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Allosteric modulation of neuronal nicotinic acetylcholine receptors requires inter-subunit movement

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Allosteric modulation of neuronal nicotinic acetylcholine receptors (nAChRs) is an increasingly important avenue of drug development for CNS cholinergic systems. We have been studying a series of compounds that modulate α3β2 receptors from a site, homologous to the canonical agonist site, at the β(+)/α(−) interface. Having established that potentiation in the system arises from enhanced gating efficacy, we have asked whether allosteric modulation requires inter-subunit movement, as is the case with stereotypical agonist activation of the channel. To answer this question, we studied point mutant α3β2 receptors, as well as α/β paired mutants for a series of residues we predicted could make contact across the interface, based on a homology model of the receptor extracellular domain. For example, α3S125, a hot spot along the ligand binding-gating transduction pathway, when substituted with tyrosine, converts the modulator and partial agonist morantel into a full agonist. This agonist activity is dramatically reduced when α3S125Y is co-expressed with β2W149A, presumably because that highly conserved tryptophan is involved in binding morantel. When the mutant α3S125C is co-expressed with β2Q39C, where this pair of residues is predicted to be adjacent across the α(+)/β(−) interface, an oxidation treatment reduces morantel potentiation of ACh-evoked currents. Similar studies on other positions in the α(+)/β(−) [agonist] and β(+)/α(−) [modulator] interfaces shed light on the extent of inter-subunit movement and receptor symmetry required for allosteric modulation of nAChRs.

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Positive and negative cooperativity of agonist and allosteric modulator binding in alpha7 nAChR: Looking for the therapeutic window

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The intrinsic open probability of α7 nAChR is very low, even under the most fully optimized conditions. Average single channel open times are on the order of 70 μs and bursts of openings are not observed. Studies of both macroscopic and single currents suggest that rapid saturation of the α7agonist binding sites may produce synchronized channel opening, but little increase in the time-averaged P-open of individual channels. In contrast, while heteromeric nAChR activate to a brief open state (O*) in the presence of low concentrations of agonist, at higher concentrations of agonist bursts of longer openings are observed, suggesting a positive co-operativity between binding two agonist molecules and the stabilization of a long-lived open state (O′). We hypothesize that in native α7 receptors, the cooperative effect of multiple agonist binding events is to promote the conversion of receptors to a unique non-conducting state (D_s) that is associated with conformational change within the ion channel. Positive allosteric modulators can greatly increase the P_{open} of α7 nAChR in multiple ways. They may change the energy barriers into and out of the open channel state such that immediately after a jump in agonist concentration there is a transient increase in P_{open} (Type I PAM). They may also alter the absolute energy differences between conducting and desensitized states, producing more current under equilibrium conditions. It has also been proposed that Type II PAMs, such as PNU-120596, convert one or more desensitized states into open channel states. Our data suggest that for α7, PNU-120596 promotes the stability of a state analogous to the O′ of heteromeric receptors and that this is coupled with a decrease in the stability of the D_s state and a conversion of D_s into a subconductance. The O′ events occur as bursts of prolonged openings, persisting sometimes for several seconds, with intraburst closures to the D_s subconductance. As in heteromeric nAChR, prolonged activation of α7 is associated with the likelihood of conversion to an alternative desensitized state (D_i) that is stable in PAM-modified receptors as long as agonist is present and reversible once agonist is removed. Responses to simultaneous co-applications of varying concentrations of agonist and PAM indicate that the stability of the D_i state in α7 is promoted by high levels of binding both agonist and PNU-120596 and that there is mutually positive cooperativity for the binding of ACh and PNU-120596. The current evoked by co-applications is biphasic when intermediate concentrations of agonist and PAM are used, with a rapid transient phase associated with the initial perturbation from the resting condition, and a slow phase as an equilibrium between the multiple conformational states is approached. The equilibrium that is approached depends on both the concentration of agonist and PAM, such that pseudo steady-state activation is optimal when concentrations of both agents are low to intermediate. When agonist concentrations are relatively high, applications of competitive antagonists (MLA or DHβE) can reduce the negative effect of high agonist occupancy and promote increased current. Our data therefore indicate how activation of α7 is achieved in a dynamic window of agonist and PAM concentrations that can determine both transient and steady-state P_{open}. Our understanding of these mechanisms will allow us to better meet the challenge of optimizing PAMs for future therapeutic development.

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1.23

Differential allosteric modulation of high- and low-sensitivity forms of the α4β2 nAChR: Evidence for distinct modulator binding sites

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Multiple lines of evidence suggest α4β2 nicotinic receptors (nAChRs) to be integral components in the neurocircuitry of cognition, suggesting this receptor to be a potential drug target for treatment of cognitive dysfunction. The α4β2 nAChR is known to exist in two distinct isoforms, caused by differential subunit stoichiometries, displaying high (HS) and low (LS) sensitivity towards acetylcholine, respectively. There is mounting evidence that both are expressed in the mammalian brain but the functional significance of each isoform is obscure. Allosteric modulation of α4β2 nAChRs presents an attractive approach towards targeting this receptor and the compound NS9283 was previously reported by our group to act as an efficacious and selective positive allosteric modulator (PAM) at the low-sensitivity α4β2 nAChR. This compound was demonstrated to produce cognitive augmentation in vivo across a broad range of behavioral pharmacology paradigms, highlighting the importance of LS-α4β2 nAChRs for cognitive function.

The present study aimed at exploring the pharmacological profile of a different compound, NS206, discovered to act as an α4β2 nAChR PAM. Using Xenopus oocyte electrophysiology, NS206 was shown to act as a potent and efficacious α4β2 nAChR PAM not only at the LS, but also at the HS-isoform. Moreover, the pattern of modulation observed at HS-α4β2 was distinct in the sense that NS206 acted by augmenting maximal ACh efficacy (peak current amplitude) without affecting ACh potency (EC_{50}). By comparison, modulation of LS-α4β2 was mediated through an increase in functional ACh potency (i.e. decreased EC_{50}), while maximal ACh efficacy was unchanged, similar to the mode-of-action observed with the LS-α4β2-selective PAM NS9283. The PAM-selectivity profile at a number of other nAChR subtypes was also characterized and will be presented. Using point mutated nAChR subunits, engineered to abolish the binding site for LS-α4β2 PAMs, loss of PAM activity was confirmed for NS9283. Interestingly, however, NS206 retained full PAM activity in the mutated receptor constructs. Collectively, these findings suggest NS206 to present a novel class of α4β2 nAChR PAM which acts through a receptor binding site separate from that of LS-α4β2 PAMs (e.g. NS9283) and to possess a distinct pattern of PAM activity, involving modulation of both LS- and HS-α4β2 nAChRs. The discovery of NS206 (and related molecules) provides an important pharmacological tool that may enable a deeper understanding of α4β2 nAChR PAM in vivo pharmacology and at broader level, insight into the physiological significance of the HS- and LS-isoforms of the α4β2 nAChR.

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Ascorbic acid is a positive modulator of α9α10 nicotinic cholinergic receptors

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Inhibitory activity of efferent cholinergic fibers projecting from the brainstem and contacting cochlear hair cells can ameliorate acoustic trauma. This inhibitory synapse is mediated by α9α10 nicotinic receptors and the subsequent activation of an SK2 type K^+ current that hyperpolarizes the hair cell. Hence, increasing α9α10-mediated responses pharmacologically could have a potential therapeutic use in noise-induced hearing loss. In this work we endeavored to identify new positive modulators for this receptor. Using the two-electrode voltage clamp technique we studied the effect of ascorbic acid (ASC) on acetylcholine (ACh) evoked responses in *Xenopus* oocytes expressing the rat α9α10 receptor. Responses to 10 μM ACh were potentiated by ASC in a concentration-dependent manner: at 3 mM ASC, an 81 ± 6% ($n = 7$) potentiation was observed. Potentiation was more pronounced at lower (305 ± 40%, 3 μM ACh, $n = 8$) than at higher (138 ± 35%, 1 mM ACh, $n = 8$) ACh concentrations. Neither 3 mM dihidroascorbate nor 3 mM D-iso-ascorbate had an effect on 10 μM ACh-evoked responses. These results suggest that the reduced L form of ASC is the active compound. The extracellular cysteines 192 and 193 (*Torpedo* α numbering) are not involved in the effect of ASC since mutating them to serine did not abolish the potentiating effect. ASC did not modify responses to ACh of rat α7 and α4β2 receptors expressed in oocytes. Altogether, our results show that ASC potentiates α9α10-mediated responses and thus has a potential therapeutic use in noise-induced hearing loss.

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A structure–activity study of 4R-cembranoid reversal of diisopropylfluorophosphate-inflicted functional impairment in hippocampal slices

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Diisopropylfluorophosphate (DFP) is an organophosphate insecticide used in many studies as a surrogate for more toxic chemical warfare nerve agents. DFP produces neurodegeneration in vivo and irreversibly decreases the area of population spikes (PS) recorded from the CA1 region of the acute hippocampal slice preparation. Tobacco-derived (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (4R) is a neuroprotective natural product that reverses DFP-induced damage both in vivo and in the hippocampal slice. The objective of this study was to define the molecular features of the cembranoid molecule that lead to high potency against DFP, concomitantly with no intrinsic toxicity, using the hippocampal slice assay. Thirteen 4R analogues were obtained by semisynthetic or bacterial biocatalytic transformations of the natural product scaffold. Acute hippocampal slices were divided into three groups: (*a*) the DFP control (slices exposed to 100 μM DFP for 10 min), (*b*) neuroprotection by the cembranoid (slices exposed to 100 μM DFP for 10 min, washed for 30 min and then exposed to 10 μM of each tested cembranoid for 1 h), and (*c*) toxicity control (slices exposed to 10 μM cembranoid for 1 h). Population spikes (PS) were measured before and after the treatment. The results are expressed as %Protection (=100 × (%Recovery in *b* − %Recovery in *a*)/(100 − %Recovery in *a*). Two analogues displayed marginal toxicity when applied in the absence of DFP; these were excluded from the subsequent analysis. Exposure to 100 μM DFP for 10 min reduced the PS to approximately 30% of the original value. Superfusion with 10 μM of the parent 4R 30 min after DFP reversed the effect of DFP by 80%. Similar protective activity was observed with the 6-keto, 9β-OH, 10α-OH and 10β-OH analogues. On the other hand, the 4*S*-epimer of 4R and 4R-*O*-methyl analogues were totally devoid of protective activity but the activity was restored in the 4R-*O*-methyl-6-keto analogue. These results suggest that the oxygens in positions 4 and 6 are crucial for the 4R binding to its target, which triggers the protection against the organophosphate toxicity in hippocampus slices.

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1.26

Different presynaptic nicotinic receptor subtypes modulate *in vivo* and *in vitro* the release of glycine in the rat hippocampus

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In the present study, using an *in vivo* approach (microdialysis technique associated to HPLC with fluorimetric detection) and in vitro purified hippocampal synaptosomes in superfusion, we investigated on the glycinergic transmission in hippocampus, focusing