been shown to express more readily in a variety of systems. The codon usage within the cDNA sequences of each subunit were optimized to increase expression in mammalian cells, areas of high GC content were altered to introduce greater AT sequence content, and predicted secondary structures were disrupted, while retaining the original protein sequence. Initially, α6/3β2 monoclones were selected, and several that expressed high levels of [^{3}H]epibatidine binding sites were identified. None of these clones produced function as measured by $^{86}Rb^+$ efflux, although single-cell patch-clamp electrophysiology identified low levels of function in one monoclone. Following introduction of the β3 subunit and further subcloning, [^{3}H]epibatidine binding site expression was increased. In an attempt to further increase nAChR expression, cells were incubated at 30 °C before testing. Multiple α6/3β2β3 nAChR monoclonal cell lines were identified as functional using $^{86}Rb^+$ efflux. The highest expresser was chosen for all further experiments. Preliminary testing was done with the agonists ACh, (−)-nicotine, carbachol, and cytisine. Each proved to be a potent agonist (EC_{50} values of 244 nM, 186 nM, 1.98 μM, and 238 nM, respectively). Cytisine was approx 50% efficacious, the others were fully efficacious. Potent antagonism was observed *vs.* 10 μM ACh activation for the α6-selective antagonists α-CtxMII and α-CtxPIA (nM IC_{50} values), while DHβE antagonism was of lower potency (7.8 μM IC_{50} value). These values closely resemble those measured at native α6β2β3* nAChRs. In addition, a set of novel compounds was tested for functional activity at α6/3β2β3, with a wide range of agonism, antagonism and potency observed. These data indicate that the new α6/3β2β3 cell line accurately reproduces native α6β2β3* agonist and antagonist pharmacology, and is well-suited for use in compound screening.

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1.6

Tethered pentamers—Low sensitivity α4β2-nicotinic acetylcholine receptors

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Nicotinic acetylcholine receptors (nAChR) containing α4 and β2 subunits appear to be expressed as two isoforms differing structurally in α4:β2 subunit ratios (3:2 and 2:3) and functionally in their sensitivity (low or high) for nicotinic agonists. When expressing α4β2-nAChR from loose subunits in *Xenopus* oocytes, variation in amounts of subunit cRNAs injected can bias expression toward a given isomer. However, no such control is possible in heterologous expression in mammalian cell lines from loose subunits. To overcome this shortcoming, we have designed and expressed cDNA constructs that encode concatenated subunits as covalently-linked or "tethered" pentamers. A construct designed to contain three α4 and two β2 subunits, when stably expressed in SH-EP1 human epithelial cells, encodes a product that conveys to cells low nicotinic agonist sensitivity for functional activation of whole-cell inward currents or $^{86}Rb^+$ efflux responses. These cells also display high affinity binding for radiolabeled nicotinic agonists. These studies suggest that the construct encodes tethered pentameric, functional and ligand binding, low sensitivity, $(\alpha4)_3(\beta2)_2$-nAChR. Further studies using this construct and cells expressing it will aid research on nAChR, help define roles played by low and high sensitivity α4β2-nAChR, and facilitate isoform-specific or -selective drug discovery with a view toward creation of novel therapeutics for treatment of psychiatric or neurological disorders.

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1.7

A methodological comparison of human α4β2 and α3β4 receptor properties using conventional and high-throughput patch-clamp electrophysiology techniques

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High-throughput screening for compounds with activity at neuronal nicotinic receptors using electrophysiology-based assays represents an important tool for biomedical research. The recent development and availability of high-throughput devices brings the need to validate these tools by demonstrating the ability to collect data that is consistent with results acquired through conventional electrophysiological methods. Population patch clamp (PPC) is a newly developed technique that allows for the simultaneous recordings from up to 64 cells per well. While PPC can greatly improve the success rate during automated electrophysiology experiments, it was not known whether the measured amplitude and kinetics from each well represented the sum of several uniform current responses from multiple individual cells, or an aggregate of varied responses resulting from different concentration transients across the cell population during application of the ligand. In this study, we compared the response properties of *h*α3β4 and *h*α4β2 nicotinic receptors to their endogenous ligand acetylcholine (ACh) using three separate electrophysiology platforms (Dynaflow, PatchXpress and IonWorks Barracuda). We found that in spite of the differences in methodological approaches among the Dynaflow (conventional electrophysiology), PatchXpress (medium-throughput electrophysiology) and IonWorks Barracuda (high-throughput electrophysiology) technologies, the values from the ACh dose-response curves (EC_{50}, Hill slope) were similar across all three platforms. In addition, we found that the decay kinetics due to desensitization of the receptors were also similar for all three applied techniques. This study provides the first data validating the consistency of results using low-, medium- and high-throughput electrophysiology platforms and supports their use for screening physiologically active compounds.

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1.8

Novel properties of neuronal nicotinic receptors revealed with brief pulses of acetylcholine

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Fast synaptic transmission within the central nervous system can occur on a sub-millisecond timescale. To effectively study these electrochemical events, ligand-gated receptors must briefly be exposed to concentrations of agonist that adequately re-create the endogenous physiological conditions. Synaptic properties such as

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-