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