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Heterogeneity of Acetylcholine Receptors in Denervated Muscle: Interactions of Receptors with Immunoglobulin from Patients with Myasthenia Gravis

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SUMMARY

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The interaction of immunoglobulin G fractions from the sera and thymus glands of patients with myasthenia gravis with acetylcholine receptors from denervated rat muscle was studied using a combination of immunoprecipitation and an assay for binding to concanavalin A–Sepharose. Results showed that myasthenic IgG distinguishes two different α-bungarotoxin-binding components, each associated with approximately half the total receptor toxin binding capacity. The receptor components can be separated as immune complexes by different properties of binding to concanavalin A and are characterized by different kinetics of toxin binding. The majority of myasthenia gravis sera contain at least two antibodies, directed against determinants on one or both components of the receptor, which do not affect the total binding capacity for α-bungarotoxin. A few sera contain a third antibody type, which prevents the binding of α-bungarotoxin to receptors.

INTRODUCTION

The occurrence of antibodies to muscle acetylcholine receptors in the serum of patients with myasthenia gravis has been clearly established by several investigators by the use of biochemical, histochemical (1), and immunological (2) techniques. The presence of an antibody capable of complexing with α-bungarotoxin-labeled receptors has been shown directly by immunoprecipitation in the sera of more than 90% of such patients (3–5). Similar antibodies can be detected by a variety of other methods, such as inhibition of the binding of [^{125}I]α-BuTX[1] to receptors (6, 7) and interference with the binding of concanavalin A to receptors (8). These findings indicate that antibody to AChR may be polyfunctional or that several types of antibody may be present.

Our recent study comparing the antireceptor properties of myasthenic serum and thymus extracts strongly suggested that more than one anti-AChR antibody was present (8). In these experiments antireceptor titer, determined by immunoprecipitation (9), was directly compared with a new assay method in which the presence

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[1] The abbreviations used are: α-BuTX, α-bungarotoxin; AChR, acetylcholine receptor; ConA, concanavalin A.

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of antireceptor factors is indicated by an apparent decrease in binding of receptors to Sepharose gels containing immobilized ConA. The sera and thymus extracts of several individual myasthenic patients gave discordant results when the two assay procedures were compared. In these cases a relatively high anti-AChR titer was associated with low inhibitory activity by the ConA assay method. This discrepancy between the two methods led to the present investigation into the mechanism of antibody inhibition of receptor binding to ConA and the possible occurrence of multiple anti-AChR immunoglobulins in myasthenia gravis.

MATERIALS AND METHODS

Patient material. Immunoglobulin G fractions were prepared by chromatography on DEAE-Sephadex (10) from nine sera and four thymus NaCl extracts from myasthenic patients classified in clinical class IIA or IIB as described previously (8). The fractions were concentrated to the original serum or extract volume and preserved at 4° with 0.02% sodium azide.

Preparation of α-bungarotoxin and acetylcholine receptors. Purified α-BuTX was prepared from freeze-dried snake venom (Sigma Chemical Company) by the method of Lee *et al.* (11). Toxin was iodinated with Sepharose-bound lactoperoxidase (Worthington) by the method of David (12), using carrier-free $Na^{125}I$ (New England Nuclear), and subsequently purified by chromatography (8). Toxin preparations had initial specific activities of 100–200 cpm/fmole (Beckman Biogamma counter at 70% efficiency). Receptors were prepared from denervated hind limbs of adult male Sprague-Dawley rats (approximately 250 g) from which sections of both sciatic nerves had been surgically removed 10–14 days previously. Excised lower-limb muscles were extracted with 10 volumes of 1 M KBr and 1 mM methanesulfonyl fluoride, homogenized with a Polytron homogenizer (setting, 2, 1 min), passed through 1-mm-mesh nylon net, and centrifuged at 5000 × *g* for 10 min. The precipitate was extracted a second time with 5 volumes of 1 M KBr–1 mM methanesulfonyl fluoride. The final pellet was extracted overnight at 4° with 2 volumes of 2% Triton X-100, 25 mM phosphate buffer, 1 mM EDTA, and 1 mM methanesulfonyl fluoride. The supernatant, after centrifugation at 39,000 × *g*, was concentrated to approximately 0.5 ml/g of original muscle weight and had a binding capacity of 15–20 pmoles of α-BuTX per milliliter.

Assays. Denervated receptors (0.2–0.4 pmole) and IgG fractions (up to 150 μl) were incubated for 24 hr at 4°, α-BuTX was then added in 1–2 mole excess, and toxin binding was allowed to proceed for 24 hr at 4°. One-third of the sample was used for immunoprecipitation by addition of excess rabbit antiserum to human IgG γ chain (Behring Diagnostics, Inc.), and two-thirds was applied to 1-ml columns of ConA–Sepharose (Pharmacia) as previously described (8). In kinetic experiments toxin binding was followed by adding aliquots of the reaction mixtures at various times to excess unlabeled α-BuTX immediately before analysis on ConA–Sepharose columns.

Data analysis. The data for the inhibition curve (Fig. 1) and the rate of toxin binding (Fig. 3) were analyzed by the PROPHET computer system, using nonlinear regression and curve-fitting programs (13).[2]

RESULTS AND DISCUSSION

Toxin-labeled receptors bind tightly to short columns of ConA–Sepharose gel. We have utilized this property as a convenient receptor assay (8) which gives results identical with the gel filtration method (15) or the DEAE binding assay (16). However, when receptors are first incubated with serum from myasthenic patients, labeled with toxin, and then applied to the ConA columns, a significant decrease in the binding of labeled receptors is found in 65% of myasthenic sera (8). We have inves-

[2] The PROPHET system is a specialized resource developed and sponsored by the National Institutes of Health. A detailed description of its features has been published (14). Further information on access to the system can be obtained from the Director, Chemical/Biological Information Handling Program, Division of Research Resources, National Institutes of Health, Bethesda, Md. 20014.

tigated the inhibitory property of myasthenic serum in more detail, using purified IgG fractions.

Table 1 shows results of the ConA assays compared with immunoprecipitation for the serum and thymus IgGs. In most cases receptors were present in excess relative to the amount of anti-AChR (8 fmoles of AChR per microliter of serum), and these assays therefore reflect maximal activities. The percentage of receptors obtained by immunoprecipitation exceeds the percentage inhibition of receptor binding to ConA–Sepharose beads to a significant extent. Activity according to the two methods of assay is independently variable. This is clearly shown for sera S_6–S_9 and three of the four thymus extracts, in which immunoprecipitation is less than 50% of the total AChR and is therefore a valid measure of titer (5) (see also Fig. 1). However, for the two most active sera, S_1 and S_2, using 50 and 150 μl, respectively, precipitation approached 100% of labeled receptors while ConA inhibition did not exceed 50%.

TABLE 1

Comparison of the ConA assay method with immunoprecipitation

Myasthenic IgG fractions prepared from serum (S) and from NaCl extracts (25%, w/v) of thymus (T) were assayed for antireceptor factors by inhibition of binding to ConA–Sepharose and by immunoprecipitation. AChR (0.4 pmole) was incubated with IgG fractions (equivalent to 50 or 150 μl of serum or thymus extract) for 18 hr and labeled with [^{125}I]α-BuTX (1.2 pmoles) for 18 hr, and the sample was divided for the two assays. Total toxin-receptor complex is set equal to 100%.

IgG fraction	Inhibition of binding to ConA[a]		Immunoprecipitation of AChR from 50 μl of serum	Apparent anti-AChR activity
	50 μl of serum	150 μl of serum		
	%	%	%	*pmoles/ml*
S_1	32	49	106	8.55
S_2	35		90	7.25
S_3	41	53	77	6.18
S_4	35		75	5.95
S_5	41		66	5.30
S_6	31		37	2.97
S_7	23		43	3.42
S_8	18		45	3.62
S_9	14		16	1.28
T_1	23		31	2.51
T_2	10	38	74	5.91
T_3	5		4	0.35
T_4	2	31	50	3.99

[a] Inhibition of receptor-bound [^{125}I]α-BuTX binding to ConA–Sepharose gels relative to ConA binding with normal human serum IgG, taken as 100%.

The different results obtained by the two assay methods are shown more clearly in Fig. 1. The myasthenic IgG caused dose-dependent inhibition of the binding of labeled AChR to ConA-gel (broken line). This curve is a composite (40 data points) from five myasthenic IgGs (S_1–S_5) prepared from sera that had previously been determined to have approximately equivalent inhibitory activity. The inhibition data were subjected to regression analysis using the PROPHET computer system (13, 14). The maximal inhibition was calculated as 54.8 ± 4.4%, and the Hill coefficient was 1.09 ± 0.14. For two of the sera, S_4 and S_5, dose-response curves for immunoprecipitation were determined in parallel with the inhibition assay (Fig. 1, solid lines). Precipitation is linear with increasing amounts of serum to about 50% of the receptors. It is therefore a valid measure of titer provided that antigen (AChR) is in more than a 2-fold excess over antibody, in agreement with the findings of Lindstrom (5) with human muscle AChR. Immunoprecipitation exceeded ConA inhibition over the entire dose range studied, as shown for sera S_4 and S_5. The complete recovery of receptors by immunoprecipitation in two cases (S_1 and S_2) showed that the toxin binding capacity was unimpaired when all receptors were complexed to myasthenic antibody. We have shown previously, by the gel filtration method for separation of free from receptor-bound toxin (15), that 90% of myasthenic sera did not impair toxin binding capacity although 72% contained antireceptor IgG (8). The observed decrease in the number of labeled AChRs binding to the ConA-gel thus appears to be caused by antibody interference with a carbohydrate moiety of the AChR, although this effect was limited to only half the toxin-labeled receptor sites.

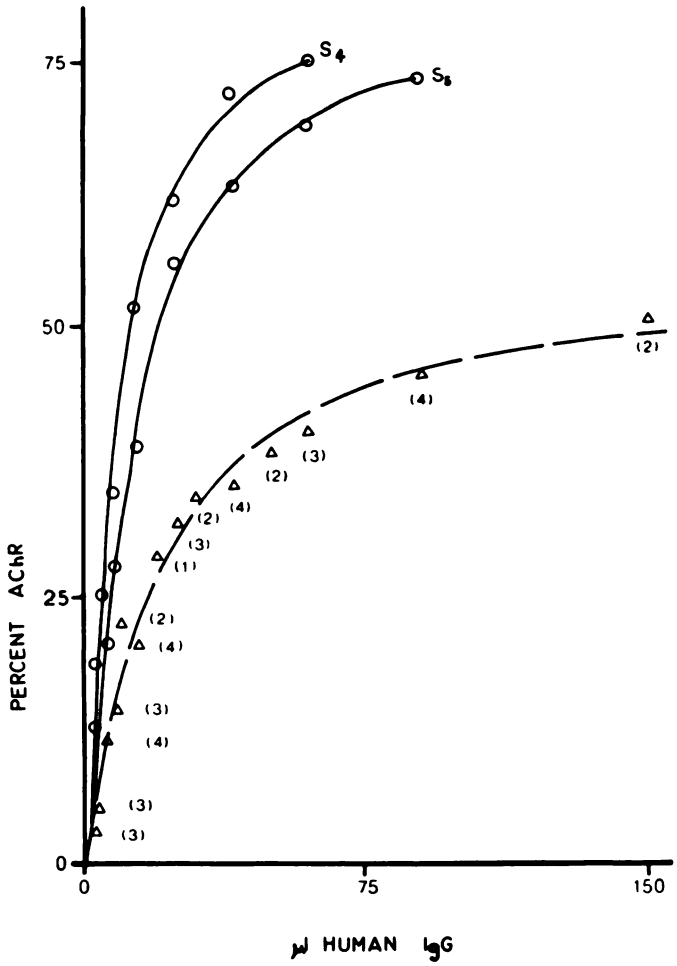

FIG. 1. *Dose-percentage response curves for inhibition of receptor binding to ConA-Sepharose* (△) *and for immunoprecipitation* (○) *of α-BuTX-labeled receptors (0.4 pmole = 100%) by myasthenic serum IgG*

The inhibition curve is a composite of data from sera S_1–S_5, with the number of points at each dose given in parentheses. Maximum inhibition of 54.8 ± 4.4% is obtained by regression analysis (Hill coefficient, 1.09 ± 0.14). The two upper curves are percentage of total toxin-labeled receptors precipitated by individual IgG fractions S_4 and S_5.

This point was investigated in detail for two sera (S_1 and S_3) that showed high activity in both the inhibition and immunoprecipitation assays and for two thymus IgGs (T_2 and T_4) that had high immunoprecipitation activity but produced only modest inhibition in the ConA binding assay (Table 1).

Receptors were first incubated with excess antibody (150 μl of IgG per 400 fmoles) 2-fold higher than the maximum used for immunoprecipitation in Fig. 1, to ensure that all receptors were complexed to anti-receptor immunoglobulin before the addition of α-BuTX. Controls were likewise incubated with normal human IgG or with NaCl, while blank incubations contained myasthenic IgG and toxin but no receptors. The incubation mixtures were applied to columns of ConA-Sepharose and Sephadex SP-C25 in series (Fig. 2). This column arrangement binds the free toxin to the Sephadex column and separates toxin-labeled receptors into ConA-binding and non-ConA-binding fractions. The distribution of specific radioactivity is

expressed in Table 2 as the percentage of the receptor-bound radioactivity determined in the control incubations. Radioactivity missing from the ConA column (relative to controls) passed through both columns (except partially for S_3) and was completely recoverable in the eluate by immunoprecipitation. The eluted radioactivity was not free toxin but was bound to immune complexes of the receptor that did not bind ConA. These receptor-IgG complexes were poorly retained by DEAE-Sephadex columns, in contrast to uncomplexed receptors.

These results require the presence of two antibodies in some myasthenic sera, and possibly two forms of AChR distinguishable by different ConA binding properties when in the form of immune complexes. Brockes and Hall (17) showed that denervated rat muscle receptors were composed of two classes of toxin binding sites, each constituting approximately half the total toxin binding capacity. The antibody experiments suggested that the two toxin binding sites might be separable in terms of their lectin binding properties. We therefore studied the kinetics of toxin binding in the presence of NaCl or normal human IgG and compared this with the kinetics observed in the presence of excess myasthenic IgG.

TABLE 2

Distribution of specific radioactivity (receptor-bound α-BuTX) in chromatographic column arrangement shown in Fig. 2 after incubation of AChR with myasthenic serum (S) or thymus (T) IgG

AChR (400 fmoles) was incubated for 18 hr with IgG equivalent to 150 μl of serum or thymus extract and labeled with 2.5 pmoles of [^{125}I]α-BuTX. Controls were incubated with NaCl or normal IgG, and receptor-bound radioactivity was set equal to 100%. Radioactivity was corrected for excess free toxin determined in the control incubations and for blank incubations (no AChR).

IgG fraction	ConA-Sepharose column	SP-C25 Sephadex column (free α-BuTX)	Immunoprecipitation of eluate	Recovery
	%	%	%	%
S_1	51	<3	50	101
S_3	47	25	28	100
T_2	62	<3	35	97
T_4	69	<3	26	95
Control	100	0	0	100

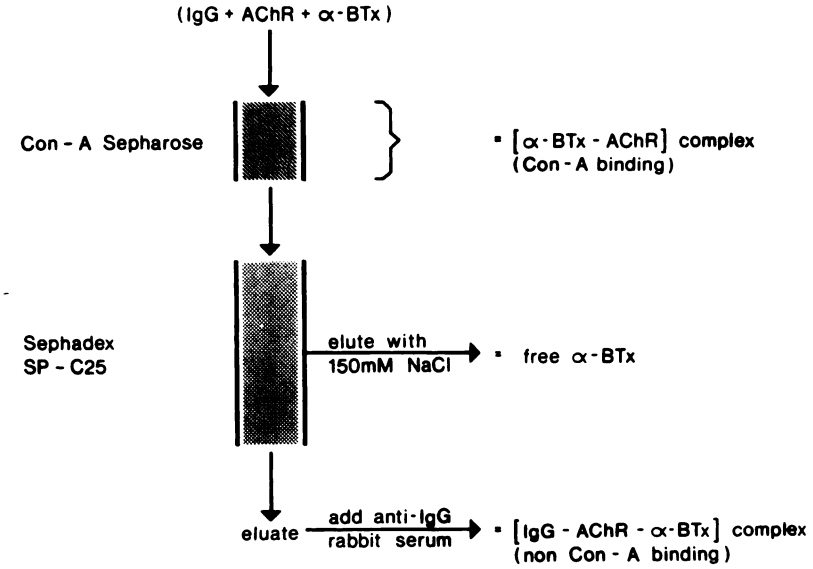

FIG. 2. *Chromatographic system for separation of free α-BuTX, ConA-binding AChR, and non-ConA-binding AChR*

The incubation mixture (200 μl) was added to columns of ConA-Sepharose (1.5 ml) and SP-C25 Sephadex (6 ml) in series and eluted with six 1-ml portions of 25 mM phosphate buffer, pH 7.2, in 1% Triton X-100. Free toxin trapped on SP-C25 columns was eluted separately with 150 mM NaCl.

Toxin binding to AChR was measured at 4° after a 24-hr incubation of aliquots of receptors with NaCl, normal human IgG, or S_5 myasthenic IgG, using ConA-gels to determine labeled receptors. The initial toxin concentration was 10 nM, and thus toxin binding would occur only at the high-affinity "specific" set of sites (K_D approximately 10^9 M^{-1}) with a negligible contribution from the low-affinity set of sites (K_D approximately 10^6 M^{-1}) also present in extracts of denervated muscle (18). This dose of myasthenic IgG (250 μl/pmole of AChR) displaced 48.3% of labeled AChR from ConA binding, which is close to the theoretical maximum displacement derived from the data in Fig. 1.

The rate of toxin-receptor complex formation was assumed to follow second-order rate equations [according to Brockes and Hall (17)], and the data were analyzed for either one kinetic component or two components in equal amounts (17), using the PROPHET nonlinear regression analysis programs (13, 14). Table 3 is a comparison of parameters for the two-component and one-component models, computed from the toxin binding curves in the presence of NaCl or normal human IgG. In both instances the two-component model provided a significantly better fit to the data at the $p < 0.001$ level. The binding curve for receptors previously incubated with normal IgG (Fig. 3, upper solid line) was therefore resolved into two components, k_1 (fast; $7.8 \pm 0.52 \times 10^4$ M^{-1} sec^{-1}) and k_2 (slow; $1.1 \pm 0.06 \times 10^4$ M^{-1} sec^{-1}), as shown in Fig. 3 (broken lines). These are comparable to values for k_1 of $5.9 \pm 0.48 \times 10^4$ and for k_2 of $0.70 \pm 0.047 \times 10^4$ M^{-1} sec^{-1} obtained with the control NaCl incubation.

The rate constants for toxin binding at 4° are smaller than the values determined by Brockes and Hall (17) at 35°, i.e., k_1 (fast) $\sim 3 \times 10^5$ and k_2 (slow) $\sim 1 \times 10^5$ M^{-1} sec^{-1}.

The toxin binding curve for the fraction of receptors retaining affinity for ConA after treatment with myasthenic IgG (Fig. 3, lower solid line) was similarly analyzed for two components (Table 4). This analysis was restricted to two components in equal amounts, as justified by the previous experiments (e.g., Fig. 1) and also by this experiment, which showed two receptor populations constituting 48% and 52% of the toxin binding sites. Based on a two-component model, the values obtained for concentration of complex or rate constants are in poor agreement with expected values based on the control binding curve.

In a similar manner, the binding curve for receptors plus myasthenic IgG was analyzed for one component (Table 5). The poorest fit to the data is obtained when the k_2 (slow) for normal receptors is specified. The other three single-component models are statistically indistinguishable with regard to best fit to the data. These

TABLE 3

Comparison of one- and two-component models for kinetics of α-BuTX binding to AChR after treatment with NaCl or normal serum IgG

Rate constants (k) and the final concentration of the AChR-α-BuTX complex were computed from the best fit to the binding curve (Fig. 3) for a single population of binding sites or two different sites in equal amounts.

Prior incubation medium	One-component model			Two-component model[a]				
	k ($M^{-1}s^{-1}$)	[AChR-α-BuTX]	Sum of squares	k_1	k_2	[AChR-α-BuTX]$_1$	[AChR-α-BuTX]$_2$	Sum of squares
		nM		*M^{-1} sec^{-1}*		*nM*	*nM*	
NaCl	18,888 ±1,056	4.84	4.33×10^{-18}	58,799 ±4,819	7,006 ±456	2.42	2.42	1.09×10^{-18}
Normal IgG	26,460 ±1,564	2.79	10.65×10^{-19}	77,832 ±5,209	10,760 ±549	1.3983	1.3983	1.79×10^{-19}

[a] Significance was tested using an F-test comparing residual variances of best-fit lines. In both NaCl and normal IgG, the two-component model provided a significantly better fit to the data than did the one-component model at the $p < 0.0001$ level.

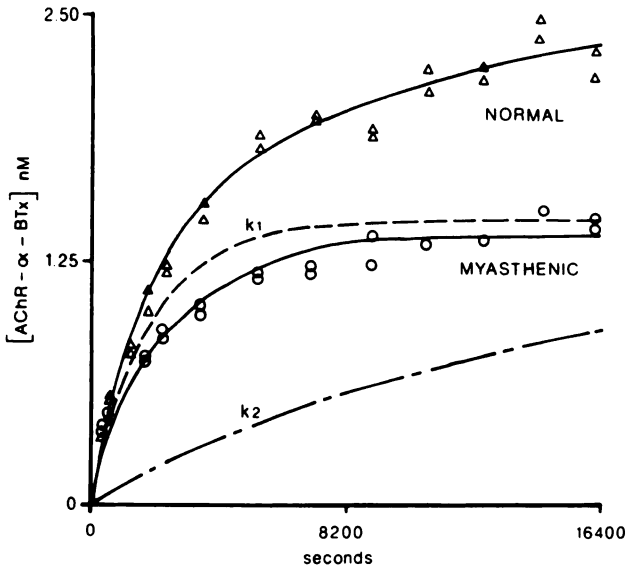

FIG. 3. *Rate of binding of α-BuTX to AChR at 4° after incubation with normal human serum IgG* (△) *or with S_5 myasthenic IgG* (○)

Toxin binding for normal IgG is shown resolved into two components with rate constants $k_1 = 7.8 \pm 0.52 \times 10^{-4}$ and $k_2 = 1.1 \pm 0.056 \times 10^4$ M^{-1} sec^{-1} and maximum concentration of complex of 1.40 nM for each component (broken lines). Toxin binding in myasthenic IgG fit best for a single binding component, $k = 5.4 \pm 0.43 \times 10^4$ M^{-1} sec^{-1}, and formed 1.39 ± 0.03 nM complex. Reaction mixtures contained 2.05 pmoles of AChR, 500 μl of serum IgG, and 6.1 pmoles of α-BuTX in 703 μl of 20 mM phosphate buffer, pH 7.8, and 2% Triton X-100. Duplicate 20-μl aliquots were assayed for toxin-receptor complex on ConA-Sepharose columns at various times over 300 min.

TABLE 4

Kinetics of α-BuTX binding to AChR incubated in myasthenic IgG (Fig. 3), analyzed according to two-component model

The parameters specified were derived from the control experiment with normal IgG (Table 3, two-component model).

Parameter specified	Parameter computed	Value expected, based on normal IgG
$k_1 = 77{,}832$ M^{-1} sec^{-1} (fast)	$[AChR\text{-}\alpha\text{-}BuTX]_1 = 0.99 \pm 0.049$	0.697 nM
$k_2 = 10{,}760$ M^{-1} sec^{-1} (slow)	$[AChR\text{-}\alpha\text{-}BuTX]_2 = 0.64 \pm 0.094$	0.697 nM
$[AChR]_1 = 0.697$ nM	$k_1 = 120{,}098 \pm 17{,}076$ M^{-1} sec^{-1}	77,832 M^{-1} sec^{-1}
$[AChR]_2 = 0.697$ nM	$k_2 = 28{,}498 \pm 3{,}031$ M^{-1} sec^{-1}	10,760 M^{-1} sec^{-1}

models all fit a binding curve for a single set of sites, approximately 1.3 nM, and with a rate constant in the range 5–8 × 10^4 M^{-1} sec^{-1}. Thus receptors that retain an affinity for ConA after treatment with myasthenic IgG consist of one set of sites with fast toxin binding kinetics, suggesting that they correspond to the fast toxin binding component of the control receptors. Receptors displaced from ConA binding should therefore correspond to the slow toxin binding component. We were unable to confirm this by analysis of toxin binding kinetics on this fraction of receptors because they are incompletely retained by DEAE columns and cannot be rapidly separated from the free toxin. These difficulties are probably caused by the low binding affinity of the AChR-IgG complexes to DEAE-gels and the large amount of protein present (3 mg of IgG per picomole of AChR).

TABLE 5

Kinetics of α-BuTX binding to AChR incubated in myasthenic IgG (Fig. 3), analyzed according to one-component model

The specified parameters for rate constants were derived from the control experiment (Table 3, two-component model) and for the final concentration of complex by measurement.

Parameter specified	Parameter computed	Sum of squares[a]
None	[AChR-α-BuTX] = 1.39 ± 0.030 nM k = 54,103 ± 4,277 M^{-1} sec^{-1}	1.96×10^{-19}
[AChR-α-BuTX] = 1.39 nM	k = 53,527 ± 3,201 M^{-1} sec^{-1}	2.01×10^{-19}
k = 77,832 M^{-1} sec^{-1}	[AChR-α-BuTX] = 1.31 ± 0.027 nM	3.37×10^{-19}
k = 10,760 M^{-1} sec^{-1}	[AChR-α-BuTX] = 2.44 ± 0.136 nM	20.36×10^{-19}

[a] Significance was determined using an F-test on residual variances after obtaining the best fit of the data to the model indicated, and comparing four models with each other. Using this criterion, the last model (k = 10,760) provided a significantly poorer fit ($p < 0.005$) than the other three models, which were found statistically indistinguishable with regard to best fit to the data, concentration of AChR-α-BuTX, and k.

The overall conclusion suggested by the data is that when receptors are complexed to antibody, two toxin binding components are identifiable. The experimental results suggest two possibilities. The first is that two species of receptor, possibly in different conformational states, are present in approximately equal amounts and both normally bind to ConA–Sepharose gel. Myasthenic antibodies form complexes with both receptor species but are able to prevent only one from binding to ConA—hence the 50% maximal inhibition. The second possibility is that the receptor protein binds two toxin molecules on different subunits, only one of which contains the site to which ConA also binds. In this case antibodies complexing to the receptor would have to cause a dissociation of receptor into two nonidentical toxin binding components. This would explain the apparent inhibition of receptor binding to ConA–gels and is in agreement with the results of the kinetic experiments (Fig. 3), which indicated that each component contains a kinetically distinct toxin binding site. A dissociation model accounts for the 50% maximum inhibition of ConA binding (Fig. 1), the physical separation of the two components by ConA–gels (Fig. 2), and the 100% recovery of the receptors by immunoprecipitation.

This possibility must be considered in the light of convincing evidence for a single receptor species in denervated rodent muscle. One molecular species of AChR has been demonstrated by physical methods such as ultrafiltration (19), gel filtration (17), and isoelectric focusing (20). Isoelectric focusing is able to distinguish the junctional AChR from extrajunctional (denervated) receptors (20). Thus denervated muscle AChRs are considered to be one molecular species containing two independent toxin binding sites (17). These results could therefore also be explained on the basis of different conformational states of the receptor. The proportions of the two states are very nearly equal in all receptor preparations used in this study and appear to be stable for at least 10 days at 4°. However, one would expect to find both fast and slow toxin binding sites in each conformational form. It is possible that when receptors are complexed to antireceptor IgG the two toxin binding sites are no longer kinetically distinguishable, although the affinity of toxin binding is reported to be unchanged by antibody (21). The question arises whether one antibody type is present in the serum and is directed against the same determinant on both conformations or components of the receptor. We have observed in individual myasthenic sera and thymus extracts that inhibition of ConA binding and the immunoprecipitation titer vary independently (Table 1). Furthermore, when IgG fractions are stored for prolonged periods (9 months) at 4°, the inhibition of ConA binding is abolished while immunoprecipitation activity against 100% of the receptors is retained. Thus a labile form of antibody is required for inhibition of ConA binding

and consequently is also necessary for physical separation of the two receptor forms. There are thus at least two antibody types, one of which appears to be directed against only one conformation while the other seems to be directed against both conformations or components of the receptor.

Both types of antibody considered here do not affect the total toxin binding capacity of AChR, and the kinetics of toxin binding at one of the two sites appears to be unaffected. However, a few myasthenic sera contain other antibody types that block toxin binding. Such antibodies were present in one of the sera (S_2) in the present study, in which toxin binding was inhibited by 25% and was accounted for by unused free toxin (Table 2). This type of antibody was found in a small number of sera in a larger group studied previously and was first reported by Almon, Andrew, and Appel (7).

At the present time the simplest interpretation of our results and those of others suggests the dissociation model. This hypothesis can be adequately confirmed only by the purification of specific forms of antibody and receptor and a careful study of the kinetics of toxin binding and other properties of the individual receptors and their antibody complexes.

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REFERENCES

1. Bender, A. N., Ringel, S. P., Engel, W. K., Daniels, M. P. & Vogel, Z. (1975) *Lancet*, 1, 607.
2. Aharonov, A., Abramsky, O., Tarrab-Hazdai, R. & Fuchs, S. (1975) *Lancet*, 2, 340.
3. Lindstrom, J., Lennon, V., Seybold, M. & Whittingham, S. (1976) *Ann. N. Y. Acad. Sci.*, 274, 254–274.
4. Appel, S. H., Almon, R. R. & Levy, N. (1975) *N. Engl. J. Med.*, 293, 760–761.
5. Lindstrom, J. (1977) *Clin. Immunol. Immunopathol.*, 7, 36–43.
6. Fambrough, D. M., Drachman, D. B. & Satyamurti, S. (1973) *Science*, 182, 293–295.
7. Almon, R. R., Andrew, C. G. & Appel, S. H. (1974) *Science*, 186, 55–57.
8. Mittag, T., Kornfeld, P., Tormay, A. & Woo, C. (1976) *N. Engl. J. Med.*, 294, 691–694.
9. Patrick, J., Lindstrom, J., Culp, B. & McMillan, J. (1973) *Proc. Natl. Acad. Sci. U. S. A.*, 70, 3334–3338.
10. Capra, J. D., Wasserman, R. L. & Kehoe, J. M. (1973) *J. Exp. Med.*, 138, 410–427.
11. Lee, C. Y., Chang, S. L. & Kan, S. T. (1972) *J. Chromatogr.*, 72, 71–82.
12. David, G. S. (1972) *Biochem. Biophys. Res. Commun.*, 48, 464–471.
13. Knott, G. *MLAB: An On-Line Modeling Laboratory* (1975) Division of Computer Research and Technology, National Institutes of Health, Bethesda, Md. 20014.
14. *Proceedings of the National Computer Conference and Exposition (Chicago)* (1974) Vol. 43, pp. 457–483, AFIPS Press, Montvale, N. J.
15. Almon, R. R., Andrew, C. G. & and Appel, S. H. (1974) *Biochemistry*, 13, 5522–5528.
16. Schmidt, J. & Raftery, M. A. (1973) *Anal. Biochem.*, 52, 349–352.
17. Brockes, J. P. & Hall, Z. W. (1974) *Biochemistry*, 14, 2092–2099.
18. Almon, R. R. & Appel, S. H. (1976) *Biochemistry* 15, 3662–3667.
19. Chiu, T. H., Dolly, J. O. & Barnard, E. A. (1973) *Biochem. Biophys. Res. Commun.*, 51, 205–209.
20. Brockes, J. P. & Hall, Z. W. (1975) *Biochemistry*, 14, 2100–2106.
21. Almon, R. R. & Appel, S. H. (1975) *Biochim. Biophys. Acta*, 393, 66–77.