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Allosteric modulation of nicotinic acetylcholine receptors

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ABSTRACT

Allosteric modulation refers to the concept that proteins could exist in multiple conformational states and that binding of allosteric ligands alters the energy barriers or ''isomerization coefficients'' between various states. In the context of ligand gated ion channels such as nicotinic acetylcholine receptors (nAChRs), it implies that endogenous ligand acetylcholine binds at the orthosteric site, and that molecules that bind elsewhere on the nAChR subunit(s) acts via allosteric interactions. For example, studies with the homomeric α7 nAChRs indicate that such ligand interactions can be well described by an allosteric model, and that positive allosteric effectors can affect energy transitions by (i) predominantly affecting the peak current response (Type I profile) or, (ii) both peak current responses and time course of agonist-evoked response (Type II profile). The recent discovery of chemically heterogeneous group of molecules capable of differentially modifying nAChR properties without interacting at the ligand binding site illustrates the adequacy of the allosteric model to predict functional consequences. In this review, we outline general principles of the allosteric concept and summarize the profiles of novel compounds that are emerging as allosteric modulators at the α7 and α4β2 nAChR subtypes.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are widely characterized transmembrane proteins involved in the physiological responses to acetylcholine. These receptors are composed of homologous subunits encoded by a large multigene family, and are expressed in muscle, nerve and sensory cells where they play critical roles in neuronal transmission and modulation. The aim of this work is to review current knowledge on allosteric modulation at nAChRs. We first briefly discuss general principles of allosteric modulation and the properties of the α7 nAChR as a prototype of allosteric ligand gated channels and then describe the profiles of novel allosteric modulators acting at the α7 and α4β2 nAChR subtypes that are increasingly being explored in terms of therapeutic potential.

1.1. *General principles of allosteric modulation*

Introduced by Wymann, Monod and Changeux in 1965, the allosteric concept proposes that a protein can have multiple conformations (or states) and that binding of a molecule preferentially stabilizes the protein in a given conformation [1]. Although initially developed for enzymatic reactions, this concept was soon proposed to adequately describe properties of membrane proteins such as the endplate nAChR [2]. A key

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Abbreviations: ACh, acetylcholine; nAChRs, neuronal nicotinic acetylcholine receptors; DMPP, dimethylphenylpiperazinium; 5-HI, 5-hydroxyindole; PAM, positive allosteric modulator; NAM, negative allosteric modulator; BSA, bovine serum albumine; LBD, ligand binding domain; CD, channel domain; SLURP-1, secreted mammalian Ly-6/uPAR related protein 1

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feature of the allosteric concept is that, due to thermal agitation, the protein spontaneously changes conformation in absence of external influences. Another feature of the allosteric concept is that binding of a ligand stabilizes the protein in a preferential conformation (see for review [3]). For example, in the case of nAChRs, agonists are ligands that preferentially stabilize the receptor in the active open state, whereas competitive antagonists are ligands that stabilize the protein in a closed conformational state. Fig. 1A (upper panel) schematically depicts the concept of a protein with two conformational states. By definition, the site occupied by the natural ligand, which is typically at the interface between subunit protomers is called the *orthosteric site*. *Allosteric sites* are distinct from the orthosteric site and can be localized elsewhere on the protein. Binding of the ligand at the orthosteric site stabilizes the protein in the active state, whereas binding of an effector at an allosteric site alters the overall properties by modifying the energy barriers, represented by isomerization coefficients, between one or more states (Fig. 1B). While a two state model may be sufficient to account for the properties of some proteins, it is insufficient for the description of nAChRs and the minimal model should include at least three states such as described in Fig. 1B.

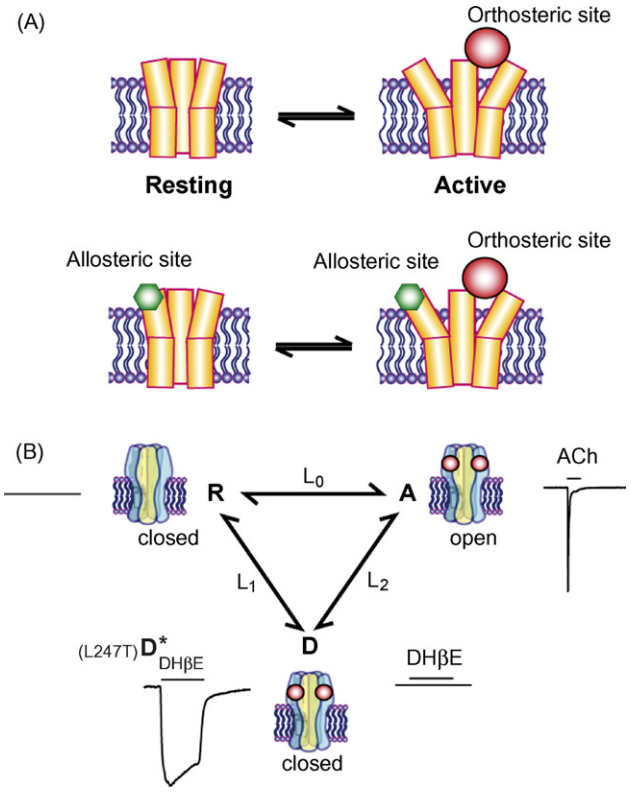

Fig. 1 – The concept of Allosteric Modulation. (A) Schematic representation of an integral membrane protein resulting from the assembly of three protomers and its transition between two different states. Upper panel represents protein conformations and the state preferentially stabilized when a ligand is bound to the orthosteric site. Lower panel symbolizes the binding of a molecule at an allosteric site. Note that allosteric sites can be localized anywhere on the protein. (B) Representative α7 ACh-evoked current and the minimal model required to describe such responses. Depicted is a typical ACh-evoked current recorded in a *Xenopus* oocyte expressing the human α7 nAChR and the minimal three state allosteric model with (R) the resting closed state, (A) the active open state and (D) the desensitized closed state. L_0, L_1, L_2 are the isomerization coefficients corresponding to the energy barriers between the three states. (D*) refers to the effect of the L247T mutation and typical currents evoked in this mutant channel by the competitive inhibitor DHβE is illustrated.

1.2. *Positive and negative allosteric modulation of nAChRs*

nAChRs are involved in a range of synaptic and extra synaptic functions [4]. In the peripheral nervous system, nAChRs mediate ganglionic neurotransmission whereas in the CNS, nicotinic cholinergic innervation mediates fast synaptic transmission and regulates processes such as transmitter release, synaptic plasticity and neuronal network integration by providing modulatory input to a range of other neurotransmitter systems. Over the years, genetic and pharmacological studies have implicated nAChR subtypes in a range of physiological and pathophysiological functions related to cognitive function, learning and memory, reward, motor control, arousal, analgesia and immune function. These and other observations have, in recent years, attracted considerable attention to nAChR subtypes as drug targets, the working hypothesis being that compounds capable of enhancing nAChR function can ameliorate pathophysiological deficits in many disease states. Many systematic reviews published on nAChRs agonists and antagonists are available that cover these aspects in greater detail [5,6].

nAChR subunits also host multiple allosteric sites. Binding of an allosteric ligand can affect the energy barrier between transitions resulting in a displacement of the equilibrium between states. Consequently, functional outcomes of the binding of an allosteric ligand (generally referred to as *effector*) can differ as a function of the transitions that are affected. Allosteric effectors that lower the energy barrier between the resting and active states increase the agonist-evoked response and are referred as positive allosteric modulators (PAM). In contrast, effectors that increase this energy barrier will cause a reduction of the agonist response and are termed negative allosteric modulators (NAM). Fig. 2A illustrates effects of lowering or increasing the L_0 coefficient on the agonist concentration–response curves. Note that for low L_0 values, a fraction of receptors remains activated even in absence of agonist. This corresponds to the spontaneous probability of transition from the resting to the active state. In other words, this implies that if a receptor displays a low isomerization coefficient between the resting and active state, a significant fraction of the receptors may be constitutively active.

1.3. *Allosteric modulation of α7 nAChRs*

Whereas it would go beyond the scope of this review to address properties of α7 nAChR in detail, it is important to recall that the α7 is the only mammalian subunit known thus

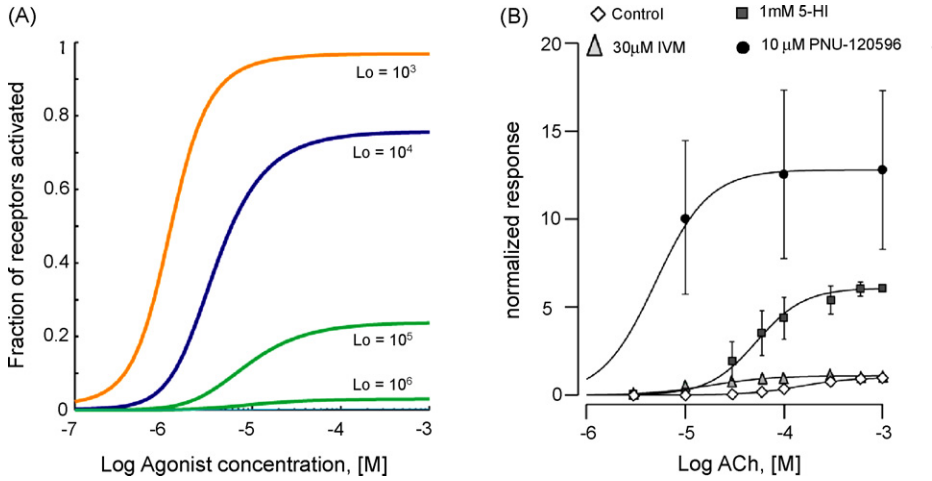

Fig. 2 – Effects of allosteric effectors at the concentration–activation curves. (A) Influence of the L_0 coefficient on the concentration–activation curve. Simulation of a two state allosteric model using four values of the L_0 coefficient. Lowering the isomerization coefficient from 10^6 to 10^3 causes an increase of the fraction of receptors that can be stabilized in the active state, reduces the concentration for half activation and increases the steepness of the curve. (B) Comparison of the effects of three positive allosteric modulators on the agonist concentration curves recorded in oocytes expressing the human α7 nAChR. Responses to ACh test pulses (3 s), were recorded first in control and after a brief exposure (30 s) to the allosteric modulator. Peak ACh-evoked currents were measured and plotted as a function of the logarithm of the agonist concentration. Cells were held at −100 mV throughout the experiment.

far, capable of forming functional nAChRs alone. The CHRNA7 gene, which encodes for the α7 subunit, is thought to be the ancestral gene from which have progressively emerged, by genetic duplication and variations, additional genes encoding the entire nAChR family [7]. In spite of the proposition that α7 can co-assemble with other subunits to make heteromeric nAChRs [8,9], it is widely accepted that majority of α7 nAChRs exist as homomeric receptors. The initial functional characterization of the chick α7 nAChR revealed that this homomeric receptor displays a rather low sensitivity to ACh and an unusually fast desensitization ([10], illustrated in Fig. 1B). Subsequently, numerous studies have confirmed that native α7 nAChRs of the mammalian central nervous system share similar functional properties [11–14]. Another salient feature is the high calcium permeability of the α7 nAChR [12,15,16].

In addition to viewing the α7 nAChR as an ancestral subunit found across species ranging from *C. elegans* to higher mammals, this receptor can also be viewed as a prototypical allosteric protein [3,17]. Functionally, α7 nAChRs are characterized by low agonist sensitivity, rapid activation and fast desensitization kinetics in the continued presence of the agonist. Kinetically, the receptor must have at least three possible states: (i) a resting closed state which is populated in absence of agonist, (ii) an active open state that is transiently stabilized upon exposure to the agonist and (iii) a desensitized closed state in which the receptor is stabilized by the sustained presence of the agonist. As functional studies reveal no rebound activity when the agonist is removed, it must be concluded that the return from desensitized-to-resting state occurs without detectable re-activation of the receptor. This suggests that the receptor can change conformation from the desensitized to the resting state without passing through the active state. The three state allosteric model initially proposed by Heidmann and Changeux for the neuromuscular junction receptor is therefore a minimal model that ought to be considered to describe properties of nAChRs [18,19].

A clear enigma in understanding the functioning of ligand gated channels is how binding of a ligand provokes the opening of the ion conducting pore formed by the transmembrane segments. Analysis of the crystal structure of the acetylcholine binding protein (AChBP) resolved, at atomic resolution, has begun to provide clues about protein ligand interactions [20–23]. These results confirmed previous studies indicating that the orthosteric ligand binds in the extracellular domain of the receptor at the interface formed by two adjacent subunits. Moreover, structure function studies carried out at the nAChRs and $GABA_A$ receptors revealed that the short amino acid segment between the second and third transmembrane domain is an important determinant in the gating process that takes place during receptor activation [24–27]. These studies indicate that binding of the ligand stabilizes the structure of the extracellular domain in a given conformational state and that coupling between the channel domain (CD) and the ligand binding domain (LBD) is facilitated by these amino acid interactions. The coordinated contribution of the LBD and CD are well illustrated by the effects observed in site directed mutagenesis studies. Amino acid exchanges in the LBD where shown to alter both receptor affinity and pharmacological properties. Mutations in the CD, however, yield more complex effects that can also affect both biophysical and pharmacological properties of the receptor. While it is easily understood that mutations in the selectivity filter of the ionic pore should yield differences in the ionic conductance, the pleiotropic effects caused by a single amino acid exchange (L247T) in the chick α7 nAChR are clearly of a

different nature. Namely, mutation of a single amino acid facing the ionic pore causes (i) suppression of receptor desensitization, (ii) some 200-fold increase in apparent affinity for the ligand, and (iii) altered pharmacological profile converting the effects of some competitive antagonists into full agonists [28,29]. Interestingly, however, all properties of the L247T mutant can be explained on the basis of the allosteric model assuming that this mutation renders a desensitized conductive state (see Fig. 1B). According to this hypothesis, properties of the L247T mutant should resemble those of the desensitized state rather than the active state, which also explains that competitive antagonists who stabilize the desensitized state activate the mutant receptor. The fact that competitive antagonists, such as methyllycaconitine or α-bungarotoxin, remain antagonists at the L247T mutant indicates that they stabilize the resting closed state. This hypothesis is supported by the fact that α-bungarotoxin binding is not affected by the L247T mutation [30].

Further analysis of the three-state model predicts that allosteric effectors that affect the energy barrier between resting and active states (L_0) should cause little or no modification of the agonist-evoked response kinetics. However, allosteric effectors affecting other energy transitions may cause detectable effects on the time course of the agonist-evoked response. Accordingly, two types of PAM may be recognized – Type I that predominately affects the apparent peak current, and Type II that increases both the apparent peak current and changes the desensitization profile of the agonist response. Other allosteric effectors, affecting additional properties of the receptors may also be expected. It should be noted that observations made at the α7 nAChR subtype could apply to heteromeric receptors as well, and a diversity of allosteric effects may be anticipated.

1.4. *First generation positive allosteric modulators of α7 nAChRs*

The anthelminthic agent, *ivermectin* (IVM) was identified as the first positive allosteric modulator of the α7 nAChR [31] (Fig. 3). Ivermectin increases maximal ACh-evoked current, modestly reduces desensitization time course, reduces the EC_{50} value of ACh and increases the slope of the concentration–activation curve (Hill coefficient). Multiple effects caused by ivermectin can be explained by a reduction of the isomerization coefficient L_0 between resting and active states. A fundamental consequence for such reduction of the energy barrier is that the pharmacological profile of the receptor will also be modified, as for example, increasing the efficacy of partial agonists. Indeed, dimethylphenylpiperazinium (DMPP), a partial agonist at the α7 nAChR, became almost a full agonist following exposure to ivermectin [31].

Subsequent to this initial observation, several molecules have been reported to enhance the ACh-evoked currents at the α7 nAChR [32–36]. For example, 5-*hydroxyindole* (5-HI) causes a significant increase of subsequent ACh-evoked current (Fig. 3). However, 5-HI is nonselective and weak, requiring high concentrations (1–20 mM) for potentiating α7 nAChR mediated current. 5-HI also modulates the 5-HT_3 receptor [32]. *Genistein*, a well-known tyrosine kinase inhibitor was found to act as an α7 PAM that like 5-HI, predominantly affected apparent peak current [33,37] (Fig. 3). As presence of Ca^{2+} in the extracellular medium was found to be necessary for proper functioning of many nAChR subtypes, it was suggested that these *divalent cations* could act as allosteric modulators. Confirmation of the allosteric nature of Ca^{2+} was made using site directed mutagenesis and construction of chimeras between the α7 nAChR and the $5HT_3$ receptor, the latter insensitive to the extracellular Ca^{2+} [38]. These studies revealed that Ca^{2+} binds in the extracellular domain of the α7 nAChR and interacts with the glutamate residue at position 172 localized in the complementary part of the ACh binding site.

The definition of an allosteric effector is also applicable to peptides and/or other proteins. This is best illustrated by the finding that *SLURP-1* (secreted mammalian Ly-6/uPAR related protein 1) secreted by human keratinocytes, is a positive allosteric modulator at the human α7 nAChR [39]. Exposure of the human α7 nAChR to SLURP-1, in the nanomolar range, causes significant increases in ACh-evoked currents, accompanied by a reduction in the EC_{50} and an increase of the slope of the concentration–activation curve [39]. The increase of the response amplitude without alteration of desensitization kinetics indicates that exposure to SLURP-1 reduces the L_0 coefficient but, otherwise, does not alter the transition equilibrium. Modulation of the native α7 nAChRs by a peptide present in the bovine serum albumin (BSA) was demonstrated in chick ciliary ganglia and rat hippocampus [40]. Fig. 2B depicts the effects of three different PAMs on concentration–activation effects of ACh at the human α7 nAChRs. Note the correspondence between theoretical (Fig. 2A) and experimental (Fig. 2B) data.

1.5. *Second generation α7 nAChR selective PAMs*

The past 3–4 years has witnessed the emergence of novel α7 nAChR PAMs although available biological data in terms of in vitro activity and selectivity profiles are somewhat limited especially those reported in patent publications. Fig. 3 presents structures of prototypical PAMs along with current traces depicting how selected PAMs modify ACh responses. (for a review, see [41]).

1.5.1. *Type I PAMs*

In addition to compounds like 5-HI and genestein described above, a number of compounds have been recently reported as positive allosteric modulators with little effect on desensitization kinetics. Ng et al. [42] recently described *compound* **6**, N-4-chlorophenyl)-α-[[(4-chloro-phenyl)amino]methylene]-3-methyl-5-isoxazole acetamide, that was derived from a chemical class previously exploited as $GABA_A$ modulators, to enhance α7 nAChR currents evoked by ACh without affecting desensitization kinetics [42] (Fig. 3). *In vivo* efficacy was demonstrated in models such as sensory gating, MK-801 evoked hyper-locomotion and eight-arm radial maze suggesting that compounds with this profile can exhibit efficacy across various cognitive domains. NS-1738, a prototypical analog from the biarylurea series was reported to enhance the potency of ACh as well as the maximal efficacy [43] (Fig. 3). NS-1738 improved recognition memory performance in rat, and further, in Morris water maze, a hippocampal-dependent

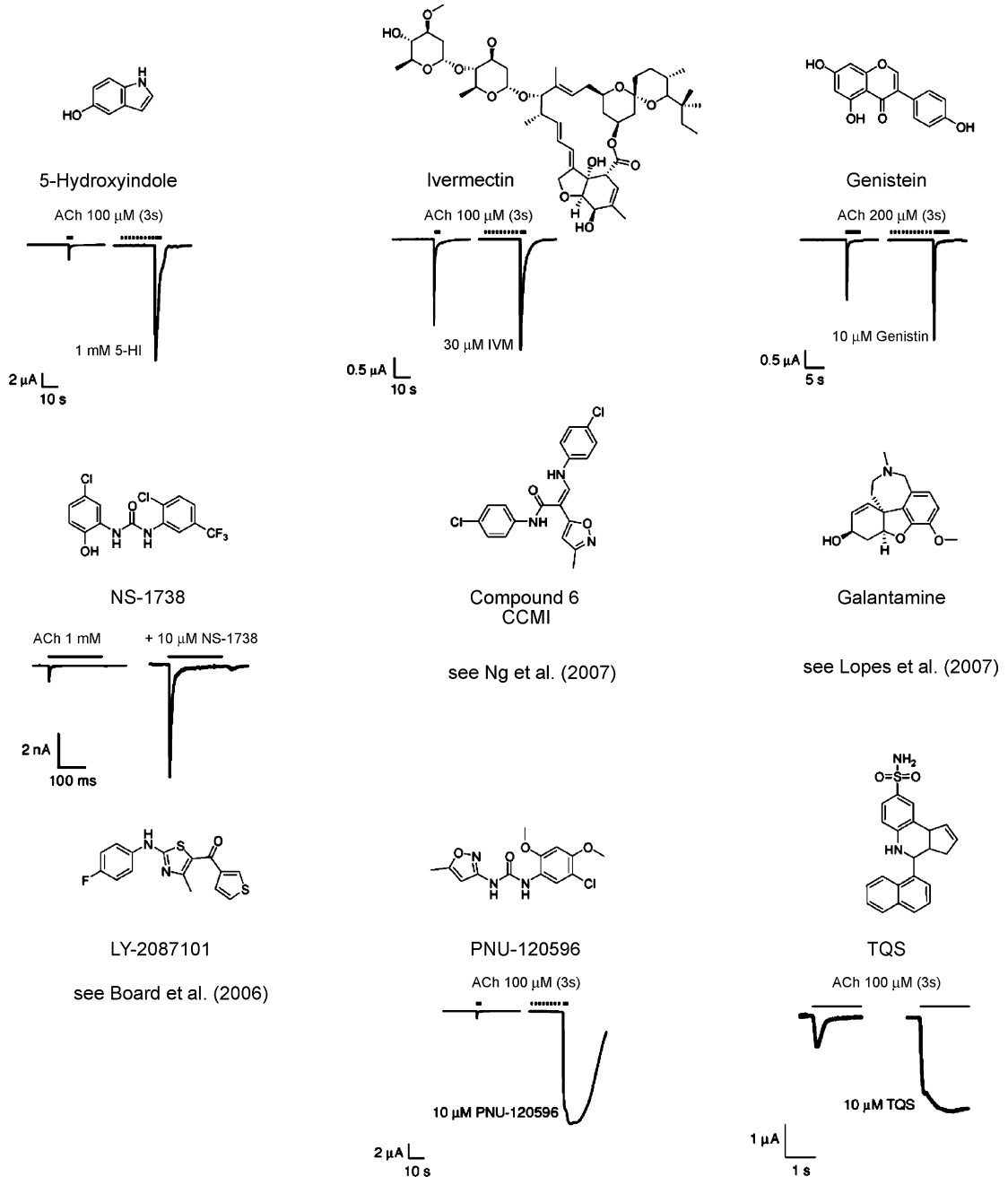

Fig. 3 – Structures and differential profiles of PAMs at the α7 nAChR. Structures of prototypical positive allosteric modulators of nAChRs and their effects at α7 nAChRs measured in *Xenopus* oocytes or in a cell lines. Note the difference in time course of the agonist-evoked currents observed following exposure to type I or type II modulators. Data for NS-1738 and TQS adapted from Timmermann et al. [43] and Gronlien et al. [37] respectively. For details of allosteric modulator profiles of compound 6, galantamine and LY-2087101, please see cited references.

model of learning and memory, the compound also reversed learning impairment induced by the muscarinic antagonist scopolamine.

The acetylcholine esterase inhibitor galantamine, currently used in the symptomatic treatment of Alzheimer's disease, showed as a function of the concentration, both a potentiating effect and an inhibitory effect of type IA currents evoked by choline in cultured hippocampal neurons [34] (Fig. 3). As these currents are thought to be mediated by the activation of α7 containing receptors it was proposed that galantamine effects may, in part, be mediated by the allosteric potentiation of α7 nAChRs. It should be noted, however, that potentiatory effects by galantamine is rather modest as compared to that observed with PAMs such as 5-HI or NS-1738. The interaction of galantamine at the *Aplysia* AchBP, was recently resolved at the atomic level revealed that this compound binds in a region distinct from acetylcholine [44].

1.5.2. *Type II PAMs*

PNU-120596 remains one of the better characterized urea analogs [45] (Fig. 3). PNU-120596 not only increased maximal amplitude and potency of ACh-evoked α7 nAChR current by several fold, but also almost suppressed desensitization. Another striking feature of PNU-120596 is its ability to restore responses in an otherwise desensitized receptor. In other words, while continuous exposure to agonist (nicotine) desensitizes the α7 nAChR and reduces the response to a non-detectable level, PNU-120596 application during continued exposure to the agonist is able to restore a current even larger than the peak current evoked by the agonist alone (see Fig. 4 in [45]). This phenomenon does not appear to be unique to PNU-120596, but may be a common feature of Type II PAMs as revealed by analysis of structurally diverse compounds [37]. These data further supports the multiple state transformations of nAChRs described earlier, and indicate that PAMs with a Type II profile preferentially stabilizes a state of the receptor that differs from the state normally stabilized by agonists. It is interesting to note that although both PNU-120956 and NS-1738 are urea derivatives (see Fig. 3), NS-1738 exhibited only marginal effects on the desensitization kinetics of α7 nAChRs unlike PNU-120596 suggesting that these molecules may have distinct mechanisms or sites of action. *In vivo* studies indicate that PNU-120596 improves sensory gating deficits induced by amphetamine in rats, a model thought to reflect neuronal network alterations associated with schizophrenia. In a separate study, it was shown that PNU-120596 improved performance in a rodent short-term recognition memory model and further increased the phosphorylation of cAMP response element-binding protein (CREB), a well-recognized biochemical process implicated in learning and memory [46]. These observations collectively demonstrate that Type II PAMs also exhibit *in vivo* efficacy in models of cognition.

Analogs belonging to the *tetrahydroquinoline* series have also been described as α7 nAChR PAMs useful for the treatment of conditions associated with reduced nAChR transmission. Like PNU-120596, a prototype analog 4-naphthalene-1-yl-3a,4,5,9b-tetrahydro-3-H-cyclopenta[c]quinoline-8-sulfonic acid amide (TQS), increased peak current and slowed desensitization of current responses [37] (Fig. 3). Concentration-responses to various agonists (ACh, GTS-21 and PNU-282987) were potentiated along with leftward shift in the EC_{50} values. In the same study, when applied at desensitized α7 nAChRs, TQS, like PNU-120596 was able to reactivate currents, unlike 5-HI or genistein.

1.6. *Allosteric modulation of α4β2 and other heteromeric nAChRs*

The finding that steroids inhibit the chick nAChRs in a non-competitive and nonsteric manner provided the first evidence of allosteric modulation of heteromeric nAChRs (reviewed [47]) and prompted further investigation of the underlying mechanisms [48]. Characterization of steroid inhibition revealed that *progesterone* exerts, in the micromolar range, a negative allosteric effect at the α4β2 nAChR [47,49]. These observations, later confirmed in many laboratories, have shown that progesterone and neurosteroids equally inhibit rat and human α4β2 nAChRs. A difference, however, was observed when examining the effects of a broader series of steroids at the human α4β2 nAChR where it was found that *17β-estradiol* actually increases ACh-evoked current [50]. Studies carried out using the rat and human α4β2 nAChRs revealed an important species difference in the effects of 17β-estradiol. While 17β-estradiol consistently increased ACh-evoked currents at the human α4β2 nAChR, this molecule was ineffective at the rat α4β2 nAChRs [51]. Studies with the water-soluble form of 17β-estradiol pointed out an interaction between this compound and the extracellular domain of the α4β2 nAChR. Subsequently, it was subsequently found that a short segment of amino acid residues (WLAGMI) in the α4-subunit is responsible for the differences observed between the rat and human receptors [50,51]. Although these studies could not demonstrate without ambiguity that steroids interact with the short C-terminal sequence, they nonetheless highlight the determinant role of this segment of the protein.

Divalent cations also modulate both homomeric and heteromeric receptors but their profile display significant differences. For example, *zinc*, which is co-released with neurotransmitters in the central nervous system, was shown to have differential effects depending on the receptor composition. While zinc, in the micromolar range, inhibited α3β2 containing receptors it causes a significant increase in ACh-evoked current at the α4β2 or α4β4 nAChRs [52]. Further analysis of zinc potentiation and inhibition revealed a determinant role of amino acids in the extracellular domain and a contribution of the histidine residue at position 162 in the N-terminal domain of the α4 subunit [53].

Developments in the identification of PAMs selective for various heteromeric nAChRs have been somewhat limited. Broad et al., recently reported *2-amino-5-ketothiazole* analogs as allosteric modulators of human nAChRs [36]. For example, thiazole analogs such as LY-2087101 increased α4β2 currents evoked by low ACh concentrations by about 8-fold. This compound showed, however, little subtype selectivity as it also increased the amplitudes of α4β4 and α7 nAChRs. Selecting LY-2087101 as an prototype and using a chimeric α7/$5HT_3$ receptor hosting the N-terminal extracellular domain and the transmembrane and C-terminal regions of the $5HT_3$ receptor, it was revealed that the site of allosteric potentiation lies in regions downstream from the N-terminus.

1.7. *Practical considerations in identifying & characterizing allosteric modulators*

Although identification of the first allosteric modulators occurred almost in a serendipitous manner, targeted methods are increasingly being utilized to screen for novel positive allosteric modulators. A range of high-throughput screening approaches based on two-electrode voltage clamp, whole cell patch clamp and fluorescence-based approaches have enabled identification of different classes of molecules acting as allosteric effectors. As noted above, two types of PAM profiles have generally been observed – one that predominately affects the apparent peak current, and the other that increases both the apparent peak current and changes the desensitization profile of the agonist response. The statement ''*one finds what* (s)*he is looking for*'' certainly applies for the

screening of allosteric effectors. Consider for example, Type II PAMs such as PNU-120596. Recalling that the ACh-evoked current at the α7 nAChR is transient, calcium influx associated with ACh-evoked responses are typically not detected using conventional fluorometric imaging plate readers such as the FLIPR. Therefore, screening assays using such technologies aimed at identifying compounds that cause an increase of the calcium influx are bound to identify molecules that predominantly modify receptor desensitization. Compounds that enhance the amplitude of the response without substantially modifying the desensitization time course are not likely to be revealed by such approaches unless modifications to the receptor or assay techniques are implemented. As positive allosteric effectors evoke larger effects at low agonist concentrations, it is to be emphasized that functional studies need to be conducted using lower range of ACh concentrations. Further, normalization of the concentration–activation curves to unity obscures an important point, i.e., variations in current amplitude responses. Finally, it is also important to determine early on that the putative effector alone does not activate the nAChR *per se*, and exhibit substantial selectivity versus other nAChRs and amongst other LGICs. Screening for negative allosteric modulators may prove somewhat more difficult than for positive modulators, as the criteria involves reduction of the agonist-evoked current and shift to the right of the concentration-response curve.

2. Conclusions

nAChRs are implicated in a range of neurological and psychiatric disorders including Alzheimer's disease, mild cognitive impairment, schizophrenia and attention deficit hyperactivity disorders. Selective positive allosteric modulation of nAChRs can be considered as a viable therapeutic approach for these disease states. As noted above, a key advantage of the PAM approach is that modulation is only revealed in the presence of endogenous agonist thereby preserving the temporal and spatial integrity of neurotransmission. In the case of α7 nAChRs, at least, two different profiles of PAMs have been described thus far: Type I modulators that predominately affects the apparent peak current, agonist sensitivity and Hill coefficient, and Type II modulators that cause, in addition, a modification of the desensitization profile of agonist response. Additional PAM profiles are expected to emerge and, once identified, may display either mixed profiles or further subtler modifications of the response profile. Some of the immediate challenges include clarification of desirable functional characteristics of PAMs and determination of their optimal selectivity profile. Whether PAMs with differential *in vitro* profiles – Type I versus Type II exhibit differences in efficacy, safety and tolerability profiles *in vivo* need to be elucidated. It also remains to be determined whether and what additional advantages PAMs offer *in vivo* compared to agonists in terms of efficacy, safety and tolerability preclinically, and ultimately in the clinic. Addressing these questions should pave the way for further exploiting the nAChR approach for novel therapeutics with potential for the treatment of diseases of unmet medical need.

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REFERENCES

[1] Monod J, Wyman J, Changeux JP. On the nature of allosteric transitions: a plausible model. J Mol Biol 1965;12:88–118.
[2] Karlin A. On the application of "a plausible model" of allosteric proteins to the receptor for acetylcholine. J Theor Biol 1967;16:306–20.
[3] Changeux JP, Edelstein SJ. Allosteric mechanisms of signal transduction. Science 2005;308:1424–8.
[4] Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 2007;47: 699–729.
[5] Jensen AA, Frolund B, Liljefors T, Krogsgaard-Larsen P. Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. J Med Chem 2005;48:4705–45.
[6] Romanelli MN, Gratteri P, Guandalini L, Martini E, Bonaccini C, Gualtieri F. Central nicotinic receptors: structure, function, ligands, and therapeutic potential. Chem Med Chem 2007;2:746–67.
[7] Le Novere N, Changeux JP. Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells. J Mol Evol 1995;40:155–72.
[8] Yu CR, Role LW. Functional contribution of the alpha7 subunit to multiple subtypes of nicotinic receptors in embryonic chick sympathetic neurones. J Physiol 1998;509(Pt 3):651–65.
[9] Khiroug SS, Harkness PC, Lamb PW, Sudweeks SN, Khiroug L, Millar NS, et al. Rat nicotinic ACh receptor alpha7 and beta2 subunits co-assemble to form functional heteromeric nicotinic receptor channels. J Physiol 2002;540:425–34.
[10] Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, et al. A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. Neuron 1990;5:847–56.
[11] Gray R, Rajan AS, Radcliffe KA, Yakehiro M, Dani JA. Hippocampal synaptic transmission enhanced by low concentrations of nicotine. Nature 1996;383:713–6.
[12] Fayuk D, Yakel JL. Ca^{2+} permeability of nicotinic acetylcholine receptors in rat hippocampal CA1 interneurones. J Physiol 2005;566:759–68.
[13] Castro NG, Albuquerque EX. alpha-Bungarotoxin-sensitive hippocampal nicotinic receptor channel has a high calcium permeability. Biophys J 1995;68:516–24.
[14] Drisdel RC, Green WN. Neuronal alpha-bungarotoxin receptors are alpha7 subunit homomers. J Neurosci 2000;20:133–9.
[15] Bertrand D, Galzi JL, Devillers-Thiery A, Bertrand S, Changeux JP. Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal alpha 7 nicotinic receptor. Proc Natl Acad Sci USA 1993;90:6971–5.
[16] Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. J Neurosci 1993;13: 596–604.
[17] Changeux JP, Edelstein SJ. Allosteric receptors after 30 years. Neuron 1998;21:959–80.

[18] Chemouilli P, Heidmann T, Changeux JP, Bachy A, Morre M. Allosteric effects of diprobutine on acetylcholine receptors. Eur J Pharmacol 1985;117:205–14.
[19] Heidmann T, Changeux JP. Characterization of the transient agonist-triggered state of the acetylcholine receptor rapidly labeled by the noncompetitive blocker [3H]chlorpromazine: additional evidence for the open channel conformation. Biochemistry 1986;25: 6109–13.
[20] Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB, et al. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 2001;411:269–76.
[21] Brejc K, van Dijk WJ, Smit AB, Sixma TK. The 2.7 A structure of AChBP, homologue of the ligand-binding domain of the nicotinic acetylcholine receptor. Novartis Found Symp 2002;245:22–9. discussion 9–32, 165-8.
[22] Celie PH, Kasheverov IE, Mordvintsev DY, Hogg RC, van Nierop P, van Elk R, et al. Crystal structure of nicotinic acetylcholine receptor homolog AChBP in complex with an alpha-conotoxin PnIA variant. Nat Struct Mol Biol 2005;12:582–8.
[23] Shi J, Koeppe JR, Komives EA, Taylor P. Ligand-induced conformational changes in the acetylcholine-binding protein analyzed by hydrogen-deuterium exchange mass spectrometry. J Biol Chem 2006;281:12170–7.
[24] Taly A, Corringer PJ, Grutter T, Prado de Carvalho L, Karplus M, Changeux JP. Implications of the quaternary twist allosteric model for the physiology and pathology of nicotinic acetylcholine receptors. Proc Natl Acad Sci USA 2006;103:16965–70.
[25] Xiu X, Hanek AP, Wang J, Lester HA, Dougherty DA. A unified view of the role of electrostatic interactions in modulating the gating of Cys loop receptors. J Biol Chem 2005;280:41655–66.
[26] Lee WY, Sine SM. Principal pathway coupling agonist binding to channel gating in nicotinic receptors. Nature 2005;438:243–7.
[27] Lummis SC, Beene DL, Lee LW, Lester HA, Broadhurst RW, Dougherty DA. *Cis–trans* isomerization at a proline opens the pore of a neurotransmitter-gated ion channel. Nature 2005;438:248–52.
[28] Revah F, Bertrand D, Galzi JL, Devillers-Thiery A, Mulle C, Hussy N, et al. Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. Nature 1991;353:846–9.
[29] Bertrand D, Devillers-Thiery A, Revah F, Galzi JL, Hussy N, Mulle C, et al. Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. Proc Natl Acad Sci USA 1992;89:1261–5.
[30] Galzi JL, Devillers-Thiery A, Hussy N, Bertrand S, Changeux JP, Bertrand D. Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. Nature 1992;359:500–5.
[31] Krause RM, Buisson B, Bertrand S, Corringer PJ, Galzi JL, Changeux JP, et al. Ivermectin: a positive allosteric effector of the alpha7 neuronal nicotinic acetylcholine receptor. Mol Pharmacol 1998;53:283–94.
[32] Zwart R, De Filippi G, Broad LM, McPhie GI, Pearson KH, Baldwinson T, et al. 5-Hydroxyindole potentiates human alpha 7 nicotinic receptor-mediated responses and enhances acetylcholine-induced glutamate release in cerebellar slices. Neuropharmacology 2002;43: 374–84.
[33] Charpantier E, Wiesner A, Huh KH, Ogier R, Hoda JC, Allaman G, et al. Alpha7 neuronal nicotinic acetylcholine receptors are negatively regulated by tyrosine phosphorylation and Src-family kinases. J Neurosci 2005;25:9836–49.
[34] Lopes C, Pereira EF, Wu HQ, Puranik P, Njar V, Schwarcz R, et al. Competitive antagonism between the nicotinic allosteric potentiating ligand galantamine and kynurenic acid at {alpha}7* nicotinic receptors. J Pharmacol Exp Ther 2007.
[35] Popa RV, Pereira EF, Lopes C, Maelicke A, Albuquerque EX. The *N*-butylcarbamate derivative of galantamine acts as an allosteric potentiating ligand on alpha7 nicotinic receptors in hippocampal neurons: clinical implications for treatment of Alzheimer's disease. J Mol Neurosci 2006;30:227–32.
[36] Broad LM, Zwart R, Pearson KH, Lee M, Wallace L, McPhie GI, et al. Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators. J Pharmacol Exp Ther 2006;318: 1108–17.
[37] Gronlien JH, Haakerud M, Ween H, Thorin-Hagene K, Briggs CA, Gopalakrishnan M, et al. Distinct profiles of {alpha}7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. Mol Pharmacol 2007;72:715–24.
[38] Galzi JL, Bertrand S, Corringer PJ, Changeux JP, Bertrand D. Identification of calcium binding sites that regulate potentiation of a neuronal nicotinic acetylcholine receptor. EMBO J 1996;15:5824–32.
[39] Chimienti F, Hogg RC, Plantard L, Lehmann C, Brakch N, Fischer J, et al. Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. Hum Mol Genet 2003;12:3017–24.
[40] Conroy WG, Liu QS, Nai Q, Margiotta JF, Berg DK. Potentiation of alpha7-containing nicotinic acetylcholine receptors by select albumins. Mol Pharmacol 2003;63:419–28.
[41] Faghih R, Gfesser G, Gopalakrishnan M. Advances in the discovery of novel positive allosteric modulators of the α7 nicotinic acetylcholine receptor. Recent Patents CNS Drug Discovery 2007;2:99–106.
[42] Ng HJ, Whittemore ER, Tran MB, Hogenkamp DJ, Broide RS, Johnstone TB, et al. Nootropic {alpha}7 nicotinic receptor allosteric modulator derived from GABAA receptor modulators. Proc Natl Acad Sci USA 2007;104:8059–64.
[43] Timmermann DB, Grønlien JH, Kohlhaas KL, Nielsen EØ, Dyhring T, Ahring PK, et al. An allosteric modulator of the α7 nicotinic acetylcholine receptor possessing cognition enhancing properties in vivo. J Pharmacol Exp Ther 2007. doi: 10.1124/ jpet.107.120436.
[44] Hansen SB, Taylor P. Galanthamine and non-competitive inhibitor binding to ACh-binding protein: evidence for a binding site on non-alpha-subunit interfaces of heteromeric neuronal nicotinic receptors. J Mol Biol 2007;369:895–901.
[45] Hurst RS, Hajos M, Raggenbass M, Wall TM, Higdon NR, Lawson JA, et al. A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J Neurosci 2005;25: 4396–405.
[46] Kohlhaas KL, Bitner RS, Robb H, Radek R, Markosyan S, Gopalakrishnan M. Improved preattention and short-term memory via positive allosteric modulation of the α7 neuronal nicotinic receptor. Int J Neuropsychopharmacol 2006;9(Suppl 1):S202.
[47] Buisson B, Bertrand D. Allosteric modulation of neuronal nicotinic acetylcholine receptors. J Physiol Paris 1998;92: 89–100.
[48] Bertrand D, Valera S, Bertrand S, Ballivet M, Rungger D. Steroids inhibit nicotinic acetylcholine receptors. Neuroreport 1991;2:277–80.
[49] Valera S, Ballivet M, Bertrand D. Progesterone modulates a neuronal nicotinic acetylcholine receptor. Proc Natl Acad Sci USA 1992;89:9949–53.

[50] Curtis L, Buisson B, Bertrand S, Bertrand D. Potentiation of human alpha4beta2 neuronal nicotinic acetylcholine receptor by estradiol. Mol Pharmacol 2002;61:127–35.
[51] Paradiso K, Zhang J, Steinbach JH. The C terminus of the human nicotinic alpha4beta2 receptor forms a binding site required for potentiation by an estrogenic steroid. J Neurosci 2001;21:6561–8.
[52] Hsiao B, Dweck D, Luetje CW. Subunit-dependent modulation of neuronal nicotinic receptors by zinc. J Neurosci 2001;21:1848–56.
[53] Hsiao B, Mihalak KB, Repicky SE, Everhart D, Mederos AH, Malhotra A, et al. Determinants of zinc potentiation on the alpha4 subunit of neuronal nicotinic receptors. Mol Pharmacol 2006;69:27–36.