Rat neuronal nicotinic acetylcholine receptors containing α7 subunit: pharmacological properties of ligand binding and function

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**Aim:** To compare pharmacological properties of heterologously expressed homomeric α7 nicotinic acetylcholine receptors (α7 nAChRs) with those of native nAChRs containing α7 subunit (α7* nAChRs) in rat hippocampus and cerebral cortex.

**Methods:** We established a stably transfected HEK-293 cell line that expresses homomeric rat α7 nAChRs. We studies ligand binding profiles and functional properties of nAChRs expressed in this cell line and native rat α7* nAChRs in rat hippocampus and cerebral cortex. We used [125I]-α-bungarotoxin to compare ligand binding profiles in these cells with those in rat hippocampus and cerebral cortex. The functional properties of the α7 nAChRs expressed in this cell line were studied using whole-cell current recording.

**Results:** The newly established cell line, KXα7R1, expresses homomeric α7 nAChRs that bind [125I]-α-bungarotoxin with a $K_d$ value of 0.38±0.06 nmol/L, similar to $K_d$ values of native rat α7* nAChRs from hippocampus ($K_d$=0.28±0.03 nmol/L) and cerebral cortex ($K_d$=0.33±0.05 nmol/L). Using whole-cell current recording, the homomeric α7 nAChRs expressed in the cells were activated by acetylcholine and (–)-nicotine with EC50 values of 280±19 µmol/L and 180±40 µmol/L, respectively. The acetylcholine activated currents were potently blocked by two selective antagonists of α7 nAChRs, α-bungarotoxin (IC50=19±2 nmol/L) and methyllycaconitine (IC50=100±10 pmol/L). A comparative study of ligand binding profiles, using 13 nicotinic ligands, showed many similarities between the homomeric α7 nAChRs and native α7* receptors in rat brain, but it also revealed several notable differences.

**Conclusion:** This newly established stable cell line should be very useful for studying the properties of homomeric α7 nAChRs and comparing these properties to native α7* nAChRs.

**Keywords:** neurotransmitter release; memory; Alzheimer’s disease

Introduction

Neuronal nicotinic acetylcholine receptors containing α7 subunits (α7* nAChRs) are widely expressed in the CNS, and have been studied extensively[2–4]. When expressed in Xenopus oocytes[9, 10] or mammalian cells[11], these receptors are highly permeable to calcium ion[12–14]. The α7 nAChRs have been implicated in a wide range of physiological functions, including neurotransmitter release[15, 16], activation of second messengers, apoptosis and neuroprotection[17, 18]. More recent evidence indicates that α7 nAChRs may be directly involved in learning and memory[19, 20], pathology of Alzheimer’s disease[21–23], and inflammation[24]. The advance in our understanding of the biological properties and roles of α7* nAChRs has suggested new therapeutic strategies and drug candidates targeting α7 nAChRs for treatment of CNS disorders, including Alzheimer’s disease[25, 26].

Stably transfected mammalian cell lines heterologously expressing neuronal nAChR subtypes have been powerful tools to study neuronal nAChRs[11, 27–32]. Several such cell lines expressing functional α7 nAChRs were successfully established in recent years, including the rat α7 subunit gene expressed in SH-SY5Y human neuroblastoma cells, which also express endogenous nAChR α3, α5, α7, β2, and β4 subunit genes[33]; the human α7 subunit gene expressed in HEK-293 cells[11] and in SH-EP1 clonal human epithelial cells[34, 35], both of which are devoid of endogenous nAChR.

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subunits; and the rat a7 subunit gene expressed in GH4C1 clonal rat pituitary cells, which endogenously express the rat nAChR β4 subunit gene.\[36, 37\].

In the present study, we established a stable clonal mammalian cell line that expresses the rat a7 gene and functional α7 receptors that mediate rapidly desensitizing currents in response to acetylcholine and nicotine. The pharmacological properties of the ligand binding site of these recombinant rat nAChRs were compared to those in native rat a7* nAChRs from hippocampus and cerebral cortex.

Materials and methods

Materials and chemicals Tissue culture medium, fetal bovine serum, antibiotics, restriction endonucleases, modifying enzymes and molecular size standards were obtained from Invitrogen Corp (Carlsbad, CA). \([^{125} I]\)-α-Bungarotoxin (\([^{125} I]\)-α−BTX, 180–260 Ci/mmol), \([α-^{32} P]\)CTP (800 Ci/mmol) and \([γ-^{32} P]\)ATP (3000 Ci/mmol) were obtained from Amersham Biosciences Corporation (Piscataway, NJ). Electrophoresis reagents were purchased from Bio-Rad Laboratories (Melville, NY). All other chemicals were purchased from Sigma Chemical Co (St Louis, MO), unless otherwise stated. Rat brain tissues were dissected from brains purchased from Zivic-Miller Laboratories (Zelienople, PA).

Cell culture and stable transfection HEK-293 cells (ATCC CRL 1573) were maintained at 37 °C with 5% CO2 in a humidified incubator. Growth medium for the HEK-293 cells was minimum essential medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin G, and 100 µg/mL streptomycin. A eukaryotic expression construct was made using the full-length cDNA of the rat α7 subunit gene (a generous gift from Dr James W. PATRICK of Baylor College of Medicine). Transfection of HEK-293 cells, and selection and establishment of stable cell lines were carried out as described previously with minor modifications. Briefly, cultured cells at >90% confluency in a humidified incubator. Growth medium for the HEK-293 cells was minimum essential medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin G, and 100 µg/mL streptomycin. A eukaryotic expression construct was made using the full-length cDNA of the rat α7 subunit gene (a generous gift from Dr James W. PATRICK of Baylor College of Medicine). Transfection of HEK-293 cells, and selection and establishment of stable cell lines were carried out as described previously with minor modifications. Briefly, transfections were carried out using Lipofectamine 2000 transfection reagent (Invitrogen Corp). From each transfected population of cells, 48 stable, G418 resistant cell clones were isolated after cultivation in selective medium for 4 weeks. These clonal cell lines were then grown in selection medium for an additional 4 weeks.

Total RNA Isolation and RNase protection assay Total cellular RNA was isolated from tissues using RNASTAT-60™, a RNA isolation reagent (TEL-TEST “B”, Inc, Friendswood, TX). DNA templates for antisense riboprobes were prepared as described previously. The size of the full length probes and the expected protected fragments of the probes are as following: rat α2, 416 and 332 bases; rat α3, 306 and 230 bases; rat α4, 496 and 408 bases; rat α5, 411 and 380 bases; rat α6, 462 and 396 bases; rat α7, 450 and 376 bases; rat β2, 322 and 263 bases; rat β3, 430 and 394 bases; rat β4, 252 and 170 bases; rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), 204 and 135 bases; human GAPDH, 220 and 130, respectively. The probes were synthesized using T7 RNA polymerase (Ambion, Austin, TX) and \([α-^{32} P]\)CTP. Specific activities of \([α-^{32} P]\)CTP used for synthesizing the probes of rat nAChR subunit genes and the probe of GAPDH were 800 Ci/mmol and 32 Ci/mmol, respectively. Approximately 20 µg total RNA was hybridized with probes overnight at 42 °C. Nonprotected probes were digested with a mixture of RNase A and RNase T1, and the samples were processed using RPA II kit (Ambion, Austin, TX). Protected fragments were separated by electrophoresis on a 6% denaturing polyacrylamide gel. Positions and densities of the fragments were visualized and analyzed by X-ray filming or phosphor imaging.

Membrane homogenate preparation and \([^{125} I]\)-α-BTX binding assay Membrane homogenates for ligand binding assays were made as described previously with minor modifications. Briefly, cultured cells at >90% confluency were removed from the culture flask (80 cm2) with a disposable cell scraper and placed in 10 mL of 50 mmol/L Tris-HCl buffer (pH 7.4, 4 °C). The cell suspension was centrifuged at 1000g for 5 min and the pellet was collected. The cell pellet was then homogenized in 10 mL buffer with a polytron homogenizer for 20 s and centrifuged at 35 000×g for 10 min at 4 °C. Rat brain tissues (500 mg tissue in 10 mL buffer) were homogenized and centrifuged similarly. Membrane pellets were resuspended in fresh buffer.

\([^{125} I]\)-α-BTX solutions were made in 50 mmol/L Tris-HCl buffer containing 0.8 mg/mL bovine serum albumin (BSA, Sigma A-7030). Aliquots of the membrane homogenates containing 30 to 200 µg protein were used for the binding assays, which were carried out in a final volume of 100 µL in borosilicate glass tubes. After incubation at 24 °C for 4 h, the samples were collected with a cell harvester (Brandel M-48) onto Whatman GF/C filter papers pre-wet with 0.5% polyethylenimine (PEI). Before harvesting the samples, about 3 mL of 50 mmol/L Tris-HCl buffer containing 0.5 mg/mL BSA and 0.5% PEI was sucked into the har -veteran and through the filter to reduce nonspecific binding of \([^{125} I]\)-α-BTX to plastic and glass surfaces. After harvesting the samples, the filters were washed three times with 5 mL of 50 mmol/L Tris-HCl buffer, and then counted in a liquid scintillation counter. Nonspecific binding was measured in samples containing 1 mmol/L (−)-nicotine incubated in parallel. Specific binding was defined as the difference between...
total binding and nonspecific binding. Data from saturation and competition binding assays were analyzed using Prism 4 (GraphPad Software, San Diego, CA).

**Whole-cell current recording** Functional expression of α7 nAChRs was evaluated in the whole-cell configuration of the patch-clamp technique using an Axopatch 200B amplifier (Molecular devices, Sunnyvale, CA). The patch electrodes, pulled from borosilicate glass capillaries (Sutter Instrument Company, Novato, CA), had a resistance of 2.5–3.5 MΩ when filled with internal solution containing 110 mmol/L Tris phosphate dibasic, 28 mmol/L Tris base, 11 mmol/L EGTA, 2 mmol/L MgCl₂, 0.1 mmol/L CaCl₂ and 4 mmol/L Na-ATP (pH adjusted to 7.3 with Tris base). Stably transfected HEK-293 cells were studied 2 to 3 d after plating cells on the 15-mm round plastic coverslips (Thermanox, Nalge Nunc, Napierville, IL). Generation of voltage-clamp protocols and acquisition of the data were carried out using pCLAMP 9.0 software (Molecular Devices). Sampling frequency was 10 kHz and current signals were filtered at 5 kHz before digitization and storage. All experiments were performed at room temperature (22–25 °C).

Cells plated on coverslips were transferred to an experimental chamber mounted on the stage of an inverted microscope (Olympus IX50, Olympus Corporation, Tokyo, Japan) and were bathed in a solution containing 140 mmol/L NaCl, 3 mmol/L KCl, 2 mmol/L MgCl₂, 25 mmol/L D-glucose, 10 mmol/L HEPES and 2 mmol/L CaCl₂ (pH adjusted to 7.4 with Tris base). The experimental chamber was constantly perfused with control bathing solution (1–2 mL/min). The high-speed solution exchange system, HSSE-2 (ALA Scientific Instruments, Westbury, NY) was used to deliver agonist or antagonist to the cell. Under optimal conditions, the delay in switching between solutions is ~10 ms. 8 out of 12 patched cells responded to agonists (8–30 pA/pF with 1 mmol/L acetylcholine at -80 mV, n>36). In experiments aimed to determine the inhibitory potency of methyllycaconitine (MLA) or α-BTX, the cell under recording was exposed initially to acetylcholine at its EC₅₀ concentration. The cell was then exposed to the antagonist at the studied concentration for 2–8 min before a co-application of acetylcholine with the antagonist. Only one concentration of the antagonist was tested on each cell, and a new coverslip with cells was used each time.

The peak amplitude of the whole-cell currents was determined using the pCLAMP 9.0 program, the leak current was subtracted. EC₅₀, IC₅₀, and the nH values were evaluated with the Origin 5.0 program (Microcal, North Hampton, MA). To determine EC₅₀ values, acetylcholine-or nicotine-induced responses were recorded at -80 mV and normalized to the amplitude of the current elicited by acetylcholine alone at its saturating concentration (1 mmol/L). Values were plotted against the concentration of acetylcholine on a logarithm scale and fitted with an equation: \( I=I_{max}/(1+(E_{C50}/[agonist])^{nH}) \), where \( I \) is the current amplitude at the agonist concentration [agonist], \( I_{max} \) is the maximum current, EC₅₀ is the concentration of the agonist eliciting a half maximum response and \( nH \) is the Hill coefficient. The acetylcholine (about EC₅₀)-evoked currents in the presence of MLA or α-BTX were measured at -80 mV and normalized to the amplitude of the current elicited by acetylcholine alone. Values were plotted against the concentrations of the inhibitor on a logarithm scale and fitted with an equation: \( I=I_{max}/(1+(IC_{50}/[antagonist])^{nH}) \), where \( I \) is the current amplitude at the antagonist concentration [antagonist], \( I_{max} \) is the maximum current, and \( nH \) is the Hill coefficient. Results are presented as the mean±SEM for the number of cells (n).

**Results**

**Stable transfection of HEK-293 cells** From each population of transfected cells, 48 stable, G418-resistant cell clones were isolated after cultivation in selection medium for 4 weeks. These clonal cell lines were then grown in selection medium. Initial screenings of the G418-resistant clonal cells were carried out by measuring mRNA and by [¹²⁵I]-α-BTX ligand binding assays. Among all cell lines examined, the clonal cell line that showed the highest expression level of the rat α7 nAChR subunit gene was used in all of the following studies. This cell line was designated KXα7R1.

**Analyses of α7 subunit mRNA of the KXα7R1 cells** Multiplex RNase protection assays were carried out to measure mRNAs coded by rat neuronal nAChR subunit genes, including a2, a3, a4, a5, a6, a7, β2, β3, and β4. Total RNA was isolated from each of the clonal cell lines as well as from the HEK-293 parent cells. As expected, the only nAChR subunit mRNA transcript detected in the KXα7R1 cells was the one coding for a7 mRNA (Figure 1).

**Specific binding of [¹²⁵I]-α-BTX to membrane preparations** The expression of the a7 nicotinic receptors in KXα7R1 cells was analyzed by measuring the binding of 2 nmol/L [¹²⁵I]-α-BTX. Untransfected parental HEK-293 cells showed no specific binding of [¹²⁵I]-α-BTX (Figure 2); whereas the KXα7R1 cells showed high specific binding (about 75 000 DPM/mg protein), which represented >70% of the total binding. We also examined the binding of 2 nmol/L [¹²⁵I]-α-BTX to membranes from several established cell lines that express combinations of an α and a β subunits, including combinations of a2β2, a2β4, a3β2, a3β4,
There was no specific binding of [125I]-α-BTX in any of these cell lines (data not shown).

Comparison of binding properties of the α7 homomeric nAChRs in KXα7R1 and native α7* nAChRs in rat hippocampus and cerebral cortex

Specific binding of [125I]-α-BTX to membrane homogenates of the KXα7R1 cells was saturable and represented more than 70% of the total binding at concentrations up to about 2 nmol/L [125I]-α-BTX (Figure 3). The density of the α7 receptor binding sites in these cells is somewhat higher than in rat hippocampus or cerebral cortex (Figure 3). Though the nAChRs expressed in the cells have a slightly higher $K_v$ value (Table 1), the differences in the binding affinities among nAChRs from cells and those from the two native tissues are not significant ($P>0.05$ by one-factor analysis of variance and Tukey’s multiple comparison test). The binding site densities ($B_{max}$ values) of the cells are similar to hippocampus but higher than cortex.

We next assessed the binding affinities of nicotinic ligands, including agonists and antagonists, in binding competition assays. All 13 compounds examined competed effectively for binding sites labeled by [125I]-α-BTX (about 1 nmol/L) in the KXα7R1 cells and the two native rat brain tissues, but the affinities ($K_i$ values) among the ligands varied widely (Figure 4 and Table 2). The affinity of α-BTX is much higher than that of any other ligand examined in all three tissues. The other 12 ligands can be divided (somewhat arbitrarily) into three groups based on their binding affinity for the α7 nAChRs expressed in the transfected cells.

First, MLA, a relatively selective competitive antagonist at α7 receptors, and epibatidine, an agonist at all nAChRs examined, showed intermediate affinities. Although MLA has much lower affinity than α-BTX for α7 receptors, its affinities to these receptors in the three tissues ($K_i$ values=6–22 nmol/L) are much higher than to the binding sites at any of the 6 heteromeric nAChR subtypes previously tested, α2β2, α2β4, α3β2, α3β4, α4β2, α4β4, where $K_i$ values of MLA were >2 µmol/L. Conversely, the affinity of epibatidine for α7 receptors is about 50-times lower than those of any heteromeric receptors. Second, six compounds, (±)-anatoxin A, anabaseine, A-85380, GTS-21, (–)-nicotine and (–)-cytisine, showed intermediate affini-

Table 1. Comparison of saturation binding of [125I]-α-BTX in membrane homogenates from KXα7R1 cells, rat hippocampus and cerebral cortex. Assays in KXα7R1 cells, hippocampus and cerebral cortex were run in parallel. Values shown are mean±SEM of 3 to 5 independent experiments. See Figure 3 for description of data analyses and curve fittings.

| Tissue                  | $K_v$ value (nmol/L) | $B_{max}$ value (fmol/mg protein) |
|-------------------------|----------------------|-----------------------------------|
| KXα7R1 cells            | 0.38±0.06            | 60±17                             |
| Rat hippocampus         | 0.28±0.03            | 52±4                              |
| Rat cerebral cortex     | 0.33±0.05            | 30±3                              |

α4β2, and α4β4. There was no specific binding of [125I]-α-BTX in any of these cell lines (data not shown).
ties with \( K_i \) values ranging from 300 nmol/L to 15 \( \mu \)mol/L. Interestingly, (−)-cytisine and (−)-nicotine displayed similar affinities at the \( \alpha7 \) receptors in the KXα7R1 cells, but nicotine’s affinity was higher than cytisine’s at the \( \alpha7 \) receptors in the two native tissues. Lastly, acetylcholine, dihydro-β-erythroidine (DHβE), carbachol and choline, had the lowest affinities, with \( K_i \) values >10 \( \mu \)mol/L. The affinities of most of the 13 ligands at \( \alpha7 \) receptors in the two native tissues were reasonably similar (Table 2) to those in the cell line heterogeneously expressing \( \alpha7 \) nAChRs (Table 2). However, there were some notable exceptions. For example, MLA, epibatidine, A-85380 and nicotine appear to have 3–9 times lower affinities for the expressed receptors than for the native receptors. Acetylcholine, the endogenous agonist at \( \alpha7 \) receptors in native tissues, appeared to have higher affinity for the receptors in the cerebral cortex than in the hippocampus or the cell line.

**Channel properties of \( \alpha7 \) nAChRs expressed in the KXα7R1 cells**

The channel function of the \( \alpha7 \) receptors expressed in KXα7R1 cells was assessed using whole-cell current recording. As shown in Figure 5A, application of acetylcholine or nicotine elicited currents in a concentration-dependent manner. Though the potencies of the two agonists in activating channel functions were similar, nicotine (EC\(_{50}\)=180±40 \( \mu \)mol/L) was slightly more potent than acetylcholine (EC\(_{50}\)=280±19 \( \mu \)mol/L). However, the slope of the nicotine curve was shallower than that of acetylcholine, and its maximal current elicited was approximately 75% of...
the peak current elicited by acetylcholine at 1 mmol/L (Figure 5A), indicating that nicotine has an apparent lower efficacy than acetylcholine in activating channel function of the α7 nAChRs. Both of these differences might reflect a greater propensity for nicotine to block the channel at concentrations less than those necessary to fully activate the channel.

To further characterize the channel properties of the receptors, the inhibitory effects of two selective α7 nAChR antagonists, α-BTX and MLA, were examined when co-applied with 280 µmol/L acetylcholine (the EC_{50} concentration). Both α-BTX and MLA blocked peak amplitudes of acetylcholine-induced currents (Figure 5B). The potency of MLA (IC_{50}=100±10 pmol/L) was much higher than that of α-BTX (IC_{50}=19±2 nmol/L) in blocking channel function of α7 nAChRs. This much greater potency (nearly 200-fold) of MLA than α-BTX in blocking α7 receptor function is the reverse of what would have been predicted from the binding affinities of the two antagonists, where α-BTX binds with an affinity 270-times greater than MLA.

Discussion

We have established a stably transfected clonal cell line, KXα7R1, that expresses rat α7 nAChRs in HEK-293 cells. The heterologously expressed α7 nAChRs bind [^{125}I]-α-BTX with a K_{d} value of 0.38 nmol/L, which is similar to K_{d} values of α7* nAChRs in rat hippocampus and cerebral cortex, 0.28 nmol/L and 0.33 nmol/L, respectively (Table 1). The K_{d} value is also similar to the K_{d} value (0.7 nmol/L) of human α7 nAChRs heterologously expressed in HEK-293 cells[11]. We have completed a comprehensive ligand competitive binding study using 13 nicotinic ligands, including the endogenous ligand, acetylcholine, two typical α7* nAChR selective ligands, α-BTX and MLA, and 10 other natural or
synthetic nicotinic ligands. The rank order of binding affinities of the a7 nAChRs from KXα7R1 cells for these ligands is as the same as that of human a7 nAChRs heterologously expressed from HEK-293.[11]. To compare binding properties of the rat a7 nAChRs in KXα7R1 cells to those of heterologously expressed human a7 nAChRs in K28 cells[34] and in SH-EP1-pCEP4-ha7 cells[38], we did correlation analyses of Kᵢ values of 4 common ligands used for all three cell lines, MLA, (-)-nicotine, (-)-cytisine and carbachol. The values of coefficients of determination (r²) from all analyses were very high: 0.96 between KXα7R1 cells and SH-EP1-pCEP4-ha7 cells; 0.99 between KXα7R1 cells and K28 cells; and 0.96 between SH-EP1-pCEP4-ha7 cells and K28 cells. These analyses indicate that binding properties of heterologously expressed rat and human homomeric a7 nAChRs are similar whether expressed in HEK cells or SH-EP-1 cells.

The channel function of the a7 receptor subtype expressed in KXα7R1 cells was assessed using whole-cell current recording. Application of acetylcholine or (-)-nicotine activated a fast, inward-directed current typical of a7 receptors at the holding potential of -80 mV (Figure 5C). Though potencies of the two agonists in activating channel functions were similar (Figure 5A), nicotine (EC₅₀=180±40 µmol/L) was slightly more potent than acetylcholine (EC₅₀=280±19 µmol/L). This is consistent with results reported for human a7 nAChRs heterologously expressed in HEK-293 cells[11] or in SH-EP1 cells[38]. As expected, the acetylcholine activated current was abolished (Figure 5B) by the two selective a7 nAChR antagonists, α-BTX and MLA. Surprisingly, however, MLA (IC₅₀=100±10 pmol/L) was nearly 20-fold more potent than α-BTX (IC₅₀=19±2 nmol/L) in blocking a7 nAChR channel function. It is conceivable that MLA may not be a “pure competitive antagonist” as widely assumed.

We further compared the binding affinities of ligands at the heterogeneously expressed homomeric nAChRs to those of native a7* nAChRs in rat hippocampus and cerebral cortex to determine the degree of similarity between the binding site of the recombinant receptor and the native site. As shown in Figure 4 and Table 2, the ligand binding profile of a7 nAChRs in KXα7R1 cells are fairly similar to those in rat hippocampus and cortex. However, the comparison did show several clear differences in ligand binding profiles, especially in the rank orders of affinities. Although it is possible these differences are due to factors not directly related to the structure of the receptors, it is also possible these differences indicate that there are structural differences between the recombinant receptors and native receptors. We speculate that the molecular basis for these differences could be one of the following: i) different transcripts of a7 subunits; ii) different posttranslational modifications in a7 subunits, such as formation of disulfide bonds, folding of protein, proteolytic cleavages, glycosylation and/or phosphorylation; iii) alterations of associated proteins; or iv) different subunit composition and subtype abundance. Among these possibilities, the most intriguing is that some native a7 receptors may be heteromeric receptors containing a7 subunits in combination with other neuronal nAChR subunits.

Since successful clonings of neuronal nAChR subunit genes (α2−α8, β2−β4) more than 20 years ago, many studies have demonstrated that functional nAChRs could be formed by heterologous expressions of a7 subunit alone[9–11,35,39] and that there are homomeric a7 nAChRs expressed in neuronal systems[40–43]. However, over the years, some experimental observations supported the notion that there may be heteromeric receptors containing a7 subunits in combination with other neuronal nAChR subunits[44–47]. A careful examination of subunit compositions of a7 receptors in bovine adrenal chromaffin cells supported the possibility of a small population of heteromeric a7 receptors expressed in chromaffin cells[48]. More recently, Liu et al reported the presence of heteromeric a7β2 receptors in rodent basal forebrain cholinergic neurons[49]; moreover, these receptor are more sensitive than are homomeric receptors to functional blockade by oligomeric Aβ₁−₄₂, which would implicate them in Alzheimer’s disease. It will be important to determine if there are heteromeric a7* receptors in rat hippocampus and cerebral cortex; and if so, how these heteromeric a7 receptors contribute to heteromeric α7* receptors in rat hippocampus and cerebral cortex;
the differences in binding profiles between homomeric α7 nAChRs in KXα7R1 cells and native α7* nAChRs.

In conclusion, we have established a stably transfected clonal cell line, KXα7R1, which expresses rat α7 nAChRs in HEK-293 cells. The α7 nAChRs bind to [125I]-α-BTX with sub nanomolar affinity. The receptors are activated by acetylcholine or (−)-nicotine, and the agonist activated currents are blocked by the two selective α7 nAChR antagonists, α-BTX and MLA. A comprehensive comparative study of ligand binding profiles of the α7 nAChRs expressed in this cell line and native α7* nAChRs expressed in rat hippocampus and cerebral cortex shows many similarities between the recombinant α7 nAChRs and native α7 receptors in rat brain. However, the comparison also shows several clear differences in ligand binding profiles, which suggest the possibility that there are structural differences between the homomeric α7 nAChRs expressed in this cell line and those native α7 receptors in rat hippocampus and cerebral cortex.

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Author contribution

Yingxian XIAO designed research; Yingxian XIAO, Galya R ABDRAKHMANOVA, Maryna BAYDYUK and Susan HERNANDEZ performed research; Yingxian XIAO, Galya R ABDRAKHMANOVA and Kenneth J KELLAR analyzed data; Yingxian XIAO, Galya R ABDRAKHMANOVA and Kenneth J KELLAR wrote the paper.

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