1.3

Effects of incorporation of α5 subunits into α4β2 nicotinic acetylcholine receptors on pharmacological functions of nicotinic ligands

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To elucidate their role in diseases and disorders such as addiction, pain and attention deficit/hyperactivity disorder, the heteromeric α4β2 nicotinic acetylcholine receptors (NNRs) have been the focus of many studies. Recently, investigations have shown that other members of the NNR family, such as α5 expressed with α4β2 in the brain, may play important roles in these diseases. Receptor functions involving activation and desensitization are influenced by many factors including subunit composition which is likely modified during the course of diseases. In the present study, we co-transfected concatenated β2–α4 dimers with single α4, β2 or α5 monomers, to express low sensitivity (LS)-α4β2, high sensitivity (HS)-α4β2 or $(\alpha4\beta2)_2\alpha5$ (α4β2α5) NNRs respectively in HEK293F cells. Subsequently the effect of α5 incorporation on the pharmacological properties of α4β2 agonists varenicline, cytisine, and sazetidine-A was investigated by a membrane potential method. For varenicline and cytisine, the activation profiles were very similar on all three α4β2 NNRs. They partially activated LS-α4β2 with comparable potencies but had no detectable activities at HS-α4β2 NNRs. At activation of α4β2α5, similar potency and efficacy were observed as compared to their respective responses at LS-α4β2 NNRs. Sazetidine-A, in contrast, was a potent full agonist at HS-α4β2 with low activity at LS-α4β2. Its partial agonistic activity at α4β2α5 was similar to that at LS-α4β2. Interestingly, when cells were pretreated with these agonists and then challenged with acetylcholine (ACh), they all displayed similar desensitization efficacy, to a complete degree, at the three α4β2 NNRs, again with comparable potencies for varenicline and cytisine. Additionally, onset of the desensitization was within minutes for all agonists tested and fastest for sazetidine-A. We also characterized the influence of α5 on sensitivities of α4β2 NNRs to the antagonists dihydro-beta-erythroidine (DHβE) and TC-5280. When co-applied with ACh, DHβE and TC-5280 displayed IC_{50} values of 114 and 177 nM at HS-α4β2, 123 and 364 nM at LS-α4β2, and 85 and 439 nM at α4β2α5, respectively. Under pre-treatment condition, TC-5280 was more potent and selective to desensitize HS-α4β2 and α4β2α5 NNRs. In summary, for three agonists tested, incorporation of α5 shifted their activation responses to LS-α4β2-like and their desensitization responses to HS-α4β2-like. Understanding the changes in pharmacological functions influenced by receptor subunit composition will help us to understand the physiological role of such receptors and to better design drugs that target the relevant receptor subtypes involved in diseases.

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1.4

Comparison of binding and functional activity profiles for a set of nicotinic acetylcholine receptor ligands across multiple α6* expression systems

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It has been proposed that modulation of the nicotinic acetylcholine receptor α6* (where the asterisk indicates additional subunits) subtype may offer therapeutic opportunities in overcoming nicotine addiction and in alleviating motoric dysfunction associated with Parkinson's disease. Progress in the identification of selective α6* ligands has previously been hampered by the lack of robust, pharmacologically relevant high-throughput assays. Until recently, the only assay amenable to high throughput *in vitro* screening was the α6/4β4 chimera. Native α6* subtypes associated with dopaminergic activity are believed to contain an α6-β2 interface which is missing in the α6/4β4 chimera, calling into question the relevance of this data for predicting *in vivo* activity in animal models of addiction and Parkinson's disease. We have recently identified a number of compounds with moderate to high affinity for α6*-containing receptors through screening of previously published nicotinic ligands and Targacept proprietary database libraries in a variety of assay formats. These include novel α6β3β4α5 construct expressed in mammalian cells, rat native tissue from α6-rich brain areas, an α6/3β2β3 chimera expressed in mammalian cells, and autoradiography in rat striatum. The affinity and, consequently, selectivity versus other nicotinic receptor subtypes (α4β2, α3β4) varied greatly depending on the assay system. We present here the *in vitro* profiles of these diverse ligands across multiple α6* affinity measures and preliminary structure–activity relationships. The relevance of α6* binding affinity to α6* function, as measured by Calcium flux in the α6/3β2β3 chimera and the α-conotoxin MII-sensitive component of striatal dopamine release, will also be discussed.

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1.5

Construction of cell line heterologously expressing the α6/3β2β3 nicotinic acetylcholine receptor (nAChR) subtype

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Natively-expressed α6* nAChRs are predominantly expressed in combination with β2 and β3 subunits (α6β2β3* nAChRs; asterisk denotes other possible subunits). Heterologous expression of functional α6* nAChRs in mammalian cell lines has historically proved difficult. In order to overcome this problem, we have applied a combination of approaches to increase expression of functional nAChRs, without using a gain-of-function mutation that could alter EC_{50} and IC_{50} values or change agonist efficacies. Native α6 subunits were replaced with α6/3 chimeric subunits, which have