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Stimulation of dopamine release by nicotinic acetylcholine receptor ligands in rat brain slices correlates with the profile of high, but not low, sensitivity α4β2 subunit combination

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ABSTRACT

α4β2 neuronal nicotinic receptors (nAChRs) can exist in high and low sensitivity states possibly due to distinct stoichiometries during subunit assembly: $(\alpha4)_2(\beta2)_3$ pentamer (high sensitivity, HS) and $(\alpha4)_3(\beta2)_2$ pentamer (low sensitivity, LS). To determine if there is a linkage between HS or LS states and receptor-mediated responses in brain, we profiled several clinically studied α4β2* nAChR agonists for the displacement of radioligand binding to α4β2 [^{3}H]-cytisine sites in rat brain membranes, effects on stimulation of [^{3}H]-dopamine release from slices of rat prefrontal cortex and striatum, and activation of HS and LS human α4β2 nAChRs expressed in *Xenopus laevis* oocytes. Binding affinities (pK_i) and potency (pEC_{50}) values for [^{3}H]-dopamine release closely correlated with a rank order: varenicline > (−)-nicotine > AZD3480 > dianicline ≅ ABT-089. Further, a good correlation was observed between [^{3}H]-dopamine release and HS α4β2 pEC_{50} values, but not between [^{3}H]-dopamine release and LS α4β2. The relative efficacies of the agonists ranged from full to partial agonists. Varenicline behaved as a partial agonist in stimulating [^{3}H]-dopamine release and activating both HS and LS α4β2 nAChRs expressed in oocytes. Conversely, ABT-089, AZD3480 and dianicline exhibited little efficacy at LS α4β2 (<5%), were more effective at HS α4β2 nAChRs, and in stimulating cortical and striatal [^{3}H]-dopamine release ≥30%. In the presence of α-conotoxin MII to block α6β2* nAChRs, the α4β2* α-conotoxin-insensitive [^{3}H]-dopamine release stimulated by these ligands correlates well with their interactions at HS, but not LS. In summary, this study provides support for HS α4β2* nAChR involvement in neurotransmitter release.

1. Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) belong to the family of pentameric acetylcholine-gated cation channels and are assembled from twelve subunits, α2–α10 and β2–β4. These subunits combine to form homomeric or heteromeric nAChRs that mediate a wide range of physiological and pharmacological effects in the nervous system. All are thought to be pentameric, comprised of a mixture of alpha-subunits (α2–α6) and beta-subunits (β2–β4) with exceptions of homomers of α7–α9 subunits, or heteromers of α9–α10 subunits [1]. A recent report provided evidence for the existence of a functional α7β2 nAChR in rodent basal forebrain [2]. The α4β2-containing (α4β2*) nAChRs account for 90% of the high affinity nicotine binding sites and are widely distributed throughout the brain including in the cortex, hippocampus, substantia nigra, and ventral tegmental area (reviewed in [3]). The last two regions are particularly important since they are rich in dopaminergic neurons whose function is regulated by various nicotinic subunits including α4β2* subtype and thought to participate in modulating the reinforcing effects of nicotine and other addictive substances (reviewed in [4]) and in CNS disorders such as Parkinson's disease.

Initially, heteromeric α4β2 subunits were thought to exist as a single subunit entity exhibiting high affinity to nicotine [5]. However, a variety of combinations and stoichiometries are possible as evidenced by injecting varying ratios of α4 and α2 cRNA or cDNA [6–9].

Combination of excess α4 with respect to β2 subunit is thought to generate predominantly $(\alpha4)_3(\beta2)_2$ pentamer with low sensitivity (LS) to ACh ($EC_{50} \sim 100\ \mu M$), whereas the reverse ratio

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is thought to favor the $(\alpha4)_2(\beta2)_3$ expression with high sensitivity (HS) to ACh ($EC_{50} \sim 1\ \mu M$). This concept is supported by studies using linked α4β2 subunits assembled with β2 subunits as $(\alpha4\beta2)_2\beta2$ pentamers and with α4 subunits as $(\alpha4\beta2)_2\alpha4$ pentamers to address the stochiometry of functional receptor assemblies [10]. Other compounds exhibiting differential profiles at HS and LS profiles include a variety of agonists (nicotine, epibatidine, cytisine, 3-Br-cytisine, 3-[2(S)-azetidinylmethoxy]-pyridine (A-85380), 5-ethoxy-metanicotine (TC-2559), A-163554 and A-168939) and some antagonists, particularly chlorisondamine [9,11]. The LS form is thought to be more permeable to Ca^{2+} than the HS form whereas the HS form is more sensitive to upregulation by chronic nicotine and reduced ambient temperature [12]. Other combinations with various degrees of sensitivities and selectivities for nAChR ligands can be formed with the substitution of other subunits such as α2, α3, α6 or β4 [3]. Like α4β2, HS and LS forms of α3β2 also have been demonstrated, suggesting this may be a general feature of β2-containing nAChR [9]. The α5 and β3 subunits do not participate in the formation of acetylcholine binding sites, but may have accessory or regulatory roles in the receptor affecting the properties of the expressed pentamer. For instance, $(\alpha4\beta2)_2$ α5 nAChRs were reported to be more permeable to Ca^{2+} than either $(\alpha4\beta2)_2\beta2$ and $(\alpha4\beta2)_2\alpha4$ pentamers [13]. One brain region that has been demonstrated to be especially rich in the variety of nAChRs present is the striatum with up to six nAChR combinations proposed [14]. Through the use of null mutant mice, immunoprecipitation, and inhibition with α-conotoxin MII, it has been ascertained that there likely exist α4β2, α4α5β2, α6β2, α4α6β2β3, and α6β2β3 combinations as well as the homomeric α7 in the striatum.

However, HS and LS forms of β2-containing nAChR are defined mainly by kinetic (EC_{50}) parameters. Whether both forms are expressed in the CNS and, if so, whether they have different functional roles is not yet fully elucidated. In mouse thalamic synaptosomes, responses to HS α4β2 nAChR selective agonists such as A-163554 and A-168939 suggest that at least the HS form is expressed functionally in that brain region [9]. Whether LS α4β2 nAChR also is expressed and whether the pattern may vary among brain regions remain open questions.

In this study, we hypothesized that there may be differential contributions of HS, $(\alpha4)_2(\beta2)_3$, and LS, $(\alpha4)_3(\beta2)_2$, nAChRs in neurotransmitter release. We addressed the contribution of HS and LS α4β2 to nAChR-mediated [^{3}H]-dopamine release from slices of rat prefrontal cortex and striatum by using a set of five agonists, and compared the pharmacological profiles of transmitter release with those of HS and LS α4β2 nAChRs expressed in Xenopus oocytes and α4β2* ([^{3}H]-cytisine) binding in rat brain. Additionally, since multiple receptor subtypes are involved in nAChR-mediated [^{3}H]-dopamine release in the striatum, α-conotoxin MII was used to distinguish the role of α4β2* from α6β2* nAChRs. Our results suggest that the HS, but not the LS, form of α4β2 nAChR is primarily involved in nicotine-stimulated dopamine release.

2. Methods

2.1. Materials

Membranes for radioligand binding studies were prepared from frozen rat brains (PelFreez, Rogers, AR, USA). For neurotransmitter release studies, male Sprague–Dawley rats were used from Charles River Laboratories (Portage, MI, USA, 250–400 g). Oocytes were obtained from adult female *Xenopus laevis* frogs (Blades Biological Ltd., Cowden, Edenbridge, Kent, UK). Animals were cared for in accordance with the Institutional Animal Care Committee guidelines that meet the guidelines of the National Institutes of Health. Acetylcholine and nicotine were obtained from Sigma (St. Louis, MO, USA or Oslo, Norway). ABT-089 (2-methyl-3-([(2S)-pyrrolidin-2-yl]methoxy)pyridine), varenicline (7,8,9,10-tetrahydro-6,10-methano-6*H*-pyrazino (2,3-h)(3) benzazepine), AZD3480 (S-E-[4-(5-isopropoxy-pyridin-3-yl)-1-methyl-but-3-enyl]-methyl-amine), and dianicline ((5aS,8S,10aR)-5a,6,9,10-tetrahydro,7H,11H-8,10a-methanopyrido[2′,3′:5,6]pyrano[2,3-d]azepine) were synthesized in house (Abbott Park, IL, USA). α-Conotoxin MII was obtained from Tocris Bioscience (Ellisville, MO, USA or Bristol, UK). [^{3}H]-cytisine and [^{3}H]-dopamine were obtained from PerkinElmer Life Sciences (Boston, MA, USA). All other chemicals and reagents were obtained from Sigma (St. Louis, MO, USA) or Fisher Scientific (Essex, UK).

2.2. [^{3}H]-cytisine binding to rat brain

[^{3}H]-Cytisine binding to α4β2 nAChRs was determined under equilibrium conditions using membrane enriched fractions from rat brain (minus cerebellum) as previously described [15]. Brains were homogenized in 15 volumes of 0.32 M sucrose and centrifuged at $1000 \times g$ at 4 °C for 10 min. Supernatants were centrifuged at $20{,}000 \times g$ for 20 min. The resulting P_2 pellets were homogenized with a Polytron (10 s at setting 7) in 15 volumes ice-cold H_2O and centrifuged at $8000 \times g$ at 4 °C for 20 min. The supernatant and loose buffy coat were centrifuged at $40{,}000 \times g$. The pellet was washed in 15 volumes ice-cold H_2O and recentrifuged before storage at −80 °C. Pellets were thawed at 4 °C, washed and resuspended with a Polytron at a setting of 7 in 30 volumes of BSS-Tris buffer (120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 50 mM Tris–Cl, pH 7.4, 4 °C) to yield 100–200 μg of protein. For concentration-inhibition assays, seven log-dilution concentrations of test compounds in duplicate, homogenate containing 100–200 μg of protein, and 0.7 nM [^{3}H]-cytisine were incubated in a final volume of 500 μl for 75 min at 4 °C. Non-specific binding was determined in the presence of 10 μM (−)-nicotine. Bound radioactivity was collected by vacuum filtration onto Millipore MultiScreen® harvest plates FB presoaked with 0.3% PEI using a PerkinElmer cell harvester and the filters were rapidly rinsed with 2 ml of ice-cold BSS-Tris buffer. PerkinElmer MicroScint-20® scintillation cocktail (40 μl) was added to each well and bound radioactivity was determined using a PerkinElmer TopCount® instrument.

2.3. Electrophysiological characterization in X. laevis oocytes

High sensitivity (HS) and low sensitivity (LS) human α4β2 nAChR were expressed in *X. laevis* oocytes by methods described previously [16,17]. In brief, three to four lobes from ovaries of female adult *X. laevis* frogs were removed and defolliculated after treatment with collagenase type 1A (2 mg/ml; Sigma) prepared in low-Ca^{2+} Barth's solution (90 mM NaCl, 1.0 mM KCl, 0.66 mM $NaNO_3$, 2.4 mM $NaHCO_3$, 10 mM HEPES, 2.5 mM sodium pyruvate, 0.82 mM $MgCl_2$, and 0.5% (v/v) penicillin–streptomycin solution, pH 7.55 (Sigma)) for 1.5–2 h at ~18 °C under constant agitation to obtain isolated oocytes. The oocytes were co-injected with human α4 and β2 nAChR cRNA (0.2–30 ng of each), kept at 18 °C in a humidified incubator in low-Ca^{2+} Barth's solution and used 2–7 days after injection. Responses were measured by two-electrode voltage clamp using parallel oocyte electrophysiology test station (Abbott, Abbott Park, IL) [18]. During recordings, the oocytes were bathed in Ba^{2+}-OR2 solution (90 mM NaCl, 2.5 mM KCl, 2.5 mM $BaCl_2$, 1.0 mM $MgCl_2$, 5.0 mM HEPES, and 0.0005 mM atropine, pH 7.4) to prevent activation of Ca^{2+}-dependent currents and held at −60 mV at room temperature (~20 °C). No acetylcholinesterase inhibitor was included in the bath buffer. To obtain either predominantly HS or LS combinations, ratios of 1:1 (HS) or 100:1 (LS) α4 and β2 cRNAs were injected. Ion current amplitudes