current onset and decay kinetics arising from the intrinsic structure of the receptor, however, have been difficult to tease apart with conventional application methods. In this study, we used a newly developed fast solution exchange technique allowing individual cultured cells expressing α7, α4β2 or α3β4 neuronal nicotinic receptors to be exposed to a range of 2.5 ms ACh applications in order to determine the latency and activation and deactivation kinetics of the evoked whole-cell currents. Our results demonstrate that the kinetics of α7-mediated responses were independent of the concentration of ACh applied, indicating the absence of receptor desensitization during rapid ACh exposures. Alternatively, the current kinetics from α4β2- and α3β4-mediated responses were dependent on concentration of ACh in a manner that suggested the presence of two kinetically-distinct populations of high and low sensitivity receptors. In addition, we applied a range of ACh concentrations at 1 Hz and 30 Hz to determine the frequency-dependent properties of the three receptor subtypes. During 1 Hz applications, all three receptor subtypes maintained a sustained level of activity at all concentrations of ACh tested. During 30 Hz burst applications however, current responses mediated by putative high-sensitivity α4β2 and α3β4 receptors showed profound facilitation, whereas α7 responses were depressed. Together, this study describes intrinsic characteristics of different neuronal nicotinic receptor subtypes and suggests a new direction for investigating these and other types of receptors under relevant physiological conditions that more closely mimic physiological conditions of neurotransmitter release at the synaptic cleft.

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1.9

Comparison of pharmacologic properties of AZD3480 and AZD1446 on neuronal nicotinic receptor subtypes

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Recent studies have indicated a rich diversity of neuronal nicotinic subtypes in the mammalian brain, based on multiple combinations of a distinct set of neuronal nicotinic receptor subunits. Although there are many similarities in the distribution of the subtypes between mammalian species, there are also important differences, for example it has been shown that the α_2 subunit shows much higher expression levels in the *Macaca* brain than in rodents. Nicotinic α4β2 and α2β2 receptors occur in high-sensitive (HS) and low-sensitive (LS) forms based on different stochiometry of the α and β subunits. The expression of concatamers in combination of single subunits allows the expression of distinct receptor subtypes. The sensitivity refers to the endogenous agonist acetylcholine (ACh) that shows high potency against human HS-α4β2 receptors (α4(2)β2(3); 2 μM) and HS-α2β2 receptors (α2(2)β2(3); 1 μM), and low potency against LS-α4β2 receptors (α4(3)β2(2); 30 μM) and LS-α2β2 receptors (α2(3)β2(2); 73 μM). AZD3480 showed high potencies against HS-α4β2 receptors (0.5 μM), HS-α2β2 receptors (1.4 μM), and LS-α4β2 receptors (0.25 μM). Potency at LS-α2β2 receptors could not be determined in view of the low magnitude of evoked current. Agonism, expressed as percent of ACh max response, was 100% at HS-α4β2 and HS-α2β2, but only 20% at LS-α4β2 and 4% at LS-α2β2 receptors. These results indicate that AZD3480 is a full agonist at high-sensitive α4β2 and α2β2 receptors but only a weak partial agonist at low-sensitive α4β2 and α2β2 receptors. AZD1446 showed a different profile, with lower potencies against HS-α4β2 receptors (15 μM), HS-α2β2 receptors (27 μM), LS-α4β2 receptors (5 μM), and LS-α2β2 receptors (60 μM). Agonism also differed, with 140% of ACh max response at HS-α4β2 receptors and 100% at LS-α4β2 receptors but only 43% at HS-α2β2 receptors and 21% at LS-α4β2 receptors. These results indicate that AZD1446 is generally less potent than AZD3480 but shows a different agonism profile by being a full agonist at high- and low-sensitive α4β2 but only a partial agonist at high- and low-sensitive α2β2 receptors. In addition, we have studied the desensitization properties of HS- and LS-α4β2 receptors, where the properties of both compounds differed markedly with less desensitization as compared to nicotine or varenicline. Furthermore, similarly to nicotine and varenicline, neither compound fully desensitized when α6 was expressed with α4β2 concatamers. In conclusion, we have found differences in the potencies as well as agonistic and desensitization properties on five distinct human neuronal nicotinic receptor subtypes of two compounds investigated in clinical studies.

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1.10

Effects of RG3487 at the α7β2 nicotinic acetylcholine receptor expressed in *Xenopus* oocytes

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The α7 nicotinic acetylcholine receptor (nAChR) plays an important role in cognitive function, and selective agonists have been proposed as novel therapeutic agents for treating cognitive impairments associated with disease. The α7nAChR exists primarily as a homopentamer in the brain, but recent reports suggest that the α7nACh subunit might co-assemble with the β2nAChR subunit to form heteromeric receptors that exhibit different pharmacological and biological properties from the homopentamer. To determine whether such receptors display differential sensitivity to the α7nAChR partial agonist, RG3487, experiments were designed to assess the properties of α7 and the putative α7β2 (1:1 ratio) expressed in *Xenopus* oocytes. In these studies, RG3487 yielded approximately equivalent current amplitude and EC_{50} values at both the α7 and α7β2 nAChRs. To further assess the possible incorporation of the β2 subunit into α7 functional nAChRs, a ratio of 1:10 α7:β2 cDNA was injected into the oocytes nuclei. A small but noticeable slowing down of the ACh-evoked current was observed in oocytes expressing the α7β2 in a 1:10 ratio. Moreover, the efficacy of RG3487 was significantly diminished in cells expressing α7β2 (1:10) subunits (41% of ACh) versus α7 alone (60% of ACh). In comparison, ACh evoked robust currents in oocytes expressing α7β2 (1:10) versus α7 alone demonstrated comparable EC_{50} and current amplitudes, and suggests the presence of the β2nACh in the receptor complex cannot be distinguished on the basis of the ACh responses. Altogether, these data suggest that expression of β2nAChR, even in an exceeding 10 fold ratio, does not prevent the formation of functional α7 receptors and causes no detectable modification of the ACh-evoked currents; however, a statistically significant lower fraction of evoked current is observed with RG3487. In a second series of experiments, we assessed the effects of the expression of the β2 subunit on the potentiation and desensitization caused by RG3487 over a broad range of concen-

trations, and compared their response to a fixed ACh test pulse. A significant reduction in the RG3487 (3 and10 nM) induced current potentiation was observed in cells expressing the α7β2 nAChRs compared to cells expressing the α7nAChR alone. These data illustrate that the presence of the β2 in the α7 receptor complex modifies the overall properties of the nAChRs and could result in a differential sensitivity to compounds as the α7 and β2 are co-expressed in some areas of the brain.

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1.11

A-582941, a pro-cognitive α7 nAChR agonist, differentially modulates mitochondrial membrane potential

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Alzheimer's disease involves multiple pathogenic processes such as abnormal amyloid deposition, tau phosphorylation, oxidative stress as well as mitochondria dysfunction leading to progressive impairment and loss of cognitive function. An assay was established in SK-N-SH cells using JC-1 dye to measure mitochondrial membrane potential (MMP), as an indicator of mitochondrial health, and the effects of compounds on MMP. A-582941, an α7 nAChR partial agonist, was able to enhance MMP with a potency of 11.4 μM and 45% efficacy after overnight serum starvation, which reflects the early stages of apoptosis based on cell viability. Dimebolin and donepezil, other pro-cognitive compounds, but not memantine, also preserved MMP with potency and efficacy values of EC_{50} = 4.6 μM (100%) and 2.2 μM (93%), respectively. Similar results were obtained using either kainic acid or ionomycin as insults. From previous studies, these four compounds exhibit either preclinical or clinical efficacy in models of memory consolidation and short-term recognition. In addition, these compounds are neuroprotective against Aβ insult or promoting neurite outgrowth in primary cortical cultures. Dimebolin and donepezil also increase the MMP over a relatively wide concentration range without compromising nuclear morphology or plasma membrane integrity, both of which are indications of irreversible cellular injury. This approach may allow for further differentiation of pro-cognitive compounds. Studies further demonstrated that concentrations of A-582941, which gave less than a 25% response in preserving MMP was significantly potentiated (to 75%) when cells were simultaneously treated with combinations of A-582941 and dimebolin. Studies are underway to compare the effects of other α7 nAChR agonists with different profiles. Preservation of MMP is an essential event in rescuing neurons from energy-depletion in neurodegenerative states and inhibiting release of pro-apoptotic components.

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1.12

Discovery of nicotinic acetylcholine receptor ligands in the chemical universe database GDB-13

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It is a dream for every medicinal chemist to examine how any possible molecule could interact with a given target. Using parallel processing, we recently reported the exhaustive computational enumeration of all possible organic molecules up to 13 non-hydrogen atoms (C, N, O, S, Cl) in form of the chemical universe database GDB-13 (www.gdb.unibe.ch) [1]. We also showed that a previous version of the database, GDB-11, could be used to design analogs of known nicotinic ligands for synthesis and testing [2]. Here we used the database GDB-13 to search for analogs of the natural product nicotine. A fast similarity classification [3] was used to select 5000 close analogs of nicotine in GDB-13. While several (ca. 150) of these analogs were known AChRs ligands, 50 compounds with no reported activity on AChRs were selected and purchased from commercial vendors. The compounds were probed at α7 neuronal nicotinic receptors expressed in *Xenopus* oocytes using the fully automated electrophysiology HiClamp (Multichannel System). Three of the most active molecules were characterized in detail by determination of the EC_{50}'s and/or IC_{50}'s. Moreover, the mode of action of inhibitors was analyzed in competition experiments. Such ligand-based similarity searching in GDB-13 should be generally useful to rapidly expand the pharmacology of acetylcholine receptors and should help to identify potent and subtype selective agonists and antagonists.

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1.13

Ligand-based QSAR modeling of neuronal nicotinic receptor data and its impact on drug design

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Neuronal nicotinic receptors (NNRs) belong to the Cys-loop family of ligand-gated ion channels and form from five subunits as homologous or heterologous, oligomeric receptors. NNRs are of interest as targets for the treatment of a variety of central and peripheral nervous system disorders, including Alzheimer's, Parkinson's, and schizophrenia, as well as for cessation of smoking and pain management. Consequently, designing subtype selective ligands, i.e., orthosteric agonists and antagonists, allosteric modulators, and channel modulators of NNRs, is an active area of pharmaceutical research. Work on membrane-bound NNR proteins has provided key information on both the structure and function of NNRs, but a lack of high resolution protein structures limits structural design efforts. However, much progress has been achieved