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.

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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.

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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