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THE ACTION OF THE NEUROMUSCULAR TRANSMITTER ON THE SLOW FIBRE MEMBRANE

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By investigating the effect of varying the membrane potential on the end-plate potential in 'twitch' fibres, Fatt & Katz (1951) and Castillo & Katz (1954) have shown that the neuromuscular transmitter produces a relatively non-selective increase in permeability at the end-plate which drives it towards an equilibrium potential some 10-20 mV below zero. In the present work an attempt has been made to find out whether the same type of process is responsible for the small-nerve junctional potential (s.j.p.) in slow fibres. A preliminary account of the experiments has been published (Burke & Ginsborg, 1955).

Depolarization of the slow fibres does not result in propagated electrical or mechanical responses. The membrane potential may therefore be set at different levels by applying 'rectangular' pulses of current, without the complication of action potentials or twitches. The effect of varying the membrane potential on the s.j.p. may then be investigated by superimposing it on different steady electrotonic potentials.

METHODS

Two microelectrodes were inserted into the same fibre (within 50μ of each other), one for polarizing the membrane and the second for recording. S.j.p.'s were evoked by selectively stimulating the small motor axons which innervate the slow fibres. The experiments were carried out on sciatic-iliofibularis preparations taken from *Rana temporaria* and *R. esculenta*. The details have been described in the preceding paper (Burke & Ginsborg, 1956).

RESULTS

The effect of varying the membrane potential on the s.j.p.

Reversal of the s.j.p. Fig. 1 A illustrates the effect on the s.j.p. of displacing the membrane potential from its resting level. When the membrane is hyperpolarized (*f, g*) the s.j.p. is greater than normal (*e*), when the membrane is depolarized (*d*) the s.j.p. is reduced, and when the depolarization exceeds a

certain level the s.j.p. is reversed (*a, b, c*). The relation between the amplitude of the s.j.p. and the membrane potential is approximately linear (Fig. 2). This experiment therefore shows that the amplitude of the s.j.p. is proportional to the displacement of the membrane potential from a critical level, and its deflexion is always directed towards this level.

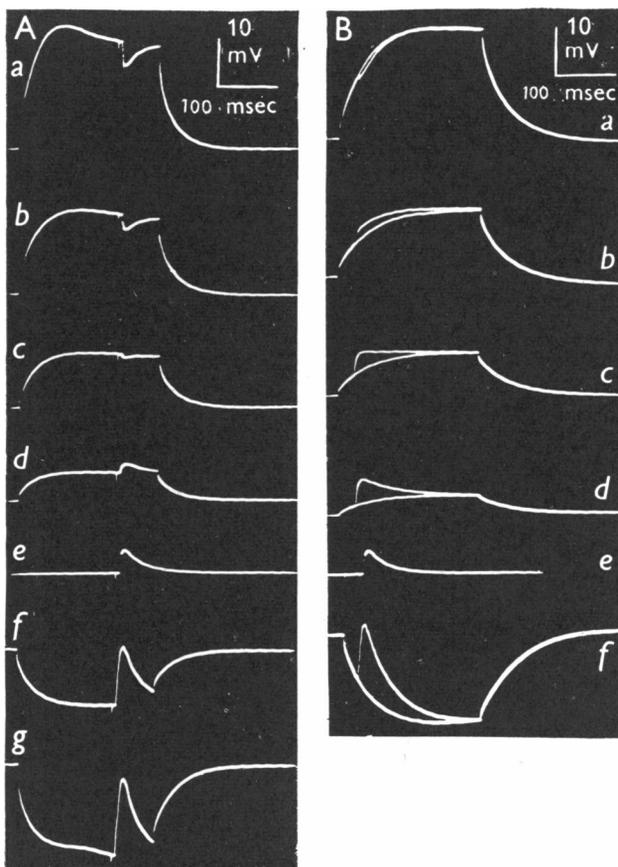


Fig. 1. The effect of changing the membrane potential on the s.j.p. The potential is altered by passing rectangular current pulses through the membrane. Depolarization upwards. In A the s.j.p. is superimposed on the steady part of the electrotonic potential. In B the s.j.p. is superimposed on the rising phase of the electrotonic potential; the second trace is of the electrotonic potential alone. The s.j.p. at the resting potential is shown in (*e*), in both A and B (fibre I).

Evidently, as in the fast fibre (Fatt & Katz, 1951; Castillo & Katz, 1954), the neuromuscular transmitter causes a reduction in the membrane resistance of the junctional regions, and shifts their potential towards a new equilibrium level. In effect, the transmitter places a shunt across the

membrane, in series with a battery of e.m.f. equal to the reversal potential. This reduces the potential difference between the initial level and the reversal level by a constant fraction and so gives rise to the observed relation between the s.j.p. and the membrane potential.

In Fig. 1 B, the reversal of the s.j.p. in the same fibre is shown in a slightly different way. Each of records (a)-(d) and (f) shows two successive traces. One (the lower in (b)-(f), the upper in (a)) is a record of an electrotonic potential, the other shows the potential produced by the same current with an

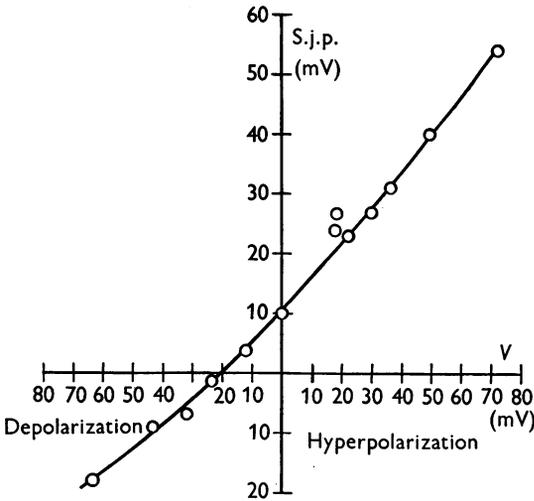


Fig. 2. The relation between the amplitude of the s.j.p. and the membrane potential for the fibre illustrated in Fig. 1. Abscissa (V): displacement of the membrane potential from its resting level (mV); ordinate: amplitude of the s.j.p. (mV).

s.j.p. superimposed on its rising phase. In (a) the effect of the transmitter is to reduce the depolarization produced by the applied current; in (b) the transmitter increases the depolarization. At some potential between the levels at which the transmitter is released in (a) and (b) its effect on the membrane potential is reversed. Within the attainable accuracy of the measurement the reversal level is the same as in Fig. 1 A.

The 'shunting action' of the transmitter is demonstrated directly in (c). The steady level of the electrotonic potential produced by the applied current alone is just below the reversal level. When the transmitter is released on the rising phase the same level is reached more quickly and then maintained. The 'time constant' of the membrane has been momentarily reduced as a result of a transient reduction in its resistance.

The short duration of the action of the transmitter is also shown in (a).

In the lower trace the level at which the transmitter is released is close to the reversal level: the reduced resistance of the membrane, due to the shunting action of the transmitter, prevents the electrotonic potential from increasing for a period equal to the rise time of the s.j.p. When this period is over the electrotonic potential continues to rise with the same time course as obtained in the absence of nerve stimulation. Evidently, as was concluded in the preceding paper, the action of the transmitter on the membrane does not outlast the rising phase of the s.j.p.

The effect of rectification. A peculiarity of the fibre used in the experiment illustrated in Figs. 1 and 2 is that it showed little sign of rectification. As a rule, however, the membrane resistance of the slow fibre increases with hyperpolarization and decreases with depolarization (Burke & Ginsborg, 1956); this must be expected to complicate the relation between the amplitude of the s.j.p. and the steady potential at which the s.j.p. is evoked (cf. Castillo & Katz, 1955). If the 'shunt' placed across the membrane by the action of the transmitter is of fixed size, it should be relatively more effective in reducing the potential difference across the membrane (strictly, the p.d. between the initial and reversal levels), the greater the membrane resistance. The s.j.p. should therefore be a progressively greater fraction of the membrane potential as the membrane is hyperpolarized, and a progressively smaller fraction as the membrane is depolarized. Fig. 2 illustrates this effect to a slight degree only, but in more typical experiments the effect was much larger. Fig. 3 shows the records obtained from a fibre which rectified to a more marked extent (the membrane resistance fell to a quarter of its resting value at a depolarization of 30 mV). The records are similar to those shown in Fig. 1A, but the relation between the s.j.p. and the membrane potential is more markedly non-linear (Fig. 4): with increasing depolarization the s.j.p. is a progressively smaller fraction of the membrane potential.

The reversal potential

Results similar to those illustrated in Figs. 1A and 3 were obtained from ten fibres: when the depolarization exceeded a critical level the s.j.p. was reversed; and the amplitude of the s.j.p. at any other level was approximately proportional to the displacement of the membrane potential from the reversal level. The experimental procedure caused a considerable decline in the resting potential of the fibres (see cols. 2 and 3, Table 1, p. 608), but this did not appear to affect the reversal level (col. 5, Table 1) in any systematic way. In six fibres this level (determined by interpolation) was within 6 mV of zero; in the remaining four it differed from zero by greater amounts (10 mV to -35 mV) (potentials in the direction of the resting potential are regarded as negative). The average value was -7 mV.

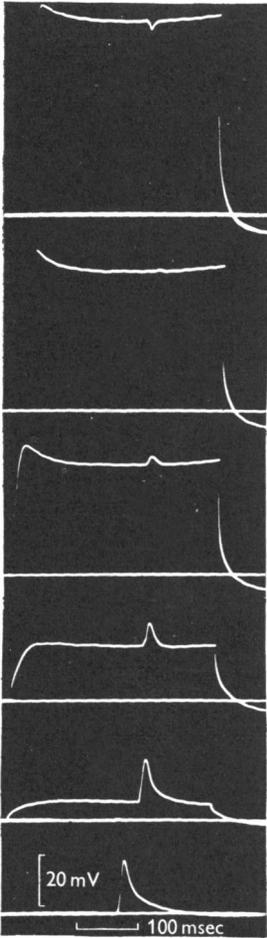


Fig. 3

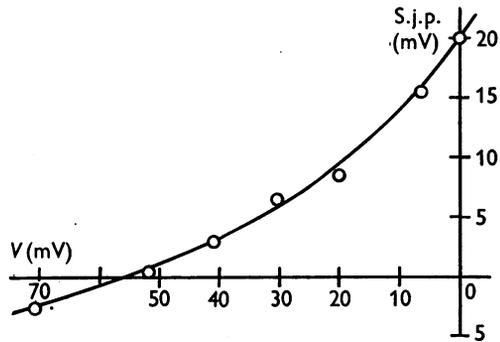


Fig. 4

Fig. 3. The effect of changing the membrane potential on the s.j.p. in a slow fibre showing marked rectification (fibre IV) (see text).

Fig. 4. The relation between the amplitude of the s.j.p. and the membrane potential for the fibre illustrated in Fig. 3. Abscissa: depolarization (mV); ordinate: amplitude of the s.j.p. (mV).

These results are consistent with the idea that the s.j.p. is the result of an increase in permeability at the junctional regions which drives their potential towards an equilibrium level near to zero. The transmitter might, for example, cause a non-selective increase in permeability; the equilibrium level would then be below zero, probably at about -15 to -20 mV (Castillo & Katz, 1954). If the transmitter caused an increase in permeability to sodium ions

alone the equilibrium potential would be above zero. To distinguish between the possible actions of the transmitter it is necessary to determine the value of the equilibrium potential, which is not, as was first thought (Burke & Ginsborg, 1955), identical with the reversal potential in the case of the present experiments.

*The relation between the equilibrium potential and
the reversal potential*

If the action of the transmitter were confined to a single point at which the current and recording electrodes were inserted, or alternatively, if the current pulse produced a uniform change in membrane potential over the length of the fibre, the potential at which the s.j.p. was annulled would be identical with the equilibrium potential. The situation in the fast fibre corresponds to the first alternative, but the slow fibre has a distributed innervation and the neuromuscular transmitter drives the membrane towards the equilibrium potential over the whole length of the fibre. Since the current is applied at a point only, the electrotonic potential falls off with distance from the polarizing electrode. Thus when the depolarization recorded at the site of the current electrode just reaches the equilibrium depolarization the electrotonic potential in regions adjacent to the electrode will be smaller than the equilibrium depolarization and will therefore not be sufficiently large to balance out the contributions towards the s.j.p. from the active junctions in these regions. It is therefore apparent that the reversal depolarization will be greater than the equilibrium depolarization.

Analysis of the effect of the distributed innervation

A. *Experimental model.* To calculate the effect of multiple innervation on the relation between the reversal and equilibrium potentials, some assumption must be made about the distribution of small nerve junctions along the slow fibre. The spatial uniformity of the s.j.p. (Kuffler & Vaughan Williams, 1953; Burke & Ginsborg, 1956) suggests that to a first approximation the junctional regions are sufficiently close together to be regarded as a continuous strip of the membrane. The electrical analogue of the fibre is then a continuous series of elements such as that shown in Fig. 5.

The experiments illustrated in Fig. 1 have been repeated on an artificial line made up of fifty such sections, with the results as shown in Fig. 6. In the model the value of the equilibrium depolarization is, of course, known, and it can be seen that it is lower than the reversal depolarization.

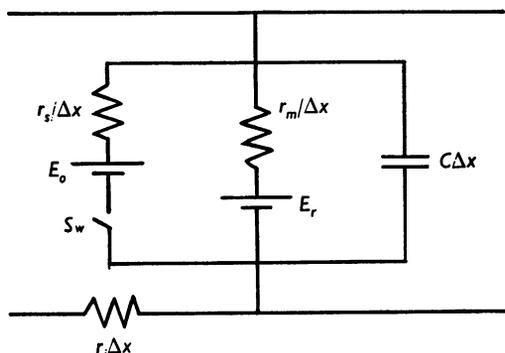


Fig. 5. Element of model of the slow fibre of length Δx . E_r , resting potential; r_m , membrane resistance \times unit length; C , membrane capacity per unit length; r_i , internal resistance per unit length; E_0 , equilibrium potential (to which the small nerve junctional regions are driven by the neuromuscular transmitter); r_s , resistance of 'activated' junctional region of the membrane \times unit length. The action of the transmitter is regarded as closing the switch S_w .

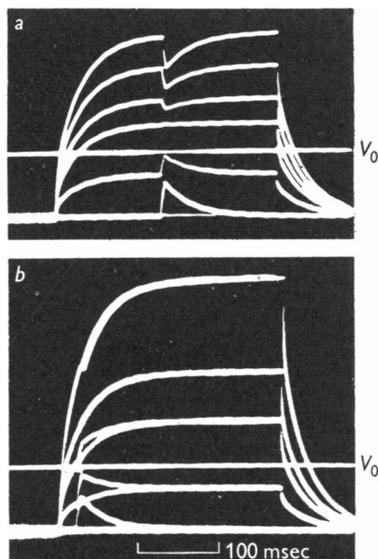


Fig. 6. The effect of electrotonic depolarization on the amplitude of the s.j.p. in an experimental model. The model consisted of fifty consecutive sections such as that shown in Fig. 5. The components were: $r_m = 6 \text{ M}\Omega$, $r_s = 1 \text{ M}\Omega$, $C = 5 \text{ m}\mu\text{F}$, $r_i = 5 \text{ k}\Omega$. The s.j.p. was produced by closing a relay for a period of 5.5 msec. The electrotonic depolarizations were produced by applying constant current pulses across one end of the line and the potential was recorded at the same point. The equilibrium depolarization, V_0 , was recorded directly. The reversal depolarization in (a) is at the level of the trace immediately above V_0 .

B. *Mathematical analysis.* It is convenient to measure potentials from the resting base-line, depolarizations being taken as positive.

Let $V_0 = E_r - E_0$, the equilibrium depolarization.

V_R = the reversal depolarization.

$k = r_m/r_s$.

t = the interval for which the switches, Sw , are closed.

$t_1 = r_m C$, the time constant with Sw open.

$t_2 = \frac{r_m r_s C}{r_m + r_s} = \frac{t_1}{1+k}$, the time constant with Sw closed.

In the absence of an applied current, the amplitude of the s.j.p., V_s , is given by

$$V_s = \frac{k}{k+1} V_0 \left[1 - \exp\left(-\frac{t}{t_2}\right) \right]. \quad (1)$$

V_s is known, and the ratio of the rise time of the s.j.p. to the decay time to $1/e$ gives an estimate for t/t_1 . Taking V_0 equal to V_R as a first approximation, eqn. (1) may be solved numerically for k . In practice, all the results were covered by the ranges: $0.2 < t/t_1 < 0.3$; $0.16 < V_s/V_R < 0.50$; whence $0.1 < k < 10$. (Using a better approximation for V_0 , i.e. $V_0 = 0.7 V_R$, determined subsequently, $1.6 < k < 6$.)

If the line is sufficiently long, when a constant current I is applied to the mid-point, the steady potential V_1 recorded at this point is given by

$$V_1 = \frac{1}{2}(r_m r_i)^{\frac{1}{2}} I. \quad (2)$$

If the switches are now closed at time $t=0$, the amplitude of the superimposed s.j.p. is equal to the change in potential after time t . This change, \bar{V}_s , is given by

$$\bar{V}_s = \frac{k}{k+1} V_0 \left[1 - \exp\left(-\frac{t}{t_2}\right) \right] - V_1 \left[1 - \psi\left(\frac{t}{t_1}\right) \exp\left(-\frac{t}{t_2}\right) - \frac{1}{\sqrt{(k+1)}} \operatorname{erf} \sqrt{\frac{t}{t_2}} \right], \quad (3)$$

where $\psi(x) = e^x \operatorname{erfc} \sqrt{x}$, a function which is tabulated in, for example, Carslaw & Jaeger (1947). We are indebted to Mr L. A. Wigglesworth who kindly derived this expression for us.

Eqn. (3) shows that the relation between the s.j.p. and the steady potential at which it is evoked is linear, the slope being given by the coefficient of V_1 . Now $V_1 = V_R$ when $\bar{V}_s = 0$; V_0/V_R is therefore equal to the ratio of the coefficient of V_1 to that of V_0 . V_0/V_R depends on the two parameters, k and t/t_1 , but is fortunately very insensitive to them. With $0.2 < t/t_1 < 0.3$ and $0.1 < k < 10$, V_0/V_R lies between 0.7 and 0.8. (It can be shown that for all values of k and t/t_1 , $0.5 < V_0/V_R < 1$.)

The theoretical model does not correspond in an exact way to the slow fibre. Probably the most serious discrepancy is that the model does not take

account of the variation in the membrane resistance, r_m , with potential. An attempt to estimate the effect of rectification on V_0/V_R has been made by assuming that r_m changes uniformly over the whole length of the fibre when V_1 is altered, but does not change further during the rising phase of the superimposed s.j.p. Allowing for a maximum reduction in r_m (at V_R) to 0.2 of its resting value, the minimum value of V_0/V_R for the present results was about 0.6. An example of the calculated effect of rectification of this type

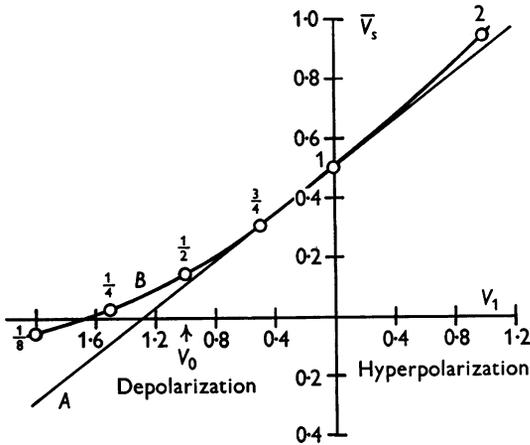


Fig. 7. Theoretical relation between the amplitude of the s.j.p. and the membrane potential. Abscissa (V_1): displacement of membrane potential from the resting level; ordinate \bar{V}_s : amplitude of the s.j.p. Both V_1 and \bar{V}_s are measured as fractions of the equilibrium depolarization, V_0 . A: non-rectifying fibre, $t/t_1 = 0.2$, $k = 4$. B: rectifying fibre; parameters at the resting potential as in A, but the membrane resistance is assumed to change with membrane potential by the factors indicated on the graph at the different points (see text).

on the relation between \bar{V}_s and V_1 is shown in Fig. 7: A would apply to a non-rectifying fibre for which $t/t_1 = 0.2$, $k = 4$; B would apply to a rectifying fibre with the same characteristics as A at the resting potential, but with a membrane resistance which varied with membrane potential in the way indicated in the figure (cf. Figs. 2 and 4).

Numerical values for the equilibrium potential

This analysis shows that the equilibrium depolarization (V_0) probably lies between 0.6 and 0.8 of the reversal depolarization (V_R). The upper value would apply to a non-rectifying fibre with a highly effective transmitter action, and the lower to a fibre showing marked rectification and a poor transmitter action. No attempt has been made to determine the appropriate factor for each experiment, but the probable range for the equilibrium potential (E_0) was determined by subtracting 0.6 V_R and 0.8 V_R from the resting potential. The individual results are shown in Table 1. There is a large scatter

(0 mV to -42 mV), but this is to be expected since the degree of rectification and of transmitter action varied from fibre to fibre. The average value of E_0 lies between -12 mV and -18 mV. The results from fibre VII appear to be anomalously low, and, if these are excluded, at least part of each individual range for E_0 lies between -7 and -21 mV.

TABLE 1. Values for the equilibrium potential. Col. 2 gives the initial resting potential on inserting the recording electrode: col. 3, the resting potential at the time the first reversed s.j.p. was recorded, estimated from the initial and final resting potential and the shift in the base line during the experiment. Col. 4, the depolarization necessary to annul the s.j.p., determined by graphical interpolation as in Figs. 2 and 4. Col. 5, the potential at which the s.j.p. was annulled, col. (3) + col. (4). Col. 6, the range in which the equilibrium potential probably lies: (a), col. (3) + $0.8 \times$ (col. 4). (b), col. (3) + $0.6 \times$ (col. 4).

| Fibre (1) | Initial resting potential (mV) (2) | Reversal resting potential (mV) (3) | V_R (mV) (4) | Reversal potential (mV) (inside- outside) (5) | E_0 (mV) (inside- outside) (6) | | |
|--------------|--|---|----------------------|--|--|--|------------|
| | | | | | (a) | (b) | |
| I | ? | -25 | 20 | -5 | -9 to -13 | -13 to -9 | |
| II | -40 | -31 | 30 | -1 | -7 to -13 | -13 to -7 | |
| III | -60 | -25 | 30 | +5 | -1 to -7 | -7 to -1 | |
| IV* | -56 | { -46 -42 -35 | { 55 56 42 | { +9 +14 +7 | +10 | { -2 to -13 +3 to -8 -1 to -10 | 0 to -10 |
| V* | -64 | { -40 -35 | { 31 23 | { -9 -12 | -10 | { -15 to -21 -17 to -21 | -16 to -21 |
| VI | -65 | -43 | 49 | +6 | | -4 to -14 | |
| VII*† | -60 | { -60 -57 -43 | { 23 21 10 | { -37 -36 -33 | -35 | { -42 to -46 -40 to -44 -35 to -37 | -39 to -42 |
| VIII | -52 | -35 | 17 | -18 | | -21 to -25 | |
| IX | -45 | -28 | 25 | -3 | | -8 to -13 | |
| X | -56 | -42 | 36 | -6 | | -13 to -20 -12 to -18 | |
| | | | | | Mean | -15 | |

* Multiple determinations; one or both electrodes were removed between each series of records. The results have not been given additional weight.

† Some of the small motor nerves were blocked to eliminate a slow movement artifact.

A second quantity of interest which the analysis provides is the additional conductance due to the active junctions ($1/r_s$) relative to the conductance of the resting membrane ($1/r_m$). The average value of r_m/r_s was 3 (varying between 1.5 and 6).

DISCUSSION

The results show that the action of the neuromuscular transmitter drives the junctional regions towards an equilibrium potential, probably between -10 and -20 mV, and produces an additional membrane conductance of about three times the resting conductance. The value of the equilibrium potential

is in agreement with that found for the end-plate in the fast fibre (-10 to -20 mV) by Castillo & Katz (1954) who have discussed its implications in detail. If the ionic concentrations in the slow fibre are similar to those in the fast fibre, the result is compatible with the idea that the transmitter causes: (a) a non-specific increase in permeability to all the diffusible ions on either side of the membrane, or (b) a specific increase to Na and either K or Cl ions. The result is not compatible with the view that the transmitter increases the permeability to Na ions alone.

Taken in conjunction with the absence of a regenerative response, the results explain why tetanic stimulation of the small motor nerves is unable to depolarize the slow fibre beyond about half its resting potential (Kuffler & Vaughan Williams, 1953). Since the equilibrium level is at about -15 mV, the maximum theoretical depolarization that nerve stimulation could produce in a fibre with a resting potential of -60 mV is 45 mV. However, this depolarization could be attained only if the resistance of the active junctional regions fell to zero; in practice the maximum depolarization must be smaller.

SUMMARY

1. The action of the neuromuscular transmitter on the slow fibre membrane has been studied by investigating the effect on the small-nerve junctional potential (s.j.p.) of displacing the membrane potential. Intracellular electrodes were used for polarizing the membrane and recording.

2. When the membrane was depolarized to a particular level, the 'reversal' level, the s.j.p. was annulled. At any other level the amplitude of the s.j.p. was proportional to the displacement from the reversal level, and its deflexion was directed towards this level. This result shows that the transmitter reduces the resistance of the junctional regions of the membrane and drives them towards a new equilibrium potential.

3. A theoretical and an experimental analysis of a model of the slow fibre membrane show that in this particular case the reversal potential and the equilibrium potential are not identical. An approximate relation between them is derived.

4. The average value of the equilibrium potential (in ten fibres) was -15 mV. This value is intermediate between the equilibrium potentials corresponding to a 'K- (or Cl-) selective' and a 'Na-selective' membrane, and therefore indicates that the s.j.p. is the result of an increase in permeability of the junctional regions of the membrane to more than one ionic species.

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