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PROPERTIES OF END-PLATE CHANNELS IN RATS IMMUNIZED AGAINST ACETYLCHOLINE RECEPTORS

BY S. ALEMÀ*, S. G. CULL-CANDY†, R. MILEDI AND A. TRAUTMANN‡

From the Department of Biophysics, University College London, Gower Street, London WC1E 6BT

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SUMMARY

1. Rats injected with purified acetylcholine receptors (AChR) extracted from electric organs of *Torpedo marmorata* showed clinical symptoms consistent with the development of experimental myasthenia gravis.

2. Sera of rats with this disease contain high levels of anti-AChR antibodies. However, no simple correlation was found between antibody titre and miniature end-plate current (m.e.p.c.) amplitude.

3. M.e.p.c.s. at the end-plates of rats injected with AChR (Anti-R), emulsified in complete Freund Adjuvant (CFA), were reduced to about one third the size of controls taken from rats injected only with CFA (Anti-CFA). Mean m.e.p.c. (Anti-R) = $0{\cdot}73 \pm 0{\cdot}06$ nA; mean m.e.p.c. (Anti-CFA) = $2{\cdot}43 \pm 0{\cdot}12$ nA ($V_m = -80$ mV, $T = 20$ °C).

4. The m.e.p.c. decay time constant, $\tau_{m.e.p.c.}$, is similar at immunized and control rat end-plates. $\tau_{m.e.p.c.}$ (Anti-R) = $1{\cdot}32 \pm 0{\cdot}06$ msec; $\tau_{m.e.p.c.}$ (Anti-CFA) = $1{\cdot}31 \pm 0{\cdot}06$ msec ($V_m = -80$ mV, $T = 20$ °C).

5. The end-plate current decay time constant, $\tau_{e.p.c.}$, is similar at immunized and control end-plates and in both cases depends exponentially on membrane potential. The change in membrane potential required to produce an e-fold change in $\tau_{e.p.c.}$ is $102{\cdot}0 \pm 5{\cdot}72$ mV at immunized (Anti-R) end-plates and $92{\cdot}3 \pm 6{\cdot}14$ mV at control (Anti-CFA) end-plates at $T = 10$ °C.

6. Acetylcholine noise was examined at immunized and control rat end-plates at 10 °C. Analysis of noise indicates that the single channel conductance, γ, and mean channel life-time, τ_{noise}, are essentially unchanged by immunization against AChR. γ (Anti-R) = $13{\cdot}15 \pm 0{\cdot}53$ pS; γ (Anti-CFA) = $12{\cdot}50 \pm 0{\cdot}50$ pS; τ_{noise} (Anti-R) = $2{\cdot}9 \pm 0{\cdot}18$ msec; τ_{noise} (Anti-CFA) = $2{\cdot}68 \pm 0{\cdot}14$ msec ($V_m = -80$ mV, $T = 10$ °C).

7. Mean quantal content and Ca^{2+} dependence of the end-plate potential are unchanged at immunized end-plates.

8. It is concluded that at immunized end-plates the number of activated receptor-

* Present address: Laboratorio di Biologia Cellulare, C.N.R., Via Romagnosi 18A, 00196 Roma, Italy.

† Beit Memorial Research Fellow, to whom reprint requests should be addressed.

‡ Present address: Laboratoire de Neurobiologie, Ecole Normale Superieure, 46, Rue d'Ulm 75005 Paris, France.

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channel complexes is reduced without modification of single channel properties. In this respect the immunized rat end-plate is a good model for myasthenia gravis affected human end-plates.

INTRODUCTION

An experimental model has been established in recent years which closely resembles human myasthenia gravis in several aspects. This model, referred to as experimental myasthenia gravis, is based on the observation that the injection of purified acetylcholine receptor (AChR) produces muscular weakness and fatigue in several animal species (Patrick, Lindstrom, Culp & MacMillan, 1973; Sugiyama, Benda, Meunier & Changeaux, 1973; Lennon, Lindstrom & Seybold, 1975; Green, Miledi, Perez & Vincent, 1975*a*; Green, Miledi & Vincent, 1975*b*; Tarrab-Hazdai, Aharanov, Silman, Fuchs & Abramsky, 1975; Granato, Fulpius & Moody, 1976; Ito, Miledi, Vincent & Newsom-Davis, 1978). Using conventional electrophysiological techniques it has been shown that neuromuscular transmission in immunized animals is severely impaired (Green *et al.* 1975*a*, *b*; Lambert, Lindstrom & Lennon, 1976; Berti, Clementi, Conti-Tronconi & Folco, 1976; Sanders, Schleifer, Elderfrawi, Norcross & Cobb, 1976; Ito *et al.* 1978; Lindstrom & Lambert, 1978). In addition, as in human myasthenia gravis, there is a decrease in the binding of α-bungarotoxin (α-BuTx) at muscle end-plates (Fambrough, Drachman & Satyamurti, 1973; Green *et al.* 1975*a*; Ito *et al.* 1978; Lindstrom & Lambert, 1978). Thus the defect in neuromuscular transmission seems mainly due to a reduction in the number of functional AChRs at the post-synaptic membrane.

Sera of immunized animals contain high titres of antibodies against heterologous AChR (generally from *Torpedo* or *Electrophorus* electric organs) and host skeletal muscle AChR (Lennon *et al.* 1975; Green *et al.* 1975*b*; Lindstrom 1976; Alemà, Miledi & Vincent, 1978). Such antibodies exhibit a direct effect on AChR function *in vitro* by reducing both miniature end-plate potential (m.e.p.p.) amplitude at frog end-plates and extrajunctional sensitivity in denervated rat muscle fibres (Green *et al.* 1975*a*, *b*; Bevan, Heinemann, Lennon & Lindstrom, 1976). Moreover, antireceptor antibodies reduce the binding of ACh and of α-BuTx to junctional receptors *in situ* (Alemà *et al.* 1978) and increase the rate of degradation of both junctional and extrajunctional receptors (Heinemann, Bevan, Kullberg, Lindstrom & Rice, 1977; Reiness, Weinberg & Hall, 1978). We have compared miniature end-plate currents and ACh noise at normal and immunized rat end-plates in order to obtain information about receptor-channel properties after immunization against the receptor.

METHODS

Purification of neurotoxins. α-bungarotoxin (α-BuTx) was purified from *Bungarus multicinctus* venom (Miami Serpentarium) as previously described (Mebs, Narita, Iwanaga, Samiejima & Lee, 1972) but with some minor modifications. After two consecutive CM-25 columns at pH 5·0, the toxin was further purified by filtration through a Sephadex G50 column at pH 7·2, followed by chromatography on a CM-25 column at pH 7·2. *Naja naja siamensis* main toxin (α-cobrotoxin) was purified according to the method of Cooper & Reich (1972) with minor modifications.

Purification of AChR. AChR was extracted from frozen electric organs of *Torpedo marmorata* with 1 % Triton X100, 20 mM-Na phosphate buffer, pH 7·4, 1 mM-EDTA, 0·1 mM-phenylmethyl sulphonyl fluoride (PMSF), 0·1 % Na azide, essentially as previously described (Green *et al.* 1975*a*; Alemà *et*

al. 1978). The receptor was purified by affinity chromatography with a cobrotoxin-sepharose 4B derivative (0·2–0·5 mg of *Naja naja siamensis* main toxin/ml. of bed) and eluted with 0·5–0·8 M-carbamylcholine in 0·1 % Triton X100 buffer. After dialysis the receptor-rich solution was further purified, concentrated and freed of contaminating carbamylcholine by adsorbing it to a small column of DEAE-cellulose and eluting it with increasing concentrations of NaCl. Fractions with the highest specific activity were pooled, extensively dialyzed and kept at 4 °C; 0·05 mM-PMSF, 0·1 % Na azide and 0·1 mM-EDTA were present throughout all the purification steps. Specific activity, expressed as nmoles of [^{125}I]α-BuTx bound/mg protein were in the range 5–7. When analysed on SDS-polyacrylamide gel electro-phoresis according to Loemmly (1970), purified receptor preparations exhibited the usual pattern of polypeptide components (Alemà *et al.* 1978; Lindstrom, Einarson & Merlie, 1978).

Immunization of rats with AChR. Female PVG (Black Hooded) and Wistar rats, approximately 150–200 g body weight, were immunized with 50–100 μg of AChR. Before emulsification with Freund complete adjuvant (Difco), AChR was briefly dialysed against 20 mM-phosphate buffer, pH 7·2, to remove excess Triton X100. Rats were injected at multiple sites both intradermally and subcutaneously with 0·25 ml. of adjuvant-receptor emulsion. Control litter-mates were injected with CFA only. A further challenge with the same amount of antigen was given after three weeks. Animals were usually sacrificed 7–10 days after the second injection.

Detection of anti-AChR antibodies. Sera were collected by cardiac puncture when animals were sacrificed to remove diaphragms, and stored at −20 °C. Sera, when analysed on double immuno-diffusion plates, gave single precipitin lines if challenged with both crude and purified receptor. Radioimmunometric quantitation of antibodies against *Torpedo* AChR was carried out by either of two methods previously described (Green *et al.* 1975*a*; Alemà *et al.* 1978; Ito *et al.* 1978). Titres are expressed as moles of iodinated α-BuTx precipitated/ml. of serum. Briefly, 1–5 p-mole of purified receptor, labelled with saturating concentrations of [^{125}I]α-BuTx were incubated in 20 mM-phosphate buffer, pH 7·4, 0·05 % Na azide, 0·1 % Triton X100 with increasing amounts of serum for 1 hr at room temperature and then left to stand overnight at 4 °C. Thereafter the immune complexes were either directly centrifuged at high speed (method *A*) or further challenged with a 10 × excess of goat anti-rat IgG serum (Miles-Yeda) (method *B*). Titres against syngeneic AChRs were measured by method *B*, using detergent-solubilized AChR from denervated rat leg muscle (10 days) as the antigen.

[^{125}*I*]*α-BuTx binding.* α-BuTx purified as described above, was iodinated by the method of Vogel, Sythowski & Nirenberg (1972) with a specific activity of about 100–150 c/m-mole. Only the di-iodinated product was used and was a gift from Dr Angela Vincent. Assay of AChR in the diaphragm of immunized and control rats was as previously described (Green *et al.* 1975*a*; Ito *et al.* 1978). Strips of diaphragm were incubated with [^{125}I]α-BuTx 1 μg/ml., extensively washed with Ringer, fixed and counted. End-plate rich areas were located under a dissection microscope and dissected out to permit an evaluation of binding to junctional and extrajunctional sites. Extrajunctional binding averaged 5–10 % junctional binding on a weight basis.

Electrophysiology. The physiological experiments were made mainly on PVG rats. Diaphragms were removed from animals under ether anesthesia. A part of the hemidiaphragm was mounted in a chamber and perfused with an oxygenated (95 % O_2/5 % CO_2) medium which contained (mM): NaCl, 113; Na_2HPO_4, 1; $NaHCO_3$, 25; KCl, 4·5; $CaCl_2$, 2; $MgSO_4$, 1; D-glucose, 11; pH 7·2. In experiments on transmitter release, which were performed on EDL muscles, the Mg^{2+} and Ca^{2+} concentrations were changed as indicated.

When temperature was being varied preparations were maintained in a bath of ~ 20 ml. volume which was not perfused. In such cases preparations were oxygenated directly in the bath via a fine capillary jet. Bath temperature was controlled at 20 or 10 °C with Peltier coolers and monitored with a thermistor placed close to the muscle.

Voltage recording micro-electrodes contained 3 M-KCl; current passing microelectrodes contained 2 M-K acetate. The two micro-electrodes were inserted in the muscle fibre near an end-plate and less than 100 μm from each other. In experiments where acetylcholine noise was investigated the ionophoretic micropipette was positioned between the recording and the current passing electrodes. M.e.p.c.s, e.p.c.s and acetylcholine induced noise were recorded through a virtual earth (500 or 1000 Hz active low pass filter) on low gain d.c. (10–100 nA/cm) and high gain a.c. (1–5 nA/cm) and stored on analogue tape (band width 0–2500 Hz). The clamp quality and analysis of data is similar to that previously described (Cull-Candy, Miledi & Trautmann, 1979).

RESULTS

Clinical symptoms

PVG (Black Hooded) rats injected with *Torpedo* AChR showed the typical clinical appearance of experimental autoimmune myasthenia gravis. Symptoms appeared approximately 1 week after the second injection and were similar to those previously described for other animal species (Green *et al.* 1975*b*; Lennon *et al.* 1975; Tarrab-Hazdai *et al.* 1975). The front limbs became weak and rats developed a characteristic posture and also an incontinence to urine. A few severely affected animals showed flaccid paralysis, loss of weight and respiratory distress. In about 30 % of the injected

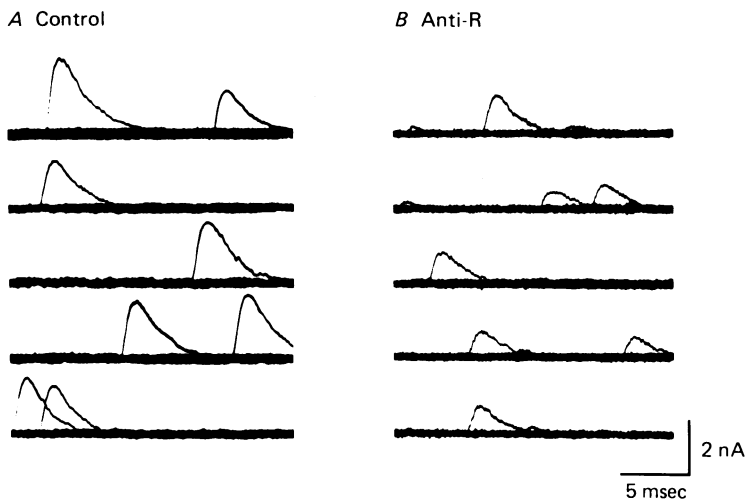


Fig. 1. Miniature end-plate currents from, *A*, a control (Anti-CFA) end-plate and *B*, an immunized (Anti-R) end-plate, at clamp potential $V_m = -80$ mV and $T = 20$ °C. Each trace shows several superimposed current sweeps. At the control end-plate (*A*) m.e.p.c.s are well above the noise level. At the immunized end-plate (*B*) the m.e.p.c.s are reduced in amplitude with some m.e.p.c.s barely visible above the background noise. The decay time of m.e.p.c.s is similar at both end-plates. Calibration 5 msec and 2 nA.

rats, not sacrificed for electrophysiological experiments, this clinical pattern progressed to death. No acute phase was observed, in contrast to Lewis rats immunized with *Electrophorus* AChR and *B. pertussis* as an additional adjuvant (Lennon *et al.* 1975). Wistar rats of comparable age and size, injected with the same *Torpedo* AChR preparations, failed to show clinical symptoms, even after several challenges with the antigen over a period of ten months (see also Green *et al.* 1975*b*). Nevertheless, in these animals m.e.p.p.s were reduced in amplitude and there was decreased α-BuTx binding to end-plates. This may simply indicate that in Wistar rats the safety margin for neuromuscular transmission is greater than in PVG rats.

Miniature end-plate currents

An example of miniature end-plate currents (m.e.p.c.s) recorded at normal and immunized rat end-plates under voltage clamp is illustrated in Fig. 1. Although at immunized end-plates the time course of m.e.p.c.s is not obviously different from normal, the amplitude undergoes a marked reduction.

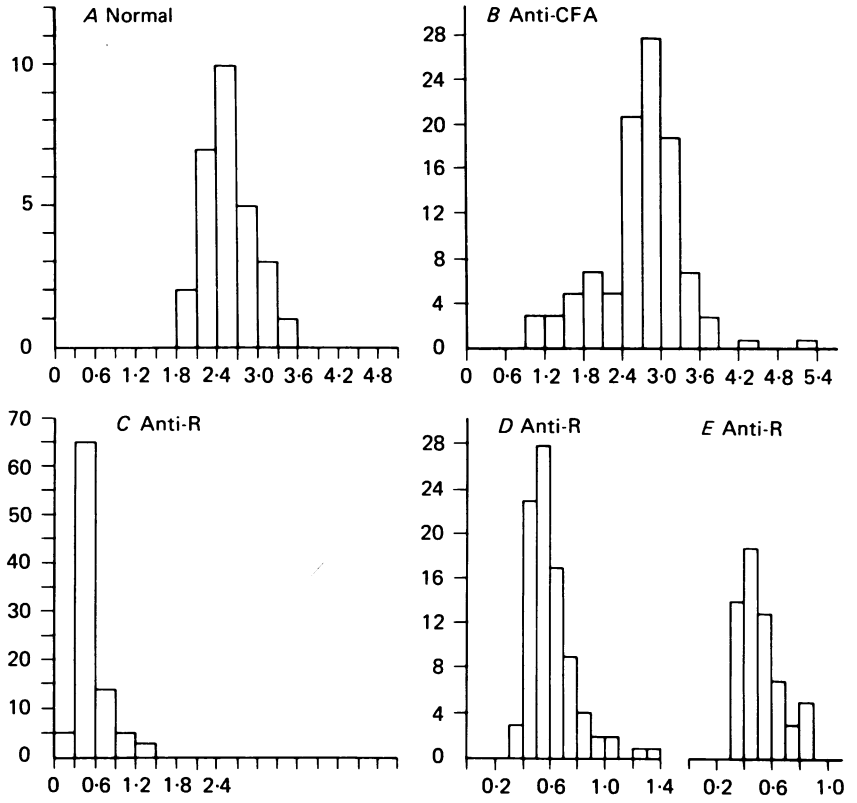


Fig. 2. Amplitude histograms of m.e.p.c.s at voltage clamped rat end-plates ($V_m = -80$ mV, $T = 20$ °C). *A*, normal end-plate, m.e.p.c. = 2·56 nA; *B*, control (Anti-CFA) end-plate, m.e.p.c. = 2·72 nA; *C*, immunized (Anti-R) end-plate m.e.p.c. = 0·52 nA; *D*, Anti-R end-plate, m.e.p.c. = 0·56 nA; *E*, Anti-R end-plate, m.e.p.c. = 0·50 nA.

M.e.p.c.s at normal and Anti-CFA end-plates (*A* and *B*) have an approximately Gaussian distribution (sub-m.e.p.c.s are present in *B*). M.e.p.c.s at Anti-R end-plates (*C*, *D*, and *E*) show a skew amplitude distribution with a reduction in mean size. Background noise in *C*, *D* and *E* masks events less than approximately 0·2–0·3 nA. Therefore, the smallest m.e.p.c.s are not seen at the Anti-R end-plates.

Examples of amplitude distributions of m.e.p.c.s at normal and immunized end-plates are shown in the histograms in Fig. 2. At normal end-plates m.e.p.c.s have a typical Gaussian distribution. Following immunization the amplitude histograms of m.e.p.c.s are shifted towards smaller mean values and their distribution is often skew as the smaller events become lost in the background noise. M.e.p.c. amplitudes were studied in rats which were untreated (normal) or immunized either with

TABLE 1. Channel properties at normal and immunized end-plates

	Normal	Anti-CFA	Anti-R
$\tau_{m.e.p.c.}$ (−80 mV, 20 °C)	1·51 ± 0·09 msec (n = 9)	1·31 ± 0·06 msec (n = 16)	1·32 ± 0·06 msec (n = 14)
Mean m.e.p.c. (−80 mV, 20 °C)	2·42 ± 0·09 nA (n = 5)	2·43 ± 0·12 nA (n = 13)	0·73 ± 0·06 nA (n = 19)
$H_{e.p.c.}$ (10 °C)	98·4 ± 5·80 mV (n = 19)	92.3 ± 6·14 mV (n = 16)	102·0 ± 5·72 mV (n = 41)
$\tau_{e.p.c.}$ (−80 mV, 10 °C)	3·10 ± 0·6 msec (n = 8)	3·17 ± 0·13 msec (n = 12)	3·5 ± 0·15 msec (n = 18)
τ_{noise} (−80 mV, 10 °C)	2·5 ± 0·5 msec (n = 2)	2·68 ± 0·14 msec (n = 8)	2·9 ± 0·18 msec (n = 7)
γ (−80 mV, 10 °C)	12·9 ± 0·4 pS (n = 2)	12·50 ± 0·50 pS (n = 8)	13·15 ± 0·53 pS (n = 8)

All values are mean ± S.E. of mean, n = number of end-plates studied. See text for further details.

complete Freund Adjuvant (anti-CFA) or with CFA and acetylcholine receptor (anti-R). The amplitude of m.e.p.c.s did not differ from normal after immunization with CFA. Thus at a clamp holding potential of -80 mV and $T = 20$ °C, mean m.e.p.c. (normal) = $2{\cdot}42 \pm 0{\cdot}09$ nA ($\pm$S.E., $n = 5$ end-plates); for rats injected with CFA, mean m.e.p.c. (anti-CFA) = $2{\cdot}43 \pm 0{\cdot}12$ nA ($n = 13$).

In animals immunized against the receptor the mean value of m.e.p.c.s varied somewhat from diaphragm to diaphragm as expected if animals are affected differently by the immunization treatment (Green *et al.* 1975*b*). However, values for

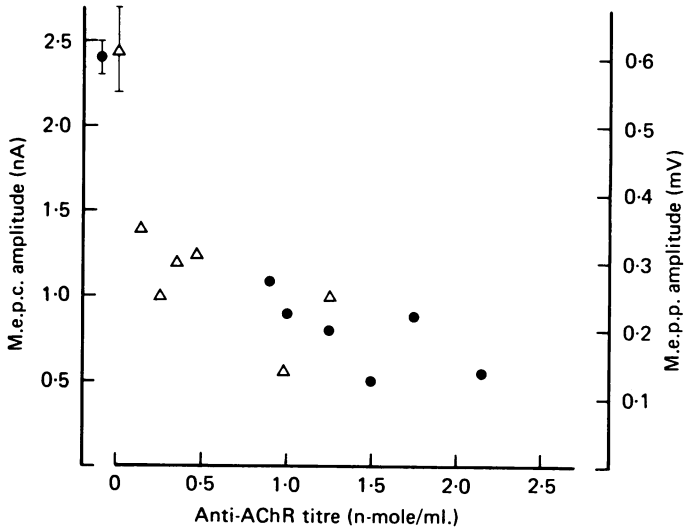


Fig. 3. Correlation between m.e.p.p. amplitude (△, Wistar rats) or m.e.p.c. amplitude (●, PVG rats) and the antibody titre. Titre of antibodies against *Torpedo* AChR was determined by method *A* (see Methods) and is expressed as moles of α-BuTx binding sites precipitated per ml. of serum. Each symbol represents data from one animal, except for the controls, where bars are $\pm$S.E.

mean m.e.p.c. amplitudes at end-plates in a given diaphragm were usually very consistent. The mean m.e.p.c. amplitudes ranged from 0·5 to 0·9 nA in different diaphragms (see Table 1). Thus immunized m.e.p.c.s were only 20–40 % of the normal m.e.p.c. amplitude; mean m.e.p.c. (anti-R) = $0{\cdot}73 \pm 06$ nA ($n = 19$). At normal and immunised end-plates the amplitude of m.e.p.c.s is markedly decreased by lowering bath temperature. For example, in two end-plates studied the mean m.e.p.c. changed from 2·40 nA at 20 °C (-80 mV) to 1·63 nA at 10 °C.

Antibody titre and amplitude of m.e.p.p.s or m.e.p.c.s

Sera of rats with experimental myasthenia gravis had antibodies to AChR in sufficient concentration to be measured by precipitation using *Torpedo* AChR labelled with [^{125}I]α-BuTx. Titres measured in this way (see Methods) were $0{\cdot}575 \pm 0{\cdot}18$ n-mole/ml. ($n = 6$) for Wistar rats and $1{\cdot}425 \pm 0{\cdot}19$ n-mole/ml. ($\pm$S.E., $n = 7$) for PVG Hooded rats (Fig. 3). Indirect immuno-precipitation gave values 3–5 times higher (Alemà *et al.* 1978). Titre of autoantibodies in PVG rats was 40–50 p-mole/ml. As

previously discussed these methods essentially measure antibodies directed against antigenic sites other than the toxin binding site(s) (Lindstrom, 1976; Alemà *et al.* 1978; Vincent, 1980) which are only a small proportion of the total antigenic determinants. As shown in Fig. 3, a correlation between anti-*Torpedo* titre and m.e.p.p. amplitude (Wistar rats) or m.e.p.c. amplitude (PVG rats) has a biphasic pattern. When the antibody titre increases, there is a moderate reduction of m.e.p.p. or m.e.p.c. amplitude. On the other hand, a marked decrease of m.e.p.p. amplitude takes place at very low levels of antibody.

A simpler correlation between antibody titre and m.e.p.p. amplitude was observed in a previous study on rabbits with the experimental disease (Green *et al.* 1975*b*; Alemà *et al.* 1978), However, poor correlation between antibody titres and weakness has been reported in mice (Granato *et al.* 1976), but in these experiments the size of m.e.p.c.s and other factors involved in neuromuscular transmission were not assessed.

Characteristics of the acetylcholine-induced channels

Analysis of voltage-clamped noise has been used to investigate the mean lift-time, τ_{noise}, and the conductance, γ, of the end-plate channels induced by acetylcholine (Katz & Miledi, 1972; Anderson & Stevens, 1973). In addition we studied the time constant of decay of m.e.p.c.s and e.p.c.s ($\tau_{m.e.p.c.}$ and $\tau_{e.p.c.}$) which gives information about the channel life-time and the rate of diffusion of ACh from the synaptic cleft (Katz & Miledi. 1973).

M.e.p.c. time course

The time constant of decay of m.e.p.c.s was obtained by one of three methods: from a semilogarithmic plot of the m.e.p.c.s; from half decay of m.e.p.c. $\times 1/\ln 2$; or from the power spectra of the m.e.p.c.s (Katz & Miledi, 1973; Cull-Candy *et al.* 1979). When these methods were applied to the same data good agreement was found between them. The time constant of decay of m.e.p.c.s at normal, at CFA-treated and at immunized end-plates was similar, thus: $\tau_{m.e.p.c.}$ (normal) = $1{\cdot}51 \pm 0{\cdot}09$ msec ($n = 9$); $\tau_{m.e.p.c.}$ (anti CFA) = $1{\cdot}31 \pm 0{\cdot}06$ msec ($n = 16$); $\tau_{m.e.p.c.}$ (anti-R) = $1{\cdot}32 \pm 0{\cdot}06$ msec ($n = 14$) at $V_m = -80$ mV, $T = 20$ °C. The life-time of the end-plate channel is therefore not markedly altered after immunization providing that the rate of diffusion of the quantum of ACh from the synaptic cleft has not changed.

E.p.c. time course

End-plate currents were obtained at 10 °C. As expected $\tau_{e.p.c.}$ at 10 °C was more than twice as long as $\tau_{m.e.p.c.}$ at 20 °C as the life-time of the channel is lengthened when the temperature is reduced (Katz & Miledi, 1972; Anderson & Stevens, 1973).

Comparison of the time constant of decay of e.p.c.s shows that $\tau_{e.p.c.}$ is slightly prolonged at the anti-receptor endplate (see Table 1) when compared to the anti-CFA end-plate. At the vertebrate neuromuscular junction the time constant of decay of the e.p.c., $\tau_{e.p.c.}$, and the duration of open state of the channel, τ_{noise}, increase exponentially as the membrane potential is hyperpolarized (see Kordaš, 1969; Magleby & Stevens, 1972).

The voltage dependence of $\tau_{e.p.c.}$ and $\tau_{m.e.p.c.}$ was estimated by measuring τ while changing the clamp holding potential usually from -60 to -160 mV in 10 or 20 mV

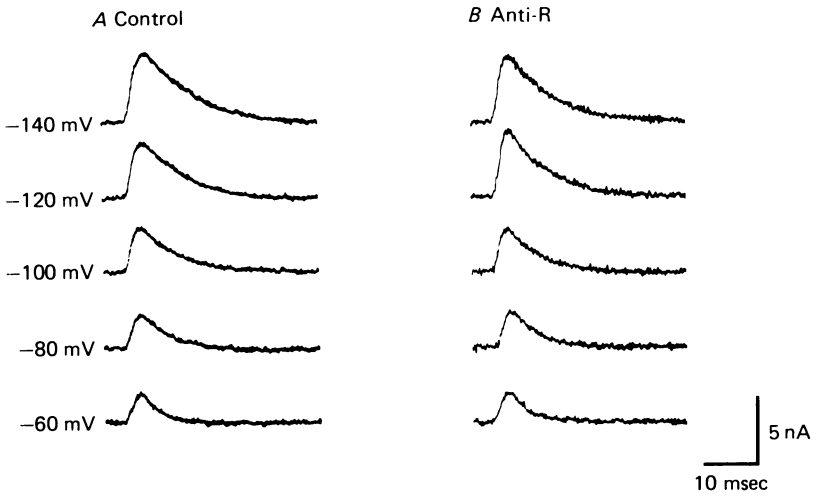


Fig. 4. Nerve impulse evoked end-plate currents from *A*, a control (Anti-CFA, curare 3×10^{-7} g/ml.) end-plate and *B*, an immunized (Anti-R, curare $3{\cdot}3 \times 10^{-8}$ g/ml.) rat end-plate over a range of clamp potentials. End-plate currents are recorded in the presence of tubocurarine to produce e.p.c.s of similar amplitude at control and immunized end-plates. Note that the increase in amplitude and prolongation of decay time accompanying hyperpolarization is similar at the two end-plates. $T = 10$ °C, Calibration 10 msec and 5 nA.

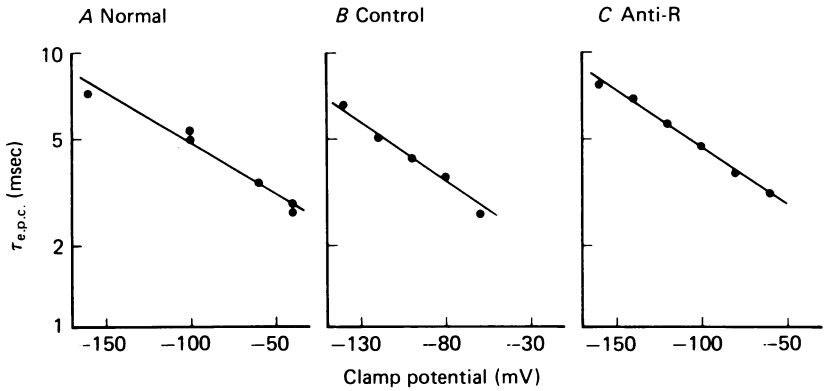


Fig. 5. Dependence of the time constant of decay of nerve-evoked e.p.c.s, $\tau_{e.p.c.}$, on clamp potential plotted for three end-plates. *A*, normal rat end-plate. *B*, control (Anti-CFA) end-plate. *C*, immunized (Anti-R) end-plate. The lines fitted to the points represent the relationship $\tau(V_m) = \tau(0)\exp(-V_m/H)$ where H(normal) = 116 mV; H(Anti-CFA) = 114 mV; H(Anti-R) = 104 mV. $T = 10$ °C.

steps. Examples of e.p.c.s over a range of clamp potentials at a control (Anti-CFA, curare 3×10^{-7} g/ml.) and an immunized (Anti-R, curare $3{\cdot}3 \times 10^{-8}$ g/ml.) rat end-plate are shown in Fig. 4. Hyperpolarization of the membrane potential increases the amplitude and decay time to a similar extent at both endplates. Fig. 5 shows $\tau_{e.p.c.}$ plotted as a function of V_m at normal, control and immunized end-plates: $\tau_{e.p.c.}$

exhibits exponential dependence on V_m. The potential change, H, required to produce an e-fold change in $\tau_{e.p.c.}$ in a series of such experiments was: H normal = 98·4 ± 5·8 mV (n = 19), H anti-CFA = 92·3 ± 6·14 mV (n = 16), and H anti-R = 102 ± 5·72 mV (n = 41). According to Student's t test these values are not different at the 5% level of significance. The magnitude of voltage sensitivity of $\tau_{e.p.c.}$ is similar to that described for frog (Kordaš, 1969) rat (Colquhoun, Large & Rang, 1977) and human end-plates (Cull-Candy *et al.* 1979).

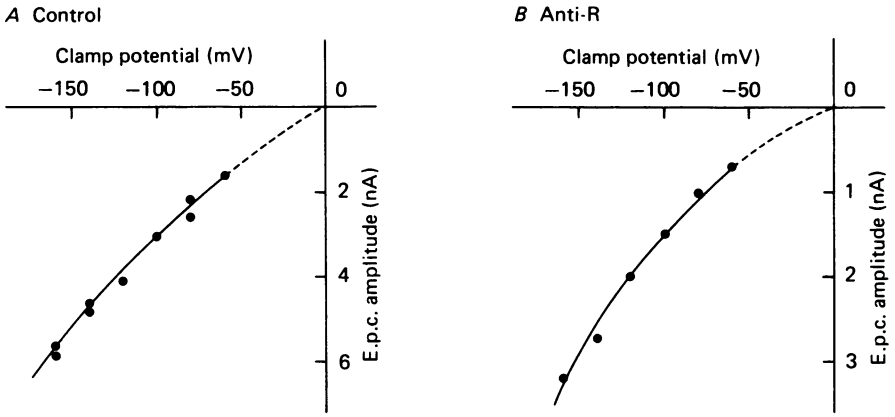
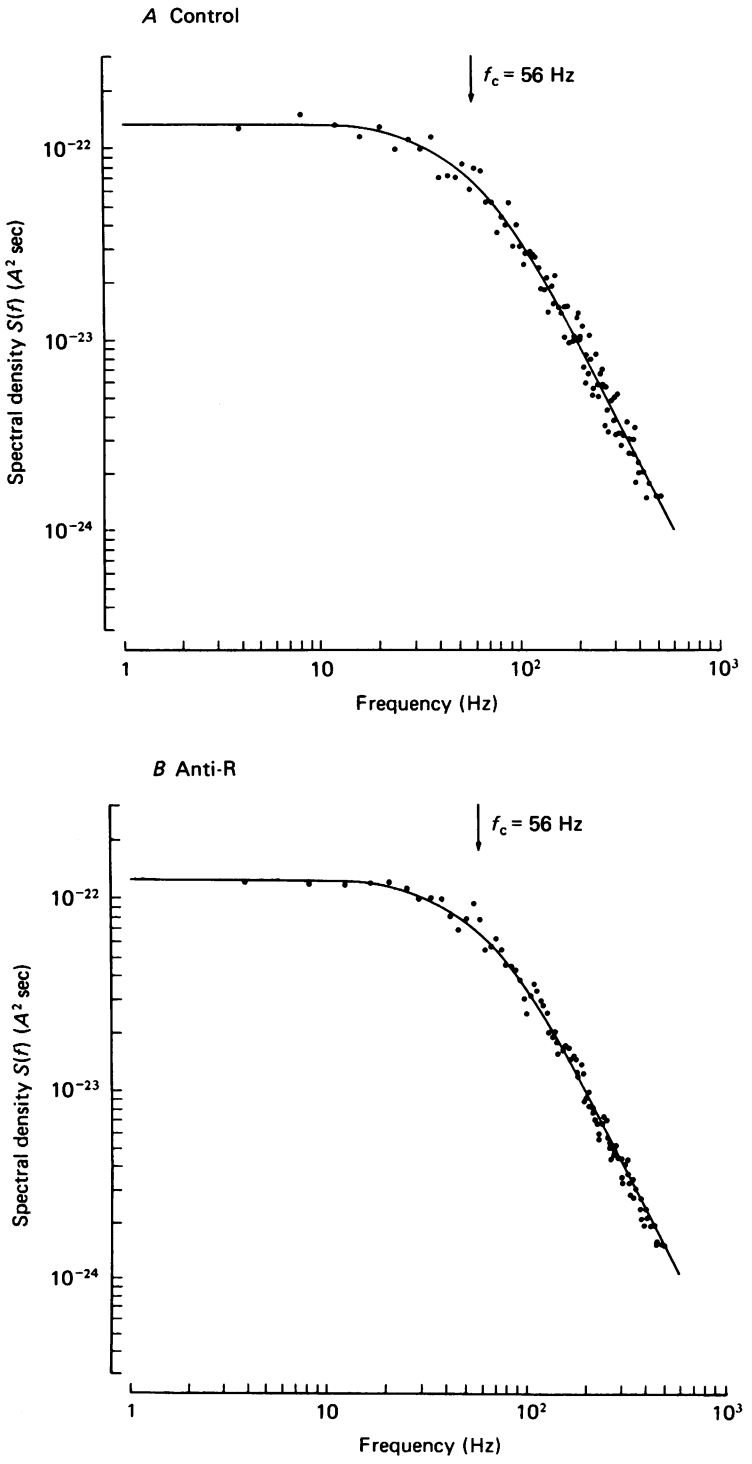


Fig. 6. Relationship between end-plate current amplitude and clamp holding potential at A, normal (Anti-CFA) end-plate and B, immunized (Anti-R) end-plate. Extrapolated equilibrium potential is approximately 0 mV at both end-plates. Tubocurarine (3×10^{-7} g/ml. in A, and 10^{-7} g/ml. in B) was present in the bathing medium.

Fig. 6 illustrates the relationship between e.p.c. amplitude and V_m at a control and an immunized end-plate. The extrapolated equilibrium potential is close to 0 mV in both examples. Since tubocurarine (3×10^{-7}–10^{-8} g/ml.) was used in some experiments it was necessary to see if there was voltage-dependent block of the ion channels (Manalis, 1977; Katz & Miledi, 1978) opened during the e.p.c. The peak conductance change of the e.p.c. at control and immunized end-plates, became progressively larger with hyperpolarization (Fig. 6). Under the conditions used in these experiments there was no evidence for a gross reduction in peak conductance or in $\tau_{e.p.c.}$ as might be expected if open channels were being blocked by curare at hyperpolarized potentials.

Fig. 7. Spectral density of current noise produced by steady ionophoretic application of acetylcholine to a voltage clamped control end-plate (Anti-CFA) and an end-plate immunized against acetylcholine receptor (Anti-R). A, control (Anti-CFA) spectrum obtained at clamp potential $V_m = -80$ mV, $T = 10$ °C, from twenty-two averaged spectra from a single noise run. Half-power frequency indicated by arrow is $f_c = 56$ Hz, hence $\tau_{noise} = 2{\cdot}84$ msec. Mean current, $\mu_I = 20{\cdot}4$ nA and the single channel conductance, calculated from the fitted curve is $\gamma = 13{\cdot}5$ pS. B, immunized (Anti-R) end-plate spectrum obtained at clamp holding potential $V_m = -80$ mV, $T = 10$ °C, from forty averaged spectra from a single noise run. Half-power frequency is $f_c = 56$ Hz, hence $\tau_{noise} = 2{\cdot}84$ msec. Mean current, $\mu_I = 26{\cdot}0$ nA and the single channel conductance calculated from the fitted curve is $\gamma = 11{\cdot}0$ pS. Data were sampled at 0·5 msec intervals through a 1000 Hz active ($1/f^8$) low pass filter. Background noise has been subtracted.


A Control
f_c = 56 Hz
10^{-22}
10^{-23}
10^{-24}
Spectral density $S(f)$ (A^2 sec)
1
10
10^2
10^3
Frequency (Hz)
B Anti-R
f_c = 56 Hz
10^{-22}
10^{-23}
10^{-24}
Spectral density $S(f)$ (A^2 sec)
1
10
10^2
10^3
Frequency (Hz)

Acetylcholine current noise

Properties of the end-plate channels, induced by acetylcholine, have been derived by analysis of the current fluctuations which occur during ionophoretic application of ACh to end-plates at 10 °C. Power-density spectra of ACh-induced current noise could be well described by a single Lorentzian, $S(f) = S(0)/[1+(f/f_c)^2]$ where $S(f)$ and $S(0)$ are the spectral densities at frequencies f and 0 Hz respectively and f_c is the cut-off frequency at which the spectral density is reduced to one half of its maximum value. Under specific assumptions about the operation of synaptic channels (Katz & Miledi, 1972; Anderson & Stevens, 1973; Colquhoun & Hawkes, 1977), the mean life-time of the channel, τ_{noise}, is derived from the cut-off frequency of the spectrum according to, $\tau_{noise} = 1/(2\pi f_c)$. In the examples shown in Fig. 7*A*, *B* the cut-off frequency (marked by an arrow) is identical at the control (anti-CFA) end-plate and at the immunized (anti-R) end-plate: $f_c = 56$ Hz, $\tau_{noise} = 2{\cdot}8$ msec (at $V_m = 80$mV, $T = 10$ °C). The mean values of noise at normal, at CFA treated and at immunized end-plates were: τ_{noise} (normal) $= 2{\cdot}5 \pm 0{\cdot}5$ msec ($n = 2$), τ_{noise} (anti-CFA) $= 2{\cdot}68 \pm 0{\cdot}14$ msec ($n = 8$), τ_{noise} (anti-R) $= 2{\cdot}9 \pm 0{\cdot}18$ msec ($n = 7$) at $V_m = 80$ mV, $T = 10$ °C. These values for the mean channel life-times are not significantly different at the 5% level.

By fitting the theoretical curve to the spectral values the single channel conductance, γ, was obtained from the zero frequency asymptote, $S(0)$, of the spectrum, as $\gamma = S(0)/[2\mu_I \tau(V_m - V_{eq})]$. In addition, γ was obtained independently from the variance of the noise as, $\gamma = \sigma^2/[\mu_I(V_m - V_{eq})]$ (where μ_I = mean membrane current, σ^2 = variance of current noise, V_m = clamp potential, V_{eq} = equilibrium potential of ACh taken as 0 mV). The mean conductance of the single channel was similar at control and immunized end-plates, γ (anti-CFA) $= 12{\cdot}5 = 0{\cdot}5$ pS ($n = 8$), γ (anti-R) $= 13{\cdot}15 \pm 0{\cdot}53$ pS ($n = 8$).

These values are reduced when compared with the values of 20–25 pS obtained at mammalian end-plates at room temperature (Colquhoun *et al.* 1977; Cull-Candy, Miledi & Trautmann, 1978). However, they are consistent with the observed ~ 30% reduction in m.e.p.c. amplitude seen when the temperature was lowered from 20 to 10 °C which probably also reflects a reduction in γ (see also, Dreyer, Muller, Peper & Sterz, 1976).

Ca^{2+} sensitivity of transmitter release

Transmitter release from nerve-terminals in EDL muscles was studied in four normal and four anti-R rats, at four or five different Ca^{2+} concentrations (0·15–7 mM; $m = 0{\cdot}15$–20) in the presence of 2 mM-Mg^{2+}. In each preparation the mean quantal content, m, was obtained by recording 50 e.p.p.s per end-plate in five to ten randomly sampled end-plates at each Ca level. On logarithmic co-ordinates the relationship between mean quantal content and Ca^{2+} concentration at both normal and immunized end-plates was linear with a slope of approximately 2·8 (Hubbard, Jones & Landau, 1968; Cull-Candy, Lundh & Thesleff, 1976). No statistically significant differences were found in the absolute level of m at the two types of endplate at any of the Ca^{2+} levels examined.

DISCUSSION

We have attempted to obtain information about the changes which occur when rats are immunized against ACh-receptor, by comparing the amplitude of m.e.p.c.s at normal and immunized rat end-plates. This gives an estimate of ACh-receptor-channel function which is independent of the input resistance of the muscle fibre. The mean amplitude of m.e.p.c.s at immunized end-plates was about 30 % of normal m.e.p.c. amplitude. The actual reduction may be greater because the mean m.e.p.c. amplitude at immunized end-plates is slightly over-estimated, due to loss of the smallest events in the background noise.

It will be recalled that major presynaptic defects have been excluded: the size of the quantum is normal, in view of the similarity between the reduction in m.e.p.p. amplitude and α-BuTx binding (Ito *et al.* 1978); the quantal content at low Ca^{2+} concentrations is unaffected by immunization; electron microscopy indicates that the position of the nerve terminal is not abnormally far from the post-synaptic membrane.

Therefore, the decrease in m.e.p.c. size is mainly of post-synaptic origin. There are several ways in which the anti-receptor antibody might interact with the receptor-channel complex so as to reduce the current passing through the membrane during an m.e.p.c. Broadly speaking the possibilities fall into two categories: a decrease in the conductance of the single channel, or simply a reduction in the number of available ACh receptor-channel complexes. As the elementary conductance of the channel is similar at normal and immunized end-plates, the reduction in amplitude of the m.e.p.c.s, brought about by immunization against ACh receptors, is probably due to a reduction in the number of available receptor-channels complexes.

The reduction in the number of available receptors could result from direct block of binding sites for ACh (and α-BuTx) perhaps by a small population of antibodies directed against junctional receptors. Evidence for this mechanism in the pathogenesis of experimental myasthenia gravis comes from *in vitro* experiments, where γ-globulins of IgGs from disease-affected rabbits and rats reduce m.e.p.p. amplitudes (Green *et al.* 1975*a*, *b*; Alemà & Miledi, unpublished). Moreover, antibodies block both the binding of ACh to *Torpedo* microsacs (Eldefrawi, 1978) and the binding of α-BuTx to frog muscle (Alemà *et al.* 1978) and mouse diaphragm junctions *in situ* (Zurn & Fulpius, 1977). These effects all have a rapid onset and a lack of temperature dependence indicating receptor block. A contributing factor to the the reduction in receptors may be their loss from the post-synaptic membrane caused by complement-mediated processes and stimulated degradation by cross-linking antibodies (Lennon, 1978; Reiness *et al.* 1978).

Binding of antibodies to the AChRs could affect the mean open time of the channels, even if their conductance is unchanged. However, analysis of ACh-induced noise showed no significant change of mean channel life-time after immunization. In view of this result, the small difference in $\tau_{e.p.c.}$, if real, could reflect a slightly slower clearance of ACh from the immunized end-plate. This could come about if the presence of anti-receptor antibody in the cleft hampered ACh-diffusion and thereby allowed more repetitive bindings to occur between ACh-molecules and receptor. In view of the similarity of $\tau_{m.e.p.c.}$ at the two types of endplate this seems unlikely. An

alternative possibility is that transmitter release is less synchronous at immunized end-plates so that $\tau_{e.p.c.}$ is slightly prolonged.

Although we found that rats immunized with the AChR retain practically normal single channel properties, antiserum against AChR slightly alters the single channel properties in cultured muscle cells obtained from rat embryonic skeletal tissue, causing a 15% reduction in the conductance and a 23% reduction in the mean open time of the channel (Heinemann *et al.* 1977). The reason for this apparent difference between *in vivo* and culture experiments is not clear although it could perhaps result from differences in the levels of antibodies in the two conditions. Alternatively it could represent a difference between adult junctional and embryonic extrajunctional ACh-induced channels in their susceptibility to antibody.

In conclusion, the immunized rat end-plate is a good model for myasthenia gravis at the human end-plate where the channel properties are also unchanged (Cull-Candy, Miledi & Trautmann, 1978, 1979). However, the similarity between normal and immunized rat end-plates in the mean quantal content of the end-plate potential, at low external Ca^{2+} levels contrasts with the situation observed at myasthenia gravis affected nerve-terminals in humans (Cull-Candy, Miledi & Trautmann, 1978; Cull-Candy, Miledi, Trautmann & Uchitel, 1980) where the quantal content is increased.

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