The Novel α7β2- Nicotinic Acetylcholine Receptor Subtype Is Expressed in Mouse and Human Basal Forebrain: Biochemical and Pharmacological Characterization

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ABSTRACT

We examined αβ2- nicotinic acetylcholine receptor (αβ2-nAChR) expression in mammalian brain and compared pharmacological profiles of homomeric α7-nAChRs and αβ2-nAChRs. α-Bungarotoxin affinity purification or immunoprecipitation with anti-α7 subunit antibodies (Abs) was used to isolate nAChRs containing α7 subunits from mouse or human brain samples. α7β2-nAChRs were detected in forebrain, but not other tested regions, from both species, based on Western blot analysis of isolates using β2 subunit–specific Abs. Ab specificity was confirmed in control studies using subunit-null mutant mice or cell lines heterologously expressing specific human nAChR subtypes and subunits. Functional expression in Xenopus oocytes of concatenated pentameric (α7)4β2, (α7)4β2β2 and (α7)3β2β2-nAChRs was confirmed using two-electrode voltage clamp recording of responses to nicotinic ligands. Importantly, pharmacological profiles were indistinguishable for concatenated (α7)5-nAChRs or for homomeric α7-nAChRs constituted from unlinked α7 subunits. Pharmacological profiles were similar for (α7)5- versus α7-nAChRs. This study represents the first direct confirmation of α7β2-nAChR expression in human and mouse forebrain, supporting previous mouse studies that suggested relevance of α7β2-nAChRs in Alzheimer disease etiopathogenesis. These data also indicate that α7β2-nAChR subunit isoforms with different α7/β2 subunit ratios have similar pharmacological profiles to each other and to α7 homopentameric nAChRs. This supports the hypothesis that α7β2-nAChR agonist activation predominantly or entirely reflects binding to α7/α7 subunit interface sites.

Introduction

Several nicotinic acetylcholine receptor (nAChR) subtypes are expressed widely along the entire neuraxis and are involved in many of the physiologic functions of the central and peripheral nervous systems (Albuquerque et al., 2009; Hurst et al., 2013). nAChR activity controls important aspects of synaptic function and brain development, including the proliferation and differentiation of neural progenitors, neuronal migration, and neuronal maturation (Griguoli and Cherubini, 2012; Picciotto et al., 2012; Yakel, 2013). Furthermore, nAChR dysfunction may play an important role in a variety of neurologic diseases, including neurodegenerative and psychiatric diseases (Gotti and Clementi, 2004; Lewis and Picciotto, 2013).

α7β2- and homomeric α7-nAChRs are the most widely expressed subtypes in mammalian brain. The latter are thought to contain five identical agonist binding sites located at subunit interfaces in extracellular domains (Gotti and Clementi, 2004; Whiteaker et al., 2007). Pharmacological hallmarks of α7-nAChRs are their high sensitivity to antagonism by snake venom–derived polypeptide toxins such as α-bungarotoxin (α-Bgtx) and α-cobratoxin (α-Cbtx), and their sensitivity to choline [a product of acetylcholine (ACh) hydrolysis] as an agonist (Albuquerque et al., 1997, 2009). α7-nAChRs are highly expressed in the cortex, hippocampus, and subcortical limbic regions, and (at lower levels) in the thalamus and basal ganglia. α7-nAChRs that are...
located on or near nerve terminals are involved in control of neurotransmitter release, whereas α7-nAChRs on dendrites or soma, as opposed to cholineric synaptic endings, play roles in classic neurotransmission. In both cases, the high calcium permeability of α7-nAChRs may also result in altered intracellular signaling and gene transcription (Dajas-Bailaron and Wonnacott, 2004; Albuquerque et al., 2009). α7-nAChRs also may be associated with extraneuronal volume transmission (Lendvai and Vizi, 2008).

Affinity purification of nAChRs using snake venom α-toxins has been performed from brain tissue of various species. Extracts from whole rat brain appear to be predominantly composed of homomeric α7-nAChRs (Drisdel and Green, 2000). However, homomeric α7- and α9-nAChRs (and heteromeric α7α8-nAChRs) have been identified in chick central nervous system extracts (Keyser et al., 1993; Gotti et al., 1994). Furthermore, studies using heterologous systems have shown that α7 subunits can form functional channels when combined with α2 (Girod et al., 1999), β2 (Khiroug et al., 2002), β3 (Palma et al., 1999), or β4 subunits (Criado et al., 2012). Fluorescently tagged nAChR α7 and β2 subunits were recently used to characterize the formation of α7β2-nAChRs, and functional differences between α7- and α7β2-nAChRs have been suggested (Murray et al., 2012). Coexpression of β2 and α7 subunits caused a significant decrease in agonist-evoked whole cell current amplitudes, but this decrease occurs without affecting the concentration-response characteristics of a range of common agonists and antagonists (Murray et al., 2012). Other studies have shown that α7 and β2 subunits are coexpressed in rat basal forebrain cholineric neurons and appear to form heteromeric α7β2-nAChRs with different biological and pharmacological properties from those of homomeric α7-nAChRs (Liu et al., 2009). In addition, interaction of these putative α7β2-nAChRs with oligomeric forms of amyloid-β (Aβ1–42) may be relevant in the etiology of Alzheimer disease (Liu et al., 2013).

These previous studies suggest that the function and pharmacology of α7*-nAChRs (where the asterisk denotes the known or possible presence of other nAChR subunits than α7; Lukas et al., 1999) may be more complex than previously thought, and that α7β2-nAChR expression may be restricted to forebrain areas. However, heteromeric α7*-nAChRs have not yet been directly detected biochemically, nor have they been definitively identified in human brain. We used the α7-nAChR–selective ligand, α-Bgtx, to affinity purify α7*-nAChRs from selected brain areas of humans or of wildtype (WT) or β2 subunit-null mutant (KO) mice. The subunit compositions of these isolated α7*-nAChRs were analyzed by Western blot analysis using subunit-specific anti-α7 or anti-β2 antibodies. The results show expression of α7β2-nAChRs in both WT mouse and human forebrain samples, but not in brains from β2 KO mice. Moreover, concatameric (linked subunit) constructs, the Xenopus oocyte system, and two-electrode voltage clamp (TEVC) recording were used to confirm functional expression of α7β2-nAChRs. This work defined α7 and β2 subunit stoichiometries that enable α7β2-nAChR function and showed similar pharmacological characteristics across α7- and α7β2-nAChR subtypes. The results confirm commonalities in expression of α7β2-nAChRs in humans and mice, and support hypotheses linking α7β2-nAChRs, cholineric signaling loss, and roles for Aβ1–42 in etiopathogenesis of at least a subset of human dementias.

### Materials and Methods

#### Animals and Materials

This study used 4- to 6-month-old male, pathogen-free, C57BL/6 WT, α2 KO, or β2 KO mice (Picciotto et al., 1995; Orr-Iturregi et al., 1997) obtained from Dr. U. Maskos (Pasteur Institute, Paris, France). All animal experiments were conducted in accordance with the European Community Council Directive (86/609/EEC) of November 24, 1986. (±)-HT[epitabidine (Epi; specific activity, 660Ci/mmol) and [125I]-α-Bgtx (specific activity of 200–216 Ci/mmol) were purchased from Perkin Elmer (Waltham, MA). Nonradioactive α-Bgtx, Epi, and nicotine were purchased from Toeris Bioscience (Bristol, UK, or Minneapolis, MN), as were dihydro-β-erythroidine (DHβE) and methyllycaconitine (MLA). Sazetidine-A (also known as AMOP-II-H) was kindly supplied by Dr. Alan Kozikowski (University of Illinois, Chicago, IL, USA). 1,2-bis-N-cytsislylethane (CC4) also was used (Riganti et al., 2005). α-CBtx and all other reagents were sourced from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

#### Human Tissues

Human cerebellum was provided by the Newcastle Brain Tissue Resource on the basis of a collaboration with Dr. Jennifer Court (Newcastle upon Tyne General Hospital, Newcastle upon Tyne, UK). Samples were all collected by the Newcastle Brain Tissue Resource with informed consent and appropriate ethical approval. Case details are shown in Table 1. The approvals and method for categorizing the subjects’ smoking status are outlined in the methods section of Court et al. (2005). Human basal forebrain tissue was provided by Dr. Emanuele Sher (Lilly Research Center, Windlesham, Surrey, UK), and was also collected with appropriate informed consent in accordance with all applicable laws and regulations.

#### Transfected Cells

Human α2, α7, β2, and β4-nAChR subunit clones in the mammalian expression vector pcDNA3 were gifts from Dr. Sergio Fucile (University of Rome, Rome, Italy). The human α7-nAChR subunit clone in pcDNA3 was a gift of Dr. Roberta Benfante (CNR Institute of Neurosciences, Milan, Italy). HEK293 and SH-SY5Y cells were transiently transfected using the Ca3(PO4)2 method or the Jet-PEI reagent (Polyplus, Euroclone, Italy) transfection. For the α2 plasmid, 1.5 × 106 cells were transfected with 6 μg plasmid using the Jet-PEI method, and was also collected with appropriate informed consent in accordance with all applicable laws and regulations.

#### Antibody Production and Characterization

We used affinity-purified, subunit-specific polyclonal antibodies (Abs), produced in rabbit against peptides derived from the C-terminal (COOH) or intracytoplasmic loop (CYT) of human or mouse nAChR subunit sequences, as previously described (Gotti et al., 2006; Grady et al., 2009). The Ab against the COOH peptide (SAPNFVEAVSKDFA) was used for α7 subunits in mouse and human tissues. Abs directed...
against the α2 mouse CYT peptide (PSPGPDLAKILEEVRYIANRFRC) or the human CYT peptide (QMDEADI$GYPNGQMDEADI$GYPNG) were used for mouse and human tissues, respectively. For the β2 subunit, we used antibodies directed against different cytoplasmic human β2 peptides: RQREREGALFFREPAGDSCTY (β2-1) and cgIADHR$EDDQQ$VREDWKC$V (β2-2).

The specificity of the affinity-purified Abs was tested by immunoprecipitation studies using α2 WT or α2 KO hippocampus and β2 WT or β2 KO mouse cortex (the results are shown in Supplemental Fig. 1). The same Abs also were tested by means of Western blotting (Supplemental Fig. 1). To exclude any cross-reactivity between nAChR subunits, anti-β2 or anti-α1 human subunit Abs were also tested by means of immunoprecipitation studies and Western blotting in HEK293 cells transfected to express human αβ2, αβ3, αβ4, or αβ7 nAChR subtypes or in SH-SYSY cells transfected to express human α7 nAChRs (see above) (results are shown in Supplemental Fig. 2).

**Purification of α-Bgtx-Binding nAChRs**

For studies using mice, approximately 100 mg of basal forebrain or hippocampus tissue microdissected from either WT or subunit-null mice were pooled in every experiment. The tissue was homogenized in 10 ml of 50 mM Na phosphate, pH 7.4, 1 M NaCl, 2 mM EDTA, 2 mM EGTA, and 2 mM phenylmethylsulfonylfluoride (to covalently inactivate serine protease activity), and the homogenates were diluted and centrifuged for 1.5 hours at 60,000g. The entire membrane homogenization, dilution, and centrifugation procedure were then repeated, and the resulting pellets were collected, rapidly rinsed with 50 mM Tris HCl, pH 7, 120 mM NaCl, 1 mM MgCl2, 2.5 mM CaCl2, and 2 mM phenylmethylsulfonylfluoride. The washed pellets were then resuspended in 2 ml of the same buffer, further supplemented with 20 μg/ml of each of the following protease inhibitors: leupeptin, bestatin, pepstatin A, and aprotinin. Triton X-100 at a final concentration of 2% was added to the washed membranes, which were extracted for 2 hours at 4°C. The extracts were centrifuged for 1.5 hours at 60,000g, recovered, and an aliquot of the supernatants was collected for protein measurement using the BCA protein assay (Pierce Biotechnology, Rockford, IL), with bovine serum albumin (BSA) as the standard. Extracts (2 ml) were incubated with 200 μl Sepharose–α-Bgtx (concentration of coupled toxin 1 mg/ml of gel) and shaken overnight at 4°C. The following day, the beads were centrifuged, the supernatant was recovered, and the resins were washed 4–6 times by resuspension followed by centrifugation. After washing, the Sepharose–α-Bgtx beads with bound nAChRs (purified α-Bgtx–binding receptors) were incubated with one to two volumes of Laemmli sample buffer (125 mM Tris phosphate, 4% SDS, 20% glycerol, 0.02% bromophenol blue, and 10% 2-mercaptoethanol, pH 6.8) and boiled for 2 minutes. The supernatant was then recovered by centrifugation.

In the case of human tissue, α-Bgtx–binding sites were purified using the same procedure as that used for mouse tissue, starting from 600 mg of tissue (see Table 1 for subject details).

**Binding Studies**

[125I]-α-Bgtx. The binding of [125I]-α-Bgtx to 2% Triton X-100 extracts of mouse tissues was determined by collection onto DEAE-Sepharose Fast Flow (GE Healthcare, Uppsala, Sweden). Triton extracts (250 μl) from each experimental group were incubated overnight with a saturating concentration (5 nM) of [125I]-α-Bgtx at 20°C in the presence of 2 mg/ml BSA. Specific radioligand binding was defined as total binding minus the nonspecific binding determined in the presence of 1 μM unlabeled α-Bgtx. Nonspecific binding averaged 30–40% of total binding. Binding to α2, α4, or α7 nAChRs could also be measured in an immunoprecipitation assay format. Receptor extracts were labeled with [125I]-α-Bgtx (5 nM in the presence or absence of 1 μM unlabeled α-Bgtx to define total and nonspecific binding). The labeled extract could then be bound to protein A beads via anti-α7 subunit Abs (described later in the Materials and Methods). Similar amounts of specific binding were recorded in either assay format, and nonspecific binding was between 10 and 15% of total binding.

[3H]Epi. Binding of [3H]Epi to nAChRs in 2% Triton X-100 brain tissue extracts obtained was also assessed. [3H]Epi binds to multiple heteromeric nAChR subtypes with picomolar affinity and to α7-nAChR with nanomolar affinity. To ensure that the α2 nAChR did not contribute to [3H]Epi binding, in solubilized extracts, binding was performed in the presence of 1 μM α-Bgtx, which specifically binds to α7-nAChRs (and thus prevents [3H]Epi binding to these sites).

As for [125I]-α-Bgtx binding assays, binding sites were captured using DEAE-Sepharose Fast Flow, after overnight incubation of 250–μl aliquots of the extracts with 1 nM [3H]Epi at 4°C. Nonspecific binding (averaging 5–10% of total binding) was determined in parallel samples containing 100 nM unlabeled Epi.

**Immunoprecipitation**

For immunoprecipitation studies of heteromeric receptors present in human tissues, we used Abs specific for α2, α3, α4, α5, β2, or β4 subunits directed against human subunit peptides as previously described (Gotti et al., 2006). For α2 and β2 subunits, we used Abs directed against peptides of mouse subunit sequences, also as previously characterized and described (Grady et al., 2009). The immunoprecipitation capacities of the anti-human subunit Abs ranged from 90 to 100% of the [3H]Epi–labeled receptors (mean of three independent experiments). For immunoprecipitation experiments, affinity-purified Abs were covalently immobilized on agarose-Protein A beads at a concentration of 4 mg/ml wet resin. Immunoprecipitation was then performed by adding 20 μl agarose-Protein A beads with bound, affinity-purified Abs to 200 μl of 1 nM [3H]Epi–labeled extracts. After overnight incubation, immunoprecipitates were recovered by centrifugation and washed three times with phosphate-buffered saline containing 0.1% Triton X-100.

**Immunoblotting and Densitometric Quantification of Western Blot Bands**

nAChR subunit contents of tissue extracts or of α-Bgtx–binding complexes were analyzed by Western blotting. For the extracts loaded before and after the purification, 10 μg of proteins were loaded, whereas for the α-Bgtx–purified receptors a constant volume (40 μl), that depending on the tissue, may represent 1/10 or 1/20 of the total recovered Laemmli sample buffer-eluted receptors was loaded onto a 9% acrylamide (Bio-Rad, Hercules, CA) gel and subjected to SDS-PAGE. After SDS-PAGE, proteins were electrophoretically transferred to nitrocellulose membranes with 0.45 mm diameter pores (Schleicher and Schuell, Dassel, Germany). The blots were blocked overnight in 5% nonfat milk in Tris-buffered saline, washed in a buffer containing 5% nonfat milk and 0.3% Tween 20 in Tris-buffered saline, incubated for 2 hours with the primary antibody (1–2.5 mg/ml), and then incubated with the appropriate peroxidase-conjugated secondary Abs (Sigma-Aldrich). After 10 washes, peroxidase was detected using a chemiluminescent substrate (Pierce Biotechnology). The signal intensity of the Western blot bands was measured using an Epson 4500 gel scanner. The developed films were scanned as a TIFF image in eight-bit grayscale format at a resolution setting of 300 dpi. All of the films obtained from the separate experiments were acquired in the same way and scanned in parallel with a calibrated optical density step tablet from Stouffer (Stouffer Graphics Arts, Mishawaka, IN).

The images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD) (Schneider et al., 2012). The pixel values of the images were transformed to optical density values by the program using the calibration curve obtained by acquiring the calibrated table with the same parameters as those used for the images. The immunoreactive bands were quantified in four separate experiments for the mouse hippocampus and basal forebrain as previously described (Grady et al., 2009).
Concatemeric α7*-nAChR Constructs

Fully pentameric nAChR concatamers were constructed from human nAChR subunit sequences. cDNAs encoding concatamers were created using the same subunit layout we have previously employed to encode high- and low-agonist-sensitivity αBβ2-αnAChR isoforms and α2β2[α3D/J[N]-nAChRs (George et al., 2012; Eaton et al., 2014). Subunits were arranged in the order α7-α2-α6-α-α7 (α7 homopentamer), α7-α2-α6-α7, or α7-β2-α7-α2-α7. Kozac and signal peptide sequences were removed from all subunit sequences with the exception of subunits expressed in the first position of the concatamer. Subunits were linked by alanine-glycine-serine repeats designed to provide a complete linker length (including the C-terminal tail of the preceding subunit) of 40 ± 2 amino acids. At the nucleotide level, linker sequences were designed to contain unique restriction sites that allow easy removal and replacement of individual α7 and β2 subunits. The protein sequences for the human nAChR subunits were encoded by synthetic nucleic sequences optimized for expression systems (GeneArt; Life Technologies, Grand Island, NY). Optimization included minimization of high GC content sequence segments, improved codon usage, reduction of predicted RNA secondary structure formation, and removal of sequence repeats and possible alternative start and splice sites. Sequences of all subunits, together with their associated partial linkers, were confirmed by DNA sequencing (GeneArt). Each concatamer was subcloned into the pSGEM oocyte high-expression vector (from Dr. Michael Hollmann, Ruhr-Universitaet, Bochum, Germany). For comparison, homomeric α7-nAChRs were also expressed from unlinked individual subunits (cDNA clone also synthesized and optimized by GeneArt). The unlinked human α7 subunit cDNA was also subcloned into the pSGEM vector.

RNA Synthesis

Plasmids containing concatemeric α7-homopentameric or α2β2-αnAChR constructs, or individual α7-nAChR subunits, were linearized with NheI (2 hours at 37°C), and the reaction mix was treated with proteinase K (30 minutes at 50°C). cRNAs were transcribed using the mMessage mMACHINE T7 kit (Applied Biosystems/Ambion, Austin, TX). Reactions were treated with TURBO DNase (1 U for 15 minutes at 37°C) and cRNAs were purified using the Qiagen RNeasy Clean-Up kit (Qiagen, Valencia, CA). cRNA purity was confirmed on a 1% agarose gel and preparations were stored at −80°C.

Xenopus Oocytes and RNA injection

Xenopus oocytes were purchased from Ecocyte Bioscience US (Austin, TX) and incubated upon arrival at 13°C. The tips of pulled glass micropipettes were broken to achieve an outer diameter of approximately 6 M (resistance of 2–6 MΩ), and pipettes were used to inject 20–60 nl containing 10 ng of cRNA/oocyte. To improve functional expression of α7*-nAChRs, 1.5 mM CaCl2 was added and preparations were stored at −80°C.

TEVC Recording of α7- and α2β2-nAChR Function

TEVC recordings were made at room temperature (20°C) in oocyte saline solution (containing 82.5 mM NaCl, 2.5 mM KCl, 5 mM HEPES, 1.8 mM CaCl2 H2O, and 1 mM MgCl2 H2O, pH 7.4). Seven to 14 days after injection, Xenopus oocytes expressing concatenated α7*-nAChRs were voltage clamped at −70 mV with an Axoclamp 900A amplifier (Molecular Devices, Sunnyvale, CA). Recordings were sampled at 10 kHz (low-pass Bessel filter, 40 Hz; high-pass filter, DC), and the resulting traces were saved to disk (Clampamp v10.2; Molecular Devices). Data from oocytes with leak currents (Ileak) > 50 nA were excluded from recordings.

Nicotinic Receptor Pharmacology

Fresh stock drug solutions (agonists: ACh, choline, nicotine, azatadine, and CC4; antagonists: DHβE, MLA, mecamylamine, and α-Btx) were made daily and diluted as required. Agonists and antagonists were applied using a 16-channel, gravity-fed perfusion system with automated valve control (AutoMate Scientific, Inc.; Berkeley, CA). All solutions were supplemented with atropine sulfate (1.5 μM) to ensure that muscarinic ACh receptor responses were blocked and thus not recorded. Oocytes expressing loose subunits and/or concatemeric α7- or α2β2-nAChRs were perfused with nAChR agonists for 5 seconds with 60-second washout times between each subsequent application. Oocytes were preincubated with nAChR antagonists for 2 minutes prior to activation with ACh (10 mM; 5 seconds). For experiments using α-Btx, bath and drug solutions were supplemented with 0.1% BSA to reduce loss of this peptide ligand by adsorption to the TEVC apparatus.

Data Analysis

The expression of [3H]Epi and [125I]-α-Bgtx receptors and the subunit contents of the [3H]Epi receptors expressed in the mouse and human samples were statistically compared using unpaired t tests. In human cerebellum samples from smokers and nonsmokers, results were compared using an unpaired t test. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA).

For TEVC data, EC50 and IC50 values were determined from nAChR-mediated peak currents through nonlinear least-squares curve fitting (GraphPad Prism 5.0) using unconstrained, monophasic logistic equations to fit all parameters, including Hill slopes. Desensitization/activation of α7*-nAChR currents in the presence of 10 mM (maximally stimulating) ACh was also analyzed by nonlinear least-squares curve fitting in Graph Pad Prism 5.0. These data were best fit by a two-phase exponential decay equation. One-way analysis of variance was used to compare parameters between multiple groups in each case. The Tukey multiple comparison test was used for post hoc analysis to compare the means of three or more groups (GraphPad Prism 5.0).

Results

α-Bgtx-Binding Sites in WT or β2 KO Mice

In preliminary experiments, we analyzed nAChR expression in 2% Triton X-100 extracts obtained from the hippocampus or basal forebrain of WT or β2 KO mice (Table 2). By two different approaches (immunoprecipitating [125I]-α-Bgtx-labeled receptors using anti-α7 subunit Abs and by [125I]-α-Bgtx binding to Triton extracts), we determined that the density of α7*-nAChRs in the mouse hippocampus is more than two times higher than that in the murine basal forebrain. During α-Bgtx binding site purification, we determined that 85–95% of those sites in Triton X-100 extracts were bound by α-Bgtx–Sepharose 4B affinity resins, whereas more than 95% of high-affinity [3H]Epi binding was recovered in the flow-through.

The densities of [3H]Epi–binding nAChRs were similar between the two regions (Table 2). Elimination of β2 subunit expression dramatically reduced expression of [3H]Epi–binding nAChRs in both regions, indicating that this binding is almost entirely due to β2*-nAChRs. By contrast, [125I]-α-Bgtx (α7*-nAChR) expression was not significantly different between WT and β2 KO mice in either the hippocampus or basal forebrain.

In addition, Western blot analysis was performed on α-Bgtx binding sites affinity purified from the hippocampus of WT or β2 KO mice and probed with anti-α7 (top) or anti-β2 (bottom) subunit Abs (Fig. 1A). Confirming results from the binding studies, Western blots also showed no significant differences in presumed α7*-nAChR levels (i.e., polypeptide labeled with...
anti-α7 subunit Abs) in whole extracts from WT or β2 KO mouse hippocampus (lane 1 in top two panels). Moreover, affinity purification on α-Bgtx affinity resins isolated comparable levels of α7*-nAChRs from WT or β2 KO mouse hippocampus (compare lanes 1 and 3 of Fig. 1A top) but did not isolate nAChRs containing β2 subunits (compare lanes 1 and 3 of Fig. 1A, bottom left), which instead were found in the flow-through fraction (lane 2; Fig. 1A, bottom left). As expected, no β2*-nAChRs were found in extracts isolated on Bgtx resins or in the flow-through from that separation for tissue taken from β2 KO mice (Fig. 1A, lower right panel lanes 1–3).

In further agreement with the findings of the binding studies, Western blot analysis showed that basal forebrain extracts contained fewer α7*-nAChRs (i.e., immunoreactive α7 protein) than hippocampal extracts, whereas amounts of immunoreactive β2 subunits were very similar across brain regions. Analysis of the Western blots probed using anti-β2 subunit Abs showed clearly detectable β2 subunit presence in α7*-nAChRs isolated on Bgtx resins from tissue derived from basal forebrain, but not from the hippocampus (compare lanes 3 of the bottom-left panels of Fig. 1A, A and B). The percentage of β2 subunit present in the α-Bgtx purified receptor was determined by western blotting by loading on the same gel 10 μg of 2% Triton X-100 extract and 1/10 of the α-Bgtx purified receptor. We found that the immunoreactivity of the β2 subunit determined in the purified α-Bgtx was 2.25% ± 0.6% (n = 4) of the total β2 subunit immunoreactivity measured in the basal forebrain extracts of WT mice.

**α-Bgtx–Binding Sites in Human Brain**

The possible presence of α7/β2-nAChRs in human brain was analyzed using post mortem samples of basal forebrain and cerebellum. In preliminary experiments, we characterized nAChR subtypes expressed in basal forebrain and cerebellum and their levels in 2% Triton extracts (Table 3). The average level of [3H]Epi–labeled (α7*)-nAChRs was higher in basal forebrain than cerebellum.

The level of [3H]Epi–binding nAChRs in cerebellum depended on smoking status. As shown in Table 3, the density of non-α7*-nAChRs measured by means of [3H]Epi binding was higher in smokers than in nonsmokers (P = 0.02). Based on immunoprecipitation using subunit-specific Abs, in both tissues the large majority of [3H]Epi–binding sites contained the β2 subunit associated with the α4 subunit (α4β2-nAChR: 75% in basal forebrain and 60 and 67% in cerebellum of smokers and nonsmokers, respectively). An additional 114% of [3H]Epi–binding sites in the basal forebrain were α3β2*-nAChRs, whereas this subtype accounted for only 7% of cerebellar [3H]Epi–binding sites.

**TABLE 3**

Levels of [3H]Epi and [125I]α-Bgtx binding to 2% Triton X-100 extracts in two different human brain regions

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>[3H]Epi</th>
<th>[125I]α-Bgtx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal forebrain</td>
<td>31.8 ± 8.5</td>
<td>80.7 ± 7.0</td>
</tr>
<tr>
<td>Cerebellum nonsmokers</td>
<td>22.5 ± 2.6</td>
<td>45.7 ± 5.1</td>
</tr>
<tr>
<td>Cerebellum smokers</td>
<td>39.7 ± 5.3*</td>
<td>48.3 ± 6.5</td>
</tr>
</tbody>
</table>

*P = 0.02 (significant differences in cerebellar membrane [3H]Epi binding between smokers and nonsmokers: unpaired t test). No significant difference was seen between [125I]α-Bgtx binding levels in cerebellar samples taken from smokers versus nonsmokers, by the same measure.
Human α7β2-nAChR Human Expression and Functional Pharmacology

Introduction

Heterologous expression has shown assembly of functional α7β2*-nAChRs (see the Introduction), but the way(s) in which α7 and β2 subunits might combine from individual, unlinked, subunits could not be defined. Accordingly, we used a linked-subunit approach to produce α7*-nAChRs with defined subunit ratios and assembly orders. Each of the three concatemeric constructs [(α7)2β2-nAChR homopentamer, (α7)β2β2-nAChR, and (α7)β2β2β2-nAChR] showed concentration-dependent ACh-evoked function (representative traces shown in Fig. 3, A–D). This function, although smaller than that measured in Xenopus oocytes expressing homeric α7-nAChRs from unlinked human α7 subunits (typically > 1 μA at 7 days after mRNA injection) was easily measurable (approximately 100–300 nA peak current response, depending on the construct). The time course of desensitization/inactivation after a peak response stimulated by 10 mM ACh (maximally stimulating concentration) was also measured for each construct. For each construct, desensitization/inactivation was best fit by a double-exponential decay model. As detailed in the legend to Fig. 3, no significant differences were seen between the fast desensitization/inactivation time constants calculated for each group. This is not surprising since the apparent time constants will likely reflect the relatively slow kinetics of agonist application in the apparatus, rather than the much faster kinetics of α7*-nAChR desensitization (Papke, 2010). Indeed, the apparent τfast values are very similar to those measured for solution exchange in our apparatus (Eaton et al., 2014). However, the τslow value calculated for the (α7)β2β2 construct was significantly slower than those associated with the other groups. Thus, despite the admitted disadvantages of measuring kinetic parameters in the Xenopus oocyte expression system, there is some evidence that α7β2*-nAChR desensitization may be slower than that of homomorphic α7-nAChRs.

Agonist and Antagonist Pharmacology of Concatemeric Human α7*-nAChRs

Pharmacological parameters of selected ligands were determined at concatenated α7*-nAChRs. Compounds chosen included the prototypical agonists, ACh and nicotine, choline (which is a relatively selective agonist of α7-nAChR; Alkondon et al., 1997), and two further agonists with established selectivity for other β2*-nAChR subtypes (sazetidine-A and CC4; Xiao et al., 2006; Kozikowski et al., 2009; Sala et al., 2013). Agonist pharmacological profiles for (α7)β2-, (α7)β2β2-, and (α7)β2β2β2-nAChR subtypes were largely indistinguishable from each other, and from that for nonconcatemeric (loose-subunit), homomeric α7-nAChRs (Fig. 4; Table 4). The only exception is that nicotine has significantly lower efficacy (normalized to that of ACh) at both α7β2*-nAChR subtypes than at concatenemeric (α7)β2-nAChRs or unlinked α7* nAChRs (which are statistically indistinguishable on this measure). There was also a trend toward lower choline potency across all concatenemeric α7*-nAChR constructs, but this did not reach statistical significance (see Table 4). The observed slight trend toward lower choline efficacy, although not significant, is suggestive of the previous observation of 50–70% efficacy of choline versus ACh at putative α7β2-nAChRs expressed from unlinked subunits (Khiroug et al., 2002; Zwart et al., 2014). Strikingly, both sazetidine-A and CC4 were very weak agonists (< 10% efficacy normalized to that of ACh) at all α7*-nAChR subtypes tested, including both α7β2-nAChRs, making it impossible to reliably calculate EC50 or Hill slope values from the resulting concentration-response data.

Fig. 2. Western blot analysis of α-Bgtx–purified nAChRs prepared from human basal forebrain and cerebellum. α-Bgtx-binding nAChRs were purified from the same volume of 2% Triton X-100 extracts of basal forebrain and cerebellum by incubating them with Sepharose 4B covalently bound with α-Bgtx. The bound receptors were eluted using sample buffer and an identical volume of purified receptors was loaded on the gel. The Western blots were probed with anti-α7 Ab (top) or anti-β2 Ab (bottom).

Middle; and anti-β2(2), bottom] targeting different epitopes within the β2 subunit. Control samples were extracts from αβ2-nAChR–expressing transfected HEK cells (lane 4) or from α-β-nAChR–expressing transfected SH-SY5Y cells (lane 8), also probed with the Abs. Levels of immunoreactivity for the α7 subunit were very similar in samples loaded in lanes 1, 2, 5, and 6, higher in the sample loaded in lane 3, and lower in the sample loaded in lane 7. Similar isolates from HEK-αβ2 cells were negative but SH-SY5Y-α7 cells contained immunoreactive α7 subunits (Fig. 2, upper panel, lanes 4 and 8, respectively). Isolation of α-Bgtx binding sites also yielded anti-β2 subunit Ab-labeled proteins from basal forebrain samples but not from the cerebellum, regardless of whether the cerebellum samples were obtained from smokers or nonsmokers. Such immunoreactivity was absent in extracts from SH-SY5Y-α7 cells but very evident in HEK-αβ2 cells (Fig. 2, middle and lower panels, lanes 8 and 4, respectively). Both the α7 and β2 subunits present in the human tissues show a slightly higher molecular weight than the corresponding transfected subunits. This is probably due to differences in glycosylation between native and transfected receptors.

Since it has been shown in a heterologous expression system that an αβ2*-nAChR subtype may be formed (Criado et al., 2012), we also probed human α-Bgtx–purified sites with anti-β2 subunit Abs with proven specificity (Supplemental Fig. 2, bottom). No specific labeling was observed in either the human basal forebrain or cerebellum samples, showing absence of αβ2*-nAChRs. Collectively, these results clearly indicate that αβ2*-nAChRs are present in the human basal forebrain but not in the cerebellum.
Concentration-response relationships were also explored for archetypal \( \alpha_7 \) antagonists (MLA and the snake venom \( \alpha \)-toxin, \( \alpha \)-Cbtx), together with the \( \beta_2 \)-selective antagonist DHB3E and the noncompetitive antagonist mecamylamine (Fig. 5). The resulting pharmacological parameters are summarized in Table 5. Similar to the agonist pharmacology, antagonist responses were statistically indistinguishable between the \( \alpha_7^* \) subtypes (including between \( \alpha_7 \)-only nAChRs expressed from either unlinked subunits, or from the concatenated \( \alpha_7 \) homopentameric construct).

**Discussion**

This study provides the first direct evidence that \( \alpha_3\beta_2\)-nAChRs are expressed in the mammalian central nervous system. This is demonstrated by isolation of Bgtx-binding or \( \alpha_7 \) subunit–containing complexes also shown to contain \( \beta_2 \) subunits from human or mouse forebrain samples. In addition, we have demonstrated for the first time that multiple human \( \alpha_3\beta_2\)-nAChR isoforms of defined subunit composition have pharmacological profiles similar to each other and to homopentameric \( \alpha_7 \)-nAChRs.

Our findings indicate that \( \alpha_3\beta_2\)-nAChRs are found in post mortem, human basal forebrain but not in the cerebellum. Note that total amounts of \( \alpha_7 \)-nAChRs are <2-fold different in the two brain regions. Specificity of the anti-\( \alpha_7 \) or anti-\( \beta_2 \) Abs used in Western blot analysis of these nAChRs is demonstrated by control studies using cell lines transfected with specific nAChR subunits, and by studies using WT and subunit-null mice. We also found \( \alpha_3\beta_2\)-nAChR expression in mouse basal forebrain but not hippocampus. Our results agree with earlier findings of \( \alpha_3\beta_2\)-nAChR expression in mouse basal forebrain (Liu et al., 2009) but not with the same investigators’ study in mouse hippocampus (Liu et al., 2012). There could be several explanations for these seemingly discrepant observations. nAChR \( \alpha_7 \) and \( \beta_2 \) subunit expression was also investigated for each nAChR construct using additional gels of oocytes. In each case, the time course was best fitted by a double-exponential decay. The fast time constant (\( \tau_{fast} \)) was statistically indistinguishable by one-way ANOVA across all four groups (unlinked \( \alpha_7 \), \( 430 \pm 55 \) milliseconds; \( \alpha_7 \)-only concatamer, \( 210 \pm 80 \) milliseconds; \( \alpha_3\beta_2\)-p3, \( 312 \pm 55 \) milliseconds; \( \alpha_3\beta_2\)-p2,4, \( 247 \pm 35 \) milliseconds; \( F_{3,11} = 2.06, P = 0.16 \) and 3 in each group). By contrast, the slow time constant (\( \tau_{slow} \)) for desensitization/inactivation of the \( \alpha_3\beta_2\)-p2,4 construct was significantly longer than that of the other groups. No other differences were detected by a Tukey post hoc comparison (\( P < 0.05 \)). Values were as follows: unlinked \( \alpha_7 \), \( 5109 \pm 800 \) milliseconds; \( \alpha_7 \)-only concatamer, \( 3130 \pm 580 \) milliseconds; \( \alpha_3\beta_2\)-p3, \( 5703 \pm 630 \) milliseconds; \( \alpha_3\beta_2\)-p2,4, \( 6318 \pm 365 \) milliseconds; \( F_{3,11} = 5.29, P = 0.02 \) and 3 in each group. (E) Summary of maximal function (\( I_{max} \)) measured in each concatemeric nAChR group by stimulation with the full agonist ACh (10 mM). Bars represent mean ± S.E.M. (n = 3). \( I_{max} \) values were as follows: \( \alpha_7 \) only, \( 83.9 \pm 18.6 \) nA; \( \alpha_3\beta_2\)-p3, \( 285 \pm 11 \) nA; and \( \alpha_3\beta_2\)-p2,4, \( 216 \pm 45 \) nA. Analysis using one-way ANOVA with a Tukey post hoc comparison showed that incorporation of \( \beta_2 \) subunits resulted in a statistically significant increase in \( I_{max} \) (\( P_{2,6} = 12.7, P = 0.007 \) (denoted by the asterisk)). The \( I_{max} \) values obtained from the two \( \alpha_3\beta_2\)-nAChR constructs were statistically indistinguishable from each other. ANOVA, analysis of variance.
et al., 2014), and \( \alpha_6\beta_2^{*}\)-nAChR (Kuryatov and Lindstrom, 2011) subtypes, introduction of appropriately sized linkers can be performed without altering nAChR functional pharmacology. Several of these previous studies also showed that concatemeric constructs were assembled correctly. To further confirm correct that concatemers were being assembled correctly and not fragmenting and rearranging into unanticipated functional forms, we also co-injected unlinked \( \beta_2 \) subunits containing a gain-of-function mutation (L9'S) in the second transmembrane domain. This additional control has previously been used by us and others (Carbone et al., 2009; Eaton et al., 2011). If concatemer fragments were contributing to the function of concatenated nAChR population, the \( \beta_2 \)-gain-of-function subunit would be seen. Such an effect was seen.

It is noted, however, that overall function was reduced when \( \alpha_7 \)-nAChR homopentamers were expressed from a concatemeric construct as opposed to unlinked subunits. This relative diminution in function of concatenated nAChR constructs has been noted in the previous publications cited above and appears to be a regular feature of using concatemeric nAChR constructs. Importantly, both (\( \alpha_7 \gamma_3 \beta_2 \)) and (\( \alpha_7 \gamma_3 \beta_2 \)) nAChR concatemeric constructs expressed more function than did the (\( \alpha_7 \gamma_3 \beta_2 \)) nAChR concatemer. This is the opposite of the situation in which loose \( \beta_2 \)-nAChR subunits are coexpressed with \( \alpha_7 \) subunits (Murray et al., 2012) and replicates an earlier finding in which coexpression of unlinked \( \alpha_5 \), \( \alpha_3 \), and \( \beta_4 \) nAChR subunits reduced function compared with expression of loose \( \beta_2 \) subunits alone, but incorporation of the \( \alpha_5 \) subunit into a concatemeric construct actually increased observed function of an \( \alpha_5\beta_2^{*}\)-nAChR pentameric concatemer (George et al., 2012). As in the previous publication, we suspect that uncontrolled assembly of an unlinked additional subunit (in this case \( \beta_2 \)) may be deleterious, but directed assembly may result in greater functional expression of the new nAChR subtype. Certainly, this study provides direct evidence that \( \beta_2 \) subunit incorporation into \( \alpha_7 \)-nAChRs is compatible with agonist-induced function.

The pharmacological profiles of \( \alpha_7\beta_2 \)-nAChRs were very similar to those of homopentameric \( \alpha_7 \)-nAChRs. Even agonists (sazetidine-A, CC4) and an antagonist (DH2E) previously shown to have significant \( \beta_2^{*}\)-nAChR selectivity had indistinguishable pharmacology across homomeric \( \alpha_7 \)-nAChRs and the two different \( \alpha_7\beta_2 \)-nAChR isoforms. Each of these findings matches those very recently published using Xenopus oocytes expressing \( \alpha_7 \) and \( \beta_2 \) subunits at a 1:10 ratio (Zwart et al., 2014). The only statistically significant difference in this study was a diminution of nicotine’s efficacy relative to that of ACh in the two \( \alpha_7\beta_2 \)-nAChR isoforms (also seen by Zwart et al., 2014). This nicotine partial agonism further confirms that \( \beta_2 \) was incorporated into \( \alpha_7\beta_2 \)-nAChR concatemers as planned and may represent a pharmacological marker for the presence of \( \alpha_7\beta_2 \)-nAChRs. The same may be true of the slower desensitization kinetics measured for the (\( \alpha_7 \gamma_3 \beta_2 \)) (Fig. 3), although it is important to note the limitations of measuring receptor kinetics in a Xenopus oocyte system (Papke, 2010). We note that the similar \( \alpha_7 \)-nAChR versus \( \alpha_5\beta_2 \)-nAChR potency of DH2E observed by us and by Zwart et al. (2014) does not match the observations made in two previous studies (Liu et al., 2009; Murray et al., 2012). The reason for this discrepancy between
TABLE 4  

<table>
<thead>
<tr>
<th>Subtype</th>
<th>ACh</th>
<th>Nicotine</th>
<th>Choline</th>
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<tr>
<td></td>
<td>IC50 (nM)</td>
<td>EC50 (nM)</td>
<td>Efficacy</td>
</tr>
<tr>
<td>α2/β2</td>
<td>6.3 ± 0.2</td>
<td>6.3 ± 0.3</td>
<td>6.3 ± 0.3</td>
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<tr>
<td>α2/β2 (unlinked)</td>
<td>6.3 ± 0.2</td>
<td>6.3 ± 0.3</td>
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</tr>
<tr>
<td>α2/β2-γ/δ/ε/ζ</td>
<td>6.3 ± 0.2</td>
<td>6.3 ± 0.3</td>
<td>6.3 ± 0.3</td>
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<tr>
<td>α2/β2-γ/δ/ε/ζ-γ/δ</td>
<td>6.3 ± 0.2</td>
<td>6.3 ± 0.3</td>
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<tr>
<td>α2/β2-γ/δ/ε/ζ-γ/δ-γ/δ</td>
<td>6.3 ± 0.2</td>
<td>6.3 ± 0.3</td>
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The functional pharmacology of α7-nAChR and α2/β2-nAChR subtypes is remarkably similar. This observation indirectly supports the concept that activation of α2/β2-nAChRs may be predominantly or exclusively mediated only through agonist binding sites at α2/α7 (not α2/β2) interfaces (Murray et al., 2012). If this is true, it seems unlikely that any competitive agonist could exhibit a significantly different potency between the α7-nAChR and α2/β2-nAChR. However, antagonists capable of disrupting the allosteric transitions required for nAChR activation (Celie et al., 2005), and of selectively binding to α2/α7 interfaces, could be valuable in this regard as could other noncompetitive ligands. In the concatemeric (α7)4(β2)1-nAChR construct (subunit order α7-α7-β2-α7-α7), only three α2/α7 subunit interfaces will be retained (between the first two subunits, the last two subunits, and between the first and last subunits that will assemble together to complete the pentameric nAChR structure). In the (α7)4(β2)1-γ-nAChR construct, only the α7/α7 interface formed between the first and last subunits will be retained. At first glance, it may seem remarkable that an α7-nAChR containing such a diminished complement of putative agonist binding sites could be effectively activated. However, elegant recent work indicates that the nAChR, including α7-α7-nAChR, can be activated effectively by as few as one agonist binding site (Rayes et al., 2009; Williams et al., 2011; Andersen et al., 2013).

That α2/β2-nAChRs are relatively scarce in basal forebrain does not imply that their role is necessarily insignificant. For example, α6β2-nAChR expression on substantia nigra/ventral tegmental area dopamine projections comprises <10% of all β2-nAChRs in dopamine terminal regions (Whiteaker et al., 2000; Gotti et al., 2005), but this subtype is extremely important in controlling local neuronal behavior and signal processing (Exley and Cragg, 2008; Exley et al., 2008). Cholinergic neurons constitute only a fraction (10–15%) of basal forebrain neurons (Semba, 2000) and the proportion of a α2/β2-nAChRs in these neurons may therefore be relatively large. The basal forebrain cholinergic system provides primary cholinergic innervations to limbic and cortical brain structures, and expresses nAChRs that participate in the cholinergic transmission
Furthermore, a recent electrophysiological study has demonstrated that Alzheimer disease is the degeneration of this cholinergic projection (Voytko et al., 1994; Hernandez et al., 2010). One of the most marked pathologic changes in the brain in Alzheimer disease is the degeneration of the cholinergic projection and the consequent reduction in the number of nAChRs (Price et al., 1985; Holtzman et al., 1992; Wenk, 1993; Dumas and Newhouse, 2011; Pinto et al., 2011). A number of studies have found that the β-amyloid (Aβ) peptide (a hallmark of Alzheimer disease) plays a critical role in neuronal degeneration and subsequent memory deficits (Price et al., 1985; Holtzman et al., 1992; Wenk, 1993; Fraser et al., 1997; Capsoni et al., 2000; Dolga et al., 2009). Furthermore, a recent electrophysiological study has demonstrated that Aβ binds with higher affinity to αβ2-nAChR than to α7-nAChR, and that this can produce hippocampal neuronal hyperexcitation (through α7-nAChR upregulation) and subsequent neurodegeneration (Liu et al., 2013).

Post mortem tissue is an underused substrate for genetic and/or preclinical studies, and provides a translational element that is difficult to recapitulate in animal models alone (McCullumsmith et al., 2014). This study’s definitive evidence that αβ2*-nAChRs are found in human as well as mouse basal forebrain provides valuable support for the concept that this subtype may be relevant to the study and etiology of Alzheimer disease. The similarities in human- and mouse-brain basal forebrain αβ2*-nAChR expression are also supportive of the use of mouse models in this context.

### Table 5

<table>
<thead>
<tr>
<th>Subtype</th>
<th>DHJ/E log IC50/M</th>
<th>MLA log IC50/M</th>
<th>Mecamylamine log IC50/M</th>
<th>a-Cbtx log IC50/M</th>
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</thead>
<tbody>
<tr>
<td>α7 (unlinked)</td>
<td>-5.2 ± 0.05</td>
<td>n.s.</td>
<td>-1.0 ± 0.1</td>
<td>-1.6 ± 0.2</td>
</tr>
<tr>
<td>α7-β7-α7-α7-α7</td>
<td>-5.3 ± 0.07</td>
<td>-0.8 ± 0.1</td>
<td>-1.6 ± 0.2</td>
<td>-1.6 ± 0.2</td>
</tr>
<tr>
<td>α7-β7-α7-α7-β7-α7</td>
<td>-5.4 ± 0.10</td>
<td>-0.6 ± 0.1</td>
<td>-1.7 ± 0.1</td>
<td>-1.7 ± 0.1</td>
</tr>
<tr>
<td>α7-β7-α7-β7-β7-α7</td>
<td>-5.4 ± 0.10</td>
<td>-0.7 ± 0.1</td>
<td>-1.8 ± 0.1</td>
<td>-1.8 ± 0.1</td>
</tr>
</tbody>
</table>

Antagonist log IC50 and Hill slope (nH) values were derived by nonlinear least-squares curve fitting of the data shown in Fig. 5 to the Hill model. Pharmacological parameters obtained for each antagonist were statistically indistinguishable between all four groups of oocytes according to analysis with one-way analysis of variance.

FIG. 5. Antagonist concentration response profiles for α7- and αβ2-nAChRs. Oocytes were injected with mRNA encoding unlinked α7 subunits (○), concatenated α7 homopentamers (●), or concatenated αβ2 pentameric concatemers (□) indicates αβ2 nAChR with the β2 subunit in position 3; ■ indicates αβ2 nAChR with the β2 subunit in positions 2 and 4. Before antagonists were applied to each oocyte, a control 10 mM ACh-evoked response was measured. Oocytes were preperfused with the following nAChR antagonists: DHJ(E) (10^-6.25 to 10^-2; n = 3) (A), methyllycaconitine (10^-10.5 to 10^-7; n = 3) (B), mecamylamine (10^-7.25 to 10^-4; n = 3) (C), or α-Cbtx (10^-10 to 10^-7; n = 3) (D). The magnitudes of subsequent 10 mM ACh stimulations were compared with that of the initial control. Data points represent the mean ± S.E.M. Drug potency and efficacy parameters were calculated by nonlinear least-squares curve fitting to the Hill equation (see Materials and Methods). The resulting pharmacological parameters and statistical analyses are summarized in Table 5.

Post mortem tissue is an underused substrate for genetic and/or preclinical studies, and provides a translational element that is difficult to recapitulate in animal models alone (McCullumsmith et al., 2014). This study’s definitive evidence that αβ2*-nAChRs are found in human as well as mouse basal forebrain provides valuable support for the concept that this subtype may be relevant to the study and etiology of Alzheimer disease. The similarities in human- and mouse-brain basal forebrain αβ2*-nAChR expression are also supportive of the use of mouse models in this context.
References
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Conducted experiments: Moretti, George, Pistillo.
Contributed new reagents or analytic tools: Pistillo, Maskos, Whiteaker.
Prepared data analysis: Moretti, George, Pistillo, Whiteaker.
Wrote or contributed to the writing of the manuscript: Zoli, Lukas, Whiteaker, Gotti.


