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# Purification and characterization of a nicotinic acetylcholine receptor from rat brain

**(monoclonal antibodies/affinity chromatography/subunit structure/receptor evolution)**

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ABSTRACT **We previously reported the immunoaffinity purification of an acetylcholine receptor from chicken brain that did not bind α-bungarotoxin but did bind nicotine and other cholinergic agonists. Antisera and monoclonal antibodies raised against this receptor crossreacted with a receptor from rat brain that had similar pharmacological properties, and also bound to functional acetylcholine receptors in chicken ciliary ganglion cells and rat PC12 cells. Here we report purification of the receptor from rat brain using monoclonal antibody (mAb) 270 raised against receptor from chicken brain. This receptor, similar in size to monomers of receptor from *Torpedo* electric organ, contained two subunits—apparent $M_r$, 51,000 and 79,000. The $M_r$ 51,000 subunit was bound by antisera to α subunits of receptor from *Torpedo* electric organ and by mAb 270, which is specific for the $M_r$ 49,000 subunit analogue of receptor from chicken brain. Both subunits were bound by mAb 286, which also binds both subunits of receptors from chicken brain. The α-bungarotoxin binding component was purified from the same extracts. It consisted of four subunits of apparent $M_r$ 44,700, 52,300, 56,600, and 65,200. The basic structure of receptors from muscle had evolved to an $(\alpha)_2\beta\gamma\delta$ subunit stoichiometry by the time of primitive elasmobranches and is now little changed in mammals. The apparent $(\alpha)_2(\beta)_2$ or $(\alpha)_3(\beta)_2$ structure of the neuronal acetylcholine receptors that we have purified may derive from an early gene duplication event in the evolution of the extended gene family, which now also includes receptors from ganglia and muscle as well as neuronal α-bungarotoxin binding sites.**

The nicotinic acetylcholine receptors from electric organ and muscle have been extensively characterized (see ref. 1). Sequence homologies between the four subunits (α, β, γ, δ) suggest that they evolved by repeated gene duplication from a primordial subunit. In comparison, little is known about the structure or function of neuronal acetylcholine receptors because of the very small amounts of protein available and the lack of a suitable biochemical probe.

α-Bungarotoxin (α-BTX) is a specific probe for receptors in electric organ and muscle, but the physiological significance of α-BTX binding sites found in the nervous system is unclear. α-BTX blocks neuronal acetylcholine receptor function in the cockroach (2), toad, and goldfish (3). However, it has no effect on cholinergic transmission in the rat locus coeruleus (4), the rat pheochromocytoma cell line PC12 (5), or chicken ciliary ganglion neurons (6). α-BTX binding proteins from both chicken (7) and rat (8) brain have been purified. Their immunological crossreaction with receptors from muscle is limited (7) or negligible (9), but the one subunit partially sequenced (7) exhibits some homology to receptor subunits. The histological binding pattern in rat brain of tritiated nicotine or acetylcholine (plus atropine to block muscarinic receptors) is different from the pattern of α-BTX (10).

In an attempt to identify a nicotinic receptor that did not bind α-BTX, a cDNA clone from a PC12 library (λPCA48) was identified by low stringency hybridization with fragments of a cDNA clone for α subunits of receptor from mouse muscle by Boulter *et al.* (11). This cDNA hybridized at low stringency with some brain regions that bind nicotine strongly but bind α-BTX little if at all (12). Complications are that PC12 cells contain both functional receptors and α-BTX binding components, both of which would be expected to have sequence homology with subunits of receptor from muscle, and *in situ* hybridization localizes RNA in cell bodies, not proteins that might be localized in distant axons and that can also be identified by their ligand binding properties. Also, we have found that nerve growth-factor treatment of PC12 cells induced functional receptors but not λPCA48 mRNA or α-BTX binding sites, which suggests that λPCA48 does not code for a receptor subunit (P.W., R. Schoepfer, and J.L., unpublished data).

There is evidence that antibodies can identify nicotinic receptors that do not bind α-BTX. Patrick and Stallcup (5) demonstrated that antisera to receptors from *Electrophorus* electric organ blocked receptor function on PC12 cells but did not bind to the α-BTX binding component. We found that nerve growth-factor treatment of PC12 cells induced binding sites for monoclonal antibody (mAb) 270 in parallel with functional receptors and that mAb 270 plus anti-antibody could down-regulate receptors, indicating that this mAb identifies functional nicotinic receptors (P.W., R. Schoepfer, and J.L., unpublished data). mAb 270 can precipitate all of the high-affinity nicotine binding sites from extracts of rat brain (2). The binding pattern of mAb 270 on sections of rat brain is identical to the binding pattern of nicotine, and many of these binding sites are on nerve endings far removed from the cell bodies (L. Swanson, D. Simmons, P.W., and J.L., unpublished data). mAb 270 was made to receptors immunoaffinity-purified from chicken brains using mAb 35, a mAb raised to receptor from *Electrophorus* (13). mAb 35 has very low affinity for receptors from rat brain (14) but high affinity for receptors in chicken brains (15, 16) and ciliary ganglia (4, 17). Antisera to receptor purified from chicken brain block the function of receptors in ciliary ganglia (18) but do not bind neuronal α-BTX binding components or receptors from muscle (15).

Here we report purification of nicotinic receptors from rat brain by using mAb 270, and we identify two kinds of subunits; we report purification of the α-BTX binding component using α-BTX and identify four kinds of subunits.

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Abbreviations: α-BTX, α-bungarotoxin; mAb, monoclonal antibody.

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## MATERIALS AND METHODS

**Antibody Preparation.** mAbs 270 and 286 were derived from rats immunized with receptors purified from chicken brain (P.W., R. Liu, F. Esch, S. Shimasaki, B. Morley, and J.L., unpublished data). Culture medium was concentrated and then 18% $Na_2SO_4$ was used to precipitate the mAb. Concentrated mAb 270 was applied in 21 ml of 25 mM Tris·HCl, pH 8.8/50 mM NaCl to a 1.5 × 8 cm column of DEAE Tris acryl M (Pharmacia) and 120 mg of pure mAb was eluted. It was coupled to cyanogen bromide-activated Sepharose CL-4B at 8 mg per ml of gel (19).

Antisera to subunits of receptors from *Torpedo* electric organ (20) and chicken brain (15) were prepared as described. Goat anti-rat IgG was affinity-purified by using rat IgG-Sepharose CL-4B and radioiodinated (19) to specific activities of 2–3 × $10^{18}$ cpm/mol.

**Purification of Rat Brain Acetylcholine Receptor.** Rat brains were obtained from Pel-Freez. Immunoaffinity purification and radioiodination of the affinity-purified receptor were essentially as described for receptor from chicken brain with mAb 35 (15).

**Purification of Rat Brain α-BTX Binding Protein.** Rat brain detergent extract, after incubation with the mAb 270 Sepharose, was passed through a 2-ml column of *Naja naja siamensis* toxin coupled to Sepharose CL-4B (0.5 mg/ml) (19) at a flow rate of 20–30 ml per hr at 4°C. The column was rapidly washed with 50 ml of phosphate-buffered saline (PBS; 10 mM Na phosphate, pH 7.5/100 mM NaCl) containing 0.5% Triton X-100, 50 ml of 1 M sodium chloride, 10 mM Na phosphate (pH 7.5) containing 0.5% Triton X-100 and again with 50 ml of PBS/0.5% Triton X-100. Bound protein was eluted by recirculating 1 M carbachol in 10 mM Na phosphate, pH 6.8/0.1% Triton X-100 through the affinity column onto 0.5 ml of hydroxylapatite (Bio-Rad Bio-Gel HTP). The hydroxylapatite was washed with 25 ml of 10 mM Na phosphate, pH 6.8/0.1% Triton X-100. Bound protein was rapidly eluted at 22°C with 300 mM Na phosphate, pH 6.8/0.05% Triton X-100, and immediately dialyzed at 4°C against 10 mM Na phosphate, pH 7.5/0.05% Triton X-100.

**Assay of Receptors.** DL-[*N-methyl*-$^3$H]nicotine (68.6 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) binding sites in brain detergent extract were determined by gel-filtration assay (14). Antibody binding to [$^3$H]nicotine binding sites was investigated by indirect immobilization of antibodies on goat anti-rat IgG Sepharose as described (14).

α-BTX binding sites were determined by DEAE assay (15) using α-BTX radioiodinated to specific activities of 3–4 × $10^{17}$ cpm/mol (19).

**Electrophoresis.** $NaDodSO_4$/10% PAGE and immunoblotting of protein samples were performed as described (15).

**Enzymatic Digestion of Receptor Polypeptides.** Treatment of $^{125}$I-labeled receptor from rat brain with endoglycosidase H (Miles) and proteases was performed as described for receptor from chicken brain (15).

**Sucrose Gradient Centrifugation.** Aliquots of rat brain detergent extract or *Torpedo* receptor (50 nM α-BTX binding sites with 5 nM $^{125}$I-labeled α-BTX were layered onto 5-ml sucrose gradients (5–20%, wt/wt, in 20 mM Na phosphate, pH 7.5/10 mM $NaN_3$/0.1% Triton X-100) and centrifuged for 70 min at 65,000 rpm in a Beckman VTi65.2 rotor. Fourteen-drop fractions were subsequently collected from the bottom of the tubes. $^{125}$I-labeled α-BTX binding to each fraction of the rat brain extract gradients was determined by DEAE assay (14). [$^3$H]Nicotine binding sites in fractions of rat brain extract gradients were determined by gently shaking overnight at 4°C with 10-μl aliquots of mAb 270-Sepharose to immobilize the brain receptor (14).

## RESULTS

**Receptor Purification and Subunit Composition.** Receptor was solubilized in Triton X-100 and affinity-purified using mAb 270-Sepharose. The concentration of receptor in detergent extracts was 0.48 ± 0.06 nM [$^3$H]nicotine binding sites, corresponding to 1.2 ± 0.2 pmol of brain per g (five preparations). The affinity column very efficiently bound the receptor. After washing, elution was achieved using 50 mM citrate (pH 3.0) containing 0.1% Triton X-100. Preliminary experiments indicated that under these conditions >98% of bound receptor protein could be eluted. The eluate was immediately neutralized with 1 M Tris (pH 8.0) to minimize acid denaturation. However, ≈70% of nicotine binding activity was lost. mAb 270 binding was also partially lost after acid denaturation, resulting in a second affinity-column step being relatively inefficient. The second purification step was nonetheless used to obtain very highly purified protein for analysis by $NaDodSO_4$/PAGE and silver staining or radioiodination. Typical results are shown in Table 1.

$NaDodSO_4$/PAGE and silver staining of the affinity-purified receptor detected two subunits (Fig. 1) of $M_r$ 51,700 ± 500 and 79,700 ± 3500 (mean ± SD of four determinations). They were also visualized by radioiodination (Fig. 1*B*). Both polypeptides were part of the same macromolecular complex, since they could be removed from solution by mAb 270-Sepharose (even though mAb 270 binds only to the $M_r$ 51,000 subunit, as shown later). In addition, both polypeptides could be removed from solution by concanavalin A Sepharose. These results also indicate that the two faint bands of $M_r$ 66,500 and 95,000 (lane 3) are trace contaminants.

**α-BTX Binding Component Subunit Composition.** Receptors from muscle and electric organ are easily proteolyzed during purification to give the appearance of only α subunits rather than the α, β, γ, and δ subunits they contain (21). The α-BTX binding component from brain was initially reported to consist of one subunit, but later studies using conditions to better control proteolysis reported three to five subunits (7). We reasoned that if the α-BTX binding component subunits could be recovered intact from our extracts, it would suggest that proteolysis was adequately controlled. Therefore, the α-BTX binding protein was affinity-purified on α toxin-Sepharose using extracts that had previously been applied to mAb 270 affinity columns (Table 2). The α-BTX binding protein preparation consisted of four polypeptides (Fig. 1*A*, lane 4), $M_r$ 44,700 ± 1200, 52,300 ± 800, 56,600 ± 700, and 65,200 ± 600 (mean ± SD of three determinations), quite similar in apparent molecular weight to α, β, γ, and δ subunits of receptors from electric organ and muscle. The faint band observed at $M_r$ 95,000 is probably the trace contaminant observed in the nicotinic receptor preparation (lane 3). In the absence of antibody probes for the toxin binding protein, we cannot unequivocally demonstrate that each of the four polypeptides is a true constituent of the same macromole-

Table 1. Purification of rat neuronal acetylcholine receptor from 100 g of rat brain

| | Volume, ml | Protein, mg | [$^3$H]Nicotine binding sites | |
|---|---|---|---|---|
| | | | pmol | % |
| Extract | 200 | 1840 | 87 | 100 |
| Unbound to first mAb 270-Sepharose column | 200 | 1840 | 6 | 8 |
| Eluate of first column | 36 | 0.245 | 25 | 31 |
| Unbound to second mAb 270-Sepharose column | 36 | — | 3.1 | 4.1 |
| Eluate of second column | 0.84 | — | 4.1 | 4.7 |

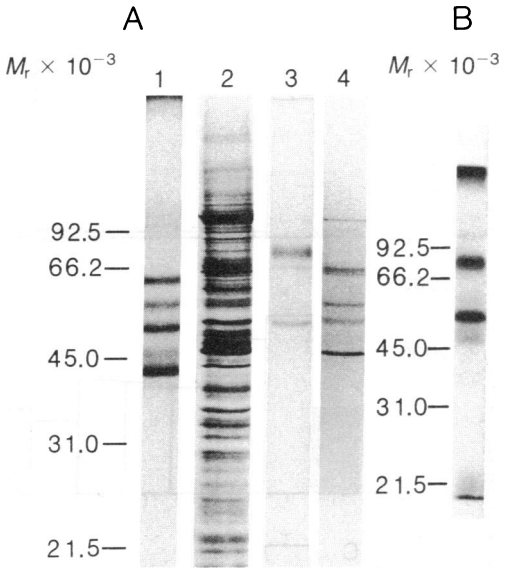


FIG. 1. Analysis of affinity-purified rat brain nicotinic receptor and α-BTX binding protein by NaDodSO$_4$/PAGE. (*A*) Lanes: 1–4, stained with silver; 1, 0.5 μg of *Torpedo* receptor; 2, 3 μg of rat brain extract; 3, ≈0.35 pmol of rat brain receptor; 4, ≈0.2 pmol of rat brain α-BTX binding protein. (*B*) $^{125}$I-labeled affinity-purified rat brain nicotinic receptor, detected by autoradiography using Kodak XAR film and Cronex intensifying screens. Positions of the molecular weight standards (low molecular weight standards, Bio-Rad) were resolved on the same gel and are indicated on the left.

cule. However, the four polypeptides do co-sediment with the α-BTX binding peak when analyzed by 5–20% sucrose gradient sedimentation (data not shown). The preservation of these labile subunits in an apparently intact form through our purification procedure suggests that the brain acetylcholine receptor subunits have also been recovered intact.

Abood and coworkers (22) reported purification of a nicotine binding protein from rat brain by affinity chromatography using a nicotine analogue as ligand. They found a single subunit of $M_r$ 56,000. If this were the receptor we have identified, proteolysis could account for the observation of a single peptide in their preparation (21). However, a single band generated by proteolysis of the same component we have observed would be expected to produce a band with an apparent molecular weight less than or equal to its smallest subunit ($M_r$, 51,000).

**Enzyme Digestion of Receptor Subunits.** The α subunits of receptors from electric organ (23), muscle (24), and chicken brain (15) have a mannose-rich oligosaccharide moiety, which can be removed by digestion with endoglycosidase H. Endoglycosidase treatment of receptor from rat brain decreased the $M_r$ of the larger subunit by 3000 and the smaller subunit by 2300 (Fig. 2*A*). Thus, both subunits have mannose-rich oligosaccharide side chains.

Digestion of isolated $^{125}$I-labeled receptor polypeptides with several proteases and subsequent analysis of the peptide maps showed that the subunits were clearly different (Fig. 2).

Table 2. Purification of rat brain α-BTX binding protein from 100 g of rat brain

| | Volume, ml | Protein, mg | [$^{125}$I]α-BTX binding sites | |
|---|---|---|---|---|
| | | | pmol | % |
| Extract | 200 | 1840 | 158 | 100 |
| Unbound to α-BTX Sepharose column | 200 | 1840 | 82 | 52 |
| Eluate | 9 | 0.216 | 38 | 25 |

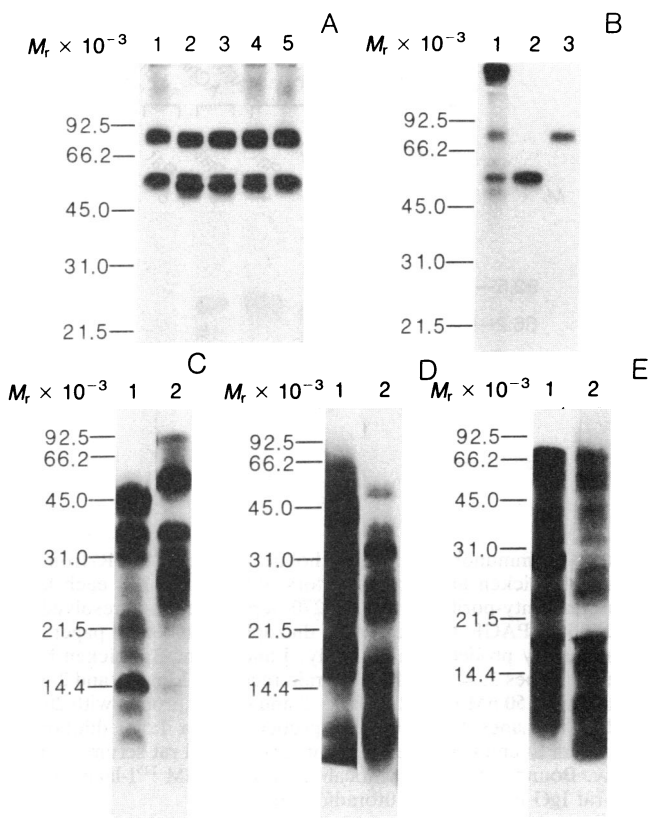


FIG. 2. (*A*) Digestion of $^{125}$I-labeled rat brain receptor with endoglycosidase H. Aliquots of radioiodinated receptor (25,000 cpm) were digested with 10, 1, 0.1, and 0.01 milliunits (lanes 2–5, respectively) of endoglycosidase H (Miles) in 25 μl of 50 mM sodium citrate (pH 5.5) containing 0.5% NaDodSO$_4$, 50 mM dithiothreitol, and 10 mM phenylmethylsulfonyl fluoride, for 15 hr at 37°C. A control incubation contained no enzyme (lane 1). Samples were resolved by NaDodSO$_4$/PAGE and located by autoradiography. (*B*) NaDodSO$_4$/PAGE and autoradiography of $^{125}$I-labeled receptor (lane 1) and isolated $M_r$ 51,000 subunit (lane 2) and $M_r$ 79,000 subunit (lane 3). (*C–E*) Peptide mapping after limited digestion of $^{125}$I-labeled receptor subunits with *Staphylococcus* V8 protease (Miles) (*C*), trypsin (Worthington) (*D*), and chymotrypsin (Calbiochem) (*E*). $^{125}$I-labeled $M_r$ 51,000 subunit (lane 1) and $^{125}$I-labeled $M_r$ 79,000 subunit (lane 2) (15,000–30,000 cpm) were digested with protease for 1 hr at 37°C, in 25 μl of 10 mM Na phosphate, pH 7.5/0.1% Triton X-100. After addition of 2.5 μl of phenylmethylsulfonyl fluoride and 25 μl of sample buffer (15), samples were resolved by NaDodSO$_4$/15% PAGE and located by autoradiography.

Comparison of these peptide maps with those previously obtained for the subunits of receptor from chicken brain (15) suggests little homology; this, however, may be misleading because subunits of receptor from *Torpedo* are clearly homologous in amino acid sequence (25) but their peptide maps differ significantly (26).

**Homologies of Receptor Subunits.** mAb 270 bound to the $M_r$ 51,000 subunit of receptor from rat brain and the $M_r$ 49,000 polypeptide of receptor from chicken brain (Fig. 3), showing that they are homologous subunits. Both the $M_r$ 51,000 subunit of receptor from rat brain (Fig. 4) and the $M_r$ 49,000 subunit of receptor from chicken brain (15) are bound by antisera to α subunits of receptor from *Torpedo* electric organ. Receptors from brains of chickens and rats can be affinity-labeled with bromoacetylcholine (14), which suggests that these receptors contain homologues of cysteines α192 and 193, which are affinity-labeled in receptor from *Torpedo* (27), but we have not yet identified which subunits of receptors from brain are affinity-labeled. The sequence of the NH$_2$-terminal 13 amino acids of the rat $M_r$ 51,000 subunit

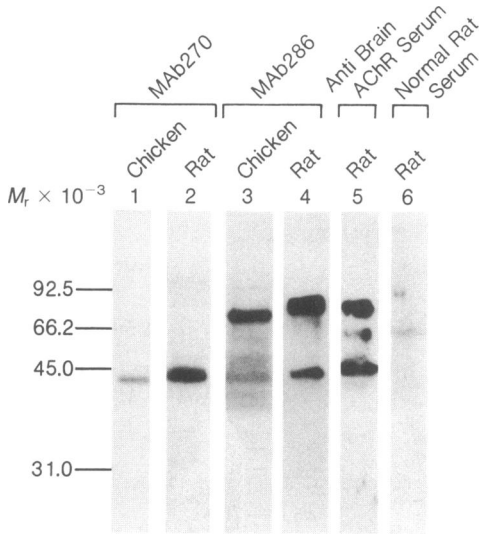


FIG. 3. Immunoblots of acetylcholine receptors (AChR) from the brains of chicken and rat. Receptors (0.5–2.0 pmol in each lane) immunoaffinity-purified on mAb 270-Sepharose were resolved by $NaDodSO_4$/PAGE transferred to diazophenylthioether paper and subsequently probed with antibody. Lanes 1 and 3, chicken brain receptor; lanes 2, 4, 5, and 6, rat brain receptor. Lanes 1 and 2 were probed with 50 nM mAb 270; lanes 3 and 4 were probed with 20 nM mAb 286. Lanes 5 and 6 were probed with a 1:500 dilution of antiserum to chicken brain receptor, and normal rat serum, respectively. Bound antibody was localized with 0.4 nM $^{125}$I-labeled goat anti-rat IgG followed by autoradiography.

has been determined (data not shown). This reveals limited sequence homology (30–40%) to published $NH_2$-terminal amino acid sequence of all of the subunits of receptors from mouse muscle.

No other subunit-specific crossreaction was detected between receptors from the brains of rats (Figs. 3 and 4) or chickens (15) and receptors from *Torpedo* electric organ. This suggests that the sequence homologies between the subunits of receptors from brain and electric organ are quite limited.

By contrast, there appears to be extensive homology between the corresponding subunits of receptors from the brains of rats and chickens. mAb 270 binds to the $M_r$ 51,000 subunit of receptor from rat and the $M_r$ 49,000 receptor from chicken (Fig. 3). mAb 286 binds to the $M_r$ 79,000 subunit of receptor from rat and the $M_r$ 75,000 subunit of receptor from chicken. Finally, antisera to receptor from chicken brain bind both subunits of receptors from rat brain (Figs. 3 and 4).

There are also homologies between the two subunit types of receptors from the brains of both rats and chickens. mAb 286 binds to both subunits of receptors from rats and chickens (Fig. 3). Crossreaction between subunits of receptor from *Torpedo* also occurs with some mAbs to receptors from *Torpedo* because of sequence homologies between subunits (26).

**Macromolecular Size of Receptor and α-BTX Binding Component.** On sucrose gradients, both the receptor and α-BTX binding protein from rat brain sedimented as single peaks, slightly larger than monomers of receptor from *Torpedo*, and therefore ≈10 S (Fig. 5). The receptor macromolecule, therefore, probably has a molecular mass of 250,000–300,000, consistent with an $(\alpha)_2(\beta)_2$ or $(\alpha)_3(\beta)_2$ subunit stoichiometry. We know that there must be more than one $M_r$ 51,000 subunit in the macromolecular complex, because brain receptor immobilized on mAb 270-Sepharose can bind additional $^{125}$I-labeled mAb 270 (data not shown). This agrees with data previously presented for the chicken neuronal receptor using mAb 35 (15). In the case of chickens, we have conducted similar experiments with a mAb specific for β subunit and obtained evidence that there is also more than one β subunit

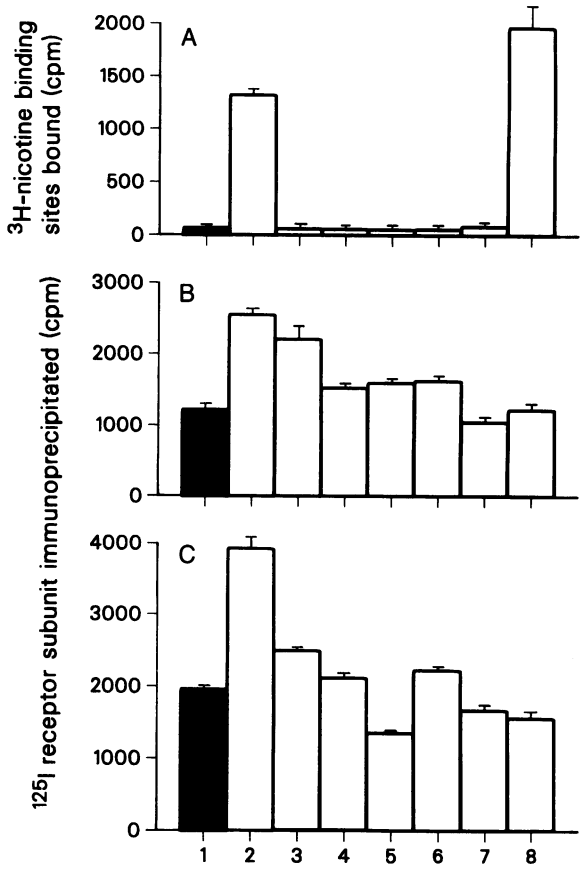


FIG. 4. Binding to receptors from rat brain of antibodies raised against receptors from electric organs and chicken brain. (*A*) Binding to native receptor. Rat brain detergent extract (200 μl; 0.44 nM [$^3$H]nicotine binding sites) was gently shaken for 15 hr at 4°C with 20 μl of goat anti-rat IgG Sepharose (gel, 10 mg/ml) and 5 μl of antiserum to chicken brain receptor [titer, 32 μM; (15)], or antisera to subunits of receptors from *Torpedo* electric organ (titers, ≈22 μM), or mAb 270 (titer, 5 μM), or mAb 35 (titer, 490 μM), or normal rat serum. [$^3$H]Nicotine binding to each aliquot was then determined as described (14). (*B*) $^{125}$I-labeled $M_r$ 51,000 subunit (13,000 cpm) and (*C*) $^{125}$I-labeled $M_r$ 79,000 subunit (11,800 cpm) were incubated for 15 hr at 4°C with 5 μl of antibody in a final vol of 100 μl of PBS/0.5% Triton X-100. The antigen–antibody complexes were precipitated with goat anti-rat IgG, pelletted and washed twice with PBS, 0.5% Triton X-100, and radioactivity was quantitated by γ counting. Bars: 1, normal rat serum; 2, antiserum to chicken brain receptor; 3, antiserum to *Torpedo* receptor α subunits; 4, antiserum to β subunits; 5, antiserum to γ subunits; 6, antiserum to δ subunits; 7, mAb 35; and 8, mAb 270.

per receptor (P.W., R. Liu, F. Esch, S. Shimasaki, B. Morley, and J.L., unpublished data).

## DISCUSSION

Receptors from brain and muscle, as well as α-BTX binding components, are probably part of an extended gene family. Receptors from electric organ and muscle have monomers of ≈9.5 S and the subunit composition $(\alpha)_2\beta\gamma\delta$ (1). Because of sequence homologies between the subunits, it is thought that α, β, γ, and δ subunits evolved from successive duplications of a primordial α subunit (25). The structure of these receptors has been highly conserved, as indicated by the 80% sequence homology between α subunits of receptors from *Torpedo* (an elasmobranch) and humans (28). Receptors of this type bind α-BTX and mAbs directed at the main immunogenic region (13), in both cases to the extracellular surface of α subunits. Recently, functional receptors have been purified from central nervous systems of locusts. These sediment at 10 S, bind α-BTX, and appear to consist of a

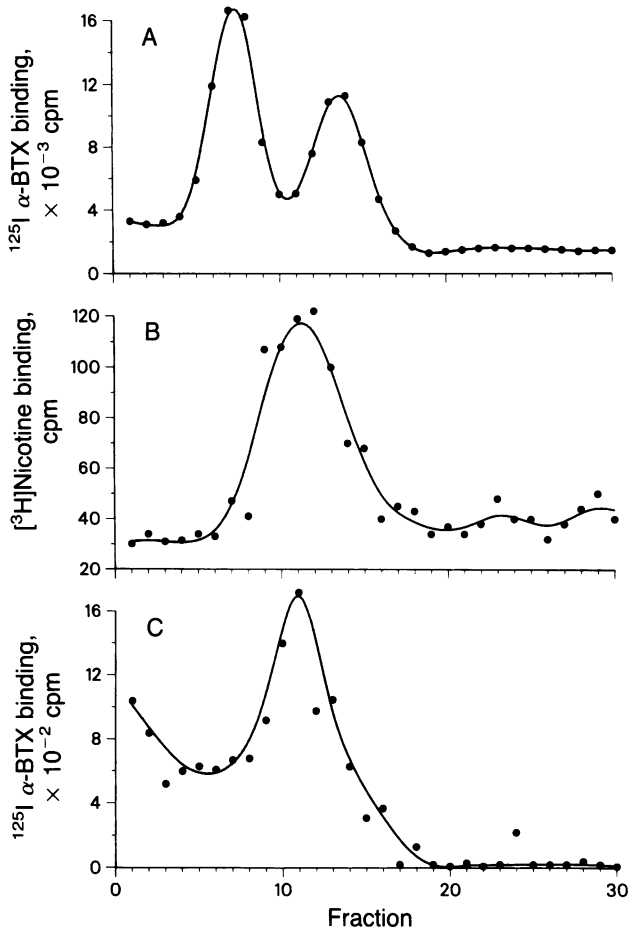


FIG. 5. Sucrose gradient centrifugation of rat brain receptor. (*A*) *Torpedo* receptor (50 nM) labeled with 5 nM [$^{125}$I]α-BTX. (*B*) Rat brain detergent extract, fractions assayed for [$^3$H]nicotine binding sites. (*C*) Rat brain detergent extract, fractions assayed for [$^{125}$I]α-BTX binding sites. Both the rat brain receptor and rat brain α-BTX binding protein peaked at fractions 11–12. *Torpedo* dimers peaked at fraction 7 and monomers peaked at fraction 13–14.

single $M_r$ 65,000 subunit (29). This may be an example of the primordial homopolymeric receptor or it may be an artifact of proteolytic degradation (30). Functional receptors in goldfish brains are ≈10 S and bind both α-BTX and some mAbs directed at the main immunogenic region, but their subunit structure is unknown (31). There are receptors from chicken brain of ≈10 S that do not bind α-BTX but do bind mAbs to the main immunogenic region, and these appear to be composed of two kinds of subunits (15). Receptors in chicken ciliary ganglia and rat PC12 cells, like receptors from brain, do not bind α-BTX (5, 6). They also share several antigenic determinants with receptors from both electric organ and chicken brain (11, 15–18). Receptors in ganglia are, however, clearly distinct from receptors in brain because ganglionic receptors do not have high affinity for acetylcholine or nicotine (32, 33). In addition, receptors in chicken brain are not a homogeneous population. We have identified two receptor subtypes that have the same high-affinity nicotine binding, and similar or identical α subunits but different β subunits and different distributions in the brain (unpublished data). Here we have shown that receptors from rat brain are 10 S, do not bind α-BTX, and have negligible affinity for mAbs to the main immunogenic region. If there really are two kinds of subunits in the receptors from chicken and rat brain, they may derive from the first gene duplication event of the primordial α subunit. The striking similarity between the four polypeptides found in the α-BTX binding component of rat brain and receptors in muscle suggests that they diverged after later gene duplication events, which produced four kinds of subunits.

The physiological role of the neuronal acetylcholine receptor described here is currently unknown. In certain pathways of the rat brain, the acetylcholine receptor may be presynaptic, in contrast to the postsynaptic role of receptors in muscle (L. Swanson, P.W., D. Simmons, and J.L., unpublished data). Similarly, it has been reported that [$^3$H]acetylcholine labeled presynaptic sites on catecholamine and serotonin axons in rat brain (34). Future studies using both mAbs and molecular genetic techniques will allow further understanding of the role of these receptors.

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