structure-guided drug design, target "templated" synthesis and computational analyses of ligand recognition. However, these efforts have been hampered by the fact that the binding proteins, while homologous with human nicotinic receptors, have low overall sequence identity and limited state changes, resulting in a pharmacology that is dissimilar to human drug targets. To address this shortcoming we have designed chimeric binding protein constructs in which the C loop and other segments of the binding site have been replaced with the amino acids corresponding to the Cys-loop receptors. In an initial step to developing surrogates of human extracellular domains amenable to crystallography, we modified the C loop and examined the ligand selectivity changes and X-ray crystal structures of these chimeras to assess the utility of the chimera approach in high throughput screening and *in situ* freeze-frame click chemistry. In addition to these constructs providing an interesting prospective on the role of the C loop in ligand recognition and specificity, they have in some cases provided X-ray crystal structures of ligands that hitherto have been difficult for us to obtain. Among these are some touchstone ligands currently on the market or under clinical investigation including varenicline (Chantix®), sazetidine A and cytisine and natural toxins, such as anatoxin A. Accordingly, the C loop and how it is configured in the ligand complex are determinants of crystal nucleation and growth. Details on the comparative structures of the above ligand complexes provide details on the determinants of ligand selectivity for receptor subtype and offer insights into the development of more selective agonists and antagonists.

Acknowledgments: This work was supported by NIH UO1-DA19372-05 and RO1-GM18360-37.

doi:10.1016/j.bcp.2011.07.018

1.17

Pharmacological properties of sazetidine A, a selective ligand of α4β2 nicotinic acetylcholine receptors

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Neuronal nicotinic acetylcholine receptors (nAChRs) serve a wide range of physiological functions and are implicated in a number of pathological processes and many pharmacological effects of nicotinic drugs. In particular, several lines of evidence indicate that the nAChRs containing both α4 and β2 subunits mediate important *in vivo* effects of nicotine, including its addictive and cognitive effects. Sazetidine-A (Saz-A) selectively binds with high affinity to α4β2 nAChRs and shows potent *in vivo* effects in animal models that include analgesia, reduction in nicotine self-administration, reduction in alcohol intake, antidepressant-like activity and reversal of attentional impairment. In *in vitro* studies, Saz-A potently inhibits nicotine-stimulated ion efflux from cells that express α4β2 nAChRs after they are pre-incubated for 10 min with Saz-A [1]. Saz-A shows full agonist activity at $(\alpha4)_2(\beta2)_3$ nAChRs but little agonist activity (<1% efficacy of that of acetylcholine) at $(\alpha4)_3(\beta2)_2$ nAChRs expressed in *Xenopus* oocytes [2]. Hence, an important question is how each of these two essentially diametrically opposed actions of Saz-A, activation and desensitization, contributes to each of the *in vivo* effects of Saz-A. We hypothesize that Saz-A converts most of the receptors to a desensitized conformational state after a brief exposure to it. Using equilibrium and kinetic binding methods, ion efflux measurements and patch-clamp electrophysiology, we compared *in vitro* pharmacological properties of Saz-A with those of nicotine, epibatidine, varenicline, 5-I-A-85380 and cytisine. Based on data from these *in vitro* studies and observations obtained from studies in behavioral animal models, we hypothesize that the long-lasting, selective desensitization of α4β2 nAChRs is the main mechanism for long lasting *in vivo* effects of Saz-A.

Acknowledgments: This work was supported by NIH grant DA027990.

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doi:10.1016/j.bcp.2011.07.019

1.18

The $(\alpha4)_3(\beta2)_2$ nAChR has a benzodiazepine-like modulatory binding site in the αα-subunit interface as revealed by studies with NS9283

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Modulating α4β2 nicotinic acetylcholine receptors through novel allosteric binding sites represents an exciting new area for pharmacological intervention of higher brain function including attention and cognition. NS9283 was originally indentified by its ability to increase agonist-evoked response amplitude of α4β2 nAChRs in Ca^{2+}-imaging as well as in electrophysiology paradigms. NS9283 did not itself produce receptor activation or displacement of [^{3}H]-epibatidine binding and the allosteric modulation was found to be selective for α2- and α4-containing nAChRs whereas no effects were observed at α1-, α3- or α7-containing nAChRs. α4β2 nAChRs are known to assemble as high- or low-sensitivity receptors dependent on subunit stoichiometry and in *Xenopus* oocyte experiments NS9283 modulation only occurred when receptors were expressed under conditions favoring a $(\alpha4)_3(\beta2)_2$-subunit stoichiometry indicating that NS9283 is selective for low-sensitivity receptors. We therefore hypothesized that the selectivity was dependent on a 3α:2β-subunit stoichiometry and in particular on the αα-subunit interface. Comparing homology models we have identified amino acids that could be involved in binding of NS9283. Of these, Histidine 142, located on the (−)-side of the α4-subunit, was particularly interesting since NS9283 is devoid of any effects up to a concentration of 31.6 μM on $(\alpha4^{H142V})_3(\beta2)_2$ receptors. Studies investigating the mode of action of NS9283 revealed that modulation of i.e. $(\alpha4)_3(\beta2)_2$ receptors could be attributed to an increase in functional agonist potency but maximal current amplitude were unaffected. Graphically, this is seen as left-shift of agonist concentration-response curves, towards higher potency of the agonist, but maximal efficacy is not affected. The key features of NS9283, i.e. subunit interface binding and left shift of agonist concentration–response curves without affecting efficacy, resembles those described for benzodiazepines at the benzodiazepine binding pocket of $GABA_A$ receptors. We therefore propose that NS9283 is a mimic of a benzodiazepine mechanism in the nicotinic system. In conclusion, NS9283 demonstrates that it is possible to find highly selective allosteric modulators of nAChRs and further

that a benzodiazepine-type mechanism of action can be found for compounds active at other Cys-loop receptors than $GABA_A$Rs.

doi:10.1016/j.bcp.2011.07.020

1.19

Electrophysiological characterization of NS9283, a novel positive allosteric modulator of α4β2 nicotinic receptors

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Neuronal nicotinic acetylcholine receptors (nAChRs) are implicated in a wide range of neurological diseases with cognitive impairments, making the members of this Cys-loop receptor subfamily highly interesting drug targets. A wide range of agonists and positive allosteric modulators (PAM) selective for neuronal nicotinic acetylcholine receptors (nAChR) has been developed over the years. Especially the homomeric α7 nAChR receptor has been targeted as this receptor is implicated in neurological diseases such as schizophrenia and Alzheimer's disease. However, recently the α4β2 nAChR has gained increasing notice as it has been demonstrated to play a key role in mediating attention [1], thereby implying an important role in schizophrenia and ADHD. Here we present NS9283, a potent and highly selective PAM of the α4β2 low sensitivity (LS) nAChR subtype. Pro-cognitive effects of NS9283 have previously been demonstrated in a wide range of *in vivo* assays, underlining the potential of α4β2 nAChR as a drug target. Using whole-cell patch-clamping with an ultrafast solution application system we performed a detailed electrophysiological characterisation of NS9283 in HEK293 cells stably expressing α4β2 nAChR. The high potency of NS9283 was confirmed by left-shifting the ACh concentration–response curve by a factor of ~40, however, ACh efficacy was unaffected. Additionally, current kinetics was addressed: by using exponential curve fitting desensitization was quantified revealing no significant effect of NS9283. Furthermore, NS9283 moderately decreased recovery from desensitization and could not reactivate desensitized receptors. In contrast to the lack of effect on desensitization, NS9283 significantly decreased receptor deactivation. A modest decrease in and slowing of activation was also observed. Finally, NS9283's effect on window current was investigated. As NS9283 left-shifted the activation curve to a higher degree than the inactivation curve, the presence of NS9283 resulted in an increased window current. In summary, the main findings of this study demonstrate that NS9283 increases ACh responsiveness and reduces receptor deactivation of α4β2 nAChR. Preliminary experiments on NS9283 binding site as well as the observed increase in potency resemble a mode-of-action similar to that of benzodiazepines on $GABA_A$ receptors. The presented findings will form the basis for a deeper mechanistic understanding of the effect of NS9283 in cognitive studies, especially with regards to its effect on synaptic level.

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doi:10.1016/j.bcp.2011.07.021

1.20

In vitro pharmacological characterization of ABT-779, a novel positive allosteric modulator of α7 nAChRs

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Targeting α7 nAChRs with subtype selective positive allosteric modulators (PAMs) may be considered an attractive approach for treating cognitive deficits associated with diseases such as schizophrenia. In this study, we describe the in vitro pharmacological properties of ABT-779, a novel α7 PAM. ABT-779 exhibited a typical Type II profile, potentiating peak current amplitude and attenuating desensitization responses to ACh at human and rat α7 NNRs (expressed in *X. oocytes* and studied by two-electrode voltage clamp) with potencies (EC_{50} values: 80–200 nM) highest among known α7 PAMs. At much higher concentrations (≥3 μM), ABT-779 alone directly evoked MLA-sensitive weakly desensitizing current. As expected for an allosteric modulator, the EC_{50} and maximal efficacy of ACh or choline was shifted to the left by ABT-779 in a concentration-dependent manner. In a cell line endogenously expressing human α7 NNRs (IMR-32), ABT-779 activated Ca^{2+} flux responses in the presence of an exogenously added agonist (EC_{50} = 80 nM) but with ~4-fold weaker maximum efficacy than A-867744 or PNU-120596 (other α7 PAMs). ABT-779 also directly evoked Ca^{2+} signals at ≥3 μM. No potentiation of agonist-evoked responses mediated by α4β2, α3β4* (IMR-32 cell line), 5-HT_{3A}, and chimeric human α7-5-HT_{3A} receptors was observed with ABT-779; instead weak inhibition of Ca^{2+} flux, membrane potential imaging or current responses were observed (IC_{50} > 450 nM) or no effect (5-HT_{3A}). As expected with allosteric type interactions, ABT-779 showed no significant displacement of binding to orthosteric sites present at α7 NNRs (K_i > 10,000 nM) or at other NNR subtypes (α4β2, α3β4*: K_i >100,000 nM). ABT-779 (10 μM) showed a clean profile in the CEREP radioligand binding panel across various receptors, ion channels and transporters, showing no inhibition greater than 80% across all targets tested. ABT-779 (10 μM) also did not show inhibition of human acetylcholinesterase activity. In rat brain hippocampal slices (CA1 region), current responses evoked by choline were amplified in the presence of ABT-779; these responses were inhibited by MLA. In dentate gyrus granule cells, ABT-779 when co-applied with the α7 nAChR agonist choline (1 mM) increased the sIPSC activity when compared to choline alone. Further, in vivo administration of ABT-779 enhanced ACh release from prefrontal cortex and hippocampus in a dose-dependent manner, with significant effects observed at doses of 0.1 and 1 μmol/kg. These studies collectively demonstrate that ABT-779 is a novel positive allosteric modulator of α7 nAChRs and can modulate native α7 NNRs controlling synaptic activity and important for cognitive processes.

doi:10.1016/j.bcp.2011.07.022