

Homomeric and native $\alpha 7$ acetylcholine receptors exhibit remarkably similar but non-identical pharmacological properties, suggesting that the native receptor is a heteromeric protein complex

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Sucrose gradient analysis of chick acetylcholine receptor (AChR) $\alpha 7$ subunits expressed in oocytes indicates that they form pharmacologically active homomers of the same size as native $\alpha 7$ AChRs, a size compatible with a complex of five $\alpha 7$ subunits. By immunisolating the [35 S]methionine-labeled $\alpha 7$ subunits we also demonstrate that they do not appear to assemble with endogenous *Xenopus* AChR subunits. Pharmacological characterization of detergent-solubilized brain $\alpha 7$ AChRs and $\alpha 7$ homomers reveals that they have similar but nonidentical properties. The pharmacological difference is most accentuated for cytosine (~50-fold). Thus, at least in F18 chicken brain, most or all of the native $\alpha 7$ AChRs do not appear to be homomeric.

Acetylcholine receptor, α -Bungarotoxin, Pharmacology; *Xenopus* oocyte; Homomeric receptor; $\alpha 7$ subunit

1. INTRODUCTION

α -Bungarotoxin (α Bgt) is a potent antagonist of the well characterized muscle-type acetylcholine receptors (AChRs). However, α Bgt also antagonizes a subset of neuronal AChRs, of which some subunits ($\alpha 7$ and $\alpha 8$) have been cloned [1,2]. The $\alpha 7$ [2] and $\alpha 8$ subunits (Gerzanich et al., in preparation) form functional homomers when expressed in oocytes. The electrophysiological properties of the homomers formed by expressing normal and mutated $\alpha 7$ subunits in oocytes have been the target of many recent investigations [3–7].

The subunit composition and function of the native neuronal AChRs which bind α Bgt are just starting to be dissected. Despite the long standing knowledge that neuronal α Bgt-sensitive proteins display nicotinic character (reviewed in [8,9]), a more detailed characterization of these AChRs was made possible using $\alpha 7$ and $\alpha 8$ subunit-specific mAbs. Using these mAbs it has been shown that neuronal AChRs which bind α Bgt are composed of at least three subtypes i.e. those containing $\alpha 7$ subunits ($\alpha 7$ AChRs), $\alpha 8$ subunits ($\alpha 8$ AChRs), and

both $\alpha 7$ and $\alpha 8$ subunits ($\alpha 7/\alpha 8$ AChRs) [1,10–12]. More recently, the contrasting pharmacological properties of the $\alpha 7$ AChRs and $\alpha 8$ AChRs immunisolated from retina have been characterized (Anand et al., manuscript submitted). At the protein level, while the results of numerous attempts to affinity purify these AChRs all show multiple bands on SDS-acrylamide gel electrophoresis, indicating that they are likely to be made up of more than one subunit [13–17], there has not been an unambiguous demonstration of the total number of subunits or the stoichiometry in which these subunits associate to form the various native neuronal AChR subtypes which bind α Bgt. At the functional level, preliminary characterization of nicotine-induced, α Bgt-sensitive currents (presumably of $\alpha 7$ AChRs) from cultured rat hippocampal neurons have been reported [18–20].

In this paper, we characterize both native brain $\alpha 7$ AChRs and $\alpha 7$ homomers expressed in *Xenopus* oocytes. Although exogenous AChR subunits translated in oocytes have been reported to assemble with endogenous *Xenopus* AChR subunits [21], we demonstrate that no additional proteins recognizable as additional bands on SDS-acrylamide gel electrophoresis are associated with $\alpha 7$ subunits expressed in *Xenopus* oocytes. We also show that the size of the $\alpha 7$ homomers is compatible with a complex of five $\alpha 7$ subunits. Furthermore, we characterize in detail the pharmacological properties of both the $\alpha 7$ homomers and the immunisolated native $\alpha 7$ AChR subtype from chick brain. Comparison of their pharmacological properties reveals that they exhibit remarkable similarities in their affini-

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Abbreviations: α Bgt, α -bungarotoxin; ACh, acetylcholine; AChR, acetylcholine receptor; Carb, carbamylcholine; Deca, decamethonium; EDTA, [ethylenedinitrilo]tetraacetic acid; EGTA, [ethylene-bis(oxyethylenenitrilo)]tetraacetic acid; Hex, hexamethonium; mAb, monoclonal antibody; PBS, phosphate-buffered saline; RIA, radioimmunoassay; TEA, tetraethylammonium; TMA, tetramethylammonium

ties for many ligands. However, differences in affinities for some ligands indicate that the native $\alpha 7$ AChR is most likely to be composed of more than one kind of subunit.

2. MATERIALS AND METHODS

2.1. mAbs

mAbs to $\alpha 7$ (318) and to $\alpha 8$ (305) have been previously described [1]. The epitope for mAb 318 was mapped using synthetic peptides to within $\alpha 7$ 380–400 [22], thus all $\alpha 7$ AChR and $\alpha 7$ homomer ligand binding studies reported here were done with subunits tethered through their putative large cytoplasmic domain, an interaction which is unlikely to alter properties of the ACh binding site on their extracellular surface. mAb 305 binding was found to depend on the native conformation of $\alpha 8$ [22]. The mAbs were affinity purified using protein G agarose.

2.2. Expression of $\alpha 7$ homomers in oocytes

The $\alpha 7$ cDNA was cloned into a modified SP64T expression vector [23] using standard DNA cloning procedures. In vitro RNA was synthesized using a standard protocol [23] and more recently using the Megascript kit (Ambion, Austin, TX). Oocytes were prepared for injections as described in [24] and injected with ~ 15 ng of cRNA per oocyte. The oocytes were incubated in semi-sterile conditions at 18°C in saline solution (96 mM NaCl, 2 mM KCl, 1 mM MgCl_2 , 1.8 mM CaCl_2 , 5 mM HEPES, pH 7.6) containing 5% heat-inactivated horse serum at 18°C for 3–4 days before use. Metabolic labeling of expressed $\alpha 7$ subunits was achieved by incubating injected oocytes in saline solution containing 0.5 mCi/ml of [^{35}S]methionine ($\sim 1,000$ Ci/mmol, Amersham) for 3–4 days.

2.3. Purification and solubilization of AChRs from oocytes and chicken brain

Oocytes expressing homomeric $\alpha 7$ AChRs were homogenized in lysis buffer (2% Triton X-100, 50 mM NaCl, 50 mM sodium phosphate (pH 7.5), 5 mM EDTA, 5 mM EGTA, 2 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine, 5 mM iodoacetamide, 1 mg/ml heat-denatured BSA) incubated at 4°C for 30 min, and centrifuged for 10 min in a microfuge to clear the cellular debris. The cleared lysate was then used for all the assays. The preparation of Triton X-100-solubilized native neuronal AChRs which bind αBgt and the method of immunoisolation of the native $\alpha 7$ AChR subtype from these extracts have previously been described [12]. Briefly, Triton X-100 extracts from brain were depleted of the $\alpha 7/\alpha 8$ and $\alpha 8$ AChR subtypes by an overnight incubation of ~ 7 ml of brain extract with ~ 1 ml of mAb 305-coupled Actigel beads (Sterogene, 5 mg/ml gel). The beads were then removed by brief centrifugation. The extent of depletion was then tested by solid-phase RIAs using microwells coated with mAb 305 in the presence of 50 nM [^{125}I] αBgt . All extracts used in RIAs were found to be depleted of $> 99\%$ of all $\alpha 8$ -containing subtypes.

2.4. Sucrose gradient sedimentation analysis

Aliquots (~ 500 μl) of extracts from either chick brain or ~ 10 oocytes were layered on to 11 ml sucrose gradients (5–20%) as previously described [25], and the sedimentation of AChR analyzed by [^{125}I] αBgt binding (at 50 nM) to the protein complexes immunoisolated on Immulon 4 microwells coated with mAb 318. Immunoisolated [^{35}S]methionine-labeled expressed $\alpha 7$ subunits from all the sucrose gradient fractions were eluted off in sample buffer and electrophoresed on 10% SDS-polyacrylamide gels. The gels were then treated for fluorography, dried and exposed to X-ray film at -70°C for 1–3 days. The gels were aligned with the X-ray films, the gel slices containing the $\alpha 7$ subunit excised, and the amount of radioactivity in the gel slice determined by liquid scintillation counting.

2.5. Pharmacological assays

Pharmacological characterization of the expressed and immunoiso-

lated brain $\alpha 7$ AChRs was performed by competitive inhibition of [^{125}I] αBgt binding by various ligands to AChRs immunoisolated on mAb 318-coated Immulon 4 microwells. Triton X-100 extracts were added to each well in the presence of various concentration of the ligands and incubated for 20 min prior to the addition of [^{125}I] αBgt . The assays in duplicate were performed in the presence of 2 nM [^{125}I] αBgt in a total volume of 100 μl . After incubation overnight at 4°C , the wells were rinsed three times with ~ 200 μl of PBS/Tween 20 buffer and then counted in a γ counter. The affinity of αBgt for the AChRs was also measured by similar solid-phase RIAs except that increasing amounts of [^{125}I] αBgt were used. Non-specific binding in all cases was determined using wells not coated with mAbs.

3. RESULTS

3.1. Immunological evidence that expressed $\alpha 7$ subunits form homomers

Using sucrose gradient sedimentation analysis of $\alpha 7$ subunits expressed in oocytes, we show that [^{125}I] αBgt binding activity of both expressed $\alpha 7$ subunits and native brain $\alpha 7$ AChRs cosediment at ~ 10 S (Fig. 1). This

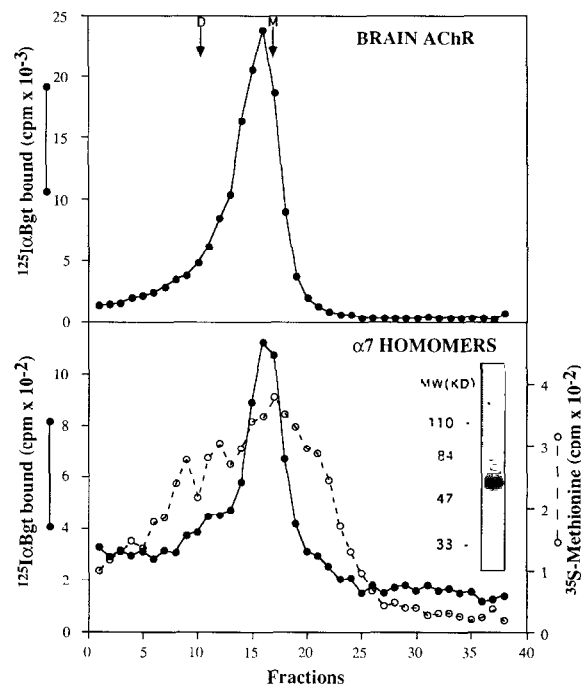


Fig. 1. Co-sedimentation of detergent-solubilized chick brain $\alpha 7$ AChRs and [^{35}S]methionine-labeled $\alpha 7$ homomers on sucrose gradients. Sedimentation profiles of AChRs solubilized with Triton X-100 on 5–20% sucrose gradients were determined by [^{125}I] αBgt binding to AChRs and $\alpha 7$ homomers immunoisolated on microwells coated with a mAb to $\alpha 7$. The top panel shows the sedimentation profile of chick brain αBgt AChRs. The arrows indicate the position of *Torpedo* AChR monomers (M) and dimers (D). The bottom panel shows the sedimentation profile of $\alpha 7$ subunits expressed in oocytes. Binding to the immunoisolated protein was measured using 50 nM [^{125}I] αBgt . The [^{35}S]methionine-labeled protein immunoisolated on the wells from the oocyte extracts was then eluted off in sample buffer and subjected to SDS-PAGE followed by fluorography. The expressed $\alpha 7$ subunit bands thus identified were excised and the amount of [^{35}S]methionine per fraction determined by liquid scintillation counting. The inset panel shows a fluorogram of the [^{35}S]methionine-labeled protein in the lane corresponding to the peak [^{125}I] αBgt binding fraction.

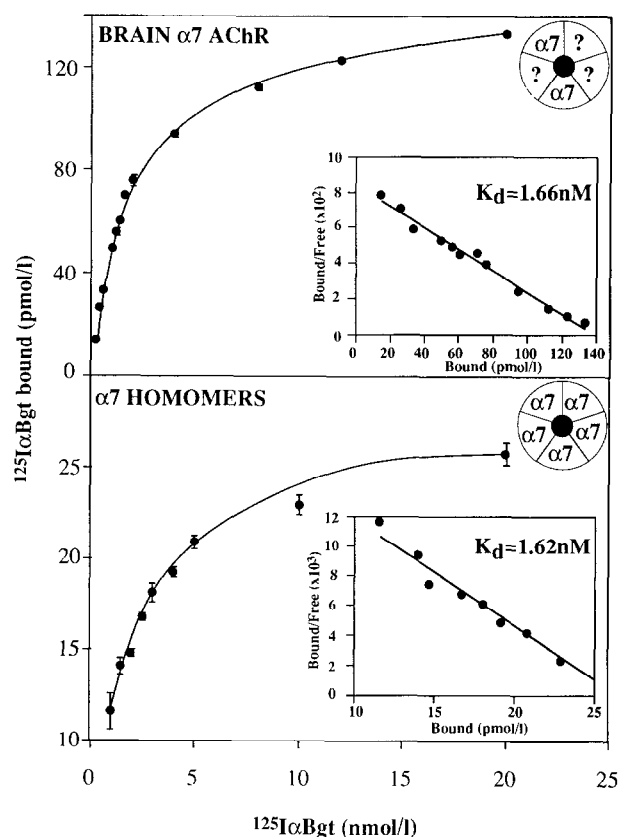


Fig. 2. Binding of [125 I] α Bgt to detergent-solubilized brain $\alpha 7$ AChRs and $\alpha 7$ homomers. Binding of [125 I] α Bgt to Triton X-100 solubilized AChRs was performed on AChRs immunoimmobilized on Immulon 4 microwells coated with a mAb to $\alpha 7$. Non-specific binding was determined using wells not coated with the mAb. Each data point is the mean of the values obtained from duplicate determinations. The insert shows Scatchard analysis of the data, displayed as bound/free (pmol/pmol) vs. bound (pmol/l). The symbols at the right top corner of each plot shows the putative subunit composition of the $\alpha 7$ AChRs, assuming that the AChRs are pentameric and that the native $\alpha 7$ AChRs have only two $\alpha 7$ ligand-binding subunits per AChR, as do $(\alpha 1)_2\beta\gamma\delta$ and $(\alpha 4)_2(\beta 2)_3$ AChRs [25,26].

indicates that native $\alpha 7$ AChRs and $\alpha 7$ homomers have the same basic size and shapes. Native $\alpha 7$ AChRs and functional $\alpha 7$ homomers might be expected to exhibit the pentagonal symmetry of other members of the AChR gene family such as muscle $\alpha_2\beta\gamma\delta$ AChR or neuronal $(\alpha 4)_2(\beta 2)_3$ AChR [25,26]. The calculated protein molecular weight of the *Torpedo* $\alpha_2\beta\gamma\delta$ AChR monomer is 267,757 Da. In comparison, the calculated protein molecular weight of the chicken $\alpha 7$ pentamer is 272,750 Da. Thus $\alpha 7$ pentamers would sediment somewhat faster than the *Torpedo* $\alpha_2\beta\gamma\delta$ AChR monomer, as is observed.

Remarkably, metabolic labeling of the expressed $\alpha 7$ protein with [35 S]methionine indicates that these subunits form a broad array of protein complexes of which only the ones assembled into homomers the size of native AChRs are capable of binding [125 I] α Bgt. Immunoisolation of the ~ 10 S [35 S]methionine-labeled protein

complex using a mAb to $\alpha 7$, followed by fractionation of this complex by SDS-PAGE reveals that it is made up of only one band of apparent molecular weight of ~ 60 kDa (shown in the inset panel of Fig. 1). It has been reported that muscle AChR subunits expressed in oocytes from cRNA could form small amounts of functional AChRs by assembling with AChR subunits endogenously expressed in *Xenopus* oocytes [21], but unless the oocytes contribute an endogenous subunit of the same apparent molecular weight as $\alpha 7$, these data argue that $\alpha 7$ subunits expressed in oocytes do not appear to assemble with other endogenously expressed subunits of the *Xenopus* AChR, but instead form homomers the size of native AChRs.

3.2. Immunoisolation of native $\alpha 7$ AChRs from detergent-solubilized chick brain extracts

We have previously shown that in E18 chick brain $\sim 68\%$ of all α Bgt-sensitive AChRs are of the $\alpha 7$ AChR subtype, $\sim 9\%$ are of the $\alpha 8$ AChR subtype, and $\sim 23\%$ are of the mixed $\alpha 7/\alpha 8$ AChR subtype [12]. To obtain extracts containing only $\alpha 7$ AChRs, we depleted Triton X-100 extracts of brain with a mAb to $\alpha 8$ coupled to agarose beads using an extract to mAb-bead ratio of 7:1 (v/v). The efficacy of depletion was then tested by solid phase RIAs using mAb microwells coated with a mAb to $\alpha 8$. Thus, we typically achieved $> 99\%$ depletion of all $\alpha 8$ -containing AChRs (including the $\alpha 8$ AChRs and the $\alpha 7/\alpha 8$ AChRs). These depleted extracts were then used for all pharmacological assays.

3.3. Scatchard analysis of [125 I] α Bgt binding to immunoisolated brain $\alpha 7$ AChRs and $\alpha 7$ homomers

Saturation binding curves shown in Fig. 2 were achieved by incubating increasing amounts [125 I] α Bgt with native $\alpha 7$ AChRs and $\alpha 7$ homomers immunoimmobilized on microwells. Scatchard analysis of these binding curves reveals that $\alpha 7$ homomers bind [125 I] α Bgt with a high affinity ($K_d = 1.62 \pm 0.08$ nM) that is nearly identical to that of the native $\alpha 7$ AChR subtype from chick brain ($K_d = 1.66 \pm 0.04$ nM).

3.4. Pharmacological characterization of native $\alpha 7$ AChRs and $\alpha 7$ homomers

Further pharmacological characterization to test the relative efficacies by which various cholinergic ligands and some atypical ligands, such as strychnine and atropine, inhibit [125 I] α Bgt binding to these AChRs was carried out using solid-phase RIAs. Fig. 3 shows the [125 I] α Bgt inhibition curves for a subset of all the ligands tested. The calculated K_i 's for all the ligands tested are shown in Table I. Thus the relative affinities of the various ligands for the $\alpha 7$ homomers was found to be α Bgt \gg cytosine \gg nicotine \gg curare \approx strychnine $>$ trimethaphan $>$ ACh $>$ TMA \approx atropine $>$ Carb $>$ Deca $>$ TEA $>$ Hexa. The affinities of the ligands for the native brain $\alpha 7$ AChRs, however, were found to be

α Bgt \gg nicotine \gg curare \approx cytisine \approx strychnine \gg trimethaphan \gg ACh \gg atropine \approx TMA \gg Deca \gg Carb \gg Hexa \gg TEA. While many ligands showed smaller differences in affinity for the $\alpha 7$ homomers compared to the native $\alpha 7$ AChR, cytisine appeared to be the most discriminatory, showing nearly a 50-fold difference in affinity (Fig. 4).

Interestingly, both the native $\alpha 7$ AChR and $\alpha 7$ homomers appeared to be sensitive to strychnine, a glycinergic antagonist, and to atropine, a muscarinic antagonist. It does not appear that strychnine or atropine have remarkably high affinity, but that several classic cholinergic ligands (e.g. curare or Carb) do not have remarkably greater affinity, or even lower affinity (ACh). Even the simplest of possible ligands, TMA, has substantial affinity as compared to curare, carbamylcholine, atropine, and strychnine. It is interesting to note that strychnine has been reported to inhibit the function of rat $\alpha 7$ homomers [7] and of α Bgt-binding AChRs on chick cochlear hair cells [27].

4. DISCUSSION

In this study we first demonstrate that $\alpha 7$ subunits expressed in *Xenopus* oocytes form α Bgt-binding $\alpha 7$ homomers that co-sediment with native brain $\alpha 7$ AChRs on sucrose gradients. The size of the homomeric AChRs is compatible with a pentamer of $\alpha 7$ subunits. Using [35 S]methionine to metabolically label the $\alpha 7$ subunits, we also show that the $\alpha 7$ subunits do not appear to assemble with endogenously expressed *Xenopus* AChR subunits. The observation that binding of [125 I] α Bgt only occurs when $\alpha 7$ subunits assemble in complexes of the size of native $\alpha 7$ AChRs suggests that the ability to bind both α Bgt and small cholinergic ligands is acquired only after assembly of the $\alpha 7$ subunit into complexes of native stoichiometry, perhaps because the binding sites are formed by distinct parts of adjacent subunits. This contrasts with the case of muscle $\alpha 1$ subunits which can bind α Bgt but acquire affinity for small cholinergic ligands only after pairing up with either the γ or δ subunits [28,29].

The native neuronal AChRs which bind α Bgt have evaded detailed characterization since they were first detected. Here, using $\alpha 7$ and $\alpha 8$ subunit-specific mAbs, we isolated the detergent-solubilized $\alpha 7$ AChR subtype from chick brain extracts. Then by characterizing the pharmacological properties of mAb-tethered, detergent-solubilized $\alpha 7$ homomers and brain $\alpha 7$ AChRs under the same assay conditions, we were able to make a meaningful comparison of their pharmacological properties for the first time.

A striking feature of this comparison is that the pharmacological properties of the $\alpha 7$ homomers are very similar to those of native $\alpha 7$ AChRs. However, the $\alpha 7$ homomers show a ~ 50 -fold higher affinity for binding cytisine than do the brain $\alpha 7$ AChRs. This feature, in

addition to the smaller differences observed with some of the other ligands, suggests that most if not all of the native $\alpha 7$ AChRs expressed in E18 chick brain do not exist as homomers, although it is conceivable that these $\alpha 7$ subunits might assemble into homomers at other times during development. While we cannot incontrovertibly exclude the possibility that some of the pharmacological differences observed are due to post-translational modifications of the $\alpha 7$ AChR expressed in oocytes, this appears unlikely to be the case because at least for *Torpedo* AChRs expressed in oocytes, despite altered patterns of N-linked glycosylation of the subunits [30], these AChRs were pharmacologically and functionally indistinguishable from the native *Torpedo* AChRs [31,32]. All published accounts of purified vertebrate brain α Bgt-binding AChRs report more than one band on SDS acrylamide gel electrophoresis [13–17], although in no case has it been proven that these components are additional AChR subunits. The pharmacological data shown here strongly support the notion that additional subunits co-assemble with the $\alpha 7$ subunits in the native AChR. The concentrations at which cytisine activates has previously been shown to be very sensitive to the type of structural subunits present. For example, co-expression of $\alpha 3$ and $\beta 2$ subunits result in a ~ 100 -fold greater efficacy for cytisine as compared to co-expression of $\alpha 3$ and $\beta 4$ subunits [33]. Thus while structural subunits do participate in the binding of, or indirectly influence the binding of, $\alpha 7$ subunits to at least some of the ligands, they appear to have a limited influence on the binding of many of the ligands by the native $\alpha 7$ AChR.

The neuronal $\alpha 7$ AChRs (the predominant brain α Bgt-sensitive AChR subtype) exhibit several different pharmacological properties from the neuronal $\alpha 4\beta 2$ AChRs (the predominant brain α Bgt-insensitive sub-

Table I
Pharmacological characterization of $\alpha 7$ AChRs

Ligand	Affinity (K_i , μ M)	
	Homomeric	Brain
Acetylcholine	24.9 ± 4.7	103 ± 8.7
Atropine	148 ± 52	198 ± 10
α -Bungarotoxin	0.00162 ± 0.00008	0.00166 ± 0.00004
Carbamylcholine	250 ± 11	580 ± 205
Curare	4.97 ± 0.5	3.40 ± 0.2
Cytisine	0.0775 ± 0.01	3.85 ± 0.6
Decamethonium	376 ± 36	124 ± 15
Hexamethonium	891 ± 174	800 ± 50
L-Nicotine	0.545 ± 0.02	1.44 ± 0.24
Strychnine	6.85 ± 0.44	5.37 ± 0.51
Tetraethylammonium	521 ± 64	1046 ± 10
Tetramethylammonium	99.9 ± 1.9	263 ± 22
Trimethaphan	18.0 ± 3.0	20.9 ± 1.8

type). We find that $\alpha 7$ AChRs show less than 10-fold lower affinity for antagonists like curare and hexamethonium, but 600- to 27,000-fold lower affinity for agonists such as nicotine, carbamylcholine, or cytosine, when compared to the affinities of the $\alpha 4\beta 2$ AChRs [34]. Another interesting feature of $\alpha 7$ homomers is that they show only a small difference in apparent affinity for the agonist, nicotine, between their activatable state ($EC_{50} \sim 7.8 \mu M$, Gerzanich et al., manuscript in preparation) and their presumably desensitized state ($K_d \sim 0.5 \mu M$). This contrasts with $\alpha 4\beta 2$ AChRs, which exhibit a large difference in their apparent affinity for nicotine between the two states with an EC_{50} of $\sim 1 \mu M$, for the activatable state, and a K_d of $\sim 3.9 nM$ [34], for the presumably desensitized state. At the functional level, it is interesting and perhaps toxicologically important to note the equipotency of nicotine and ACh (~ 1

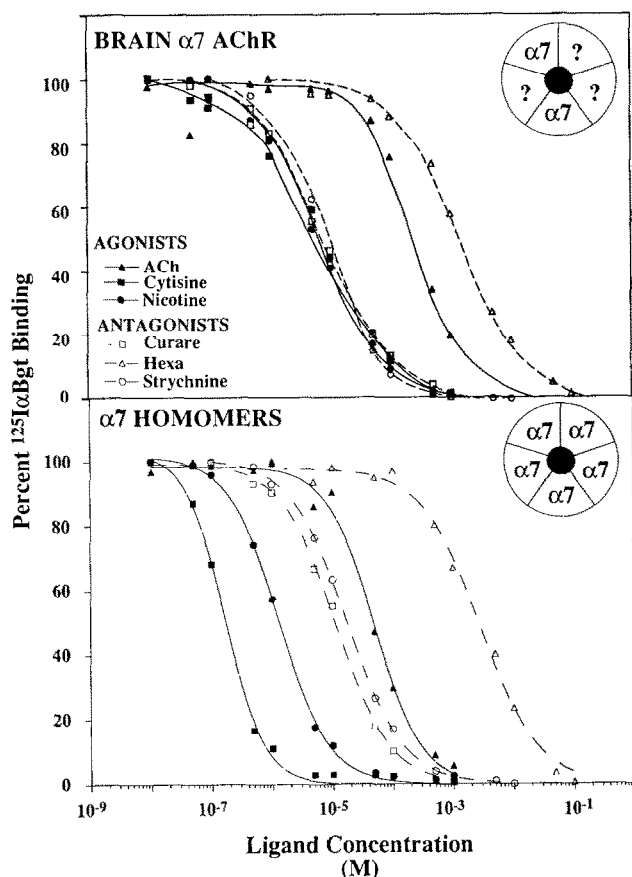


Fig. 3. Pharmacological characterization of detergent-solubilized brain $\alpha 7$ AChRs and $\alpha 7$ homomers. Pharmacological characterization was performed by competitive inhibition of [^{125}I] α Bgt binding by various ligands to AChRs solubilized with Triton X-100 and then immunomobilized on mAb 318-coated Immulon 4 microwells. Competitive inhibition was performed in the presence of 2 nM [^{125}I] α Bgt. Representative dose-response curves from one experiment are shown. Each data point is the mean of the values obtained from duplicate determinations.

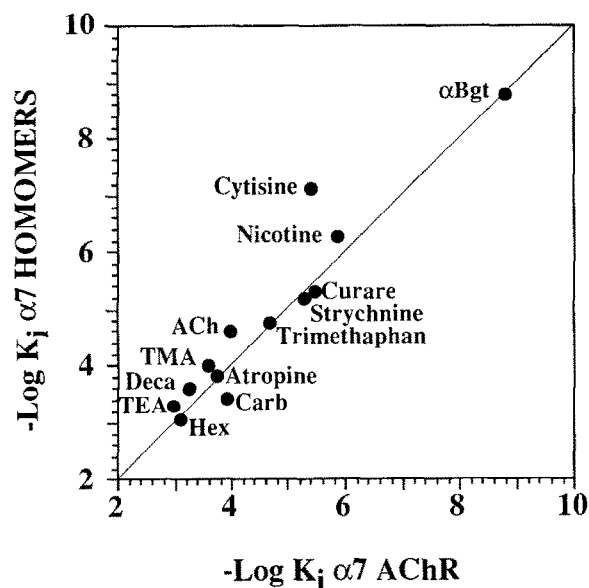


Fig. 4. Correlation of affinities for ligands between the native brain $\alpha 7$ AChRs and $\alpha 7$ homomers. A comparison of the affinities of native brain $\alpha 7$ AChRs for various ligands to that of the $\alpha 7$ homomers expressed in oocytes is shown as a log-log plot. The $-\log K_1$ (for the various ligands) of the brain $\alpha 7$ AChRs is plotted on the x-axis and the $\alpha 7$ homomers is plotted on the y-axis.

μM) for $\alpha 4\beta 2$ AChRs expressed in oocytes [35,36] compared to the 14:1 potency ratio of nicotine vs. ACh seen for $\alpha 7$ homomers ([2]; Gerzanich et al., manuscript in preparation), and the even greater (72-fold) selectivity of native $\alpha 7$ AChRs for nicotine vs. ACh (Table I).

Cloning of AChR subunit cDNAs has expanded the repertoire of AChR subtypes that might be expressed in the central and peripheral nervous system. However, thus far biochemical techniques have not uniquely identified structural subunits in $\alpha 7$ AChRs and no candidate cDNAs have been identified for structural subunits for α Bgt-binding AChRs. In this study, by comparing and contrasting the properties of the native $\alpha 7$ AChRs and $\alpha 7$ homomers, we have provided pharmacological tools which will help authenticate the cloning of structural subunit cDNAs which should then allow the reconstitution and study of native $\alpha 7$ AChRs in expression systems devoid of other AChRs.

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REFERENCES

- [1] Schoepfer, R., Conroy, W.G., Whiting, P., Gore, M. and Lindstrom, J. (1990) *Neuron* 5, 35–48.
- [2] Couturier, S., Bertrand, D., Matter, J.-M., Hernandez, M.-C., Bertrand, S., Millar, N., Valera, S., Barkas, T., and Ballivet, M. (1990) *Neuron* 5, 847–856.
- [3] Revah, F., Bertrand, D., Galzi, J.-L., Devillers-Thiery, A., Mulle, C., Hussy, N., Bertrand, S., Ballivet, M. and Changeux, J.-P. (1991) *Nature* 353, 846–849.
- [4] Galzi, J.-L., Bertrand, D., Devillers-Thiery, A., Revah, F., Bertrand, S. and Changeux, J.-P. (1991) *FEBS Lett.* 294, 198–202.
- [5] Bertrand, D., Devillers-Thiery, A., Revah, F., Galzi, J.-L., Hussy, N., Mulle, C., Bertrand, S., Ballivet, M. and Changeux, J.-P. (1992) *Proc. Natl. Acad. Sci. USA* 89, 1261–1265.
- [6] Bertrand, D., Bertrand, S. and Ballivet, M. (1992) *Neuro. Sci. Lett.* 146, 87–90.
- [7] Sèguèla, P., Wadiche, J., Dineley-Miller, K., Dani, J.A. and Patrick, J.W. (1993) *J. Neurosci.* 13, 596–604.
- [8] Schmidt, J. (1988) *Int. Rev. Neurobiol.* 30, 1–38.
- [9] Clarke, P.B.S. (1992) *Trends Pharmacol. Sci.* 13, 407–413.
- [10] Britto, L.R.G., Keyser, K.T., Lindstrom, J.M. and Karten, H.J. (1992) *J. Comp. Neurol.* 317, 325–340.
- [11] Britto, L.R.G., Hamassaki-Britto, D.E., Ferro, E.S., Keyser, K.T., Karten, H.J. and Lindstrom, J.M. (1992) *Brain Res.* 590, 193–200.
- [12] Keyser, K.T., Britto, L.R.G., Schoepfer, R., Whiting, P., Cooper, J., Conroy, W., Brozowska-Precht, A., Karten, J.H. and Lindstrom, J. (1992) *J. Neurosci.* 13, 442–454.
- [13] Whiting, P. and Lindstrom, J. (1987) *Proc. Natl. Acad. Sci. USA* 84, 595–599.
- [14] Conti-Tronconi, B.M., Dunn, S.M.J., Barnard, E.A., Dolly, J.O., Lai, F.A., Ray, N. and Raftery, M.A. (1985) *Proc. Natl. Acad. Sci. USA* 82, 5208–5212.
- [15] Hermans-Borgmeyer, I., Sawruk, E., Schloss, P., Gundelfinger, E.D. and Betz, H. (1988) in: *Nicotinic Acetylcholine Receptors in the Nervous System* (F. Clementi, C. Gotti and E. Sher, eds.) pp. 77–88. Springer-Verlag, Berlin.
- [16] Gotti, C., Ogando, A.E., Hanke, W., Schlue, R., Moretti, M. and Clementi, F. (1991) *Proc. Natl. Acad. Sci. USA* 88, 3258–3262.
- [17] Gotti, C., Hanke, W., Schlue, W.-R., Briscini, L., Moretti, M. and Clementi, F. (1992) *Neuroscience* 50, 117–127.
- [18] Alkondon, M. and Albuquerque, E.X. (1991) *J. Receptor Res.* 11, 1001–1022.
- [19] Alkondon, M. and Albuquerque, E.X. (1993) *J. Pharmacol. Exp. Ther.* (in press).
- [20] Zorumski, C.F., Thio, L.L., Isenberg, K.E. and Clifford, D.B. (1992) *Mol. Pharmacol.* 41, 931–936.
- [21] Buller, A.L. and White, M.M. (1990) *Mol. Pharmacol.* 37, 423–428.
- [22] McLane, K.E., Wu, X., Lindstrom, J.M. and Conti-Tronconi, B.M. (1992) *Neuroimmunology* 38, 115–128.
- [23] Melton, D., Krieg, P., Rebagliati, M., Maniatis, T., Zinn, K. and Green, M. (1984) *Nucleic Acids Res.* 12, 7035–7056.
- [24] Colman, A. (1984) in: *Transcription and Translation: A Practical Approach* (Hames, B.D. and Higgins, S.J., eds.) pp. 271–302. IRL press, Oxford.
- [25] Anand, R., Conroy, W.G., Schoepfer, R., Whiting, P. and Lindstrom, J. (1991) *J. Biol. Chem.* 266, 11192–11198.
- [26] Cooper, E., Couturier, S. and Ballivet, M. (1991) *Nature* 350, 235–238.
- [27] Fuchs, P. and Murrow, B. (1992) *Proc. R. Soc. London B* 248, 35–40.
- [28] Blount, P. and Merlie, J.P. (1988) *J. Biol. Chem.* 263, 1072–1080.
- [29] Saedi, M.S., Conroy, W.G. and Lindstrom, J. (1991) *J. Cell Biol.* 112, 1007–1016.
- [30] Buller, A.L. and White, M.M. (1990) *J. Membrane Biol.* 115, 179–189.
- [31] Mishina, M., Tobimatsu, T., Imoto, K., Tanaka, K., Fujita, Y., Fukuda, K., Kurasaki, M., Takahashi, H., Morimoto, Y., Hirose, T., Inayama, S., Takahashi, T., Kuno, M. and Numa, S. (1985) *Nature* 313, 364–369.
- [32] Sakmann, B., Methfessel, C., Mishina, M., Takahashi, T., Takai, T., Kurasaki, M., Fukada, K. and Numa, S. (1985) *Nature* 318, 538–543.
- [33] Luetje, C.W. and Patrick, J. (1991) *J. Neurosci.* 11, 837–845.
- [34] Whiting, P., Schoepfer, R., Lindstrom, J. and Priestley, T. (1991) *Mol. Pharmacol.* 40, 463–472.
- [35] Bertrand, D., Ballivet, M. and Rungger, D. (1990) *Proc. Natl. Acad. Sci. USA* 87, 1933–1997.
- [36] Charnet, P., Labarca, C., Cohen, B.N., Davidson, N. and Lester, H.A. (1992) *J. Physiol.* 450, 375–394.