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NON-LINEAR SUMMATION OF END-PLATE POTENTIALS IN THE FROG AND MOUSE

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SUMMARY

1. End-plate potentials (e.p.p.s) and end-plate currents (e.p.c.s) were recorded intracellularly from muscle fibres of frog and mouse at various levels of curarization to determine the relation between the potential change and the underlying synaptic conductance change over a wide range of e.p.p. amplitudes.

2. In frog muscle fibres the e.p.p.–e.p.c. relation was linear for e.p.p. amplitudes up to about 10 mV, beyond which the rate of increase of e.p.p. amplitude became progressively smaller as the e.p.c. amplitude increased. The equation proposed by Martin (1955) to correct for this non-linearity consistently over-corrected the e.p.p. amplitudes.

3. When synaptic potentials and currents of long duration were produced by ionophoresis of ACh onto the end-plate, the voltage–current relation showed greater non-linearity than with nerve-evoked responses, and correction of the synaptic potential amplitudes resulted in a linear relation.

4. The relation between e.p.p. and e.p.c. amplitudes in mouse muscle showed a greater non-linearity than in frog muscle and over-correction by the equation was correspondingly smaller.

5. Theoretical voltage–current relations were calculated for various membrane models and compared with the relations observed experimentally. The results from mouse muscle agreed with those expected for a point synaptic contact on an infinite cable; those from frog muscle were consistent with a simple resistive-capacitative model with no cable extending from the synaptic region.

6. The applicability to the experimental results of several correction factors for non-linear summation is discussed.

INTRODUCTION

In electrophysiological studies of synaptic transmission, the mean number of quanta (m) of transmitter released by a presynaptic stimulus is often obtained by dividing the mean amplitude of the post-synaptic response ($\bar{v}$) by the mean amplitude of the single quantal response ($\bar{v}_1$); i.e. $m = \bar{v}/\bar{v}_1$ (delCastillo & Katz, 1954*a*). For

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large quantal releases this method underestimates m because of non-linear summation of the quantal units. The non-linearity arises because the effect of each quantum is to produce a fixed increment of leakage conductance, rather than a fixed increment of potential, across the post-synaptic membrane (Fatt & Katz, 1951; delCastillo & Katz, 1954*b*). It follows that in a multiquantal response the increment of potential contributed by each additional quantum becomes less and less as the total number of quanta (i.e. the total existing leakage) increases. A correction for this effect was proposed by Martin (1955), based on a model of the post-synaptic membrane at the frog neuromuscular junction in which the input resistance of the muscle fibre was shunted by a variable synaptic conductance. Using this model, the 'corrected' mean quantum content was given by

$$m = \bar{v}/\bar{v}_1(1-\bar{v}/E),$$

where E is the difference between the resting membrane potential and the reversal potential for the end-plate response. The equation may be rewritten

$$\bar{v}' = m\bar{v}_1 = \bar{v}/(1-\bar{v}/E), \tag{1}$$

where $\bar{v}'$ is the synaptic potential amplitude expected if the units summed linearly. If the correction were accurate, $\bar{v}'$ would then be proportional to the peak increase in conductance of the post-junctional membrane during the action of the transmitter.

One deficiency of this model was that membrane capacitance was neglected. As noted by Martin (1955), this is of no consequence if the duration of transmitter action is sufficiently long for the synaptic potential to reach a steady-state (i.e. for the capacitance to become fully charged to the new level determined by the conductance change). However, for durations shorter than this another factor is introduced, namely the reduction in the time constant of the membrane by the added synaptic conductance. During a small response the membrane will be discharged toward its steady-state level at a rate only slightly greater than that determined by the resting membrane time constant. During a large response, however, the discharge will be relatively more rapid because of the reduced membrane time constant. Consequently, during a brief application of transmitter the amplitude of the larger response will be closer to its steady-state level than that of the smaller response. The effect of membrane capacitance, then, is to compensate at least partially for the non-linearity expected in a purely resistive model. Because of this effect, use of eqn. (1) can be expected to lead to over correction of synaptic potential amplitudes.

Numerous more recent theoretical treatments of this problem (Auerbach, 1972; Adams, 1976; Bennett, Florin & Pettigrew, 1976; Edwards, Hirst & Silinsky, 1976; Martin, 1976; Stevens, 1976) suffer from two major limitations: first, most of them apply only to simple resistive-capacitative models and not to cable-like structures such as muscle fibres; secondly, the models do not take into account other factors, such as spatial dispersion of the motor end-plate in frog muscle (Couteaux, 1955) or possible potential gradients in synaptic clefts which might introduce other non-linearities (Hubbard, Llinàs & Quastel, 1969). In view of these limitations, the present experiments were undertaken to determine directly the extent of non-linear summation at the neuromuscular junction by comparing end-plate potential (e.p.p.) amplitudes with the amplitudes of corresponding end-plate currents (e.p.c.s) obtained

while voltage clamping the end-plate region (Takeuchi & Takeuchi, 1959). Under voltage clamp conditions, the e.p.c. provides a direct measure of the magnitude and time course of the underlying synaptic conductance change. The results of two individual experiments of this nature published previously by Takeuchi & Takeuchi (1960) suggested that no correction for non-linearity was required for e.p.p. amplitudes up to about 15 mV in frog muscle. A more recent experimental approach to the problem by Miyamoto (1978) led to a similar conclusion but relied on indirect measurement of non-linear summation which depended on assumptions about the time course of facilitation of transmitter release.

METHODS

End-plate responses to nerve stimulation were examined in muscles from both frogs and mice, bathed in solutions containing (+)-tubocurarine chloride, 0·6–13 μM. In addition, in frog muscle, responses were produced by ionophoretic application of acetylcholine (ACh) to the end-plate regions of individual fibres (Nastuk, 1953; delCastillo & Katz, 1955). Frog muscles were obtained from *Rana temporaria*. The m.ext.l.dig.IV was used for most experiments involving nerve stimulation; sartorius muscle was used for a few such experiments and for all experiments involving ionophoresis. Muscles were mounted in a lucite chamber perfused at room temperature (18–22 °C) with saline of the following composition (mM): NaCl, 120; KCl, 2·0; $CaCl_2$, 6·0–8·0; Tris-HCl, 5·0; adjusted to pH 7·4. In the experiments on mammalian muscle, the dorsal halves of hemidiaphragms from young adult female mice were mounted in a small chamber (1·5 ml. volume) within a 35 mm tissue culture dish and perfused at room temperature (20–24 °C) with a modified Krebs solution containing (mM): NaCl, 133; KCl, 4·7; $CaCl_2$, 7·2; $MgCl_2$, 1·2; NaH_2PO_4, 1·3; $NaHCO_3$, 16·3; glucose, 7·8; equilibrated with 95% O_2–5% CO_2 at pH 7·4. Many of the intramuscular nerve branches in the diaphragm were cut in order to reduce the number of innervated muscle fibres; this helped reduce both movement of the muscle at low levels of curarization and distortion of e.p.p.s by extracellular field potentials from neighbouring fibres.

Conventional techniques were used for nerve stimulation and for intracellular recording of e.p.p.s with glass micropipettes. The latter were filled with 3 M-KAc and had resistances between 10 and 20 MΩ for recording from frog muscle and 30 and 40 MΩ for mouse muscle. In frog fibres, end-plates were localized by repeated insertions of the pipettes to obtain maximum amplitude and minimum time to peak of the e.p.p. or (in the ionophoresis experiments) of miniature e.p.p.s. In mouse fibres, which were often damaged by repeated impalements, electrode insertions were made close to terminal nerve branches, seen in an inverted compound microscope.

For voltage clamping, a current passing electrode of 5–15 MΩ resistance was inserted closer than 50 μm to the voltage recording electrode. The shaft of the current electrode was surrounded by an earthed metal shield and the fluid depth around its tip kept at a minimum, the fluid surface usually being covered with paraffin oil. The clamping amplifier had d.c. gain of 10^4, falling to about $3{\cdot}5 \times 10^3$ at 500 Hz. The theoretical clamping efficiency at 250 Hz was greater than 95% and, experimentally, small unclamped signals on the voltage record during the rising phase of the e.p.c. never exceeded 3% of the corresponding e.p.p. amplitude and terminated before the peak of the e.p.c. E.p.c.s were monitored by a current-to-voltage converter between the bath and earth.

For ionophoresis, micropipettes of about 30 MΩ resistance were filled with 3 M-ACh. ACh potentials were deliberately prolonged (rise time 150–200 msec) by backing the pipette away from a focal position and using long (5–50 msec) ionophoretic pulses. Steady-state depolarizations and desensitization were avoided by applying braking currents of 3–7 nA to the pipettes.

Input resistances and time constants of muscle fibres were measured in the usual way by passing rectangular current pulses through the intracellular current electrode and recording the resulting voltage changes (Fatt & Katz, 1951). All records were displayed on an oscilloscope and photographed for subsequent measurement.

Fibres in frog muscles were used only if their resting membrane potentials were 80 mV or

larger after insertion of both intracellular pipettes; mouse muscle fibres were required to have resting potentials of at least 60 mV. However, mouse fibres were routinely hyperpolarized to at least 80 mV by applied d.c. currents (usually less than 20 nA) for the duration of the experiment in order to increase the range of e.p.p. amplitudes which could be examined without reaching threshold for action potential initiation.

When the end-plates were adequately clamped, e.p.c.s produced by nerve stimulation reached a peak in 0·8 msec or less and decayed exponentially with a time constant of 0·7–1·2 msec in frog fibres. In mouse fibres, currents reached a peak in 0·6 msec or less and decayed with a time constant of 0·5–0·9 msec. All records in which the decay was not exponential, having either a long 'tail' or, conversely, a rapid decline followed by an overshoot were taken as signs of inadequate clamping of the end-plate region and such records were rejected. The adequacy of this criterion was examined in frog muscle fibres by recording membrane potential along the clamped region with a third intracellular pipette. When the decay of the e.p.c. was exponential, no unclamped voltage signals could be detected at any distance up to 300 μm from the point of clamping other than small capacitative transients due to interelectrode coupling which occurred whether the exploring electrode was inside or outside the fibre. Even small deviations from exponential decay of the e.p.c. were associated with loss of voltage control within 100 μm of the clamping. It was concluded that the requirement of exponential current decay was an adequate criterion to ensure proper voltage control of the end-plate region during the clamp.

For histological examination muscles of both types were fixed briefly and stained for cholinesterase (Karnovsky & Roots, 1964). End-plate length and muscle fibre diameter were measured from individual fibres after teasing in glycerol.

RESULTS

Frog muscle

After both voltage and current electrodes were in place, recorded membrane potentials in frog muscle fibres ranged from 80 to 105 mV, remaining relatively constant ($\pm$ 3 mV) in any given fibre during the period of recording. Input resistances varied from one fibre to the next in the range of 0·35–1·4 MΩ and input time constants were between 15 and 30 msec. The procedure used to obtain e.p.p.–e.p.c. relations was to adjust the level of curarization until the majority of fibres in the muscle had just ceased to twitch. An end-plate with a large response was located and a number (usually 10 or more) of paired voltage and current records obtained. Additional curare was then added to the perfusion fluid to reduce the e.p.p. amplitude to a few mV over the next 10–20 min. As the amplitude decreased several series of paired e.p.p.–e.p.c. records were taken. The curare was then washed from the bath and recording continued during the subsequent growth of the response until the muscle twitched.

Typical records from one such experiment are shown in Fig. 1*A*, and the corresponding relation between e.p.p. and e.p.c. amplitudes are plotted in Fig. 1*B*. The relation appeared to be linear for e.p.p.s up to 10–15 mV in amplitude, confirming the previous observation of Takeuchi & Takeuchi (1960). The interrupted line was drawn from the origin through the group mean of all e.p.p. amplitudes up to 10% of the resting potential and the corresponding e.p.c. mean, and represents the slope of the initial portion of the e.p.p.–e.p.c. relation. E.p.p. amplitudes greater than 10–15 mV fell progressively farther below the line as the amplitudes increased, as would be expected if the quantal units making up the responses summed non-linearly. When eqn. (1) was applied to the e.p.p. amplitudes the corrected points (open circles) fell above the line, rather than on it, and the corrected e.p.p.–e.p.c.

relation was concave upward, rather than linear as desired. Thus use of eqn. (1) resulted in marked over-correction for non-linear summation. In applying the correction, the reversal potential was assumed to be near zero (personal observations; cf. Mallart, Dreyer & Peper, 1976); i.e. E was taken as equal to the resting potential. If -15 mV had been used for the reversal potential (Takeuchi & Takeuchi, 1960), even greater over-correction would have resulted.

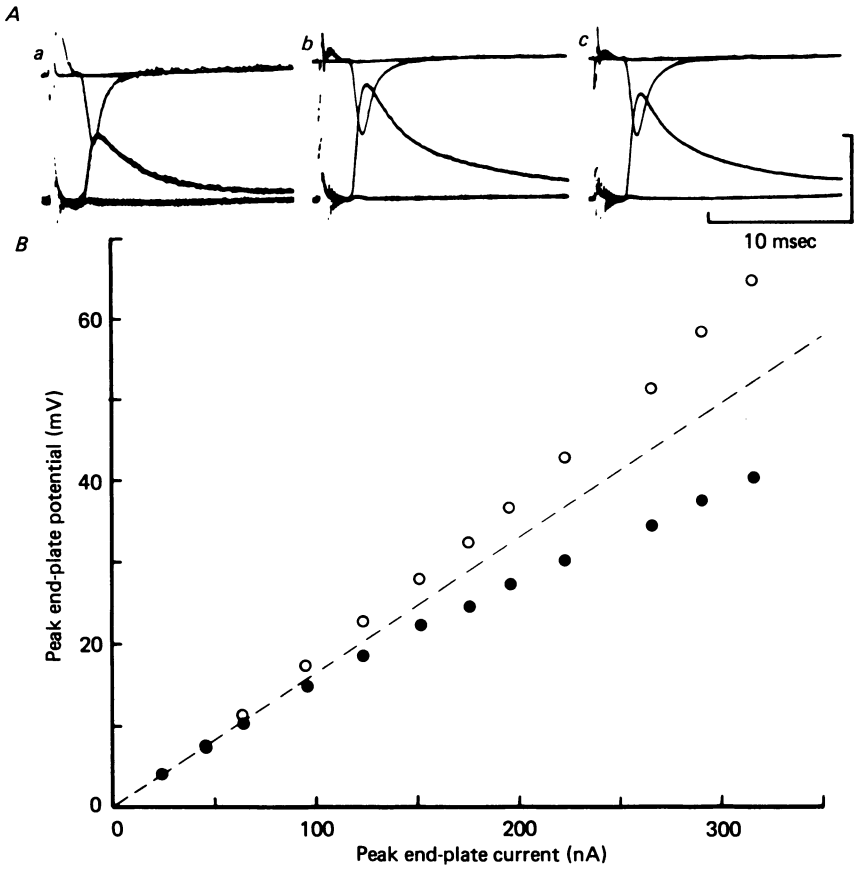

Fig. 1. A, e.p.c.s (upper traces) and e.p.p.s (lower traces) recorded from a frog muscle fibre at various levels of curarization. Vertical calibration: 6 mV, 30 nA (a); 15 mV, 150 nA (b); 30 mV, 300 nA (c). B, relation between e.p.p. and e.p.c. amplitudes (filled circles). Each point is mean of 16 or more paired responses. Interrupted line indicates initial slope of relation. When e.p.p. amplitudes are corrected by eqn. (1) (open circles) points lie above the line. Resting potential 100 mV, input resistance 0·72 MΩ, membrane time constant 17 msec.

Results from two additional cells are shown in Fig. 2A, which illustrates in greater detail the initial part of the voltage current relation. It can be seen that there is little or no deviation from linearity for e.p.p.s up to about 10 mV in amplitude or, in other words, to 10% or more of the resting potential. Consequently, the regression line from the origin through the average of the experimental points corresponding to e.p.p.s up to 10% limit provides a reasonable estimate of the initial slope of the relation. To allow grouping of results from different fibres, e.p.p. amplitudes were

normalized by expressing them as fractions of the driving potential; i.e. as v/E. The regression line was then used to determine the e.p.c. amplitude, i_0, corresponding to $v/E = 0{\cdot}05$ and all e.p.c. amplitudes expressed as i/i_0. The results from six experiments, normalized in this way, are shown in Fig. 2*B*. As with the three individual experiments, the e.p.p.–e.p.c. relation began to deviate from linearity when e.p.p.

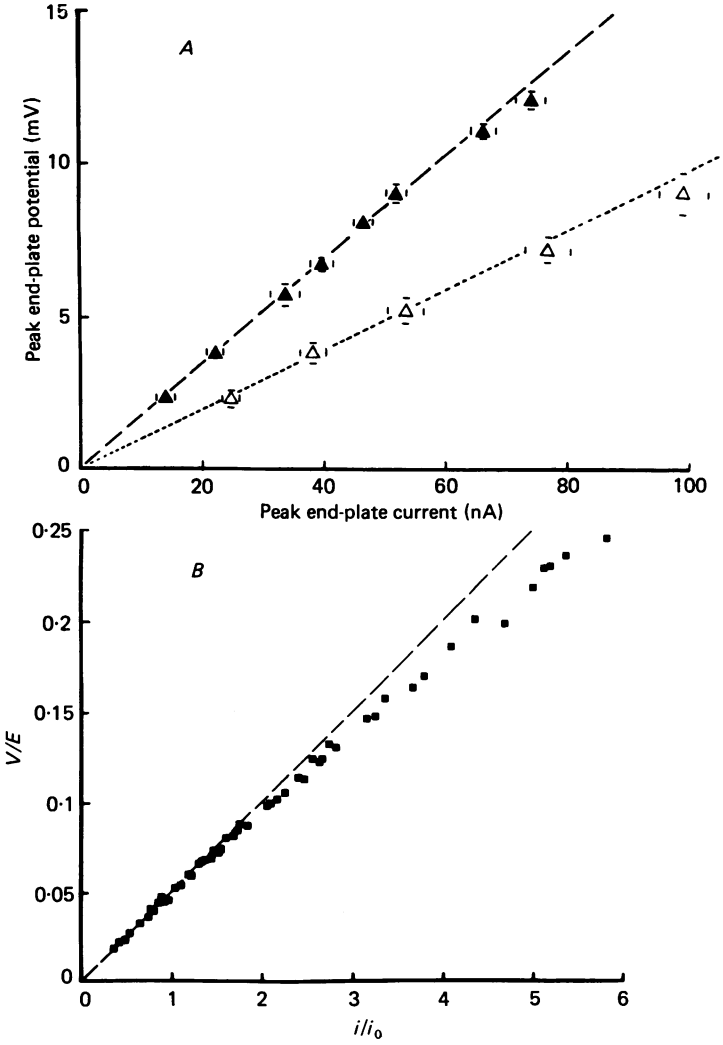

Fig. 2. *A*, initial e.p.p.–e.p.c. relation from two frog muscle fibres. Each point is mean of twelve or more measurements, bars indicate ± 1 S.D. Filled triangles: resting potential 97 mV, input resistance 0·71 MΩ, time constant 22 msec. Open triangles: resting potential 80 mV, input resistance 0·88 MΩ, time constant 30 msec. Lines are drawn from the origin through the mean of all points with e.p.p. amplitudes less than 10% of the resting potential to indicate slope of the relation. *B*, pooled results from seven experiments, Ordinate: e.p.p. amplitude (v) as a fraction of the resting potential (E). Abscissa: e.p.c. amplitude as a fraction or multiple of the peak current (i_0) corresponding to $v/E = 0{\cdot}05$. In each experiments i_0 was taken from the regression line defining the initial slope, as shown in *A*.

amplitudes exceeded 10–15 % of the resting potential. However, the deviation was much less than expected from eqn. (1); e.p.p.s reaching 20 % of the resting potential required an increase of about 10 % to restore linearity, or less than half the correction (25 %) given by the equation.

Effect of anticholinesterase. It was expected that prolongation of the e.p.c. might increase the non-linearity of the relation between potential and current because the increased duration of transmitter action would allow the membrane capacitance to be more fully discharged (see Introduction). To test this idea further experiments were done with neostigmine added to the bathing solution. Records from one such experiment are shown in Fig. 3*A* and the corresponding voltage–current relation in Fig. 3*B*. Although neostigmine more than doubled the time to peak of the e.p.c. and

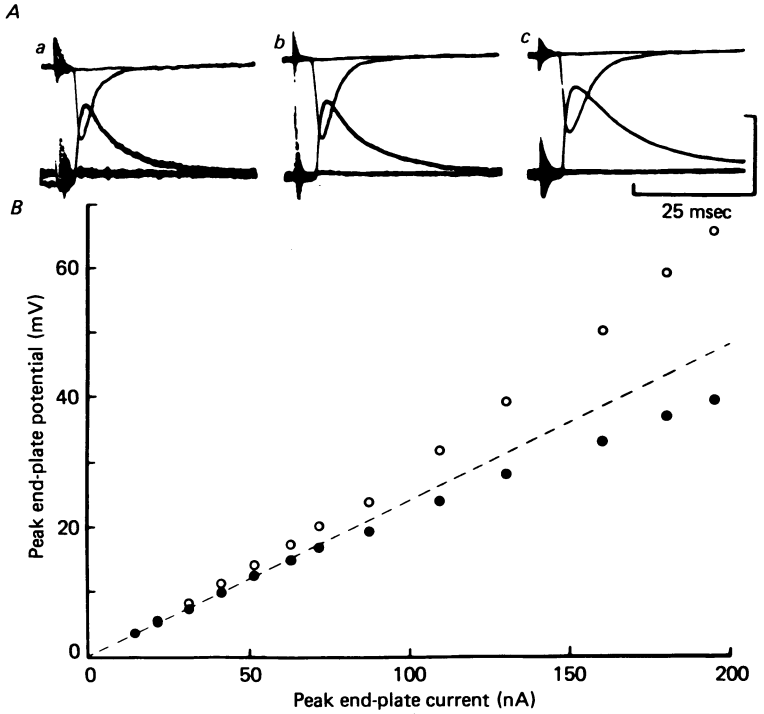

Fig. 3. E.p.p.–e.p.c. relation in presence of neostigmine (3·3 μM). *A*, records obtained at various levels of curarization as in Fig. 1. Vertical calibration: 3 mV, 15 nA (*a*); 15 mV, 60 nA (*b*); 30 mV, 150 nA (*c*). Note increase in duration of both e.p.p. and e.p.c. with increasing amplitude. *B*, plot of experimental results before (filled circles) and after (open circles) correction of e.p.p. amplitudes by eqn. (1). Interrupted line is initial slope of uncorrected relation. Resting potential 98 mV, input resistance 0·65 MΩ, time constant 22 msec.

increased its time constant of decay by a factor of about 3, the relation between e.p.p. and e.p.c. was linear over a greater range than in normal solution. Similar results, contrary to those expected theoretically, were obtained in four other experiments. The increase in linearity, rather than the decrease expected, was due to the fact that the duration of the e.p.c. was not constant throughout the amplitude range. For example, in the experiment illustrated in Fig. 3, the smallest e.p.c.s rose to a peak in about 1·3 msec and decayed with a time constant of 2·5 msec. When the level of

curarization was reduced, the largest responses were further prolonged, rising to peak in 1·7 msec and decaying with a time constant of 4·3 msec. This effect of curare on e.p.c. duration in preparations treated with anticholinesterase has been observed previously (Katz & Miledi, 1973; Magleby & Terrar, 1975) and accounts for the apparent increase in the range over which the voltage–current relation was linear when compared with the experiments in control solutions where no such changes in time course occurred. Because the e.p.c. duration was not constant the experiments in neostigmine were not included in the subsequent analysis.

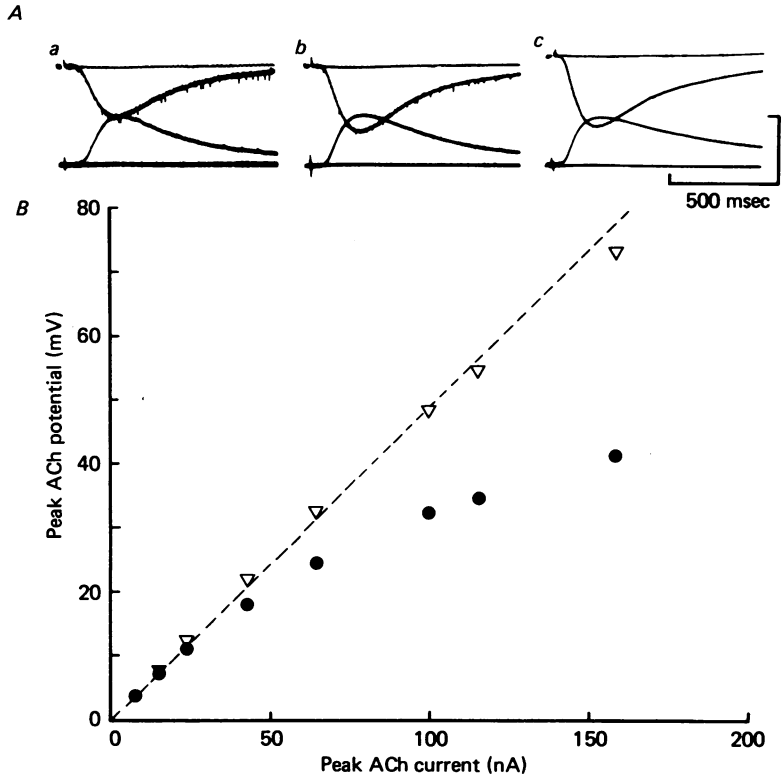

Fig. 4. *A*, end-plate currents (upper traces) and potentials (lower traces) produced by ionophoresis of increasing amounts of ACh onto the end-plate. Vertical calibration: 15 mV, 30 nA (*a*); 30 mV, 60 nA (*b*); 60 mV, 150 nA (*c*). *B*, voltage–current relation. Experimental points (filled circles) represent means of nine or more measurements. When e.p.p. amplitudes are corrected by eqn. (1) (triangles), points fall on straight line corresponding to the initial slope of the relation. Resting potential 95 mV, input resistance 0·61 MΩ, time constant 30 msec.

ACh potentials. To determine whether eqn. (1) was applicable to end-plate responses of very long duration, voltage–current relations were obtained for ACh potentials produced by ionophoresis of the drug onto the end-plate. ACh potentials and currents are shown in Fig. 4, together with the corresponding relation between voltage and current. As illustrated in Fig. 4*B* (filled circles), there was much greater non-linearity than obtained with nerve-evoked responses. Correction of the ACh potential amplitudes by eqn. (1) (open triangles) resulted in a reasonable linear relation. Similar results were obtained in two other experiments, indicating that the

correction factor given by eqn. (1) is suitable for post-junctional conductance changes of long duration.

Mouse muscle

Resting potentials in mouse muscle ranged from 60 to 85 mV with both electrodes in place. Input resistances were between 0·8 and 2·5 MΩ and input time constants between 1·8 and 6·0 msec. In order to obtain a wider range of e.p.p. amplitudes, fibres were routinely hyperpolarized to 80 mV or greater during the duration of an experiment. The procedure followed to obtain the e.p.p.–e.p.c. relation was the same as with frog muscle.

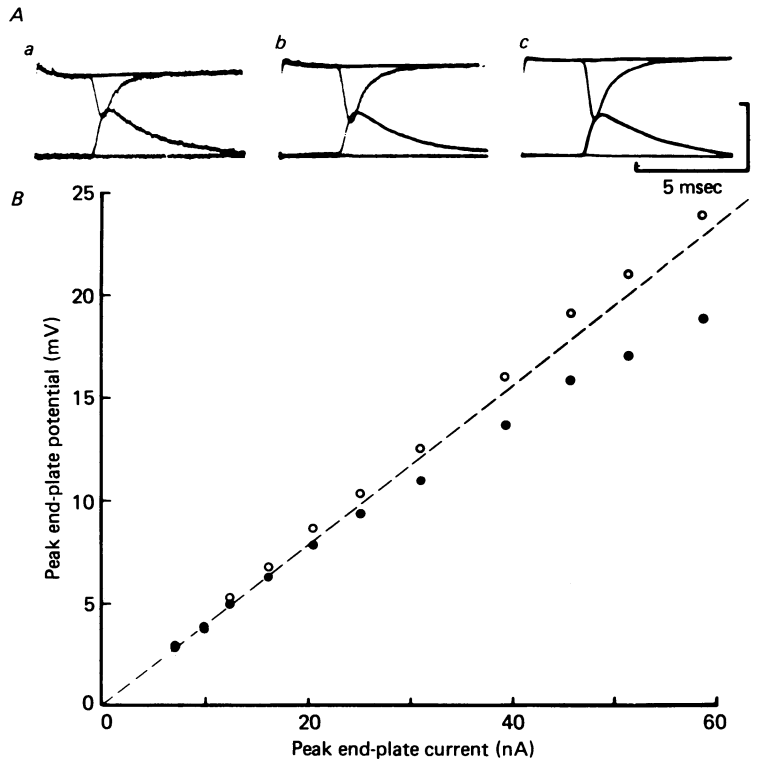

Fig. 5. *A*, e.p.c.s (upper traces) and e.p.p.s (lower traces) recorded from a mouse fibre at various levels of curarization. Vertical calibration: 6 mV, 15 nA (*a*); 15 mV, 30 nA (*b*); 30 mV, 60 nA (*c*). *B*, relation between e.p.p. and e.p.c. amplitudes (filled circles). Each point is mean of seventeen or more paired responses. Interrupted line indicates initial slope of relation. Over-correction of e.p.p. amplitudes by eqn. (1) (open circles) is less severe than with frog muscle (cf. Fig. 1). Membrane potential held at 90 mV input resistance 1·1 MΩ, time constant 3·5 msec.

Typical records from the mouse diaphragm are shown in Fig. 5*A* and results from the same experiment plotted in Fig. 5*B*. It was apparent in this and five similar experiments that deviation from linearity occurred at relatively smaller e.p.p. amplitudes than in frog fibres. Consequently the initial slope of the relation was determined by drawing a line from the origin through the group mean of all e.p.p.s of amplitude up to 5% of the resting potential (rather than 10% used previously) and the corresponding e.p.c. mean. Application of eqn. (1), using a reversal potential of zero (Linder & Quastel, 1978), again resulted in over-correction but to a lesser

extent than at the frog junction (cf. Fig. 1). The pooled results from six fibres are shown in Fig. 6, with same normalization procedure as before, but using e.p.p. amplitudes up to only 5% of the resting potential to determine the initial slope of the voltage current relation (Fig. 6*A*). Deviation from linearity with increased e.p.p. amplitude (Fig. 6*B*) was clearly greater than in frog fibres; e.p.p.s reaching 20% of the resting potential would have to be increased by about 18% to restore linearity, as compared with the 10% increase required in frog muscle. Thus the over-correction by eqn. (1) was less severe.

Histology

End-plates in both frog and mouse muscles were stained for cholinesterase in order to determine the longitudinal extent of the end-plate regions. In forty-three fibres from one frog muscle, end-plates extended for distances ranging between 180 and 570 μm with a mean ($\pm$ S.D.) of 380 ± 110 μm. End-plate lengths were correlated roughly with fibre diameters, which were between 24 and 60 μm with a mean of 36 ± 7 μm. Thus the ratio of end-plate length to fibre diameter was about 11. In seven other frog muscles the ratio was always greater than 9·5. In five mouse muscles the ratio of end-plate length to fibre diameter ranged between 1·3 and 1·7. In thirty-one fibres from one muscle the mean fibre diameter was 21 ± 4 μm, with values ranging between 13 and 39 μm. End-plates were between 15 and 60 μm in length, averaging 34 ± 9 μm.

Theory

The correction factor represented by eqn. (1) was derived from a model of the muscle fibre membrane equivalent to that illustrated in Fig. 7*A*. The input conductance of the fibre (G) is shunted by the synaptic conductance (g) when the switch is closed. For convenience the resting membrane potential is represented as being zero and the synaptic driving potential represented by the battery, E. When the switch is closed a synaptic potential of amplitude v will appear across the membrane, given by

$$v = Eg/(G+g). \tag{2}$$

When the cell is clamped at the resting potential, the synaptic current (i) is simply

$$i = Eg. \tag{3}$$

The relation between the voltage and current given by this model is shown in Fig. 8 by the curve labelled 'DC'. The abscissa and ordinate have been normalized as in Figs. 2 and 6. When points on this curve are corrected by applying eqn. (1) to the synaptic potential amplitudes, the corrected points lie on the broken line indicating a linear relation between voltage and current and hence between synaptic potential amplitude and synaptic conductance. In other words, if the model were appropriate eqn. (1) would provide the desired correction.

The model shown in Fig. 7*B* is equivalent to that used by Martin (1976) to derive a revised correction for non-linear summation taking the cell membrane capacitance, C, into consideration. The amplitude of the synaptic potential now depends on the duration (Δt) of the synaptic conductance change relative to the membrane time constant ($\tau = C/G$) and is given by:

$$v = \frac{Eg}{G+g}\left\{1 - \exp\left[-T(1+g/G)\right]\right\}, \tag{4}$$

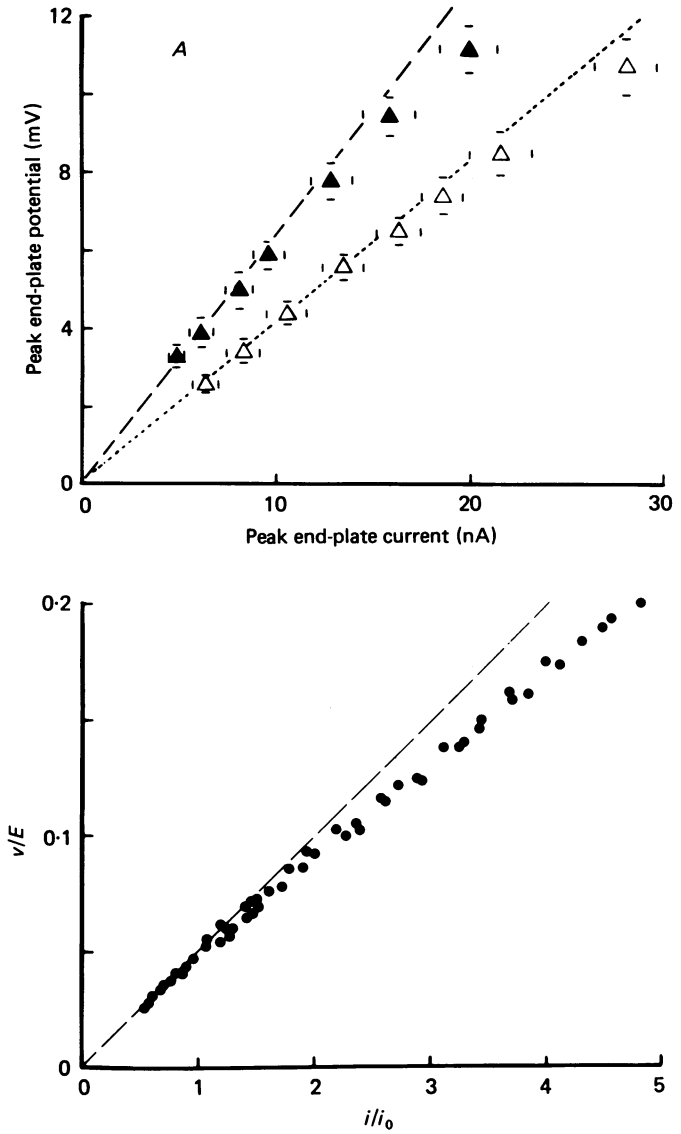

Fig. 6. *A*, initial e.p.p.–e.p.c. relation from two mouse fibres. Each point is mean of ten or more measurements, bars indicate ± 1 S.D. Filled triangles: input resistance 1·1 MΩ, time constant 2·5 msec. Open triangles: input resistance 1·6 MΩ, time constant 3·6 msec. Membrane potential in both experiments held at 90 mV. Lines are drawn from the origin through the mean of all points with e.p.p. amplitudes less than 5% of the resting potential to indicate initial slope of the relation. *B*, pooled results from 6 experiments. Ordinate: e.p.p. amplitude (v) as fraction of the resting potential (E). Abscissa: e.p.c. amplitude as a fraction or multiple of the peak current (i_0) corresponding to $v/E = 0{\cdot}05$. In each experiment i_0 was taken from the regression line defining the initial slope, as shown in *A*.

where $T = \Delta t/\tau$. As before, the synaptic current when the cell is clamped at the resting potential is given by eqn. (3). When the duration of transmitter action is sufficiently long for the membrane capacitance to become fully charged the relation between synaptic potential and synaptic current is the same as for the DC model. Thus the DC curve in Fig. 8 represents a lower limit for the voltage–current relation given by this model. When Δt is very short the current–voltage relation approaches an upper limit shown by the curve labelled 'RC lim' in Fig. 8. Details of the correction factor arising from this model are discussed by Martin (1976). The model and the associated correction factor are presumably appropriate for nerve cells without extensive processes, but not applicable to a restricted synaptic contact on an extended cable-like cell such as a muscle fibre. On the other hand, the model might be appropriate for frog muscle fibres in which the synaptic contact extends over a considerable length of the cable.

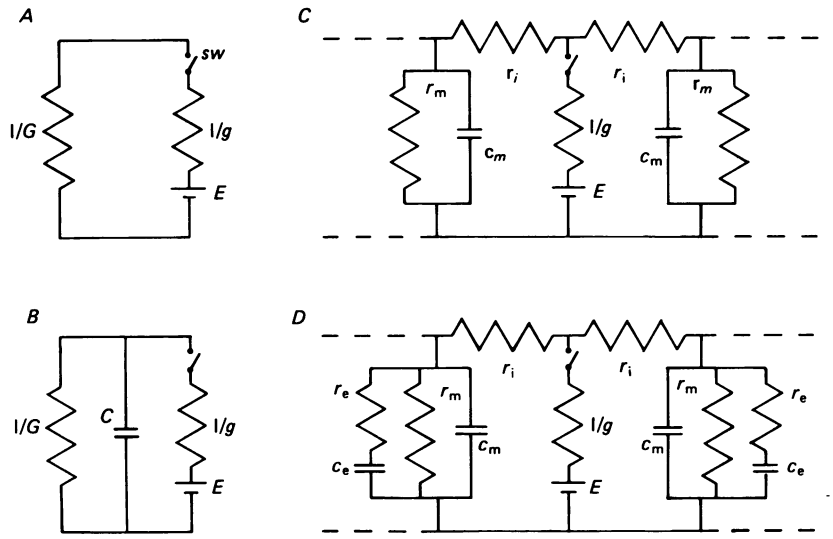

Fig. 7. Electrical models of synapse. *A*, 'DC' model on which eqn. (1) is based. Resting membrane conductance, G, is shunted by synaptic conductance, g, in series with driving potential, E. Switch (*sw*) is closed for time Δt. *B*, 'RC' model, with added membrane capacitance, C. *C*, cable model, with internal longitudinal resistance r_i and transverse membrane resistance and capacitance per unit length r_m and c_m. *D*, dual time constant cable with additional transverse elements r_e and c_e. Input conductance of cables is $G = 2/\sqrt{r_m r_i}$.

The remaining models in Fig. 7 represent muscle fibres in which synaptic contact is made at a single point near their middle. Fig. 7*C* shows a conventional cable model with internal longitudinal resistance r_i, and transverse membrane resistance and capacitance r_m and c_m respectively. The cable is assumed to extend for a large distance in both directions from the synaptic contact. Fig. 7*D* represents a dual time-constant cable (Falk & Fatt, 1964), with additional transverse elements r_e and c_e. For the conventional cable, the synaptic potential amplitude is given by

$$v = \frac{E}{1-(G/g)^2}\left\{1-\frac{G}{g}\operatorname{erf}\sqrt{T}-\exp\left[-T(1-g^2/G^2)\right]\operatorname{erf} c(g\sqrt{T/G})\right\}, \qquad (5)$$

where $G = 2/\sqrt{(r_m r_i)}$ and $T = \Delta t/r_m c_m$ (see Jack, Noble & Tsien, 1975). When the cable is clamped at the point of synaptic contact, the synaptic current is again given by eqn. (3). As with the RC model the voltage-current relation depends on the duration of transmitter action, Δt, and the lower limit is given by the DC curve in Fig. 8. The upper limit, when Δt approaches zero, is shown by the curve labelled 'cable lim'.

An expression equivalent to eqn. (5) for the synaptic potential amplitude in the dual time constant cable has been derived and solutions kindly provided by Professor B. L. Ginsborg. Details of the derivation and the solution, which requires numerical integration, will be presented elsewhere. In addition to terms in $\tau = r_m c_m$, the expression contains two other time constants, $\tau_e = r_e c_e$ and $\tau_x = r_m c_e$. The limits of the voltage–current relation are the same as those for a conventional cable,

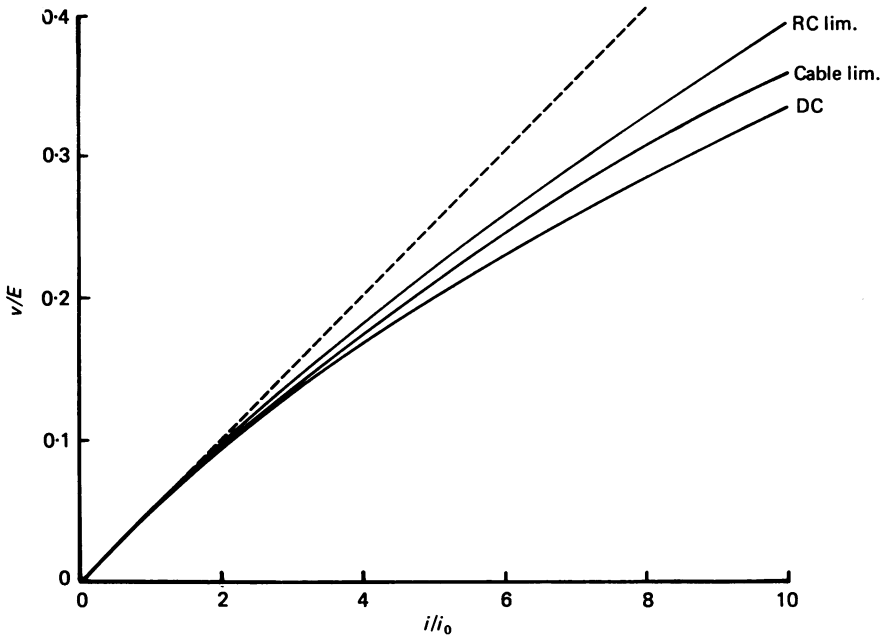

Fig. 8. Theoretical e.p.p.–e.p.c. relations for models shown in Fig. 7. Abscissa and ordinate normalized as in Figs. 2 and 6. Interrupted line indicates linear relation. DC: relation for DC model. This is also the theoretical lower limit for all other models when duration of transmitter action, Δt, is infinitely long. RC lim: limiting value of the relation for the RC model when $\Delta t \to 0$. Cable lim: limiting value for both cable models when $\Delta t \to 0$.

but with intermediate values of Δt curves from the two cable models differ slightly. With both cable models and the RC model it is implicit that the synaptic conductance change is rectangular with amplitude g and duration Δt. However the actual wave form does not seem to be significant in determining the shape of the normalized voltage–current relations (Martin, 1976; J. Searl & B. L. Ginsborg, unpublished observations). Within practical limits, the results obtained with other waveforms are indistinguishable from those obtained with rectangular conductance changes of duration $\Delta t = A/g_p$, where A is the area under the more complex waveform and g_p is its peak amplitude.

In Fig. 9 the experimental results for frog and mouse fibres are compared with those expected theoretically from the RC and cable models. In order to make the comparisons it was necessary to choose a value for Δt in each preparation and to select values for the membrane time constants. For frog muscle, Δt was taken as 1·5 msec; the membrane time constant, τ, for the RC and conventional cable models was taken as 22·5 msec so that $T = \Delta t/\tau$ was 0·067. For the dual time constant cable

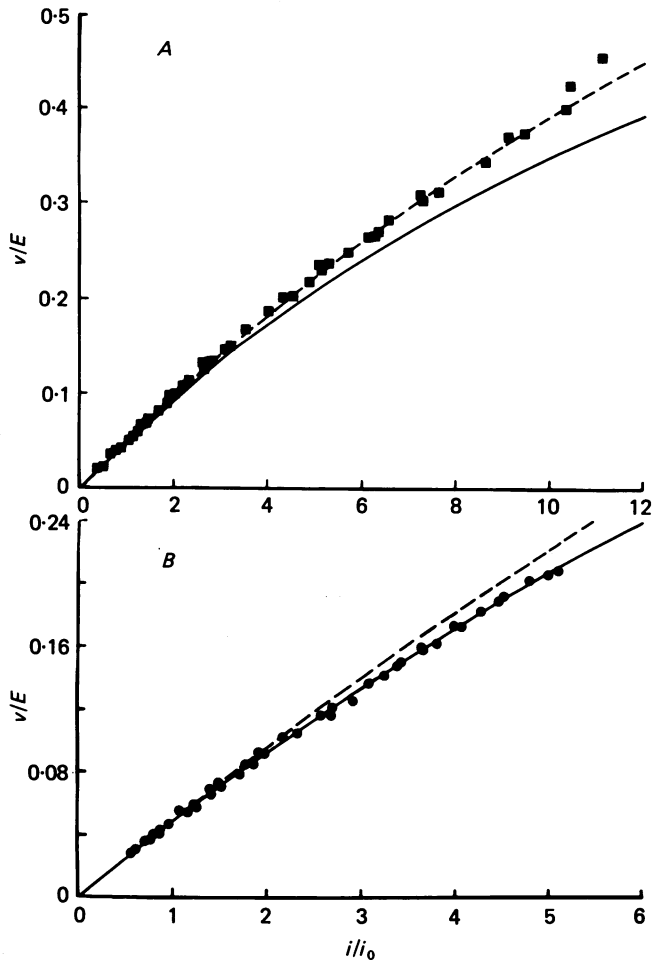

Fig. 9. Comparison of experimental results from frog muscle (*A*) and mouse muscle (*B*) with theoretical predictions from RC model (interrupted line) and cable models (continuous line). Theoretical curves calculated using duration of transmitter action, Δt = 1·5 msec for frog muscle and 1·0 msec for mouse muscle (see text). Abscissa and ordinate normalized as in Figs. 2 and 6. Results from frog muscle agree closely with RC model, those from mouse muscle with cable models.

the following values were selected: τ = 11 msec, τ_e = 1·3 msec and τ_x = 10 msec. It can be seen in Fig. 9*A* that the experimental points were in good agreement with the RC model and fell consistently above the cable predictions over the entire range of amplitudes. It is important to note that no selection of cable parameters could improve significantly the agreement with the experimental points, as the points were

well above the 'cable limit' over most of the amplitude range. On the other hand, with $T = 0{\cdot}067$, the RC curve is near its upper limit and is relatively insensitive to changes in parameters. In other words, the agreement of the experimental points with the RC curve and lack of agreement with the cable curves was not critically dependent on the selection of T.

The theoretical curves for mouse muscle (Fig. 9*B*) were determined by the following parameters: $\Delta t = 1{\cdot}0$ msec, $\tau = 3{\cdot}0$ msec for the RC and conventional cable ($T = 0{\cdot}33$), and for the dual time constant cable, $\tau = 1{\cdot}6$ msec, $\tau_e = 0{\cdot}2$ msec and $\tau_x = 1{\cdot}5$ msec. The experimental results clearly agree best with the cable models. In order to bring the RC model into equally good agreement an unrealistic value of 1·25 for T would be required, rather than the value of 0·33 selected.

DISCUSSION

The results presented here for the relation between e.p.p. and e.p.c. amplitudes in the mouse muscle are consistent with those expected for a synaptic contact of restricted length on a cable (Fig. 9*B*) and present no particular theoretical problem. The results for nerve-evoked responses in the frog muscle, on the other hand, fall outside the expected limits for a cable and agree more closely with those expected for a simple RC circuit. It seems likely that this apparent deviation from cable theory is related to the fact that the frog neuromuscular junction extends along the fibre for distances up to almost 600 μm, or approximately one third of the resting space constant of the fibre. Although we have no mathematical treatment of this situation at the moment, it might be expected intuitively that when a significant length of cable is shunted by the synaptic conductance the RC model would be more appropriate than the cable model. Certainly if the synapse extended over the *entire* length of the fibre all parts of the membrane would be discharged simultaneously and the RC model would apply exactly.

A second possible explanation of the behaviour of frog fibres requires consideration. This is related to the fact that the end-plate currents may be dependent upon membrane potential. Such dependence would have no effect on our e.p.c. measurements, as these were all made at the same potential in any given experiment, but could affect end-plate currents underlying large e.p.p.s and hence the amplitude of the e.p.p.s themselves. Specifically, three effects must be considered. The first two are direct effects of potential on e.p.c. amplitude and time course (Kordaš, 1969; Magleby & Stevens, 1972; Dionne & Stevens, 1975): (*a*) the relation between e.p.c. amplitude and membrane potential becomes non-linear as the membrane potential is increased; (*b*) the time constant of decay of the e.p.p. decreases with depolarization. For nerve-evoked e.p.c.s the first effect appears as a decrease in peak conductance with hyperpolarization which becomes apparent only at membrane potentials greater than about 90 mV, both in previously published experiments and in control experiments carried out in our laboratory. It is therefore of little significance for the present argument. The second effect is in the wrong direction to account for the deviation of the results from those expected theoretically. Thus the depolarization occurring during the e.p.p. would, if anything, hasten the decay of the underlying current. The

resulting decrease in charge transfer might result in an otherwise unexpected decrease in amplitude of the larger e.p.p.s and introduce greater, not less, non-linearity into the voltage–current relation. The third effect is an indirect one arising from the voltage dependence of the putative 'channel blocking' action of curare. This blocking might be expected to be smaller for large e.p.p.s than for corresponding e.p.c.s because of its voltage dependence. Suppose, for example, that the larger responses are obtained with a curare concentration of about 2 μM. Then at a holding potential of 90 mV the association rate constant will be about 20 sec^{-1} (Colquhoun, Dreyer & Sheridan, 1979) and at the peak of the e.p.c. (0·8 msec) about 1·5 % of the channels will be blocked. In the unclamped condition depolarization by the e.p.p. to say 60 mV would reduce this fraction to about 1 % if the rate constants followed the voltage change instantaneously. This is a very small effect which becomes negligible if the relaxation time is considered as well.

Finally, it is necessary to consider that the experimental results from the frog muscle may be in error because of incomplete clamping of the extended end-plates. The observed e.p.c.s would then be smaller than the 'true' synaptic currents required to clamp the entire end-plate, allowing voltage to escape from the clamp at regions of the synapse distant from the electrodes. This would have no effect on the shape of the voltage–current relation if the *fraction* of current reduction were independent of e.p.p. amplitude; i.e. all e.p.c.s would simply be scaled by a constant factor. Preliminary calculations suggest that this is very nearly the case. Suppose, for example, that an end-plate occupies a length equal to half the space constant of the muscle fibre and is clamped in the middle. Then one can estimate that when an e.p.p. of say 25 mV is clamped, residual voltages of about 10 % of the unclamped amplitude will occur at either end of the synaptic region and the recorded e.p.c. amplitude will be approximately 85 % of the true synaptic current amplitude. This figure increases to about 87 % when a 5 mV e.p.p. is clamped and decreases to about 83 % for a 40 mV e.p.p. Thus, for the larger currents to correspond to the smaller ones, successively increasing increments should be added to the recorded e.p.c.s as the e.p.p. amplitude increases, the maximum correction being about 5 %. Such correction is not sufficient to account for the disparity between the experimental results and those expected for a cable and, in any case, exploration of the synaptic region with a third microelectrode failed to reveal any unclamped voltages of this magnitude when the criteria for adequate clamping were satisfied (see Methods).

Given that the experimental results are accurate, it is clear that eqn. (1) overcorrects for non-linear summation in both preparations, although the error is not as large with the mammalian muscle. The remaining question is what correction factor should be used? The most prudent advice would be to avoid situations where correction is required. At the frog junction, this involves rejecting e.p.p.s of greater than say 15 % of the resting potential and leaving smaller responses uncorrected. In the mouse, however, the range over which correction is not required is more restricted. Perhaps in general an empirical correction of the form suggested by Martin (1976) is most useful, namely $\bar{v}' = \bar{v}/(1 - f\bar{v}/E)$, where f is dependent on the duration of transmitter action relative to the membrane time constant. For mouse muscle, $f = 0{\cdot}8$ gives a good fit to the results; i.e. the corrected points fall on the straight line determined by the initial slope of the voltage–current relation. For frog muscle, $f = 0{\cdot}55$

(equivalent to the 'RC limit') provides an accurate correction. At this limit the correction proposed by Stevens (1976), namely $\bar{v}' = E \ln [E/(E-\bar{v})]$, gives virtually identical results. We have no information about the corrections necessary for glycerinated or for 'cut muscle' preparations. Presumably in such preparations larger values of f would be required because of smaller membrane time constants.

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