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END-PLATE CURRENTS AND ACETYLCHOLINE NOISE AT NORMAL AND MYASTHENIC HUMAN END-PLATES

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SUMMARY

1. The amplitudes and time courses of miniature end-plate currents (m.e.p.c.s) have been compared at normal and myasthenic (MG) human end-plates studied under voltage clamp. The m.e.p.c. amplitude at MG end-plates is reduced to about one third normal; mean m.e.p.c. (normal) = $2{\cdot}6 \pm 0{\cdot}2$ nA, mean m.e.p.c. (MG) = $1{\cdot}0 \pm 0{\cdot}1$ nA. The decay time constant of m.e.p.c.s ($\tau_{m.e.p.c.}$) is very similar at normal and MG end-plates; $\tau_{m.e.p.c.}$ (normal) = $1{\cdot}70 \pm 0{\cdot}1$ msec, $\tau_{m.e.p.c.}$ (MG) = $1{\cdot}80 \pm 0{\cdot}13$ msec ($V_m = -80$ mV, $T = 23$ °C).

2. The equilibrium potential of the end-plate current (e.p.c.) at normal and myasthenic human end-plates is close to 0 mV.

3. Decay time constants $\tau_{e.p.c.}$ and $\tau_{m.e.p.c.}$ increase exponentially with membrane hyperpolarization. The voltage sensitivity of the time constants was similar at normal and MG end-plates.

4. Both normal and myasthenic e.p.c.s are greatly prolonged in the presence of neostigmine (10^{-6} g/ml.). At the same time the voltage sensitivity of $\tau_{e.p.c.}$ is slightly reduced.

5. In response to steady ionophoretically applied ACh the mean membrane currents obtained at MG end-plates were smaller than the normal under similar conditions.

6. Analysis of end-plate current noise obtained during the steady application of acetylcholine (ACh) to voltage clamped normal and MG human end-plates showed that the amplitude of the elementary current event (γ) and the average channel life-time (τ_{noise}) was similar at the two sites: τ_{noise} (normal) = $1{\cdot}54 \pm 0{\cdot}04$ msec, τ_{noise} (MG) = $1{\cdot}63 \pm 0{\cdot}11$ msec; γ(normal) = $22{\cdot}3 \pm 1{\cdot}57$ pS, γ (MG) = $20{\cdot}25 \pm 1{\cdot}93$ pS ($V_m = -80$ mV, $T = 23$ °C). The voltage sensitivity of the channel life time, measured from end-plate current noise, was similar at normal and MG end-plates.

7. At normal human end-plates a packet of transmitter opens about 1500 channels whereas at MG end-plates a packet opens only about 600 channels. It is calculated that the size of the transmitter packets released from MG-terminals is at least as large as the packet of the ACh released from normal human nerve terminals.

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INTRODUCTION

Earlier studies have demonstrated that at human end-plates affected by the disease myasthenia gravis (MG) the spontaneous miniature end-plate potentials (m.e.p.p.s) and impulse evoked end-plate potentials (e.p.p.s) are unusually small (Elmqvist, Hofmann, Kugelberg & Quastel, 1964; Albuquerque, Rash, Mayer & Satterfield, 1976; Ito, Miledi, Molenaar, Vincent, Polak, van Gelder & Newsom Davis, 1976). In addition the over-all binding of labelled α-bungarotoxin (α-BuTx) at the MG end-plate is diminished, in some cases, down to 10% of the normal (Fambrough, Drachman & Satyamurti, 1973; Green, Miledi, Perez de la Mora & Vincent, 1975; Ito, Miledi, Vincent & Newsom Davis, 1978) and the post-synaptic sensitivity to ionophoretically applied ACh is markedly reduced (Albuquerque *et al.* 1976). The presence in the sera of MG patients of anti-receptor antibodies (Almon, Andrew & Appel, 1974), which are capable of reducing the ACh sensitivity of tissue cultured human and rat muscle cells (Bevan, Kullberg & Heinemann, 1977; Anwyl, Appel & Narahashi, 1977) and the presence of an immune complex on the post-synaptic membrane (Engel, Lambert & Howard, 1977) suggests that a proportion of receptors at MG end-plates will be either blocked or modified by the antibody. This would seem to be sufficient to explain the reduction in m.e.p.p. amplitude and in α-BuTx binding. However, there are also ultrastructural changes at the MG end-plate including widening of the synaptic cleft and alterations of the junctional folds (Engel & Santa, 1971; Santa, Engel & Lambert, 1972) which could affect m.e.p.p.s. For instance there is evidence that, at frog end-plates, the decay of the miniature end-plate current (m.e.p.c.) is influenced by the rate of diffusion of ACh from the cleft (Katz & Miledi, 1973; Magleby & Terrar, 1975) and hence depends on the geometry of the cleft and the receptor density. In addition it remained possible that the combination of antibodies with the receptors could affect the characteristics of the membrane channel opened by ACh (see Katz & Miledi, 1972; Anderson & Stevens, 1973).

It was of interest, therefore, to determine whether the post-synaptic changes which occur in MG involve modification in the time course and amplitude of the elementary conductance increase which results from the action of ACh on the receptors. A preliminary report of some of these results has appeared (Cull-Candy, Miledi & Trautmann, 1978).

METHODS

Intercostal muscles which were from patients clinically diagnosed to have myasthenia gravis (MG), were obtained either during thymectomy or as a biopsy; control muscles were obtained during thoracotomy from patients without neuromuscular disease. All MG patients in this series had previously undergone anticholinesterase therapy.

Preparation. Each muscle was dissected to give several bundles of about twenty to one hundred fibres with tendon insertions at each end and with a nerve supply. They were stretched and pinned on Sylgard resin and transilluminated through a condenser to give dark or bright field illumination. Stimulation of the nerve was via a capillary suction electrode with a fine glass tip chosen to fit the particular nerve branch. Muscles were investigated at 23 °C and remained in good condition for 2 days.

Medium. Muscles were usually perfused continuously with an oxygenated (95% O_2/5% CO_2) medium of the following composition (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 some cases preparations were oxygenated

directly in the bath via a fine capillary jet which produced only a slight disturbance of the bathing fluid. In many experiments, used to record e.p.c.s evoked by nerve stimulation, the Mg concentration was doubled and the Ca concentration reduced. On a few occasions (+)-tubocurarine (TC) was added to the normal perfusing medium to reduce the amplitude of the e.p.c.s. Neostigmine (Prostigmin, Roche) was used to inhibit cholinesterase.

Micro-electrodes and micropipettes. Voltage recording electrodes were filled with 3 M-KCl; current passing electrodes contained 2 M-K citrate slightly acidified with citric acid. Electrodes were selected for low noise and clean penetration of cells. Ionophoretic micropipettes were filled with 1 M-ACh.

Voltage clamping. Fine nerve branches were followed and the voltage micro-electrode inserted close to this site. Spontaneous m.e.p.p.s with a rise time < 1·5 msec were considered acceptable. The current passing micro-electrode was then inserted less than 100 μm from the recording electrode and fast rise time m.e.p.p.s were recorded simultaneously with both electrodes. For noise experiments the ionophoretic micropipette was positioned between the recording and current passing electrodes usually at sufficient distance from the junction to produce a gradual rather than a rapid increase in ACh concentration at the receptors to minimize artifacts arising from fluctuation in the ejection rate. Drug ejection currents, which were from an isolated source connected to a separate bath electrode, were monitored on low and high gain. Under voltage-clamp the gain of the clamp amplifier was adjusted so that a voltage change produced by a d.c. command was within 1% of the actual command value and the residual voltage from m.e.p.c.s was undetectable at 1 mV/cm. M.e.p.c.s, e.p.c.s and ACh-induced current fluctuations were recorded through an active virtual earth (with 500 or 1000 Hz low pass filter) on a low gain d.c. channel (10–100 nA/cm) and a high gain a.c. channel (1–2 nA/cm) and stored on analogue tape (band width 1–2·5 kHz). For m.e.p.c.s with actual rise times in the range 0·4–0·7 msec the observed rise times are artificially prolonged by approximately 0·3 msec if recorded via the 500 Hz low pass filter of the virtual earth, but the slower time constant of decay of the m.e.p.c.s is not significantly altered. Thus, experiments in which the virtual earth filter was used in both the 500 and 1000 Hz configurations at the same end-plate showed that the measured time constants of m.e.p.c.s and of ACh noise were unchanged. However, the resulting enhancement of the signal to noise ratio facilitated measurement of the decay time constant of m.e.p.c.s particularly at myasthenic end-plates and the m.e.p.c.s presented in Fig. 1 were recorded via the 500 Hz filter.

Spectral analysis. Records were fed via a Kemo active filter (1000 Hz low pass, $1/f^8$ roll-off) into a PDP-11 and digitalized at 2000 Hz sample rate. The Fast Fourier transform which yielded a two-sided spectral density function, $S(f)$, was calculated for 512 point segments; fifty to one hundred and fifty spectra were averaged to produce a final power spectrum. An averaged control spectrum of background noise taken immediately before application of ACh (or between segments which contained m.e.p.c.s – for m.e.p.c. spectra) was subtracted to remove background contributions from non-receptor membrane currents and electronic noise. At low frequencies the power density of the background noise was usually 2–3 orders of magnitude below the power density of ACh-induced noise. In some cases a smoothing of the resulting spectrum was performed according to the weighting $x_n = \frac{1}{4}(x_{n-1}) + \frac{1}{2}(x_n) + \frac{1}{4}(x_{n+1})$.

RESULTS

Miniature end-plate currents

Examples of miniature end-plate currents recorded under voltage-clamp at normal and MG end-plates are shown in Fig. 1*A*, *B*. Usually the m.e.p.c.s have a rapid rising phase followed by a slower, exponential decay. However, a small proportion of the m.e.p.c.s at normal and MG end-plates do not conform to this simple form. Examples of m.e.p.c.s of abnormal time course recorded at normal end-plates are shown in Fig. 1*C*. Such m.e.p.c.s have previously been observed at other mammalian end-plates (Green *et al.* 1975). The small percentage of m.e.p.c.s which did not decay exponentially were not used in the subsequent analysis.

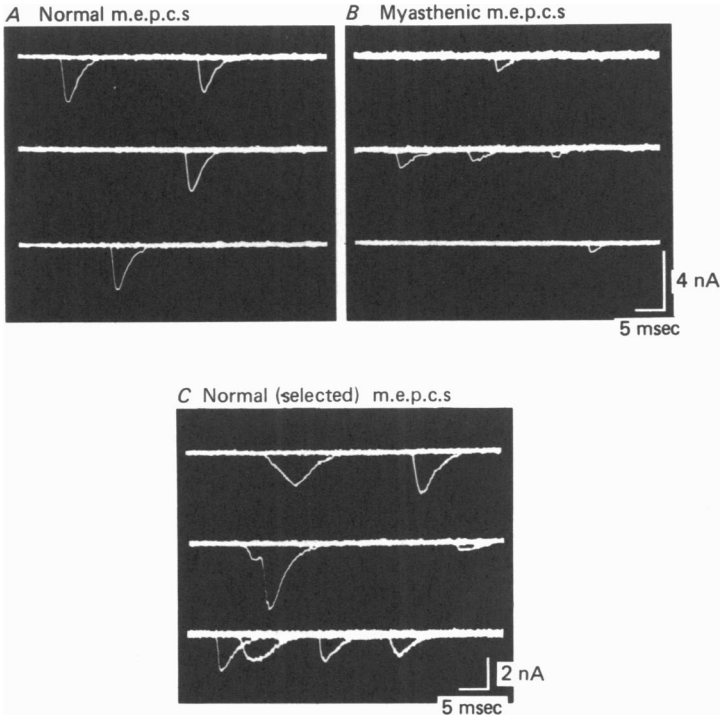

Fig. 1. Examples of miniature end-plate currents from normal (*A*), and myasthenia gravis (*B*), end-plates. Each trace is of several superimposed sweeps photographed during replay from analogue tape; m.e.p.c.s were recorded under voltage-clamp through a 500 Hz low pass filter which slightly prolongs the rise time but does not appreciably alter the decay time constant, $\tau_{m.e.p.c.}$. Normal m.e.p.c.s are all from the same end-plate. The three traces of myasthenic m.e.p.c. are from different end-plates. Clamp holding potential $V_m = -80$ mV, $T = 24$ °C. *C*, selected miniature end-plate currents from three normal end-plates. Examples shown include m.e.p.c.s with prolonged rise, prolonged decay and a composite m.e.p.c. as well as 'normal' m.e.p.c.s. Clamp holding potential, $V_m = -80$ mV, $T = 23$ °C.

Fig. 2. Amplitude distributions of m.e.p.c.s at two normal end-plates *A* and *C* and at two myasthenic end-plates *B* and *D* at clamp holding potential $V_m = -80$ mV and $T = 23$ °C. The m.e.p.c.s at the normal end-plates show a typical Gaussian distribution. The smallest m.e.p.c.s recorded were clearly above the background noise level (indicated by arrow on the abscissa). In *C* some sub-m.e.p.c.s were present, with a mean amplitude of 1·2 nA. M.e.p.c.s at the myasthenic end-plate show a skew distribution with the smallest m.e.p.c.s disappearing into the background noise thus making the apparent means at the myasthenic end-plates (*B* and *D*) over-estimates of the true means.

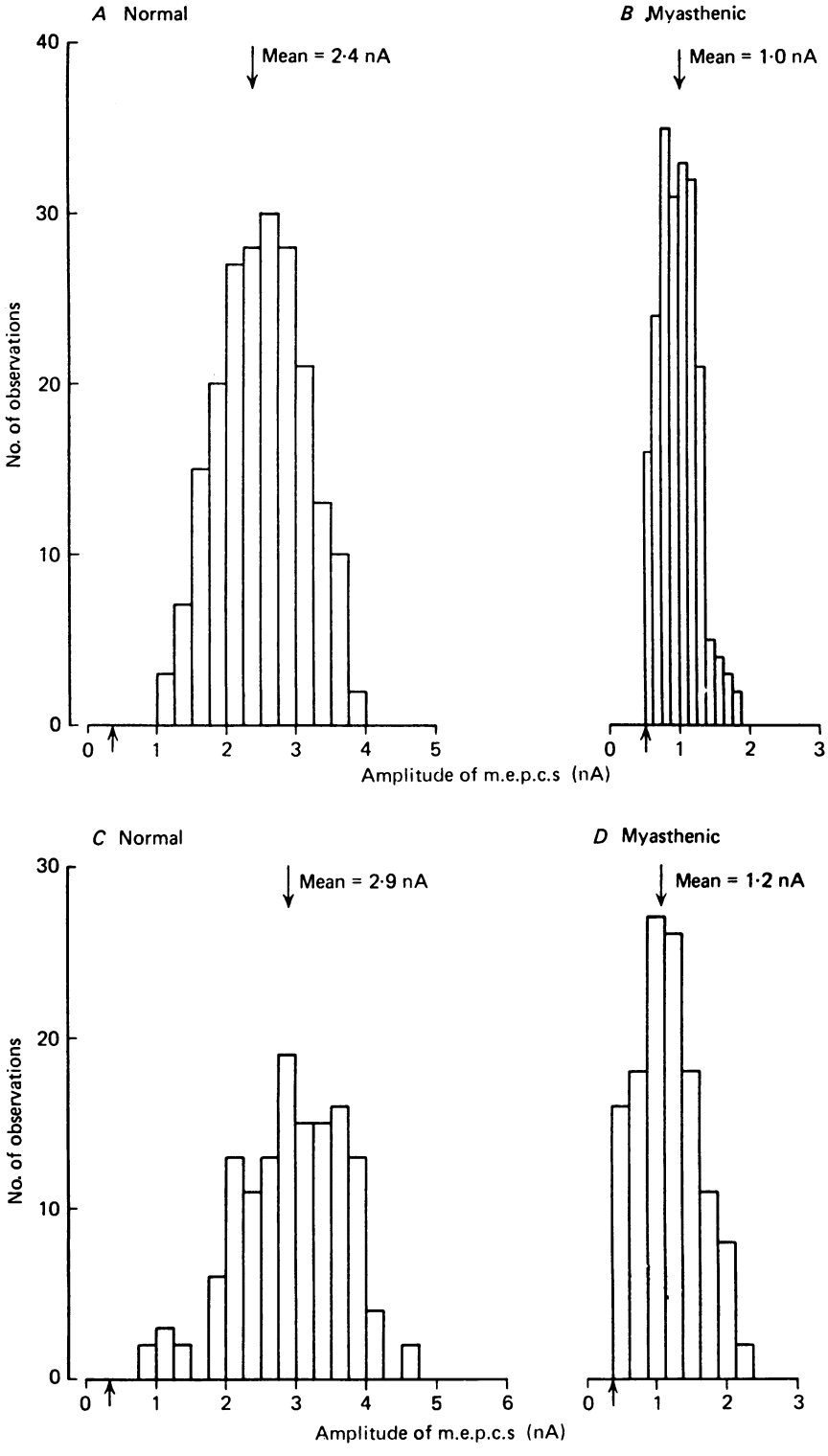

Fig. 2. For legend see facing page.

M.e.p.c. amplitude distributions

M.e.p.c.s at MG end-plates are markedly reduced in amplitude when compared with normal. Amplitude histograms of m.e.p.c.s at normal and MG end-plates are shown in Fig. 2. At a clamp holding potential of -80 mV the mean m.e.p.c. amplitude at normal end-plates = $2{\cdot}6 \pm 0{\cdot}2$ nA (mean $\pm$ S.E. of mean, for fourteen end-plates in three muscles). At myasthenic end-plates, the mean amplitude = $1{\cdot}0 \pm 0{\cdot}1$ nA (for twenty-five end-plates, in five muscles). However, in one case (not included in the values used to derive the mean amplitude) the m.e.p.c. amplitude was $2{\cdot}6 \pm 0{\cdot}35$ nA although this patient had the typical symptoms of MG and had a high titre of antibodies directed against the nicotinic receptor.

During experiments where end-plates were studied under voltage-clamp the input resistance (R_{in}) of normal and MG muscle fibres was obtained by measuring the current required to jump the clamp potential from -80 to -90 mV. The input resistance is not markedly affected by MG; R_{in} (normal) = $1{\cdot}2 \pm 0{\cdot}10$ MΩ ($n = 10$), R_{in} (MG) = $1{\cdot}1 \pm 0{\cdot}15$ MΩ ($n = 10$).

At normal human end-plates the distribution of m.e.p.c.s is Gaussian and the smallest potentials are well above the noise level of the recording system except for those cases where a population of sub-m.e.p.c.s, similar to that described at frog, mouse and rat end-plates (Kriebel & Gross, 1974; Bevan, 1976; Cooke & Quastel, 1973; Cull-Candy, Lundh & Thesleff, 1976) is also detectable (see Fig. 2*C*). In contrast, at MG end-plates the amplitude histogram of m.e.p.c.s is skew with the smaller events disappearing in the background noise (see Fig. 2*B*, *D*).

The difference in the mean amplitude of m.e.p.c.s at normal and MG end-plates is therefore underestimated as the value for MG end-plates does not include all the smaller events. The mean amplitude of m.e.p.c.s appears at least 3 times smaller at MG end-plates than at normal ones. This compares with the reduction in mean m.e.p.p. amplitude previously seen at MG end-plates (Elmqvist *et al.* 1964; Albuquerque *et al.* 1976; Ito *et al.* 1976, 1978).

M.e.p.c. time course

The time constant of decay of the m.e.p.c., $\tau_{m.e.p.c.}$, was obtained either from a semilogarithmic plot of the decay phase of the m.e.p.c.s or, when sufficient m.e.p.c.s were available at a given end-plate, from power spectra of the m.e.p.c.s such as illustrated in Fig. 3. Spectral analysis allowed convenient and rapid processing of a large number of m.e.p.c.s.

If the m.e.p.c. time course has an instantaneous rise followed by an exponential decay, their spectra should follow the form of a single Lorentzian, $S(f) = S(0)/(1+(f/f_c)^2)$, represented in Fig. 3 by a continuous curve (Bendat & Piersol, 1971). $\tau_{m.e.p.c.}$ could then be obtained from the cut-off frequency, f_c of the power spectrum (i.e. the frequency at which the power is reduced to one half of the zero frequency asymptote) where $\tau_{m.e.p.c.} = 1/(2\pi f_c)$. As the rising phase of the m.e.p.c. is not instantaneous, the high frequency components of the spectrum are attenuated compared to a Lorentzian, but the low frequency components should be little affected, so that f_c gives a reasonable estimate of $\tau_{m.e.p.c.}$ (see also Katz & Miledi, 1973).

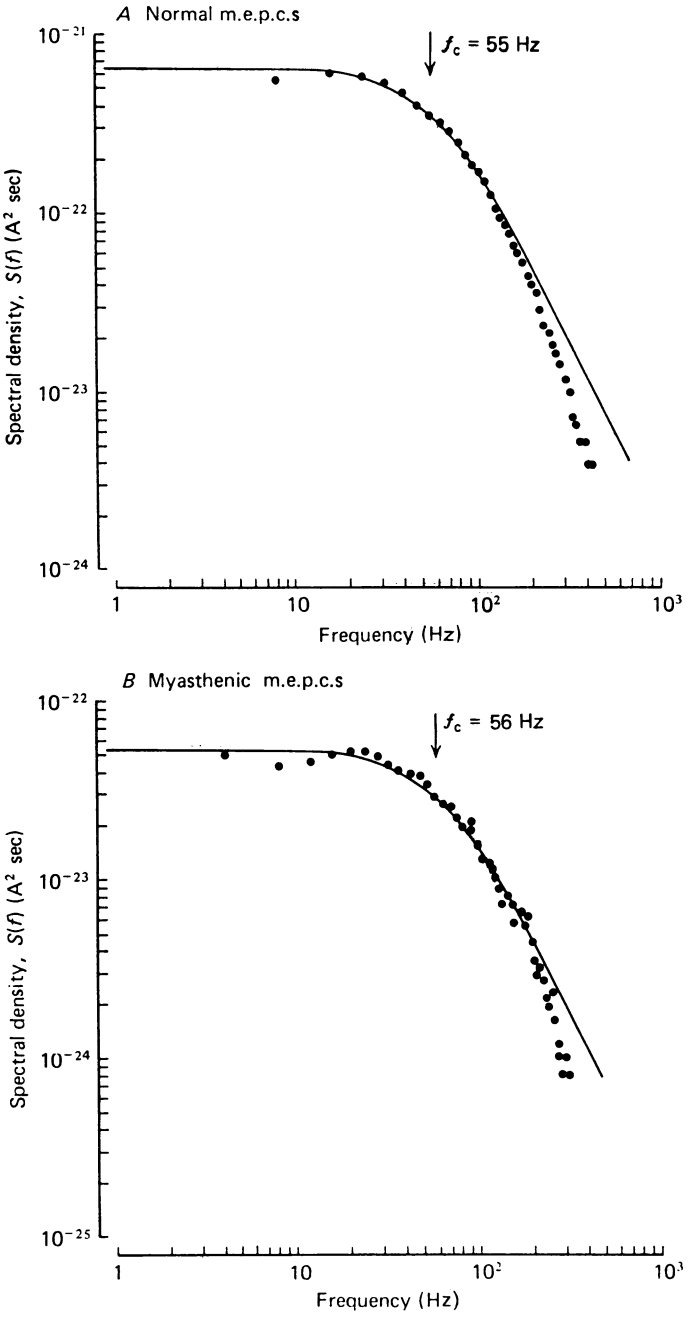

Fig. 3. Spectral density of m.e.p.c.s at a normal and a myasthenic end-plate. *A*, normal end-plate spectrum obtained at clamp holding potential $V_m = -110$ mV, $T = 23$ °C. Averaged spectrum of forty-three individual m.e.p.c. spectra. Cut-off frequency, indicated by arrow, is $f_c = 55$ Hz, hence $\tau_{m.e.p.c.} = 2{\cdot}9$ msec. *B*, myasthenic end-plate spectrum obtained at clamp holding potential $V_m = -110$ mV, $T = 23$ °C. Averaged spectrum of thirteen individual m.e.p.c. spectra. Cut-off frequency indicated by arrow is $f_c = 56$ Hz, hence $\tau_{m.e.p.c.} = 2{\cdot}8$ msec. The zero frequency asymptote $S(0)$ is approximately 12 times smaller in the myasthenic spectrum than in the normal which indicates a ratio 3·5:1 for the m.e.p.c. amplitudes. Note that at high frequencies the spectral density is less than predicted by the $1/f^2$ continuous curve.

In order to obtain an accurate estimate of the time constant of decay, $\tau_{m.e.p.c.}$, miniature currents should be clamped as close as possible to the site of the end-plate (see Takeuchi & Takeuchi, 1959). Because optical identification of end-plates was sometimes difficult in human muscle biopsies it was necessary to consider the effect of inaccurate location on the time course of synaptic events. The space constant of the fibre decreases as the frequency of the command signal increases. Therefore, a displacement of the electrodes away from the end-plate region will affect the rising phase of the m.e.p.c. more than its decay.

The relationship between the duration of the rising phase and the time constant of decay of m.e.p.c.s and e.p.c.s was compared at thirty-six normal and thirty myasthenic end-plates. M.e.p.c.s with rise times which varied between approximately 0·4–0·7 msec (1000 Hz filter, see Methods), presumably due to varying distances between electrodes and end-plates, all had similar decay times. M.e.p.c.s with rise times > 0·7 msec had decay times which increased in relation to the rise time, presumably as both rise and decay phases were distorted by larger displacement of the electrodes; these m.e.p.c.s were rejected.

$\tau_{m.e.p.c.}$ At end-plates where the clamp quality was considered adequate (i.e. rise time < 0·7 msec, 1000 Hz filter or < 1 msec, 500 Hz filter) normal and MG m.e.p.c.s had very similar decay time constants; normal $\tau_{m.e.p.c.} = 1{\cdot}70 \pm 0{\cdot}10$ msec (mean ± S.E. of mean for ten end-plates); myasthenic $\tau_{m.e.p.c.} = 1{\cdot}80 \pm 0{\cdot}13$ msec (for fourteen end-plates), at $V_m = -80$ mV, $T = 23$ °C.

End-plate currents

End-plate current amplitude

One of the factors which determine the amplitude of synaptic currents is the electrochemical gradient for the ions involved in the post-synaptic conductance change, i.e. the value of the equilibrium potential (V_{eq}) of the end-plate current (e.p.c.). The value of the equilibrium potential was obtained by driving the membrane potential (V_m) beyond V_{eq} (Fig. 5) so that the direction of the current was reversed; V_{eq} was then interpolated from a least-squares fit to the peak e.p.c. amplitudes taken at 10 mV intervals (over the range +50 to −140 mV). Fig. 4 shows an example of the relationship between peak e.p.c. amplitude and membrane potential determined in this way for a normal and MG end-plate. At both end-plates the e.p.c. is anulled at close to 0 mV. In all cases the equilibrium potential was found to be practically the same at normal and MG end-plates; V_{eq} (normal) = $+0{\cdot}8 \pm 3{\cdot}1$ mV (mean ± S.E. of mean, for four end-plates), V_{eq} (MG) = $+2{\cdot}8 \pm 2{\cdot}8$ mV (for four end-plates). The reduction in amplitude of m.e.p.c. and e.p.c. in myasthenia gravis cannot, therefore, be attributed to a change in the ionic driving force. For convenience in calculations of the single channel conductance (see below) the equilibrium potential for ACh is taken as 0 mV in both situations. The equilibrium potential of the e.p.c. is usually considered to be in the range 0 to −15 mV at frog (del Castillo & Katz, 1954; Takeuchi & Takeuchi, 1960; Kordaš, 1969; Katz & Miledi, 1977), cat (Axelsson & Thesleff, 1959), rat and mouse (Magazanik & Potopova, 1969; Dreyer, Müller, Peper & Sterz, 1976) motor end-plates.

There is a linear relationship between end-plate current amplitude and membrane potential in the examples shown in Fig. 4. However, at some end-plates studied, the

peak conductance change of the e.p.c. both at normal and MG human end-plates is not constant but becomes progressively *larger* as the membrane potential is hyperpolarized. This is in contrast with the situation at frog end-plates where the peak conductance of the e.p.c. decreases with hyperpolarization of the membrane potential (Kordaš, 1969; Dionne & Stevens, 1975), although it should be noted that at the frog end-plate the peak e.p.c. conductance may also be independent of V_m (Takeuchi & Takeuchi, 1960; Trautmann & Zilber-Gachelin, 1976). We found no consistent differences between normal and MG end-plates in the dependence of the peak conductance of the e.p.c. on V_m.

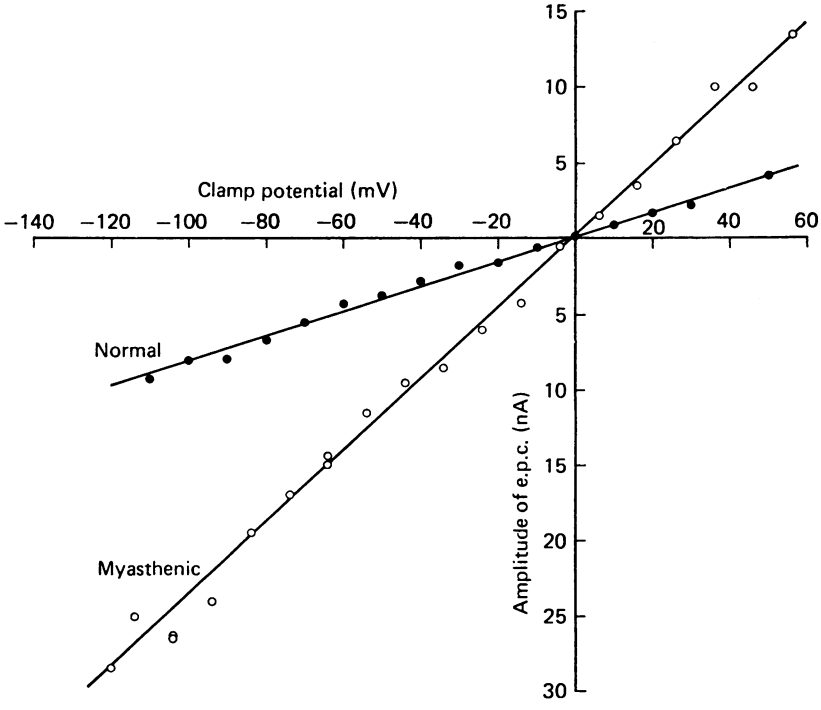

Fig. 4. Relationship between e.p.c. amplitude and membrane potential at a myasthenic (○) and a normal (●) end-plate. Each point represents the averaged amplitude of at least five e.p.c.s at each membrane potential. The equilibrium potential for the e.p.c. is close to zero mV at both end-plates; E_{Eq} (myasthenic) = −1 mV E_{Eq} (normal = −2 mV. In both cases a regression line (least-square method) has been fitted through the points. Normal and myasthenic e.p.c.s were obtained by stimulating the nerve at 0·5 Hz in normal medium; for the normal e.p.c. TC 2×10^{-7} g/ml. was added to the medium to avoid eliciting action potentials and contractions in the muscle.

Membrane potential dependence of end-plate current decay

At human (normal and MG) end-plates there is a logarithmic relationship between the time constant of decay of the e.p.c., $\tau_{e.p.c.}$, and the clamp potential indicating that $\tau_{e.p.c.}$ increases exponentially with membrane hyperpolarization. The dependence of $\tau_{e.p.c.}$ on membrane potential may then be expressed as (Magleby & Stevens, 1972; Gage & McBurney, 1975)

$$\tau_{e.p.c.}(V_m) = \tau(0) \exp(-V_m/H),$$

where $\tau_{e.p.c.}$ is the time constant at membrane potential V_m, $\tau(0)$ is the time constant at membrane potential 0 mV and H is the constant which characterizes the voltage dependence. Values for H were very similar at normal and MG end-plates: $H_{e.p.c.}$ (normal) $= 93 \pm 8$ mV (n = four end-plates); $H_{e.p.c.}$ (MG) $= 92 \pm 17$ mV (n = four end-plates). The magnitude of the voltage sensitivity of $\tau_{e.p.c.}$ at human end-plates

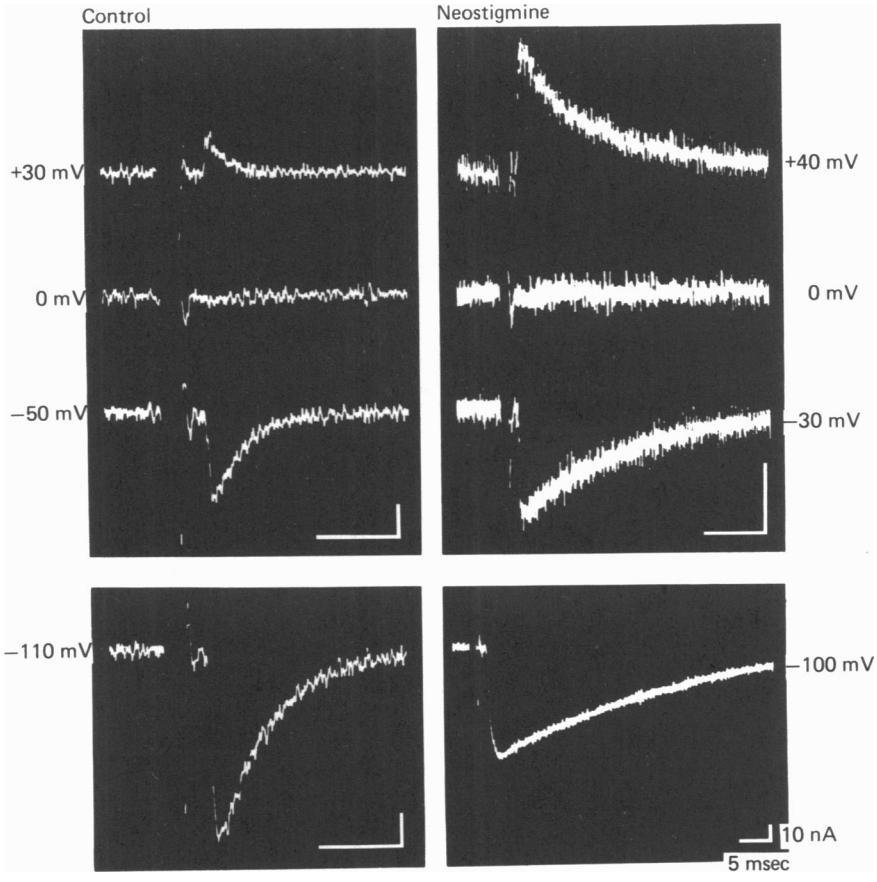

Fig. 5. Nerve-evoked end-plate currents, from a normal end-plate over a range of clamp holding potentials, in normal medium (plus TC 10^{-7} g/ml.) and in the presence of neostigmine 10^{-6} g/ml. (TC 10^{-7} g/ml.). The equilibrium potential for transmitter action is close to 0 mV both in presence and absence of neostigmine. Note that the time constant for decay, $\tau_{e.p.c.}$, is markedly prolonged by hyperpolarization of the membrane in both cases. T = 24 °C. Calibration 10 nA and 5 msec.

is very similar to that previously described for frog, toad and rat end-plates (Magleby & Stevens, 1972; Gage & McBurney, 1975; Colquhoun, Large & Rang, 1977). Similar values of H have also been obtained for $\tau_{m.e.p.c.}$ at normal and MG end-plates (see Fig. 8A); $H_{m.e.p.c.}$ (normal) $= 105{\cdot}8 \pm 5{\cdot}7$ mV (n = eight end-plates), $H_{m.e.p.c.}$ = $116{\cdot}6 \pm 8{\cdot}3$ mV (n = nine end-plates).

Because of the wide use of neostigmine in the treatment of MG, we have also examined some properties of the normal e.p.c. in the presence of neostigmine. Results from an experiment at a normal end-plate in which $\tau_{e.p.c.}$ is examined before

and after treatment with neostigmine 10^{-6} g/ml. are illustrated in Figs. 5 and 6. In this example the presence of neostigmine is accompanied by an approximately tenfold increase in $\tau_{e.p.c.}$. At the same time its voltage sensitivity is slightly decreased (H changing from 100 to 147 mV). As already suggested (Katz & Miledi, 1973; Magleby & Terrar, 1975), the primary effect of neostigmine may be to increase repetitive binding of ACh molecules in the synaptic cleft. Our results favour such an interpretation (see also Kordaš, 1977).

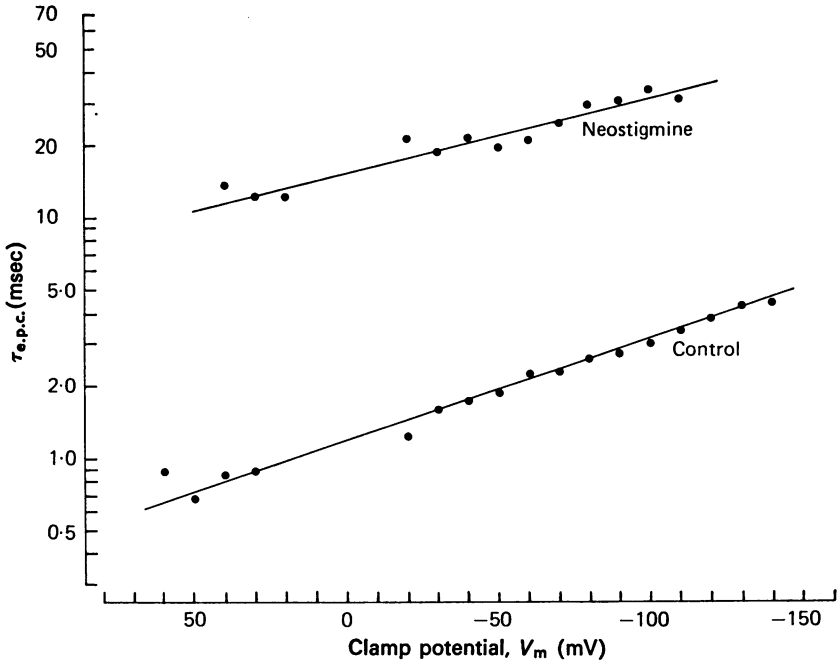

Fig. 6. Dependence of the time constant of decay of the e.p.c. ($\tau_{e.p.c.}$) on clamped membrane potential at a curarized normal end-plate (TC 10^{-7} g/ml.) before and after the addition of neostigmine, 10^{-7} g/ml. In this cell $\tau_{e.p.c.}$ = 2·0 msec at V_m = −53 mV in normal conditions and $\tau_{e.p.c.}$ = 22·0 msec after the addition of neostigmine. Straight lines through the points are fitted by the least-squares method (on log-linear coordinates) and are drawn according to the equation $\tau(V) = \tau(0).\exp(-V/H)$. In this cell H = 100 mV under normal conditions and H = 147 mV after the addition of neostigmine. T = 23 °C.

In the presence of neostigmine $\tau_{e.p.c.}$ is also prolonged at MG end-plates, but the variability of the effect of prostigmine from end-plate to end-plate did not allow us so far to examine this quantitatively.

ACh current noise

The properties of the single end-plate channel have been studied by analysing the voltage-clamped noise which occurs during steady ionophoretic application of ACh to the human end-plate. When normal and MG end-plates are compared under similar conditions consistently smaller mean membrane currents were obtained in response to ACh, indicating a reduced post-synaptic sensitivity if no additional

diffusion barrier is present at MG end-plates. A reduction in the ACh-induced potential change at MG end-plate has previously been reported (Albuquerque *et al.* 1976).

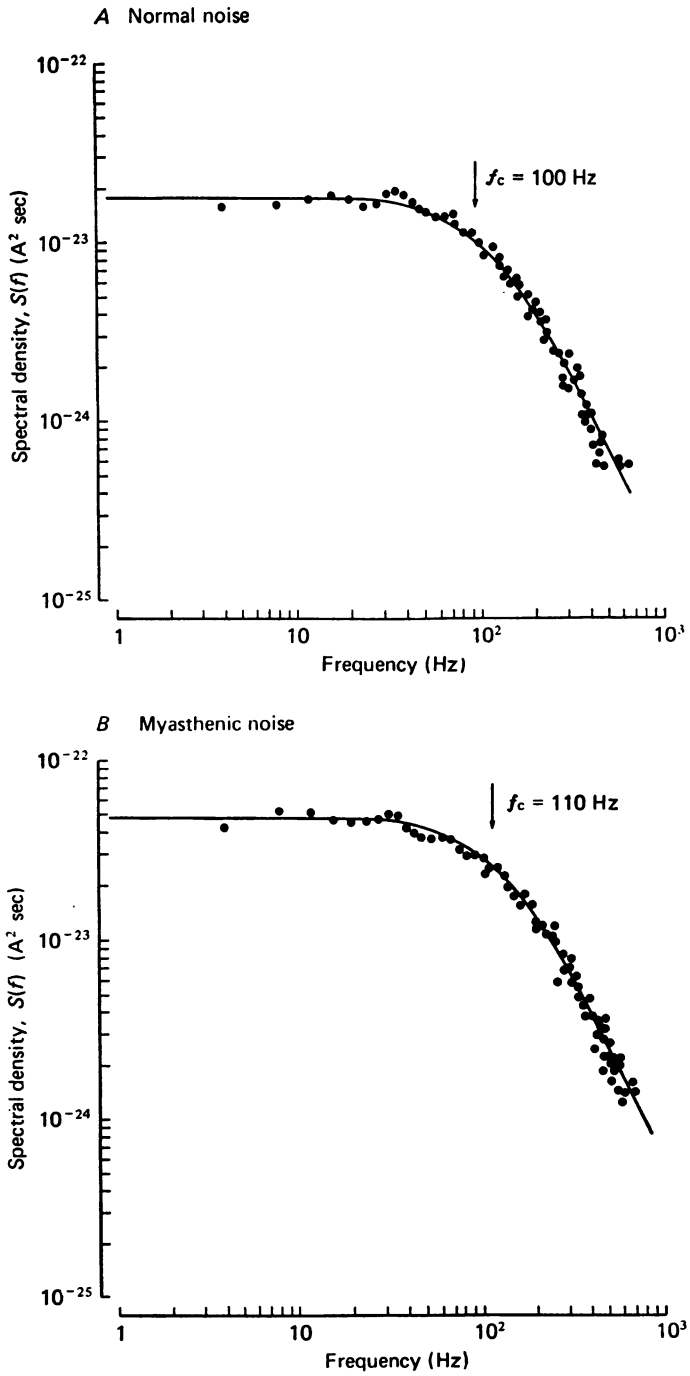

Fig. 7. For legend see facing page.

Although not studied systematically it appeared that receptors in the MG muscle membrane are desensitized more readily than those at normal end-plates in response to prolonged ionophoretic ACh application. This probably results from the larger doses of ACh needed to produce an adequate response at MG end-plates, as the rate of desensitization is known to increase with agonist concentration (Katz & Thesleff, 1957; Magazanik & Vyskočil, 1975). To minimize desensitization and maintain a steady membrane current for noise analysis we used moderate doses of ACh and worked with small membrane currents. Segments of response were selected for noise analysis when an approximately steady plateau of the response had been attained. The membrane currents during the ACh application were usually in the range of 2–30 nA although occasionally currents of up to 100 nA were studied at normal end-plates. As the area of membrane activated by these ACh doses was probably large compared with that activated by a single transmitter packet, it may be assumed that the ACh dose remained within the 'low concentration limit' (Anderson & Stevens, 1973).

Single channel parameters

Fig. 7 illustrates examples of spectra of current fluctuations induced by ACh at a normal and an MG end-plate. The spectra were fitted with a single Lorentzian component $S(f) = S(0)/(1+(f/f_c)^2)$; 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 spectrum has decreased to half of the zero frequency asymptote.

Under specific assumptions for the operation of synaptic channels, details of which have been considered elsewhere (see Katz & Miledi, 1972, 1976; Anderson & Stevens, 1973; Colquhoun & Hawkes, 1977; Neher & Stevens, 1977), the mean life time of the channel, τ_{noise}, was obtained from the cut-off frequency of the spectra (indicated by arrows in Fig. 9*A*, *B*) according to $\tau_{noise} = 1/(2\pi f_c)$. In the examples illustrated in Fig. 7 for the normal end-plate $f_c = 100$ Hz, $\tau_{noise} = 1{\cdot}58$ msec, for the myasthenic end-plate $f_c = 110$ Hz, $\tau_{noise} = 1{\cdot}44$ msec.

The mean value for the open time of ACh-induced end-plate channels in *normal* muscle membrane was $\tau_{noise} = 1{\cdot}54 \pm 0{\cdot}04$ msec (for n = thirteen end-plates, τ estimated from thirty-five separate noise-runs in four muscles) the comparable value for end-plate channels in *myasthenic* muscle membrane was $\tau_{noise} = 1{\cdot}63 \pm 0{\cdot}11$ msec (for n = eleven end-plates, τ estimated from twenty-eight separate noise-runs in four

Fig. 7. Spectral density of current fluctuations produced by steady ionophoretic application of ACh to a voltage clamped normal end-plate and a myasthenic end-plate. *A*, normal end-plate spectrum obtained at clamp holding potential $V_m = -80$ mV, $T = 23$ °C from fifty-two averaged spectra from a single ACh application. Cut-off frequency indicated by arrow is $f_c = 100$ Hz, hence $\tau_{noise} = 1{\cdot}58$ msec. Mean current $\mu_I = 2{\cdot}7$ nA and the single channel conductance, calculated from the fitted Lorentzian curve, is $\gamma = 23{\cdot}5$ pS. *B*, myasthenic end-plate spectrum obtained at clamp holding potential $V_m = -80$ mV, $T = 23$ °C from 138 averaged spectra from a single ACh application. Cut-off frequency indicated by arrow is $f_c = 110$ Hz, hence $\tau_{noise} = 1{\cdot}44$ msec. Mean current $\mu_I = 11$ nA and the single channel conductance calculated from the fitted curve is $\gamma = 18{\cdot}5$ pS. Data were sampled at 2000 Hz through a 1000 Hz active $(1/f^8)$ low pass filter and in the examples shown a final smoothing was applied according to $x_n = \frac{1}{4}x_{n-1} + \frac{1}{2}x_n + \frac{1}{4}x_{n+1}$. Background noise has been subtracted.

muscles) at $V_m = -80$ mV, $T = 23$ °C. Thus, there was no significant difference in the life time of the ACh-induced channels in normal and myasthenic end-plates.

The channel life time obtained from ACh noise increased as the membrane was hyperpolarized. Fig. 8*B* shows an example of τ_{noise} examined as a function of the clamp potential at a normal and at a myasthenic end-plate. The voltage sensitivity of the channel life time of myasthenic and normal ACh receptors was very similar; for myasthenic end-plates $H_{noise} = 108{\cdot}4 \pm 8{\cdot}3$ mV (for eight end-plates), for normal end-plates $H_{noise} = 107{\cdot}5 \pm 6{\cdot}4$ mV (for ten end-plates).

The conductance, γ, of the single open end-plate channel was determined both from the variance of the current noise and also from the zero frequency asymptote

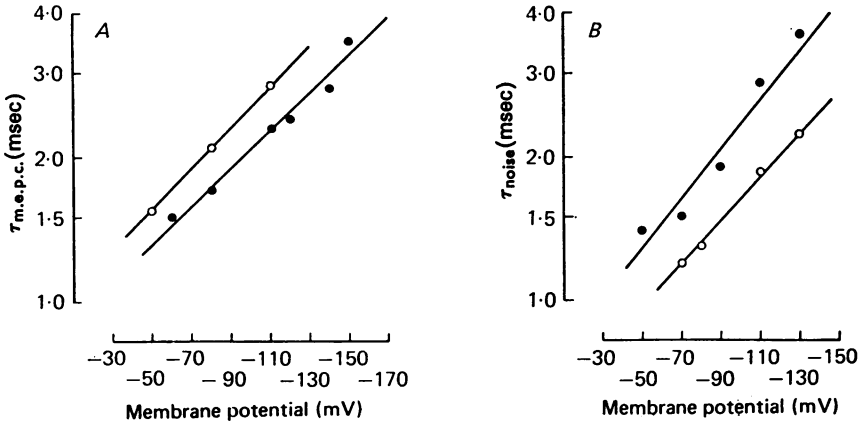

Fig. 8. *A*, dependence of the time constant of decay of the m.e.p.c., $\tau_{m.e.p.c.}$, on clamped membrane potential at a typical myasthenic (○) and a typical normal (●) end-plate. Straight lines through the points are fitted by the least squares method (on log-linear co-ordinates) and are drawn according to the equation $\tau(V) = \tau(0).\exp(-V/H)$, using slope values of $H(MG) = 102{\cdot}8$ mV, H(normal) = 110·5 mV. *B*, dependence of the mean channel life time, τ_{noise} (obtained from the cut-off frequency of the ACh power spectrum), on clamped membrane potential at a typical myasthenic (○) and a typical normal (●) end-plate (different from those illustrated in *A*). Straight lines through the points are fitted by the least-squares method using slope values of H(MG) = 96·2 mV, H(normal) = 85·4 mV.

of the theoretical curve fitted to the spectra ($\gamma = \sigma^2/[\mu_I(V_m - V_{eq})]$ and $\gamma = S(0)/[2\mu_I\tau(V_m - V_{eq})]$, where μ_I = mean membrane current, V_m = holding membrane potential, V_{eq} = equilibrium potential for ACh, taken as zero mV, σ^2 = variance of the membrane current fluctuations). These two methods of estimating γ gave similar values. The mean conductance of the single channel induced by ACh at normal end-plates was, γ (normal) = $22{\cdot}30 \pm 1{\cdot}57$ pS (for n = twenty end-plates, calculated from forty separate 'noise runs', four muscles); at myasthenic end-plates, γ (myasthenic) = $20{\cdot}35 \pm 1{\cdot}93$ pS (for n = ten end-plates, calculated from twenty-eight separate 'noise runs', four muscles) at $V_m = -80$ mV, $T = 23$ °C. γ was calculated for ACh-induced noise over a range of membrane potentials (−50 to −130 mV) and approximately the same values were obtained in all cases indicating

no apparent dependence of γ on V_m. The values of γ for ACh channels at the human end-plate are not very different from those observed in mouse and rat end-plates (see Dreyer *et al.* 1976; Colquhoun *et al.* 1977).

DISCUSSION

Duration of m.e.p.c. and of channel life time. Similarity in the mean time constant of decay of m.e.p.c.s at normal and MG end-plates may be taken to reflect a similarity in the life time of the transmitter-induced channels at the two sites, providing other factors, such as the time required for diffusion of ACh from the synaptic cleft (see Katz & Miledi, 1973), and the size of the packets of transmitter are reasonably similar at the two sites. From the analysis of ACh induced current noise the life time of the channels opened by ionophoretically applied ACh was found to be virtually identical in normal and MG muscle membranes. The channel life time of about 1·5 msec at -80 mV and 23 °C at the human end-plate is slightly longer than that of end-plate channels in mouse (Dreyer *et al.* 1976) or rat (Colquhoun *et al.* 1977) under approximately the same conditions and also longer than in frog (Katz & Miledi, 1972; Anderson & Stevens, 1973). This difference may reflect differences in the lipid environment around the receptor or differences in the receptor molecules themselves.

Provided that the ACh concentration during an m.e.p.c. subsides rapidly compared with the life time of the open channel then the time constant of decay of the m.e.p.c. should closely approximate τ_{noise}. This was the case at some end-plates investigated. However, the ratio $\tau_{m.e.p.c.}/\tau_{noise}$ was usually slightly greater than unity when determined at the same end-plate. The ratio of the *mean* values was $\tau_{m.e.p.c.}/\tau_{noise} = 1{\cdot}1$ both at normal and at MG human end-plates. It therefore seems that the 10% difference between the two time constants may reflect the delayed clearance of transmitter from the synaptic cleft (see Katz & Miledi, 1973; Magleby & Terrar, 1975; Colquhoun *et al.* 1977).

Since at myasthenic end-plates the number of functional ACh receptors is diminished the m.e.p.c.s might be expected to decay faster than at normal end-plates as repetitive binding of ACh to receptors, which slows down its diffusion from the cleft, is reduced (see Katz & Miledi, 1973). No such difference between $\tau_{m.e.p.c.}$ at normal and MG end-plates was observed in the present experiments. Perhaps with more accurate localization of the end-plates a small difference will be detected. However, when comparing $\tau_{m.e.p.c.}$ at the two sites other factors which may retard or accelerate the decay of the m.e.p.c. still need to be considered. For instance, in some segments of myasthenic end-plates the synaptic cleft appears wider than normal. Because of the increase in the distance between the source of the ACh in the terminals and the ACh receptors in the muscle membrane the time course of ACh action would be expected to be slowed down although this would be counteracted by a faster diffusion of ACh from the widened cleft and simplified postsynaptic membrane (Engel & Santa, 1971; Engel, Tsujihata, Lindstrom & Lennon, 1976). Two additional factors that could slow down the decay of m.e.p.c.s at myasthenic end-plates would be an increase in the amount of ACh contained in the packages responsible for the m.e.p.c.s and retarded diffusion of ACh resulting from

the presence of antibody-receptor complexes around the remaining functional receptors (Rash, Albuquerque, Hudson, Mayer & Satterfield, 1976; Engel *et al.* 1977). Further experiments are required to decide to what extent these factors may be involved in determining the time course of the myasthenic m.e.p.c. However, so far our results indicate that there is not an over-all modification in the time course of decay of ACh, released from a single packet, at the myasthenic end-plate when compared with the normal.

TABLE 1. Mean m.e.p.c. amplitudes ($V_m = -80$ mV, $T = 23$ °C) and corresponding numbers of α-bungarotoxin binding sites per end-plate from five patients with myasthenia gravis. The values are means ± S.E.; values in parentheses are the number of end-plates studied for m.e.p.c.s or the number of bundles studied for binding measurements.

Muscle	M.e.p.c. amplitude (nA)	α-BuTx binding sites ($\times 10^{-7}$)
1	0·85 ± 0·10 (4)	0·36 ± 0·50 (2)
2	1·20 ± 0·13 (7)	0·30 ± 0·10 (5)
3	0·75 ± 0·11 (5)	0·52 ± 0·25 (8)
4	1·36 ± 0·10 (7)	0·64 ± 0·29 (8)
5	0·70 ± 0·13 (2)	0·22 ± 0·02 (3)

Mean m.e.p.c. = 1·00 ± 0·13 nA; mean binding = 0·41 ± 0·08 × 10^{-7} sites/end-plate.

Amplitude of m.e.p.c.s and the single channel conductance. The mean m.e.p.c. amplitude at myasthenic end-plates is reduced to about one third of the normal value (Table 1). On the other hand, the equilibrium potential for the action of ACh and the conductance of the single channel are similar at the two sites.

For a mean channel conductance at normal end-plates of 22 pS (single channel current = 1·76 × 10^{-12} A, at $V_m = -80$ mV) and a mean m.e.p.c. amplitude of 2·6 nA at clamp potential of −80 mV approximately 1500 channels would be opened by the action of one transmitter packet. This agrees well with previous estimates (Katz & Miledi, 1972; Anderson & Stevens, 1973; Ito *et al.* 1978). At the MG end-plate the mean m.e.p.c. is estimated to be at most 1·0 nA at −80 mV (Table 1), but since some miniature currents are lost in the noise the true mean must be smaller. Therefore, with a mean channel conductance of say 20 pS a single packet of transmitter opens less than 600 post-synaptic channels.

It has been estimated that the number of α-BuTx binding sites at the myasthenic junction may be only 10–50 % of that at the normal junction (Fambrough *et al.* 1973; Green *et al.* 1975) despite the fact that the length of the myasthenic junction is almost twice normal (Cöers & Telerman-Toppet, 1976; Ito *et al.* 1978). Table 1 shows typical mean m.e.p.c. amplitudes and the corresponding numbers of α-bungarotoxin binding sites per end-plate for some of the muscles used in this study. The α-BuTx binding was reduced from control levels of 1·3–1·4 × 10^{-7} sites per end-plate in normal human muscle to 0·41 × 10^7 sites at MG end-plates which means that binding is only 30 % of that at the normal junction. In the same muscles m.e.p.c.s were on average 35–40 % of the control amplitudes.

We have confirmed qualitatively previous observations describing a reduced sensitivity to ionophoretically applied ACh at the MG end-plate (see Albuquerque *et al.* 1976; Cull-Candy *et al.* 1978; Ito *et al.* 1978). This contrasts with the earlier claim

that MG end-plates show apparently normal sensitivity in response to slow bath application of carbachol (Elmqvist *et al.* 1965). It therefore appears that the method of slow bath application, previously used, may not have been sufficient to allow detection of a difference in sensitivity between normal and MG end-plates. The over-all sensitivity to ionophoretically applied ACh at MG end-plates is approximately 3 times less than normal (Albuquerque *et al.* 1976). As we estimate that at the myasthenic end-plate the number of channels opened by a packet of transmitter is reduced to about one third and the number of functional receptors is reduced to a similar amount (see Table 1), then the number of ACh molecules per quantum at myasthenic end-plates is at least as large as normal provided that the affinity of the receptors is not changed. This eliminates the possibility (Elmqvist *et al.* 1964) that the size of the packet of ACh may be reduced at MG end-plates which is also contradicted by biochemical studies showing an increased ACh content of MG human muscle (Ito *et al.* 1976). The possibility that enlarged packages of ACh may be released, from myasthenic nerve terminals, has not been eliminated.

It may be concluded that the properties of ACh-induced channels in myasthenia gravis are virtually unchanged. Thus the reduction in α-BuTx binding sites, in post-synaptic sensitivity and in the mean conductance change in response to a packet of ACh result mainly from a loss of functional receptors rather than a modification of individual ionic channels.

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