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IMMUNOLOGICAL STUDIES OF ACETYLCHOLINE RECEPTORS

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Immunochemical techniques for the study of acetylcholine receptors are described. Immunization of rabbits, rats, guinea pigs, and goats with acetylcholine receptor protein purified from Electrophorus electric organ tissue results in muscular weakness and death due to impaired neuromuscular transmission. Serum from immunized animals contains high concentrations of antibodies directed at receptors from the electric organ and low concentrations of antibodies directed at receptors from skeletal muscle. The detailed similarities between the disease of receptor-immunized animals, "experimental autoimmune myasthenia gravis" (EAMG), and myasthenia gravis are compared. Reactions of antisera from animals with EAMG with receptor from Electrophorus and Torpedo are studied. Antireceptor antibodies in these antisera are directed predominantly at determinants other than the acetylcholine-binding site.

INTRODUCTION

The nicotinic acetylcholine receptor is an integral protein of the postsynaptic membrane. Receptor binds acetylcholine released from the nerve and triggers a depolarization of the postsynaptic membrane. At the nerve-muscle junction on frog sartorius muscle, for example, during the millisecond that a receptor is activated by the binding of an acetylcholine molecule, a cation selective pore is opened through the membrane allowing passage of about 5×10^4 ions (1). The receptor macromolecule thus has two functionally important components – the stereospecific binding site for acetylcholine, and the pore or "ionophore" that it regulates. There is an abundance of drugs and toxins capable of binding to the acetylcholine binding site. By contrast, there is almost (2) a complete lack of drugs or toxins which affect the ionophore or the control of the ionophore by the regulatory portion of the receptor. Antibodies recognizing determinants on the receptor protein, in principle, could be used as probes for sites on the receptor molecule other than the acetylcholine-binding site. Antibodies are known to inhibit receptor activity on cells in vitro (3, 4) when antisera are applied, and in vivo when animals are autoimmune against receptor (3, 4, 5). Thus some, or all of the sites on receptor to which antibodies bind are important to its physiological function. This paper describes studies of receptor by immunological methods.

METHODS

Acetylcholine receptor was purified by affinity chromatography on cobra toxin-agarose conjugates by modifications of the method previously described (6). These

modifications consist of batch application of receptor extract to toxin agarose (0.5 mg toxin ml of epichlorohydrin cross-linked agarose A50M), batch washing of the gel by centrifugation, and batch elution with 0.5 M carbamylcholine followed by DEAE chromatography to concentrate receptor and remove carbamylcholine. These modifications eliminate the necessity for further purification on sucrose gradients and virtually eliminate contamination of purified receptor with toxin. Receptor is usually diluted in a solution containing 0.5% Triton X-100, 100 mM NaCl, 10 mM Na phosphate pH 7.0, 10 mM NaN_3. Receptor concentration, expressed as moles toxin-binding sites per liter, is determined by separation of I^{125}-toxin-receptor complexes from excess toxin on sucrose gradients (6), chromatography on a 1 × 10-cm G200 column, by immunoprecipitation, or by DEAE filter paper (7). The DEAE method gave twofold higher values than the others and was not routinely used.

Naja naja siamensis toxin was purified according to Karlsson (8) and uniformly iodinated using chloramine T.

Immunoprecipitation assays were performed essentially as previously described for rabbit antireceptor sera (4), though other animals and various serum fractions including pure gamma globulin were used in different experiments as indicated in the figure legends. In all cases sufficient antigamma globulin was used to maximize the size of the precipitate. Precipitates were washed once with 1 ml 0.5% Triton buffer before being dissolved in 1% acetic acid (200 μl) and placed in scintillation fluid.

Rabbits were immunized with receptor protein in complete Freund's adjuvant as previously described (3). A female goat was immunized twice at a 2-week interval with 1.4 mg of receptor in 4.1 ml of an adjuvant-buffer emulsion. Six days after the second injection the animal showed signs of myasthenia which responded to intramuscular neostigmine. By 11 days after the second injection the goat could stand only with great difficulty and was exsanguinated. Female Lewis rats were immunized (5) with a single injection of 0.1 mg of receptor in 0.2 ml of adjuvant emulsion at several intradermal sites, and at separate sites were injected subcutaneously with B. pertussis vaccine as an additional adjuvant (10^{10} organisms, a special product of Eli Lilly & Company).

Acetylcholine receptors were solubilized from rat and human muscle as a crude extract. Muscle was homogenized at 4°C with 4 vol of 0.1 M Na phosphate, pH 7.0. The pellet from centrifugation for 30 min at 10^5 × g was extracted with 2 vol of 2% Triton X-100 in 0.1 M NaCl, 0.01 M Na phosphate, pH 7.0, 0.01 M NaN_3 by agitation at 4°C for 1 hr. The supernatant obtained after centrifugation for 30 min at 10^5 × g contained receptor at about 2×10^{-10} M as demonstrated by benzoquinonium-protectable labeling with I^{125}-toxin.

Linear sucrose gradients (5 ml 5–20% sucrose, 0.5% Triton, 0.1 M NaCl, 0.01 M Na phosphate, pH 7.0) were layered with 0.1 ml samples and centrifuged in an SW50.1 rotor at 50,000 rpm for 9 hr before being fractionated by puncturing the bottom of the tube (6). Double diffusion in agar was conducted as described (3).

Experimental Autoimmune Myasthenia Gravis

Sera from animals immunized with receptor protein purified from Electrophorus contained precipitating antireceptor antibodies. Sera from even different species of immunized animals formed only a single precipitin line on double diffusion in agar against pure receptor (Fig. 1), indicating homogeneity of the receptor preparation used for immunization.

Animals immunized with receptor protein from Electrophorus (3, 4, 5, 9) or

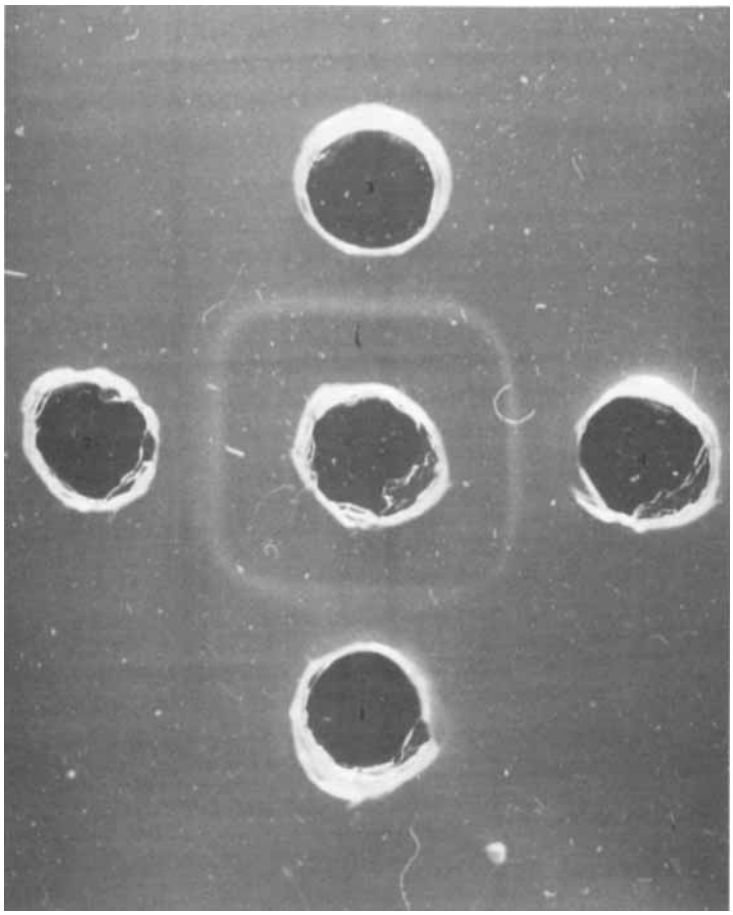

Fig. 1. Double diffusion in agar of receptor against antisera from rabbit and goat. The center well contains a 1 mg/ml solution of purified receptor from Electrophorus. Wells 1 and 2 contain goat anti-receptor sera. Wells 3 and 4 contain rabbit antireceptor sera.

Torpedo (10, 5) develop muscular weakness characterized by easy fatigability, and may die in flaccid paralysis. All species thus far tested, including rabbits (3, 9, 10), goats, rats (5), and guinea pigs (5), succumb to this disease which we have termed "experimental autoimmune myasthenia gravis" (EAMG) (5). [1] Demonstration of a decreasing muscle response to repetitive stimulation by electromyography, and repair of this decrementing response by administration of anticholinesterase agents, confirmed that neuromuscular transmission was defective in these animals (3, 5). Further, demonstration that immune rabbit serum could block the response of electroplax to carbamylcholine (4) and of cultured myofibers to ionotophoretic application of acetylcholine (3), suggested that the immunized animals' myasthenia resulted from an autoimmune response against the acetylcholine receptors of their skeletal muscles. Direct electrophysiological evidence of receptor blockage in rats with EAMG has been obtained by Dr. Ed Lambert of the Mayo Clinic (Fig. 2). In EAMG the amplitude of miniature endplate potentials is greatly reduced,

[1] Abbreviations: EAMG, experimental autoimmune myasthenia gravis; MG, myasthenia gravis.

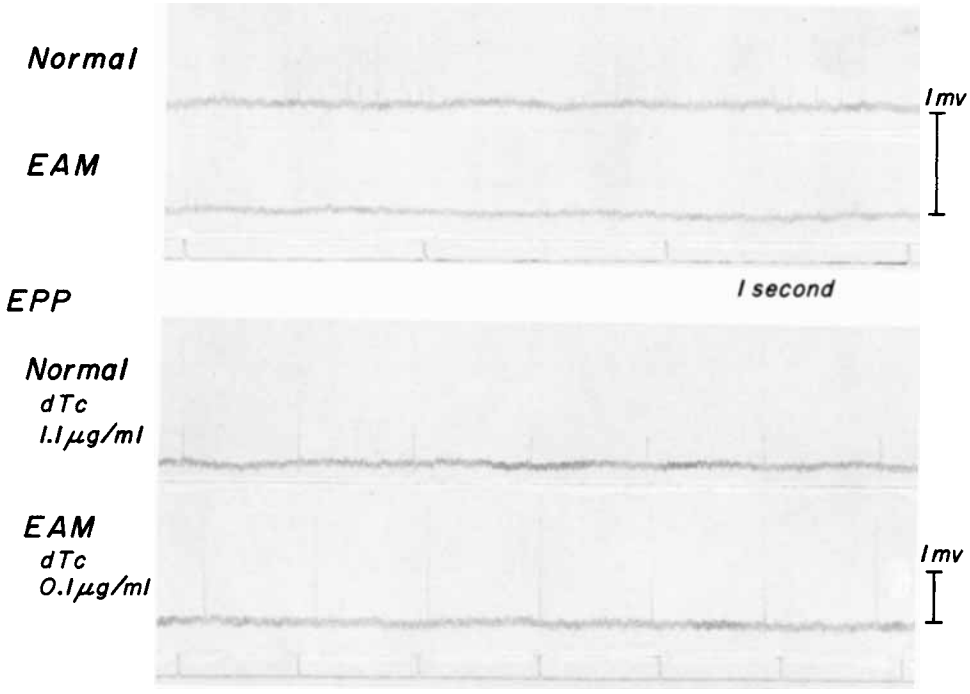

Fig. 2. Endplate potentials in rat diaphragm. The upper portion of the figure compares intracellular microelectrode recordings of miniature endplate potentials (MEPP) from a nerve muscle junction of a normal muscle and a rat with EAMG. The lower portion of the figure compares curare concentrations required to reduce the amplitude of neurally evoked endplate potentials (EPP) to the same value below the threshold necessary to evoke a muscle action potential. The rat used in this figure was injected at The Salk Institute in collaboration with Vanda Lennon and Marjorie Seybold and studied at the Mayo Clinic by Ed Lambert.

and the sensitivity to curare increased, yet the total number of quanta available for release are unaltered (11). These are precisely the results expected if the antibodies against rat muscle acetylcholine receptors present in these animals (see below) were inhibiting receptor activity. After binding of antibodies, reduced numbers of active receptors or large numbers of partially inactivated receptors would reduce the endplate potential triggered by the acetylcholine in single spontaneously released quanta, and a smaller number of receptors would have to be blocked by curare to reduce the amplitude of the endplate potential below the point where it could trigger a muscle action potential.

Assay of Antibodies to Acetylcholine Receptor

Antibodies to receptor are routinely assayed using as antigen receptor labeled at acetylcholine binding sites with I^{125}-toxin (4). We have shown that superimposable precipitation curves are obtained using receptor reversibly labeled at acetylcholine binding sites with I^{125}-toxin or covalently labeled at other sites with [^{3}H] acetic anhydride (4). We have also shown that binding of antibody to receptor does not greatly alter affinity of receptor for either I^{125}-toxin or benzoquinonium (12).

Labeling of receptor with I^{125}-toxin is achieved by first adding excess I^{125}-toxin for several hours, and then adding antireceptor serum. Since the acetylcholine-binding site is occupied by I^{125}-toxin, only antibodies directed at other determinants can bind. Then complexes of I^{125}-toxin-receptor-antibody are precipitated by the addition of carrier antibody and antiantibody and the I^{125} in the pellet is measured. Controls are performed to allow correction for the small amount of I^{125} trapped in a pellet formed from normal serum, and to test for the trace amounts of antitoxin antibody occasionally present.

Figure 3 shows precipitin curves obtained by adding increasing amounts of goat antiserum to fixed amounts of labeled receptor. In one curve the receptor used was a crude extract of Electrophorus electric organ, and in the other curve an equivalent amount of pure receptor protein was used. Curvature in the plots probably results from binding of more than one antibody per receptor at concentration ratios of antibody to receptor near 1. Single point determinations of antibody titer are made in the presence of substantial excesses of receptor in order to reflect the initial slope. The nearly identical slopes of these curves suggest that the purified receptor is immunologically indistinguishable from receptor in the initial extract, thus unaltered during purification.

Antibodies to Receptors from Rat Muscle in EAMG

Antibodies against rat skeletal muscle acetylcholine receptors are present in the sera of rats immunized against receptor from Electrophorus (Fig. 4). The slope of this curve indicates a titer of antirat acetylcholine receptor antibody of 1.4×10^{-8} moles

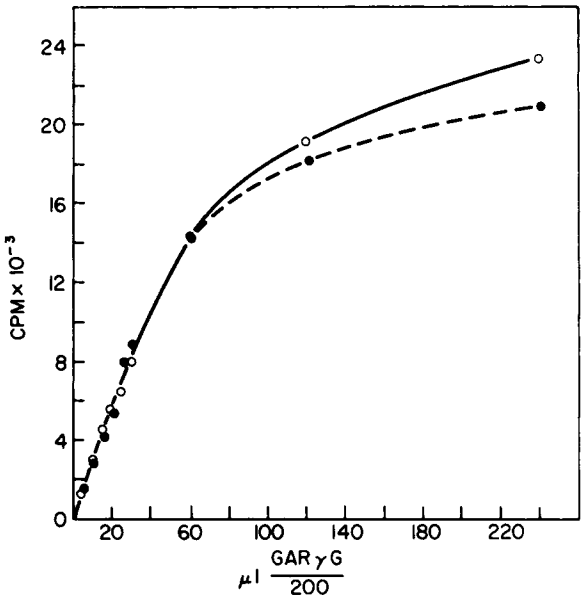

Fig. 3. Immune precipitation of I^{125}-toxin labeled receptor from crude extracts and after purification. Receptor at 1.5×10^{-7} M in toxin-binding sites diluted from a crude extract (○) or after purification (●) was incubated with a five fold excess of I^{125}-toxin. Triplicate aliquots (200 μl) of each were incubated overnight at 4°C with the indicated amounts of goat antireceptor gamma globulin from a 0.102 mg/ml stock and 70 μg of control goat gamma globulin. Antibodies were precipitated by overnight incubation with 0.49 mg of affinity purified rabbit antigoat gamma globulin.

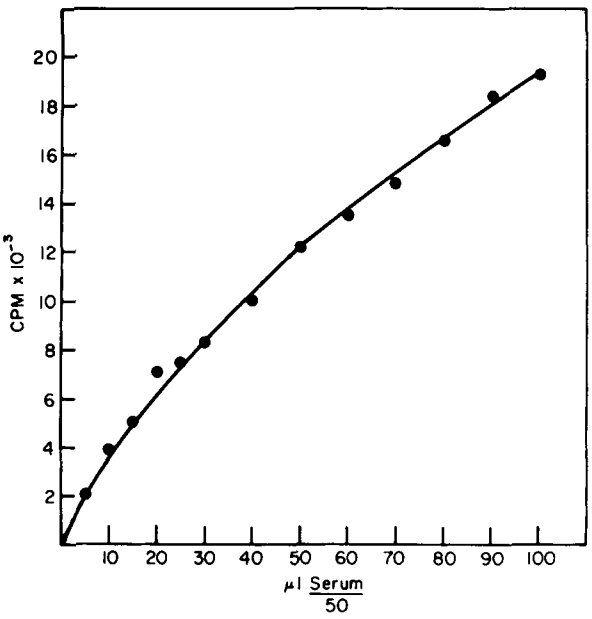

Fig. 4. Immune precipitation of rat muscle acetylcholine receptor by serum from a rat with EAMG. Duplicate tubes containing 1 ml of rat muscle acetylcholine receptor (2×10^{-10} M) previously labeled using I^{125}-toxin (4×10^{-9} M) were incubated overnight with the indicated amounts of serum, and sufficient normal serum to make 100 μl total serum/50. Rabbit antirat gamma globulin serum was used for precipitation.

toxin-binding sites precipitated per liter. Since receptor is a multimer (6), and only one antibody molecule need bind to produce precipitation in this assay, the molar concentration of antibody is one-half to one-third this amount. Assay in the presence of excess receptor from Electrophorus indicated a titer of antibodies against receptor from Electrophorus of 1.0×10^{-5} M, or about 10^3 times the titer of antibodies against rat muscle acetylcholine receptor. Thus, although homology between these two nicotinic receptors is sufficient to induce an autoimmune response, the degree of homology demonstrable serologically is quite small. The concentration of antibodies recognizing solubilized rat receptors is still substantial. If all of the antibodies present in the serum capable of binding to receptors solubilized from rat muscle were capable of binding to and partially or completely inhibiting the activity of receptors at nerve muscle junctions, it seems likely that an adaptive response, for example increased receptor turnover, might be necessary to allow even the marginally effective neuromuscular transmission observed in these animals.

Antibodies to Receptors from Human Muscle in MG

Sera from humans with myasthenia gravis (MG)[1] contain antibodies against acetylcholine receptors from human skeletal muscle (Table I). Using the double precipitin assay to detect antibodies against determinants other than the acetylcholine-binding site, anti-human muscle acetylcholine receptor antibodies have been found in 82 of 84 sera from MG patients tested (12). Under the assay conditions used, reaction with Electrophorus receptor was negligible (12). Only a few of these sera prevented labeling of receptor with

TABLE I. Antireceptor Antibody Concentration in Myasthenia Gravis and Experimental Autoimmune Myasthenia

	Titers of antireceptor from muscle	Titers of antireceptor from eel
Rats with chronic autoimmune myasthenia	1.3×10^{-8} M ($0.45 - 1.9 \times 10^{-8}$ M)	7.7×10^{-6} M ($5.7 - 9.2 \times 10^{-6}$ M)
Humans with myasthenia gravis	8.5×10^{-9} M ($0.026 - 41 \times 10^{-9}$ M)	$< 10^{-10}$ M

Titers are expressed as moles toxin-binding sites precipitated per titer of serum using as antigen I^{125}-toxin labeled receptor from rat or human muscle or Electrophorus electric organ as indicated. The figures for rats are the average and range of four animals with severe myasthenia 30 days after immunization with 1.5 μg of pure receptor. The figures for humans are the average and range of 50 myasthenia gravis patients (12).

I^{125}-toxin, indicating that few of the antibodies present were directed at the acetylcholine-binding site. It is known that MG patients have fewer I^{125}-toxin binding sites at their endplates (13). In MG, endplate morphology is simplified and there is evidence of degeneration and regeneration of synapses (14), so it does not necessarily follow that this loss of receptor sites is due to occlusion of binding to receptors by antibodies at their acetylcholine-binding sites.

Myasthenia gravis appears to result from an autoimmune response to skeletal muscle acetylcholine receptors. There are a number of similarities between MG and EAMG, the most striking of which is the presence of antireceptor antibody. Other similarities include weakness, fatigability, decrementing electromyograms, alleviation of symptoms by anticholinesterase drugs, small miniature endplate potentials, increased curare sensitivity, and simplified folding of the postsynaptic membrane (see references 14, 15 and 16 for evidence of these defects in MG; see references 11, 12, 19, 20, 21 for evidence of these defects in EAMG). Since in both cases the antibodies in the sera are directed predominantly at receptor determinants other than the acetylcholine-binding site, blockage of receptor activity in the intact membrane may result either from an allosteric effect on acetylcholine binding or interference with the regulation or function of the ionophore.

Absence of Antibodies to Toxin-Binding Site of Receptor

Only a small fraction of the antibodies formed against receptor are directed at the acetylcholine binding site. Antibody binding to receptor can be detected without I^{125}-toxin labeling the receptor, by labeling the receptor with [^{3}H] acetic anhydride (4). This does not alter the antigenic properties of the receptor (4). Toxin-binding activity of receptor remains after acetylation (Fig. 5). Patrick et al. (4) demonstrated that precipitin curves against ^{3}H-Ac-labeled receptor using sera from receptor-immunized rabbits were only slightly altered in slope by the presence of excess unlabeled toxin to protect the acetylcholine-binding site. A similar experiment with immune goat serum (Fig. 6) was unable to detect any antisite antibody. No measurable fraction of the antibodies were inhibited from binding to the receptor by the toxin covering the acetylcholine-binding site. This is somewhat surprising since we know this site is exposed on the receptor. Observation of very little cross-reaction between acetylcholine receptors of rats and Electrophorus at sites other than the acetylcholine binding site argues against extensive cross-reaction at this site, but if only small amounts of antisite antibody were produced and it cross-reacted extensively, it might all be adsorbed on receptors of the immunized animal. It may simply

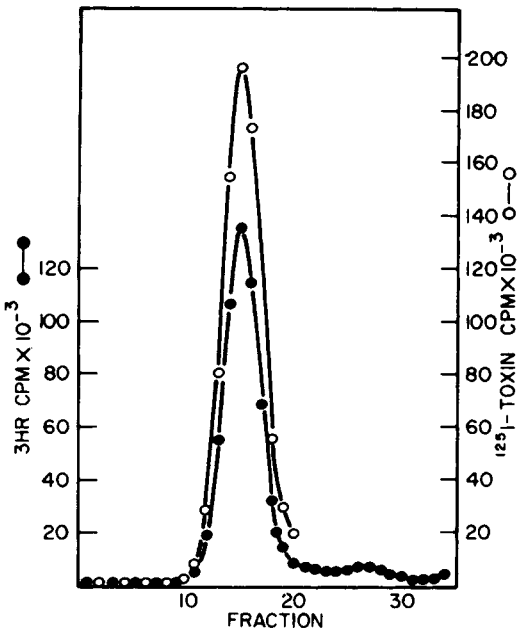

Fig. 5. Sedimentation of ^{3}H Ac-receptor labeled with I^{125}-toxin. A sample of ^{3}H Ac-receptor incubated with excess I^{125}-toxin was sedimented on a sucrose gradient as described in Methods. Fraction 1 is the bottom of the tube. Fractions were assayed both by differential scintillation counting and by gamma counting. The peak at the top of the tube containing excess free toxin is not shown.

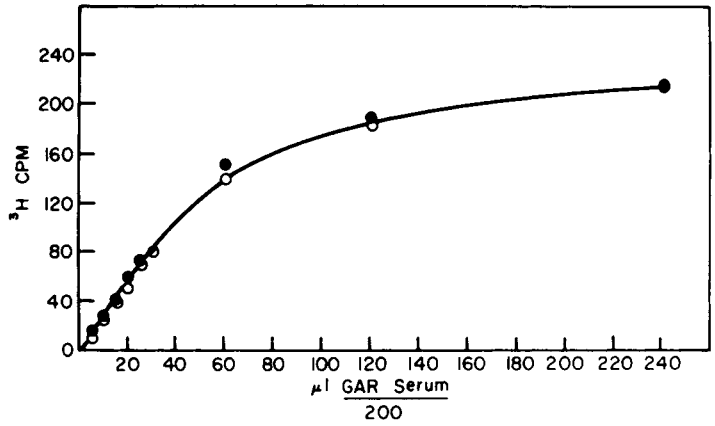

Fig. 6. Immune precipitation of receptor ± toxin. Pure receptor from Electrophorus was labeled with ^{3}H Ac. Labeled receptor at 2×10^{-8} M in toxin-binding sites (○) and an identical preparation incubated for 1 hr at 4°C with 4×10^{-8} M unlabeled toxin (●) were dispensed in triplicate 200-μl aliquots. Then the indicated aliquots of diluted goat antireceptor serum (initial titer 3.8×10^{-6} M toxin-binding sites precipitated per liter) were added for 8 hr at 4°C. Control goat gamma globulin (48 μg) was added to each tube along with sufficient rabbit antigoat serum for precipitation for 8 more hr.

be that the acetylcholine-binding site does not contribute to the antigenicity of the receptor greatly out of proportion to the small fraction of the surface area of the receptor that it comprises.

How is receptor activity blocked by antibodies? A direct antagonistic action preventing acetylcholine binding is one possibility. Table II shows that immune goat serum can inhibit I^{125}-toxin binding to electric organ cells. But the stoichiometry between antibody binding and blockage of toxin binding is unknown, since even with 10-fold diluted serum there is a 10^4 excess of antibodies over receptor sites on exposed cells. Since most of these antibodies do not bind to the toxin-binding site directly, their blockage of toxin binding and their blockage of receptor activity might be allosteric and incomplete or result from steric inhibition of a trivial sort due to the huge excess of antibodies over receptor.

TABLE II. Toxin Binding to Electroplaques After Exposure to Antireceptor Serum

Sample	cpm	Specific sites labeled/cell
(A) Cells + I^{125}-toxin	2493 ± 164	4.2×10^{10}
(B) Cells + antiserum + I^{125}-toxin	1226 ± 185	1.8×10^{10}
(C) Cells + benzoquinonium + I^{125}	273 ± 18	

Cells dissected from the organ of Sachs were incubated at room temperature for 20 min in 10 ml of (A) Ringer's solution, or (B) a 10-fold dilution in Ringer's of a 15% Na_2SO_4 out of goat anti-receptor serum having a final titer of 3×10^{-7} M toxin-binding sites precipitated per liter, or (C) 10^{-3} M benzoquinonium in Ringer's. Then all solutions were made 10^{-8} M in I^{125}-toxin for 20 min. After washing quickly four times with 10 ml Ringer's, and for 2 hr with another 10 ml, each cell was lysed with 0.2 ml of 2% SDS at 37°C for 30 min before I^{125} was measured by scintillation counting.

Immunological Comparison of Receptors from Electrophorus and Torpedo

Acetylcholine receptors from Electrophorus electricus electric organ can be compared immunologically with those of Torpedo californica. The extent of similarity to expect between the nicotinic acetylcholine receptors from the electric organs of a fresh water teleost such as Electrophorus and a marine elasmobranch such as Torpedo is not evident. Even the molecular size of receptor from Electrophorus and Torpedo differs. Receptor extracted from fresh Torpedo electric organ sediments on sucrose gradients mostly as a dimer of the size extracted from Electrophorus, but can be converted to the size of Electrophorus by mild proteolysis of the membranes prior to solubilization of the receptor (Fig. 7). About 10 times as much goat anti-Electrophorus receptor serum is required to precipitate an equivalent amount of Torpedo receptor (Fig. 8).

Only a small fraction of goat anti-Electrophorus receptor antibodies recognize receptor from Torpedo as shown by quantitative precipitin curves. Figure 9A shows that the titer of goat anti-Electrophorus receptor against receptor from Torpedo (as measured by the slope of the curve in terms of moles of toxin-binding sites precipitated per liter of serum) is only 3% of its titer against receptor from Electrophorus. Adsorption of goat anti-eel receptor serum with a 10-fold excess of Torpedo receptor in membrane fragments reduces its titer against Torpedo receptor 60% (Fig. 9B), whereas the titer against eel is reduced only 13% (Fig. 10). Some of the loss on adsorption is probably nonspecific, and adsorption may not be complete, despite the excess of Torpedo receptor. Thus, it is uncertain whether the 40% titer against Torpedo remaining after adsorption with Torpedo

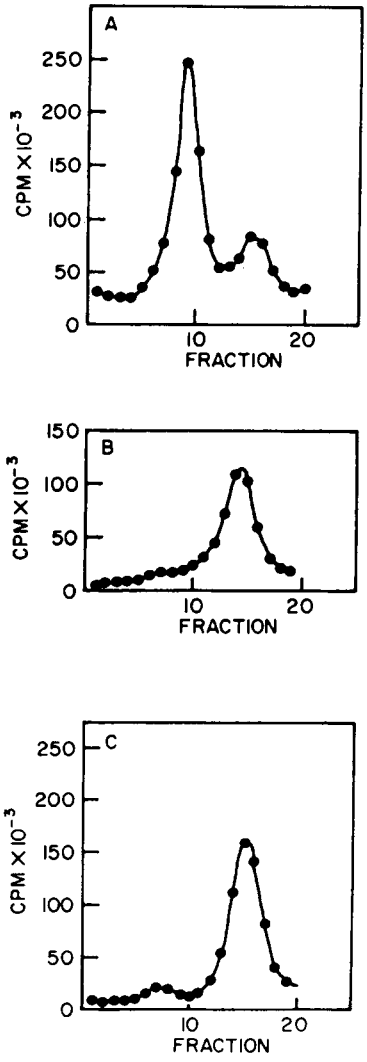

Fig. 7. Sedimentation of receptor from Torpedo and Electrophorus on sucrose gradients. Sucrose gradients of I^{125}-toxin-labeled receptor fractions were centrifuged as described in Methods. The first fraction is from the bottom of the tube. Only the first 20 of 34 fractions are shown to eliminate the peak of excess I^{125}-toxin at the top of the tube. Torpedo membrane fractions used in this experiment were obtained from Palmer Taylor (18). (A) Torpedo receptor crude extract from membrane pellet of fresh electric organ treated with 1 M NaCl to remove acetylcholinesterase (0.1 ml sample of 3.6×10^{-6} M in toxin-binding sites). (B) Electrophorus purified receptor (0.1 ml sample of 1.8×10^{-6} M). (C) Torpedo receptor crude extract from aliquot of membrane pellet used in (A) after trypsin treatment to remove acetylcholinesterase (5 μg/ml 5 min at 25°C terminated by 10 μg/ml soybean trypsin inhibitor).

is due to incomplete adsorption or is due to antibodies recognizing determinants on the receptor which are not revealed when it is in the membrane.

Antibodies adsorbed to Torpedo membranes can be eluted with 3 M KSCN (Fig. 11). The amount recovered in the eluate is 6.6% of that initially exposed to the membranes, or

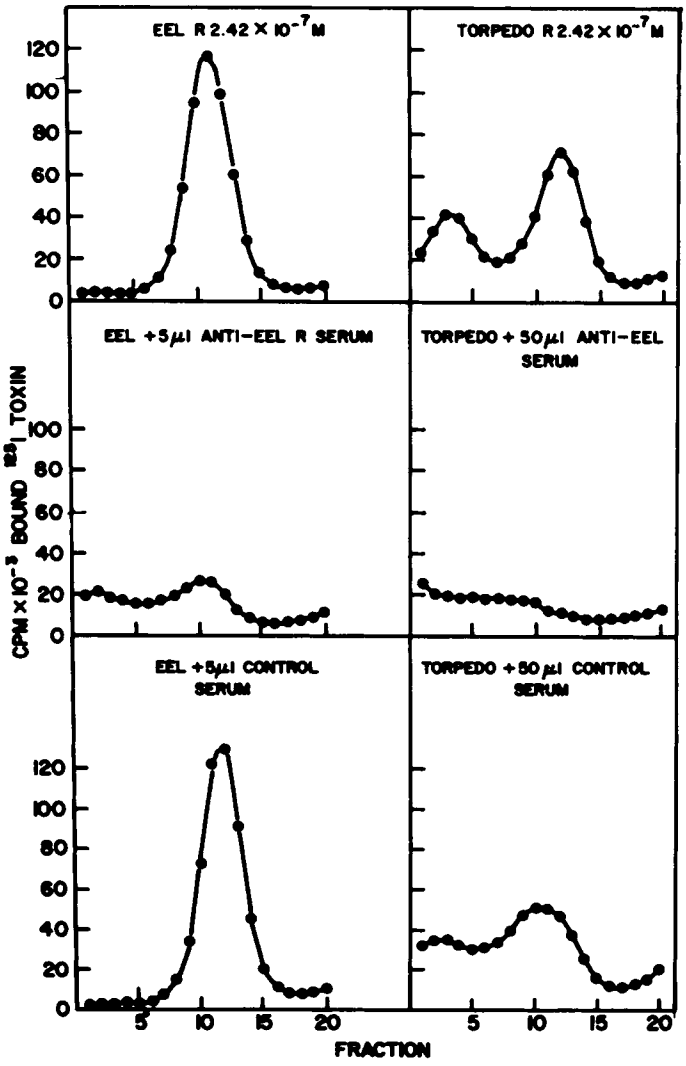

Fig. 8. Precipitation of I^{125}-toxin-labeled receptor from sucrose gradients by goat antiserum against Electrophorus receptor. Sucrose gradients were centrifuged as described in Methods. Fraction 1 is at the bottom of the tube. Fractions between 20 and 34 which contain the excess toxin peak are not shown. Aliquots of Electrophorus or Torpedo receptor (0.2 ml) incubated with excess I^{125}-toxin were incubated overnight with the indicated amounts of serum and then sedimented on sucrose gradients.

about half of that bound. This is all antibody directed at determinants other than the acetylcholine-binding site. It might be expected that if the acetylcholine-binding site were a very conservative portion of the receptor, a larger fraction of the eluted antibody would be directed at this site than in the initial antiserum. Yet when the eluted antibody was assayed for antibody against the acetylcholine binding site by precipitin curves against ^3H-acetylated receptor in the presence or absence of unlabeled toxin, no antibody against the site was detectable (Fig. 12). Since none was detectable by this method initially, these

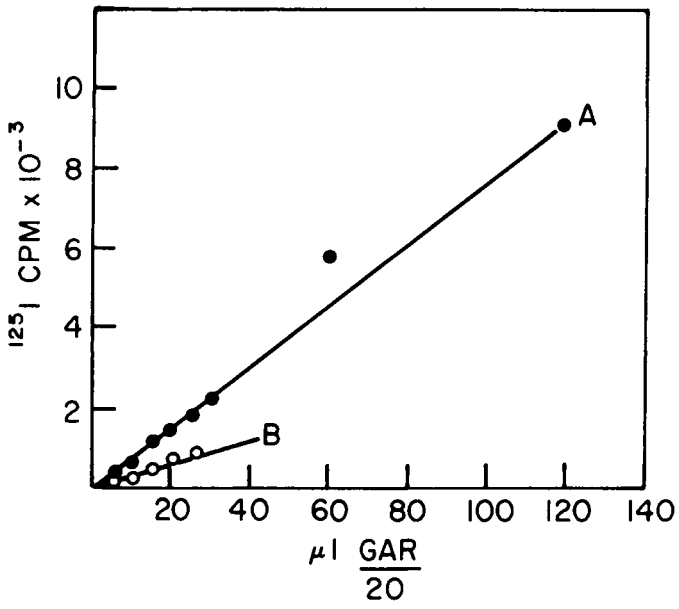

Fig. 9. Immune precipitation of I^{125}-toxin-labeled Torpedo receptor by goat antiserum against Electrophorus receptor before and after adsorption with Torpedo electric organ membranes. Immunoprecipitation is conducted as in Fig. 3 except I^{125}-toxin-labeled Torpedo receptor is used. (A) Shows precipitation by goat serum raised against receptor from Electrophorus. (B) Shows precipitation after adsorption of the serum with a 10-fold excess of Torpedo toxin-binding sites over its precipitating titer in the form of electric organ membrane fragments. Serum (2 ml 3×10^{-6} M) in anti-Electrophorus receptor was adsorbed with 5 ml of membrane fragments containing 64 nmoles Triton X-100-extractable toxin-binding sites overnight with shaking. The supernatant and two 9-ml saline washes were pooled to give 20 ml of serum/10.

antibodies might be enriched several-fold in the eluate, and still be undetectable. At least all of the determinants detectable in common between receptor from Electrophorus and Torpedo are located outside the acetylcholine-binding site.

CONCLUSIONS

All antisera available against acetylcholine receptor, whether obtained by direct immunization with purified receptor, by experimental induction of an autoimmune response, or by unknown pathological causes (in the case of MG), are directed mostly at determinants other than the acetylcholine-binding site. Studies with antibodies combining biochemical and electrophysiological approaches should allow definition of the determinants on the receptor to which these antibodies bind, and aid in defining the role of these structures in receptor function.

Experimental autoimmune myasthenia gravis should prove of value not only as a model for the study of myasthenia gravis, but as an experimental technique to allow in vivo experimentation with the effects of immune modification on receptor activity, on innervation, on denervation, and on reinnervation. It is very likely that disease resulting from an autoimmune response to receptor is not restricted to the receptor for acetylcholine

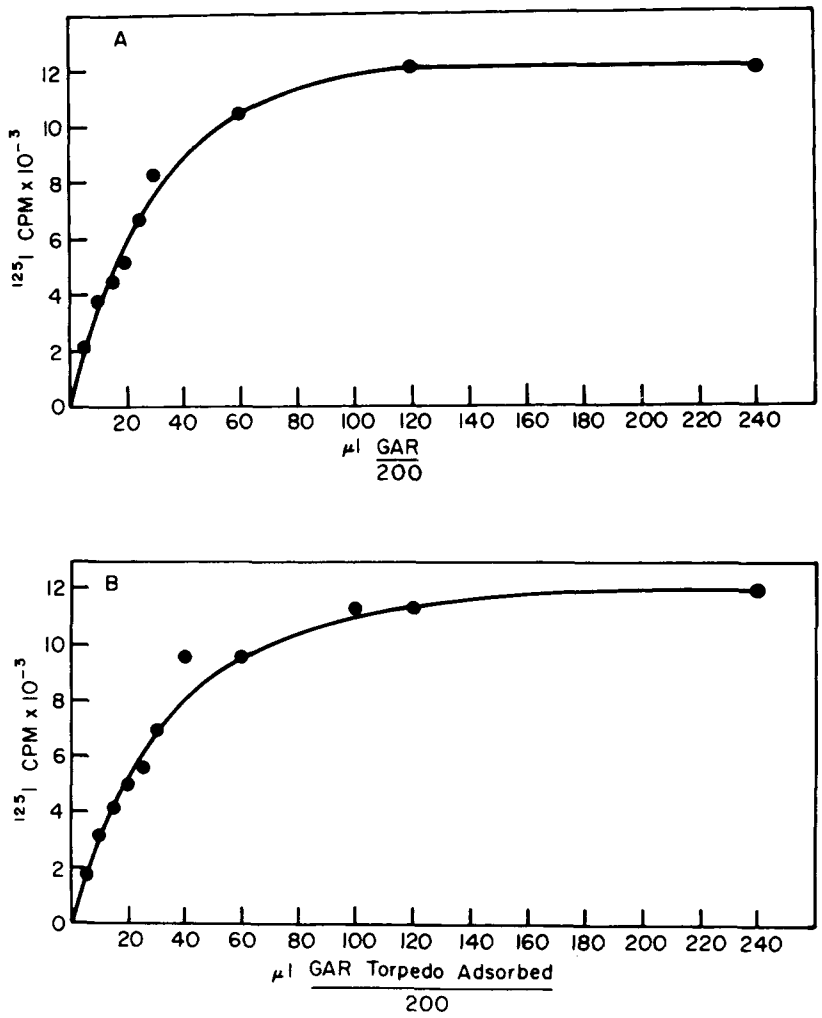

Fig. 10. Immunoprecipitation of Electrophorus receptor by goat anti-Electrophorus receptor serum before and after adsorption with Torpedo electric organ membranes. Immunoprecipitation is conducted as described in Fig. 3. (A) Shows precipitation of Electrophorus receptor by antiserum raised against it. (B) Shows precipitation after adsorption of this serum with Torpedo membranes as described in Fig. 9.

(17). We can expect that studies of EAMG and MG will be instructive in the study of such diseases, and that immunological approaches may well prove valuable in the study of other receptors.

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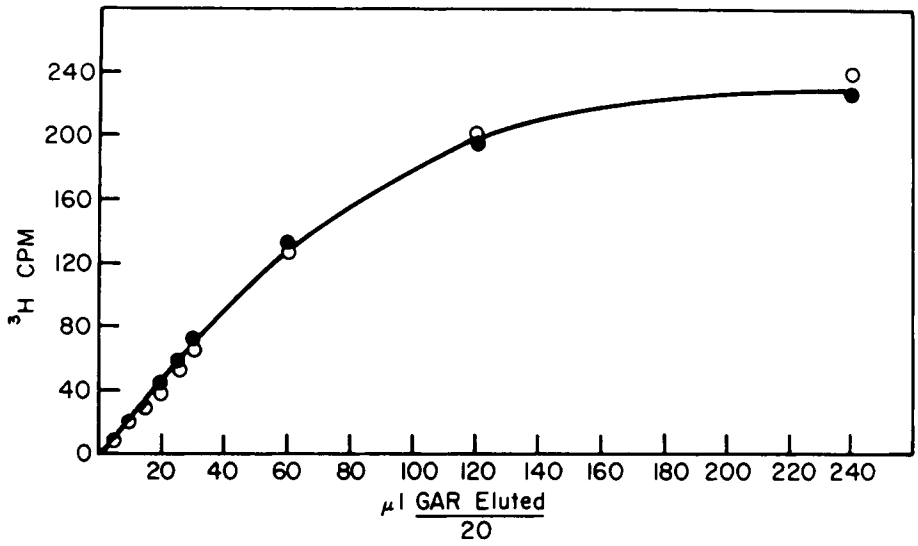

Fig. 11. Immunoprecipitation of receptor from Electrophorus by goat antireceptor serum eluted from Torpedo membrane. Goat antiserum raised against receptor from Electrophorus was adsorbed with Torpedo electric organ membranes as described in Fig. 9. Bound antibodies were eluted by shaking with 3 M KSCN twice for 1 hr with 5 ml, with the supernatant of 30 min at $10^5 \times g$ collected each time. The pooled supernatants were dialyzed against normal saline, centrifuged again to clarify them, and defined as a five fold dilution of the eluate.

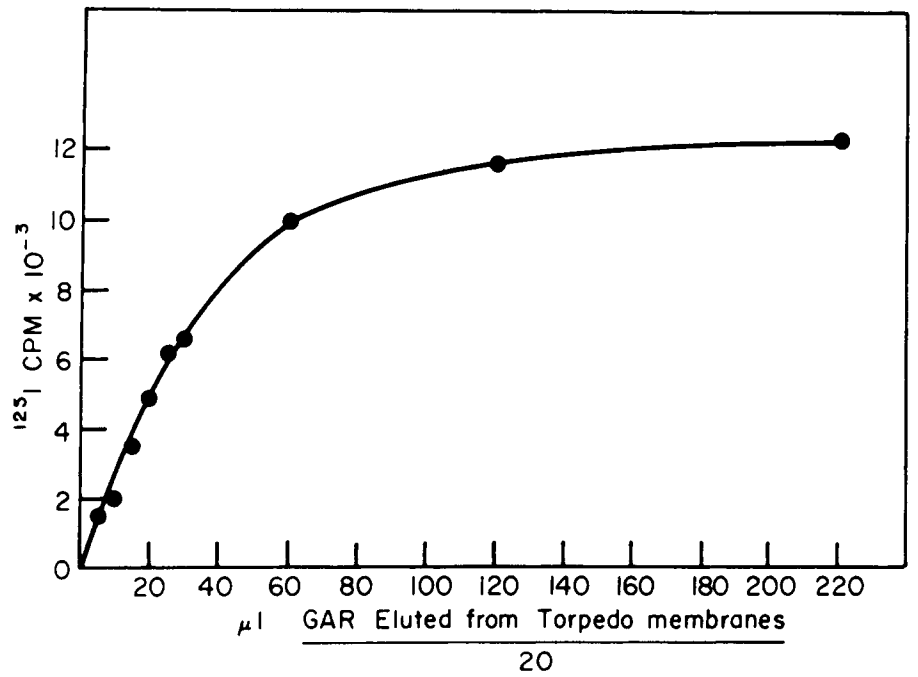

Fig. 12. Immunoprecipitation of receptor ± toxin with antiserum eluted from Torpedo electric organ membranes. Immunoprecipitation of ^{3}H Ac-labeled receptor from Electrophorus was conducted as described in Fig. 6 using goat antireceptor serum raised against receptor from Electrophorus which was eluted from Torpedo electric organ membranes as described in Fig. 10.

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