>
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<td>PHA-709829,N(-[3R]-1-azabicyclo[3.2.1]oct-3-yl)furo[2,3-c]pyridine-5-carboxamide</td>
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<td>PNU-282987,N(-[3R]-1-azabicyclo[2.2.2]octan-3-yl)-4-chlorobenzamide</td>
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<td>PNU-120596,1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea</td>
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<tr>
<td>RIC-5</td>
<td>Resistance to cholinesterase 3</td>
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<tr>
<td>STAT3</td>
<td>Signal transducer and activator of transcription 3</td>
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<tr>
<td>TFN</td>
<td>Tumor necrosis factor-α</td>
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1988; Wada et al., 1988; Duvoisin et al., 1989; Papke et al., 1989a,b; Luetje et al., 1990; Papke, 2014), for a number of years the nature of the α-BTX binding sites in the brain remained a mystery (Carbonetto et al., 1978; Hunt and Schmidt, 1978; Oswald and Freeman, 1981; Marks et al., 1986; Wonnacott, 1986; Schoepfer et al., 1990) until the cloning of the z7-subunit gene (CHRNA7) (Bertrand et al., 1992; Seguela et al., 1993). An additional α-BTX neuronal nAChR subunit, z8, was also discovered (Gotti et al., 1994). It is expressed in chick retina where it forms functional receptors, but there is no mammalian homolog.

One of the first unique properties noted for z7 receptors was that they formed functional receptors without the coexpression of additional complementary subunits, suggesting the potential presence of five low-affinity ACh binding sites at the z7–z7 subunit interfaces (Palma et al., 1996). It has been shown that z7 receptors have intrinsically low probability of opening in response to ACh alone because of the existence of desensitized states associated with high levels of agonist occupancy (Uteshev et al., 2002; Williams et al., 2011a,b; Williams et al., 2012; Andersen et al., 2013), as reviewed in Papke and Lindstrom (2020). When activated by ACh alone, the z7 nAChR has other unique physiologic and pharmacological properties that distinguish it, including a high permeability to calcium (ratio of calcium to sodium permeability ≈ 10), rapid and reversible desensitization, and pronounced inward rectification (Seguela et al., 1993). In contrast, the ratio of calcium to sodium permeability of the nAChR in rat ganglionic neurons (Adams and Nutter, 1992) has been shown to be only 0.65:1.

The z7 subunit is highly expressed in the hippocampus and hypothalamus (Seguela et al., 1993; Dominguez del Toro et al., 1994) and has functionally important expression in non-neuronal tissues, such as cells of the immune system (Wang et al., 2003). z7 receptors are also selectively activated by choline (Papke et al., 1996) and are therefore ideally suited to respond to manifestly different kinds of signals, including localized tissue damage and paracrine signals. Human z7 receptors expressed in Xenopus oocytes have functional properties that correspond well to those of z7 responses of cultured hippocampal neurons (Lindstrom et al., 1984; Alkondon et al., 1994; Alkondon and Albuquerque, 1995; Papke and Porter Papke, 2002) and native neuronal tissues (Uteshev et al., 2002). However, functional expression of z7 receptors in transfected cells was found to be difficult to achieve until the discovery of the molecular chaperone resistance to cholinesterase 3 (RIC-3) (Halevi et al., 2003), which allowed for functional expression in a variety of cell lines (Williams et al., 2005). Subsequently, NACHO, an alternative chaperone protein, was discovered (Gu et al., 2016), which may be at least as important as RIC-3 for nAChR function in the brain (Matta et al., 2017; Deshpande et al., 2020).

In this review, we focus primarily on pharmacological tools used to study z7 nAChRs. However, it should also be noted that transgenic animals and gene-delivery methodology provide alternative supplementary approaches for the study of z7 function in vivo. z7 knockout mice have widely been used, both for the study of z7 in the central nervous system (Stoker and Markou, 2013; Koukouli et al., 2016) and in the periphery (Alsharari et al., 2013). Additionally, conditional knockouts of z7 have been generated using the Cre-Lox approach (Hernandez et al., 2014). z7 has also been studied with animals made suitable for optogenetic stimulation of cholinergic fibers (Grybko et al., 2011) and with z7 gene delivery to increase z7 expression in specific brain regions (Ren et al., 2007). Immunohistochemistry is a common tool used to sort out the roles for specific receptor subtypes, but the use of the z7 knockout mice has revealed that z7 antibodies should be used with caution since they detect putative z7 protein signals in knockout animals (Herber et al., 2004; Garg and Loring, 2017). As z7 antibodies have questionable reliability, fluorescently tagged z7 proteins (Palma et al., 2002) have been shown to be useful tools (Lee et al., 2009; Rogers et al., 2012).

III. z7 Receptors as Therapeutic Targets

Alzheimer disease, Parkinson disease, Lewy-body dementia, and schizophrenia are all characterized by decreased expression of nAChRs in the brain (Schröder et al., 1991a,b; Lange et al., 1993; Freedman et al., 1995; James and Nordberg, 1995; Perry et al., 1995; Nordberg et al., 1997; Spurden et al., 1997; Gotti et al., 2006). Normal aging results in a loss of cholinergic function and an impairment in normal learning ability that can be temporarily modulated by nicotine or nicotinic compounds (Arendash et al., 1995; Levin and Torry, 1996; Prendergast et al., 1997). Based on these types of data, a number of attempts are ongoing to develop clinical strategies for treatment of both disease-related and senile dementia that target neuronal nAChRs (Bhat et al., 1990; Weinstock, 1995; Wilson et al., 1995; Snaedal et al., 1996; Kihara et al., 1997; Robbins et al., 1997; Woodruff-Pak and Hinchliffe, 1997; Zamani et al., 1997; Russo et al., 2012, 2014). Unfortunately, to date, no trials have been successful at bringing a drug to market. In some cases, this may have been due to lack of efficacy, and in other cases it may have been due to unforeseen adverse effects (Yang et al., 2017; Manetti et al., 2018; Terry and Callahan, 2019, 2020). It remains to be the case that new discoveries and research directions are required to provide some hope that future trial outcomes might be improved.

Drugs that appear active in preclinical models for cognitive disorders typically have significant efficacy
for activation of the α7 ion channel (Briggs et al., 2009; Pieschl et al., 2017). A second major new direction for the development of α7-based therapeutics is for the treatment of inflammatory diseases and pain (Wang et al., 2003). Research in this area began with the discovery of the role of α7 nAChR in the vagal-mediated cholinergic anti-inflammatory pathways (CAPs) (Borovikova et al., 2000; van Westerloo et al., 2006; Pavlov et al., 2007; Rosas-Ballina et al., 2009; Rosas-Ballina and Tracey, 2009). Discovery of the CAPs provided impetus to discover drugs for inflammatory diseases and inflammation-related pain. This also gave compelling motivation to reconsider our view of α7 and other nAChRs strictly as mediators of transmembrane signal transduction. The non-neuronal cells that mediate α7’s control of inflammation have not been shown to generate α7-mediated currents. Moreover, some α7-targeting ligands that can effectively control inflammation are “silent agonists,” ligands with little or no efficacy for ion-channel activation but the ability to induce nonconduction states that may be associated with signal transduction (Thomsen and Mikkelsen, 2012a; Clark et al., 2014; Papke et al., 2015a; van Maanen et al., 2015; Quadri et al., 2018a). The role of α7 in CAP involves signaling through the Jak2/STAT3 pathway; decreasing levels of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 through inhibition of nuclear factor κB activation; and increasing levels of anti-inflammatory cytokines, such as IL-10 (de Jonge et al., 2005; Chatterjee et al., 2009; Marrero and Bencherif, 2009; Egea et al., 2015; Zhang et al., 2017). Evidence for the role of the Jak2/STAT3 signaling in CAP has come primarily from studies that have shown a correlation between the effects of nicotine (Li et al., 2020b) or α7-selective agonists (Kraft et al., 2017; Zhang et al., 2020b) on inflammation-associated cytokines and the relative levels of phosphorylated and nonphosphorylated Jak2 and STAT3 with Western blot analyses. These effects were shown to be sensitive to α7 antagonists (de Jonge et al., 2005; Li et al., 2020b; Zhang et al., 2020b), small interfering RNA knockdown of α7 (Fei et al., 2017), or the Jak2 antagonist AG490 (de Jonge et al., 2005; Fei et al., 2017; Kraft et al., 2017). However, α7 nAChR has a large and diverse intracellular interactome (Paulo et al., 2009), and it remains to be determined whether there is a direct interaction of the α7 nAChR protein itself with the Jak2/STAT3 pathway or whether the effects rely on other intracellular intermediates.

Even the α7 agonists that are most efficacious for producing channel activation elicit only brief and infrequent ion-channel currents and are far more effective at inducing and, in some cases, maintaining the receptors in nonconducting states, which have traditionally been dismissed as desensitized and functionally unimportant (Williams et al., 2011b). However, accumulating data suggest that the prejudice that the ligand-bound nonconducting states of nAChRs are all functionally unimportant should be discarded. Just as conformational changes promoted by ligand binding extend through the transmembrane domains, they must also extend into the intracellular domain and likely regulate signal-transduction processes in both neuronal and non-neuronal cells.

In this review, we will cover multiple pharmacological approaches to the therapeutic targeting of α7 nAChRs and how they have evolved as our perspectives have improved over the last 2 decades to include targeting the orthosteric agonist (i.e., ACh) binding site as well as more recently discovered sites for allosteric modulators (Williams et al., 2011c) and activators (Horenstein et al., 2016; Gulsevin et al., 2019; Toma et al., 2019), also considering metabotropic as well as ionotropic signaling.

IV. α7-Selective Agonists

A. Older Ligands and Structures. The first and arguably most direct approach for the selective targeting of α7 was with the identification of α7-selective agonists that activated α7 receptors but not other nAChR subtypes. One of the first such agents to be identified was GTS-21 (3-(2,4-Dimethoxy)benzylidene)-anabaseine, GTS-21 is a benzylidene anabaseine, Fig. 2, top right, where R1 and R2 are OCH3 (methoxy) groups) (Meyer et al., 1997). GTS-21 is a partial agonist for α7 receptors that has remained one of the standard drugs in the field, with more than 20 PubMed citations in 2020 alone. However, it should be noted that GTS-21 is something of a complicated drug in that it inhibits 5HT3 receptors (Gurley and Lanthorn, 1998) and other nAChR subtypes (Briggs et al., 1997) and produces protracted desensitization of α7 receptors after activation (Papke et al., 2009). As we will discuss later, some of these unusual properties may very well be why the drug continues to be useful as the field is expanding the extent of potential indications.

The range of α7-selective agonists widened rapidly after the identification of GTS-21, as numerous drug companies established programs in the area. Progress in the field was presented in a paper published in 2008 (Horenstein et al., 2008) that discussed numerous published structures (Fig. 1A) and, by comparing selective and nonselective drugs of multiple structural families, identified three structural motifs that could be applied to a nonselective agonist to produce an analog that was α7-selective. One motif was associated with the hydroxyl group that was present in the α7-selective agonist choline but not present in the nonselective agonist ethyl-trimethyl-ammonium (Papke et al., 1996). A second was identified as the “tropane...
Fig. 1. Compounds used to determine the structural motifs of \( \alpha_7 \)-selective agonists as presented in (Horenstein et al. 2008). (A) \( \alpha_7 \)-Selective agonists are in red boxes compared with related compounds that are not selective for \( \alpha_7 \). The highlighted compounds are tilorone (2,7-bis[2-(diethylamino)ethoxy]fluoren-9-one dihydrochloride) (Briggs et al., 2008); A-844606 (2-(5-methyl-hexahydro-pyrrolo[3,4-c]pyrrol-2-yl)-xanthen-9-one) (Briggs et al., 2008); ACME (cis-1-methyl-2,3,3a,4,5,9,6-hexahydro-1H-pyrrolo[3,2-a]isoquinoline) (Papke et al., 2005b); S 24795 (2-[2-(4-bromophenyl)-2-oxoethyl]-1-methyl pyridinium) (Lopez-Hernandez et al., 2007); tropamine \((\text{1R,5S})-8\text{-methyl-8-azabicyclo[3.2.1]octan-3-yl})\) (Papke et al., 2005a); tropisemine \((\text{1R,5S})-8\text{-methyl-8-azabicyclo[3.2.1]octan-3-yl})\) (Papke et al., 2005a); tropane \((\text{1R,5S})-8\text{-methyl-8-azabicyclo[3.2.1]octan-3-yl})\) (Papke et al., 2005a); cocaine methiodide, \((\text{methyl (1R,2R,3S,5S)-3-benzoyloxy-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane-2-carboxylate})\) (Francis et al., 2001); AR-R17779 \((\text{(-)-Spiro-1-azoniabicyclo[2.2.2]octane-3,5\text{-oxazolidin-2\text{-one})})\) (Mullen et al., 2000; Papke et al., 2004); JN403 \((\text{(S)-(1-azoniabicyclo[2.2.2]octan-3-yl})\text{-carbamic acid (S)-1-2-fluoro-phenyl\text{-ethyl ester}})\) (Feuerbach et al., 2007); ABBF \((\text{N-(3R)-1-azoniabicyclo[2.2.2]octan-3-yl})\text{-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxylic acid})\) (Boess et al., 2007); PNU-282987 (Bodnar et al., 2005); PHA-543,613 (Acker et al., 2008); compound 15b \((\text{1-3-[2-(benzo[b]thiophen-2-yl)-2-oxoethyl]-1-azoniabicyclo[2.2.2]octane})\) (Tatsumi et al., 2004); compound 25 \((\text{R})\text{-3\text{-[5-chlorothiophen-2-yl]spiro-1-azoniabicyclo[2.2.2]octane-3,5\text{-1',3\text{'oxazolidin-2\text{-one})})})\) (Tatsumi et al., 2004); compound 23 \((\text{(R)-3\text{-[5-iodothiophen-2-yl]spiro-1-azoniabicyclo[2.2.2]octane-3,5\text{-1',3\text{'oxazolidin-2\text{-one})})})\) (Tatsumi et al., 2004); PSAB-OFP, \((\text{(R)-5-phenylspiro-1-azoniabicyclo[2.2.2]octane-3,5\text{-1',3\text{'oxazolidin-2\text{-one})})})\) (Tatsumi et al., 2004); compound 22 \((\text{N-3[2-fluoro-phenyl\text{-ethyl ester}})\) (Biton et al., 2007); SSR-180711 (1,4-diazabicyclo[3.2.2]nonane-4-carboxylic acid, 4-bromophenyl ester) (Biton et al., 2007); TC-1698 (2-(3-pyridyl)-1-azabicyclo[3.2.2]nonane) (Marrero et al., 2004); and PHA-709,829 (Acker et al., 2008). (B) Modified quinuclidine \( \alpha_7 \)-selective agonists: quinuclidinol \((1\text{-azoniabicyclo[2.2.2]octane})\) (Horenstein et al., 2008); methyl-quinuclidine \((1\text{-azoniabicyclo[2.2.2]octane iodide})\) (Horenstein et al., 2008); and BQNE \((\text{(E)-3-benzylidene-1-azoniabicyclo[2.2.2]octane chloride})\) (Horenstein et al., 2008).
motif” based on the structural dissection of tropisetron (Papke et al., 2005a). The third, “benzylidene motif,” was identified in distinguishing the α7-selective GTS-21 from the parent compound anabaseine, which activates multiple nAChR subtypes (Kem et al., 1997). In the 2008 study, it was shown that the nonselective agonist quinuclidine could be modified with any of the three motifs identified to generate a new α7-selective compound (Fig. 1B) (Horenstein et al., 2008).

B. Identification via Compound Screening. The process of identifying selective agonists typically involves many steps, and with large-scale programs the first step is running radioligand screens with cells or tissues expressing the target receptor and off-target receptors of interest. This first step, which identifies high-affinity ligands but does not distinguish between agonist and antagonist, must then be followed up with functional assays. Large-scale programs have generally relied on high-throughput screening with automated measurements using transfected cell lines and fluorescent indicators that typically measure changes in intracellular calcium, which is presumed to be a downstream reporter of receptor activation. In some cases, especially in smaller studies, these are followed up with patch-clamp or voltage-clamp studies. However, in most large-scale studies no actual raw data are provided, only tabulated summaries. Although these approaches are generally thought to be amenable to the study of heteromeric receptors expressed in cell lines, they are less suitable for the study of α7 receptors. Even when applied to heteromeric receptors, these approaches can lead to erroneous conclusions due to the pharmacological differences in receptors with varying subunit stoichiometry, a factor that cannot be directly controlled in transfected cells. For example, the initial characterization of Sazetidine-A (Xiao et al., 2006) claimed that it desensitized α4β2 nAChRs without activating them. However, it was later shown that this was only the case for the receptor configuration with three α subunits and two β subunits (Zwart et al., 2008). For receptors with the reverse subunit ratio, Sazetidine-A is a potent full agonist.

Because of its special properties discussed above, α7 nAChRs remain difficult to study with high-throughput cell-based assays, which has often led to compromised approaches, such as the use of nondesensitizing α7-5HT3 chimeric receptors (Craig et al., 2004; O’Donnell et al., 2010) or by amplifying responses with an allosteric modulator (Arunangvisalan et al., 2015; Kaczanowska et al., 2017). However, both of these approaches yield receptors with properties typical of native α7 receptors activated by ACh (Dinklo et al., 2006; Gee et al., 2007; Miller et al., 2020; Papke and Lindstrom, 2020). Likewise, high-throughput Fluorescent Imaging Plate Reader (FLIPR) assays (Dunlop et al., 2007), which rely on calcium signals (Skidmore et al., 2012; Zanaletti et al., 2012b; Hill et al., 2016; Iwuagwu et al., 2017), are most likely reporting downstream signaling and not ion-channel currents (King et al., 2018; Miller et al., 2020) and may suggest a significantly higher potency than what may be obtained with traditional electrophysiological methods (Haydar et al., 2009). Because of these limitations, many of both older studies (Horenstein et al., 2008) and more recent work (Tietje et al., 2008; Malysz et al., 2010; Marrero et al., 2010; Prickaerts et al., 2012; Yamauchi et al., 2012; Zanaletti et al., 2012a; Feuerbach et al., 2015; Tang et al., 2015) identifying α7-selective agonists rely on receptors expressed in Xenopus oocytes. Although α7 receptors give large reliable responses when expressed in oocytes, there are nonetheless also special concerns that are not always well addressed in these studies. For example, most often responses are measured in terms of peak currents only, and in the case of α7 receptor responses, the amplitude of peak currents is more a function of the synchronization to receptor activation that occurs in advance of the full drug application than it is a measure of the concentration dependence of receptor activation (Papke and Thinschmidt, 1998; Papke and Porter Papke, 2002). Additionally, the reversibility of drug-induced desensitization and the cumulative effects of desensitization with repeated drug applications are concerns that are seldom well addressed or even considered (for example see (Prickaerts et al. 2012)).

The basic methods and conclusions of the studies that characterized the compounds in Fig. 1 have been previously summarized (Horenstein et al., 2008). Although some of these compounds like cis-1-methyl-2,3,3a,4,5,9b,-hexahydro-1H-pyrrololo[3,2-h]isoquinoline (Papke et al., 2005b), PHA-709829 (Acker et al., 2008), and the cinnamylidene anabaseines (de Fiebre et al., 1995; Meyer et al., 1998) have proven to be useful experimental tools and are cited in 129 and 165 papers, respectively. Additionally, as a drug already approved for use in humans, tropisetron has been tested with humans suffering from schizophrenia for its ability to improve deficiencies in auditory gating (Koike et al., 2005; Zhang et al., 2012). As will be discussed in detail below, two forms of α7 activity, channel-based and signal-transduction, may point separately to cognitive functions and regulation of the immune system, respectively (Briggs et al., 2009; Horenstein and Papke, 2017). One application that may fall in between is in regard to the symptomatic management of schizophrenia, in which the desensitizing partial agonist GTS-21 has received particular attention (Martin et al., 2004; Martin and Freedman, 2007; Kem et al., 2018). Although smoking is on a slow decline in the general population, the incidence of smoking remains especially high in people...
with schizophrenia (Mallet et al., 2017), in which it seems that smoking serves as a sort of self-mediation, providing some of the relief that might be obtained with \( \sigma \)7-based therapies (Mackowick et al., 2012). Unfortunately, the population of schizophrenics that smoke probably have developed the same kind of dependence that normal smokers must deal with, a dependence that is normally associated with the effects of nicotine on the heteromeric receptors in the brain (Papke et al., 2020a). Therefore, the management of the smoking behavior in schizophrenics may require novel cessation therapies that address both \( \sigma \)7 stimulation and attenuation of the dependence that is due to the heteromeric nAChRs.

**C. New Compounds and Structures.** Shown in Fig. 2 are \( \sigma \)7-selective agonists that have been identified since the 2008 study. Data related to these compounds are summarized in Table 1. It should be noted that this survey omits two agents that are reputed to be \( \sigma \)7-selective agonists and have actually been used in clinical trials, (4\( \sigma \))-4-(5-phenyl-1, 3, 4-thiadiazol-2-yloxy)-1-azatricyclo[3.3.1.1\( ^{3, 7} \)]decane (Haig et al., 2018) and R3487/MEM3454 (Huang et al., 2014), because there are no published structures or basic research published to establish their \( \sigma \)7 activity. It should also be noted that many of the compounds in Fig. 2 are the leads from studies of multiple compounds in the studies referenced in Table 1, as indicated. The 19 compounds shown and listed were drawn from a total of roughly 400 actually reported. A common structural feature of \( \sigma \)7-selective agonists is the presence of a nitrogen center that is sufficiently basic to be protonated. The resulting ammonium group is what traditionally has been considered the minimal pharmacophoric element. However, a few possible exceptions to this “rule” have emerged with the DPP compounds discussed below. Some of the members of this family feature a core aminopyrimidine ring, which has been considered to have sufficiently weak basicity based on NMR titrations, that they may bind to the receptor in unprotonated form. In addition to those compounds presented in Fig. 2 and described in Table 1, there have been several other notable medicinal chemistry characterizations, including an in situ click-chemistry study using acetylcholine binding protein (AChBP) (Yamauchi et al., 2012), a family of 4-heteroarylaminol-1’-azaspirocoloxazole-5,3’-bicyclo[2.2.2]octanes (Hill et al., 2016), a series of spirocyclic quinuclidinyl-d2-isoxazoline derivatives (Dallanoce et al., 2011), spiroguanidine-derived \( \sigma \)7 neuronal nicotinic receptor partial agonists (Hill et al., 2017), and a series of agonists with a 1,3,4-oxadiazol-2-amine core. These studies account for an additional 124 compounds. With so many potential compounds available, an important question is whether any of them really stand out as major new discoveries.

**D. Functional Properties of \( \sigma \)7-Selective Agonists.** One compound that has drawn a fair amount of attention since it was first published in 2012 and actually advanced to clinical trials for Alzheimer disease (Barbier et al., 2015) and schizophrenia (Preskorn et al., 2014) is EVP-6124. One thing that made EVP-6124 stand out in its initial characterization was the claim that EVP-6124 (as well as its derivative FRM-17874), based on the study of peak currents in Xenopus oocytes in addition to its acting as an agonist of \( \sigma \)7, could at low concentration potentiate the activity of the normal neurotransmitter ACh. As noted above, there are caveats and limitations to the analysis of \( \sigma \)7 peak currents that are not always appreciated. In the case of the putative potentiation of ACh responses by EVP-6124, as shown in Fig. 3, this is not a special property of EVP-6124 but rather a special property of \( \sigma \)7 receptors. Essentially the same effect can be obtained by priming the ACh responses with a low concentration of ACh to give a larger (i.e., more synchronized) peak current response.

For the most part, all of the recent characterizations of \( \sigma \)7 agonists have focused solely on receptor ion-channel activation. One lesson that might be learned from GTS-21, a compound used in well over 200 studies, is that there is more to a potentially useful drug than how well it produces transient activation of the channel. Like all nAChRs, \( \sigma \)7 receptors have multiple conformational states, including several nonconducting states that, although classified as desensitized, may be associated with the signal-transduction processes that underlie CAP, which is something that will later be discussed in greater detail under the topic of silent agonists. As noted above, in addition to activating \( \sigma \)7 receptors, GTS-21 produces desensitization that persists for a significant period of time (Papke et al., 2009). Of all of the studies referenced in Table 1, the desensitizing properties of the agents were only considered of interest with the DPP compounds (Camacho-Hernandez et al., 2019). Note that these compounds were originally introduced as 4,6-disubstituted-2-aminopyrimidines; however, with further consideration of their structures, (P. Taylor personal communication) the nomenclature of these compounds should be based on their core structure as N,N-dipicolyl amino pyrimidines. The family can further be divided into “DPP” compounds and “2-amino-dipicolylaminopyrimidine” compounds, wherein the prefix stands for an additional amino substitution at position 2 of the pyrimidine ring.

**E. Translational Development.** Notwithstanding the DPP compounds, which certainly merit more detailed studies and evaluation with in vivo models, it is unclear whether the hundreds of new \( \sigma \)7-selective agonists identified since 2008 have really advanced the field very far. None have really proven themselves in clinical trials, and as experimental tools, it remains to be seen whether any will surpass the utility of agents
Fig. 2. Recently identified putative α7-selective agonists (see Table 1): A-582941 (2-methyl-5-[6-phenylpyridazin-3-yl]octa- hydropyrrole[3,4-c]pyrrole) (Tietje et al., 2008); ABT-107, 5-6-(3R)-1-azabicyclo[2.2.2]oct-3-yl[4-(5-phenyl-1H-1,3,4-thiadiazol-2-yl)oxazol-2-amine] (Haig et al., 2018); AQW051, (R)-2-(6-p-tolyl-pyrindin-3-yl)-1-aza-4H-pyrrole (2.2.2)octane (Feuerbach et al., 2015); AZD0328, (20 R)-spiro[1-azabicyclo[2.2.2]octane]-2,20(30 H)-furo[2,3-b]pyridine n-tartrate (Sydserff et al., 2009); BMS-933043, (2R)-N-(6-H-imidazol-1-yl)-4-pyrimidinyl-4'H-spiro[4-azabicyclo[2.2.2]octane-2,5'-[1,3]oxazol]-2'-amine (Cook et al., 2016; Pieschl et al., 2017); BMS-910731, N-(6-methyl-1,3-benzoxazol-2-yl)-3,5-dihydro-4-azaspiro[2.2.2]octane-2,4-imidazol-2'-amine (Hill et al., 2017); BMS-902483, (1S,2R,4S)-(2H)-isoquinolin-3-yl-4'-azaspiro[2.2.2]octane-2,5'-[1,3]oxazol]-2'-amine (Hill et al., 2016, 28105289) (Cook et al., 2016); Br-IQ17B, N-(3R)-1-azabicyclo[2.2.2]octane-2-yl]-1H-imidazol-1-yl-5-bromoindolizine-2-carboxamide (Tang et al., 2015); CP-810,123, 4-(5-methyl)[4,5-b]pyridin-2-yl)-1,4-diazabicyclo[3.2.2]nonane (ODonnell et al., 2010); EVP-6124 (Prickaerts et al., 2012); FRM-17874 (Stoiljkovic et al., 2015); NS6784, 2(1,4-diazabicyclo[3.2.2]nonan-4-yl)-5-phenyl-1,3,4-oxadiazole (Briggs et al., 2009); SEN12333, WAY-317538 5-morpholin-4-yl-pentanoic acid (4-pyridin-3-yl-phenyl)-amide (Roncarati et al., 2009); SEN15924, WAY-361789, 5-(4-acetyl[1,4]diazepan-1-yl)pentanoic acid (5-(4-methoxyphenyl)-1H-pyrrozol-3-yl) amide (Zanaletti et al., 2012b); SEN78702, WYE-308775, N-[5-(5-fluoropyrindin-3-yl)-1H-pyrrozol-3-yl]-4-piperidin-1-ylbutyramide (Zanaletti et al., 2012a); TC-7020, [5-methyl-N-(3-pyridin-3-yl)methyl]-1-azabicyclo[2.2.2]oct-3-yl|thiophene-2-carboxamide (Marrero et al., 2010); and 5-1-(1S,3R)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl)-1H-1,2,3-triazol-4-yl)-1H-indole (TTIn-1) and related compounds (Arunrungvichian et al., 2015).
TABLE 1
Putative z7-selective agonists (see Figure 2 for structures)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Summary*</th>
<th>Reference</th>
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<tr>
<td>A-582941</td>
<td>Expression system: <em>Xenopus</em> oocytes and GH4C1 cells for z7, z3* and z4* receptors in HEK cells with Ca2+ FLIPR assay. Binding studies with human brain membranes. Effects on 5HT3 receptors: not studied. Summary: partial agonist of z7 with relatively little activation of other nAChR tested. Positive cognitive effects (inhibitory avoidance) in rats blocked by NS6740.</td>
<td>(Tietje et al., 2008)</td>
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<tr>
<td>ABT-107</td>
<td>Expression system: oocyte z7 compared with z3/z4/z4/2 and z4/4 in cell lines. Also tested in brain slices. Effects on 5HT3 receptors: no activity. Summary: efficacious (86%) partial agonist for human z7 (EC50 = 50–90 nM). Protected cultured cortical neurons from glutamate toxicity. Numerous follow-up studies.</td>
<td>(Malsys et al., 2010)</td>
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<td>AZD0328</td>
<td>Expression system: receptor binding with transfected HEK cells compared with nicotine binding in rat brain, PC12 cells, or muscle-type BC3H1 cells. <em>Xenopus</em> oocytes rat and human z7, rat and human z4/z4, and human z3/z4. Effects on 5HT3 receptors: partial agonist (12%) EC50 = 474 ± 173 nM. Summary: efficacious (64%) partial agonist for human z7 (EC50 = 150 ± 40 nM). Low efficacy on z4/z4 receptors. Positive effects in NOR. Increases activity of midbrain dopamine neurons. Some follow-up studies on memory and dopaminergic denervation.</td>
<td>(Sydserff et al., 2009)</td>
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<tr>
<td>AQW051</td>
<td>Expression system: binding studies in SH-SY5Y cells and rat brain membranes. <em>Xenopus</em> oocytes voltage clamp for z7, all others FLIPR from cell lines. No actual data shown. nAChR subtypes studied: z7, z2/z2, z3/z4, z3/z4, z2/z4, z3/z4, z4/z4. Effects on 5HT3 receptors: nature of activity ill-defined, claimed 500-fold less potent than for z7. Summary: claimed efficacy for z7 of 75%, but no data shown, claimed EC50 = 40 nM. Positive effects with NOR and water-maze performance with aged rats. Pharmacokinetics and tolerability were evaluated in three phase I placebo-controlled studies in 180 healthy subjects with relatively few adverse effects. Numerous follow-up studies.</td>
<td>(Feuerbach et al., 2015)</td>
</tr>
<tr>
<td>BMS-933043</td>
<td>Expression system: cell line FLIPR. Methods described only in supplemental material, and actual no data shown in manuscript or supplement. Binding in HEK cell membranes. Electrophysiology with patch-clamp and dynaflow (Cellectrion) perfusion system. nAChR subtypes studied: z1/z1/z4, z3/z4, z4/z2, z7. Effects on 5HT3 receptors: putatively low potency compared with z7. Summary: impossible to evaluate the quality of the data. This is a particular concern of the z7 electrophysiology. Positive effects reported with NOR.</td>
<td>(King et al., 2017a)</td>
</tr>
<tr>
<td>BMS-902483</td>
<td>Expression system: Binding with z7-transfected cells. Electrophysiology on z7 with patch-clamp and dynaflow (Cellectrion) perfusion system. Limited data shown. nAChR subtypes studied: z1/z1/z4, z3/z4, z4/z4, z7. Binding data only for non-z7. Effects on 5HT3 receptors: antagonist IC50 = 0.51 nM. Summary: z7 partial agonist (62%) EC50 = 0.24 μM. Limited data on selectivity. Positive effects on NOR, auditory gating, and other behavioral tests. Augmented LTP. NOR effects blocked by NS6740.</td>
<td>(Cook et al., 2016)</td>
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<tr>
<td>BrIQ17B</td>
<td>Expression system: <em>Xenopus</em> oocytes for z7 (peak currents) and other subtypes. Radioisotope ligand binding. Western blots, whole-cell recordings of hippocampal culture neurons also used. nAChR subtypes studied: z3/z4, z4/z2, z7, and GABA_A receptors. Effects on 5HT3 receptors: inhibition only at high conc. Summary: partial (64%) agonist, EC50 1.8 ± 0.2 (based on peak currents). Lower conc. inhibited ACh-evoked responses. Inconsistency in data since in one case 0.3 μM produced no apparent response when applied prior to ACh, yet in CRC study 0.3 μM produced approximately 7% maximal response.</td>
<td>(Tang et al., 2015)</td>
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<td>CP-810,123</td>
<td>Expression system: binding assay for rat z7 nAChRs expressed in GH4C1 cells using [125I]BTX as the radioligand. High-throughput FLIPR-based functional assay that used SH-EP1 cells expressing the z7/5-HT3 chimera. nAChR subtypes studied: only z7/5HT3 chimera studied directly z4/z2, and z3/z4 inferred from binding studies with rat brain or IMR32 cells, respectively. Effects on 5HT3 receptors: binding assay for human 5-HT3 receptors expressed in HEK293 cells using [3H]LY271882 as the radioligand. Summary: Large family off compounds studies with CP-810,123 identified as most promising lead. Data based on chimera reported an EC50 on this unnatural receptor of 16.4 nM with an Imax 105% that of 50 μM nicotine. No data are shown. Tested in auditory gating yielded inconclusive results.</td>
<td>(O'Donnell et al., 2010)</td>
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<td>DPP compounds</td>
<td>Expression system: Binding with AChBPs, transfected cells, and <em>Xenopus</em> oocytes. nAChR subtypes studied: z7/z4/z2. Effects on 5HT3 receptors: confirmed no activity with cell-based neurotransmitter fluorescent engineered reporters. Summary: Compounds have been described as “noncanonical agonists” since their structures defy normal models of the nAChR pharmacore. They were initially identified by their binding to molluscan AChBP. Activity and selectivity confirmed with cell-based fluorescence activity with the PAM PNU-120596 used to increase z7 signals. Selective activation of z7 confirmed with TEVC in <em>Xenopus</em> oocytes for a subset of the compounds. Efficacy ranged</td>
<td>(Kaczanowska et al., 2017; Camacho-Hernandez et al., 2019)</td>
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TABLE 1—Continued

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<tr>
<th>Compound</th>
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EVP-6124 (encenicline)  
Expression system: binding with rat brain membranes, TEVC in *Xenopus* oocytes.  
*nAChR* subtypes studied: *α7, α4β2, α5β4, and muscle-type receptors.*  
Effects on 5HT3 receptors: Binding studies showed that EVP-6124 inhibited the 5-HT3 receptor by 51% at 10 nM.  
Summary: a reported EC$_{50}$ of 0.16 μM based on peak currents, suggesting more potent activity on 5HT receptors than *α7*. *I$_{max}$* estimation limited by protocol, which permitted cumulative desensitization. Claimed to have potentiating activity at low conc.; however, see Figure 3. Active in NOR and other cognitive tests. Numerous follow-up studies.  

FRM-17874  
Expression systems: binding studies and *Xenopus* oocytes TEVC.  
*nAChR* subtypes studied: *α7 only.*  
Effects on 5HT3 receptors: Evaluated in binding studies that showed significant inhibition.  
Summary: analog of EVP-6124, also reputed to have a potentiating effect at low conc. TEVC in oocytes indicated a EC$_{50}$ of 0.42 ± 0.17 μM, but data were of insufficient quality to estimate an *I$_{max}$*. FRM-17874 improved novel object recognition in rats and enhanced memory acquisition and reversal learning in the mouse water T-maze and enhanced hippocampal LTP.  

Iwuagwu et al.  
Compound 31  
Expression systems: FLIPR assays of transfected cells and patch clamp for HERG and reportedly for *α7*, although no patch data are shown, and no patch-clamp results reported for *α7*.  
*nAChR* subtypes studied: *α7 only.*  
Effects on 5HT3 receptors: *IC$_{50}$* for *α7* = 9.2 μM from FLIPR.  
Summary: Lead compound from a study of 4-heteroarylamino-10-azaspiro [oxazole-5,3-bicyclo[2.2.2]octanes]. EC$_{50}$ for *α7* of 11 nM from FLIPR. Positive effect in NOR.  
No apparent follow-up publications.  

(S)SEN12333 (WAY-317538)  
Expression system: GH4C1 cell line for *α7* FLIPR and patch-clamp studies. Binding studies with transfected cells.  
*nAChR* subtypes studied: putative *α3*, putative *α4*.  
Effects on 5HT3 receptors: claimed inactive, no data shown.  
Summary: EC$_{50}$ = 667 nM in FLIPR assay and 42 μM in patch-clamp study of peak currents. Data on numerous other analogs reported.  

(S)SEN15924 (WAY-361789)  
Expression system: FLIPR assays GH4C1 cells for *α7*. Transfected HEK cells for 5HT3, SH-SY5Y for putative ganglionic (*α3*) receptors, and TE671 for muscle-type.  
*nAChR* subtypes studied: putative *α3*, *α1β1δ*, and *α7*.  
Effects on 5HT3 receptors: inhibitory activity at ~30 μM.  
Summary: Large study of numerous analogs. Lead compound does something in FLIPR assay (no actual data shown) EC$_{50}$ = 0.18 μM ± 0.01. Positive effects in NOR and auditory gating reported.  

(S)SEN78702 (WYE-308775)  
Expression system: FLIPR assays: GH4C1 cells for *α7*. Transfected HEK cells for 5HT3, SH-SY5Y for putative ganglionic (*α3*) receptors and TE671 for muscle-type transfected CHO cells for HERG channels.  
*nAChR* subtypes studied: putative *α1β1δ*, *α3*4, *α7*.  
Effects on 5HT3 receptors: reportedly no agonist activity. Antagonist activity not studied.  
Summary: hypothetically, a full agonist in FLIPR assay, but relative to what standard is not clear EC$_{50}$ = 125 ± 70 nM. Potency values from such assays are typically at least 10-fold higher than those from electrophysiology. No agonist activity detected on other subtypes. Antagonist activity not studied. Positive effects in NOR, and acoustic startle response reported.  

(2R)-7-methyl-N-quinuclidin-3-ylpyrrolo[1,2-a]quinoline-2-carboxamide (Compound 10a)  
Expression system: *Xenopus* oocytes.  
*nAChR* subtypes studied: *α3*, *α4β2, and *α7*.  
Effects on 5HT3 receptors: very effective agonist of 5HT3a expressed in oocytes.  
Summary: Very little data presented. MLA, *EC$_{50}$* for *α7* of approximately 2 μM with roughly 70% efficacy (peak currents). MLA blocks, PNU-120596 potentiates, and MLA. Preapplications of low conc. inhibited ACh responses with an *I$_{max}$* of 21, 2 ± 1.3 nM. No apparent follow-up publications at the time of this writing.  

TC-7020  
Expression system: *Xenopus* oocytes for *α7*, TE671 and SH-SY5Y and SH-EP1 cells for other *nAChRs* in FLIPR assays. Also, brain membranes for binding studies.  
*nAChR* subtypes studied: *α1β1δ* (TE671 cells), putative *α3* (SH-SY5Y) *α4* (SH-EP1), and *α7* in oocytes.  
Effects on 5HT3 receptors: not studied.  
Summary: Authors state that TC-7020 is an efficacious partial (68%) agonist for *α7* net charge responses, but data are not shown, nor is an EC$_{50}$ provided. Oocyte work was done in the laboratory of an author of this review (R.L.P.), and although Marrero et al. say that an

(continued)
like PNU-282987, PHA-543,613, and GTS-21, which are already commonly used. Moreover, it is rumored that many of the programs in this area by the large pharmaceutical companies like Pfizer (Malyasz et al., 2010; O’Donnell et al., 2010; Zanaletti et al., 2012b), Abbott (Tietje et al., 2008; Briggs et al., 2009), Astrazeneca (Sydserff et al., 2009), Bristol-Myers Squibb (Cook et al., 2016; Hill et al., 2016, 2017; Iwuagwu et al., 2016; O’Donnell et al., 2010; Zanaletti et al., 2012b), Novartis (Feuerbach et al., 2015), Bayer (in partneship with EnVivo) (Prickaerts et al., 2012), Targacept (Marrero and Bencherif, 2009), GlaxoSmithKline (Skidmore et al., 2012), and Servier (Beracochea et al., 2008) have been discontinued. Although some have left the field of nicotinic receptor research entirely, others have shifted their efforts away from targeting the orthosteric agonist binding site and toward allosteric modulators.

V. \(\alpha 7\)-Positive Allosteric Modulators

A. Functional Modulation and \(\alpha 7\) Nicotinic Acetylcholine Receptor Structure. Like all nAChRs, the \(\alpha 7\) receptor is an allosteric protein [reviewed in (Papke and Lindstrom 2020)] with multiple ligand binding sites that interact to determine the conformational and functional dynamics of the receptor. Considering first the ACh or orthosteric binding sites, as mentioned earlier, these are configured in the extracellular domain at the subunit interfaces. Early mutagenesis studies with heteromeric muscle-type nAChRs [reviewed in (Papke 2014)] inferred the existence of three critical subdomains on the primary face of the ligand binding site in the \(\alpha \) subunits, which are referred to as the A, B, and C loops. A pair of disulfide-linked vicinal cysteines at the tip of the C-loop is a defining feature of all \(\alpha \) subunits. In heteromeric nAChRs, subunits that lack these vicinal cysteines form the complementary face of the orthosteric binding sites. Specialized subdomains referred to as the D, E, and F loops are present in the muscle subunits that provide the complementary surface of the ACh binding sites (\(\delta \), \(\gamma \), and \(\varepsilon \) the \(\varepsilon \) subunit substituting for \(\gamma \) in adult muscle-type receptors). In heteromeric neuronal receptors these specialized subdomains are present in the \(\beta 2 \) and \(\beta 4 \) subunits (Papke and Lindstrom, 2020). Early electron micrographic studies of the nAChR of the Torpedo electric ray homologous to muscle-type receptors supported the presence of these functional subdomains (Unwin, 1993; 2005) that more recently have been definitively identified in high-resolution structures on neuronal \(\alpha 4/\beta 2 \) (Morales-Perez et al., 2016) and \(\alpha 3/\beta 4 \) (Morales-Perez et al., 2016) receptor subunit complexes. Support for the hypothesis that the \(\alpha 7\) subunits of homomeric \(\alpha 7\) receptors contain homologs of both the primary and complementary surfaces of the orthosteric binding sites at alternating subunit interfaces has come from mutation analyses...
(Papke, 2014) and the crystal structures of molluscan AChBPs (Brejc et al., 2001). AChBPs are soluble proteins secreted by the glial cells in the ganglia of various invertebrates, and they are formed as pentamers of proteins that are homologous to the extracellular domain of nAChR α subunits (Camacho-Hernandez and Taylor, 2020).

Since no crystal structures of z7 receptors are available at present, homomeric pentamers of AChBP mutants have been developed as models for z7 (Gulsevin, 2020; Gulsevin et al., 2020a,b). Even if we begin with the parsimonious and possibly naive assumption that each z7 receptor has five functionally equivalent orthosteric activation (OA) agonist binding sites (Palma et al., 1996), early studies with the AChBPs suggested that as ligands begin to bind, at least in regard to some ligands, the binding sites become nonequivalent (Hibbs et al., 2009). Crystal structures with the z7-selective partial agonist GTS-21 (see above) showed that the ligand crystallized in different orientations at some interfaces compared with others. Although those studies could not determine whether the difference between binding sites represented a starting condition or was an emergent property of the crystallization process, recent in silico studies that begin with symmetrically configured subunits suggest that when these are allowed dynamic relaxations, the subunit interfaces quickly become asymmetric (Henchman et al., 2003; Gulsevin, 2020; Gulsevin et al., 2020a,b).

Considering what we know about the dynamics of z7 activation by orthosteric agonists (Papke and Lindstrom, 2020), regardless of whether all of the five subunit interfaces start out as functionally equivalent, as long as one or more of them bind agonist, it is clear that dynamic conformational changes affect the entire receptor. The activation of the z7 ion channel by orthosteric agonist occurs at low probability and only with low levels of agonist site occupancy (Uteshev et al., 2002; Williams et al., 2011a; Williams et al., 2012). Further levels of agonist binding serve only to induce the concentration-dependent form of desensitization that is unique to z7 (Papke and Lindstrom, 2020).

B. Desensitization and Allostereicism. Desensitization (Katz and Thesleff, 1957) is a feature common to all nAChR, and for heteromeric nAChR, coincident with desensitization, the orthosteric binding site adopts a conformation that binds agonists with high affinity (Papke, 2014). It was this feature that allowed the early radioligand binding studies to identify the heteromeric nAChR as high-affinity receptors for ACh and nicotine (Clarke et al., 1985). Although z7 receptors desensitize so rapidly that the currents evoked by the application of high concentrations of AChs are terminated before the drug application can even be completed (Papke and Porter Papke, 2002; Papke, 2010; Williams et al., 2012), the orthosteric binding sites do not adopt a conformation with high affinity for ACh, and, in general, z7 receptor desensitization is rapidly reversible. There are, however, exceptions to this in which a particular ligand like GTS-21 can induce relatively stable desensitization. The possible functional significance of this will be discussed further in the section on silent agonists.

As noted above, nAChRs have a long history of being considered allosteric proteins (Changeux, 1981), and as such, their function is regulated by ligands binding to allosteric sites as well as the sites for orthosteric agonists (Changeux and Revah, 1987; Papke, 2014). In recent years, some of the most striking effects for allosteric ligands have been described for positive allosteric modulators (PAMs) of z7 receptors (Williams et al., 2011c). As noted above, in general, z7 receptors have only a low probability of ion-channel activation by ACh or other agonists working through the orthosteric binding sites. Two basic types of PAMs have been identified that differ in the degree to which they synergize with orthosteric agonists to overcome the intrinsic limitations on z7-channel activation (Fig. 4) (Gronlien et al., 2007). Type I PAMs like NS-1738 (Timmermann et al., 2007) increase channel activation during the phase that precedes the induction of more stable desensitized states, so that responses are increased in amplitude but not very much in duration (Fig. 5A). Type II PAMs like PNU-120596 (Hurst et al., 2005; Gronlien et al., 2007) (Fig. 5B) increase channel currents by additionally destabilizing conformations associated with desensitized states of the receptor (Williams et al., 2011b). PAMs of this type when coapplied with agonist will not only stimulate prolonged currents during the coapplication if receptors have been desensitized by a previous drug application, but type II PAMs applied alone will reactivate receptors (Papke et al., 2009) (see also discussion of allosteric antagonists below).

As shown in a schematic representation of the conformational dynamics of z7 activation and desensitization as regulated by agonists and PAMs [Fig. 6, adapted from (Williams et al., 2011b) and modified based on (Quadri et al. 2019)], when bound by orthosteric agonist alone, site occupancy is low, and the receptor has only a low probability of entering a relatively unstable open state for brief durations. The effects of a type I PAM would be consistent with an increase in single-channel conductance; however, single-channel studies (Andersen et al., 2016) have shown that the primary effects are to stabilize the open state and to permit reopening when the OA site occupancy is low (box in Fig. 6) without changing the transitions to the desensitized states that develop over time or with changes in OA site occupancy.

Single-channel studies of z7 receptors potentiated by type II PAMs (Williams et al., 2011b; Williams et
al., 2012; Peng et al., 2013; Andersen et al., 2016; Quadri et al., 2019) have indicated that the increased channel activation is associated with transitions between desensitized states and an unstable intermediate flip state (Lape et al., 2008) that is then able to convert repeatedly between two or more novel open-channel states in bursts that can persist for many seconds. These bursts represent bouts of single-channel activation typically more than a hundred thousand times greater than the single-channel currents stimulated by ACh alone. Comparing then the PAM effects on the macroscopic (whole-cell) current, which are increased on the scale of 50–100-fold, with the single-channel effects, we see that the net effects of these PAMs is to generate very large bursts of currents from a very limited fraction of the channels at any one time. Because of this stochastic nature of the large effects on a small fraction of channels typically in a given experiment, there is a great deal of variability among the responses in a group of cells.

Studies of mutants and chimeras localized the binding sites for α7 PAMs (Bertrand et al., 2008; Young et al., 2008) to the upper portion of the second transmembrane domain, with an especially important role attributed to a methionine residue in the 15’ position (Young et al., 2008). The presence of a methionine residue in this position is unique to α7 among all the nAChR subunits, and not only does mutation of this residue to leucine (the most common residue in other subunits) lead to a loss of sensitivity for α7 to PAM potentiation, but substitution of this residue into the sequence of β2 or β4 subunits generates heteromeric receptors that are sensitive to potentiation by many α7 PAMs (Stokes et al., 2019).

With a relatively large potentiating ligand bound within one or more of the transmembrane domains, it is perhaps not surprising that the ion conduction pathways that form in PAM-potentiated receptors are qualitatively different from the channels formed when the receptor is activated by ACh alone. Channels activated by ACh have relatively high calcium permeability and inward rectifying current-voltage relations, which are features that are not typical of PAM-potentiated currents (Sitzia et al., 2011; Miller et al., 2020). Specific PAMs may each generate their own unique conduction pathway (Miller et al., 2020), a differing set of full and subconductance states, and varying sensitivity to channel-blocking antagonists (Quadri et al., 2019).
C. Ligands and Structures. Compounds identified as $\alpha_7$ PAMs are listed in Table 2, and the structures of the most commonly used ones are shown in Fig. 4. Earlier known compounds are described in more detail in a previous review (Williams et al., 2011c). The first $\alpha_7$ PAM to be identified, 5-hydroxyindole (Gurley et al., 2000), is classified as type I but has not been widely used since it works with very low potency. The effects of NS1738, a more potent type I PAM, are shown in Fig. 5 compared with the effects of the widely used type II PAM, PNU-120596. The cholinesterase inhibitor, galantamine, which was approved for the treatment of Alzheimer disease, was initially claimed to be an $\alpha_7$ PAM (Samochcki et al., 2003); however, this claim has recently been shown to be invalid (Kowal et al., 2018).

$\alpha_7$ PAMs have been shown to be active in many of the same animal models that have been used with the identification of $\alpha_7$-selective agonists. For example, LL-00066471 (Verma et al., 2021) and BNC375 (Wang et al., 2020b) were shown to improve performance in novel object recognition (NOR) and other cognitive tests. Likewise, RO5126946 (Sahdeo et al., 2014), NS1738 (Timmermann et al., 2007), and Lu AF58801, (1S,2S)-2-phenyl-cyclopropanecarboxylic acid [((R)-4-ethoxy-phenyl)-2-hydroxy-ethyl]-amide (Eskildsen et al., 2014) were also active in cognitive tests, and BNC375 enhanced long-term potentiation (Wang et al., 2020b). LL-00066471, JWX-A0108 (Sun et al., 2019), and JNJ-1930942 (Dinklo et al., 2011) improved acoustic startle reflex or genetic defects believed to be associated with hippocampal auditory gating. PAM2 (Arias et al., 2020), 1-(2',5'-dihydroxyphenyl)-3-(2-fluoro-4-hydroxyphenyl)-1-propanone (Perez de Vega et al., 2019), TQS (Abbas et al., 2017), and PNU-120596 (Bagdas et al., 2018b) were effective in models of inflammatory or neuropathic pain. Some PAMs are advancing toward
clinical trials [(Gee et al. 2017), reviewed in (Yang et al. 2017)]. In 1997, AVL-3288 advanced into a phase I clinical trial for schizophrenia and schizoaffective disorder. However, more recently it has been reported that primary clinical outcomes were negative in follow-up trials (Kantrowitz et al., 2020).

Because of the large currents promoted by $\alpha_7$ PAMs and the reportedly high calcium permeability of $\alpha_7$ receptors when activated by ACh alone (Miller et al., 2020) and the high calcium permeability reported for $\alpha_7$ receptors activated by ACh alone (Miller et al., 2020) and the high calcium permeability of $\alpha_7$ receptors, there is an interest in using $\alpha_7$ PAMs as potential therapeutic agents. However, it is important to note that there may be a potential for cytotoxic increases in intracellular calcium (Sitzia et al., 2013; Stokes et al., 2019; Papke et al., 2020b). The separation of the TQS isomers, for example, revealed that the (+) isomer behaves like a type II PAM but only at relatively high concentrations, whereas the (−) isomer is much more potent and functions more distinctly different PAMs. To date, only two other TQS-related compounds that we have been able to use for our studies of allosteric mechanisms (Horenstein et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016). The basic syntheses for compounds in this family generates racemic mixtures of stereoisomers, and Dr. Thakur’s work has brought to light that the isomers of these TQS analogs can differ greatly in their biologic activity (Thakur et al., 2016).
related compounds have had their stereoisomers studied separately, and those isomers too were shown to have distinctly different activity profiles (discussed below), suggesting that there is more room for discovery in the characterization of the compounds in this structural family.

**D. Allosteric Activators (Ago–Positive Allosteric Modulator).** By definition, a PAM is an agent that does not activate the (wild-type) \(\alpha_7\) receptor when applied alone but does increase the activation produced by an orthosteric agonist when the two are coapplied or otherwise work in concert (perhaps by preapplication of the PAM). Among the compounds described by the Millar group were agents that behaved as allosteric agonists—that is, they produced channel activation when applied alone without the coapplication of an orthosteric agonist (Gill et al., 2012; Pałczyńska et al., 2012). This activation appeared to rely on the same putative binding site in the second transmembrane domain required for PAM activity (Gill et al., 2011). Additionally, the agents increased activation by orthosteric agonists, supporting their classification as “ago-PAMs,” a term first

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**Fig. 7.** TQS isomers. Shown on top are the structures of the two isomers of TQS (Stokes et al., 2019). The upper traces are averaged normalized responses (±S.E.M.) of oocytes expressing human \(\alpha_7\) to 60 \(\mu\)M ACh or 60 \(\mu\)M ACh coapplied with 10 \(\mu\)M (+)TQS or (−)TQS (n equal to 3 and 4, respectively). The lower traces are averaged normalized responses (±S.E.M.) of oocytes (n = 8) expressing human \(\alpha_7\) to 60 \(\mu\)M ACh or 60 \(\mu\)M ACh coapplied with 0.3 \(\mu\)M (+)TQS or (−)TQS. The data for the 0.3 \(\mu\)M responses have previously been published in bar graph format (Stokes et al., 2019).

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applied to activators of metabotropic glutamate receptors (Noetzel et al., 2012). One of the first compounds of this type to be identified was 4BP-TQS (Gill et al., 2011). The Thakur laboratory subsequently isolated the isomers of 4BP-TQS (Thakur et al., 2013), and it was shown that all of the activity was accounted for by the (+) isomer, which has subsequently been identified in the literature as “GAT107” (Papke et al., 2014b) (Fig. 8). Additional allosteric activators utilizing the TQS scaffold were identified by the Millar laboratory (Gill-Thind et al., 2015), but these have not been studied in detail, nor have their isomers been separated. More recently, B-973 (Fig. 8) was identified as an ago-PAM (Post-Munson et al., 2017) with a structure that is not related to the TQS scaffold. The isomers of B-973 were isolated, and “B-973B” was identified as the active form (Garai et al., 2018).

We have characterized three forms of GAT107 activity (Fig. 9). When the compound is applied alone at a sufficiently high concentration, there is “direct allosteric activation” (Fig. 9A). This activity is transient and decays with the washout of free compound from the bath. However, GAT107 appears to remain bound to the PAM binding sites in the transmembrane domains so that after an application of GAT107 alone, a subsequent application of ACh is greatly increased in amplitude, a phenomenon we refer to as “primed potentiation” (Papke et al., 2014b) (Fig. 9A, second ACh response). In oocyte experiments, GAT107-primed potentiation can persist for up to an hour. Responses are, of course, also very large when GAT107 is coapplied with agonist (Fig. 9B), in which case it directly potentiates the ACh response, acting like a typical PAM (“direct potentiation”). The potency of GAT107 as a PAM is greater than its potency as an allosteric agonist so that the application of 1 µM alone does not activate receptors and produces relatively little primed potentiation (Fig. 9C). However, when coapplied with ACh, it does very effectively activate receptors and potentiate the ACh response (Fig. 9D). It should also be noted that there is relatively little primed potentiation after an episode of direct potentiation compared with after the application of GAT107 alone. This suggests that after activation by the simultaneous application of ACh and GAT107 either the receptor adopts a PAM-insensitive state (Williams et al., 2011b) or the GAT107 is less tightly bound to the transmembrane PAM sites during this form of activation. Although differing somewhat in duration and concentration dependence, the functional properties of B-973B are basically similar to those of GAT107 on the level of macroscopic current (Quadri et al., 2019). On the microscopic level, however, although the two ago-PAMs each promote protracted bursts of single-channel opening, each have their own distinct fingerprint of full and subconductance states, and the agents differ in their sensitivity to the noncompetitive antagonist mecamylamine (Quadri et al., 2019; Miller et al., 2020).

Although it was originally proposed that 4BP-TQS produced allosteric activation solely by binding to the transmembrane PAM site (Gill et al., 2011), several lines of evidence argue for GAT107 binding to additional allosteric sites in the extracellular domain as well as the transmembrane PAM site (Papke et al., 2014b; Horenstein et al., 2016). As mentioned above, there is a clear kinetic difference in transient allosteric activation by GAT107 and its prolonged effects as a PAM. There are also distinct structural epitopes in the receptor that affect allosteric activation without major effects on the PAM activity of GAT107. For example, z7D101A mutants show virtually no allosteric activation by GAT107, whereas the ACh responses are still well potentiated (Horenstein et al., 2016). The TQS analog, 2,3,5,6TMP-TQS (TMP-TQS) was first identified by the Millar laboratory as a silent allosteric modulator because it had no apparent PAM activity yet was also able to antagonize allosteric activation. Subsequent isolation of the TMP-TQS isomers showed that although the (+) isomer was a weak PAM, the (−) isomer was a potent antagonist of GAT107 allosteric activation with relatively little effect on the primed potentiation produced by a GAT107 application (Papke et al., 2020b) (Fig. 10A). This pharmacological and structural separation of the two forms of GAT107 and TMP-TQS supports the existence of specific binding sites for allosteric activation. Therefore, another way in which z7 receptors may be developed as pharmacologic targets is by identifying small ligands that would bind to these allosteric sites and couple with conventional PAMs to produce activation (Gulsevin et al., 2019) or induce other conformational changes. This concept will be discussed in more detail in the section on silent agonists below.

The blockade of GAT107 allosteric activation by (−)TMP-TQS (Fig. 10A), would be consistent with this analog functioning as a competitive antagonist at the allosteric activation binding sites. However, it is actually likely that it is also capable of functioning as an inverse agonist at that site. As mentioned earlier, GTS-21 is a partial agonist that produces a significant amount
of residual desensitization. Applications of PNU-120596 alone after GTS-21 applications can reactivate channels and produce a current (Papke et al., 2009) (Fig. 10B). Applications of (-)TMP-TQS suppress this reactivation of desensitized channels (Fig. 10B).

VI. Silent Agonists

A. Conditional Activation of α7. In addition to the effects of agonists and allosteric ligands, the conformational states of α7 nAChR can be regulated by the binding of agents identified as “silent agonists” (Chojnacka et al., 2013; Papke et al., 2014a). Even efficacious agonists are relatively inefficient at inducing the open-channel state of α7 and are far more effective at stabilizing agonist-dependent nonconducting states, which are traditionally referred to as “desensitized” (Katz and Thesleff, 1957), a term that may be correct only when referring to ionotropic function. As evidence accumulates for α7 having metabotropic activity (Horenstein and Papke, 2017; Kabbani and Nichols, 2018), we see with ligands like NS6740 a dissociation between channel activation and metabotropic function so that receptors with “desensitized” ion channels may be metabotropically active (Thomsen and Mikkelsen, 2012a; Papke et al., 2015a).

Although there are likely to be more, we can distinguish two classes of agonist-induced nonconducting states: D0, which can be converted to open-channel states with PAMs, and D1, which is insensitive to PAMs (Williams et al., 2011b). Extending the concept of full and partial agonists, silent agonists bind competitively with efficacious agonists, but with such low probability of inducing channel activation that they appear as antagonists unless coapplied with a PAM. Although silent agonists are relatively ineffective at activating the

Fig. 9. Concentration and protocol dependence of responses to GAT107. (A) The traces are the averaged normalized responses (±S.E.M.) of oocytes expressing human α7 to 10 μM GAT107 applied alone and followed by an application of 60 μM, as compared with the initial responses to ACh alone (n = 5). (B) The traces shown are the averaged normalized responses (±S.E.M.) of oocytes expressing human α7 to 10 μM GAT107 coapplied with 60 μM ACh and followed by an application of 60 μM, as compared with the initial responses to ACh alone (n = 7). (C) The traces shown are the averaged normalized responses (±S.E.M.) of oocytes expressing human α7 to 1 μM GAT107 applied by itself and followed by an application of 60 μM, as compared with the initial responses to ACh alone (n = 7). (D) The traces shown are the averaged normalized responses (±S.E.M.) of oocytes expressing human α7 to 1 μM GAT107 coapplied with 60 μM ACh and followed by an application of 60 μM, as compared with the initial responses to ACh alone (n = 7).
channel, they do induce Ds and Di unlike the classic competitive antagonist methyllycaconitine (MLA). Signal-transduction studies in non-neuronal cells (Thom- sen and Mikkelsen, 2012a; Boulet et al., 2015; Papke et al., 2015a; Yue et al., 2015; Zanetti et al., 2016; King et al., 2017b; Maldifassi et al., 2018) have implicated silent agonists and the Ds and/or Di states of the receptor as likely to mediate channel-independent signaling. Developing specific therapeutics for the treatment of inflammatory diseases and pain may therefore come from defining the structural features of drugs that predict silent agonism. It is not sufficient merely to detect the induction of Ds and Di states but to appreciate the receptor’s dynamic nature and how the distribution of conformational states evolves and changes over time as agonist and/or PAM applications perturb the population of receptors (Papke et al., 2015a; Papke et al., 2018b). Although Di is ultimately favored by high concentrations of PAM and agonists (Fig. 6), Ds may be favored only intermittently, and these dynamics can be differentially regulated by specific ligands (Williams et al., 2011b).

B. Ligands and Structures. Just as there are multiple motifs that may make ligands selective activators of the α7 ion channel (Horenstein et al., 2008), we (Chojnacka et al., 2013; Papke et al., 2014a, 2015a; van Maanen et al., 2015; Quadri et al., 2016, 2017a,b, 2018b) and others (Briggs et al., 2009) identified multiple classes of structurally distinct silent agonists (Fig. 11). One of the first silent agonists to be identified, NS6740 (Briggs et al., 2009), is also arguably one of the most interesting and may point to a fundamental dichotomy in the modes of α7 receptor function for therapeutic purposes both ion channel–mediated and ion channel–independent, corresponding to cognitive function and the CAP, respectively. The efficacy of NS6740 for channel activation is no more than 20% that of ACh (Pismataro et al., 2020), but it very effectively induces nonconducting states that have been shown in oocyte studies to be stable for long periods of time after a single application of NS6740. While this desensitization persists, the receptors are unable to be activated by more efficacious agonists. Throughout this period the desensitization can be perturbed by applications of a PAM like PNU-120596, and interestingly, sequential applications of NS6740 and the long-acting ago-PAM GAT107 can generate large currents persistently for an hour (Papke et al., 2018b).

A negative effect of NS6740 on the α7-mediated cognitive effects of a more efficacious α7 agonist was shown by (Briggs et al. 2009), who used NS6740 to block the effects of A-582941 in a mouse model of inhibitory avoidance. Likewise, it was later shown that NS6740 could block the effects of BMS-902483 in NOR (Pieschl et al., 2017). In rat hippocampal slices,
NS6740 reduced synaptic plasticity (Papke et al., 2018a). However, in regard to CAP, NS6740 and the desensitizing partial agonist GTS-21 were both shown to effectively reduce the release of TNF-α from microglial cells exposed to the bacterial endotoxin lipopolysaccharide (Thomsen and Mikkelsen, 2012a). Numerous in vivo and in vitro studies have confirmed the activity of GTS-21 as a regulator of the CAP (Kox et al., 2011; Yue et al., 2015; Kashiwagi et al., 2017; Kong et al., 2018; Schaller et al., 2018; Wang et al., 2019; Sitapara et al., 2020; Wang et al., 2020a), and although it is less well studied, NS6740 was also shown to induce significant dose- and time-dependent antinociceptive activity in formalin- and acetic acid-induced nociceptive behaviors as well as in the chronic constriction nerve injury model for neuropathic pain (Papke et al., 2015a).

C. Function In Vivo and In Vitro. Results like those described above have motivated other studies to identify other silent agonists for potential development as treatments for inflammatory disease and neuropathic pain (Horenstein and Papke, 2017; Bagdas et al., 2018a; Manetti et al., 2018). The compound identified as “KC-1” (5-phenylanabaseine, 6’-phenyl-3,4,5,6-tetrahydro-2,2’-bipyridine) (Chojnacka et al., 2013) was developed in the laboratory of Nicole Horenstein using an anabaseine scaffold related to GTS-21. In a systematic analysis of linear amines, we identified triethyl methylammonium as a minimally sized silent agonist (Papke et al., 2014a) created by the addition of a methyl group to the to the minimally sized α7-selective agonist ethyl dimethyl ammonium (Horenstein et al., 2008). A similar approach was used to generate additional families of silent agonists and to implicate a critical difference in the size of the cationic nitrogen group to produce a shift from active partial agonism to silent agonism (Papke et al., 2014a).

One particularly interesting group was the diEPP family based on the ganglionic agonist dimethylphenylpiperazinium with the switch from methyl to larger ethyl subgroups. This family was further developed (Quadri et al., 2016) and led to the identification of two analogs that were subsequently shown to be active in vivo for reducing inflammatory pain [para trifluoromethyl N,N-diethyl-N’-phenylpiperazine (Quadri et al., 2016)] and attenuating inflammation in an animal model of multiple sclerosis [1-ethyl-4-(3-bromo)phenyl)piperazine (Godin et al., 2020)].

Although the basic assumption based on the pharmacophore studies (Papke et al., 2014a) was that silent agonists work primarily through an extension of the site for orthosteric agonists that is more permissive of the somewhat larger ammonium group, we also investigated the hypothesis that silent agonism, as revealed by the application of PAMs, might also come from ligands that bound to the allosteric activation site implicated in our studies of GAT107. Using in silico screening of our library of diEPP compounds, we identified 1,1-diethyl-4(naphthalene-2-yl)piperazinium-1-ium iodide (2-NDEP) as a candidate allosteric silent agonist and...
confirmed that it generated PNU-120596–dependent currents in the α7C190A mutant, which has an inactivated orthostERIC agonist binding site (Gulsevin et al., 2019). Testing the hypothesis that a sulfonium could function as a surrogate for ammonium in a nicotinic agonist led to the identification of 1-ethyl-4-phenylthiomorpholin-1-ium triflate as a silent agonist (Quadri et al., 2017b).

The compound R-47 (also PMP-072) has an interesting history. It was first developed as a proprietary compound that was passed on as intellectual property through a series of now defunct or inactive companies, eventually ending up with Targacept. Once it was finally released, it was published as “R-47” by the team of chemists who originally synthesized it (Clark et al., 2014) and as “PMP-072” by groups who also collaborated with the company that first developed it (van Maanen et al., 2015). The data on PMP-072’s activity in a collagen-induced model of rheumatoid arthritis were actually published as part of a Ph.D. thesis 6 years prior to the time when it was permitted to publish the structure. The paper by (Clark et al., 2014) showed that R-47 significantly inhibited the cellular infiltration in a murine model of allergic lung inflammation. More recently, R-47 has been shown to prevent and reverse paclitaxel-induced peripheral neuropathy (Toma et al., 2019).

The compound identified as “31b” is the lead compound from a study of compounds with a methyl-quinuclidine core pharmacophore (Quadri et al., 2018b). This compound can be classified as a silent agonist based on its electrophysiological properties but has not yet been tested with in vivo models. Arecoline, on the other hand, is a silent agonist (Papke et al., 2015b) that is self-administered by hundreds of millions of people on a daily basis because it is probably the most active alkaloid in the areca nut (Gupta et al., 2020), the key ingredient in betel quids (betel nut). Betel (areca) is the fourth most commonly used addictive substance in the world (World Health Organization, 2004; Papke et al., 2020c; Singh et al., 2020).

**D. Other Novel Silent Agonists.** The functional and structural diversity of conotoxins is enormous, and several have been identified as selective antagonists of α7 nAChRs (see below). Interestingly though, conotoxin MrIC has been implicated to be an α7 silent agonist in cell-based assays (Jin et al., 2014; Mueller et al., 2015). Although it has been reported to be an antagonist of α7 expressed in oocytes (Jin et al., 2014), using a commercially available sample of MrIC (Alomone Laboratories, Jerusalem, Israel) we saw that, although 50 μM MrIC applied alone did not activate α7 receptors, when it was coapplied with 30 μM PNU-120596, substantial currents were stimulated (Fig. 12A). Presumably, if MrIC were coapplied with a standard agonist to α7-expressing cells, it would behave as a competitive antagonist since this a basic property of silent agonists (Papke et al., 2014a). It may be the case that other conotoxins that have been classified as antagonists might have similar silent agonist properties if they were tested with PAM coapplications.

It is interesting to consider what other foods in our diet might also have silent effects of α7 receptors. For example, we made the somewhat serendipitous observation that, although coffee had no apparent effects on α7 receptors, responses observed when coffee was coapplied to α7 receptors with PNU-120596 (Fig. 12B, upper traces) suggest that...
there are also previously unknown silent agonists in this widely consumed beverage. There are many biologically active molecules in coffee, and we confirmed that neither caffeine nor the alkaloid trigonelline were \( \alpha7 \) silent agonists (not shown). However, \( N \)-methylpyridinium, another plentiful alkaloid in coffee (Burton et al., 2020) that is a urinary biomarker for coffee consumption (Lang et al., 2011), is an effective silent agonist (Fig. 12B, lower traces).

VII. \( \alpha7 \) Antagonists

A. Snake Toxin Antagonists and Their Analogs. Prior to the cloning of the \( \alpha7 \) gene, the associated receptors in brain were identified simply as “\( \alpha \)-BTX binding sites” (Jumblatt et al., 1981; Schulz et al., 1991), “\( \alpha \)-BTX receptors” (Clarke et al., 1991), or “\( \alpha \)-BTX sensitive neuronal nAChR” (Zorumski et al., 1992; Castro and Albuquerque, 1995), and \( \alpha \)-BTX is, of course, an excellent antagonist for \( \alpha7 \)-mediated responses (Uteshev et al., 1996; Alkondon et al., 1998; Kempshall et al., 1999; Drisdel and Green, 2000; Kaiser and Wonnacott, 2000; Xiao et al., 2009). However, \( \alpha \)-BTX is also a potent and nearly irreversible antagonist of the nAChR at the neuromuscular junction (Servay et al., 1978), so although it has utility for the sorts of binding studies that were used to identify \( \alpha7 \)-selective agonists (see above) and confirm the presence of \( \alpha7 \) receptors in cell lines (Williams et al., 2012) and tissues (Rasmussen and Perry, 2006; Xiao et al., 2009), it has no utility for in vivo studies.

A search for mammalian homologs to the snake toxin that bind to muscle-type and \( \alpha7 \) nAChRs brought to light the existence of a large family identified as “three-finger proteins” based on structures that are homologous to important domains in the snake toxins (Nirthanan, 2020). One analog, secreted mammalian Ly-6/urokinase plasminogen activator receptor related protein-1, is secreted by epithelial cells; related proteins in the brain are membrane-tethered via glycosylphatidilinositol anchors. They appear to function as endogenous regulators of nAChRs, but the details remain somewhat uncertain [reviewed in Vasilyeva et al. 2017; Tsetlin et al. 2020]. Soluble synthetic proteins of the toxin-like domains of several of these proteins have been shown to be able to modulate the function of numerous proteins, including \( \alpha7 \) receptors. Although the secreted mammalian Ly-6/urokinase plasminogen activator receptor related protein-1 protein appears to antagonize \( \alpha7 \) function (Shulepko et al., 2020), the water-soluble synthetic variant of human Lynx1 has been reported to upregulate \( \alpha7 \) function (Shenkarev et al., 2020).

The conotoxin \( \alpha \) CTx ImII has been reported to be a selective antagonist of \( \alpha7 \) nAChR (Ellison et al., 2003). Additional \( \alpha7 \)-selective conotoxins have been developed by making mutations in the \( \alpha \)-conotoxins PnLA (Hopping et al., 2014), ImI (Armishaw et al., 2006), or ArIB (Innocent et al., 2008). Structures of the AChBPs alone or complexed with either an agonist or the conotoxin ImI suggest that although the binding of agonists led to the closing down of the C-loop over the orthosteric ligand binding site from the more open “apo” (resting) configuration, binding of the conotoxin had the effect of pushing the C-loop further back, in the opposite direction as what occurs with the binding of agonists, in addition to blocking the binding site itself (Hansen et al., 2005).

B. \( \alpha7 \) Channel Blockers. \( \alpha7 \) receptors are sensitive to a variety of open-channel blockers, including the local anesthetics QX-314 and tetracaine as well as the larger slowly reversible antagonists bis-(2,6,6-tetramethyl-4-piperidinyl) sebacate and 2,2,6,6-tetramethylpiperidin-4-yl heptanoate. Inhibition by these agents, however, varied depending on whether the channels were activated by ACh alone or ACh in combination with the PAM PNU-120596 (Peng et al., 2013). The anticholinesterase ASS234 (Fig. 13), which was developed as a therapeutic for Alzheimer disease (Romero et al., 2020), was shown to be a noncompetitive antagonist of \( \alpha7 \) and was used as a starting point to develop additional antagonists, with compound 38 proposed as a new lead compound.
(Criado et al., 2016). Compound 7i (Fig. 13) was identified as a lead compound in a study to identify \( z7 \) antagonists that might have utility as antidotes for organophosphorus nerve agent intoxication (Peng et al., 2010). However, there has not been much follow-up on either of these studies. A more recent study of piperidine-spirooxadiazole derivatives (Zhang et al., 2020a) identified compound B10 (Fig. 13) as a noncompetitive antagonist of \( z7 \) with an IC\(_{50}\) of 5.4 \( \mu \)M and reasonably good selectivity relative to \( z4/2 \) and \( z3/4 \) nAChRs.

The widely used, relatively nonselective nAChR antagonist mecamylamine (Fig. 13) inhibits \( z7 \) currents activated by ACh alone, with an IC\(_{50}\) of 10 ± 1 \( \mu \)M and currents activated by ACh coapplied with PNU-120596 with an IC\(_{50}\) of 4.8 ± 0.7 \( \mu \)M (Peng et al., 2013), which was roughly an order of magnitude lower than its potency for inhibiting heteromeric neuronal nAChR (Papke et al., 2013). As noted earlier, \( z7 \) currents activated by the ago-PAM B-973B are largely insensitive to mecamylamine (Quadri et al., 2019). 1,2,4,5-Tetrahydromandrene \( \mathrm{B} \) is a potent \( z7 \)-selective noncompetitive antagonist that produces a slowly reversible block of both open and closed channels. It has an IC\(_{50}\) of 1.0 ± 0.1 \( \mu \)M for \( z7 \) and a time constant for recovery of 26 minutes. It is 48-fold less potent for blocking \( z4/2 \) nAChRs and 10-fold less potent for \( z3/4 \) nAChRs, and the block of these heteromeric receptors is readily reversible (Lopez-Hernandez et al., 2009). It may be noted, though, that with the exception of mecamylamine, which is readily available and widely used, the other \( z7 \)-selective noncompetitive antagonists are neither easily available nor commonly used.

**C. Methyllycaconitine.** By far the most commonly used \( z7 \) antagonist is MLA (methyllycaconitine), which was first isolated from _Delphinium brownii_ (Aiyar et al., 1979). Although initially described as having low nanomolar potency for inhibiting the \( z7 \) responses of cultured hippocampal neurons (Alkondon et al., 1992) and of approximately 100 nM in brain slices (Frazier et al., 1998), in oocyte studies MLA has a potency of 1.2 ± 0.2 \( \mu \)M for the inhibition of \( z7 \) and was a 30-fold less potent antagonist of \( z4/2 \) nAChRs but only 2-fold less potent for inhibiting \( z3/4 \) nAChRs (Lopez-Hernandez et al., 2009). As well as being active in vitro (Alkondon et al., 1992; Donnelly-Roberts et al., 1996; Alkondon et al., 1998; Virginio et al., 2002; Lopez-Hernandez et al., 2009), MLA has also been used in many in vivo studies to evaluate the role of \( z7 \) receptors in various central nervous system functions (Rao et al., 1996; Felix and Levin, 1997; Damaj et al., 1999; Klink et al., 2001; Markou and Paterson, 2001; Levin et al., 2002; Andreassen et al., 2009). These differences in the apparent potency of MLA are curious but may relate to differences in the methodology used. The high potencies reported for the hippocampal culture and slice experiments (Alkondon et al., 1992; Frazier et al., 1998; 2880) were associated with prolonged bath applications of MLA at low concentration, whereas in the oocyte studies it was acutely applied without preincubation. It may be the case that the receptors acquire high affinity for the ligand with prolonged exposure. This would be analogous to the behavior of nicotine with its “high-affinity receptors” that only bind nicotine with nanomolar affinity after the receptors equilibrate into desensitized states over time. Nicotine’s potency for transient activation of \( z4/2 \) receptors is 2.5 \( \mu \)M (Papke et al., 2007), which is much lower than the 4.6 nM affinity reported in binding studies of \( z4/2 \) receptors expressed in oocytes (Parker et al., 1998).

MLA is commonly used to confirm the role of \( z7 \) receptors in CAP (Tasaka et al., 2015; Bagdas et al., 2016; Donvito et al., 2017; Krafft et al., 2017; Gao et al., 2018; Papke et al., 2018b; Quadri et al., 2018a; Yin et al., 2019; Li et al., 2020a; Pinheiro et al., 2020). The dosage used in these studies has typically been around 3 mg/kg (Gao et al., 2018; Yin et al., 2019; Li et al., 2020a) but in some cases was as low as 1 mg/kg (Tasaka et al., 2015; Pinheiro et al., 2020) and, by one group, was as high as 10 mg/kg (Bagdas et al., 2016; Donvito et al., 2017; Papke et al., 2018b; Quadri et al., 2018a), wherein it likely had significant effects on the \( z3/4 \) receptors of autonomic ganglia as well as on the \( z7 \) receptors on cells of the immune system. An alternative approach for showing the critical role of \( z7 \) in CAP has been the use of \( z7 \)-knockout mice (Bagdas et al., 2016; Li et al., 2018; Fang et al., 2019; Shao et al., 2019).

MLA is generally considered a competitive antagonist of \( z7 \) activation by orthosteric agonists, and it has been used as an alternative ligand to identify neuronal \( \alpha \)-BTX binding sites (Yum et al., 1996) shown to bind in the same sites as \( \alpha \)-BTX but with more rapid kinetics of association and disassociation (Davies et al., 1999). However, the binding of MLA appears to do more than simply block the access of orthosteric agonists to the binding sites, especially in regard to CAP and the allosteric activation of \( z7 \). Like NS6740 and GTS-21, MLA was also shown to decrease the microglia response to lipopolysaccharide stimulation of TNF-\( \alpha \) (Thomsen and Mikkelsen, 2012a), which to some degree might confound its use as an antagonist in the in vivo studies of CAP.

In regard to allosterically modulated receptors, MLA appears to be more of an inverse agonist than a simple blocker of the ACh binding site. When \( z7 \) receptors in outside-out patch-clamp experiments were activated by a solution containing ACh and PNU-120596, the long bursts stimulated by the drug exposure continued on average for another 2.57 seconds after the drugs were removed, suggesting that no further binding was required to maintain the bursting behavior. However, when
bursting channels were exposed to a solution containing MLA, the bursts ceased on average in 220 milliseconds, suggesting that MLA actively suppressed channel reopening (Williams et al., 2011b). Under conditions in which persistent activation of α7 receptors was achieved by application of PNU-120596 to receptors that had covalently bound (tethered) agonists, applications of MLA nonetheless produced transient, concentration-dependent decreases in current. When persistent currents were generated by sequential applications of NS6740 and GAT107, applications of MLA at high concentrations (≥100 nM) reduced current, whereas lower concentrations actually produced concentration-dependent increases in current (Papke et al., 2018b).

VIII. Discussion and Conclusions

α7 nAChRs are marvelously complex and challenging drug targets with a rich array of conformational states regulated by both orthosteric and allosteric binding sites (Fig. 14). Although ligands working at the orthosteric sites give us a mere glimpse at ion-channel activation, and silent agonists binding in the extended orthosteric site give us not even that much, both classes of ligands take the receptors into nonconducting states that may function in ways that we are only beginning to understand. The drugs binding to the allosteric modulator sites have revealed something of the complex conformational landscape associated with the nonconducting states, which themselves provide an entirely new dimension of potentially functional states. On top of this already complex matrix of interacting states, we have yet to appreciate in any real detail how this matrix expands as ligands bind at multiple sites that may initially be similar but dynamically change with increasing levels of agonist occupancy.

In this review, we have necessarily focused on channel activation as a reporter of α7 conformational dynamics. However, the addition of CAP to the profile of α7 therapeutics means that one of the greatest challenges will be to understand how conformational changes regulated by ligand binding to extracellular and transmembrane sites translate to intracellular systems of signal transduction that exist in α7-expressing cells of the immune system, some of which apparently do not even have the capacity for channel activation. We are only beginning to understand the mechanisms connecting ligand binding to channel activation in heteromeric receptors for which structures of extracellular and transmembrane domains are available (Morales-Perez et al., 2016; Walsh et al., 2018; Gharpure et al., 2019). However, each nAChR has a unique intracellular domain, and we have very limited understanding (Stokes et al., 2015) of their functions. The intracellular domain of α7 receptor subunits has features that have been well conserved through evolution and hold promise for bettering our understanding of the function of α7 receptors.

Although the α7-selective agonists discussed are clearly active in the various assays that were used to identify them, continued work in this area would benefit from more consideration of how they will be presented to the receptors in vivo. In most of the systems used, detection of any response at all required the rapid application of relatively high concentrations of agonist to evoke a coordinated response from a significant fraction of the receptors. This mode of delivery and synchronized activation of receptors are largely irrelevant to the therapeutic delivery of drugs, which would typically be associated with slow delivery of low concentrations of drug (Papke et al., 2011). Although an attempt was made to model this with EVP-6124 and perhaps gave misleading results (Fig. 3), it should be kept in mind for all fast-acting, nondenaturing agonists that their activity in vivo should be characterized with more relevant protocols.

As noted above, all of the recently characterized α7 agonists, the DPP family stands out as the most novel both in structure and functional diversity. They present a challenge to our conventional models of the nAChR pharmacophore, and because of their wide range of desensitizing activities, studies of their activity in vivo may help determine the relative significance of desensitization for specific indications. Another area in which additional work would be beneficial is in regard to separation of racemic compounds into component isomers of differing activity. This will not only provide for more selective drugs with specific activities, but also, as structural models continue to be developed, knowledge of the

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**Fig. 14.** Binding sites for therapeutic ligands on α7 nAChRs. Cartoon of a cut-away view of an α7 receptor subunit complex (two subunits removed) and the approximate locations of the binding sites for the ligands discussed in this review. Located in the extracellular vestibule are putative binding sites (A) for allosteric ligands, such as ago-PAMs, allosteric agonists (2NDEP), and allosteric antagonists/inverse agonists, such as (-/TMP-TQS. Located at subunit interfaces on the outer surface of the extracellular domain are the binding sites (O) for ACh and other orthosteric agonists. These sites will also overlap the sites (S) that bind somewhat larger silent agonists. The binding site for PAMs and other allosteric modulators (M) is within the transmembrane domain and requires specific residues at the outer end of the second transmembrane domain. This figure is adapted from (Papke and Lindstrom 2020).
stereochemistry of active versus inactive compounds will permit more productive structure-based design of new drugs.

Clearly the study of α7 nAChR presents unique challenges that when appreciated and met will allow for greatly improved therapeutic targeting for particular indications. We need to better understand the conformational dynamics of the receptor as regulated by ligand binding. This can be accomplished by continuing to generate new chemical tools and characterizing the time- and concentration-dependent effects of those ligands on both the conducting and nonconducting states of the receptor. Those data can then also be used to inform the experimental design in a variety of functional assays. Through understanding how specific ligands manipulate all of the conformational states of α7, we will be able to target individual elements in the intracellular cascades associated with inflammatory disease using drugs, such as silent agonists and our recently discovered class of allosteric agonists, as well as to hopefully further develop new therapeutics for cognitive disorders and dementias.

Authorship Contributions

Participated in research design: Papke, Horenstein.
Conducted experiments: Papke.
Contributed new reagents or analytic tools: Horenstein.
Performed data analysis: Papke.
Wrote or contributed to the writing of the manuscript: Papke, Horenstein.

Appendix

Figure preparation: To illustrate the functional properties of α7-targeting ligands discussed in this review, we have drawn upon the large archive of data in the Papke laboratory. Except when noted, much of the data were used in the preparation of papers that are cited in the review or come from unpublished experiments with the same protocols. Therefore, these figures are to a degree adapted from those prior papers, and when appropriate, statistical analyses are provided in the original papers. However, the data for the illustrations in this review were all generated from fresh analyses of the original pClamp data files.

Expression in Xenopus Oocytes

The human α7 nAChR clone was obtained from Dr. J. Lindstrom (University of Pennsylvania, Philadelphia, PA). The human resistance to cholinesterase 3 clone was obtained from Dr. M. Treinin (Hebrew University, Jerusalem, Israel) and coexpressed with α7 to improve the level and speed of α7 receptor expression without affecting the pharmacological properties of the receptors (Halevi et al., 2003). Subsequent to linearization and purification of the plasmid cDNAs, complementary RNAs were prepared using the mMessage mMachine in vitro RNA transcription kit (Ambion, Austin, TX).

Oocytes were surgically removed from mature female Xenopus laevis frogs (Nasco, Ft. Atkinson, WI). Frogs were maintained in the Animal Care Service facility of the University of Florida, and all procedures were approved by the University of Florida Institutional Animal Care and Use Committee. In brief, the frog was first anesthetized for 15–20 minutes in 1.5-liter frog tank water containing 1 g of 3-aminobenzoate methanesulfonate buffered with sodium bicarbonate. The harvested oocytes were treated with 1.4 mg/mL type I collagenase (Worthington Biochemicals, Freehold NJ) for 2–4 hours at room temperature in calcium-free Barth’s solution (88 mM NaCl, 1 mM KCl, 2.38 mM NaHCO3, 0.82 mM MgSO4, 15 mM HEPES, and 12 mg/L tetracycline, pH 7.6) to remove the ovarian tissue and the follicular layers. Stage V oocytes were subsequently isolated and injected with 4–6 ng α7 RNA and 2–3 ng RIC-3 RNA (2:1 ratio) in 50 nl water. Oocytes were maintained in Barth’s solution with calcium (additional 0.32 mM Ca(NO3)2) and 0.41 mM CaCl2, and recordings were carried out 1–4 days after injection.

Two-Electrode Voltage-Clamp Electrophysiology

Experiments were conducted using OpusXpress 6000A (Molecular Devices, Union City, CA) (Papke and Stokes, 2010). Both the voltage and current electrodes were filled with 3 M KCl. Oocytes were voltage-clamped at −60 mV at room temperature (14 °C). The oocytes were bath-perfused with Ringer’s solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl2, 10 mM HEPES, and 1 μM atropine, pH 7.2) at 1 ml/min. The control ACh concentrations were 60 μM.

Solutions were applied from g6-well plates via disposable tips. Drug applications were 12 seconds in duration followed by 85-second washout periods. The responses were calculated as both peak current amplitudes and net charge, as previously described (Papke and Porter Papke, 2002). Data were collected at 50 Hz, filtered at 20 Hz, and analyzed by Clampfit 9.2 or 10.0 (Molecular Devices) and Excel (Microsoft, Redmond, WA). Data were expressed as mean ± S.E.M. from at least five oocytes for each experiment and plotted with Kalegraph 4.52 (Abelbeck Software, Reading, PA). Multicell averages were calculated for comparisons of complex responses. Averages of the normalized data were calculated for each of the 10.322 points in each of the 206.44-second traces (acquired at 50 Hz) as well as the S.E. for those averages.

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References


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