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Biochemistry

Immunization of rats with polypeptide chains from torpedo acetylcholine receptor causes an autoimmune response to receptors in rat muscle

(myasthenia gravis/membrane/neurotransmission disease model)

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ABSTRACT **Four polypeptide chains were purified from acetylchloline receptor of *Torpedo californica* electric organ. Their apparent molecular weights were 64,000, 57,000, 49,500, and 38,000. Rats immunized with any of the four chains produced antibodies that crossreacted with rat muscle receptors *in vivo*. Specificities of anti-chain sera were evaluated *in vitro* by reaction with native receptor solubilized from electric organs and muscles of several species and by inhibition of this reaction with the purified polypeptide chains. The chains are immunologically distinct from one another. Antigenic determinants comparable to each chain of torpedo receptor are found in receptor from both rat and human muscle. At least part of each of these determinants is exposed on the extracellular surface of the muscle membrane. The most immunogenic determinants on native receptor are lost on denaturation to polypeptide chains. Its component peptides are much less immunogenic than native receptor, and induce antibodies of different specificity. Anti-receptor antibodies of many specificities can cause experimental autoimmune myasthenia gravis.**

Acetylcholine receptor (AChR) purified from *Torpedo californica* electric organs in mild detergents consists of a macromolecular component sedimenting on sucrose gradients at ~9 S and a dimer of this size sedimenting at ~13 S (1–3). After reduction and dissociation of either form in sodium dodecyl sulfate ($NaDodSO_4$) and electrophoresis on acrylamide gels, a pattern of four bands has been observed (1–3). Apparent molecular weights (M_r) reported for these bands approximate 39,000, 48,000, 58,000, and 64,000 (1, 2). Similar band patterns were observed with AChR purified from several species of torpedo (4). Claudio and Raftery (4) showed that antibodies prepared against the polypeptide chains corresponding to each of the bands from *T. californica* specifically crossreacted with the corresponding bands from other torpedo species, showing that subunit antigenic determinants were conserved in the four species of ray examined. The polypeptide chain of lowest M_r (39,000) could be affinity labeled with an acetylcholine (ACh) analogue (1), showing that the chain contributes to the ACh binding site of the AChR. The function of the other polypeptide chains found in AChR purified from torpedo electric organ is unknown.

AChR purified from rat muscle consists of a macromolecular component of ~9 S (5). The band pattern obtained after reduction and dissociation in $NaDodSO_4$ and electrophoresis on acrylamide gels shows major polypeptide chains with apparent M_r of 45,000 and 51,000, along with minor components of M_r 49,000, 56,000, 62,000, and 110,000 (5, 6). Two subunits of AChR from rat muscle (M_r 45,000 and 49,000) can be affinity labeled (6), suggesting that a second site for ACh binding is labeled that is not labeled in receptor from electric organ. AChR purified from a mouse muscle cell line also shows a pattern of four bands (M_r 72,000, 65,000, 53,000, and 44,000) on electrophoresis in $NaDodSO_4$ (7).

In contrast to these reports showing a complex subunit structure in AChR purified from electric organs and muscle, it has also been reported that AChRs purified from *T. marmorata* (8) and fetal calf muscle cells in tissue culture (9) consist of polypeptide chains of only a single size, M_r 40,000–41,000. AChR is very susceptible to proteolysis, and proteolysis may be an important source of the variation in apparent subunit composition of purified AChR (10).

There are antigenic similarities between AChR from electric organ and muscle: immunization of animals with AChR purified from electric organs results in the formation of antibodies that crossreact with muscle AChR (11–15) and immunization with AChR purified from muscle results in antibodies that crossreact with electric organ AChR (12, 13). *In vivo*, the crossreaction of antibodies with muscle AChR results in inhibition of AChR activity (16), loss of AChR (14), and alteration of the structure of the postsynaptic membrane (17), all of which impair neuromuscular transmission (18). The features of animals immunized with purified AChR so closely resemble those of patients with myasthenia gravis that these animals are said to have "experimental autoimmune myasthenia gravis" (EAMG) (15, 19).

We report here experiments that show which of the polypeptide chains composing AChR from *T. californica* are responsible for inducing crossreaction with AChR from muscle. Binding specificities of antisera prepared against the polypeptide chains from torpedo AChR are characterized, so that these antibodies can be used as templates to compare the structures of AChR from electric organ and muscle.

MATERIALS AND METHODS

T. californica were obtained from Pacific Biomarine, Venice, CA.

AChR was purified by affinity chromatography with toxin agarose as described (13), except that tissue was solubilized in 1% Na cholate (Interchem) instead of 1% Triton X-100. Subsequent dilutions of AChR were made in "0.2% cholate buffer" (0.2% Na cholate/10 mM Na phosphate buffer, pH 7.5/10 mM NaN_3).

AChR polypeptide chains were purified by preparative electrophoresis. Characterization of the purified chains will be described elsewhere. The method of purification of the chains

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Abbreviations: ACh, acetylcholine; M_r, molecular weight; AChR, acetylcholine receptor; αBGT, α-bungarotoxin; EAMG, experimental autoimmune myasthenia gravis; $NaDodSO_4$, sodium dodecyl sulfate.

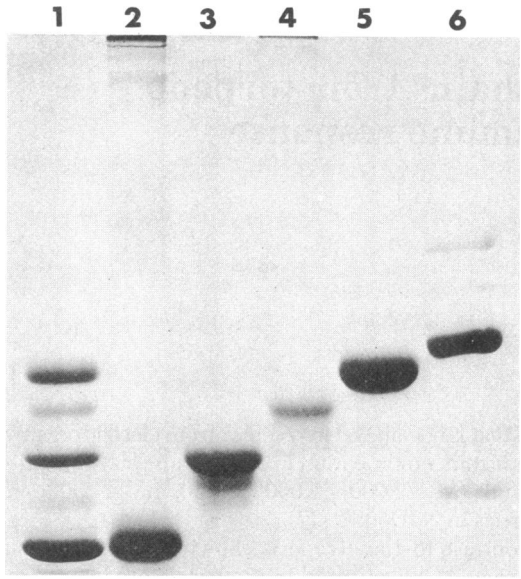

FIG. 1. Electrophoresis of AChR from torpedo and purified AChR chains. Electrophoresis in 0.1% $NaDodSO_4$ on a 10% acrylamide slab gel by the discontinuous buffer system of Laemmli (20) was followed by staining with Coomassie brilliant blue R. (*1*) AChR purified by affinity chromatography (17 μg). (*2*) Chain of M_r 38,000 (9.7 μg) purified from this preparation of cholate-solubilized AChR by preparative slab gel electrophoresis in $NaDodSO_4$. Note the pattern of aggregation characteristic of this chain. (*3*) Chain of M_r 49,500 (7 μg). Note the presence of faster migrating material generated during purification often observed with this chain. (*4*) Chain of M_r 57,000 (4.2 μg). (*5*) Chain of M_r 64,000 (6.1 μg). Note the absence of aggregation or breakdown and intense staining characteristic of this chain. (*6*) M_r standards, from the top: H chain dimers of IgG (M_r 100,000), ovalbumin dimers (M_r 87,000), bovine serum albumin (M_r 68,000), and ovalbumin (M_r 43,500).

follows. Freshly purified AChR in 0.2% cholate buffer plus 0.5 M NaCl was concentrated to 10–20 mg/ml by an Amicon apparatus with an XM100 filter. Typically, 30–40 mg in about 2 ml were diluted to 13.5 ml with sample buffer (2.3% $NaDodSO_4$/5% mercaptoethanol/10% glycerol/62.5 mM Tris·HCl, pH 6.8). Half of this was applied to each of two 10% acrylamide gel slabs (6 × 155 × 160 mm) in a trough (6 × 14 × 115 mm) formed in the 4.75% stacking gel. Gels containing 0.1% $NaDodSO_4$ were made by the method of Laemmli (20). Electrophoresis was for 16 hr at 30 mA. During electrophoresis, separated chains were visible by refractive index as lines across the gel. Gels were stained for 2 min with 0.25% Coomassie brilliant blue R in 40% methanol/7% acetic acid, destained for 20 min or less in 40% methanol/7% acetic acid, and rinsed with water. The stained bands were excised with a razor blade. Gel slices were homogenized for 15 s in a Waring blender with 200 ml of H_2O. Two milliliters of 10% $NaDodSO_4$ were added to the homogenate, which was then agitated overnight at room temperature. Gel fragments were removed by vacuum filtration and the extract was concentrated by lyophilization. After solubilization in 10 ml of H_2O, extracts were again lyophilized in smaller tubes. Coomassie dye and $NaDodSO_4$ were extracted from the resulting powder with two 5-ml aliquots of methanol. The remaining precipitate was dissolved in 2 ml of 1% $NaDodSO_4$ and dialyzed against 0.1% $NaDodSO_4$ in 100 mM NaCl/10 mM Na phosphate, pH 7.5/10 mM NaN_3. Total yield of purified chains approximated 30% of the starting AChR protein.

RESULTS

A typical preparation of chains purified from torpedo AChR analyzed by analytical $NaDodSO_4$/polyacrylamide gel electrophoresis is shown in Fig. 1. Recovery of chains appeared proportional to their composition in the initial AChR preparation. Their apparent M_r are approximately 38,000, 49,500, 57,000, and 64,000. The purified 38,000 M_r chain characteristically showed some aggregation. A faint band sometimes observed between the 38,000 and 49,500 M_r bands was discarded. The purified 49,500 M_r chain often showed some breakdown to an apparent M_r ~46,000. The purified 64,000 M_r chain characteristically electrophoresed as a single sharp band without aggregation or degradation.

In order to test whether purified chains could induce EAMG, rats were immunized at 2-week intervals with 50-μg doses emulsified in complete Freund's adjuvant (Table 1). All three rats immunized with the 38,000 M_r chain developed obvious muscular weakness, the first on day 64. Decrease in AChR content of muscle was measured to provide an objective criterion for the occurrence of EAMG. At sacrifice, these rats contained only 32% of the muscle AChR extractable from control

Table 1. Crossreaction with rat muscle AChR induced *in vivo* by immunization with torpedo AChR polypeptides

Group	Total dose, μg	Day killed	Weakness	Serum anti-AChR titer, nM		Total muscle AChR, pmol/rat	Antibody-bound AChR, % of total
				Anti-rat	Anti-torpedo		
Control rats	—	—	—	—	—	43.5 ± 0.3	0 ± 0
Control gel immunized	—	77, 89, 89	0, 0, 0	0.058 ± 0.06	0.059 ± 0.06	42.3 ± 2.6	0.33 ± 0.58
M_r 64,000 immunized	200, 250, 250	69, 89, 89	++, 0, 0	28.5 ± 19.4	4450 ± 385	22.3 ± 6.1	36.4 ± 15
M_r 57,000 immunized	250, 250, 250	77, 89, 89	0, ++, 0	5.91 ± 2.82	3210 ± 273	20.0 ± 1.2	27.6 ± 9.5
M_r 49,500 immunized	250, 250, 250	77, 89, 89	0, 0, 0	0.34 ± 0.17	1460 ± 456	25.8 ± 3.0	1.64 ± 0.47
M_r 38,000 immunized	200, 200, 250	64, 69, 77	++, ++, +	32.3 ± 13.5	4950 ± 2680	13.8 ± 2.2	44.3 ± 7.3

Groups of three female Lewis rats were injected with nothing (control rats), an extract of an unused acrylamide gel prepared like the extracts containing the purified polypeptide chains (control gel), or the various polypeptide chains. Rats were injected intradermally with 50-μg doses of chains in complete Freund's adjuvant at 2-week intervals and killed on the day indicated after the first injection. Visible signs of muscular weakness characteristic of EAMG were scored as described (21). Titers of serum antibodies to AChR were measured (13) on sera obtained by sacrifice. AChR was extracted from the decapitated carcasses, and the content of AChR and antibody-bound AChR was determined as described (14). All values are expressed as mean ± SEM.

Table 2. Crossreaction of antisera to polypeptide chains from torpedo AChR with AChR solubilized from other species

Immunogen; Animal	Serum antibody titer against AChR from various sources, nM					
	Torpedo	Electrophorus	Rat	Fetal calf	Squirrel monkey	Human
M_r 64,000						
Rat	5,180	53.1	65.8	72.6	96.1	52.4
Rat	3,870	146	≤1.2	10.8	3.2	7.2
Rat	4,310	1,030	18.4	34.4	7.1	12.1
Rabbit	700	13.3	1.7	6.1	≤0.7	5.6
M_r 57,000						
Rat	3,740	523	≤2.4	10.0	1.7	9.6
Rat	2,840	132	3.8	7.8	4.0	11.9
Rat	3,040	245	11.5	7.9	≤1.4	10.4
Rabbit	417	18.1	1.9	1.1	≤0.4	5.4
M_r 49,500						
Rat	626	10.3	0.0	≤0.5	0.0	5.9
Rat	1,570	26.9	≤0.5	0.9	0.0	5.3
Rat	3,200	3.2	≤0.5	≤0.2	0.0	5.9
Rabbit	407	0.7	≤1.5	0.7	≤0.4	4.2
M_r 38,000						
Rat	10,300	259	50.5	72.5	13.6	224
Rat	2,100	0.2	6.0	16.6	1.04	17.7
Rat	2,440	15.6	40.1	131	31.7	60.2
Rabbit	792	90.2	36.7	24	21.5	24.3

Sera taken at sacrifice from the rats described in Table 1 were tested. Rabbits given injections of 250 μg of polypeptide chain on days 0, 14, and 29 were bled on day 43 for the sera tested here. Detergent extracts of AChR (1 nM) labeled with 2 nM ^{125}I-labeled α-bungarotoxin (αBGT) were used as antigens in these assays, as described (13). Antibody titers are expressed as moles of αBGT binding sites precipitated per liter of serum. All titers not derived from assay values at least 2-fold greater than normal serum blanks are prefixed by ≤.

rats, and 44% of this AChR had antibodies bound. Titer of serum antibodies crossreacting with native torpedo AChR was quite high, 4.95 μM, and, as is typical of rats with EAMG induced by immunization with native torpedo AChR, only about 0.6% of these antibodies crossreacted with AChR solubilized from rat muscle.

For comparison, four rats given a single dose of 1 μg of native torpedo AChR after 43 days showed no obvious weakness, but their AChR content was 36% of normal, and 18% of these AChRs had antibodies bound. Titer of serum antibodies to torpedo AChR averaged 0.55 μM. A group given 0.3 μg of native AChR had 60% of normal AChR content, and only 1.6% of these were bound with antibodies. At this low dose of AChR, titer of serum antibodies or torpedo AChR was only 75 nM, and no titer against rat was detectable. Rats immunized with 2–64 μg of torpedo AChR showed a maximum decrease in AChR content to 28% of normal, with 55–100% of this AChR bound with antibodies. As doses increased from 2 to 64 μg of torpedo AChR, titers of serum antibodies to torpedo AChR increased from 1 to 5 μM, while titers of serum antibodies to rat AChR increased from 20 to 50 nM. Thus, although the 38,000 M_r chain could induce EAMG, it was much less effective than native AChR. A total of 200–250 μg over 64–77 days was required to produce EAMG equivalent to that produced by 1–2 μg of native AChR in 43 days.

Rats immunized with the 64,000 and 57,000 M_r chains also developed EAMG, though less severely than rats immunized with the 38,000 M_r chain (Table 1). Rats immunized with the 49,500 M_r chain averaged only ½–⅓ the serum antibody titer to native torpedo AChR of rats immunized with the other chains, and serum antibody to rat AChR was negligible. Decrease in AChR content was only to 60% of normal, and only 1.6% of this was bound with antibodies.

Rabbits were also immunized with purified chains. None of the four rabbits given 250-μg doses totaling 2500 μg over 186 days developed obvious signs of muscular weakness. Correspondingly, their titers of serum antibodies to AChR were much lower than those of rats immunized with purified chains (Table 2).

Torpedo AChR polypeptide chains were tested for crossreaction with native AChR extracted from other species (Table 2). Like antibodies to native AChR, antibodies to the polypeptide chains were quite species specific. As with animals immunized with native torpedo AChR (unpublished results), crossreaction was usually most extensive with electrophorus AChR and less extensive with AChR from the muscles of other species. The degree of crossreaction with AChR from other species varied widely between rats in a group. As examples, the degree of crossreaction between rat sera to the 64,000 M_r chain and native electrophorus AChR varied from 1–24% of the titer against native torpedo AChR, and rat antisera to the 38,000 M_r chain titered against squirrel monkey AChR gave 0.05–53% of the titer observed against human AChR.

Rat sera with high titers against native torpedo AChR were obtained with all four chains (Table 2). However, much higher doses of chains were required to achieve these titers than were required of native torpedo AChR. Previously we had observed that denaturation of electrophorus AChR with $NaDodSO_4$ (13), heat, or urea (unpublished) greatly reduced the immunogenicity of determinants crossreacting with native AChR. Thus, with native AChR from both torpedo and electrophorus the most immunogenic determinants are formed by the native tertiary conformation of these proteins. Because antibodies to native torpedo AChR are not inhibited by substantial excesses of polypeptide chains (Table 3), it is clear that less than 1% of the antibodies formed against native torpedo AChR recognize determinants formed by the amino acid or carbohydrate sequences in $NaDodSO_4$-denatured chains.

Table 3. Failure of polypeptide chains to inhibit antibodies prepared against native torpedo AChR

Sample	AChR bound, cpm × 10^{-5}
Control rat anti-torpedo AChR	1.62 ± 0.06
+38,000 M_r chain	1.62 ± 0.03
+49,500 M_r chain	1.62 ± 0.04
+57,000 M_r chain	1.64 ± 0.03
+64,000 M_r chain	1.64 ± 0.01
+ All four chains	1.63 ± 0.03

Triplicate 1-ml aliquots of 0.7 nM rat anti-torpedo AChR serum dilution were incubated for 24 hr at 4° alone or with 0.51 μg of the indicated chains in 0.2% cholate buffer. Then torpedo AChR labeled with ^{125}I-labeled αBGT was added for an additional 12 hr. Final concentration of AChR was 1.5 nM; that of ^{125}I-labeled αBGT, 2 nM. Assuming a specific activity of 10^4 nmol/g, 0.15 μg of native AChR was present in each tube. Thus, chains were present in 3.4-fold weight excess, and >10-fold molar excess over their content in native AChR. Assuming slopes for inhibition similar to those in Fig. 2, chains were present in >26-fold excess over antibody.

The antibodies formed against $NaDodSO_4$-denatured chains that crossreacted with native torpedo AChR were efficiently inhibited from crossreaction by polypeptide chains (Fig. 2). Thus, these antibodies must be directed at determinants on native AChR that are not normally immunogenic and whose antigenicity is not conformationally dependent. These experiments would not detect antibodies that recognized determinants available only on denatured chains. Addition of excess antiserum to any chain resulted in precipitation of all native torpedo AChR present (data not shown); thus all native AChR molecules contained determinants reacting with each of the anti-chain antisera.

Inhibition of anti-chain antisera by homologous chains (Fig. 2) was initially directly proportional to the amount of chain added. Each mole of chain protected an average of 2.9 ± 0.6 mol of AChR ^{125}I-labeled αBGT binding sites from antibody. This is consistent with the observation that AChR has several toxin-binding sites per molecule (1, 2, 10). The steep slope of these inhibition curves indicates that either there was no significant amount of antibodies in the sera that bound to only denatured chains and did not bind to native AChR, or if any such antibodies were present, they bound to the chains without interfering with the binding of antibodies to crossreacting determinants.

Antiserum raised against each polypeptide chain was inhibited little by the other polypeptide chains (Fig. 2). The small amount of crossinhibition observed can be explained by slight crosscontamination with adjacent bands in the gel during purification or by crossreaction of similar determinants on different chains. The 38,000 M_r chain is best resolved on the gel and appears to be obtained at greater than 99% purity, whereas the other chains appear to be obtained at around 95% purity, by the one-step purification method used. To any extent that the chains might crossreact, estimates of purity would increase.

Antisera raised against each chain from torpedo AChR crossreacted with native AChR from human muscle. Crossreaction with human AChR was efficiently inhibited by the homologous chain in each case (Table 4). The small amounts of AChR in human muscle [~1 pmol/g (22)] have thus far precluded its purification and direct analysis of its polypeptide composition. These studies indicate that immunological and structural similarities between human and torpedo AChR occur at several different sites.

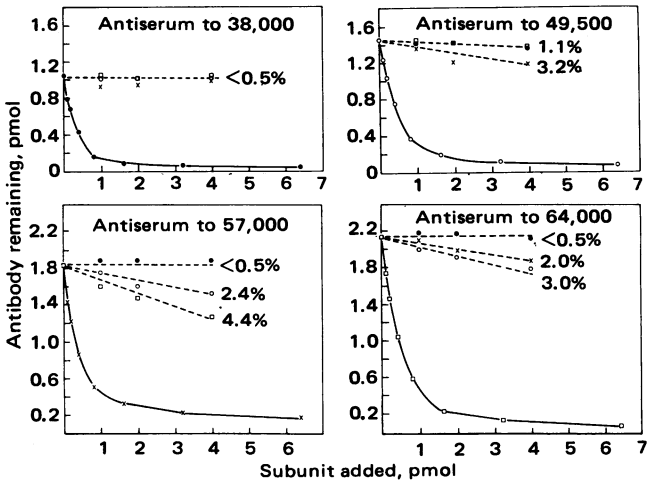

FIG. 2. Inhibition by polypeptide chains of the reaction of antisera to chains with native torpedo AChR. Constant amounts of rat antisera to torpedo AChR polypeptide chains were incubated with increasing amounts of torpedo AChR chains (from the preparation shown in Fig. 1) to inhibit antibodies binding to the chains. Then excess native torpedo AChR labeled with ^{125}I-labeled αBGT was added to react with the remaining antibodies. Some inhibition was caused by addition of chains other than the chain to which the serum was raised. The percent contamination of the other chain by the homologous chain necessary to produce this inhibition is noted at the end of each line. ●, M_r 38,000; ○, M_r 49,500; ×, M_r 57,000; □, M_r 64,000. Triplicate assays were averaged for each point. Chains were diluted in 0.8 ml of 0.2% cholate buffer. Protein concentration was estimated by the method of Lowry, and molar concentration was calculated using values for apparent M_r obtained by $NaDodSO_4$ acrylamide gel electrophoresis. Normal rat serum (5 μl) was added to each tube. Antisera (diluted 1/200–1/1000 in 0.2% cholate buffer) were added in 100-μl aliquots. After 24 hr at 4°, 100 μl of torpedo AChR labeled with ^{125}I-labeled αBGT (100 and 50 nM, respectively) was added for an additional 15 hr. Then goat anti-rat IgG (10 μl) was added to each tube. After 4 hr, tubes were centrifuged and ^{125}I in the washed pellets was measured. Antibody was measured in mol of ^{125}I-labeled αBGT binding sites of AChR precipitated.

DISCUSSION

The experiments described provide important information about structure of the AChR molecule: (*i*) All four polypeptide chains (M_r 38,000, 49,500, 57,000, and 64,000) are part of each macromolecular complex, since excess antibodies against any chain caused precipitation of all torpedo AChR labeled with ^{125}I-labeled αBGT. (*ii*) The four polypeptide chains are immunologically distinct. (*iii*) Chains in addition to the 38,000 M_r chain [which forms part of the ACh binding site (1)] are conserved through evolution, and so must be important to the physiological function of AChR. (*iv*) AChR from both human and rat muscle contain determinants specifically crossreacting with determinants characteristic of all four chains of torpedo AChR. (*v*) In rat muscle AChR, at least part of the determinants crossreacting with the 64,000, 57,000, 49,500, and 38,000 M_r chains are exposed on the extra-cellular surface of the AChR molecule, since antibodies crossreacting with these determinants bound to rat muscle AChR *in vivo* and caused a decrease in muscle AChR content.

Some of the data reported are of particular relevance to EAMG and myasthenia gravis. Most importantly, these data clearly demonstrate that no single antigenic determinant on AChR is required to induce EAMG, since four different polypeptide chains composing the AChR macromolecule induce EAMG. Furthermore, since antibodies to these chains are directed at determinants on the AChR molecule that were not

Table 4. Inhibition by polypeptide chains of crossreaction of antibodies to torpedo polypeptides with native human muscle AChR

Sample	AChR bound (cpm × 10^{-3} ± SEM) to human muscle AChR
Anti-64,000 serum	3.90 ± 0.33
+64,000 M_r chain	0.00 ± 0.20
+57,000 M_r chain	3.99 ± 0.17
+49,500 M_r chain	3.54 ± 0.08
+38,000 M_r chain	4.63 ± 0.23
Anti-57,000 serum	9.26 ± 0.65
+64,000 M_r chain	6.00 ± 0.07
+57,000 M_r chain	1.45 ± 0.23
+49,500 M_r chain	6.63 ± 0.08
+38,000 M_r chain	8.94 ± 0.15
Anti-49,500 serum	7.12 ± 0.27
+64,000 M_r chain	5.17 ± 0.11
+57,000 M_r chain	4.54 ± 0.35
+49,500 M_r chain	1.14 ± 0.16
+38,000 M_r chain	6.41 ± 0.22
Anti-38,000 serum	8.68 ± 0.35
+64,000 M_r chain	8.43 ± 0.28
+57,000 M_r chain	8.68 ± 0.17
+49,500 M_r chain	8.54 ± 0.41
+38,000 M_r chain	2.45 ± 0.15

The rat anti-torpedo polypeptide chain antisera used in Fig. 2 (the first, fifth, eleventh, and thirteenth samples in Table 2) were diluted to 13–39 pM in antibodies crossreacting with human muscle AChR. Triplicate 375-μl samples of diluted serum were incubated at 4° for 36 hr alone or with aliquots of the indicated polypeptide chain. The homologous chain to each antiserum was added equal to 0.67-fold the moles of anti-native torpedo AChR antibodies present in the mixture. Equal molar amounts of the other chains were also added. To detect antibodies not inhibited by preincubation with chains, we added toxin-labeled AChR (125 μl of 4 nM AChR + 8 nM ^{125}I-labeled αBGT) overnight to all tubes. Bound toxin-labeled AChR was then precipitated with goat anti-rat IgG, and ^{125}I in the washed pellets was measured. A normal serum blank value was subtracted from all samples.

immunogenic on the native AChR molecule, it appears that an immune response to one or more of many antigenic sites will induce EAMG. This is not surprising, since loss of AChR rather than direct inhibition of AChR activity appears to be the most important factor causing impairment of neuromuscular transmission in EAMG and myasthenia gravis (23). Antibody-dependent, complement-mediated damage of the postsynaptic membrane (24, 25), for example, should not depend critically on which determinant of the AChR molecule is bound with antibody, nor would loss of AChR through antigenic modulation (16, 26, 27) be expected to depend strongly on where antibodies bind to AChR. The most immunogenic determinants on the native AChR molecule are formed by the tertiary conformation of its polypeptide chains. EAMG was induced in rats through immunization with the less immunogenic denatured polypeptide chains comprising AChR by administering large doses. Our previous attempts, using chains from electrophorus AChR (13), were not successful due to the small amounts of material available. Like Claudio and Raftery (4), we have not yet induced obvious muscular weakness in our immunized rabbits. This is probably because immunization of rabbits with large amounts of chain failed to produce high titers of antibody crossreacting with native torpedo AChR or muscle AChR.

Specific anti-chain sera like those we have prepared provide templates for comparing specific structures on AChR from torpedo with structures on AChR from other sources, especially muscle AChR, which are difficult to study directly. However, it remains to be determined which of the polypeptide chains observed in muscle AChR (5–7) will crossreact with each of the chains from torpedo. This may be a complex problem, since there appear to be differences in the polypeptide chain pattern even between electric organ AChR from torpedo and eel (10).

Studies of the effects of specific anti-chain sera on the activity of AChR in electric organ and muscle should help determine the functions of these chains in the AChR molecule.

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1. Karlin, A., Weill, C. L., McNammee, M. G. & Valderrama, R. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **40,** 203–210.
2. Raftery, M. A., Vandlen, R. L., Reed, K. L. & Lee, T. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **40,** 193–202.
3. Chang, H. W. & Bock, E. (1977) *Biochemistry* **16,** 4513–4520.
4. Claudio, T. & Raftery, M. A. (1977) *Arch. Biochem. Biophys.* **181,** 484–489.
5. Froehner, S. C., Reiness, C. G. & Hall, Z. (1977) *J. Biol. Chem.* **252,** 8589–8596.
6. Froehner, S., Karlin, A. & Hall, Z. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4685–4688.
7. Boulter, J. & Patrick, J. (1977) *Biochemistry* **16,** 4900–4908.
8. Sobel, A. & Changeux, J. P. (1977) *Biochem. Soc. Trans.* **5,** 511–514.
9. Merlie, J. P., Changeux, J. P. & Gros, F. (1977) *J. Biol. Chem.*, in press.
10. Karlin, A. (1977) in *Pathogenesis of Human Muscular Dystrophies*, ed. Rowland, Ed. (Excerpta Medica, Amsterdam-Oxford), pp. 73–84.
11. Patrick, J. & Lindstrom, J. (1973) *Science* **180,** 871–872.
12. Patrick, J., Lindstrom, J., Culp, B. & McMillan, J. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3334–3338.
13. Lindstrom, J., Lennon, V., Seybold, M. & Whittingham, S. (1976) *Ann. N.Y. Acad. Sci.* **274,** 254–274.
14. Lindstrom, J. M., Einarson, B., Lennon, V. A. & Seybold, M. E. (1976) *J. Exp. Med.* **144,** 726–738.
15. Lindstrom, J. M., Engel, A. G., Seybold, M. E., Lennon, V. A. & Lambert, E. H. (1976) *J. Exp. Med.* **144,** 739–753.
16. Heinemann, S., Bevan, S., Kullberg, R., Lindstrom, J. & Rice, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3090–3094.
17. Engel, A., Tsujihata, M., Lindstrom, J. & Lennon, V. (1976) *Ann. N.Y. Acad. Sci.* **274,** 60–79.
18. Lambert, E., Lindstrom, J. & Lennon, V. (1976) *Ann. N.Y. Acad. Sci.* **274,** 300–318.
19. Lindstrom, J. (1977) in *The Biochemistry of Myasthenia Gravis and Muscular Dystrophy*, eds. Marchbanks, R. & Lunt, G. (Academic Press, New York), pp. 135–156.
20. Laemmli, U. (1970) *Nature* **227,** 680–685.
21. Lennon, V. A., Lindstrom, J. M. & Seybold, M. E. (1975) *J. Exp. Med.* **141,** 1365–1375.
22. Lindstrom, J. (1977) *J. Clin. Immunol. Immunopath.* **7,** 36–73.
23. Lindstrom, J. & Lambert, E. (1977) *Neurology*, in press.
24. Sahashi, K., Engle, A. G., Lindstrom, J. M., Lambert, E. H. & Lennon, V. A. (1977) *J. Neuropathol. Exp. Neurol.* **36,** 626.
25. Engel, A., Lambert, E. & Howard, G. (1977) *Mayo Clin. Proc.* **52,** 267–280.
26. Kao, I. & Drachman, D. B. (1977) *Science* **196,** 527–529.
27. Appel, S. A., Anwyl, R., McAdams, M. W. & Elias, S. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2130–2134.