Antibodies from patients with myasthenia gravis recognize determinants unique to extrajunctional acetylcholine receptors (junctional acetylcholine receptors/skeletal muscle/embryonic acetylcholine receptors)

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Communicated by Stephen W. Kuffler, October 13, 1978

ABSTRACT We have examined the interaction between sera from patients with myasthenia gravis and acetylcholine receptor (AcChoR) purified from normal and denervated rat skeletal muscles (junctional receptor (JR) and extrajunctional receptor (EJR), respectively). Eight of ten myasthenic sera had titers against EJR that were significantly higher (1.1–2.4 times) than their titers against JR. The antigenic receptors of these sera ranged from 2 to 102 nM. Although activities of three other sera were too low (<1 nM) to allow accurate titrations, provisional measurements with these sera gave titers against EJR that were at least as high (1.0–1.4 times) as those against JR. Competition experiments with myasthenic sera demonstrated two classes of determinants on rat AcChoR: those that are common to JR and EJR and those that are present or exposed only on EJR. Myasthenic sera did not recognize any determinants unique to JR. Several antisera raised to purified AcChoR from eel or Torpedo electric organs or denervated rat skeletal muscle had equal titers against the two forms of receptor. Treatment of JR and EJR by various enzymatic or chemical procedures designed to alter prosthetic groups on the proteins failed to affect their antigenic reactivity. AcChoR from embryonic rats was indistinguishable immunologically from JR of adult muscle.

Myasthenia gravis is an autoimmune disease characterized by muscle weakness and rapidly fatiguing neuromuscular transmission (1, 2). These symptoms are probably due to a reduced number of acetylcholine receptors (AcChoR) at the neuromuscular junction (3). Sera from patients with myasthenia contain antibodies to AcChoR (4–7), and animals immunized with purified AcChoR (8–13) develop symptoms similar to those of myasthenia gravis. Moreover, repeated injection of IgG from myasthenic patients produces symptoms of the disease in mice (14). These observations indicate that the interaction of antibodies with AcChoR plays an important role in the pathogenesis of the disease.

Two molecular forms of AcChoR are found in mammalian skeletal muscles. Junctional receptor (JR) is densely packed at adult neuromuscular junctions, while extrajunctional receptor (EJR) is found at lower density over the entire surface of embryonic or denervated adult muscle fibers (15–18). JR and EJR are almost identical glycoproteins that are indistinguishable by gel filtration, sucrose gradient sedimentation, and reaction with a rabbit antiserum to eel AcChoR (19). Both receptors have the same subunit composition, and the major subunits have identical peptide maps (ref. 20; N. Nathanson, personal communication). However, JR and EJR are not identical. In situ, they can be distinguished by curare sensitivity (21), channel open time and conductance (22), and metabolic turnover time (23, 24); after solubilization and purification, they differ in reaction with α-bungarotoxin (a-BuTx) and curare and in isoelectric point (18, 25).

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Experiments by Almon and Appel (26), with myasthenic sera and extracts of normal and denervated muscle, suggested that the two receptor types might also be immunologically different (see also ref. 27). By using highly purified preparations of JR and EJR, we have found the EJR has determinants that are not present on JR; these determinants are detected with myasthenia sera but not with several antisera raised in animals to AcChoR from various sources. These results demonstrate a further, presumably molecular, difference between JR and EJR.

MATERIALS AND METHODS

Materials. White male Sprague–Dawley rats were obtained from Simonsen. Bacterial alkaline phosphatase and partially purified snake venom phosphodiesterase were obtained from Worthington Biochemical Corp.; calf intestine alkaline phosphatase (type VII) and neuraminidase (Clostridium perfringens, type IX) were from Sigma. Highly purified (protease-free) snake venom phosphodiesterase was the generous gift of Michael Laskowski, Sr., and Arthur E. Pritchard. Phosphoprotein phosphatase was prepared from rabbit muscle by the procedure of Kato and Bishop (28) by Neil M. Nathanson in this laboratory. a-BuTx and 122I-labeled a-BuTx (125I-a-BuTx, specific activity 200–500 cpm/μg at 70% efficiency) were prepared as described (19).

AcChoR. For most experiments, JR was prepared from normal rat hind leg muscle and EJR from leg muscle that had been denervated 10–14 days earlier by bilateral sciatic nerve section. Receptor was purified and assayed as described (20), except that Triton X-100 was used in the elution from cobrotoxin-Sepharose. In a few experiments, JR was obtained from the endplate regions of normal rat diaphragms and EJR from endplate-free regions of diaphragms denervated 7–14 days previously by phrenic nerve section (29).

Toxin–receptor complexes were prepared by incubating JR or EJR with a 5.5- to 2-fold excess of a-BuTx or 125I-a-BuTx for 24–36 hr at 4°C, followed by centrifugation at 15,000 × g for 15 min to remove aggregates. The amount of toxin–receptor complex formed was assayed by DEAE-cellulose filtration (19). JR and EJR, labeled directly with 125I, were prepared by Stanley C. Froehner as described (20). A Triton X-100 extract of a crude membrane fraction from decapitated, eviscerated 18-day rat embryos was prepared as described in the legend to Table 1 by C. Gary Reiness in this laboratory. Antisera. Goat anti-human IgG, rabbit anti-goat IgG, rabbit anti-rat IgG, and goat anti-rabbit IgG were obtained from Cappel Laboratories, Inc. Sera from patients with myasthenia gravis were obtained by David Dawson (Department of Neurology, Peter Bent Brigham Hospital). A consent form approved by the Human Experimentation Committee of Harvard Medical School was used.

Abbreviations: AcChoR, acetylcholine receptor(s); JR, junctional AcChoR; EJR, extrajunctional AcChoR; α-BuTx, α-bungarotoxin.
Antiserum from a goat immunized with AcChoR from eel, *Electrophorus electricus*, and rabbit anti-giant IgG were the generous gifts of Jon Lindstrom. Antiserum from rabbits immunized with native or denatured AcChoR from *Torpedo* were donated by Stanley C. Froehner. Antiserum from a rabbit and a goat immunized with *Torpedo* AcChoR were kindly provided by Adrienne Gordon. Rabbit antiserum against rat muscle AcChoR were prepared by subcutaneous injection at multiple sites of 6–8 μg of purified EJR in complete Freund’s adjuvant, followed by a second identical injection 6–8 weeks later. Blood was obtained from the ear vein or by cardiac puncture upon death of the rabbits 10 days after the second immunization. Rat antiserum against purified rat muscle EJR were prepared by injection of 2 μg of purified EJR in complete Freund’s adjuvant, followed by a second injection 2 weeks later. Blood was obtained from the femoral artery under ether anesthesia.

IgG was prepared by precipitation of serum with 40% ammonium sulfate, followed by passage over a DEAE-cellulose column in 20 mM phosphate buffer (pH 8.0).

**Antibody Precipitation Assay.** The assay was modified from that of Patrick and Lindstrom (8). Various amounts of 125I-α-BuTx-receptor complex were incubated with a given volume of serum (usually 2 μl) for 4 hr at room temperature in a total volume of 20 μl. The second antibody (30 μl for 2 μl of serum) and 10 mM sodium phosphate, pH 7.1/0.9% NaCl/1% Triton X-100 were added to a final volume of 200 μl. After incubation for 12 hr at 4°C, the immune precipitate was collected by centrifugation and washed twice by resuspension in 0.3 ml of the same buffer followed by centrifugation. The radioactivity of the pellets was then measured in a gamma counter.

**Enzyme Treatments.** JR or EJR (5 fmol) was incubated with various enzymes at 25°C in 10 μl of buffer containing 1% Triton X-100. The enzymes and buffers used were: snake venom phosphodiesterase, 0.4 unit ( Worthington) or 0.01 unit (protease-free) in 10 mM MgCl₂/10 mM Tris acetate, pH 8.8; rabbit muscle phosphoprotein phosphatase, 6 × 10⁻⁵ unit in 50 mM Tris-HCl, pH 7.4/5 mM MnCl₂/15 mM dithiothreitol; alkaline phosphatase, 1 unit ( bacterial) or 3 units ( calf intestine), in 10 mM Tris-HCl, pH 8.0/10 mM MgCl₂ and neuraminidase, 0.15 unit in 10 mM sodium acetate (pH 5.0). Each enzyme preparation was shown to be active in separate experiments. Control tubes contained buffer and receptor only. The incubations were stopped by addition of 40 μl of ice-cold 75 mM Tris-HCl, pH 7.4/1% Triton X-100/3 mM EDTA. The treated and control samples of JR and EJR were then incubated with 125I-α-BuTx and assayed for toxin binding and immunoprecipitation.

### Table 1. Binding of AcChoR in muscle extracts by myasthenic serum

<table>
<thead>
<tr>
<th>Serum</th>
<th>Myasthenic</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denervated muscle AcChoR, fmol</td>
<td>2.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Innervated muscle AcChoR, fmol</td>
<td>1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Free 125I-α-BuTx, fmol</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Innervated or denervated lower leg muscles of rats were homogenized (100 mg of tissue wet weight per ml) in 50 mM Tris-HCl, pH 7.4/100 mM NaCl at 4°C. A crude membrane fraction was prepared by centrifuging the homogenate at 25,000 × g for 30 min at 4°C and resuspending the pellet in the original volume of buffer. Triton X-100 was added to a concentration of 1% and the extract was incubated for 80 min at 4°C. After centrifugation at 25,000 × g for 15 min, AcChoR in the extracts was labeled by incubation with 125I-α-BuTx at 4°C for 24 hr. Toxin–receptor complex or free toxin (10 fmol) was incubated with 2 μl of serum for 4 hr at 20°C. Bound radioactivity was determined by precipitation with a second antibody and measurement in a gamma counter. Each value is the average of duplicates that were reproducible to within ±0.2 fmol.

**Periodate Treatment.** The method was modified from that of Spiro (30). JR or EJR (10 fmol) in 50 mM sodium acetate, pH 5.0/1% Triton X-100 was incubated with 10 mM NaIO₄ at 4°C in the dark for 5 min–4 hr. The reaction was stopped by addition of excess ethylene glycol, and the solution was neutralized with 2 vol of 80 mM Tris-HCl, pH 7.4/1% Triton X-100. Toxin binding was assayed and the binding to myasthenic serum was determined either by immunoprecipitation or by the competition experiment (radioimmunoassay) described below. Both untreated and neuraminidase-treated receptors were oxidized with periodate.

**RESULTS**

**Interaction of Myasthenic Sera with AcChoR in Muscle Extracts.** In initial experiments, a series of human sera were examined for their ability to bind AcChoR in detergent extracts of crude membrane fractions of normal and denervated rat muscles. The results of one such experiment are shown in Table 1. As reported by others (4–7), the myasthenic serum bound more toxin–receptor complex from denervated muscles than did serum from a normal subject. In addition, we found that AcChoR in extracts of normal muscle was bound by myasthenic serum, although to a lesser extent than AcChoR from denervated muscle. The binding activity was retained in the IgG fraction after ammonium sulfate precipitation and DEAE-cellulose chromatography. Free 125I-α-BuTx was not precipitated (Table 1). Qualitatively similar results were obtained with several other myasthenic sera; in each case more AcChoR from denervated muscle than from normal muscle was bound by a given volume of serum. However, we were never able to achieve more than 40–50% precipitation of the AcChoR, and the normal serum blank values were always a substantial fraction (5–10%) of the total radioactivity added.

**Interaction of Myasthenic Serum with Purified JR and EJR.** To overcome these difficulties and to ensure that the observed differences were due to differences in receptor, we performed further experiments with highly purified preparations of AcChoR from normal and denervated rat leg muscle. The proportion of protein that was receptor in these preparations was estimated at ≈60% (normal muscle) and at over 90% (denervered muscle) (20). Analysis by isoelectric focusing established that contamination of each form of the receptor by the other (i.e., JR by EJR and vice versa) was less than 25% (N. Nathanson, personal communication).

A complete precipitation curve in which equal amounts of purified JR and EJR complexed with toxin were incubated with variable amounts of serum (serum I in Table 2) is shown in Fig. 1A. Greater binding of EJR occurred over a wide range of serum concentrations, and maximum precipitation of JR required a higher serum concentration than for EJR. These results could be explained if the JR preparation contained receptor that was antigenically active but had lost toxin-binding activity. This possibility was ruled out, however, by the finding that a rabbit antiserum raised to purified rat EJR failed to differentiate the

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1 The failure to observe binding to AcChoR from innervated muscles in earlier experiments (4) was probably due to the use of extracts of whole muscle rather than of crude membrane fractions. When we repeated the experiments in Table 1 with whole muscle extracts, the blank values with control sera were increased and obscured the binding of AcChoR from normal muscle by myasthenic sera. The high blank values seen with extracts of whole innervated muscle are presumably due to their high content of protein relative to AcChoR. When the protein to AcChoR ratio in extracts of denervated muscle was adjusted to the same value by addition of extracts of endplate-free regions of normal diaphragm (containing no AcChoR), the blank values were raised and binding of AcChoR in these denervated extracts was barely detectable (unpublished experiments).
two receptor preparations (Fig. 1B). The difference in binding of the two receptors could not be explained by differential displacement of toxin from the receptor, since nearly complete (>80%) precipitation of both toxin–receptor complexes could be obtained. The difference was also observed with receptor labeled directly with 125I and not complexed with α-BuTx.

A series of myasthenic sera was then examined by incubating a fixed amount of serum with increasing amounts of purified JR and EJR. One example is illustrated in Fig. 2. In 7 of the 10 sera examined, a plateau value of receptor binding was obtained in the presence of a large excess of toxin–EJR that was clearly higher than the level obtained for toxin–JR. The ratios of these plateau values (EJR/JR) ranged from 1.1 to 2.4. The three re-

Table 2. Anti-AcChoR titers of sera from patients with myasthenia gravis

<table>
<thead>
<tr>
<th>Serum</th>
<th>EJR, nM ± SEM</th>
<th>JR, nM ± SEM</th>
<th>EJR/JR</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>102 ± 2</td>
<td>62 ± 2</td>
<td>1.6*</td>
</tr>
<tr>
<td>II</td>
<td>17 ± 0.2</td>
<td>12 ± 0.3</td>
<td>1.4*</td>
</tr>
<tr>
<td>III</td>
<td>15 ± 0.2</td>
<td>11 ± 0.2</td>
<td>1.4*</td>
</tr>
<tr>
<td>IV</td>
<td>7.6 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>2.0*</td>
</tr>
<tr>
<td>V</td>
<td>4.5 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.4*</td>
</tr>
<tr>
<td>VI</td>
<td>4.2 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>1.1*</td>
</tr>
<tr>
<td>VII</td>
<td>3.0 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>1.2*</td>
</tr>
<tr>
<td>VIII</td>
<td>0.53 ± 0.02</td>
<td>0.54 ± 0.03</td>
<td>1.0</td>
</tr>
<tr>
<td>IX</td>
<td>0.47 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>1.4*</td>
</tr>
<tr>
<td>X</td>
<td>0.07 ± 0.02</td>
<td>0.06 ± 0.03</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The titers were determined from titration curves against purified JR and EJR as in Fig. 2. The titers are expressed as concentration representing the amount of AcChoR bound in the plateau region (receptor excess) of the titration curve by a given volume of serum. Each value is an average of three to six determinations.

* Ratio significantly different from 1.0; P < 0.006 (Student’s t-test).

maining sera had low antireceptor titers (<1 nM) so that we were unable to obtain accurate titrations. Provisional measurements gave ratios of 1.0, 1.2, and 1.4 for these sera, but these could be in error by as much as 25%. The results of these titration curves are summarized in Table 2.

Sera that have higher plateau values for EJR must contain antibodies directed against determinants that are unique to EJR in an amount that is equal to or greater than the difference between these values for JR and EJR. To determine whether any antibodies in the myasthenic serum were directed against determinants common to the two receptors, we performed competition experiments similar to those used in a radioi-

Fig. 2. Titration of anti-AcChoR activity of a serum from a patient with myasthenia gravis (strain IV). Various amounts of 125I-α-BuTx–receptor complex were incubated with 2 μL of antiserum and the amount of receptor bound was determined. ●, JR; ○, EJR. Normal human sera gave linear increase of radioactivity precipitated (about 1% of initial radioactivity), which probably represents non-

munoassay with four different sera (I, III, IV, and VI in Table 2). JR or EJR complexed with unlabeled α-BuTx was incubated with the myasthenic serum prior to incubation with a saturating amount of either the homologous or heterologous receptor bound to 125I-α-BuTx. Fig. 3 illustrates the results of a competition experiment with serum IV. Both receptors blocked binding to the heterologous species, indicating that determinants are shared between them. For the 125I-toxin–JR complex, both JR and EJR were equally effective in blocking activity, suggesting that all the determinants present on JR that were recognized by this serum were also present on EJR. As expected from the experiments described above, JR was less effective than EJR in blocking binding to the 125I-toxin–EJR complex (Fig. 3B). Similar results were obtained with the other three sera.

Interaction of AcChoR with Antisera from Animals with Experimental Autoimmune Myasthenia Gravis. Animals injected with purified AcChoR from the same or different species produce antibodies to AcChoR and develop symptoms resembling those of myasthenia gravis (8–13). We examined the interaction of such antibodies with rat JR and EJR. In contrast to the results with human myasthenic sera, the two forms of AcChoR were not distinguished by any of the antisera we tested, including the rabbit antiserum shown in Fig. 1B, a second rabbit antiserum and three rat antisera to purified rat muscle EJR, a goat and a rabbit antiserum to oel AcChoR, and three rabbit antisera and a goat antiserum to Torpedo receptor (Table 3).

Attempts to Alter Antigenicity of Receptors. We tried to obtain information about the nature of the antigenic determi-
amounts of 15 incubated were activity treatments receptor was the of receptors of intestinal phosphatase, neuraminidase, in muscle phosphatase, neuraminidase, by monoprecipitation tion in the periodate their inidase. FIG. Receptor. Embryonic ability Incubation to toxin-binding of the treatment of each by enzymatic groups from bacterial alkaline phosphatase, intestinal phosphatase, phosphoprotein phosphatase, neurominidase, or snake venom phosphodiesterase produced no change in either toxin-binding activity or immunoprecipitation by a myasthenic serum (IV). When the receptors were treated with periodate for 4 hr, a 40-50% reduction in toxin-binding activity occurred, but there was no change in their ability to block binding of labeled toxin-receptor complex in the competition assay. Similar results were obtained when periodate treatment followed incubation with neuraminidase.

Embryonic Receptor. To see whether AcChoR from embryonic muscle shared determinants with JR and EJR derived from adult muscle, we tested labeled and unlabeled toxin-receptor complexes from 18-day rat embryos with a myasthenic serum (IV) whose titer against EJR was twice that against JR. The titer against embryonic receptor was the same as against EJR. Competition experiments (Fig. 4) showed further that all of the determinants on AcChoR from embryonic muscle recognized by the myasthenic serum were present on EJR and confirmed that the embryonic receptor had determinants not present on JR.

**DISCUSSION**

Antibodies in sera from patients with myasthenia gravis bind to toxin-AcChoR complexes both in purified preparations and in crude extracts of denervated and innervated rat muscles. The use of purified JR and EJR in these experiments offered several advantages. First, it was possible to establish that the greater binding to EJR than to JR preparations was due to differences between the two forms of receptor and not to differences in other components of the extracts. Second, the 20- to 50-fold higher concentration of receptor in purified preparations permitted complete binding of the anti-AcChoR activity in a given volume of serum and thus enabled us to measure true antibody titers. Finally, since the maximum binding obtained with a large excess of toxin-receptor complex was higher for EJR than for JR, one can conclude that there is a population of antibodies in myasthenic serum that reacts only with EJR. Thus, the difference in binding of the two forms seen at lower receptor concentrations occurs because JR lacks certain determinants and not because it has fewer copies of common de-

![Graph](image)

**FIG. 3.** Competition experiment between JR and EJR. Various amounts of unlabeled α-BuTx-receptor (●, JR; □, EJR) complex were incubated with 2 μl of a myasthenic serum (IV) for 4 hr at 23°C. Then 15 fmol of 125I-α-BuTx-labeled EJR (A) and JR (B) was added and the mixture was incubated for an additional 4 hr at 23°C. Bound receptor was then determined. The percentage of anti-AcChoR activity not blocked by the unlabeled AcChoR is shown as a function of the amount of unlabeled AcChoR used.

**Table 3.** Titers of antisera from animals immunized with AcChoR

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Titer against</th>
<th>Torpedo AcChoR, μM</th>
<th>Eel AcChoR, μM</th>
<th>Rat EJR, M × 10⁸</th>
<th>Rat JR, M × 10⁸</th>
<th>% crossreaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torpedo AcChoR</td>
<td>Goat</td>
<td>&gt;5</td>
<td>—</td>
<td>8</td>
<td>8</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Torpedo AcChoR</td>
<td>Rabbit</td>
<td>1.0</td>
<td>—</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Torpedo AcChoR</td>
<td>Rabbit</td>
<td>2.5</td>
<td>—</td>
<td>1</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Torpedo AcChoR (denatured)</td>
<td>Rabbit</td>
<td>0.7</td>
<td>7.1</td>
<td>0.1</td>
<td>0.1</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Eel AcChoR</td>
<td>Goat</td>
<td>—</td>
<td>—</td>
<td>49</td>
<td>48</td>
<td>—</td>
</tr>
<tr>
<td>Eel AcChoR</td>
<td>Rabbit</td>
<td>—</td>
<td>2.4</td>
<td>4</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>Rat EJR</td>
<td>Rabbit</td>
<td>—</td>
<td>—</td>
<td>32</td>
<td>32</td>
<td>—</td>
</tr>
<tr>
<td>Rat EJR</td>
<td>Rat</td>
<td>—</td>
<td>—</td>
<td>0.2</td>
<td>0.2</td>
<td>—</td>
</tr>
<tr>
<td>Rat EJR</td>
<td>Rat</td>
<td>—</td>
<td>—</td>
<td>0.03</td>
<td>0.03</td>
<td>—</td>
</tr>
<tr>
<td>Rat EJR</td>
<td>Rat</td>
<td>—</td>
<td>—</td>
<td>0.02</td>
<td>0.02</td>
<td>—</td>
</tr>
</tbody>
</table>

Titers were obtained and expressed as in Table 2, with the appropriate AcChoR and second antibody.
terminants or determinants that react with lower affinity. The failure of even high concentrations of JR to bind as much antibody as does EJR means that less than 10% of the receptor in the JR preparations is contaminating EJR.

Of the various differences between JR and EJR that have been observed, only that in isoelectric point (25) has indicated that the two receptors might be different molecular forms. The demonstration of determinants that are unique to EJR provides independent evidence that the two receptors are structurally different. Post-translational modifications involving the addition or removal of sugar, phosphate, or nucleotidyl groups are possible sources of the difference (31). However, various chemical and enzymatic treatments of the receptors that might remove such groups failed to affect either the unique or the shared determinants of rat EJR and JR. It has also not been possible to alter the isoelectric point of *Torpedo* or muscle AccHoR by treatment with bacterial alkaline phosphatase, intestinal phosphatase, phosphoprotein phosphatase, or phospholipase (N. Nathanson, personal communication). Since any of the prosthetic groups, if present, might have been resistant to the treatments used, these results do not rigorously exclude them as possible sources of the molecular differences between the two receptor forms.

The competition studies with JR and EJR reported here demonstrate that myasthenic sera contain two classes of antibodies: those directed against determinants that are present on both JR and EJR and those directed against determinants that occur only on EJR. Since EJR is as effective as JR in competing for the binding of JR by myasthenic sera, all of the determinants of JR that are recognized by these sera appear to be on EJR. Thus, we are unable to detect any antibodies directed against unique JR determinants.

Most, and possibly all, myasthenia sera contain antibodies to unique determinants on EJR. Each of the 7 antisera described in Table 2 for which an unambiguous comparison could be made, and each of 10 additional sera that we have recently tested (data not shown), has a higher titer to EJR than to JR. The prevalence of antibodies specific for EJR and the failure to detect antibodies against determinants unique to JR suggest that at least one of the immunogens responsible for the development of myasthenia gravis may be more closely related to EJR than to JR.

The presence of antibodies directed only against EJR is not a general property of antisera to the receptor since sera from several animals immunized with rat EJR or eel or *Torpedo* AccHoR failed to distinguish the two forms. In this respect, experimental autoimmune myasthenia gravis may be an incomplete model of the human disease.

The significance of antibodies against EJR determinants in myasthenic sera may lie in the resemblance between EJR in adult denervated muscle and the AccHoR in embryonic muscle. The immune system develops when most of the muscle AccHoR is not localized at endplates; also, it has been suggested that myoid cells in the thymus, whose receptors are presumably extrajunctional, may play a role in the pathogenesis of the disease (32). The immunological similarity of embryonic AccHoR and adult EJR that we have found extends other studies that have shown that AccHoR from embryonic muscle has the properties of adult EJR (17, 25, 33, 34) rather than of JR. However, recent physiological studies have shown that embryonic AccHoR and adult EJR may not be identical (35).

Thus, our present studies confirm and extend the finding that JR and EJR are different molecular species. The nature of this difference and its role in the pathogenesis of myasthenia gravis are problems for future investigations. The ability of myasthenic sera to distinguish the two types of AccHoR make it an important tool for such studies.

We thank Dr. David Dawson for helpful discussions and for providing sera from patients with myasthenia gravis. We are indebted to Steve Rowe for expert technical assistance and to Liz Neville for assistance in preparation of this manuscript. We also thank our colleagues for valuable comments on the manuscript. This work was supported by grants from the Muscular Dystrophy Association and the National Institutes of Health. C.B.W. was a recipient of a National Science Foundation Graduate Fellowship.