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Understanding the Bases of Function and Modulation of α7 Nicotinic Receptors: Implications for Drug Discovery

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ABSTRACT

The nicotinic acetylcholine receptor (nAChR) belongs to a superfamily of pentameric ligand-gated ion channels involved in many physiologic and pathologic processes. Among nAChRs, receptors comprising the α7 subunit are unique because of their high Ca\(^{2+}\) permeability and fast desensitization. nAChR agonists elicit a transient ion flux response that is further sustained by the release of calcium from intracellular sources. Owing to the dual ionotropic/metabotropic nature of α7 receptors, signaling pathways are activated. The α7 subunit is highly expressed in the nervous system, mostly in regions implicated in cognition and memory and has therefore attracted attention as a novel drug target. Additionally, its dysfunction is associated with several neuropsychiatric and neurologic disorders, such as schizophrenia and Alzheimer’s disease. α7 is also expressed in non-neuronal cells, particularly immune cells, where it plays a role in immunity, inflammation, and neuroprotection. Thus, α7 potentiation has emerged as a therapeutic strategy for several neurologic and inflammatory disorders. With unique activation properties, the receptor is a sensitive drug target carrying different potential binding sites for chemical modulators, particularly agonists and positive allosteric modulators. Although macroscopic and single-channel recordings have provided significant information about the underlying molecular mechanisms and binding sites of modulatory compounds, we know just the tip of the iceberg. Further concerted efforts are necessary to effectively exploit α7 as a drug target for each pathologic situation. In this article, we focus mainly on the molecular basis of activation and drug modulation of α7, key pillars for rational drug design.

Introduction

Nicotine has been a key molecule for the advancement of pharmacology since the beginning of the 20th century, when Langley (1905), through fundamental experiments, concluded that muscle contraction was mediated by a “receptive substance” present on the muscle. The muscle nicotinic acetylcholine receptor (nAChR) was thus a pillar in the discovery of neurotransmitter receptors (Langley, 1905). Still, it was not until 1970 that the first neurotransmitter receptor, nAChR, was identified (Changeux et al., 1970; Miledi and Potter, 1971). With the later advent of the molecular biology revolution in the 1980s, the nAChR family was first identified and an extended family of homologous pentameric receptors was revealed (Patrick et al., 1983; Le Novère and Changeux, 1995). This class of receptors was first known as Cys-loop receptors because all family subunits contain a conserved pair of disulfide-bonded cysteines separated by 13 residues. The discovery of orthologs in prokaryotes (Tasneem et al., 2005), which lack the double cysteines, has extended the Cys-loop family to the superfamily of pentameric ligand-gated ion channels (pLGIC).

In vertebrates, the pLGIC superfamily includes cationic channels, nAChRs and serotonin 5-HT\(_3\) receptors, and anionic channels activated by GABA or glycine (Le Novère and Changeux, 2001; Lester et al., 2004; Sine and Engel, 2006; Bartos et al., 2009). Their vital role in converting chemical recognition into an electrical impulse makes these receptors prime loci for learning, memory, and disease processes, as well as targets for clinically relevant drugs.

The nAChR is widely distributed throughout the animal kingdom, from nematodes to humans (Le Novère and Changeux, 1995). nAChRs are expressed in many regions of the central nervous system, mostly in regions implicated in cognition and memory. Additionally, nAChRs have emerged as a therapeutic strategy for several neurologic and inflammatory disorders. With unique activation properties, the receptor is a sensitive drug target carrying different potential binding sites for chemical modulators, particularly agonists and positive allosteric modulators. Although macroscopic and single-channel recordings have provided significant information about the underlying molecular mechanisms and binding sites of modulatory compounds, we know just the tip of the iceberg. Further concerted efforts are necessary to effectively exploit α7 as a drug target for each pathologic situation. In this article, we focus mainly on the molecular basis of activation and drug modulation of α7, key pillars for rational drug design.

Abbreviations:
ACh, acetylcholine; α-BTX, α-bungarotoxin; ECD, extracellular domain; JAK, Janus kinase; LY-2087101, (2-amino-5-keto-thiazole), [2-[(4-fluorophenylamino)-4-methyl-5-thiazolyl]-3-thienyl-methanone; nAChR, nicotinic acetylcholine receptor; NAM, negative allosteric modulators; NS-1738, 1-(5-chloro-2-hydroxyphenyl)-3-(2-chloro-5-trifluoromethylphenyl)urea; PAM, positive allosteric modulator; pLGIC, pentameric ligand-gated ion channels; PNU-120596, 1-(5-chloro-2,4-dimethoxyphenyl)-3-(5-methylisoxazol-3-yl)urea; SAM, silent allosteric modulator; STAT, signal transducer and activator of transcription; TMD, transmembrane domain; TQS, 4-(naphthalen-1-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide.
nAChR Structure and Function

nAChR subunits are classified as two types, α and non-α, with the α-type containing a disulfide bridge in the agonist binding site. Five muscular (α1, β, γ, ε, and δ) and eleven neuronal (α2–α7, α9, α10, and β2–β4) nAChR subunits have been identified in the mammalian genome (ligand-gated ion channel database, http://www.ebi.ac.uk/compneur-srv/LGICdb/cys-loop.php).

nAChRs are assembled from five identical (α7 or α9) or different subunits (at least two α-type subunits), and can form a variety of different heteromeric receptors with a broad spectrum of pharmacological and kinetic properties (Fig. 1). The resolution of the three-dimensional structures of pLGICs has been the subject of intense efforts over the last decade (Brejc et al., 2001; Dellasanti et al., 2007; Hilf and Dutzler, 2008, 2009; Boquet et al., 2009; Hibbs and Gouaux, 2011; Corringer et al., 2012; Hassaine et al., 2014; Miller and Aricescu, 2014; Sauguet et al., 2014; Cecchini and Changeux, 2015). However, no high-resolution structure of the full length α7 has been reported to date; an extracellular domain of α7/AChBP chimera (Li et al., 2011; Nemez and Taylor, 2011) and a nuclear magnetic resonance (NMR) structure of α7 transmembrane domain have been described (Bondarenko et al., 2014).

All pLGICs share a conserved organization with five subunits symmetrically arranged around a central ion pore (Fig. 2). Functional domains include the extracellular domain (ECD), which carries the agonist binding sites at subunit interfaces; the transmembrane domain (TMD), which contains the ion pore and the gate; and the intracellular domain (ICD), which contains determinants of channel conductance and sites for regulation and intracellular signaling (Paulo et al., 2009; Jones et al., 2010; King et al., 2015) (Fig. 2). The interface between the ECD and TMD, also referred to as the coupling region, is important for coupling agonist binding to channel opening (Bouzat et al., 2004; Lee and Sine, 2005; Castillo et al., 2006; Bartos et al., 2009), as well as for determining open channel lifetime and rate of desensitization (Bouzat et al., 2008; Yan et al., 2015) (Fig. 2).

The possible structural events that translate neurotransmitter binding at the ECD into opening of the transmembrane ion channel 60 Å away is an issue of intense research that has been discussed in recent reviews (Corringer et al., 2012; Althoff et al., 2014; Sauguet et al., 2014; Cecchini and Changeux, 2015). On the basis of the Monod-Wyman-Changeux model (Monod et al., 1965), the functional response of a pLGIC can be interpreted as a selection from a few global and discrete conformations elicited by the binding of agonist: closed, open, and desensitized, the latter showing high agonist affinity at the same time being impermeable to ions (Zhang et al., 2013) (Fig. 3). Intermediate states between closed and open or open and desensitized states have been proposed for nAChRs and several pLGICs (Lape et al., 2008; Corradi et al., 2009; Mukhtasimova et al., 2009; Cecchini and Changeux, 2015). Therefore, the number of states in the main conformations and the rate of transition between states determine receptor kinetics, and this tunes each receptor to its physiologic role. In turn, drugs, by binding to different states, can differently modulate receptor function.

α7 in the Nervous System in Healthy and Disease States

α7 is one of the most abundant nAChRs in the central nervous system. It is particularly expressed in regions implicated in cognitive function and memory, such as hippocampus, cortex, and several subcortical limbic regions (see Lendvai et al., 2006; Bartos et al., 2009) (Fig. 1). The possible structural events that translate neurotransmitter binding at the ECD into opening of the transmembrane ion channel 60 Å away is an issue of intense research that has been discussed in recent reviews (Corringer et al., 2012; Althoff et al., 2014; Sauguet et al., 2014; Cecchini and Changeux, 2015). On the basis of the Monod-Wyman-Changeux model (Monod et al., 1965), the functional response of a pLGIC can be interpreted as a selection from a few global and discrete conformations elicited by the binding of agonist: closed, open, and desensitized, the latter showing high agonist affinity at the same time being impermeable to ions (Zhang et al., 2013) (Fig. 3). Intermediate states between closed and open or open and desensitized states have been proposed for nAChRs and several pLGICs (Lape et al., 2008; Corradi et al., 2009; Mukhtasimova et al., 2009; Cecchini and Changeux, 2015). Therefore, the number of states in the main conformations and the rate of transition between states determine receptor kinetics, and this tunes each receptor to its physiologic role. In turn, drugs, by binding to different states, can differently modulate receptor function.

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et al., 2013). It is also expressed on non-neuronal cells, including astrocytes, microglia, oligodendrocyte precursor cells, and endothelial cells, where it plays a role in immunity, inflammation, and neuroprotection (Shytle et al., 2004; Shen and Yakel, 2012; Dineley et al., 2015).

In neurons, $\alpha_7$ receptors localize presynaptically on GABAergic and glutamatergic terminals in the hippocampus and other regions to facilitate release of neurotransmitters. Postsynaptically, $\alpha_7$ receptors mediate fast synaptic transmission, and in perisynaptic locations they modulate other inputs to neurons and activate a variety of signaling pathways through volume transmission (Gotti and Clementi, 2004; Jones and Wonnacott, 2004; Dani and Bertrand, 2007; Dickinson et al., 2008; Albuquerque et al., 2009; Sinkus et al., 2015) (Fig. 4).

$\alpha_7$ contributes to cognitive functioning, sensory processing information, attention, working memory, and reward pathways, and a large body of evidence shows that enhancing $\alpha_7$ activity improves attention, cognitive performance, and neuronal resistance to injury (reviewed in Uteshev, 2014). Significant reduction of $\alpha_7$ in the brain, particularly in the hippocampus, has been reported in Alzheimer disease (Guan et al., 2000; Kadir et al., 2006) and schizophrenic patients (Schaaf, 2014; Dineley et al., 2015; Deutsch et al., 2016). A partial duplication of CHRNA7 resulting in the chimeric gene CHRFAM7A, which is present only in humans, has been associated with schizophrenia (Sinkus et al., 2009; Neri et al., 2012). Its gene product, dup$\alpha_7$, lacks part of the binding site and may act as a negative modulator of $\alpha_7$ (Wang et al., 2014).

Despite its homomorphic character, $\alpha_7$ can assemble with other subunits to form heteromeric receptors. In particular, $\alpha_7\beta_2$ heteromeric receptors have been detected in several deficit hyperactivity disorder, epilepsy, Alzheimer disease, and sensory processing deficit (Sinkus et al., 2009, 2015; Schaaf, 2014; Dineley et al., 2015; Deutsch et al., 2016). A partial duplication of CHRNA7 resulting in the chimeric gene CHRFAM7A, which is present only in humans, has been associated with schizophrenia (Sinkus et al., 2009; Neri et al., 2012). Its gene product, dup$\alpha_7$, lacks part of the binding site and may act as a negative modulator of $\alpha_7$ (Wang et al., 2014).

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Therefore, $\alpha 7$ nAChR is emerging as an important drug target for the modulation of inflammation in different pathological contexts, including sepsis, ischemia/reperfusion, rheumatoid arthritis, and pancreatitis.

$\alpha 7$ Pharmacology and Ion Selectivity

Hallmark features of $\alpha 7$ receptors include high Ca$^{2+}$ permeability, relatively low sensitivity to ACh, full activation by choline, high-affinity for $\alpha$-bungarotoxin (\(\alpha\)-BTX), relatively low affinity for nicotine, and fast desensitization that occurs on the submillisecond time scale.

Dose-response curves show EC$_{50}$ values of $\sim$100–200 $\mu$M for ACh [Hill coefficient (nH) $\sim$1] (Andersen et al., 2013), 0.4–1.6 $\mu$M for choline, and 18–91 $\mu$M for nicotine (Wonnacott and Barik, 2007). Several $\alpha 7$-specific agonists have been synthesized, including PNU-282987 (EC$_{50}$ 128 nM), AR-R17779 (EC$_{50}$ $\sim$10–20 $\mu$M), compound A (EC$_{50}$ $\sim$14 nM to 0.95 $\mu$M), and partial agonists GTS-21 (EC$_{50}$ $\sim$6–26 $\mu$M), and SSR180711 (EC$_{50}$ $\sim$1–4 $\mu$M). Selective competitive antagonists are $\alpha$-BTX (IC$_{50}$ $\sim$100 $\mu$M), which has been widely used to detect $\alpha 7$ in tissues, and methylcholanthrene (MLA, IC$_{50}$ 10–200 $\mu$M) (Wonnacott and Barik, 2007).

$\alpha 7$ allows flux of Na$^+$ and K$^+$ and is highly permeable to Ca$^{2+}$. The PCa$^{2+}$/PNa$^+$ ratio is $\sim$10–20, which is greater than that of other nAChRs and similar to N-methyl-d-aspartate receptors (Séguela et al., 1993; Albuquerque et al., 1997). The high Ca$^{2+}$ permeability underlies most of $\alpha 7$ functions: facilitation of neurotransmitter release, depolarization of postsynaptic cells, and initiation of many cellular processes through its action as a second messenger (Gotti and Clementi, 2004). The transient increase in intracellular Ca$^{2+}$ is converted into a sustained, wide-ranging phenomenon by calcium release from intracellular stores through a calcium-induced calcium release mechanism, a process involving IP$_3$ and ryanodine receptors (Tsuneki et al., 2000; Dajas-Bailador et al., 2002; Guerra-Alvarez et al., 2015) (Fig. 5).

The concept of $\alpha 7$ as a dual metabotropic/ionotropic receptor is attracting increasing attention (Kabbani et al., 2013). $\alpha 7$ binds both G$\alpha$ and G$\beta\gamma$ proteins through the M3–M4 loop and enables a downstream calcium signaling response that can persist beyond the expected time course of channel activation (Fig. 5) (Kabbani et al., 2013; King et al., 2015). Moreover, in lymphocyte T cells, mobilization of Ca$^{2+}$ through the $\alpha 7$ channel is not necessarily required for the nicotine-induced release of Ca$^{2+}$ from the internal stores (Razani-Boroujerdi et al., 2007). Channel-independent signal transduction has been related to the role of $\alpha 7$ in inflammation (de Jonge and Ulloa, 2007; Thomsen and Mikkelsen, 2012) and in neurite growth (Nordman and Kabbani, 2012; Kabbani et al., 2013).

$\alpha 7$ is not only permeable to Ca$^{2+}$ but is also modulated by the ion; Ca$^{2+}$ has been shown to regulate agonist efficacy and cooperativity (Bonfante-Cabarcas et al., 1996; Albuquerque et al., 1997). As in other pLGICs (Zimmermann et al., 2012), the divergent modulatory sites may be located at the ECD.

$\alpha 7$ Channel Kinetics

Heterologous expression of $\alpha 7$ in oocytes and mammalian cells combined with electrophysiological experiments has provided information about the receptor’s unique activation properties. The surface expression of recombinant $\alpha 7$ requires...
coexpression of the chaperone RIC-3 (Williams et al., 2005), a transmembrane protein that is required for efficient receptor folding, assembly, and functional expression (Castillo et al., 2005; Millar, 2008). Recently, another chaperone, NACHO, has been identified. NACHO is a transmembrane protein of neuronal endoplasmic reticulum that mediates assembly of a7 by promoting protein folding, maturation through the Golgi complex, and expression at the cell surface (Gu et al., 2016).

Typical a7 receptor-mediated currents decay rapidly in the presence of agonist as a result of desensitization (Fig. 6A). Given the fast kinetics, the temporal resolution of agonist exchange and most recording systems limit the accurate estimation of the true desensitization rate (Zhou et al., 1998; Lovinger et al., 2002; Bouzat et al., 2008), which may partially account for the great variability of desensitization rates found in the literature. Outside-out patches rapidly perfused with ACh, which allows for a more accurate determination of the desensitization rate, show current decay time constants of ∼0.4 milliseconds (Bouzat et al., 2008). Owing to their fast decay, a7 peak responses occur in advance of complete solution exchange. Therefore, more accurate EC50 values are obtained if the net charge, which represents the time integration of all channel activation, rather than the peak current, is used for the analysis of the concentration-effect relationship (Papke and Porter Papke, 2002) (Fig. 6A).

At the single-channel level, channel activity appears as isolated brief pulses (0.1–0.3 milliseconds) flanked by long closed periods and, less often, as two or three brief pulses in quick succession (bursts) (Fig. 6B). Thus, a7 has a very low open probability. Single-channel openings exhibit a broad distribution of current amplitudes, probably owing to limited time resolution of the brief openings, with a maximum of ∼10 pA at −70 mV (Mike et al., 2000; Bouzat et al., 2008; Andersen et al., 2013; daCosta and Sine, 2013; Yan et al., 2015).

Although there is no general consensus for an a7 kinetic model, an interesting aspect is that the temporal pattern of single ACh-activated currents is similar at 10 μM or 1 mM ACh (Bouzat et al., 2008) (Fig. 6B). This lack of concentration-dependence combined with the fact that most receptor activation episodes consist of a single opening with a duration similar to the desensitization time constant suggests that desensitization is the predominant pathway for channel closing, a unique feature among nAChRs. Control of open-channel lifetime through desensitization has potential consequences for inter-response latency at a synapse where the neurotransmitter pulse is transient. Recovery from desensitization depends on agonist concentration and exposure duration, since different desensitized states may exist. Desensitized a7 receptors expressed in human embryonic kidney cells recover with a time constant of ∼1 second (Bouzat et al., 2008), whereas 15–30 seconds are required for full recovery in the hippocampus (Frazier et al., 1998). Thus, after a7 brief response, a latency of several seconds is required to generate another response of full amplitude. The fast desensitization and brief open duration may avoid cell toxicity caused by increased intracellular Ca2+ owing to a7 overstimulation.
Another intriguing aspect of $\alpha 7$ activation is the relationship between ACh occupancy and activation. We set out to determine how many of the five identical agonist binding sites are required to activate $\alpha 7$ by developing a strategy that utilizes coexpression of an inactivated binding-site subunit and a reporter amplitude subunit. This allows for the determination of the number of ACh-occupied sites from the amplitude of each individual single-channel opening (Rayes et al., 2009; Andersen et al., 2011, 2013). The results revealed that ACh occupancy of only one of five $\alpha 7$ binding sites is necessary for activation, and that open-channel lifetime of a single-occupied receptor is indistinguishable from that of receptors containing five intact binding sites (Andersen et al., 2013). Also, occupancy of a single site by the antagonist methylcaconitine (Palma et al., 1996) or $\alpha$-BTX (daCosta et al., 2011, 2015) will inhibit $\alpha 7$ function.

Whole-cell experiments have revealed the physiologic consequence of having more sites than those required for activation; saturation of receptors in cells expressing a wild-type and a binding-site mutant subunit ($\alpha 7$Y188T) is achieved at higher ACh concentrations when the proportion of receptors with fewer functional binding sites increases (Andersen et al., 2013; Williams et al., 2011a). This suggests that $\alpha 7$ is a highly sensitive sensor of ACh and is therefore adapted to function with submaximal occupancy of its sites, a property appropriate for volume transmission.

The unique activation properties of $\alpha 7$ also suggest that any slight change in the energy barriers between active, closed, and/or desensitized states may have a deep impact on receptor function, which makes these receptors very sensitive drug targets.

**$\alpha 7$ Modulation as a Therapeutic Strategy**

In addition to agonists and antagonists that bind to the orthosteric site, a large number of compounds modulate $\alpha 7$ function by binding to allosteric sites. These compounds may act as: 1) positive allosteric modulators (PAMs) that potentiate currents only in the presence of the agonist; 2) allosteric agonists that activate receptors from nonorthosteric sites; 3) negative allosteric modulators (NAMs), which either act as open-channel blockers by binding to the pore or inhibit activation allosterically; and 4) silent allosteric modulators (SAMs) that have no effect on orthosteric agonist responses but block allosteric modulation (Fig. 7).

Since stimulation of $\alpha 7$ improves attention, cognitive performance, and neuronal resistance to injury in addition to eliciting robust analgesic and anti-inflammatory effects, $\alpha 7$ potentiation has emerged as a potential therapeutic strategy, and the search for novel potentiaters is an active research field. The potential therapeutic use of several $\alpha 7$ partial agonists and PAMs on animals and humans has been documented in several recent reviews (e.g., Wallace and Porter, 2011; Thomsen and Mikkelsen, 2012; Lendvai et al., 2013; Dineley et al., 2015) (Table 1). Still, no drug has reached phase III clinical stage.

Compared with agonists, PAMs are promising therapeutic tools because they: 1) better maintain the temporal spatial characteristics of endogenous activation; 2) show higher selectivity, since the orthosteric site is more conserved among nAChRs than allosteric sites (Yang et al., 2012); 3) allow greater diversity in structure and final effects; 4) reduce tolerance attributable to $\alpha 7$ desensitization; and 5) act as neuronal protectors (Kalappa et al., 2013; Sun et al., 2013; Uteshev, 2014). In particular, because neuronal damage elevates choline levels near the site of injury, the presence of a PAM may not require coapplication of an agonist for the mediation of a local neuroprotective effect (Uteshev, 2014). Therefore, it is increasingly accepted that targeting allosteric sites can provide novel medications with greater structural diversity and specificity.

PAMs have been classified on the basis of their macroscopic effects as type I or type II (Fig. 8). Type I PAMs mainly enhance agonist-induced peak currents without significantly affecting current decay and do not reactivate desensitized receptors, whereas type II PAMs delay desensitization and reactivate desensitized receptors (Bertrand and Gopalakrishnan, 2007; Arias and Bouzat, 2010; Williams et al., 2011b). The ratio of the changes in net charge/peak current induced by type I PAMs is close to one, whereas it is higher than one in the presence of type II PAMs (Andersen et al., 2016; Williams et al., 2011c).

Single-channel recordings provide an invaluable tool for understanding the foundations of these macroscopic effects. In the presence of either type I or type II PAMs, ACh-activated $\alpha 7$ channels show prolonged open durations and appear in longer activation episodes, revealing that both PAM types affect activation kinetics (Andersen et al., 2016) (Fig. 8). The most efficacious PAM to date is PNU-120596, a type II PAM (Hurst et al., 2005). This compound elicits significantly prolonged openings that appear grouped in bursts, which in turn coalesce into long activation periods of several seconds, referred to as clusters (daCosta et al., 2011, 2015; Williams et al., 2011b; Palczyńska et al., 2012; Andersen et al., 2016). However, the
Fig. 7. Allosteric modulatory sites in \( \alpha7 \). Structural model of the \( \alpha7 \) receptor viewed from the side representing several potential sites for allosteric modulators. At the TMD, the intrasubunit transmembrane cavity is a site for a great variety of compounds that may elicit different pharmacological effects (potentiation (PAMs), inhibition (NAMs), no effect (SAMs), or activation (allosteric agonists)). PNU-120596 docked into this cavity is shown. Open-channel blockers inhibit response by transiently blocking the flux of ions through the pore. At the ECD, different potential sites for inhibitors and potentiators have been proposed (Ludwig et al., 2010; Spurny et al., 2015).

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**α7 Modulation**

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**Fig. 8.** Macroscopic and single-channel current profiles of human α7 in the presence of typical type I and type II PAMs. Macroscopic current profiles have been used to classify PAMs into type II (i.e., PNU-120596 and PAM-2), which increase the decay time constant, or type I, which only increase the peak current (5-HI and NS-1738). Left: Macroscopic currents elicited by ACh in the absence (black) and presence of the specified PAM (gray traces). Right: Traces of 50–100 μM ACh-activated single α7 channels in the absence or in the presence of 1 μM PNU-120596, 5 μM PAM-2, 2 mM 5-HI, 10 μM NS-1738. Membrane potential: −70 mV. Channel openings are shown as upward deflections. All PAMs enhance open channel lifetime and elicit activation episodes formed by successive opening events.

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**α7 Allosteric Binding Sites**

Computational studies (Dey and Chen, 2011), electrophysiological studies from mutant or chimeric receptors (Bertrand et al., 2008; Young et al., 2008; Collins et al., 2011; daCosta and Sine, 2013), crystallographic studies, (Spurny et al., 2015), and NMR studies (Bondarenko et al., 2014) have suggested the existence of several allosteric binding sites, some of which are common to other pLGICs (Spurny et al., 2015). Thus, the intrasubunit transmembrane cavity (Hibbs and Gouaux, 2011).

The macrocyclic lactone ivermectin (a type I PAM) also appears to be the result of either slight changes in desensitization that are more, this cavity may be a common modulatory site within the pLGIC superfamily from which a large variety of different compounds that bind to and mediate a great spectrum of structural changes and displaying distinct pharmacological effects (typical of type I PAMs, type II PAMs, NAMs, SAMs, and allosteric agonists) may bind to this common site (Gill et al., 2011, 2012, 2013; Palczynska et al., 2012). Single-channel activity of α7 in the presence of allosteric agonists resembles that of the receptor in the presence of ACh and a PAM. Both are characterized by long bursts instead of isolated brief ACh-elicited openings, indicating activation with significantly reduced desensitization (Palczynska et al., 2012).

Moreover, α7-selective allosteric modulators showing subtle structural changes and displaying distinct pharmacological effects (typical of type I PAMs, type II PAMs, NAMs, SAMs, and allosteric agonists) may bind to this common site (Gill et al., 2013; Gill-Thind et al., 2015). Thus, the intrasubunit transmembrane cavity appears to be a promiscuous binding site with a strategic location for allosteric modulation. Furthermore, this cavity may be a common modulatory site within the pLGIC superfamily from which a large variety of different compounds that bind to and mediate a great spectrum of allosteric effects (Corradi et al., 2011; Nury et al., 2011; Jayakar et al., 2013; Sauguet et al., 2014).
**Extracellular Allosteric Binding Sites.** The putative binding site of galantamine, an α7 potentiator, is located at the outer surface of the ECD in the vicinity of the ACh site (Hansen and Taylor, 2007; Ludwig et al., 2010). Three different allosteric sites in the ECD of the α7/AChBP chimeras were also identified by X-ray crystallography (Spurny et al., 2015; Fig. 7). Although all the allosteric binders behaved on human α7 as negative allosteric modulators, it was proposed that their chemical modification could lead to a change in functional activity.

Binding to potential ECD sites has been proposed for the type I PAMs 5-HI and NS-1738, although other reports support a TMD location (Placzek et al., 2004; Bertrand et al., 2008; Hu and Lvinger, 2008; Gronljen et al., 2010; Collins et al., 2011; Andersen et al., 2016). A virtual screening revealed that some PAMs that bind to the TMD, such as PNU-120596 and TQS, also dock into potential allosteric sites at the ECD (Dey and Chen, 2011). Therefore, for any given PAM, multiple binding sites or domains may be involved in the conformational changes associated with potentiation, which could account for these controversial results. Also, until the location of an allosteric binding site is unequivocally defined, it is advisable to refer to structural determinants of potentiation instead of a binding site.

Despite the large body of experimental evidence supporting α7 potentiation as a promising therapeutic strategy, there are still many unsolved challenges: 1) Potentiation by exogenous agonists may inhibit α7 response owing to desensitization. Thus, PAMs might have therapeutic benefits in situations where stronger agonist responses are desirable. 2) Given the presence of other nAChRs and homologous receptors, high PAM selectivity is required. However, PAMs targeting multiple receptors might show better efficacy (Iturriaga-Vásquez et al., 2015; Möller-Acuña et al., 2015). 3) Excessive receptor activation, particularly with efficacious nondesensitizing PAMs, might lead to cytotoxicity, which is an issue of concern and controversy (Ng et al., 2007; Liu et al., 2009; Williams et al., 2012; Guerra-Álvarez et al., 2015; Uteshev, 2016). 4) The broad distribution of α7 and its interplay with other signal pathways could make the cell response to a given PAM variable among cell types or conditions. Given the broad spectrum of effects and molecular mechanisms of PAMs, it is probable that each patient or pathologic situation could require a unique PAM.

**Concluding Remarks**

α7 has emerged as an important drug target for improving cognition and memory in several neuropsychiatric disorders, and as a target for inflammatory processes. α7 is unique owing to its high calcium permeability and fast desensitization, and it behaves as a ACh-sensitive sensor harboring a built-in filtering mechanism against excessive stimulation. Transient calcium responses are further sustained by the release of calcium from intracellular sources, and several signaling pathways are also activated because α7 has a dual ionotropic/metabotropic nature. Its ubiquitous location and pleiotropic effects make α7 an interesting but complex drug target. A better understanding of the molecular basis underlying allosteric modulation and its wide spectrum of effects, as well as the availability of high resolution structures of α7, will help in the rational design of therapeutics for the receptor.

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**Authorship Contributions**

Wrote or contributed to the writing of the manuscript: Bouzat, Corradi.

**References**


a7 Modulation


