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

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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.

Reference

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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.

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