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.

doi:10.1016/j.bcp.2011.07.010

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.

doi:10.1016/j.bcp.2011.07.011

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-