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EFFECTS OF A MONOCLONAL ANTI-ACETYLCHOLINE RECEPTOR ANTIBODY ON THE AVIAN END-PLATE

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SUMMARY

1. The effects of anti-acetylcholine receptor (AChR) monoclonal antibodies (mAbs) 370 and 132A on miniature end-plate potentials (MEPPs) and end-plate currents (EPCs) in the posterior latissimus dorsi muscle of adult chickens were investigated.

2. After incubation of the electrophysiological preparation with mAb 370 (5–50 μg/ml), which blocks both agonist (carbamylcholine) and α-bungarotoxin (α-BTX) binding and induces a hyperacute form of experimental autoimmune myasthenia gravis (EAMG), MEPP and EPC amplitudes were irreversibly reduced.

3. This effect was not associated with any significant change in the time constant describing EPC decay (τ_{EPC}), current reversal potential, or the voltage dependence of τ_{EPC}. The τ_{EPC} at -80 mV was $5{\cdot}9 \pm 0{\cdot}6$ ms before incubation with mAb 370 (50 μg/ml) and $6{\cdot}0 \pm 0{\cdot}9$ ms afterwards. Current reversal potential was $-3{\cdot}9 \pm 0{\cdot}4$ mV before mAb incubation and $-4{\cdot}8 \pm 1{\cdot}5$ mV afterwards. The change in membrane potential required to produce an e-fold change in τ_{EPC} was $128 \pm 2{\cdot}3$ mV before antibody incubation compared to $125 \pm 6{\cdot}6$ mV after incubation.

4. A second anti-AChR mAb, 132A (50 μg/ml), which is capable of inducing the classically described form of EAMG without blocking agonist or α-BTX binding, or inducing hyperacute EAMG, produced no significant change in MEPP amplitude, EPC amplitude, τ_{EPC} or EPC reversal potentials.

5. The mAb 370 (50 μg/ml) induced a partially reversible decrease of the quantal content of the neurally evoked end-plate potential (EPP). This effect was not observed with mAb 132A, (+)tubocurarine (10^{-7}–10^{-5} g/ml) or an irrelevant anti-oestrogen receptor mAb.

6. These data suggest that the rapid onset of weakness observed in chicken hatchlings after the injection of mAb 370 (Gomez & Richman, 1983) can be attributed to a combined effect of a block of acetylcholine (ACh)-induced ion channel activity in the postsynaptic membrane and a reduction of the neurally evoked release of acetylcholine from the nerve terminal.

INTRODUCTION

The defect in neuromuscular transmission in the animal model of myasthenia gravis (MG), EAMG, induced by immunization with acetylcholine receptor (AChR; Patrick & Lindstrom, 1973; Lennon, Lindstrom & Seybold, 1975), has been attributed primarily to a reduction in the number of functional AChRs in the postsynaptic membrane (Lindstrom, Einarson, Lennon & Seybold, 1976) without change in channel properties (Alema, Cull-Candy, Miledi & Trautmann, 1981; Hohlfeld, Sterz, Kalies, Peper & Wekerle, 1981). In contrast, the hyperacute form of EAMG (Gomez & Richman, 1983), which is characterized by profound weakness and death within an hour of injection of anti-AChR monoclonal antibodies (mAbs) directed against the α-bungarotoxin (α-BTX) binding region, could result from mAb-induced alterations in functional ion channel activation. At variance with the findings in classical EAMG (Lennon, Seybold, Lindstrom, Cochrane & Ulevitch, 1978; Sahashi, Engel, Lindstrom, Lambert & Lennon, 1978; Engel, Sakakibara, Sahashi, Lindstrom, Lambert & Lennon, 1979), animals with hyperacute EAMG develop paralysis in the absence of an accompanying inflammatory response or of end-plate membrane destruction. The lack of histological change supports the view that this class of mAbs exerts its effect through a direct inhibition of agonist-induced ion channel activity. That a similar mechanism may play a role in MG is suggested by the observations that antibodies directed against the α-BTX site are often present in that disease (Drachman, Adams, Josifek & Self, 1982; Schuetze, Vicini & Hall, 1985; Tzartos, Hochschwender, Vasquez & Lindstrom, 1987).

Sera from animals with EAMG induced by immunization with purified electric organ AChR contain high titres of antibodies directed against the heterologous (*Torpedo*) AChR as well as the host muscle AChR. These sera have been shown to block AChR channel activation *in vitro* as evidenced by a reduction in extrajunctional sensitivity in denervated rabbit muscle fibres (Niemi, Nastuk, Chang, Plescia & Plescia, 1981) and cultured human myotubes (Bevan, Kullberg & Heinemann, 1977). Recently, a highly specific serum from a patient with MG has been shown to contain antibodies that selectively block the slow extrajunctional class of AChRs (Schuetze *et al.* 1985). We have demonstrated that the mAbs that induce hyperacute EAMG are capable of blocking both agonist and α-BTX binding and can suppress carbamylcholine-induced cation influx into AChR-rich vesicles (Donnelly, Mihovilovic, Gonzalez-Ros, Ferragut, Richman & Martinez-Carrion, 1984).

We now report that following incubation of chicken muscle fibres with one of these mAbs, there was a significant decrement in both miniature end-plate potential (MEPP) and end-plate current (EPC) amplitudes, without a change in EPC decay. In addition, the antibody reduced the quantal content of the neurally evoked EPP. A preliminary account of these experiments has appeared in abstract form (Maselli, Gomez, Nelson & Richman, 1987).

METHODS

Monoclonal antibodies

Hybridomas secreting anti-AChR mAbs were formed by fusion of spleen cells from rats injected with AChR purified from *Torpedo californica* with cells of the SP2/O–Ag14 mutant hybridoma cell line as previously described (Gomez & Richman, 1983). We studied mAb 370, which is capable of blocking α-BTX binding and inducing hyperacute EAMG. The mAb 132A, which binds to the main immunogenic region of the α-subunit and does not block α-BTX or ligand binding, or induce hyperacute EAMG, was used as a control (Gomez & Richman, 1983; Mihovilovic & Richman, 1987). The mAb D547 to extranuclear oestrogen receptor (gift of G. Green), which does not cross-react with any structure of the neuromuscular junction (Gomez & Richman, 1987), was used as an irrelevant control antibody.

Preparation

Experiments were performed on the posterior latissimus dorsi muscle from chickens between 4 and 12 weeks after birth. This muscle is composed almost exclusively of fast-twitch, singly innervated fibres. The medial part of the muscle is thin enough to allow end-plate visualization (Ginsborg, 1960; Lebeda & Albuquerque, 1975).

Chickens were killed with an overdose of sodium pentobarbitone (75 mg/kg) and the muscle with its nerve supply was removed from the animal. Dissections were performed in a chamber which allowed for continuous superfusion of the preparation with oxygenated Tyrode solution.

Following dissection, muscles were pinned to a layer of cured Sylgard resin (Dow Corning Corp. Midland, MI, USA) in a Plexiglass chamber that had been pre-incubated for 20 min with 1% bovine serum albumin (BSA) in Ringer solution. Pre-incubation with BSA was necessary to avoid non-specific mAb binding to the recording chamber itself. The preparations were then transferred to the stage of an upright microscope (Leitz Laborlux 12) equipped with Hoffman interference contrast optics and observed at a total magnification of 250×.

Electrophysiology

Intracellular MEPP and EPP recording was carried out using standard electrophysiological techniques as described by del Castillo & Katz (1954). Microelectrodes were filled with 3 M-KCl and had resistances in the range of 10–25 MΩ. The physiological salt solution used during the recordings had the following composition (mM): NaCl, 140; KCl, 3·5; $MgCl_2$, 2·7; $CaCl_2$, 1·8; Na_2HPO_4, 0·5; $NaHCO_3$, 16; and glucose, 10. The bathing solution was continuously bubbled with a 95% O_2:5% CO_2 gas mixture. The pH of the solution was maintained between 7·3 and 7·4 and the bath temperature was held at approximately 30 °C during the entire experiment.

EPCs were recorded using a Dagan model 8500 intracellular preamp-clamp (Dagan Corp., Minneapolis, MN, USA). The voltage-clamp feed-back gain (total $2{\cdot}5 \times 10^4$) was maximally adjusted, avoiding oscillation. At the peak of the EPC the maximum potential changes at the voltage electrode site were less than 2–5% of the net driving force. Both voltage and current electrodes were filled with 3 M-KCl and had resistances ranging from 4 to 10 MΩ. EPCs were elicited by a supramaximal stimulation at 1 Hz applied to the motor nerve via a concentric bipolar electrode. In order to uncouple excitation from contraction, muscles were bathed for 25–30 min in normal Ringer solution containing 2 M-formamide and 15 mM-$NaHCO_3$ at a pH of 7·4 (Herrera, 1984). The preparations were subsequently washed and the recordings made in Tyrode solution. Experiments were carried out at room temperature (26·5 °C).

Data acquisition and analysis

Spontaneous MEPPs and neurally evoked EPPs were recorded using a WPI-750 electrometer (WPI Inc., Hamden, CT, USA). The output of the electrometer was amplified, filtered and sampled at 5–10 kHz by a 12-bit A/D converter (Data Translation 2818, Data Translation, MA, USA) using an IBM/AT. MEPPs from individual fibres were captured on-line using an adjustable-threshold, automated pattern-recognition routine and written into data files for subsequent analysis. The amplitudes of MEPPs and EPPs were corrected for the resting membrane potential (RMP), assuming a constant input resistance and a current reversal potential at O mV:

$$\mathrm{MEPP}_{\mathrm{corrected}} = \mathrm{MEPP} \times (-77/\mathrm{RMP}). \quad (1)$$

EPCs were elicited via computer-controlled voltage pulses delivered to the nerve via a Grass S-22 stimulator (Grass Corp. Quincy, MA, USA). Current recordings were sampled and stored on the IBM-AT. Exponential fits to the EPC decays were performed using a non-linear least-squares fitting routine.

For calculation of the quantal content (m), the preparations were dissected and the entire experiment performed in the presence of Tyrode solution containing 10 mM-Mg^{2+} in order to decrease the probability of the stimulus-induced release of ACh, thus allowing recording of subthreshold EPPs and MEPPs from the same end-plates. EPPs were obtained by supramaximal stimulation of the nerve at 1 Hz. The mean amplitude of 100 EPPs, excluding the first ten EPPs, was corrected for RMP and non-linear summation (Martin, 1955). The end-plate quantal content was calculated from EPP and MEPP data files using the indirect variance method where $m_i = (EPP/\sigma_{EPP})^2$. Where a multiple number of fibres were recorded for a given experimental condition, values are reported as the mean ± S.E.M. with the number of fibres in parentheses.

RESULTS

Effect of mAb 370 and mAb 132A on MEPP amplitudes

MEPPs were recorded from 255 chicken latissimus dorsi muscle fibres before and after 3 h of incubation with either mAb 370 or mAb 132A. A total of twenty to fifty MEPPs were detected and recorded on-line from each fibre, stored in a data file, and averaged at the end of each experiment. Examples of MEPP recordings are illustrated in Fig. 1*A*. In preparations incubated with 50 μg/ml of mAb 370, it was very difficult to record MEPPs despite normal resting potentials and direct placement of the electrode near the end-plate region, suggesting complete receptor blockade. This effect was formally quantified in one experiment which showed that MEPPs were present in 56% of impaled fibres before antibody incubation, whereas only 17% of impaled fibres showed MEPPs after incubation. However, in fibres where MEPPs could be recorded, the mean MEPP amplitude (results summarized in Fig. 1*B* and *C*) was reduced by 34% over control values in muscles incubated with 5 μg/ml of mAb 370, and 48% in muscles incubated with 50 μg/ml of mAb 370. These concentrations exceed the K_d of the mAbs (approximately 1 nM) by three orders of magnitude. In contrast, no reduction in MEPP amplitude was observed in muscles incubated with the mAb 132A (50 μg/ml, Table 1).

Following the 3 h antibody incubation and initial MEPP recording, the preparations were perfused with Tyrode solution for a period of at least 1 h and no

TABLE 1. Amplitudes of MEPPs recorded from posterior lattissimus dorsi muscles before and after incubation with mAbs 370 and 132A

	Before incubation	After incubation	P
mAb 370			
MEPP amplitude (mV)	0·76 ± 0·02 (73)	—	—
5 μg/ml mAb 370	—	0·50 ± 0·03 (17)	< 0·001
50 μg/ml mAb 370	—	0·40 ± 0·03 (37)	< 0·0001
mAb 132A			
MEPP amplitude (mV)	0·79 ± 0·03 (58)	0·81 ± 0·04 (70)	—

Data are displayed as mean ± S.E.M. with the number of fibres in parentheses. P values determined by Student's t test.

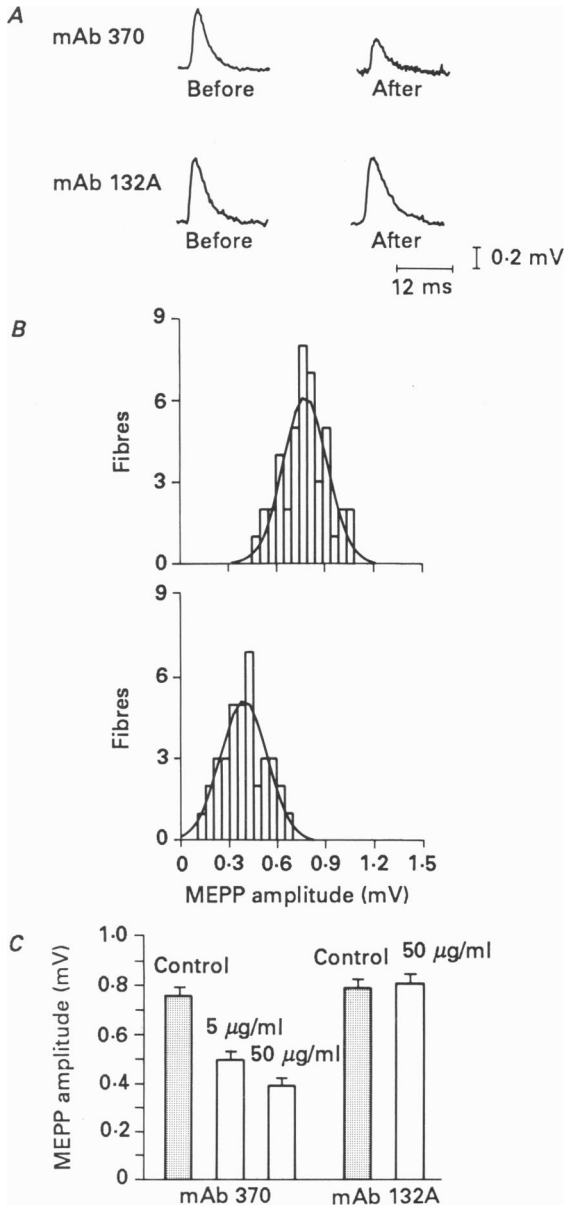

Fig. 1. The effect of mAb 370 and mAb 132A on chicken posterior latissimus dorsi MEPP amplitude. *A*, reduction in MEPP amplitude after incubation with mAb 370. Example of MEPPs recorded from representative fibres before and after incubation with either mAb 370 (50 μg/ml) or mAb 132A (50 μg/ml). Each signal is the average of twenty to fifty MEPPs obtained from the same fibre. *B*, MEPP amplitude distribution before and after incubation with mAb 370 (50 μg/ml). *C*, MEPP amplitude recorded from 255 fibres in a total of thirteen posterior latissimus dorsi preparations before and after incubation with either mAb 370 or mAb 132A. Bars represent the mean amplitude ($\pm$S.E.M.) of MEPPs recorded under the following conditions: (1) control conditions; (2) after incubation with mAb 370 (5 μg/ml); (3) after incubation with mAb 370 (50 μg/ml); (4) control conditions; and (5) after incubation with mAb 132A (50 μg/ml).

reversal in the mAb 370-induced reduction of MEPP amplitude was observed. The mean MEPP amplitude recorded after incubation with 50 μg/ml of mAb 370 was $0{\cdot}42 \pm 0{\cdot}04$ mV ($n = 20$ fibres) with mAb present, and $0{\cdot}38 \pm 0{\cdot}03$ mV ($n = 17$ fibres) during the wash-out. The mean resting potential for the fibres examined was minimally decreased following exposure to either mAb. Hence, it is unlikely that the decrement in MEPP amplitude induced by mAb 370 can be accounted for by a simple decrease in driving force during the course of the experiment. The mean frequency of MEPPs recorded before incubation with 50 μg/ml of mAb 370 was $0{\cdot}13 \pm 0{\cdot}02$/s ($n = 45$ fibres) and before incubation with 50 μg/ml of mAb 132A was $0{\cdot}20 \pm 0{\cdot}02$/s ($n = 31$ fibres). The mean frequency of MEPPs recorded after incubation with either monoclonal antibody was slightly increased being $0{\cdot}59 \pm 0{\cdot}13$/s ($n = 35$ fibres) and $0{\cdot}73 \pm 0{\cdot}08$/s ($n = 58$ fibres) for mAb 370 and mAb 132A, respectively. This increase in MEPP frequency is unlikely to be a specific antibody effect since it was also observed after 3 h of incubation with the irrelevant antibody mAb D547.

Effect of mAb 370 and mAb 132A on EPC amplitude

EPCs were recorded in ten preparations before and after a 3 h incubation of the tissue with either mAb 370 or mAb 132A (six and four preparations, respectively). EPCs were elicited via neural stimulation at the frequency of 1 Hz.

The mean EPC amplitude recorded at -80 mV was 125 ± 26 nA ($n = 14$) before incubation with mAb 370 (50 μg/ml) and 46 ± 8 nA ($n = 32$) following incubation ($P < 0{\cdot}001$). On the other hand, the mean peak amplitude of EPCs recorded at -80 mV after incubation with mAb 132A (50 μg/ml) was essentially unchanged: 102 ± 21 nA ($n = 12$) before and 92 ± 5 nA ($n = 10$) after incubation with mAb 132A. Typical EPC recordings obtained at different membrane potentials from a fibre prior to mAb 370 exposure and from a fibre following antibody incubation can be seen in Fig. 2*A*. Peak currents obtained from end-plates prior to incubation with mAb were characterized by a linear current–voltage relationship (Fig. 2*B*) with a reversal potential near 0 ($-3{\cdot}6$ mV). In this experiment, mean peak EPC amplitudes were reduced more than 10-fold after incubation of the muscle with mAb 370; however, the *I–V* relation remained linear with the reversal potential also near 0 ($-4{\cdot}8$ mV). The reversal potential before incubation with mAb 370 and 132A was $-3{\cdot}9 \pm 0{\cdot}4$ mV ($n = 7$) and $-5{\cdot}5 \pm 1{\cdot}7$ mV ($n = 10$), respectively. These values remained constant following mAb incubation and were determined to be $-4{\cdot}8 \pm 1{\cdot}5$ mV ($n = 8$) for mAb 370 and $-4{\cdot}9 \pm 1{\cdot}8$ mV ($n = 18$) for mAb 132. The absence of a change in reversal potential suggested that the mAb 370 did not significantly affect the channel ionic selectivity.

Effect of mAb 370 and mAb 132A on the time constant and voltage dependence of EPC decay

All of the EPCs decayed with a time course which could be adequately fitted with a single exponential, characteristic of first-order kinetics. After incubation with either mAb, the EPC decay course remained mono-exponential and the mean τ_{EPC} was not significantly changed.

Monoclonal antibody 370 reduced the peak amplitude without altering τ_{EPC} throughout the voltage range tested. This effect can be seen in Fig. 3*A*, which

displays the mean peak EPC amplitude as a function of voltage in five control fibres and five fibres recorded after incubation with mAb 370. Although there was a marked reduction of the mean EPC amplitude after muscle exposure to mAb 370, Fig. 3*B* shows that τ_{EPC} and its dependence on voltage was not affected by the antibody. The τ_{EPC} recorded at −80 mV was 5·9±0·6 ms ($n = 10$) before incubation

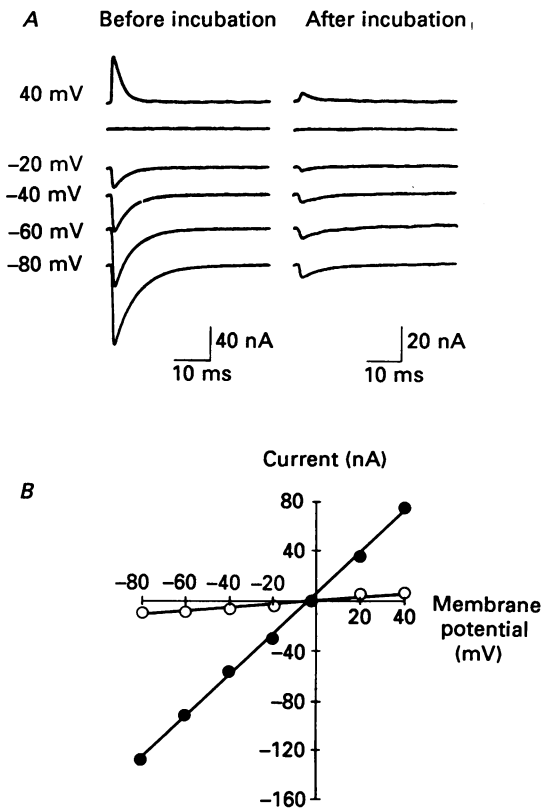

Fig. 2. Neurally evoked EPCs in the presence and absence of mAb 370. *A*, EPCs before and after incubation with mAb 30 (50 μg/ml). Each trace is the average of at least six EPCs. Note different current scales. *B*, peak EPC amplitudes plotted as a function of voltage before incubation with mAb 370 (●) and after incubation (○). Note that the EPC reversal potential after incubation with mAb 370 remains unchanged.

with mAb 370 (50 μg/ml) and 6·0±0·9 ms ($n = 28$) after incubation. Similarly, τ_{EPC} was 6·0±0·5 ms ($n = 10$) before and 6·2±0·5 ms ($n = 9$) after muscle incubation with mAb 132A. The change in voltage producing an e-fold change in τ_{EPC} was 128±2·3 mV ($n = 6$) before mAb 370 incubation compared to 125±6·6 mV ($n = 24$) after incubation.

Effect of mAb 370 and mAb 132A on the quantal content of the EPP

Since the decrease in EPC peak amplitude induced by mAb 370 could have been in part related to an additional presynaptic effect, the quantal content of EPPs obtained in the presence of high Mg^{2+} concentrations was measured before and after

mAb incubation. The results show that mAb 370 reduced the quantal content (m). This effect was not observed with mAb 132A, the non-active mAb D547 or concentrations of (+)tubocurarine which induced reduction of EPP amplitudes comparable to that induced by mAb 370. These results are summarized in Table 2. Interestingly, the presynaptic effect of quantal content reduction induced by mAb 370 was partially reversed during the wash-out period but the postsynaptic effect, i.e. reduction of MEPP amplitudes, was irreversible.

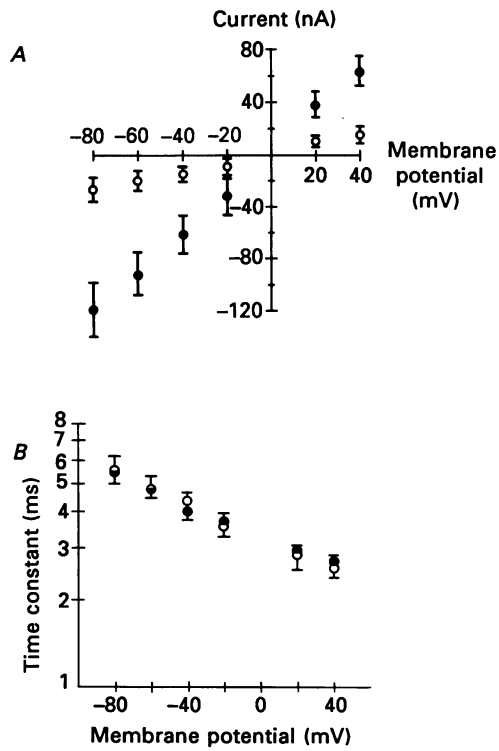

Fig. 3. EPC current–voltage relationship and voltage dependence of τ_{EPC} in the presence and absence of mAb 370. *A*, averaged values of peak EPC amplitudes from five fibres plotted as a function of voltage before (●) and after incubation (○) with mAb 370 (50 μg/ml). *B*, semilogarithmic plot of the mean τ_{EPC} as a function of voltage for the same five fibres as in *A* before (●) and after incubation (○) with mAb 370. The error bars represent the S.E.M.

DISCUSSION

Monoclonal antibodies acting as probes targeted to specific regions of the AChR molecule can induce profound modifications in AChR function (Mochly-Rosen & Fuchs, 1981; Watters & Maelicke, 1983; Donnelly *et al.* 1984; Mihovilovic & Richman, 1984, 1987; Blatt, Montal, Lindstrom & Montal, 1986; Dolly, Gwilt, Lacey, Newsom-Davis, Vincent, Whiting & W.-Wray, 1988). Our results indicate that, at the level of the intact end-plate, the effect of mAb 370 consists of a marked reduction in the sensitivity of the postsynaptic membrane to both the spontaneous

TABLE 2. Amplitudes and quantal content of neurally evoked EPPs before and after incubation with mAbs 370, 132A and D547 and (+)tubocurarine (10^{-7}–10^{-5} g/ml)

	Before incubation	After incubation	Wash-out
mAb 370 (50 μg/ml)			
EPP amplitude (mV)	1·46 ± 0·1 (25)	0·77 ± 0·04 (18)*	0·96 ± 0·1 (4)
Quantal content (m_i)	3·85 ± 0·3 (25)	1·97 ± 0·3 (23)*	3·40 ± 0·9 (4)
mAb 132A (50 μg/ml)			
EPP amplitude (mV)	2·4 ± 0·4 (6)	2·2 ± 0·3 (5)	2·6 (2)
Quantal content (m_i)	6·37 ± 0·6 (6)	5·79 ± 0·7 (5)	6·86 (2)
mAb D547 (50 μg/ml)			
EPP amplitude (mV)	2·06 ± 0·2 (16)	1·95 ± 0·2 (12)	2·86 ± 0·5 (4)
Quantal content (m_i)	5·76 ± 0·3 (16)	5·64 ± 0·7 (12)	5·39 ± 0·7 (4)
(+)Tubocurarine	Control	10^{-7} g/ml	10^{-5} g/ml
EPP amplitude (mV)	1·58 ± 0·1 (10)	1·09 ± 0·1 (4)	0·68 ± 0·08 (6)*
Quantal content (m_i)	4·65 ± 0·5 (10)	5·12 ± 0·7 (4)	4·99 ± 0·6 (6)

*$P < 0·001$.

quantal release of ACh and the release induced by nerve stimulation. In addition, our experiments show that mAb 370 also decreases the quantal content of the stimulus-induced release of ACh.

Postsynaptically, the effects of the anti-AChR mAb 370 could be explained by either a lowering of the AChR channel conductance, a decrease in the mean channel open time, or the prevention of channel opening. Previous studies of AChR function carried out at the single-channel level using both polyclonal and monoclonal anti-AChR antibodies have shown a wide range of results. Using noise analysis on ACh-activated currents, Goldberg, Mochly-Rosen, Fuchs & Lass (1983) studied the effects of a mAb directed against the cholinergic binding site and found a decrease in the channel conductance and an increase in the mean channel open time in cultured chick myoballs. A modest decrease in mean channel conductance and open time (10%) was found by Heinemann, Bevan, Kullberg, Lindstrom & Rice (1977), again using noise analysis on ACh-activated currents recorded in embryonic skeletal muscle cells treated with rat polyclonal anti-AChR sera. More recent studies at the single-channel level using patch-clamp techniques have failed to demonstrate a significant decrease in channel conductance after exposure of the receptor to either monoclonal or polyclonal anti-AChR Abs (Schuetze *et al.* 1985). Using a highly specific serum from a patient with MG, Schuetze and colleagues (1985) found decreased MEPP amplitudes in developing rat end-plates but normal gating properties of the AChR channels that remained active after antibody treatment. Blatt *et al.* (1986), using *Torpedo* AChRs reconstituted in planar lipid bilayers, found that mAbs that bind to the extracellular domains of α- and β-subunits and mAbs that bind to the cytoplasmic portion of β- and γ-subunits, all inhibited single-channel activity without changing channel conductance. Similar results were found by Maelicke, Fels, Plumer-Wilk, Wolff, Covarrubias & Methfessel (1986) in single-channel recordings obtained from rat myotubes treated with an anti-AChR mAb.

At the level of the end-plate, our results are similar to the single-channel experiments carried out in other laboratories using other anti-AChR mAbs. We did not find that mAb 370 either induced changes in channel gating or selectivity. Such changes would have been detected as changes in the EPC reversal potential or alterations in the rate constants describing EPC decay. Therefore, the most likely explanation for our findings is that mAb 370 prevents the opening of postsynaptic channels. The blockade of neuromuscular transmission induced by mAb 370 resembles that induced by α-BTX (Katz & Miledi, 1978) in that there is an apparent block of channels to which the antibody is bound, but unmodified function of unbound channels. Our results also demonstrate that a complete blockade of neuromuscular transmission, as would have been indicated by total disappearance of MEPPs and EPCs, apparently was not achieved even at the highest mAb concentration. However, since MEPP detection was exceedingly difficult after incubation with mAb 370, it appears that total block did occur at most of the individual end-plates and that perhaps a limited accessibility of the mAb might have prevented complete blockade at other end-plates. Alternatively, it can be speculated that a subpopulation of postsynaptic receptor channels may have been resistant to the blocking effect of this mAb. This is less likely since (1) chicken end-plates maintain a homogeneous population of channels throughout development (Schuetze, 1980) and (2) a double-exponential EPC decay, suggestive of a dual population of channels with different kinetics, was never observed either before or after the incubation of the preparations with either mAb.

While EPCs at the amphibian neuromuscular junction have been characterized (Magleby & Stevens, 1972*a*, *b*; Magleby & Terrar, 1974), there is little characterization of EPCs recorded at the end-plates of adult chickens. We have observed that under the same experimental conditions (-60 mV holding potential), the time course of EPC decay in adult chickens was approximately four times longer than the time course of EPC decay recorded in amphibian end-plates (τ_{EPC} in chickens was $4{\cdot}77 \pm 0{\cdot}24$ ms ($n = 9$); τ_{EPC} in frogs was $1{\cdot}32 \pm 0{\cdot}02$ ms ($n = 15$); R. A. Maselli & S. Palombi, unpublished data). On the other hand, the mean EPC amplitude in chickens was three to four times smaller than the mean EPC amplitude recorded at the frog neuromuscular junction (mean EPC amplitude in chickens was $93{\cdot}4 \pm 12{\cdot}2$ nA ($n = 9$); mean EPC amplitude in frogs was $303{\cdot}3 \pm 32$ nA ($n = 15$); R. A. Maselli & S. Palombi, unpublished data). Since it has been demonstrated that EPC decay is an index of mean end-plate channel lifetime (Katz & Miledi, 1973; Colquhoun, Large & Rang, 1977; Gage & Van Helden, 1979), our results are in agreement with those reported by Schuetze (1980) indicating the persistence of AChR channels with slow gating characteristics in the adult chicken. In view of the channel block produced by mAb 370 on chicken end-plates, it is tempting to postulate a selective specificity of the antibody for channels with slow gating characteristics.

In addition to the striking postsynaptic effects, mAb 370 reversibly decreases quantal content, raising the possibility that the antibody may bind to a receptor involved in the regulation of transmitter release. The functionally reversible effects may represent low-affinity mAb binding to putative neuronal nicotinic receptors, and, hence, these observations are consistent with the interesting, although not universally accepted, model of modulation of nerve impulse-evoked release of

neurotransmitters by feed-back control through autoreceptors at presynaptic nerve endings (see, for example, Boulter, Connolly, Deneris, Goldman, Heinemann & Patrick, 1987). In control experiments, neither anti-AChR mAb 132A, the irrelevant anti-oestrogen receptor mAb, nor (+)tubocurarine caused a reduction in quantal content. Taken together, these data suggest that perhaps the presynaptic response may be related to presynaptic receptor block similar to that obtained in the postsynaptic membrane. In addition, the mAb-induced reduction in quantal content may explain the difference in percentage reduction in MEPP (48%) *versus* EPC (63%) amplitude in the presence of mAb 370 which we observed experimentally.

It should be noted that a number of studies have demonstrated cross-reactivity of anti-*Torpedo* AChR mAbs with neuronal receptors (Lukas, 1986; Halvorsen & Berg, 1987; Chase, Holliday, Reese, Chun & Hawrot, 1987; Deutch, Holliday, Roth, Chun & Hawrot, 1987). However, none of those mAbs have had effects on neurone receptor function.

In summary, the mAb 370-induced weakness in the hyperacute animal model of MG can be attributed to a combined effect of a block of ACh-induced ion channel activity in the postsynaptic membrane and an impairment in the release of ACh from the nerve terminal as seen in the *in vitro* studies. The selectivity and gating of those postsynaptic receptor channels that remain activatable in the presence of antibody is unmodified. Since the gating of AChR channels in MG is also unchanged (Cull-Candy, Miledi & Trautmann, 1979), it is possible that antibodies of this type play a role in this disease. Furthermore, the results of this study strengthen the view that the disordered neuromuscular transmission in MG is the result of a combination of antibody-mediated mechanisms.

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