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THE EFFECT OF POLARIZING CURRENTS ON UNITARY Ia EXCITATORY POST-SYNAPTIC POTENTIALS EVOKED IN SPINAL MOTONEURONES

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SUMMARY

1. Depolarizing and hyperpolarizing currents were applied to motoneurones in which unitary Ia e.p.s.p.s were evoked. The results concentrate on those e.p.s.p.s which had time courses which were compatible with somatically located synapses.

2. No reversal of these e.p.s.p.s was observed. Depolarizing currents up to 150 nA simply reduced the peak amplitude.

3. Hyperpolarizing currents caused little, if any, increase in the peak amplitude of the e.p.s.p. The time course of decay became briefer as the membrane was hyperpolarized.

4. Changes in decay time course of the e.p.s.p. which accompanied depolarization and hyperpolarization could be attributed to changes in membrane conductances, rather than to changes in synaptic current time course.

5. The failure of the e.p.s.p. to increase with hyperpolarization was shown to be due to the failure of the synaptic current to increase, rather than to the shunting of anomalous rectification.

6. Chemical and electrical transmission are evaluated against these results and those of the preceding papers.

INTRODUCTION

Two previous papers (Edwards, Redman & Walmsley, 1976*a*, *b*) have presented results and explanations on the variability in the net inward charge of unitary, evoked, Ia excitatory post-synaptic potentials (e.p.s.p.s). The results of this investigation left open the question of how failures in

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transmission arose. Because of this, it was not possible to differentiate between a junctional mechanism of an unusual chemical type and electrical coupling.

A crucial test for chemical transmission is to demonstrate that a membrane potential exists at which the synaptic current (generating the synaptic potential) is zero over its complete time course. With further depolarization this current must reverse in polarity. (In central neurones where voltage clamping is technically difficult, observations are usually restricted to the synaptic potential.) Previous investigations on the effect of membrane potential on the amplitude of Ia e.p.s.p.s (Coombs, Eccles & Fatt, 1955; Smith, Wuerker & Frank, 1967; Kuno & Llinás, 1970; Marshall & Engberg, 1973; Shapovalov & Kurchavyi, 1974; P. Carlen & R. Werman, personal communication) have been complicated by the use of composite e.p.s.p.s. With a spatially dispersed synaptic input, all synapses are not subjected to the same membrane potential when the current is applied at a single point, i.e. the soma (Calvin, 1969). With composite e.p.s.p.s which are chemically mediated, reversal should occur with the rising phase of the potential reversing at a lower membrane potential than the falling phase. This type of reversal has recently been shown to occur for the climbing fibre e.p.s.p. in Purkinje cells (Llinás & Nicholson, 1975*b*). However, in all published results on composite Ia e.p.s.p.s, the falling phase of the e.p.s.p. has reversed first, and in most cases a clear reversal of the rising phase has not been demonstrated. In the published results of Coombs *et al.* (1955) only the first 1 msec of the e.p.s.p. is displayed. Such e.p.s.p.s could possibly be contaminated by inhibitory and disynaptic excitatory components which are mediated by synapses with different junctional mechanisms to those which apply to the Ia synapse. Reversal of the rising phase of the Ia e.p.s.p. has occurred at a membrane potential of approximately 0 mV (Coombs *et al.* 1955; Marshall & Engberg, 1973, and private communication).

The results in this paper describe the effect of transmembrane current applied at the soma, on the peak amplitude of unitary Ia e.p.s.p.s. The various synaptic boutons arising from a single Ia afferent nerve may still be spatially dispersed. However, it is possible to use the time course of the e.p.s.p., and the cable properties of the motoneurone, to select e.p.s.p.s which originate from a highly localized region of the dendritic tree (Jack & Redman, 1971; Jack, Miller, Porter & Redman, 1971; Iansek & Redman, 1973). Moreover, it is possible to choose from this subset a small group of e.p.s.p.s whose time course indicates a somatic, or very near somatic, synaptic location. These somatic e.p.s.p.s are of special interest, because no calculation is required to compensate for an electrotonic decrement of the soma membrane potential.

Somatic e.p.s.p.s could not be reversed with depolarizing currents up to 150 nA. Nor did the change in amplitude of the e.p.s.p. with membrane potential changes indicate that a linear relationship existed between synaptic current and membrane potential. While these results do not prove that the Ia e.p.s.p. cannot be reversed, they do indicate that the junctional mechanisms at these synapses are more complicated than those of a classical chemical synapse.

METHODS

The experiments were conducted in the same series from which the results of the two previous papers were obtained. The dissection, and much of the recording arrangements, were identical.

The method used to alter the membrane potential was either to apply continuous current while averaging the e.p.s.p., or to apply the current in a sequence of pulses of 400 msec duration, using a stimulus repetition interval of 500 msec. When using current pulses a stimulation cycle took three stimulus intervals (and three oscilloscope sweeps) to complete. In the first interval no current was applied although the e.p.s.p. was evoked. In the second and third intervals the e.p.s.p. was evoked and current was applied. The current in the second and third intervals could be different and usually it was arranged to be hyperpolarizing for one interval, and of equal magnitude but depolarizing during the next interval. The e.p.s.p. was evoked at 300–350 msec after the start of the current pulse, and several msec after the start of the sweep and digitizing period. By this means the automatic d.c. offset control (Edwards *et al.* 1976*a*) kept the trace within the linear range of the A–D converter while a high d.c. gain was used. The sequence of three intervals was repeated 200 times. Three separate averages were simultaneously computed. These were for the e.p.s.p. without current (control), and for the e.p.s.p. with the two different currents in the other two intervals. This method had a number of advantages over the use of continuous current. Electrode balance is best checked at the make and break of the current pulse, by temporarily overcompensating with capacitance neutralization. The membrane potential several hundred milliseconds after a current pulse is applied (the time the e.p.s.p. was evoked) is approximately the same for each successive current pulse. The base line for this measurement could be obtained in the interval when no current was applied. The disadvantage of using current pulses was that for large currents ($>$ 10–20 nA), action potentials were often generated on the make of depolarizing currents, and to a lesser extent on the break of hyperpolarizing currents. If these action potentials were only generated in the first 100–200 msec following the make of depolarizing current, then the current pulse method could be used. If the action potentials persisted, either the duration of the current pulse could be increased, or continuous current applied while a conventional e.p.s.p. average was taken. In such circumstances, impulse generation often ceased after a short period of continuous current. No simultaneous average of a control e.p.s.p. was available with this method, nor was there any guarantee that the membrane potential change remained stable during such prolonged periods of depolarizing current. (Marshall & Engberg (1973) have reported instabilities in membrane conductance during prolonged depolarizing currents.)

Currents in excess of 100 nA were generally required to produce large membrane potential changes. Accurate use of the bridge technique requires a stable tip resistance over the entire range of currents applied. For these large currents, such

electrode properties could not be achieved. Generally, the electrodes blocked, or developed a high resistance. Most success was obtained with electrodes with a tip resistance in the range of 2–4 MΩ, after being sharply bevelled. Electrode balance for small currents was checked against the change in amplitude of the antidromic action potential at these currents (Frank & Fuortes, 1956). By this means the input resistance at low currents can be reliably determined. There is no reliable way that the membrane potential can be measured at large currents using the bridge, although we routinely attempted to measure it.

The results presented were obtained from motoneurcnes that (i) had stable membrane potentials in excess of 50 mV upon completion of the averages at each current, (ii) generated full antidromic action potentials throughout the experiment, and (iii) were penetrated by electrodes with fairly stable tip resistances. Current–voltage plots were attempted wherever possible. The membrane voltage change was measured at the time the e.p.s.p. was evoked.

The methods for measuring cable parameters for the motoneurone, and for determining synaptic location, were as previously described (Edwards *et al.* 1976*a*).

RESULTS

A total of sixty-five e.p.s.p.s were subjected to membrane potential changes. Of those for which distance estimates could be made, six were somatic, or within one fifth of a space constant from the soma. Current–voltage curves and averages over a wide range of applied currents were obtained for only four of these results. Partial results were obtained for the remainder of the e.p.s.p.s due mainly to electrode blockage at high currents, unstable tip resistances and deterioration of cell membrane potential.

Results for one somatic e.p.s.p. are shown in Figs. 1 and 2. The 10–90 % rise time and the half-width were 0·22 and 1·6 msec, respectively. The cell time constant, measured from the decay of the e.p.s.p. and from the decay of the intracellular current pulse, was 7·1 msec. These shape indices locate the site of origin of the e.p.s.p. at the soma (see Fig. 8 in Jack *et al.* 1971 and Fig. 1 in Iansek & Redman, 1973). Fig. 1 shows the average time course and amplitude for this e.p.s.p. for a variety of depolarizing and hyperpolarizing currents. With progressive hyperpolarization, there is a small increase in e.p.s.p. peak amplitude (Fig. 2*B*) and a marked decrease in the decay time of the e.p.s.p. The half-width at 70 nA hyperpolarizing is 35 % of the control half-width, compared to 85 % of the control at 10 nA hyperpolarizing. The peak amplitude decreases with increasing depolarizing current as indicated in Fig. 2*B*. The decay of the e.p.s.p. shows complex changes with depolarization. At 10 nA the decay is prolonged. An undershoot is evident at larger currents. The undershoot, and the prolonged decay, disappear at 60–70 nA. The electrode came out of the cell when a current larger than 150 nA was applied.

The current–voltage relationship for this cell was measured, as described

in Methods. The result is shown in Fig. 2*A*. The extension of the *I–V* curve to larger currents is not shown, as the balance could be incorrect. The steady-state input resistance at resting membrane potential was 4 MΩ. For depolarizing and hyperpolarizing currents in the range 50–100 nA, the slope input resistance dropped to much less than 1 MΩ. Delayed K rectification on depolarization (Ito & Oshima, 1965) and

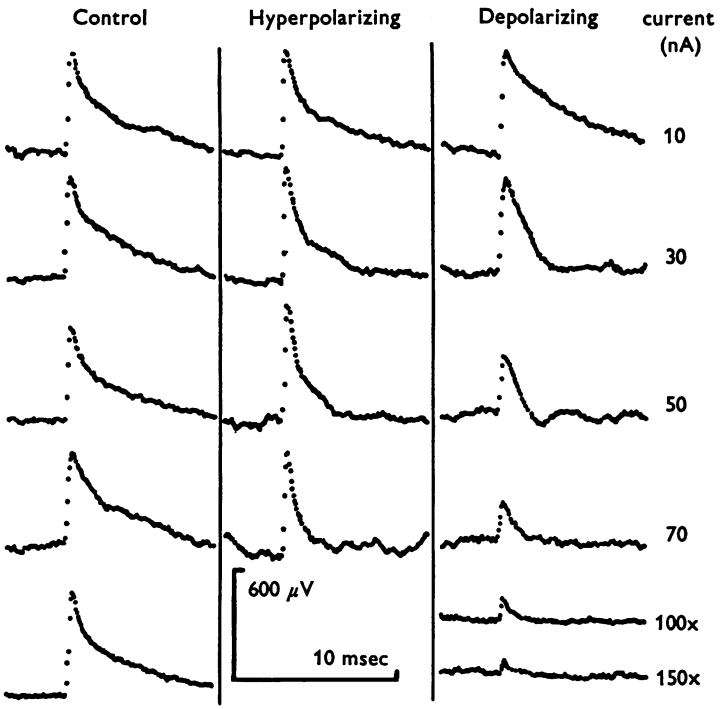

Fig. 1. Currents were applied as long duration pulses in the sequence no current (control), hyperpolarizing current and depolarizing current. This sequence was repeated 200 times while the three averages were computed. This sequence of three averages is shown for currents in the range 10–70 nA. Depolarizing currents of 100 and 150 nA were applied as continuous current, and for each of these currents the consecutive evoked e.p.s.p.s were averaged. The lower control e.p.s.p. average was then obtained. × indicates continuous current.

anomalous rectification on hyperpolarization (Nelson & Frank, 1967) could explain these decreases. The resting membrane potential for this cell was 75 mV throughout the recording.

The *I–V* curve of Fig. 2*A* may be used to examine changes in membrane resistivity and incremental input resistance at different membrane

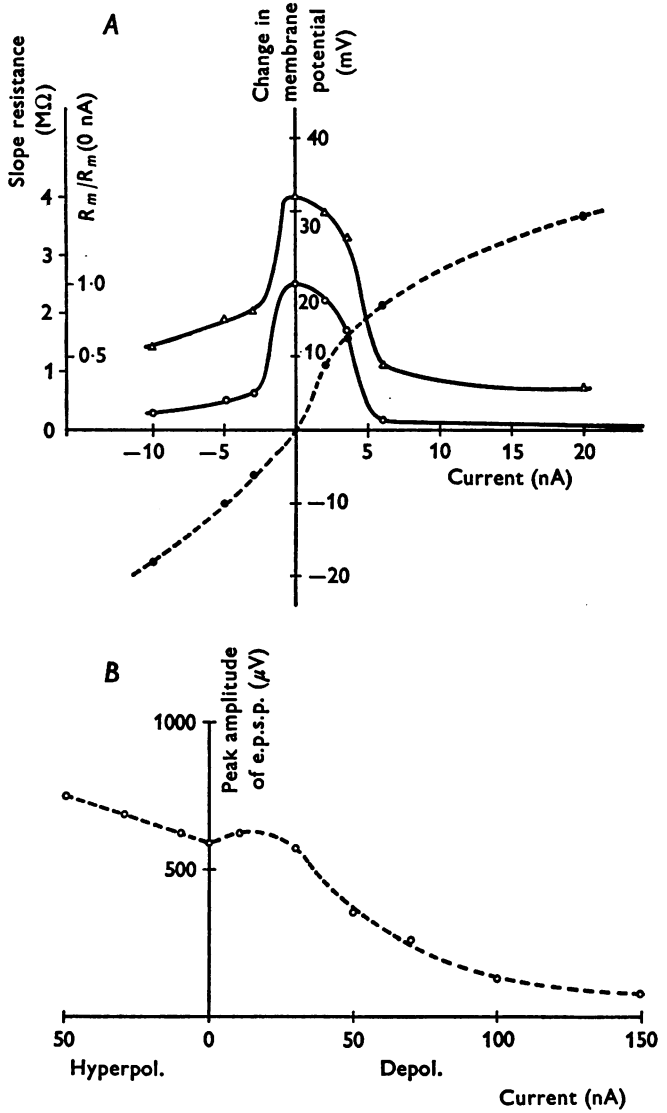

Fig. 2. *A*, current–voltage curve for cell from which Fig. 1 results were obtained (●). Slope resistance in MΩ measured from the *I–V* curve (△). Specific membrane resistivity normalized by the value at resting membrane potential (○). *B*, peak amplitude of e.p.s.p. (in μV) as a function of electrode current, measured from the averages in Fig. 1. Depolarizing currents are positive.

potentials. The slope resistance of this curve at any membrane potential is the input resistance presented to a small e.p.s.p. generated at a synapse on the soma. This input resistance is determined by a number of dendrites in parallel, with a further contribution from the soma membrane. Neglecting the latter as small, and assuming N equal diameter dendrites with sufficiently large electrical lengths such that end effects can be ignored then

$$R_{in} = \frac{1}{N}\frac{1}{\pi a^{\frac{3}{2}}}\left(\frac{R_m R_a}{2}\right)^{\frac{1}{2}},$$

where a is the radius of each dendrite (reduced to an equivalent cylinder) R_m is the specific membrane resistivity and R_a is the specific resistivity of the cytoplasm. R_{in} is measured as the slope resistance on the I–V curve at different membrane potentials. Uniform resistivity is assumed.

Voltage-dependent membrane conductance changes will be greatest near the point of current application, and using this equation to calculate R_m will result in an over-estimate of R_m in proximal dendritic membrane, and it will underestimate R_m for more distal regions. Rather than guess values for a, R_a, and N, in Fig. 2*A* we have plotted the ratio of R_m at the particular membrane potential, to the value of R_m at the resting membrane potential. The large relative decrease in R_m with depolarization illustrates the difficulty in supplying sufficient current to produce large changes in membrane potential.

Although the e.p.s.p. amplitude was considerably reduced, it could not be reversed at these large depolarizing currents. Nor can the curve of e.p.s.p. amplitude against depolarizing current (Fig. 2*B*) be extrapolated to predict a current at which the e.p.s.p. becomes zero. This asymptotic curve does not imply that such a current does not exist. Two factors must be considered. One is that the large decrease in R_m which accompanies depolarizing currents means that equal increments of depolarizing current do not cause equal decreases of membrane potential. So if there did exist a linear relationship between peak synaptic current and membrane potential, this would introduce a non-linearity in the relationship with depolarizing current. The other factor is that peak synaptic current and peak synaptic potential are related by the input impedance at the soma. Most of the synaptic current is capacitive for this e.p.s.p. (see Discussion and Fig. 7). A reduction of greater than 75% in incremental input resistance is necessary before the peak synaptic potential is affected, for the same synaptic current. Such a reduction in input resistance occurs for depolarizing currents in excess of 6 nA, but does not occur for the range of hyperpolarizing currents shown. Accordingly Fig. 2*B* may be modified within the hyperpolarizing current range of 0–10 nA (using the I–V curve of Fig. 2*A*) to give a relationship between

peak potential and hyperpolarization. This is linear, and an extrapolation of this curve to find the potential at which the e.p.s.p. becomes zero (Takeuchi & Takeuchi, 1959; Martin & Pilar, 1963, Ginsborg, 1967) gives a reversal potential of approximately +175 mV. This potential is vastly in excess of the potential at which composite e.p.s.p.s have been reversed

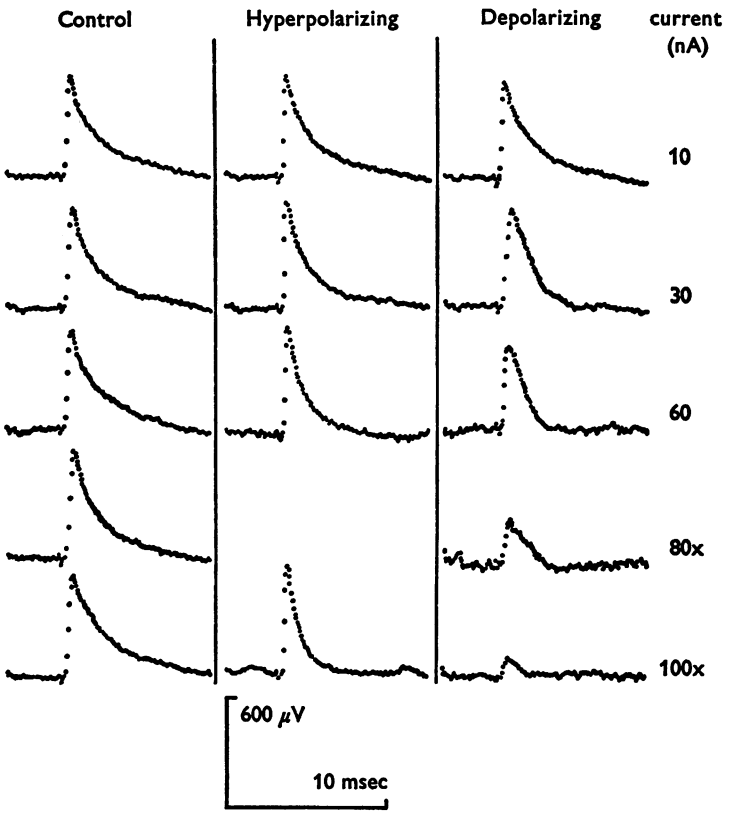

Fig. 3. Currents were applied as long duration pulses in the sequence no current (control), hyperpolarizing current and depolarizing current. This sequence was repeated 200 times while the three averages were computed. This sequence of three averages is shown for currents in the range 10–60 nA. Depolarizing currents of 80 and 100 nA were applied as continuous current, as was the 100 nA hyperpolarizing current. For each of these currents, consecutive evoked e.p.s.p.s were averaged. The final control e.p.s.p. average was then obtained. × indicates continuous current.

(Coombs *et al.* 1955; Marshall & Engberg, 1973). Either a linear relationship does not exist between synaptic current and membrane potential for small hyperpolarizations, or the reported reversal potential is incorrect.

A second series of records in shown is Fig. 3. This e.p.s.p. had a rise

time of 0·24 msec and a half-width of 1·5 msec. The membrane time constant for the cell was 5 msec. These shape indices placed the synapse at the soma. The changes in this e.p.s.p. in response to both depolarizing and hyperpolarizing currents were similar to those for the first result. Hyperpolarization caused little change in peak amplitude while depolarization decreased it (Fig. 4*B*). The rate of decay was increased with hyperpolarization.

The *I–V* characteristic for this motoneurone is shown in Fig. 4*A*. The input resistance for small depolarizing currents was 0·7 MΩ. The resting membrane potential was stable at 70 mV. Also shown in Fig. 4*A* is the specific membrane resistance obtained from a similar calculation to that used in connexion with Fig. 2*A*. In this case there is little change in incremental input resistance for small depolarizations and hyperpolarizations. If voltage is substituted for current (in the range of ± 10 nA) on the abscissa of Fig. 4*B*, extrapolation of the peak synaptic potential curve gives a reversal potential in excess of 400 mV. The peak synaptic potential is almost independent of membrane potential over a large range of hyperpolarizing current.

In several cells containing e.p.s.p.s of somatic origin, currents of the order of 200 and 300 nA were applied. The electrode invariably blocked before an average could be completed. However no sign of a reversal could be observed in the individual responses before this occurred. At these large currents the e.p.s.p. appeared to be non-existent. It could have been obscured by the high noise level which develops at such currents.

Fig. 5 shows a series of records in which time course changes in the decay of the e.p.s.p. are apparent as the cell is progressively hyperpolarized or depolarized. The input resistance of this motoneurone was 0·9 MΩ for small voltage variations about the resting potential. The changes with increasing hyperpolarization are consistent with those in the two previous results. The halfwidth decreased to 30 % of control at 100 nA. Indeed, it was rare not to observe a decrease in decay time in the e.p.s.p. while the membrane was hyperpolarized. The changes in decay time course with depolarization were more variable. In this cell, currents between 10 and 40 nA (depolarizing) caused active responses to accompany the e.p.s.p. and prolonged the time to peak. The results in Fig. 5 are averages, and mask the great variety of time courses which occurred when individual e.p.s.p.s triggered off active responses. The undershoot potential, which is apparent at 35 nA, was often associated with active responses in some range of membrane depolarization.

Most of the e.p.s.p.s which generated active responses originated at synapses located on or close to the soma (0–0·4 λ). For e.p.s.p.s generated at distances greater than 0·4 λ, one of the e.p.s.p.s (out of a total of 8)

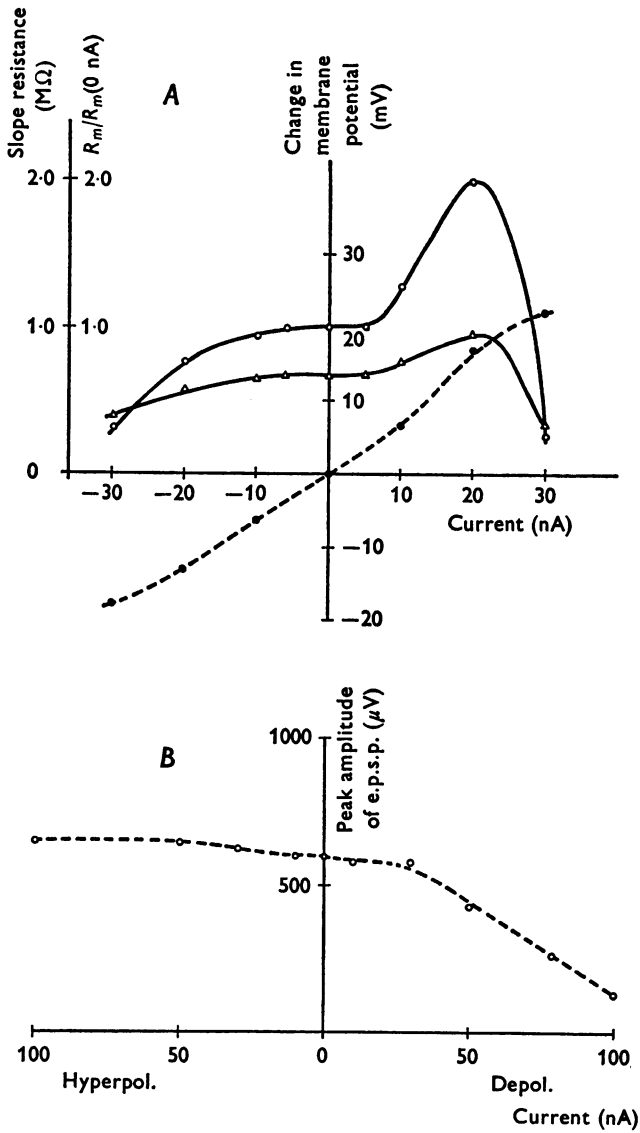

Fig. 4. *A*, current–voltage curve for cell from which Fig. 3 results were obtained (●). Slope resistance (MΩ) measured from the *I–V* curve (△). Specific membrane resistivity normalized by the value at resting membrane potential (○). *B*, peak amplitude of e.p.s.p. (μV) as a function of electrode current, measured from the averages in Fig. 3. Depolarizing currents are positive.

generated an active response with depolarizing current. Insufficient information was obtained to specify the conditions leading to an active response. These conditions involve the level of membrane depolarization, the site of origin of the e.p.s.p. and its peak amplitude, and whether the

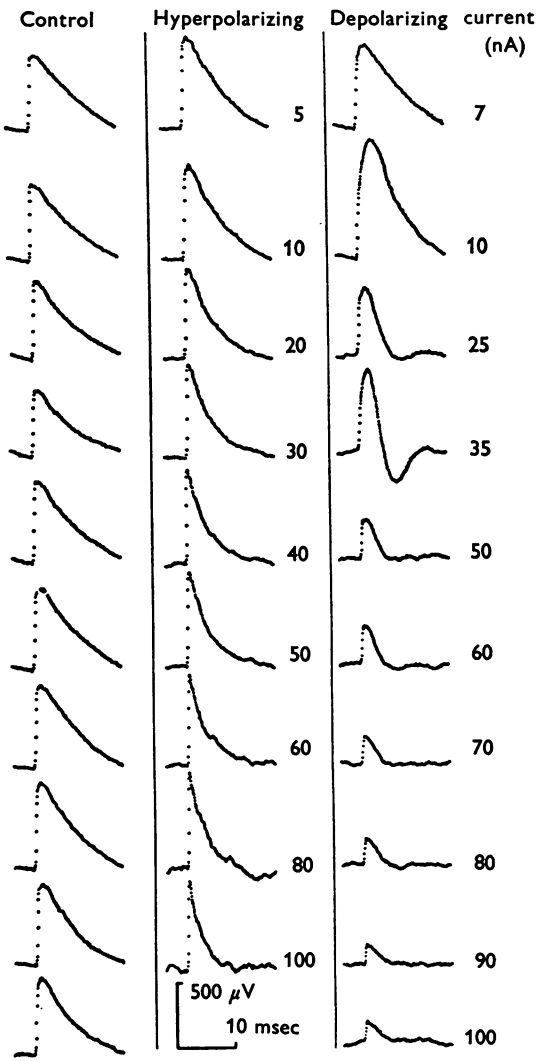

Fig. 5. Averaged e.p.s.p. recorded during long duration current pulses. The sequence was no current (control), hyperpolarizing current, then depolarizing current. This sequence was repeated 200 times to compute the three averages. In any one sequence, the hyperpolarizing current and depolarizing current were occasionally of different magnitude.

depolarization was caused by continuous current or by long duration current pulses.

In some motoneurones, brief current pulses (200 μsec duration) were superimposed on the long duration current pulse causing the membrane potential shift. Fig. 6 shows the effect of a hyperpolarizing and depolarizing current on the time course of the voltage transient produced by the brief current pulse. An e.p.s.p. with a time course indicative of

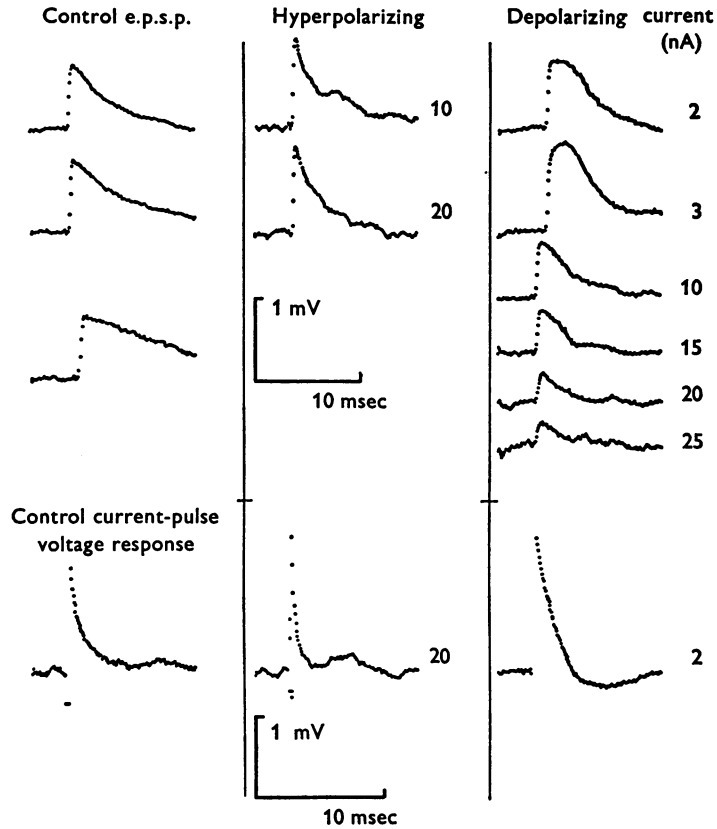

Fig. 6. Averaged e.p.s.p. recorded during long duration current pulses. As before, the sequence was no current (control), hyperpolarizing current and depolarizing current, with this sequence repeating 200 times. Not all the control responses and the hyperpolarizing responses are shown, as there was little change in these averages. In the same cell, sequences of three averages were recorded when a brief current pulse, of 200 μsec duration, and 5 nA depolarizing current, was superimposed on the long duration current pulse. No e.p.s.p. was evoked during this procedure. At the bottom of the Figure is the response to the brief pulse when the long pulse is 20 nA hyperpolarizing and 2 nA depolarizing. The control response (no long duration current pulse) is also shown.

a proximal synaptic location was also subjected to these same polarizing currents in the small cell. At 20 nA hyperpolarizing current the half-width, of the e.p.s.p. was 58% of the control half-width and the half-decay of the current response was 50% of the corresponding control value. A 2 nA depolarizing current increased the half decay time of the current pulse response, and a significant undershoot potential developed. Complex changes occurred in the e.p.s.p. time course. A prolonged peak potential appeared, followed by a rapid decay to a large undershoot potential. The half-width of the e.p.s.p. was prolonged by a few percent.

DISCUSSION

Many of the qualifications associated with previous investigations on the reversal potential for the Ia e.p.s.p. in motoneurones have been removed in these experiments. The possibility of contaminating the e.p.s.p. with inhibitory and disynaptic excitatory components is remote. The somatic origin of the e.p.s.p.s studied has removed the distorting effects of a non-uniform membrane polarization (Rall, 1967; Calvin, 1969). The use of bevelled micro-electrodes has alleviated some of the difficulties associated with increasing tip resistance at high currents (Anderson, Kleinhaus, Manuelidis & Pritchards, 1974). For all of these reasons, a clear reversal of the e.p.s.p. should have been easier to achieve in these experiments than in the experiments of Coombs *et al.* (1955), Smith *et al.* (1967) and Kuno & Llinás (1970). However, no reversal of the e.p.s.p. could be demonstrated. Moreover, the asymptotic form of the curve relating depolarizing currents to e.p.s.p. amplitude (Fig. 2*B*) makes extrapolation to a reversal current impossible. Extrapolation of the relationship between peak synaptic potential and membrane potential for small hyperpolarizations indicates a reversal potential of the order of 200 mV or greater. A reversal potential of this magnitude is probably well in excess of the equilibrium potential for Ca ions (Katz & Miledi, 1967; Llinás & Nicholson, 1975*a*). It thus appears that the extrapolation procedure is not valid and that the junctional mechanisms do not conform to those of a classical chemical synapse for small membrane hyperpolarizations. It is shown below that the failure of the peak synaptic potential to significantly increase with membrane hyperpolarization cannot be explained by the anomalous rectification in the membrane shunting an increasing synaptic current.

Amplitude and time course dependence on membrane hyperpolarization

There is general agreement in the literature that when the membrane is hyperpolarized, the e.p.s.p. peak amplitude shows little increase, and

that the rate of rise and decay of the e.p.s.p. is increased (Coombs *et al.* 1955; Nelson & Frank, 1967). These effects have generally been attributed to anomalous rectification (Nelson & Frank, 1967). Anomalous rectification cannot always be detected by steady-state resistance measurements, yet an apparent increase in rate of decay of potential transients invariably occurs with hyperpolarization. A possible explanation for this is that the

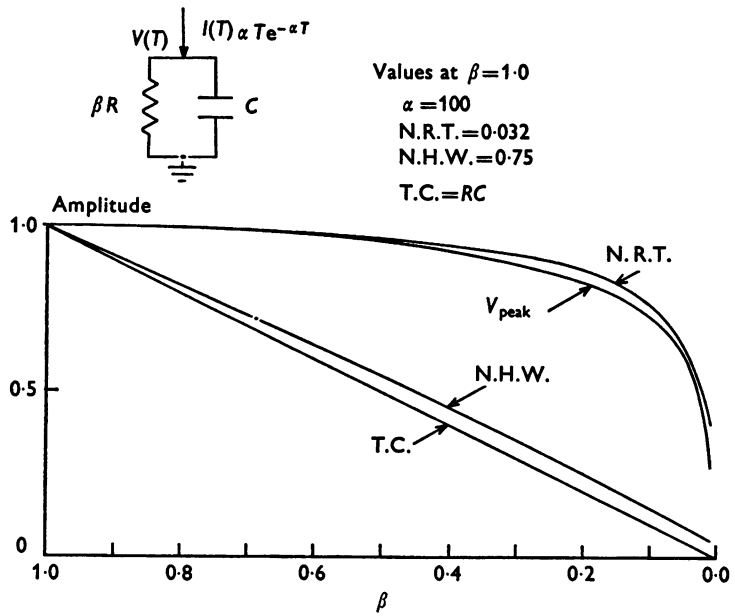

Fig. 7. Measurements of peak voltage (V_{peak}), normalized rise time (N.R.T.) and normalized half-width (N.H.W.) of a voltage transient recorded across a parallel *RC* network. The network is excited by a current source with time course $Te^{-\alpha T}$, and the resistance varies between R and zero as β varies between 1 and 0. All measured quantities are normalized against their maximum values. The value of α is 100, to match the time course of the e.p.s.p. in Figs. 1 and 3.

soma and proximal dendritic membrane contains the inward rectifying channels. The large dendritic input conductance (under steady-state conditions) compared to the soma conductance, causes the $I-V$ curves measured for steady-state currents to reflect the dendritic membrane properties. Also, steady membrane currents may disturb the normal extracellular potassium concentration.

The small change observed in peak amplitude with hyperpolarization is unlikely to be explained by anomalous rectification. The peak amplitude of the e.p.s.p. is determined by the time course and magnitude of the synaptic current, and the input impedance at the synaptic site (Rall &

Rinzel, 1974). The time courses of the synaptic current generating the e.p.s.p. in Figs. 1 and 3 were calculated. Both time courses were assumed to be of the form $Te^{-\alpha T}$, where T is normalized time, and α is a constant (Rall, 1967; Jack & Redman, 1971; Iansek & Redman, 1973). Both results were fitted with $\alpha = 100$. For the e.p.s.p.s in Figs. 1 and 3, this gives a time to peak synaptic current of 71 and 50 μsec, respectively. Fig. 7 illustrates some measurements taken of a potential transient rise time, half-width and peak amplitude generated across a simple parallel *RC* network as the resistance was varied. A current of constant peak amplitude, and with $\alpha = 100$ (defined at the maximum of *RC*) was applied. Although the half-width is very sensitive to changes in R, the rise time and peak amplitude are not affected until the resistance is reduced to about one tenth of its initial value.

The rise time, peak amplitude and early decay of somatic e.p.s.p.s generated by brief synaptic currents of somatic origin will follow the same pattern as in Fig. 7. Although a parallel *RC* network is, in general, a most inadequate electrical description of the motoneurone, the electrical effects of dendritic cables need not be considered in this example. A factor of 2 change in half-width in the results of Figs. 1 and 3 over the full range of hyperpolarization requires halving of the incremental input resistance. A decrease in this resistance by up to a factor of 3 (Fig. 2*A*) occurred for small hyperpolarizing currents. This decrease will have a negligible effect on peak amplitude of the synaptic potential. Thus the peak amplitude of synaptic current is relatively independent of membrane potential for small hyperpolarizations. The dependence which is revealed implies a reversal potential of the order of 200 mV greater than that demonstrated with composite e.p.s.p.s.

Amplitude changes with membrane depolarization

The inability to demonstrate a reversal of the Ia e.p.s.p. does not imply that a reversal potential does not exist. The kinetics and *I–V* characteristics of the delayed rectifier appear to be complicated (Marshall & Engberg, 1973) and very much larger currents than those achieved in these experiments may be necessary to demonstrate a reversal. We mentioned in Results observations at 200–300 nA before electrode block occurred. At these currents a very high noise level prevailed, but no e.p.s.p. could be observed. Furthermore, the most complete data (Figs. 1 and 2) were obtained from a motoneurone whose input resistance for small depolarizations was relatively large (Burke, 1967). The currents applied exceeded those of Smith *et al.* (1967) and Kuno & Llinás (1970) when they observed reversals, and of course, the synapses in the present experiments were somatic.

These results conflict with the results of experiments in which composite e.p.s.p.s have been used. The biphasic reversals of e.p.s.p.s observed by Smith *et al.* (1967), Kuno & Llinás (1970), Shapovalov & Kurchavyi (1974) could indicate an inhibitory component in the e.p.s.p. or a delayed excitatory component with a reversal potential well within range of the induced depolarization. No biphasic reversals were observed in these experiments.

Junctional mechanisms at Ia synapses

Membrane hyperpolarizations do not increase the peak amplitude of a somatically generated e.p.s.p. in a manner consistent with a reversal potential of about 0 mV. Nor is the extrapolated reversal potential less positive than the generally accepted equilibrium for either Na or Ca ions. The relative insensitivity of the synaptic potential to membrane hyperpolarization cannot be explained by anomalous rectification. It could be explained if the conductance modulation were voltage dependent. Such a mechanism has been proposed by Dudel (1974) who showed that end-plate synaptic currents at the crayfish neuromuscular junction did not increase with hyperpolarization. Also, a variable contribution to synaptic current from K^+ ions following membrane potential changes should be considered (Takeuchi & Takeuchi, 1961). Hyperpolarizing membrane currents may deplete extracellular potassium, increasing the gradient for K^+ ions across the synapse. The reversal potential would become more negative as the membrane was hyperpolarized.

At the conclusion of a major investigation of transmission at Ia synapses in motoneurones, Rall, Burke, Smith, Nelson & Frank (1967) were unable to unequivocally distinguish between a low resistance coupling model and chemical transmission. Most relevant data were reviewed in their discussion. To that discussion we now add the results of these papers. Transmission at a single synapse does not occur in discrete quantal steps with Poisson or binomial statistics. An explanation has been given for post-tetanic potentiation, when it occurs, which does not require chemical transmission. The model for a chemical junction, which best fits these further results, is one where the charge transferred is 'all-or-nothing' (Edwards *et al.* 1976*b*). If it could be conclusively shown that the failures in transmission do not result from failure of the impulse to invade the terminal this would provide support for chemical transmission. The strong evidence for chemical transmission is the demonstrated reversal of the leading edge of a composite Ia e.p.s.p. by Coombs *et al.* (1955) and by K. C. Marshall & I. Engberg (private communication).

The electrical requirements of low resistance coupling are difficult to reconcile with available anatomical data for Ia terminals. The network

which couples the presynaptic terminal to the post-synaptic neurone (Bennett, 1966; Rall *et al.* 1967) consists of three main resistors: R_1 which couples the presynaptic terminal to the synaptic cleft, the cleft leakage resistance (R_c) and the resistance across the subsynaptic membrane (R_2). Walmsley (1975) has calculated bounds for these resistances such that the following conditions are met: (i) the peak synaptic current is 1 nA (Iansek & Redman, 1973) for a 100 mV action potential in the presynaptic terminal; (ii) the shunt resistance of the terminal on the presynaptic axon is not less than one fifth of the input resistance of the axon, assumed to be 100 MΩ (Katz, 1966); (iii) no synaptic current flows when the post-synaptic membrane is depolarized to +150 mV (this paper); (iv) the terminal depolarization appears as a resistance shunt of 400 MΩ (Kuno, 1964); and (v) the currents through the coupling capacitances in parallel with R_1 and R_2 are small compared to the currents through these resistors (Walmsley, 1975). A number of sets of values of R_1, R_2 and R_c are possible for these requirements. The bounds are (in MΩ) $25 \leqslant R_c \leqslant 80$, $0 < R_1 \leqslant 20$ and $55 \leqslant R_2 \leqslant 100$. These bounds are interdependent and the choice of one value narrows the range for the other two. A representative solution is $R_1 = 20$, $R_2 = 55$ and $R_c = 40$. This value of R_c is eight-times greater than that calculated by Katz (1966) for a cleft of comparable dimensions with normal extracellular volume resistivity in and around the cleft. The subsynaptic membrane resistivity must be of the order of 100 Ω cm², which is about two orders of magnitude less than the specific membrane resistivity. None of these resistance values are impossible. However, they do require specialization of specific resistivities.

It could be expected that the junctional mechanisms at Ia synapses on motoneurones and DSCT neurones are the same. The post-synaptic potentials in DSCT neurones following activation of a single Ia fibre have similar electrophysiological properties to the Ia e.p.s.p. in motoneurones. There is no increase in e.p.s.p. with membrane hyperpolarization (Kuno, Muñoz-Martinez & Randic, 1973). Fluctuations in peak amplitude are small (Eide, Fedina, Jansen, Lundberg & Vyklický, 1969; Kuno, 1971). However, any comparison of electrophysiological properties of Ia e.p.s.p.s would need to take into account the different geometrical arrangements of the terminals arising from a single afferent on these cell types.

The results of this series of experiments are insufficiently penetrating to specify a detailed mechanism of synaptic transmission. They raise more questions than they answer. It is difficult to suggest experiments through which a reasonable understanding of transmission at Ia synapses can be reached. Experiments in which Ca^{2+} and Mg^{2+} are applied to the cord (in various ways) are unlikely to give unequivocal results, because increased concentrations of these ions can interfere with impulse propagation into the terminals. Also, diffusion barriers may prevent these ions

reaching the synapse in sufficient concentrations. Obviously, further experiments on the problem of reversing unitary e.p.s.p.s are required, with improved techniques. Marshall & Engberg (1973) have achieved a twin intracellular impalement using independently manipulated electrodes. This powerful technique, applied to e.p.s.p.s of somatic origin, could be used to voltage clamp the soma, and provide a direct measure of the synaptic current generating these e.p.s.p.s. Until such information is available, and until the reason for failure of transmission can be determined, the junctional mechanisms at the most studied synapse in the central nervous system remain obscure.

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