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THE EFFECT OF ELECTRIC POLARIZATION OF THE SPINAL CORD ON CENTRAL AFFERENT FIBRES AND ON THEIR EXCITATORY SYNAPTIC ACTION

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Several factors influence the amount of transmitter that is liberated when an impulse propagates down to a presynaptic nerve terminal (Eccles, 1961). There is much indirect evidence (Lloyd, 1949; Eccles & Rall, 1951; del Castillo & Katz, 1954*a*; Liley, 1956; Eccles & Krnjević, 1959*a*, *b*) that one of the important factors is the amplitude of the presynaptic action potential. But more important is the direct evidence that has been produced by taking advantage of the very large size of both the presynaptic and post-synaptic components of the giant synapses of the squid stellate ganglion (Hagiwara & Tasaki, 1958; Takeuchi & Takeuchi, 1962). The presynaptic spike potentials were measured by an intracellular electrode close to the synaptic region, and the excitatory post-synaptic potential (EPSP) was recorded by a second micro-electrode inserted post-synaptically. Polarizing current was applied to the presynaptic fibre by a third intracellular electrode and the changes in the presynaptic spike potential thereby induced were found to produce profound changes in the size of the EPSP. Qualitatively similar results were obtained at the neuromuscular junctions of frog (del Castillo & Katz, 1954*a*) and rat (Hubbard & Willis, 1961) skeletal muscle, the transmitter output in both cases being increased by hyperpolarization and in the rat being decreased by depolarization of the presynaptic endings. There is also an indication of a similar mechanism in the cat's spinal cord (Eccles & Krnjević, 1959*b*), and in addition many observations justify the hypothesis that phenomena like 'post-tetanic potentiation' (Lloyd, 1949; Eccles & Krnjević, 1959*b*), and 'presynaptic inhibition' (Eccles, Eccles & Magni, 1961; Eccles, Magni & Willis, 1962; Eccles, 1961) are due, respectively, to hyperpolarization and depolarization of the presynaptic endings. The experiments here described lend further support to the hypothesis that the size of the presynaptic spike potential determines the amount of transmitter output at excitatory synapses in the cat spinal cord, by demonstrating (*a*) that the passage of a polarizing current across the spinal cord produces membrane potential changes of primary afferent fibres and (*b*) that these changes are accompanied by changes in the monosynaptic excitation of motoneurones.

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METHODS

The experiments were performed on the lumbar spinal cord of lightly anaesthetized (pentobarbital sodium) cats with spinal cords severed at the second lumbar segment. The general stimulating and recording arrangements were the same as those of concurrent investigations described elsewhere (Eccles, Kostyuk & Schmidt, 1962*a*, *b*).

The polarizing current was applied through two silver–silver chloride electrodes covered by a thin layer of cotton soaked in agar Ringer Locke solution. The dorsal electrode was situated medial to the dorsal root entrance; the ventral electrode was just lateral from the ventral roots. In order to minimize the blocking of impulse conduction in the dorsal parts of the afferent fibres and also to increase the polarizing effect of the current on the afferent terminals in the motor nucleus region, the dorsal electrode had a much larger surface than the ventral one (Fig. 1), so that the current density was higher in the ventral part of the spinal cord than in the dorsal. Batteries provided the current source and the current was turned on and off manually, being continued for several seconds during each current step. On a few occasions current pulses with durations of 0·3–1·0 sec were delivered from a Grass Stimulator. Currents up to 1 mA have been passed in both directions without appreciable polarization of the electrodes. Usually, however, currents in excess of 0·5 mA have been avoided because such currents caused distortion of the results by conduction block in the primary afferent fibres. A similar method of polarization has previously been used for studying changes in ventral root potentials (Semonyutin, 1960) and monosynaptic reflexes (Frank & Gerard, 1951).

When testing the excitability of primary afferent fibres in the spinal cord, the corresponding ventral roots were severed, and brief pulses (0·2–0·6 msec) from a Grass Stimulator were applied through a glass micro-electrode filled with 4 M-NaCl and inserted in close proximity to the afferent fibres. The antidromic spikes recorded from the appropriate peripheral nerves gave a measure of the number of afferent fibres excited by a stimulus of any particular strength (cf. Wall, 1958). As a consequence of the applied polarizing current there were changes in excitability of the primary afferent fibres, which were signalled by alterations of the size of the antidromic spike produced by a test stimulus; the percentage change in excitability was measured as described by Eccles, Magni & Willis (1962).

RESULTS

Effects of polarization on the membrane potential of central afferent fibres

Figure 2 B shows intracellular records from a primary afferent fibre at about 0·5 mm below the dorsal surface of the cord. Currents of varying strengths in one or the other direction were applied between the two polarizing electrodes (Fig. 1), each current step lasting for several seconds. The amplitude of the spike potential followed almost linearly the amount

of polarizing current (Fig. 2A), except for the very low value at −0·5 mA. Presumably this current caused blockage of the impulse at a node just before it reached the site of impalement. The membrane potential changes which were associated with these changes in spike potential amplitude were more difficult to evaluate, since they were distorted by the large field potentials created by the current; therefore, the spike potential was used as an indicator of the changes in membrane potential.

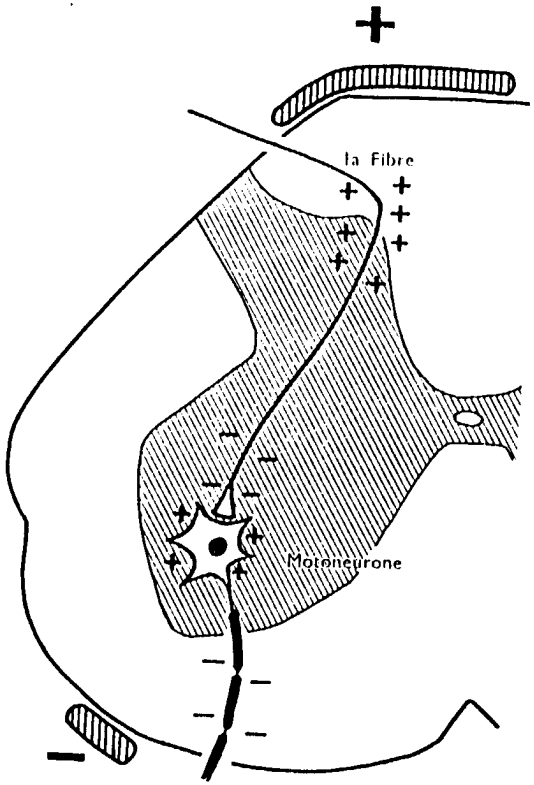

Fig. 1. Schematic diagram illustrating the approximate relative sizes and the situations of the two polarizing electrodes on the spinal cord. In addition, there are indicated the changes in membrane potential which occur in different parts of a primary afferent fibre and a motoneurone when there is a polarizing current with the dorsal electrode positive. The positive signs (+) indicate an increase in membrane potential (hyperpolarization), the negative signs (−) a decrease (depolarization). The membrane potential changes are produced by the entrance and exit of that fraction of the applied current that flows along the core conductors both of the primary afferent fibres and of the motoneurone and its axon. Reversing the direction of the polarizing current would result in the opposite membrane potential changes.

It would be expected that changes in the membrane potential of a nerve fibre would be accompanied by changes in the threshold for electrical stimulation (see Wall, 1958). The specimen records of Fig. 3 were taken after a NaCl-filled glass micro-electrode was inserted 0·5 mm below the cord dorsum in the region of the L7 root entrance zone so that it was in an extracellular position, but adjacent to primary afferent fibres. Brief stimulating pulses (0·2 msec duration) of increasing strength (Fig. 3A) were then applied through the micro-electrode and the antidromic spikes were recorded in the nerve to the flexor digitorum longus and plantaris muscles. Thereafter the pulse strength was kept constant at 80 V and the cord was polarized by application of steady currents of several seconds duration between the polarizing electrodes. The electrode on the cord dorsum was negative in series B and positive in C. D shows the approximate changes

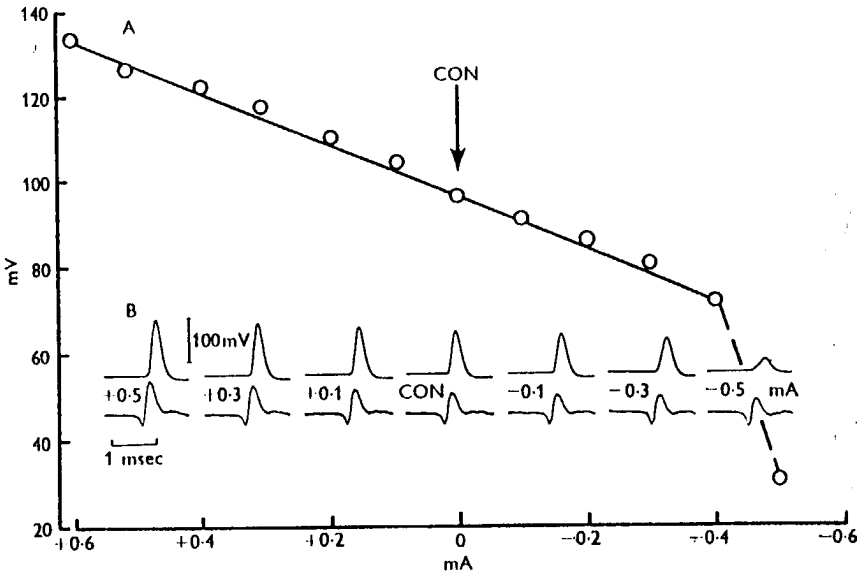

Fig. 2. Changes in spike potential amplitude which the polarizing currents produce in a primary afferent fibre. The afferent fibre from the nerve to flexor digitorum longus muscle was impaled by a micro-electrode at about 0·5 mm below the dorsal cord surface. In A the amplitude of the spike potential (in mV) is plotted against the size and direction of the polarizing current (in mA). The signs (+) and (−) indicate the polarity of the dorsal surface electrode (see Fig. 1). B shows specimen records of the series plotted in A. The current strength is indicated for each record of B, CON being the control response in the absence of a polarizing current. Such controls were taken before and after both the + and − polarizing series. The upper traces in B are the intracellular spike potentials, the lower the cord-dorsum potentials recorded in the vicinity of the micro-electrode at the zone of the dorsal root entry. The voltage calibration is for the intracellular records only.

in excitability, calculated for the complete series partly shown in B and C by using the calibration responses of series A (cf. Eccles, Magni & Willis, 1962).

It is observed that, when the polarizing current was applied by making the electrode on the cord dorsum positive, there was a decrease in excitability of the primary afferent fibres down to as low as 40 % of the control; hence it can be inferred that there was a considerable increase in membrane potential, an effect which correlates well with the increase in spike potential observed under similar conditions in Fig. 2. Similarly, when the dorsal cord electrode was negative, the increase in excitability of up to 150 % of the control indicates a depolarization that correlates well with the decreased spike to the right of Fig. 2. It can be concluded that when currents are passed in one or other direction across the cord the excitability changes can be employed as an index of changes in the membrane potential (cf. Wall, 1958).

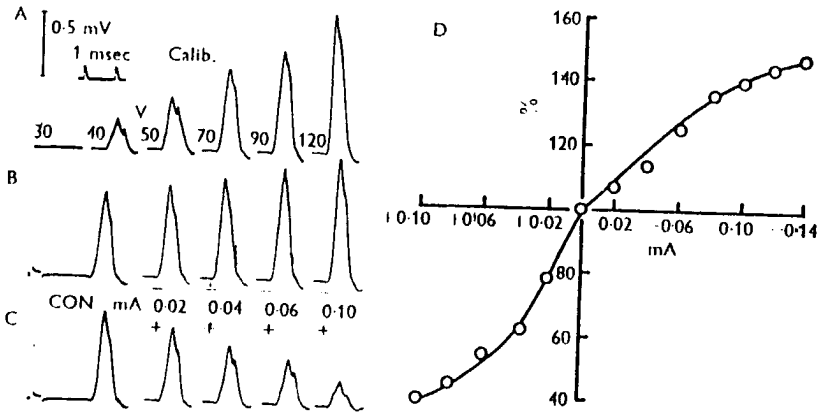

Fig. 3. Excitability changes which polarizing currents produce in the primary afferent fibres in the dorsal region of the spinal cord. An extracellular stimulating electrode (glass micro-electrode filled with 4 M-NaCl, resistance about 1 MΩ) was inserted to about 0·5 mm from the cord dorsum at L7 level. Stimulating current pulses of 0·2 msec were used and the antidromic spike was recorded in the nerve to plantaris and flexor digitorum longus muscles, the ventral roots being severed. A shows specimen records of the monophasic spikes in the nerve that are produced by stimulus pulses driven by the voltages indicated on each record and gives the calibrating series (Calib.) for evaluating the excitability changes in B and C. In B and C the stimulus strength was kept constant at 80 V and the cord was polarized with increasing currents as indicated in mA between B and C, CON being the control values. The polarity of the dorsal electrode was negative in B and positive in C. The approximate excitabilities during the polarization are shown in D as percentages of the control value that is observed in the absence of polarization. The calculation of these percentages is based upon series A by a method described by Eccles, Magni & Willis (1961).

In Fig. 4 A–C a stimulating electrode was inserted into the gastrocnemius motor nucleus and the antidromic spike in the primary afferent fibres was recorded from the gastrocnemius nerve. In A are specimen records from the calibration series of the spikes evoked by brief pulses of increasing voltage as indicated. In the B series the stimulus was kept constant at 85 V, and the cord was polarized with steady currents of various strengths, as in Fig. 3 B, C. In C the approximate excitability changes of the complete series are plotted against the strength and direction of the polarizing current. The direction of the current which caused a hyperpolarization and

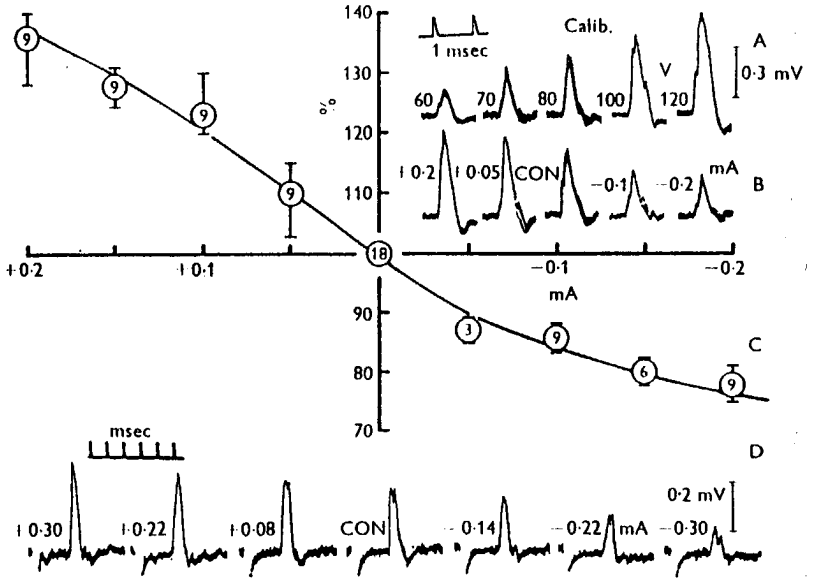

Fig. 4. Excitability changes which the polarizing currents produce in the primary afferent fibres within the motoneuronal nucleus. In A, B and C the stimulating electrode was inserted in the gastrocnemius motor nucleus at L7 level and the antidromic spikes evoked by pulses of 0·2 msec duration were recorded in the nerve to the gastrocnemius muscle. Figure 4 A resembles Fig. 3 A, and Fig. 4 B (at 85 V fixed stimulus) Fig. 3 B plus C, so that no special description is required; those figures (3 and 4) refer to the same experiment. The plotted curve, Fig. 4 C, resembles in its general arrangement that of Fig. 3 D, the relative excitabilities (ordinates) being similarly calculated. In Fig. 4 C each point is the mean value of a certain number of measurements (written in each circle) the range of which is indicated by the vertical bars. The range of the control value is not indicated since it was smaller than the diameter of the circle. D shows records from a similar experiment where the peripheral recording was from the nerve to flexor digitorum longus muscle. In this experiment the cord was polarized with current pulses of 300 msec duration. The stimulus strength was the same for all records and the currents are indicated. The stimulus was given about 250 msec after the onset of the current.

an associated decrease in excitability of the afferent fibres in the dorsal part of the cord (Fig. 3C, D) increased the excitability of these fibres in the ventral horn, thus demonstrating a depolarization there (cf. Fig. 1), though it could not be directly measured because intracellular recording from the fine terminal fibres was not possible in the ventral horn. The reverse changes were produced by reversing the direction of the polarizing current.

The excitability changes observed in the ventral horn varied considerably with the position of the stimulating electrode. Often several adjustments were necessary before increasing stimulus strength gave a suitably graded increase in the population of afferent fibres excited (cf. Fig. 4A) together with a smoothly graded series of responses to variations in polarization (Fig. 4B, C).

The duration of the polarizing current did not influence the results appreciably. For example, Fig. 4D shows specimen records of an experiment where the stimulating pulse was timed 250 msec after the onset of the polarizing current. The results were essentially the same as in the experiment of Fig. 4A–C, where the polarization always lasted several seconds before stimulation. This minimum time allowance of 250 msec was always made in order to avoid complications by excitability changes resulting from any excitation of the fibres at the onset of the polarizing currents.

In general, the change in excitability for any given amount of current was smaller in the ventral than in the dorsal parts of afferent fibres. It would be expected that, on account of its large surface area, the terminal ramification of an afferent fibre in the ventral horn would offer a considerably lower resistance than the myelinated dorsally-lying segment of the same fibre. Consequently, the membrane potential and excitability changes produced by a given current would be smaller in the ventral ramification.

Effects of polarization on motoneurones and monosynaptic EPSPs

The influence of polarizing currents on monosynaptic transmission has been studied with intracellular recordings from 30 motoneurones. The changes of the monosynaptically produced EPSP were measured and compared with the changes in the antidromic spike potential. In a few cases the changes in the membrane potential of the motoneurone were also measured by subtracting the intracellular potentials from the field potentials recorded with the micro-electrode just outside the cell for corresponding strengths of polarizing currents.

A typical experiment is illustrated in Fig. 5. A shows specimen records of EPSPs recorded in a motoneurone innervating the anterior biceps

muscle. CON is the control EPSP, whereas the other EPSPs were recorded during polarization of the cord by currents of intensities indicated on each record. The complete series is plotted in B, and shows an approximately linear relationship of EPSP to current. Current which depolarized the afferent endings (Fig. 4) decreased the EPSP size, whereas hyperpolarizing current increased the EPSP.

It is important to establish that these changes in the size of the EPSP were not a consequence of changes which the polarizing current would produce in the motoneuronal membrane potential, for it has been shown that depolarization effects a decrease in the EPSP and hyperpolarization an increase (Coombs, Eccles & Fatt, 1955). As was stated above, two methods were employed in assessing the effects of the polarizing currents

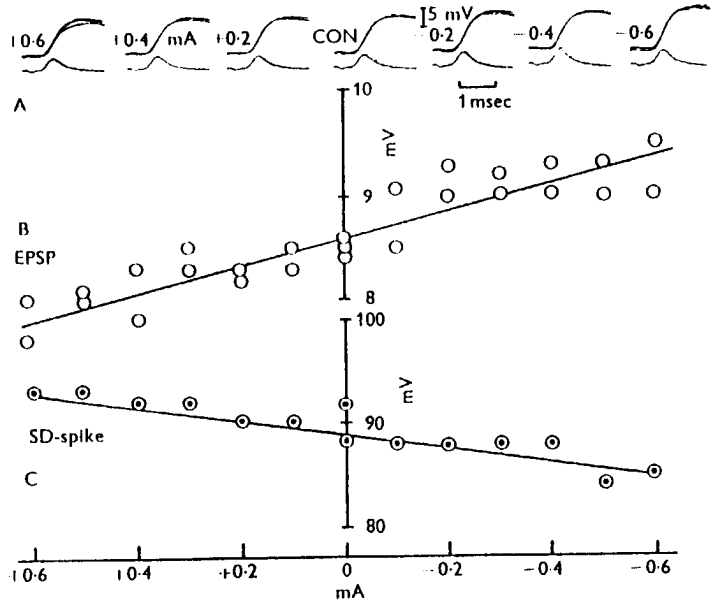

Fig. 5. Changes produced in monosynaptic EPSPs by polarizing current across the cord. Intracellular recording from a motoneurone supplying the anterior biceps muscle, the membrane potential being −70 mV. A shows specimen records of EPSPs under the influence of increasing currents in both directions, as indicated (in mA) for each record, CON being the control value. Each record consists of many superimposed faint traces. The upper traces are the intracellular records, which are differentiated in the lower traces. In B the amplitudes of the EPSPs (in mV) for the series partly shown in A are plotted against the direction and strength of the polarizing currents. C shows the amplitudes of antidromically evoked SD-spikes measured under the influence of the same currents. In A, B and C (+) and (−) indicate the polarity of the dorsal electrode. Voltage calibration in A is for intracellular recording only.

on the motoneuronal membrane potential. In Fig. 5C it is seen that the antidromic soma-dendritic spike potential (SD spike) was increased when the polarizing electrode on the cord dorsum was positive, and decreased when it was negative. This indicates that in the former condition the motoneuronal membrane was hyperpolarized (as depicted in Fig. 1), and in the latter depolarized. Similar changes were also observed on the few occasions when the motoneuronal membrane potentials were directly measured as described above. Thus the changes which the polarizing current induced in the motoneuronal membrane potential would have an action on the EPSP opposite to that actually observed, as may be readily appreciated in Fig. 5. The observed effect of the polarizing current on the EPSP must therefore be produced by its action on the presynaptic terminals, their depolarization resulting in diminution of the synaptic excitatory action producing the EPSP, while their hyperpolarization caused an increase. The change in the motoneurone membrane potential was small (± 5 mV on the average), so allowance for its influence on the EPSP size would increase the slope of curves such as that of Fig. 5B at the most by about 50%.

In these experiments a decrease in the EPSP might also be caused by a conduction block in some of the fibres conveying the afferent volley. Therefore an increase in EPSP was the only reliable indication that alteration in the membrane potential of the presynaptic terminals effected a change in their synaptic excitatory action. However, in most cases conduction block could easily be detected by the sudden decrease both of the EPSP size and of the spike potential of the afferent volley as it entered the cord. When, as in Fig. 5B, the plotted points for the diminished EPSP lay on the same straight line as for the increased EPSP, conduction block need not be considered for the range of polarizing currents there illustrated. Depolarization of the presynaptic terminals was just as effective in diminishing their synaptic excitatory action as hyperpolarization was in increasing it. Of the 30 motoneurones observed, 12 showed an increased EPSP of 10–15% of the control size, 11 showed an increase of up to 10% and the other 7 motoneurones showed no appreciable increase during polarization. The maximal decrease of the EPSP which could be achieved before the onset of conduction block was generally 10–15%. In a further 14 motoneurones a spike was superimposed on the EPSP, so the size of the EPSP was measured by the slope of its rising phase. In all cases the steepness increased with presynaptic hyperpolarization.

Recording from a motor axon ventrally from the motor nucleus (Fig. 6) reveals that the size of the antidromic spike potential was very effectively changed by polarizing currents, as would be expected for the membrane potentials depicted in Fig. 1; for any given direction of the current the changes were opposite to the changes in the soma-dendritic spike

(Fig. 5C). Again, as in the intracellular recording from single afferent fibres (Fig. 2), the spike amplitude varied nearly linearly over a wide range of current until the antidromic spike was blocked (Fig. 6A, B at +0·5 mA). The changes in spike potential amplitude were usually much larger in the axon than in the soma, which is presumably a consequence of the relatively larger conductance of the much more extensive soma membrane.

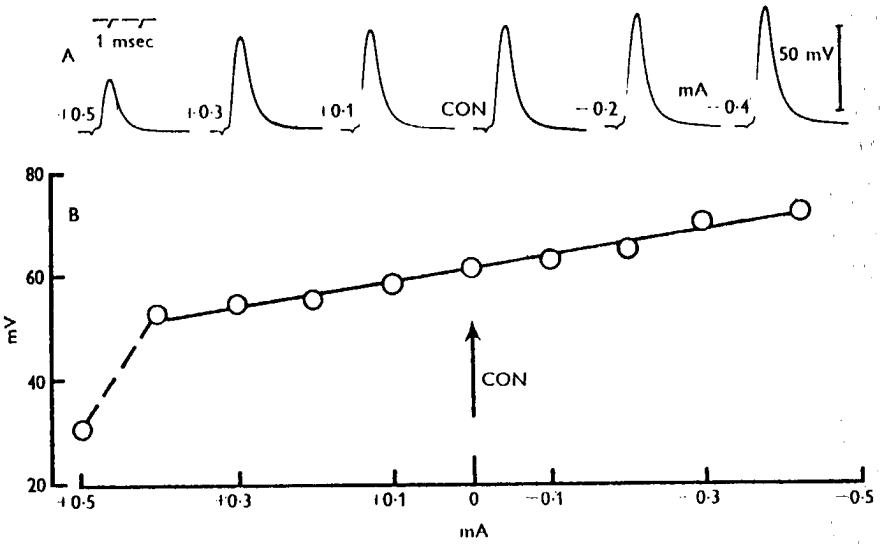

Fig. 6. Changes in action potential amplitude of a motor axon by polarizing currents applied across the spinal cord. The motor axon was impaled on its pathway from the L7 motor nucleus to the ventral root. A gives specimen records of the series plotted in B. The polarity of the dorsal surface electrode is indicated by (+) or (−) and the current strengths are given on each specimen record, CON being the unpolarized control value.

DISCUSSION

The experiments have demonstrated that the passage of a polarizing current across the spinal cord has the effects on the membrane potentials of Group Ia primary afferent fibres and of motoneurones that would be expected from their geometrical arrangements (Fig. 1). The testing of excitability of the terminal branches of the Group Ia fibres in the motoneurone nucleus (Fig. 4) shows that the polarizing currents exert an appreciable effect on the membrane potentials close to the presynaptic terminals of these fibres. In the dorsal part of the spinal cord the change in membrane potential was also revealed by the variation in size of the spike potential recorded by an intracellular electrode (Fig. 2). Comparable results have been observed when the membrane potential was changed by

electrotonic spread from polarization applied to the afferent fibres in the dorsal root close to their entry into the spinal cord (Eccles & Krnjević, 1959*b*). These latter results showed that the size of the spike potential was usually a little more than compensatory for the membrane potential change; for example, with hyperpolarization there was an increased overshoot.

The important new observation is that the application of the polarizing currents to the presynaptic fibres produced a relatively large variation in the size of their synaptic excitatory action. This was seen only rarely and at most was very small when the polarizing currents were applied to the dorsal roots, presumably because the electrotonic decrement to the terminals in the motoneurone nucleus was so great (Eccles & Krnjević, 1959*b*). Possibly the observations of Bonnet (1956, Fig. 6) with ventro-dorsal polarization of the frog's spinal cord show the same effect, but certainly most of the ventral root potential there recorded would be polysynaptic and hence much more complex in interpretation. In the present method of applying the polarizing currents to the presynaptic fibres, excitability measurements revealed that there was a considerable change of the membrane potential in the afferent terminals in the motoneurone nucleus (Fig. 4); and, correspondingly, there was a significant change in the synaptic excitatory action of these fibres, as revealed by the EPSP (Fig. 5). If it be assumed that the spike potential of the afferent fibres varied with the membrane potential in the same manner as occurs for their main shafts in the dorsum of the cord (Figs. 2, 3), the present results give qualitatively the same results as those obtained with the squid giant synapse (Hagiwara & Tasaki, 1958; Takeuchi & Takeuchi, 1962). However, it is not known if the changed size of the EPSP results from the change in the total height of the presynaptic spike, or only in the overshoot.

The alterations produced in the size of the EPSP (Fig. 5) provide the first direct evidence in support of hypotheses that attribute increases or decreases of the synaptic excitatory action on motoneurones to changes in the presynaptic spike potential. Increases of synaptic excitation characterize post-tetanic potentiation (Lloyd, 1949; Eccles & Rall, 1951; Eccles, Krnjević & Miledi, 1959; Curtis & Eccles, 1960); and it has been shown that after tetanic stimulation both the after-hyperpolarization and the increased spike potential of the presynaptic fibres exhibit a time course comparable with the post-tetanic increase in the EPSP (Eccles & Krnjević, 1959*b*). Decrease of synaptic excitation characterizes presynaptic inhibition (Frank & Fuortes, 1957; Eccles, Eccles & Magni, 1961; Eccles, Schmidt & Willis, 1962); and it has been shown that, during presynaptic inhibition, the presynaptic depolarization exhibits a time course comparable

with the decrease in the EPSP (Eccles, Eccles & Magni, 1961; Eccles, Magni & Willis, 1962).

The reduction of the EPSP during strong presynaptic inhibition (Eccles, Eccles & Magni, 1961) was always larger than that found in the polarizing experiments. There are two possible explanations of this difference. In the first place, in presynaptic inhibition the depolarization of the presynaptic terminals may be larger. Secondly, since with presynaptic inhibition the depolarization of the afferent fibres is actively produced by a transmitter substance, the presynaptic spike would be decreased not only by the diminution of the membrane potential (as occurs with the polarizing current), but also as a consequence of the increased ionic conductance produced by the transmitter substance, just as occurs for example with the muscle impulse at an activated motor end-plate (Fatt & Katz, 1951; del Castillo & Katz, 1954*b*).

SUMMARY

1. Polarizing currents have been passed across the cat spinal cord in a dorsal–ventral direction so as to modify both the membrane and the spike potentials of the primary afferent fibres from muscles.

2. Changes in membrane potential were inferred from the changed excitability of the afferent fibres, which indicated the expected variations—hyperpolarization dorsally and depolarization ventrally when the dorsal polarizing electrode was positive, and vice versa when it was negative. Intracellular recording from the fibres in the cord dorsum showed the expected large increase of spike potential with hyperpolarization and decrease with depolarization.

3. Intracellular recording from motoneurones showed that the monosynaptic excitatory action of the primary afferent fibres was increased when the membrane potentials of their presynaptic terminals were increased by the polarizing currents. Conversely, there was depressed excitatory action with presynaptic depolarization.

4. It is pointed out that these findings are in agreement with investigations on other synapses and provide direct evidence relating to the mechanisms concerned in post-tetanic potentiation and presynaptic inhibition.

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THE ROLE OF LACTIC ACID IN THE VASODILATOR ACTION OF ADRENALINE IN THE HUMAN LIMB

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Adrenaline given intravenously exerts a dilator action on the muscle vessels of the forearm in man (Allen, Barcroft & Edholm, 1946) and a local vasodilator effect is also seen when adrenaline is infused intra-arterially in the presence of antagonists (de la Lande & Whelan, 1959). The dilator effect is confined to the muscle vessels and on intra-arterial infusion the response may be masked by reduction in skin blood flow (Skinner & Whelan, unpublished).

Lundholm (1956) attributes the dilator action of adrenaline in the cat to the release of lactic acid from the muscles and Barcroft & Cobbold (1956) suggest that products of carbohydrate metabolism may be involved. The recent demonstration of a local metabolic effect of adrenaline on human skeletal muscle, with an increased concentration of lactic acid in the venous effluent (de la Lande, Manson, Parks, Sandison, Skinner & Whelan, 1961) prompted the investigation of the role which lactic acid might play in the adrenaline dilatation in human skeletal muscle.

METHODS

Infusions of sodium lactate in doses ranging from 1·3 to 20 mg, made up in NaCl solution 0·9 g/100 ml., were given into the brachial artery of one side in five normal subjects. The blood flow through both forearms was followed by the technique of venous occlusion plethysmography, by means of water-filled temperature-controlled plethysmographs at 34° C. The subjects lay supine on a couch and the laboratory temperature was 22–24° C. 0·9% NaCl was infused at a constant rate throughout the control periods preceding and following the infusion of lactate. In two subjects lactic acid, buffered to a pH of 4·2, 3·8 and 3·3 was infused.

The level of lactate in the venous effluent from the muscles of the forearm was determined during sodium lactate and lactic acid infusions. Muscle blood samples were withdrawn from a nylon catheter inserted into a deep branch of the veins in the antecubital fossa; its position was checked by observing the changes in oxygen saturation of samples withdrawn during a period of rhythmic exercise of the forearm muscles (de la Lande *et al.* 1961). Correspondence of the infused area with the area drained by the vein from which samples were withdrawn was checked by the introduction of Evans Blue dye (0·5 mg/min) into the artery and its recovery in the venous samples. Blood lactic acid was determined by the method of Barker & Summerson (1941).