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THE EFFECTS OF DEPOLARIZATION OF MOTOR NERVE TERMINALS UPON THE RELEASE OF TRANSMITTER BY NERVE IMPULSES

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SUMMARY

1. Depolarizing currents were applied to motor nerve terminals in the rat phrenic nerve–diaphragm muscle preparation *in vitro*.

2. During the passage of depolarizing currents the amplitude of the presynaptic nerve action potentials and of end-plate potentials (e.p.p.s) was reduced in proportion to the current strength.

3. The reduction in e.p.p. amplitudes was shown to be due to a reduction in the number of quanta released.

4. An excess of Mg ions or the previous application of a hyperpolarizing current could prevent the reduction of e.p.p. amplitudes and quantal contents by depolarizing current.

5. Depolarizing current application prevented later hyperpolarizing currents affecting e.p.p. amplitudes.

INTRODUCTION

The central point of the hypothesis suggested to explain presynaptic inhibition in the feline spinal cord is that the presynaptic action potential is reduced in amplitude and therefore less effective in releasing transmitter, as a consequence of a depolarization of the nerve terminals (Eccles, 1964). The present investigation had its origin in an attempt to examine the relationship between nerve terminal depolarization and transmitter release under the controlled conditions of an *in vitro* preparation. Previous attempts *in vitro* to examine the effect of depolarization of nerve terminals upon release of transmitter by nerve impulses had been unsuccessful

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because of technical difficulties (del Castillo & Katz, 1954*a*). The techniques of focal polarization and recording (Hubbard & Willis, 1962*a*; Hubbard & Schmidt, 1963) however allowed the observation of the presynaptic nerve action potential (n.a.p.) and of the post-synaptic effect, the end-plate potential (e.p.p.) during the application of a depolarizing current to the terminals.

A preliminary account of part of this investigation has been published (Hubbard & Willis, 1962*b*).

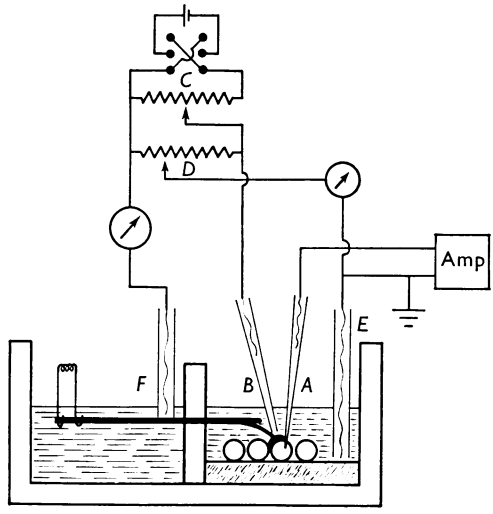

Fig. 1. Schematic diagram of focal depolarization of presynaptic terminals at the rat neuromuscular junction. The muscle (represented by circles) was placed in the right-hand side of the divided recording chamber. The nerve passed beneath the partition into the left compartment where it was mounted upon stimulating electrodes. A micro-electrode (*A*) was used to record intracellular potentials from muscle fibres. Polarizing currents were passed between a micropipette (*B*) placed near an end-plate region and an electrode (*F*) in contact with the nerve in the paraffin pool in the left compartment. The source of current was a battery connected to a variable resistance through a reversible switch (*C*). A bridge circuit was established by shunting the polarizing circuit with a potentiometer (*D*) with the sliding contact connected to earth (*E*). Currents were monitored by microammeters as shown. Further details in text.

METHODS

The rat hemidiaphragm phrenic nerve preparation was used in all experiments. The techniques of intracellular recording and the method of focally polarizing the nerve terminals using a focal electrode and a bridge circuit were those of Hubbard & Willis (1962*a*) and are indicated diagrammatically in Fig. 1. In view of the complexity of the effects of depolarizing current it seems worthwhile to outline certain features of this technique. As Fig. 1 shows, polarizing currents were obtained from a 45 V battery; the amount of current was controlled by a potentiometer and the direction by a reversing switch (Fig. 1*C*). The currents were passed between an electrode on the phrenic nerve (Fig. 1*F*) and an electrode located near

the presynaptic terminals (Fig. 1*B*). The first electrode (*F*) was a Ag–AgCl wire which made contact with the nerve through an agar-Ringer column. The other electrode (*B*) was a Ag–AgCl lead inserted into a glass micropipette filled with agar-Ringer solution. The micropipette was prepared by breaking the tip of a finely drawn micro-electrode until the outside diameter was 30–100 μ; the best results were obtained when the tip was squarely broken rather than obliquely, and when the resistance was about 2 MΩ. The range of resistances used was 0·5–4MΩ. This micropipette was mounted on a micromanipulator and the junctional region was located by using the pipette to record foci of extracellular e.p.p.s; the pipette was then switched into the polarizing circuit. Current was passed from the micropipette

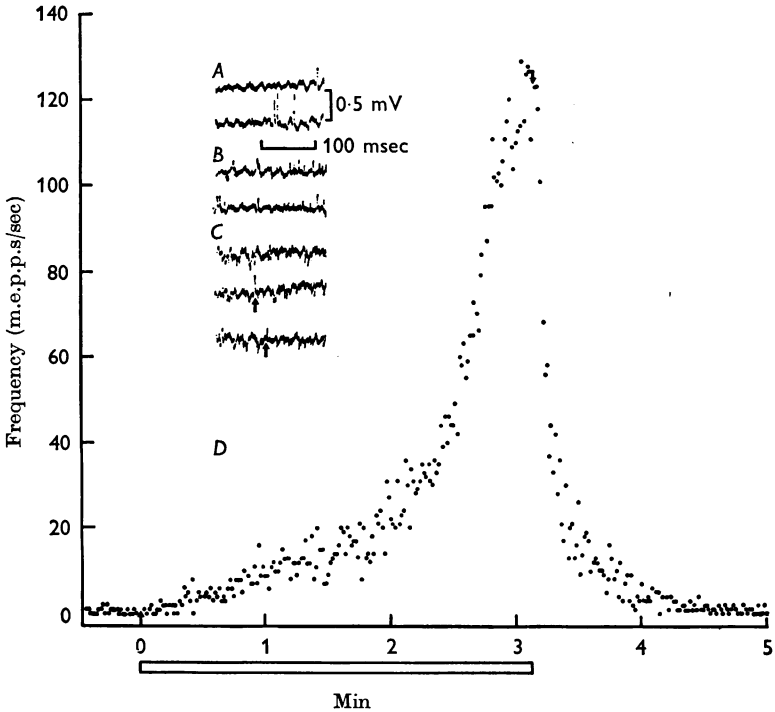

Fig. 2. M.e.p.p. frequency and amplitude changes induced by focal depolarization of the post-synaptic (muscle) membrane. In *A* sample records are shown from an experiment in which focal depolarizing currents of increasing strength were applied to a small area of the post-synaptic membrane. *A* shows the control frequency and amplitude, *B* shows increased frequency but reduced amplitude under the influence of depolarizing current and *C* shows reversal of the amplitude of most m.e.p.p.s and a further increase in frequency, upon a further increase in the depolarizing current strength. Note that not all m.e.p.p.s are reversed in amplitude (arrows in *C*). *D* shows the m.e.p.p. frequency increase which followed application of a 0·9 μA depolarizing current without using a bridge circuit. The muscle membrane potential fell by 0·5 mV, the change being complete at 1 min. The current application is indicated by the bar below the abscissa and the arrows in the graph. Recording was continuous, the C.R.O. sweep being stopped and the m.e.p.p.s recorded on moving film. Each point indicates the number of m.e.p.p.s recorded in that sec. Temperature, 33° C.

along the nerve to the electrode in the paraffin pool, thus hyperpolarizing the nerve terminals or in the reverse direction, depolarizing the terminals. The current in this circuit was monitored by a micro-ammeter; the values of current used ranged from 0·1 to 20 μA. When currents in excess of about 7–10 μA were used the nerve impulse was often blocked, but miniature end-plate potentials (m.e.p.p.s) could still be recorded. There was no evidence that current flow through the micropipette was affected by electrode polarization.

On two occasions the polarizing electrode (Fig. 1*B*) was located by using it to record extracellular m.e.p.p.s rather than foci of extracellular e.p.p.s. This procedure was abandoned when it was found that a current which depolarized nerve endings, as shown by an increase in the frequency of intracellularly recorded m.e.p.p.s, could also reduce the amplitude of the potentials (Fig. 2*B*) and at greater strengths even reverse their sign (Fig. 2*C*), without producing any change in the muscle fibre membrane potential.

Similar findings have been reported by Katz & Miledi (1965*a*) at the frog nerve muscle junction polarized in the same way. These effects were explained by an intense depolarization of a very localized post-synaptic receptor area so that the membrane potential of this particular area is driven beyond the m.e.p.p. equilibrium level. The unchanged membrane potential recorded by the intracellular electrode was attributed to the high membrane resistance under the depolarizing electrode so that little current entered the fibre during the intervals between m.e.p.p.s. Our results support this interpretation. It will be noted that some m.e.p.p.s are not reversed (arrows in Fig. 2*C*) and hence it must be concluded that the depolarized area was localized to only a portion of the compact receptor area.

In preliminary experiments it was found that large currents produced a potential drop in the bath which altered the post-synaptic membrane potential by as much as 10 mV. When the amplitudes of m.e.p.p.s were measured under these conditions, the mean amplitude varied linearly with the membrane potential and with the current; during a steady current, the amplitude did not vary with time. These changes in m.e.p.p. amplitude could be ascribed to passive alterations in the post-synaptic membrane potential. Changes in m.e.p.p. frequency and e.p.p. amplitude also occurred under these circumstances which were analogous to those described by Takeuchi & Takeuchi (1961) at the frog neuromuscular junction and ascribed by them to changes in the K concentration [K] round nerve terminals. In these experiments Takeuchi & Takeuchi (1961) passed inward currents across muscle fibre membranes and observed a fall in e.p.p. quantal content and also a fall in m.e.p.p. frequency which took 5–10 sec to approach its final value. The inward current was thought to be carried largely by K so that the [K] around nerve terminals would fall.

In our experiments the membrane was passively depolarized during current flow and presumably there would then be an outwardly directed flow of K ions which would increase the [K] around nerve terminals. Certainly our observations of m.e.p.p. frequency and e.p.p. amplitude were completely in accord with this view. For instance, Fig. 2*D* shows a striking example of the effects of a small (0·9 μA) current applied without using the bridge circuit (Fig. 1*D*) so that the muscle membrane potential fell by about 0·5 mV. It will be noticed that m.e.p.p. frequency was initially unchanged and then increased at what appeared to be an exponential rate. When the current was turned off the frequency fell exponentially to control levels. This behaviour—a delayed onset, apparent exponential increase and rapid decline upon turning off the current is characteristic of the effects of increasing and later decreasing the [K] round nerve terminals (Gage & Quastel, 1965) and may be contrasted with the sudden onset, maintained frequency and immediate fall upon cessation of current characteristic of the effects of depolarizing nerve terminals by current (del Castillo & Katz, 1954*a*; Liley, 1956*a*). Identification of the frequency changes as due to an increased [K] round nerve terminals was afforded by a recently found characteristic of the effects of a raised [K] on nerve terminals (J. I. Hubbard, S. F. Jones & E. M. Landau, unpublished observations). The finding was that the effects of increasing the bathing [K] from 5 to

16 mM, if repeated within 10–30 min, were much more rapid in onset and the effect on frequency for the same time of exposure was much larger than in the first trial. As Fig. 3 shows, exactly the same result was produced by three successive applications of 1·3 μA to the neuromuscular junction of the same muscle fibre shown in Fig. 2*D*. With each application the muscle fibre was depolarized by the same amount (4 mV), but the m.e.p.p. frequency change was more rapid and the final effect greater for each repetition.

As well as the m.e.p.p. frequency changes illustrated in Figs. 2*D* and 3, small increases in the quantal content of e.p.p.s were occasionally found in both curarized and Mg paralysed preparations which is a known effect of raising the extracellular [K] (Liley, 1956*b*; Takeuchi & Takeuchi, 1961). These effects were never very large and presented as an increase in the quantal content of e.p.p.s some time after the initial reduction brought about by the depolarizing current (see Results). Alternatively, in preparations paralysed with 12·5 mM-Mg in the bathing solution depolarizing currents were normally without effect (see Results), but occasionally a small increase in quantal content was detected during current flow.

To prevent a potential drop in the bath during the application of polarizing currents the bridge circuit shown in Fig. 1*D* was employed. A potentiometer was inserted across the

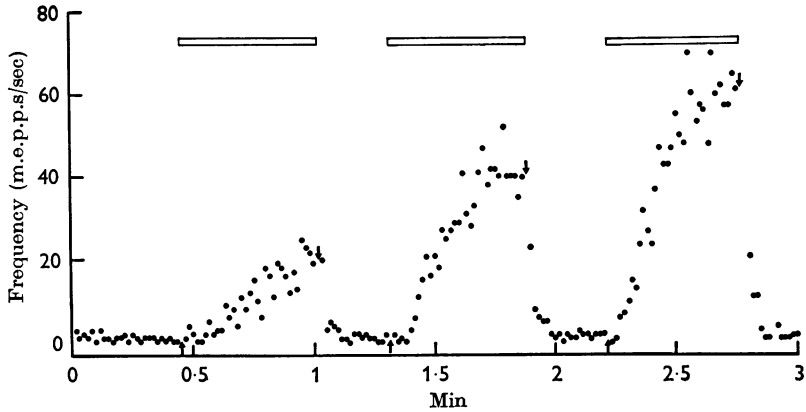

Fig. 3. The m.e.p.p. frequency changes following repeated application of the same depolarizing current in the absence of the bridge circuit. In the experiment illustrated 1·3 μA was applied 3 times as indicated by the horizontal bars in the graph (on indicated by arrows pointing upward, off by arrows pointing downward). The muscle membrane potential fell by 4 mV during each application, the change being complete in about 0·5 min. The circles show m.e.p.p.s/sec as in Fig. 2. The method of recording was also the same as in the experiment illustrated in Fig. 2 and the experiment was performed at a later time at the same junction. Temperature, 33° C.

polarizing circuit, with the sliding contact connected to the bath earth (Fig. 1*E*). By an appropriate adjustment of the position of the sliding contact, a ratio between the two sides of the potentiometer could be found at which no potential drop was detected by the recording electrode in the bath near the tip of the polarizing electrode. In effect, the tip of the polarizing electrode was at the same potential as the bath earth (Fig. 1*E*). With this procedure, the amount of current which crossed the muscle membrane was minimized, as shown by the lack of significant changes in muscle resting potential and in m.e.p.p. amplitude during the flow of large polarizing currents. Current flowing between the sliding contact of the potentiometer and the bath earth was monitored in a few experiments by a second micro-ammeter, as shown in Fig. 1. The current in this arm of the circuit never exceeded 1 μA when the bridge was balanced to eliminate changes in the post-synaptic membrane

potential. In any case, this current had no effect on the changes produced in the e.p.p. by currents in the polarizing circuit; for example, it was possible to reverse the direction of this current without influencing the e.p.p. changes.

The proper balancing of the bridge generally prevented any m.e.p.p. frequency changes of the type shown in Figs. 2*D* and 3 or any increases in e.p.p. quantal content. Occasionally however, even with the bridge balanced these effects could be detected after 10–30 sec of current passing, perhaps due to depolarization of, and K release from, nearby fibres.

In a few experiments, a Grass stimulator (Model S 4), isolated from earth, was employed to pass rectangular pulses of current with durations up to 1 sec and also direct current. The bridge circuit was balanced during the passage of the direct current, and the value of current was read on the micro-ammeter. Then pulses of current (200–1000 msec) were passed at the same settings of the potentiometers and of the Grass stimulator. Finally, the direct current was again passed, and the balance point and current value were checked.

In some experiments the presynaptic n.a.p. was recorded together with the extracellular e.p.p. before and during the application of polarizing current. In these experiments the methods of Hubbard & Schmidt (1963) were used. The preparations were paralysed by raising the magnesium concentration [Mg] of the bathing solution and extracellular recording was carried out using a low resistance 4 M-NaCl filled electrode, which replaced the intracellular electrode shown in Fig. 1*A*.

The temperature of the bathing fluid was kept constant by a thermistor controlled bath heater circuit. Experiments were carried out either at room temperature (22–25° C) or between 33–36° C. In any particular experiment the temperature was constant to within ±0·5° C.

RESULTS

Extensive investigations were made only in those junctions where the effects of depolarization were constant for the period of current flow. It was found that these actions could be divided into those actions which were apparent only during the period of current flow (*a*) and those actions which outlasted this period (*b*).

(*a*) *Effects accompanying current flow*

In curarized preparations e.p.p.s evoked during the passage of depolarizing current were reduced in amplitude (Fig. 4*A*, *B*). The effect was qualitatively similar at room temperature (22–25° C) and 33–36° C. This reduction in amplitude was accompanied by a small increase in latency, proportional to the strength of the applied current (Fig. 5*A*). The latency was increased for the first e.p.p.s evoked during current flow, generally remained constant at the new value for subsequent e.p.p.s, and reverted to the control value when the current ceased to flow. A progressive increase in the latency of consecutive e.p.p.s during current flow was occasionally found and was a precursor of complete neuromuscular block, reversed by turning off the current (cf. del Castello & Katz, 1954*a*).

Measurements of the rate of rise, of the time to peak and the half decay time had the same value in control e.p.p.s and e.p.p.s reduced in amplitude by depolarizing current. It may thus be concluded that no appreciable change in e.p.p. time course occurred during current flow.

Varying durations of current. At many junctions when the duration of application of a given current strength was varied, the depression of e.p.p. amplitude was of the same size regardless of the durations of current flow. For example, Fig. 4*A* shows the e.p.p.s evoked every 4 sec before, during and after the passage of 1·8 μA for 20 sec. During the current flow the e.p.p. amplitudes were depressed to some 56% of the control value (Fig. 4*B* open circles) and reverted to the control value when the current was

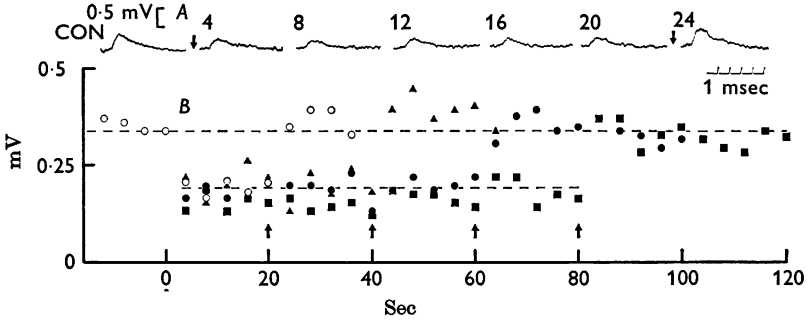

Fig. 4. The effect of varying the duration of a depolarizing current. The records in *A* show e.p.p.s evoked at 4 sec intervals before (CON) during and after (arrows) the passage of a 1·8 μA current for 20 sec. The full experiment is shown in *B* in which e.p.p. amplitudes are plotted in mV. After the 20 sec current application shown in *A* and in *B* (open circles) and the subsequent recovery of e.p.p. amplitude, the same current was passed for 40 sec (triangles). Again, after e.p.p. amplitudes had returned to the control value (upper dotted line) the current was passed for 60 sec (filled circles). After recovery from this application the effects of 80 sec of current flow (squares) were assessed. The lower dotted line in *B* shows the average e.p.p. amplitude during the first 20 sec current application. Arrows in *B* indicate the turning off of the current. It will be noticed that e.p.p. amplitudes during successive current applications are reduced by the same amount. The preparation was curarized and at room temperature.

turned off (Fig. 4*A*, second arrow and 4*B* first arrow). Figure 4*B* shows the e.p.p.s evoked at the same junction during the passage of the same current for 20 (open circles), 40 (triangles), 60 (filled circles) and 80 sec (squares), time being allowed between each application for the e.p.p. size to return to the control value. The dotted line in the graph shows the average depression of these e.p.p.s during the first 20 sec period (open circles). The amplitude of the e.p.p.s evoked during the subsequent longer applications (triangles, filled circles, squares) are plotted on the same graph and it can be seen that the depression remains of much the same size for even the 80 sec current application (squares).

In some experiments the depolarizing current was applied as a pulse lasting 200 or 1000 msec. The amplitude of e.p.p.s evoked during such pulses was often constant for a given current strength, whether evoked

early or late in relation to the pulse onset and was the same as after 4 sec of current flow of the same strength.

Varying strengths of current. At any particular junction e.p.p. amplitude during current flow was linearly proportional to the strength of the applied current over most of a wide range of current strengths. Figure 5 illustrates this relationship at a heavily curarized junction. The sample e.p.p.s (Fig. 5*A*) show a progressive increase in latency and reduction in amplitude as

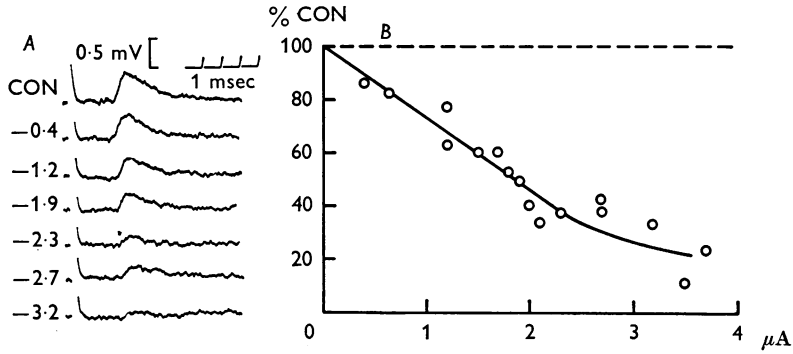

Fig. 5. The effect of variation of depolarizing current strength upon e.p.p. amplitude. In this experiment a range of depolarizing currents was applied for 8 sec. The records in *A* show representative e.p.p.s evoked during a selection of current strengths. Note the increased latency of e.p.p.s as the strength of the current increases. Stimulation was at 4 sec intervals throughout. In *B* the average amplitude of the pair of e.p.p.s evoked during the current is plotted as a percentage of the average amplitude of the five e.p.p.s evoked before the onset of current. This experiment and that illustrated in Fig. 4 were carried out at the same junction.

the current strength was increased. In the graph the continuous line indicating the relationship between applied current and amplitude deviates from linearity at the highest current strengths. This deviation was a feature of many experiments in which a large range of currents was employed indicating that the relationship between current strength and e.p.p. amplitudes was closer to an exponential form than the linear relationship deduced from consideration of a smaller range of current strengths (Hubbard & Willis, 1962*b*). Indeed such a deviation would be expected if the release of transmitter was an event which occurred with diminishing probability as the current strength was increased.

Effects of Mg. If the [Mg] of the bathing medium was raised to 12·5 m-moles/l. and the [Ca] kept at 2 m-moles/l., the reduction of e.p.p. amplitudes by depolarizing currents was completely prevented. A similar susceptibility to depression by a raised [Mg] of the bathing medium has been noted for the effects of depolarization, by current or a raised extracellular [K], upon m.e.p.p. frequency (del Castillo & Katz, 1954*a*; Liley, 1956*a*).

It was, however, possible to demonstrate effects of depolarizing current in preparations paralysed by less drastic alteration of the bathing Mg and Ca concentration ratio, indicating that the action of depolarization was not a decurarizing effect. For instance, Fig. 6*A* shows the e.p.p.s evoked at 2 sec intervals in a preparation paralysed by raising the [Mg] of the bathing medium to 6 m-moles/l. and lowering the [Ca] to 1 m-mole/l. It can be seen that during a current flow of 2·3 μA the depression was constant and recovery occurred within 2 sec of the cessation of current. In the

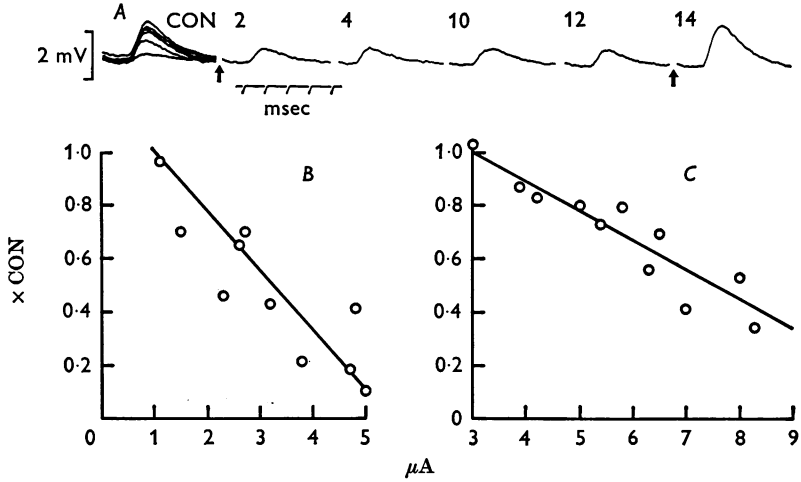

Fig. 6. The effect of depolarizing currents upon e.p.p. amplitudes in a Mg paralysed preparation. In *A* after the superimposed records (CON) had been taken a depolarizing current of 2·3 μA was applied for 12 sec. Stimulation continued at 2 sec intervals before, during and after the current flow. The sample e.p.p.s were elicited at the marked times. Arrows in *A* mark the onset and turning off for the current. In *B* the amplitudes of the first six responses during the depolarizing current taken from this and other experiments at the same junction are plotted as multiples of the average control amplitude against the strength of the applied current. In *A* and *B* the bathing solution contained 6 mM-$MgCl_2$ and 1 mM-$CaCl_2$ and the preparation was at room temperature. *C* illustrates another experiment. The graph was constructed as in *B* but the points are the average of 10–20 responses elicited at 4 sec intervals compared with the average control response and the preparation was paralysed with 15 mM-$MgCl_2$ and 4 mM-$CaCl_2$. The temperature was 36° C. The line in *C* is the calculated regression line of e.p.p. amplitude as a percentage of control upon current strength in μA.

graph (Fig. 6*B*) the mean amplitude of e.p.p.s elicited 2 sec after the onset of current is plotted as a fraction of the control size, against the current strength in μA. It will be noted that there was an apparent threshold in that only currents in excess of 1 μA were effective. The similar graph in Fig. 6*C* was constructed from the results of an experiment in which the e.p.p.s were larger and more constant in amplitude (Ca 4 mM, Mg 15 mM).

There was a more convincing linear relationship between e.p.p. amplitude reduction and current strength, the line drawn through the points being the regression line for percentage of control e.p.p. amplitude on current strength, but only currents greater than 3 μA were effective. The finding that a certain current strength was required before any effect on e.p.p. amplitude was detected is attributable, at least in part, to the variability of e.p.p. amplitudes in these preparations and the consequent difficulty in detecting a small reduction of e.p.p. amplitudes when using small samples. A further factor may have been the variation in the relationship of the polarized terminal and the polarizing electrode. Presumably the greater the separating distance, the larger was the current required to achieve a given amplitude reduction.

Proof of presynaptic action. The inhibition of the effects of depolarizing currents upon e.p.p. amplitude in the presence of a raised [Mg] was a strong indication that the effects were upon nerve terminals, i.e. presynaptic. Confirmation of this presynaptic locus of depolarizing current was afforded by measuring the quantal content of e.p.p.s before, during and after the passage of depolarizing current. All the usual means of measuring quantal content were employed, with the limitations imposed by the blockage of depolarizing action by Mg, and the difficulty of obtaining records during long periods of current flow which were free from post-synaptic effects. For instance, quantum size was measured from the variance of the amplitude of successive e.p.p.s during the application of current (del Castillo & Katz, 1954*b*; Martin, 1955). These experiments required long exposure to current before sufficient data accumulated for the analysis. Successful trials showing no change in quantum size, together with reduction of quantal content during current flow, were however carried out on several occasions.

One such experiment was that illustrated in Fig. 4, where there were 50 e.p.p. responses during the successive periods of current application. The mean quantum size estimated by pooling the estimates obtained from ten groups of five e.p.p.s was 5·6 μV. The control quantum size estimated from a similar number of groups was 5·2 μV. Evidently there had been no significant change in the post-synaptic effect of a quantum of transmitter and it must be concluded therefore that the depression of e.p.p. amplitudes demonstrated in Fig. 4 was due to a fall in the quantal content of these e.p.p.s.

Another method was the simultaneous measurement of m.e.p.p. and e.p.p. amplitudes during the passage of a depolarizing current. An example of the results of this method in a preparation paralysed by raising the [Mg] (to 6 mM) and lowering the [Ca] (to 1 mM) of the bathing medium is shown in Fig. 7. In this experiment a current of 9·3 μA was passed for

55 sec. The amplitude of the e.p.p.s evoked every second throughout this period was reduced on the average to two thirds of the mean control amplitude. The amplitude of spontaneously occurring m.e.p.p.s averaged over the same periods was only slightly reduced, in parallel with a slight fall in muscle membrane potential. Comparison of e.p.p. and m.e.p.p.

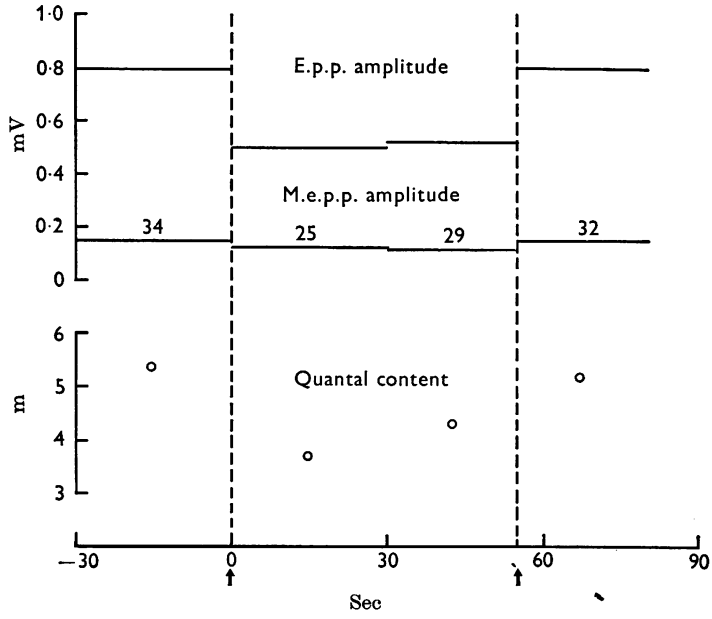

Fig. 7. The effect of depolarizing currents upon m.e.p.p. and e.p.p. amplitudes simultaneously observed. The preparation was paralysed with 6 mM-$MgCl_2$ and 1 mM-$CaCl_2$ and was at room temperature. E.p.p.s were evoked at 1 sec intervals and their amplitude shown in the upper lines on the graph was averaged for 25 or 30 sec periods. A current of 9·3 μA was passed for 55 sec, shown by the vertical dashed lines. Spontaneously occurring m.e.p.p.s were measured from the same recorded sweeps of the oscilloscope and their average amplitudes over the same periods for which the e.p.p. amplitudes were measured are shown by the lower continuous lines. Arrows mark the turning on and off of the current. The number of m.e.p.p.s in each period is shown above the line indicating the average amplitude in that period. In the lowest graph the average quantal content (m) of responses in each time block is shown (open circles). m was calculated from the ratio of e.p.p. to m.e.p.p. amplitudes and the points are placed in the middle of the relevant time period.

amplitudes showed the expected fall in quantal content (m). No significant m.e.p.p. frequency changes were provoked by the current in these experiments, nor would any be expected in the presence of an increased concentration of Mg (del Castillo & Katz, 1954*a*; Liley, 1956*a*).

A third method of measuring quantal content—the alteration of the bathing [Ca] and/or [Mg] to a level where there were intermittent failures

of the e.p.p. response to stimulation, and the calculation of m from the proportion of trials in which there was no response (del Castillo & Katz, 1954*b*) was also tried on several occasions. As expected (effects of Mg) no reduction of quantal content was ever found in the presence of Mg in concentrations of or above 12·5 mM. In a few experiments with 11 mM-$MgCl_2$ in the bathing solution a very sensitive indicator of quantal content changes was used. This was the examination of the e.p.p. responses to tetanic stimuli in Mg-paralysed preparations. Typically e.p.p. amplitudes increase progressively during a tetanus in such a preparation, and this increase is due to a progressive increase in the quantal content of e.p.p.s. (del Castillo & Katz, 1954*c*; Liley, 1956*c*). It was found that if the [Mg], was adjusted so that there were occasional failures to respond to the 1st few stimuli of a train of ten stimuli at 100/sec, then when current was applied the number of failures to all members of the train increased, that is, quantal content decreased. For instance, in one experiment there was one failure to the 1st and three to the second stimuli in ten trials of the train repeated at 1 sec intervals. During the passage of a 4·8 μA depolarizing current there were failures to respond to the 2nd, 3rd, 4th, 5th, 6th, 9th and 10th members of the train as well as an increased number of failures (6) to the 1st stimulus. After the current was turned off there was apparently some lingering effect for in the ten post-current trials the proportion of failures to the 1st five stimuli was still above the precurrent level. It could be concluded that depolarizing currents in the first 10 sec of applicatcn reduced the quantal content of responses.

The reduction of the quantal content (m) of responses evoked during depolarization of nerve terminals can be considered in the light of the concept (recently reviewed by Martin, 1966) that

$$m = np,$$

where n is the available store of quanta and p is the fraction released. It is not known how the terminal n.a.p. affects these parameters when it initiates the release of transmitter. Conventionally, following del Castillo & Katz (1954*c*), the terminal action potential has been assumed to effect p. In the present investigation, as a reduction in quantal content (m) had been demonstrated, it was of some interest to determine whether p or n had been altered. This was particularly the case as in a previous investigation hyperpolarizing current applied to nerve terminals had been found to increase n (Hubbard & Willis, 1962*a*).

The method of measuring n and p employed in the present investigation was to measure the magnitude of neuromuscular depression. E.p.p.s were evoked in curarized preparations by paired stimuli spearated by a 1 sec interval, the paired shocks being delivered every 5 sec. Under these cir-

cumstances as shown in Fig. 8*B* (filled circles) the second e.p.p. is smaller than the first, the mean ratio in the experiment of Fig. 8 being 84 % for the ten control observations. This diminution in size of the second e.p.p. is thought to be due to a decrease in the amount of transmitter liberated from the nerve endings because of a partial exhaustion of the store of

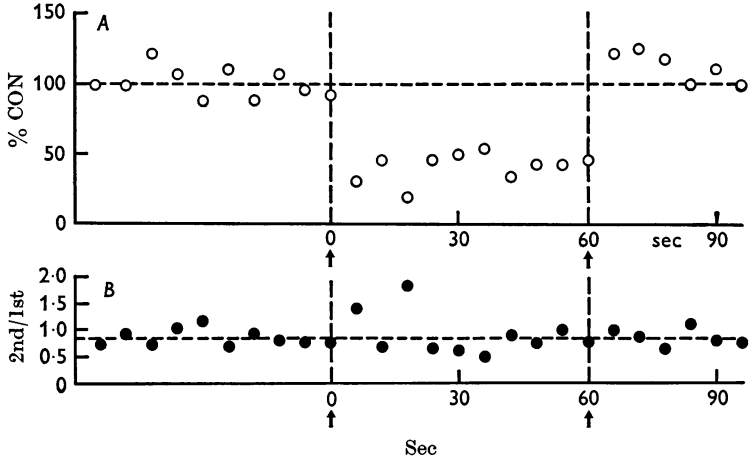

Fig. 8. The effect of depolarizing currents upon neuromuscular depression. Pairs of e.p.p.s separated by a 1 sec interval were evoked every 5 sec before, during and after the passage of a current of 4·1 μA for 60 sec. The amplitude of the first e.p.p. of the pair is shown in *A* (open circles) and the ratio of the amplitude of the second e.p.p. to that of the first e.p.p. in *B* (filled circles). The arrows and dashed broken lines mark the turning on and off of the current. In *A* the horizontal dashed line marks the average control e.p.p. amplitude. In *B* the similar line marks the average value for the ratio of amplitudes of the pair of e.p.p.s. The preparation was curarized and at room temperature.

available transmitter brought about by the first impulse. If depolarizing nerve terminals reduced the fraction of this store released by the first impulse then the second impulse would act on a larger store than in the control situation. The amplitude of the evoked e.p.p. would then be larger relative to the first e.p.p. of the pair, than in the control situation.

Alternatively, if the depolarizing current acted by reducing the store (i.e. reducing n), the depression would be unchanged (Liley & North, 1953; Otsuka & Endo, 1960). In the experiment illustrated a depolarizing current of 4·1 μA reduced the amplitude of the first e.p.p. (Fig. 8*A*, open circles) to about 50 % of the control size for the ten repetitions of the sequence; it recovered again when the current was switched off. The ratio of e.p.p.s (Fig. 8*B* filled circles) did not, however, alter greatly, the ratios being clustered around the dotted line indicating the control value of the ratio of amplitudes. It may be noted, however, that on two occasions shortly

after the onset of the current the second e.p.p. was larger than the first. This tendency to relief of the depression soon after the onset of current was noticed in nine of sixteen experiments. The effect was not large and for the sixteen experiments the mean depression (± 1 S.E.) of the second e.p.p. before the application of current was $13 \pm 2\%$. During the passage of the current it was $12 \pm 2{\cdot}5\%$. There was thus over-all no significant change in the amount of depression. The reduction in e.p.p. amplitude during the current ranged between 13 and 60% of the control amplitude. The possibility that the experiments were confounded by a predominance of small effects was eliminated by considering separately the seven experiments in which the depolarizing current brought about an e.p.p. amplitude reduction of 25% or more. There were again no significant changes in neuromuscular depression during the flow of depolarizing current.

These results suggest that the effect of a depolarizing current is to reduce n, the store of available quanta. The occasional finding of some relief of the neuromucular depression shortly after the current was turned on indicates that there was then also a decrease in p, the probability of release.

(*b*) *Prolonged effects of depolarizing current flow*

During the present and a previous series of experiments concerned with the application of hyperpolarizing and depolarizing currents to nerve terminals (Hubbard & Willis, 1962*a*) certain anomalies occurred. Hyperpolarizing currents occasionally failed to cause the expected progressive increase in e.p.p. amplitudes (Hubbard & Willis, 1962*a*) while depolarizing currents were occasionally equally ineffective in causing the expected reduction in e.p.p. amplitudes (Figs. 4–8). These effects were not caused by electrode polarization for the current flowing through the electrodes was continuously monitored (see Methods). A systematic investigation of these anomalies revealed a new phenomenon, the mutual inhibition of the appropriate effect of one direction of current after suitable conditioning by passage of current in the other direction.

Figure 9 illustrates the experiments in which the inhibition of depolarization by previous hyperpolarization was uncovered. In this experiment in a curarized preparation hyperpolarizing currents of 1·6–6·6 μA had been passed for periods of 40–90 sec, with similar periods of time in between applications. Progressive increases in e.p.p. amplitude of the order of 50–100% occurred as previously described (Hubbard & Willis, 1962*a*). When a similar range of depolarizing currents was applied, there was no effect upon e.p.p. amplitudes. Figure 9*A* and the graph in 9*C* show the lack of effect of a current of 5·0 μA applied for 80 sec.

The range of depolarizing currents was then repeated again, and now

currents in excess of 4 μA had a small depressant effect upon e.p.p. amplitude. A third application of the same range of currents had much more effect. For instance, the same depolarizing current of 5·0 μA, which on first application had no effect on e.p.p. amplitude (Fig. 9*A* and graph 9*C*), now depressed e.p.p. amplitudes to 60 % of the control value (Fig. 9*B*) there being a post current exaltation of e.p.p. amplitude when the current was turned off.

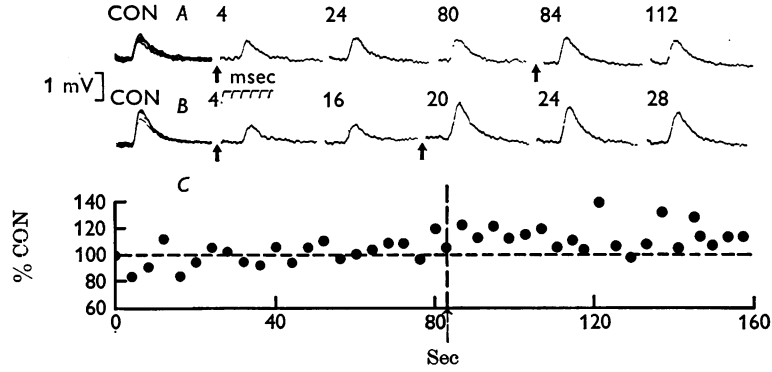

Fig. 9. Effect of previous hyperpolarization upon depolarizing action. The sample records in *A* were taken at the indicated times after a depolarizing current of 5·0 μA was turned on (first arrow) and off (second arrow). In *C* the amplitudes of all the e.p.p.s, elicited at 4 sec intervals during current flow, are shown as multiples of the control amplitude (*A* CON). *B* shows sample records from the same junction several min later when the same current was reapplied (arrows). There was then a considerable depression of e.p.p. amplitudes. The preparation was curarized and at room temperature (23·5° C).

At the conclusion of the third application of depolarizing current in the experiment illustrated in Fig. 9, a range of hyperpolarizing currents was again applied. There was no hyperpolarizing effect upon e.p.p. amplitude. Obviously depolarizing and hyperpolarizing currents were mutually inhibitory in their actions upon e.p.p. amplitude.

A systematic investigation was then made of this interaction between depolarizing current and hyperpolarization (Fig. 10). In the experiment a depolarizing current of 3·4 μA had been passed for 40 sec on two ocassions separated by 48 sec. Forty seconds later a hyperpolarizing current of 2·2 μA was passed for 40 sec. In Fig. 10*E* (dotted circles), the small increase in e.p.p. amplitudes is represented. A further 136 sec later the procedure was repeated. The current now had a greater effect (Fig. 10*E*, half filled circles). Two hundred and forty-four seconds later a further application of the same current (Fig. 10*E*, open circles) had a yet greater effect. Three-hundred and seventy six seconds later the effect reached even

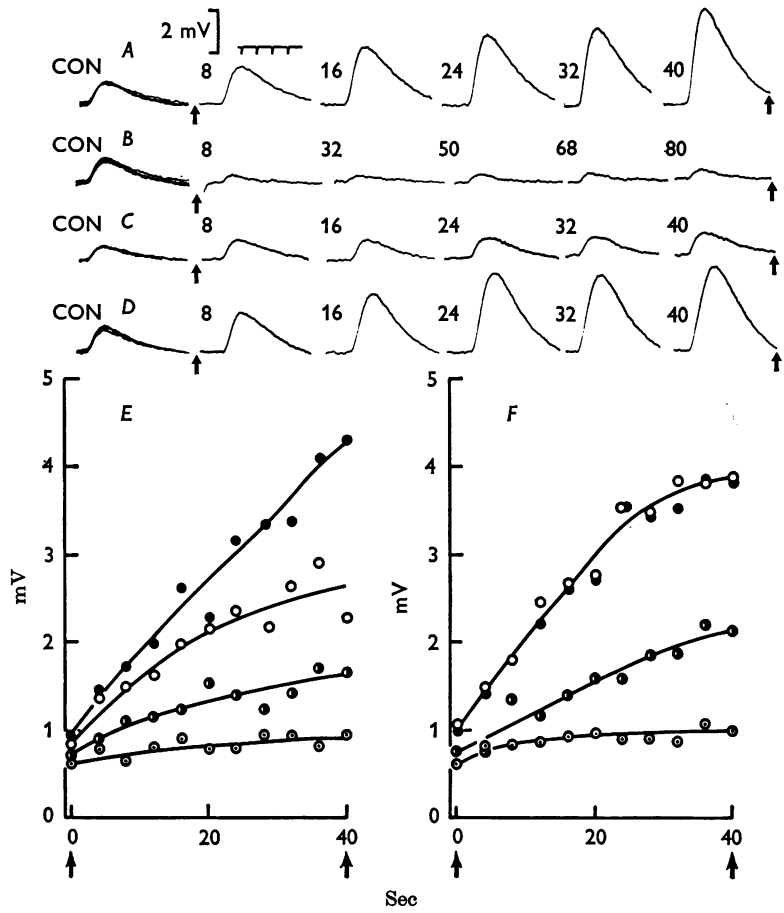

Fig. 10. Inhibition of hyperpolarizing effects by previous depolarization. These records and graphs illustrate an experiment in which the sequence, depolarization and then repeated hyperpolarizations, until the hyperpolarizing effect upon e.p.p. amplitude was approximately constant was applied twice. The first sequence is shown in *E*. In *E* the amplitudes of e.p.p.s (mV) elicited during the application of hyperpolarizing current (2·2 μA) applied for 40 sec, are shown 40 sec (dotted circles), 136 sec (half-filled circles), 240 sec (open circles) and 376 sec (filled circles) after the first application of depolarizing current. Sample records from this last application of current are shown in *A*. Immediately after the effects shown in *A* had passed off a second application of depolarizing current (3·2 μA for 80 sec) was made. Sample records are shown in *B*. In *F* the effect of the 2·2 μA hyperpolarizing current is shown at various times after the passage of depolarizing current. The current was passed 48 (dotted circles), 124 (half-filled circles), 272 (open circles) and 502 (filled circles) sec later. The sample records in *C* were taken from the application at 48 sec and in *D* from the application at 272 sec. Arrows in *A*, *B*, *C* and *D* indicate turning on and off of current. The preparation was curarized and at room temperature.

greater heights when the same current was applied (Fig. 10*E* filled circles). Sample pictures of e.p.p.s in this most successful application of current are shown in Fig. 10*A*. At this point, when the e.p.p. amplitudes had declined to the control size, a depolarizing current, again of 3·4 μA, was passed for 80 sec, causing the profound depression of e.p.p. amplitudes partly illustrated in Fig. 10*B*. The hyperpolarizing current, again of 2·2 μA, was applied 48, 124, 272 and 502 sec later on each occasion for 40 sec. The sample pictures (Fig. 10*C*) and graph (Fig. 10*F*, dotted circles) show the very small effect found at the first application at 48 sec. Later applications at 124 sec (Fig. 10*F* half circles) and 272 sec (Fig. 10*D* and 10*F*, open circles) were progressively more effective, the effect at 502 sec (Fig. 10*F*, filled circles) being the same as that at 272 sec.

In the absence of a preceding depolarization, the effects of a given value of hyperpolarizing current are readily repeatable (Hubbard & Willis 1962*a*, Fig. 3). It must therefore be concluded that after 80 sec of depolarization the hyperpolarizing current did not reattain its maximum effect for about 4 min. Other experiments indicated that this effect of depolarizing current could last even longer than the 4 min found in this experiment. For instance, at another junction in a curarized preparation equal currents in opposite directions were used. After 4·2 μA had been passed in the depolarizing direction for 20 sec, trials were made with 4·2 μA in the hyperpolarizing direction at 72, 132, 184 and 244 sec after the depolarizing current. Only with the last application was the hyperpolarizing current as effective as before conditioning. A further application of depolarizing current now blocked the action of the hyperpolarizing current, applied every 2 min for 20 sec, for between 6 and 8 min.

It was also possible to demonstrate the inhibitory effect of previous depolarization in Mg-paralysed preparations even though the [Mg] was such that there was no actual depression of e.p.p. amplitude by the depolarizing current. A striking illustration of this ability to abolish the effect of hyperpolarizing current in a Mg-paralysed preparation was found in the experiment illustrated in Fig. 11. In this experiment e.p.p.s were evoked by nine stimuli at 100 sec. After the control records had been taken (Fig. 11*A*), a hyperpolarizing current of 6·0 μA was applied for 60 sec. The sample records show the very greatly increased amplitude of all the e.p.p.s during the current (Fig. 11*B*, *C*) and the 30 sec time course of the return to the control level (Fig. 11*D*, *E*). In contrast, after a depolarizing current of the same strength had been applied for 40 sec, the same hyperpolarizing current was ineffective when applied 40 sec later (Fig. 11*F–J*). Later applications were fully effective, as was a higher strength of current.

Other experiments showed that the after-effects of hyperpolarization upon e.p.p. amplitude (Hubbard & Willis, 1962*a*) could be greatly re-

duced in amplitude and duration by the application of a depolarizing current. In one experiment, for instance, the preparation was paralysed with 11 mM-$MgCl_2$ in the bathing solution. When a current of 4·0 μA was applied, e.p.p. amplitudes increased until, after 20 sec, the amplitude was more than 10 times the control size. The current was then turned off and

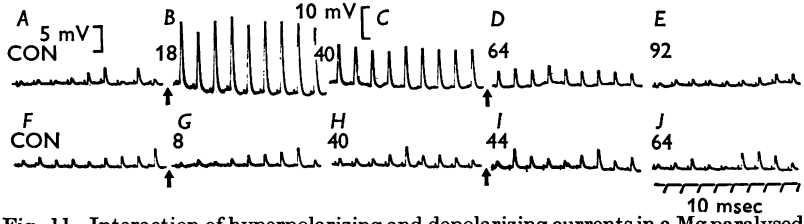

Fig. 11. Interaction of hyperpolarizing and depolarizing currents in a Mg paralysed preparation. Records show e.p.p. responses to a tetanus of nine impulses at 100 sec before (*A*) and during (*B*, *C*) the application of a 6·0 μA current for 60 sec. *D*, *E* show recovery of control amplitude. N.B. 10 mV calibration applies to *C* and *D*, 5 mV to *A*, *B*, *E* and *F*–*J*. In *F*–*J* the sample records are taken from a similar shorter current application at the same junction as *A*–*E* but 40 sec after a depolarizing current had been applied. Mg, 11 mM. Temperature 33° C.

e.p.p. amplitudes, evoked every 2 sec, declined to reach the control size in about 30 sec. The hyperpolarizing current was then reapplied with the same effectiveness but during the first 6 sec after current flow a depolarizing current of 4·0 μA was applied. The decline in e.p.p. amplitude was very rapid, the control level being reached 8 sec after the turning off the hyperpolarizing current (i.e. 2 sec after turning off the depolarizing current). Thirty-two seconds later the hyperpolarizing current was applied again. Again it was very effective in increasing e.p.p. amplitudes and after the turning off of the current the e.p.p. amplitudes took some 30 sec to reach the control level.

It may thus be concluded that depolarizing currents, though not affecting e.p.p. amplitudes *per se* yet could block any effect of hyperpolarizing current. This blocking action appeared to have a duration determined by the duration of the depolarizing current and could be overcome by increasing the strength of the hyperpolarizing current. As both the effects of depolarizing and hyperpolarizing current upon e.p.p. amplitude are mediated by changes in quantal content their interaction must also involve quantal content changes. It was possible to demonstrate this directly by examining quantal content in high Mg containing solutions. Hyperpolarizing nerve terminals under these conditions can still increase the quantal content of responses and this is manifest as a reduction of the proportion of stimuli which fail to elicit an e.p.p. response (Hubbard & Willis, 1963). It was possible by previous passage of a depolarizing current to completely prevent this hyperpolarizing action.

The presynaptic n.a.p. during depolarization. The reduction of the excitatory post-synaptic potential during presynaptic inhibition has been attributed to a reduction of the amplitude of the n.a.p. in the nerve terminal due to depolarization of the terminals. It was therefore of interest to examine the presynaptic n.a.p. at the neuromuscular junction during the passage of depolarizing current.

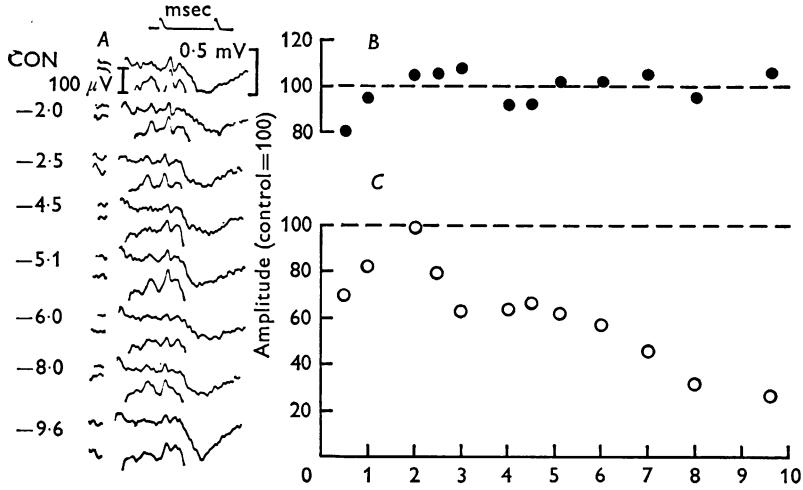

Fig. 12. The effect of depolarizing currents upon the amplitude of e.p.p.s and of presynaptic n.a.p.s in solutions containing 12 mM-Mg. In *A* sample records of e.p.p.s and presynaptic n.a.p.s are shown. The n.a.p. and e.p.p. were recorded at high gain (lower trace of pair, 100 μV calibration) and also at a lower gain (upper trace, 0·5 mV calibration). N.a.p. amplitudes were measured from the lower trace and e.p.p. amplitudes from the upper trace. The sample records were chosen because the amplitude of the n.a.p. was close to the mean size found during the application of the stated depolarizing currents. The filled circles in *B* indicate the mean amplitude of the 10–20 e.p.p.s recorded sequentially at 1 sec intervals during the application of the current compared with the pooled mean amplitude of the 10 e.p.p.s recorded both before and after the application of current. The control level (100 %) is indicated by the horizontal dotted line. The open circles in *C* indicate the mean amplitude of the presynaptic n.a.p.s recorded simultaneously with the e.p.p.s. The mean n.a.p. amplitudes during the application of current are expressed as a percentage of the mean amplitude of the similar number of n.a.p.s recorded before and after the current application. N.a.p. amplitudes were measured between the positive and negative peaks. The temperature was 35° C.

As Fig. 12 shows, the amplitude of the extracellularly recorded presynaptic n.a.p. was reduced and its latency increased during the passage of depolarizing currents. The reduction and the concomitant increase in latency appeared upon the turning on of the current and were not progressive. The amplitude and latency returned to control values immediately after the current was turned off. The magnitude of the reduction of

amplitude could be shown to be proportional to the magnitude of the applied current (Fig. 12*A*, *C*) when the effects of a range of currents were explored. The small size of the potentials made it necessary to average 10–20 records to obtain a reliable measure of the effects of current. Samples even of this size showed some fluctuation in mean amplitude. The mean n.a.p. amplitude for the experiment of Fig. 12 in the absence of current appeared constant throughout the experiment, but the mean of individual groups of ten ranged between 109 and 73 % of the mean of all such samples. Presumably this variation is responsible for the fluctuation of average spike amplitude in Fig. 12*C*, in the presence of depolarizing currents 0·5, 1 and 2 μA. With larger currents and presumably larger mean effects a more consistent relationship was obtained.

The variability of the recorded n.a.p. amplitude again raised the question (cf. Hubbard & Schmidt, 1963; Katz & Miledi, 1965*b*) whether part of the variability in e.p.p. amplitude (and quantal content) in the presence of 12 mM-Mg might be due to a variation in spike amplitude. The present experiments provided a further opportunity for testing this relationship in experiments where the mean n.a.p. amplitude remained constant over the period of examination and the extracellularly recorded e.p.p. had little contamination from 'non specific' e.p.p.s. For this purpose long (2 min) series of e.p.p. and n.a.p. were recorded at 1 sec intervals at the same high gain. The relationship between e.p.p. and n.a.p. amplitude was examined statistically. The correlation coefficient (r) in one experiment was 0·1704 ($n = 123$) and as $P\{r < 0{\cdot}1704\}$ was 0·03 this experiment gave reasonable evidence of correlation. The other experiment however gave $r = 0{\cdot}0937$ ($n = 125$) which is not significant at the 5 % level. The question thus remains undecided.

In the experiment illustrated there was no change in e.p.p. amplitude during the application of depolarizing current (Fig. 12*B*) to parallel the reduction in n.a.p. (Fig. 12*C*). This was presumably because the preparation was paralysed with 12 mM-$MgCl_2$. In similar experiments with preparations paralysed with 11 mM-$MgCl_2$ there was sometimes a parallel reduction of e.p.p. amplitude and n.a.p., particularly with the larger currents of a range of currents. In one such experiment, for instance, currents of 2·1, 2·8 and 4·0 μA were without consistent effect upon e.p.p. amplitude although spike potential amplitudes fell. Application of 5·1 μA caused a 70 % fall in average e.p.p. amplitude and a similar fall in average n.a.p. amplitude. An even greater fall in both amplitudes was caused by 6 μA, but after 4 sec nerve block ensued.

Interestingly, previous depolarization of nerve terminals did not prevent a hyperpolarizing current applied immediately after the termination of depolarization, causing the expected increase in spike size (Hubbard

& Schmidt, 1963). The changes in e.p.p. amplitude were, however, prevented as previously demonstrated (Fig. 9). For instance, a hyperpolarizing current of 9·6 μA was applied immediately after the experiment illustrated in Fig. 12 was completed. Insignificant changes in e.p.p. amplitude occurred during the 10 sec period of application. Five minutes later application of the same current produced an immediate and progressive increase in amplitude culminating within 5 sec in the development of a muscle fibre action potential. In both cases there was the same increase in the presynaptic spike potential amplitude. In other experiments the effects of a range of hyperpolarizing currents upon n.a.p. amplitudes were similar immediately before and immediately after depolarizing experiments of the type illustrated in Fig. 12.

DISCUSSION

The reduction of e.p.p. amplitudes during the focal application of depolarizing currents to nerve terminals (Figs. 4–8) has also been reported at the frog neuromuscular junction by Russian workers using the same method of polarization (Vladimirova, 1963). Unfortunately their report is less extensive than our own preliminary report (Hubbard & Willis, 1962*b*). It does, however, suggest that the previous failure to find reduction of e.p.p. amplitudes during depolarization of frog terminals (del Castillo & Katz, 1954*a*) was due to technical difficulties. The occasional increase in e.p.p. amplitudes upon strong depolarization found in this latter investigation may perhaps be accounted for by K release from depolarized muscle fibres.

The results of the present investigation are fully compatible with the proposed hypothesis for presynaptic inhibition. During depolarization of the terminals the n.a.p. is reduced in amplitude (Fig. 12) and the number of quanta of transmitter released is reduced (Fig. 7), exactly as has been reported during presynaptic inhibition in the feline spinal cord (Eccles, Schmidt & Willis, 1963; Kuno, 1964).

Depolarizing currents have been applied to the giant synapses of the squid (Hagiwara & Tasaki, 1958; Takeuchi & Takeuchi, 1962; Bloedel, Gage, Llinas & Quastel, 1966; Katz & Miledi, 1966) and to the cat spinal cord (Eccles, Kostyuk & Schmidt, 1962). In all these experiments intracellularly recorded post-synaptic potentials were reduced in amplitude during the current flow and in the squid experiments, as in the present investigation, it was also possible to correlate this reduction with a diminution in the amplitude of the presynaptic action potential.

The recent demonstration that quantal release in tetrodotoxin poisoned preparations *in vitro* can be varied by varying the intensity of a brief

depolarizing pulse applied to nerve terminals (Katz & Miledi, 1967), strongly suggests that the observed reduction in n.a.p. amplitude during depolarizing current flow is directly responsible for the reduction of transmitter release observed in the present and previous investigations. This conclusion then implies that the reduction in the number of quanta in the available presynaptic store (n) during depolarization (Fig. 8) is brought about by a reduction in n.a.p. amplitude. Further investigations upon the effects of depolarizing current upon n and p (the fraction of n released by an impulse) during tetanic stimulation confirm the reduction of n shown in Fig. 8 (J. I. Hubbard & E. M. Landau, unpublished observations) and by implication the correlation of n and spike amplitude. Our present results thus support our preliminary conclusion that the depolarizing current acts by reducing the amount of transmitter available for release by nerve impulses (Hubbard & Willis, 1962*b*), but our suggestion at that time, that the reduction might be due to movement of vesicles away from the nerve terminal membrane now appears untenable. Indeed, there is now evidence that in the presence of 20 mM-KCl, vesicle numbers in nerve terminal are decreased (Hubbard & Kwanbunbumpen, 1968) yet it is known that n is increased (Parsons, Hofmann & Feigen, 1965; J. I. Hubbard, S. F. Jones & E. M. Landau, unpublished observations). It thus appears unlikely that n can be identified with vesicle numbers.

Some of the present results raise questions which require for their answer an intimate and as yet unavailable knowledge of what Katz & Miledi (1966) have called the 'electrosecretory' process. One concept of this process is that a depolarization of nerve terminals allows the inward movement of a charged Ca complex to initiate release (Katz & Miledi, 1966; Hubbard, Jones & Landau, 1967). Mg ions appear to compete with Ca for this complex, but are ineffective in initiating release (Jenkinson, 1957). The inability of depolarization of nerve terminals to affect transmitter release in the presence of high [Mg] may thus arise because most or all of the complex is in the Mg form which is ineffective in the release process.

The mutual inhibition of the action of depolarizing and hyperpolarizing currents demonstrated in Figs. 9, 10 and 11 cannot be present at explained by such schemes. Indeed this apparent 'memory' of previous depolarization or hyperpolarization has not previously been described at any synapse. A methodological artifact appears to be excluded by the finding that the terminal n.a.p. is affected by current while e.p.p.s are unaffected (Results). A close parallel, although of much shorter time course, is the prolongation of n.a.p.s in frog ganglion cells treated with tetraethyl ammonium (TEA) salts which can be influenced by preceding polarization. For instance, a depolarization applied for a brief period as long as

100 msec before the n.a.p., will shorten the n.a.p. duration (Riker, 1964). Riker explained his striking finding by suggesting that depolarization produced an alteration in the rate of dissociation of a TEA membrane complex. He further suggested that TEA acted by deplacing Ca. Dissociation of the TEA complex then involved restoration of Ca. The much longer effects found in the present investigation (Fig. 10) probably do not involve Ca for we have found that varying the [Ca] of the bathing medium does not greatly affect the absolute magnitude of the e.p.p. amplitude increase produced by a given strength and duration of hyperpolarizing current (J. I. Hubbard & W. D. Willis, unpublished results). This suggests that Ca is not directly involved in the changes produced by hyperpolarization. Furthermore, there is good evidence that Ca changes affect p, the fraction of transmitter released by a nerve impulse (Elmqvist & Quastel, 1965), rather than the magnitude of the available quantal store, which is the effect of both hyper- and depolarizing currents applied to nerve terminals (Hubbard & Willis, 1962*a*, and Fig. 8 this investigation).

An alternative to persistent membrane changes is Elul's (1966) suggestion that a hyperpolarizing current in the vicinity of a synapse would attract the ionic envelope of the nerve terminal and constrict the synapse. Depolarizing currents would have the opposite effect. This explanation would require that the synaptic constrictions altered the amount of transmitter reaching the subsynaptic membrane, which seems plausible, (Eccles & Jaeger, 1957) and that the constrictions or widenings had a long relaxation time, during which currents in the opposite sense to which they had responded, were ineffective. This hypothesis if speculative is at least testable.

One practical result of this strange finding of interaction of the effects of depolarizing and hyperpolarizing current is that great caution must be used in identifying behavioural changes induced or prevented by long lasting hyper- and depolarizations of animal cortices (e.g. Morrell, 1961*a*, *b*; Albert 1966) with synaptic mechanisms. It is clear that following such treatment a variety of pre- and post-synaptic results may be expected at synapses. For instance, depolarization would release K, thus perhaps facilitating synaptic transmission (Figs. 2, 3), as well as having a direct depressant effect (Figs. 4, 8). Furthermore, it would appear that a hyperpolarizing current applied after depolarization might have a different effect from the same current applied beforehand (Figs. 10, 11).

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