Monoclonal Anti-Acetylcholine-Receptor Antibodies Directed against the Cholinergic Binding Site†

Daria Mochly-Rosen and Sara Fuchs*

ABSTRACT: We have isolated 32 hybridoma cell lines producing monoclonal antibodies against the acetylcholine receptor from *Torpedo californica*. One of these lines, designated 5.5.G.12, secretes antibodies which are directed against the cholinergic binding site of the acetylcholine receptor. This specific antibody blocked the binding of α-bungarotoxin to the acetylcholine receptor. The binding of monoclonal antibody 5.5.G.12 to acetylcholine receptor was inhibited by α-neurotoxins and by other cholinergic ligands in accordance with their affinities to the nicotinic acetylcholine receptor. None of the other monoclonal antibodies obtained inhibited the binding of α-bungarotoxin to acetylcholine receptor, nor was their binding to the acetylcholine receptor inhibited by cholinergic ligands. The monoclonal antibody elicited against the binding site of *Torpedo* acetylcholine receptor bound also to acetylcholine receptors of various species and organs, demonstrating the wide structural homology between the cholinergic sites of various acetylcholine receptors.

Antibodies elicited in animals against purified acetylcholine receptor (AChR)[1] from electric organs of electric fish inhibit the physiological activity of AChR as well as the binding of α-bungarotoxin (α-Bgt) to the receptor (Sugiyama et al., 1973; Patrick et al., 1973; Green et al., 1975; Aharonov et al., 1977). Sera from patients with myasthenia gravis, which contain antibodies against self-AChR, were also demonstrated to block the binding of α-Bgt to AChR (Bender et al., 1976; Almon & Appel, 1976). However, these data are not a direct indication for the presence of antibodies against the cholinergic binding site. Moreover, there are many reports which demonstrate that the binding of α-Bgt or other cholinergic ligands to AChR does not interfere with the binding of antibodies to the AChR (Lindstrom, 1976; Aharonov et al., 1977). It was thus suggested that the binding site of the AChR is not immunogenic and that the blocking of α-Bgt binding by anti-AChR antibodies is due to a steric hindrance by antibodies which are not necessarily directed against the cholinergic binding site (Aharonov et al., 1977). However, antibodies against the cholinergic binding site of AChR might be present in low amounts in sera of immunized animals and myasthenic patients, but the experimental procedures employed were not appropriate to detect this specificity.

The question of whether antibodies to the cholinergic binding site of AChR are elicited by immunization or by spontaneous autosensitization is of crucial interest both for evolutionary and immunological aspects. If the binding site is structurally conserved through evolution, it can be expected not to be immunogenic. However, if antibodies against this site are obtained, they may be involved in the pathogenesis of myasthenia gravis and experimental autoimmune myasthenia gravis. As mentioned above, studies on sera containing heterogeneous populations of antibodies have not led to conclusive results concerning this point. We have chosen to produce monoclonal antibodies (mcAbs) to the AChR since this technique, in which a single antibody specificity, even a minor one, can be augmented and analyzed separately, is very helpful in such a case. Using this technique, we have isolated a cell line which produces monoclonal antibodies specifically directed against the cholinergic binding site of the AChR. In this report we describe the preparation and characterization of these mcAbs. We found that these antibodies have binding properties similar to cholinergic nicotinic ligands. A preliminary report of this research has been previously published (Mochly-Rosen et al., 1980).

Materials and Methods

AChR was solubilized by Triton X-100 from the electric organ of *Torpedo californica* (Pacific Bio-Marine, Venice, CA) and was purified as described by Aharonov et al. (1977). AChR-enriched membrane fragments were purified following the method of Cohen et al. (1972), with some modifications described by Tarrab-Hazdai et al. (1980). Reduced carboxymethylated AChR (RCM-AChR) was prepared by reduction and carboxymethylation of AChR in 6 M guanidine hydrochloride (Bartfeld & Fuchs, 1977); trypsin-digested AChR (T-AChR) was prepared as described by Bartfeld & Fuchs (1979).

Chicken (Prives et al., 1976), mouse, and rat (Yaffe, 1973) primary muscle cultures were used for the preparation of muscle cell membranes. The cells in the cultures were collected with a rubber policeman in 0.01 M Tris-HCl buffer, pH 7.4, containing 10^{-3} M EDTA and 10^{-5} M PMSF. The cells were then homogenized, and the homogenate was centrifuged (1000*g*) at 4 °C for 10 min. The supernatant was centrifuged at 100000*g* at 4 °C for 1 h. This pellet was resuspended and is referred to as "muscle cell membranes". An enriched membrane fraction of mouse whole brain was prepared according to the following procedure. Mouse brain was homogenized in phosphate-buffered saline (PBS) containing 10^{-5} M PMSF at 100 mg wet weight/mL. The homogenate was centrifuged at 1000*g* for 10 min and 4 °C, and the supernatant was layered on a discontinuous sucrose gradient of 42%, 37%, and 30% sucrose, respectively, and centrifuged overnight at 20000 rpm in a Beckman ultracentrifuge SW-50 rotor. The 37% sucrose layer was used for further assays.

α-Bungarotoxin (α-Bgt) was prepared according to Clark et al. (1972). Iodination of proteins with ^{125}I was performed

† From the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel. *Received March 13, 1981.* This research was supported by grants from the Muscular Dystrophy Association of America and the Los Angeles Chapter of the Myasthenia Gravis Foundation.

[1] Abbreviations used: AChR, acetylcholine receptor; α-Bgt, α-bungarotoxin; mcAb, monoclonal antibody; RCM-AChR, reduced and carboxymethylated acetylcholine receptor; T-AChR, trypsinated acetylcholine receptor; PBS, phosphate-buffered saline; BSA, bovine serum albumin; GaMIg, goat anti-mouse immunoglobulins; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride.

0006-2960/81/0420-5920$01.25/0

by the chloramine-T method (Hunter, 1978).

Monoclonal antibodies with anti-AChR activity were formed as described previously (Mochly-Rosen et al., 1979) by using the cell hybridization technique of Köhler & Milstein (1975). In principle, C57BL/6 mice were injected twice each with 10 μg of purified AChR. Spleen cells of these mice were subjected to adoptive transfer (Eshhar et al., 1979) in order to enrich the specific antibody-forming cells. The spleens of the recipient mice were fused with P3-NSI/1-Ag4-1 (NS1) nonsecreting plasmacytoma cells in the presence of poly(ethylene glycol). The hybrid lines having anti-AChR activity (tested by radioimmunoassay with [^{125}I]AChR) were cloned on agar and further propagated in vitro and in vivo as ascitic tumors.

For radioimmunoassay, 25-μL aliquots of the ^{125}I-labeled antigen containing 2.5 ng of [^{125}I]AChR, [^{125}I]RCM-AChR, or [^{125}I]T-AChR or 50 ng of [^{125}I]Bgt–AChR complex in 0.01 M Tris-HCl buffer, pH 7.4, and 0.1% Triton X-100, containing bovine serum albumin (BSA) (1 mg/mL), were incubated for 30 min at 37 °C with 25 μL of the test antibody, diluted in PBS containing 5% normal mouse serum. Goat anti-mouse immunoglobulin (GaMIg) (100 μL) was added, and the test tubes were incubated for 30 min at 37 °C and then overnight at 4 °C. The precipitates formed were centrifuged, washed twice, and counted. For inhibition of the radioimmunoassay, [^{125}I]AChR was incubated with increasing concentrations of different cholinergic ligands for 40 min at room temperature, before the addition of the test antibodies (in a dilution that bound ~30% of the antigen), and the assay was continued as described above. The binding of anti-AChR antibodies to membrane preparations was measured by solid-phase radioimmunoassay (Klinman et al., 1976). Wells of microtiter plates were coated with 100 μL of either *Torpedo* membranes containing 20 μg of protein/mL and 2 nmol of α-Bgt sites/mg of protein or muscle membranes containing 500 μg of protein/mL and 2 pmol of α-Bgt sites/mg of protein and incubated for 2 h at room temperature or overnight at 4 °C. Unbound antigen was removed and the plates were washed 3 times with PBS containing 1% BSA (diluent) and incubated for an additional 1 h at room temperature with this solution in order to saturate free sites in the plastic wells. The solution was flicked out, and 50 μL of the test antibody was added and incubated for 2 h at room temperature. This solution was flicked out, and then the wells were washed 4 times with the diluent. Radiolabeled goat anti-mouse immunoglobulin (25 μL, [^{125}I]GaMIg, 50 000–100 000 cpm, 5–10 ng of protein) was added to each well and the plates were incubated overnight at 4 °C. After 5 washes with PBS, the plates were dried, and each well was cut out and counted in the γ counter. For inhibition of solid phase radioimmunoassay, increasing amounts of the inhibitor were added to the membrane-coated wells for 40 min at room temperature before the addition of the antibodies.

Biosynthetic labeling of mcAb 5.5.G.12 was carried out by the addition of [^{75}Se]methionine (Amersham, 100 μCi) to the hybridoma cell culture [(5–10) × 10^6 cells in 5 mL of methionine-free Dulbecco modified Eagle's medium containing 15% horse serum] and incubation for 5–7 h. The cells were pelleted and discarded, and the supernatant was dialyzed against PBS to remove unbound radiolabeled methionine. Aliquots of [^{75}Se]Met-mcAb 5.5.G.12 (15 000 cpm, ~300 ng of mcAb 5.5.G.12) were incubated with different amounts of membranes for 30 min at 37 °C in Eppendorf test tubes. The tubes were centrifuged for 15 min at 4 °C and washed once with cold PBS, and the pellets were counted in a γ counter. Specificity of the binding was verified by the ability of unlabeled mcAb 5.5.G.12 to compete with the labeled antibodies. For inhibition of the binding of [^{75}Se]Met-mcAb 5.5.G.12, various unlabeled anti-AChR antibodies were added to the membrane preparations and incubated with them for 30 min at 37 °C before the addition of the labeled antibodies. In experiments of inhibition by cholinergic ligands, the ligand was incubated with the membranes for 40 min at room temperature prior to the addition of the labeled antibodies.

α-[^{125}I]Bungarotoxin binding was determined as described by Aharonov et al. (1975) and by Olsen et al. (1972). Inhibition of the binding of α-[^{125}I]Bgt by different cholinergic ligands was performed according to Aharonov et al. (1977). Inhibition of α-Bgt binding by mouse anti-AChR antibodies or mcAbs was done in a similar procedure described elsewhere for rabbit anti-AChR antibodies (Bartfeld & Fuchs, 1977): AChR was incubated with increasing amounts of the test antibodies for 15 min at room temperature. α-[^{125}I]Bgt (~ 50 000 cpm, 5 ng of α-Bgt) was then added, and the reaction mixtures were further incubated for 15 min at room temperature for binding at equilibrium or for 30 s at 4 °C for initial rate studies. The degree of inhibition of toxin binding by antibodies was determined relative to the 0% inhibition measured by the addition of α-[^{125}I]Bgt to AChR before the addition of antibody.

Results

Production of Anti-AChR mcAb and Their Antigenic Characterization. Hybridization of spleen cells from AChR-immunized C57BL/6 mice with NS1 plasmacytoma cells and the preparation of monoclonal antibodies were performed as described under Materials and Methods. Specific antibody producing lines were determined by radioimmunoassay with radiolabeled *Torpedo* AChR ([^{125}I]AChR). The antigenic specificity of the various anti-AChR antibody lines was assessed by radioimmunoassays with various preparations of *Torpedo* AChR such as membraneous AChR, Triton-solubilized AChR, the α-Bgt–AChR complex, denatured AChR (RCM-AChR), and trypsinated AChR (T-AChR). The monoclonal antibodies were also assayed for their binding capacity to various muscle AChR preparations of different species. As we have observed in previous hybridizations (Mochly-Rosen et al., 1979), some anti-AChR monoclonal antibodies bound trypsinated AChR and did not bind denatured AChR, whereas other monoclonal antibodies bound denatured AChR and not trypsinated AChR. The former group represents antibodies directed against conformational antigenic determinants of AChR, and the latter represents antibodies directed against nonstructural antigenic determinants of AChR, which are present also on the denatured receptor. It is of interest to point out that we also found that all the mcAbs bound to membraneous *Torpedo* AChR at higher antibody dilutions when compared to the Triton-solubilized AChR. Some of the mcAbs cross-reacted also with membrane preparations of rat, mouse, and chick primary muscle cultures (Souroujon et al., 1981).

All but one of the mcAbs bound equally to [^{125}I]AChR and to α-[^{125}I]Bgt–AChR. The mcAB designated 5.5.G.12, which did not bind to the α-Bgt–AChR complex, belongs to the family of mcAbs directed against conformational antigenic determinants; it bound selectively T-AChR and did not bind RCM-AChR (Table I).

Similar to the other mcAbs obtained, mcAb 5.5.G.12 bound to *Torpedo* AChR-enriched membranes (Table II). This binding was inhibited by unlabeled α-Bgt and other cholinergic ligands (see Table IV), indicating that the antibodies bound specifically to the AChR in the membranes. For these ex-

Table I: Specificity of 5.5.G.12 Monoclonal Antibody

antibody	antibody titer (M) toward[a] AChR	T-AChR	RCM-AChR	α-Bgt-AChR
5.5.G.12	6.4×10^{-9}	4.9×10^{-9}	-	-
mouse anti-AChR	1.9×10^{-7}	1.9×10^{-7}	4.1×10^{-7}	3.2×10^{-7}

[a] Antibody titers were determined by radioimmunoassay using goat anti-mouse Ig for the precipitation of the Ag-Ab complex. Titers are expressed as moles of antigen precipitated per liter of serum.

Table II: Binding of mcAb 5.5.G.12 to Membraneous AChR (Solid-Phase Radioimmunoassay)

membrane preparation	antibody dilution	antibody binding (cpm) 5.5.G.12	URS[a]
Torpedo AChR-enriched membranes	1/100	3697	1076
	1/1000	3751	244
	1/10000	2976	136
membranes from chick primary muscle cell culture	1/10	2553	1043
	1/20	1917	1005
membranes from rat primary muscle culture	1/50	5077	1257
mouse brain membranes	1/2	5363	1871
	1/5	4975	882
	1/10	3753	582

[a] URS, monoclonal antibody with an unrelated specificity.

periments the solid-phase radioimmunoassay was used, utilizing ^{125}I-labeled goat anti-mouse Ig to quantitate the binding of the antibodies to the membraneous AChR (see Materials and Methods).

As shown in Table III the binding of [^{75}Se]Met-mcAb 5.5.G.12 was inhibited by the unlabeled mcAb 5.5.G.12 and by mouse anti-AChR antibodies. Only limited inhibition was obtained with other anti-AChR mcAbs suggesting that they are directed against different antigenic sites in AChR, sufficiently remote from the site to which mcAb 5.5.G.12 is bound. Moreover, it is possible that the inhibition obtained by mouse anti-AChR serum is due to an antibody population with a specificity similar to that of mcAb 5.5.G.12. In addition to the binding of mcAb 5.5.G.12 to membraneous *Torpedo* AChR, it also bound membrane preparations of rat, mouse, and chicken muscle when tested by the solid-phase radioimmunoassay (Table II). Specific binding was also observed with membranes of mouse brain (Table II).

Inhibition of Binding of α-Bgt to AChR by mcAb 5.5.G.12. Mouse anti-AChR serum, like rabbit anti-AChR serum (Aharonov et al., 1977), inhibited the binding of α-Bgt to AChR. Since mcAb 5.5.G.12 appeared to be directed to the α-Bgt binding site of AChR and since it bound specifically only to AChR preparations which retained their α-Bgt binding activity (AChR and T-AChR, Table I), it was of interest to test the effect of this mcAb on the binding of α-Bgt. As shown in Figure 1 mcAb 5.5.G.12 inhibited the binding of α-Bgt to AChR at least as effectively as mouse anti-AChR serum did. Similar inhibition of the binding of α-Bgt to T-AChR and to chicken and rat primary muscle cultures was also obtained. It should be noted that the titer of anti-AChR antibodies as measured by radioimmunoassay with [^{125}I]AChR was at least 10 times higher in anti-AChR serum than in mcAb 5.5.G.12. None of the other mcAb with anti-AChR activity had a significant inhibitory effect (data not shown) on the binding of α-Bgt to AChR, nor did a mixture of 10 of such antibodies (Figure 1). The inhibition of the binding of α-Bgt to AChR

Table III: Inhibition of the Binding of [^{75}Se]Methionine mcAb 5.5.G.12 to *Torpedo* AChR-Rich Membranes by Various Anti-AChR Antibodies

antibody	antibody dilution	inhibition of binding[a] (%)
mouse anti-AChR serum	1/10	84 ± 3
	1/100	59 ± 6
normal mouse serum	1/10	22 ± 1.5
	1/100	32 ± 12
5.5.G.12 ascitic fluid	1/10	98 ± 3
	1/100	83 ± 11
5.14 ascitic fluid	1/10	35 ± 6
	1/100	38 ± 22
5.33 ascitic fluid	1/10	6 ± 10
	1/100	2 ± 1
5.34 ascitic fluid	1/10	19 ± 5
	1/100	17 ± 18
1.17-K ascitic fluid	1/10	23 ± 10
	1/100	1 ± 8
URS[b] ascitic fluid	1/10	17 ± 6
	1/100	6 ± 7

[a] Average of three experiments. [b] URS, mcAb with an unrelated specificity.

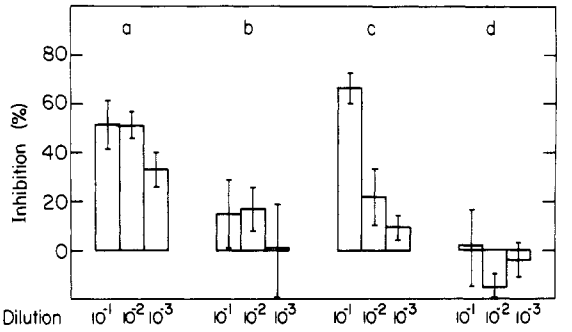

FIGURE 1: Inhibition of the binding of α-[^{125}I]Bgt to AChR by (a) mcAb 5.5.G.12, (b) a mixture of 10 different anti-AChR mcAbs other than mcAb 5.5.G.12, (c) mouse anti-AChR serum, and (d) normal mouse serum. The experiment was performed under equilibrium conditions (see Materials and Methods), and α-[^{125}I]Bgt was incubated for 15 min at room temperature with AChR following the preincubation with the tested antibodies. The data represent average values of three different experiments.

or to muscle cultures by mcAb 5.5.G.12 or by mouse anti-AChR serum has never exceeded 60% when the inhibition experiments were performed under equilibrium conditions, allowing a long incubation with α-Bgt (15 min, see Materials and Methods). This could be due to an exchange of the bound antibodies to AChR by the high-affinity α-Bgt, during the long incubation with the latter. Indeed, when the inhibition test was determined at initial rates (i.e., incubation with α-Bgt for 30 s at 4 °C, see Materials and Methods), a complete inhibition of α-Bgt binding was obtained with 100 μg of mcAb 5.5.G.12 immunoglobulins (Figure 2). Fifty percent inhibition was achieved by an immunoglobulin concentration of 3×10^{-8} M.

Inhibition of Binding of mcAb 5.5.G.12 to AChR by Cholinergic Ligands. As demonstrated above, mcAb 5.5.G.12 did not bind to AChR when complexed with α-Bgt (Table I). In order to analyze the nature and specificity of the antigenic determinant of the AChR reacting with these antibodies, we tested the effect of several cholinergic ligands on the binding of mcAb 5.5.G.12 to AChR. As can be seen in Figure 3, unlabeled α-bungarotoxin, *Naja naja siamensis* α-neurotoxin, as well as agonists and antagonists for the nicotinic AChR, inhibited the binding of mcAb 5.5.G.12 to AChR according to their binding affinities for the nicotinic receptor. The ligand concentrations required for 50% inhibition were similar to the

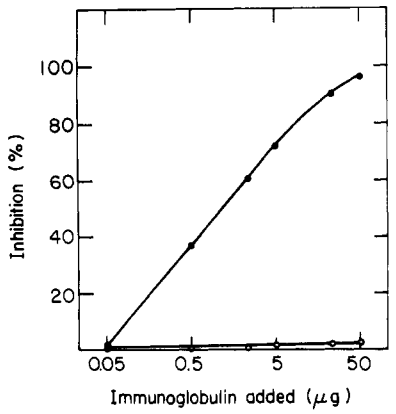

FIGURE 2: Inhibition of binding of α-[^{125}I]Bgt to AChR by mcAb 5.5.G.12 (●) and by normal mouse immunoglobulins (○). α-[^{125}I]Bgt was incubated with the AChR for 30 s at 4 °C following preincubation with the immunoglobulins.

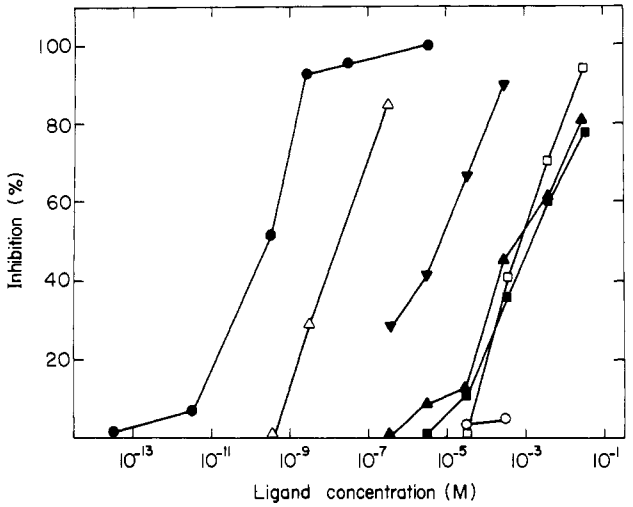

FIGURE 3: Inhibition of binding of [^{125}I]AChR to mcAb 5.5.G.12 by α-bungarotoxin (●), *N. naja siamensis* α-neurotoxin (△), *d*-tubocurarine (▼), decamethonium (▲), carbamoylcholine (■), acetylcholine (□), and atropine (○).

concentrations required for 50% inhibition of the binding of α-[^{125}I]Bgt to AChR (Table IV). Similar effects of the cholinergic ligands were observed also on the binding of mcAb 5.5.G.12 to *Torpedo* AChR-rich membranes (Table IV). Atropine, a muscarinic cholinergic agonist, did not inhibit significantly the binding of mcAb 5.5.G.12 to AChR, nor did the two local anasthetics, procaine, or tetracaine.

Discussion

Monoclonal antibodies which do not bind to AChR in the presence of α-Bgt were described by Gomez et al. (1979) and by James et al. (1980). Whereas the binding of the mcAb described by Gomez et al. was not inhibited by ligands other than α-Bgt, the binding of one of the mcAbs described by James et al. was also inhibited by *d*-tubocurarine and carbamoylcholine. In this report we describe the properties of a monoclonal antibody (5.5.G.12) directed against the cholinergic binding site of AChR. The binding of this antibody to AChR is inhibited by cholinergic ligands, in accordance with their binding affinities to the receptor. Moreover, this antibody binds to the AChR in primary cultures of rat, chick, and mouse muscle and to mouse brain. This is to be expected from an evolutionarily conserved site.

The cholinergic binding site of AChR is conformation dependent, and denaturation of the receptor abolishes its binding

Table IV: Inhibition of the Binding of mcAb 5.5.G.12 and of α-Bungarotoxin to AChR by Cholinergic Ligands

	ligand concn (M) at 50% inhibition for inhibition of		
ligand	α-bungarotoxin binding to AChR[a]	mcAb 5.5.G.12 binding to AChR[a]	mcAb 5.5.G.12 binding to membranal AChR[b]
α-bungarotoxin	1.5×10^{-10}	3.0×10^{-10}	4.0×10^{-9}
N. naja siamensis toxin	1.0×10^{-9}	1.3×10^{-8}	9.0×10^{-9}
d-tubocurarine	9.0×10^{-6}	6.2×10^{-6}	5.0×10^{-6}
decamethonium	3.0×10^{-5}	3.3×10^{-4}	2.0×10^{-4}
carbamoylcholine	1.7×10^{-4}	5.0×10^{-4}	2.8×10^{-5}
acetylcholine	1.0×10^{-5}	6.1×10^{-4}	ND[c]
atropine	1.2×10^{-2}	$>10^{-2}$	$>10^{-2}$

[a] Triton X-100 solubilized AChR. [b] AChR-enriched membranes. [c] ND, not determined.

capacity (Bartfeld & Fuchs, 1977). This is compatible with the finding that mcAb 5.5.G.12, which is directed against the cholinergic binding site, did not bind to denatured AChR (Table I) or to any of the isolated denatured subunits (M. Souroujon, D. Mochly-Rosen, and S. Fuchs, unpublished experiments). Other monoclonal antibodies which were shown to be directed against nonstructural antigenic determinants, reacted with denatured receptor and with the isolated subunits (M. Souroujon, D. Mochly-Rosen, and S. Fuchs, unpublished experiments).

The specificity of mcAb 5.5.G.12 was verified by two kinds of inhibition experiments: (a) inhibition of α-Bgt binding to AChR by the antibodies and (b) inhibition of the binding of the antibodies to AChR by α-neurotoxins and by cholinergic ligands. Both these assays demonstrated that mcAb 5.5.G.12 is directed against the cholinergic binding site or at least against a site very closely associated with it. The I_{50} values obtained for the various cholinergic ligands for inhibition of 5.5.G.12 binding to AChR are similar to those for inhibition of α-Bgt binding to the AChR [*N. naja siamensis* toxin > *d*-tubocurarine > carbamoylcholine ≫ atropine (Table IV)]. Anti-AChR sera inhibited the binding of α-Bgt to AChR as did mcAb 5.5.G.12, but the binding of anti-AChR sera to AChR was not affected significantly by α-Bgt or other cholinergic ligands. Since we were able to isolate a clone producing antibodies specifically directed against the acetylcholine binding site, anti-AChR sera must also contain antibodies of this specificity. The failure to observe inhibition of antiserum binding to AChR by cholinergic ligands could be explained if relatively small amounts of antibodies of this particular specificity are present. Out of the 32 anti-AChR mcAbs tested, only mcAb 5.5.G.12 inhibited α-Bgt binding to AChR. When the other mcAbs were tested for their ability to inhibit the binding of mcAb 5.5.G.12 to AChR, only one clone, 5.14, showed any significant inhibition (Table III). This antibody, which is specific for the denatured form of the α subunit of the AChR (M. Souroujon, D. Mochly-Rosen, and S. Fuchs, unpublished experiments), did not affect the binding of α-Bgt to the receptor. The differential response to mcAb 5.14 in inhibiting α-Bgt binding as compared to mcAb 5.5.G.12 binding to AChR is probably due to the difference in the molecular weights of antibodies (150 000) and α-Bgt (8000). Thus, the binding of mcAb 5.5.G.12 (anti-binding-site antibodies) to the cholinergic site can also be inhibited by antibodies not directed against the same site, provided that they are against a site which is in close proximity to the cholinergic site (e.g., on the same polypeptide chain).

In the light of our results, the role and involvement of antibodies against the cholinergic binding site in the pathology of anti-AChR antibodies should be considered. Anti-AChR antibodies are detected in the sera of most myasthenic patients, but there is no good correlation between antibody titer and clinical state. The assay of anti-AChR antibodies in myasthenic patients is based on the use of human AChR complexed with labeled α-Bgt. This assay cannot detect antibodies against the cholinergic binding site although they may be present and relevant to the disease. Therefore, testing for blocking antibodies and for antibodies which affect the turnover of AChR may be more significant. It is possible that antibodies of several defined specificities may be involved in the pathogenesis of the disease and that antibodies to the binding site are just one of them. Reports from two other groups (Lennon & Lambert, 1980; Richman et al., 1980) have demonstrated that experimental myasthenia was passively transferred by mcAb directed against determinants not associated with the cholinergic binding site.

Monoclonal antibodies provide a powerful tool for studies of the structure and function of the AChR. The ability to select for one particular antibody specificity, even a minor one, is one of the advantages offered by cell hybridization and monoclonal antibodies. The availability of a particular mcAb directed against the cholinergic binding site enables us to compare the binding sites of the AChR from different species and different tissues. Moreover, these antibodies may be used to evaluate the biological importance of such a site-directed antibody in experimental and spontaneous autosensitization against AChR.

Acknowledgments

We thank Dr. Zelig Eshhar and Dr. Adrienne Gordon for fruitful discussions.

References

Aharonov, A., Kalderon, N., Silman, I., & Fuchs, S. (1975) *Immunochemistry 12*, 765.

Aharonov, A., Tarrab-Hazadai, R., Silman, I., & Fuchs, S. (1977) *Immunochemistry 14*, 129.

Almon, R., & Appel, S. H. (1976) *Ann. N.Y. Acad. Sci. 274*, 235.

Bartfeld, D., & Fuchs, S. (1977) *FEBS Lett. 77*, 214.

Bartfeld, D., & Fuchs, S. (1979) *Biochem. Biophys. Res. Commun. 89*, 512.

Bender, A. N., Ringel, S. P., Engel, W. K., Vogel, Z., & Daniels, M. P. (1976) *Ann. N.Y. Acad. Sci. 274*, 20.

Clark, D. G., McMurchie, D. D., Elliot, E., Wolcott, R. G., Landel, A. M., & Raftery, M. A. (1972) *Biochemistry 11*, 1663.

Cohen, J. B., Weber, M., Huchet, M., & Changeux, J.-P. (1972) *FEBS Lett. 26*, 43.

Eshhar, Z., Blatt, C., Bergman, Y., & Haimovich, J. (1979) *J. Immunol. 122*, 2430.

Gomez, C. M., Richman, D. P., Berman, P. W., Burres, S. A., Arnason, B. G. W., & Fitch, F. W. (1979) *Biochem. Biophys. Res. Commun. 88*, 575.

Green, D. P. L., Miledi, R., & Vincent, A. (1975) *Proc. R. Soc. London, Ser. B. 189*, 57.

Hunter, W. M. (1978) in *Handbook of Experimental Immunology* (Weir, D. M., Ed.) pp 14.1–14.40, Blackwell, Oxford.

James, R. W., Kato, A. C., Rey, M. J., & Fulpius, B. W. (1980) *FEBS Lett. 120*, 145.

Klinman, N. R., Pickard, A. R., Segal, N. H., Gerhart, T. J., Netcalf, E. S., & Pierce, S. K. (1976) *Ann. Immunol. (Paris) 127C*, 489.

Köhler, G., & Milstein, C. (1975) *Nature (London) 256*, 495.

Lennon, V. A., & Lambert, E. H. (1980) *Nature (London) 285*, 238.

Lindstrom, J. (1976) *J. Supramol. Struct. 4*, 389.

Mochly-Rosen, D., Fuchs, S. & Eshhar, Z. (1979) *FEBS Lett. 106*, 389.

Mochly-Rosen, D., Bartfeld, D., Eshhar, Z., Tarrab-Hazdai, R., & Fuchs, S. (1980) *Isr. J. Med. Sci. 16*, 805.

Olsen, R. W., Meunier, J. C., & Changeux, J. P. (1972) *FEBS Lett. 28*, 96.

Patrick, J., Lindstrom, J., Culp, B., & McMillan, J. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 3334.

Prives, J., Silman, I., & Amsterdam, A. (1976) *Cell (Cambridge, Mass.) 7*, 543.

Richman, D. P., Gomez, C. M., Berman, P. W., Burres, S. A., Fitch, F. W., & Arnason, B. G. W. (1980) *Nature (London) 286*, 738.

Souroujon, C. M., Mochly-Rosen, D., & Fuchs, S. (1981) *Isr. J. Med. Sci.* (in press).

Sugiyama, H., Benda, P., Meunier, J. C., & Changeux, J. P. (1973) *FEBS Lett. 35*, 124.

Tarrab-Hazdai, R., Bercovici, T., Goldfarb, V., & Gitler, C. (1980) *J. Biol. Chem. 255*, 1204.

Yaffe, D. (1973) in *Tissue Culture Methods and Applications* (Kruse, P. F., & Patterson, M. K., Eds.) pp 106–114, Academic Press, New York.