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EXCITABILITY FOLLOWING ANTIDROMIC ACTIVATION IN SPINAL MOTONEURONES SUPPLYING RED MUSCLES

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It is well known that red muscles may be distinguished from pale muscles by the slowness of their contraction (Fulton, 1926; Denny-Brown, 1929*b*). In addition, Denny-Brown (1929*a*) found that red muscle has a lower threshold for postural reflexes, including the stretch reflex, than fast pale muscle. A histological study of fibre diameter has also revealed that the average diameter of motor fibres to the slow red extensors is smaller than for those to the pale extensors, although there is a considerable overlap (Eccles & Sherrington, 1930). While these findings might suggest some specific differences in properties between motoneurones supplying red and pale muscles, no systematic approach has been made toward this problem until recently.

By studying stretch reflexes in the state of post-tetanic potentiation it has recently been suggested that spinal motoneurones innervating extrafusal muscle fibres may be classified into two subgroups (Granit, Henatsch & Steg, 1956; Granit, Phillips, Skoglund & Steg, 1957). The tonic type is characterized by a long-lasting reflex discharge to sustained muscle stretch and may also be distinguished from the other, phasic, type of motoneurones by its slower frequency of discharge. Intracellular recording from spinal motoneurones has shown that the motoneurones of slow red muscles have after-hyperpolarization of greater duration than those of fast pale muscles (Eccles, Eccles & Lundberg, 1957*a*). In the latter study it was postulated that the discharge frequency of motoneurones under continuous synaptic excitation is controlled by the duration of after-hyperpolarization. It appears that tonic motoneurones in general tend to activate red muscles. As would be expected from the diameter spectrum of motor fibres to the ankle extensors (Eccles & Sherrington, 1930), soleus motoneurones have generally shown a slower axonal conduction velocity than gastrocnemius motoneurones (Eccles *et al.* 1957*a*). The observation that the phasic axonal spike is large and the tonic small when recorded in the same

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ventral root filament (Granit *et al.* 1956) may be explained on this basis. However, when the duration of after-hyperpolarization is plotted against axonal conduction velocity, the points for various motoneurones are distributed contiguously along a straight line (Eccles *et al.* 1957*a*). This relationship indicates a continuous gradation between the two types of motoneurones, and fails to show a separation of large motoneurones into two distinct categories. This does not preclude the possibility that motoneurones may, in response to afferent stimulation, show a more distinct separation into tonic and phasic types.

Another feature of tonic motoneurones is that they are more strongly influenced by inhibitory action through axon collaterals than are phasic motoneurones (Granit, Pascoe & Steg, 1957; Henatsch & Schulte, 1958) Tonic and phasic characteristics may therefore be distinguished, after anti dromic activation of spinal motoneurones, by the duration of after-hyperpolarization and by the strength of antidromic inhibition. The present study evaluates in detail these factors that may be important determinants of tonic or phasic behaviour of motoneurones.

METHODS

Adult cats were made 'spinal' by a section through the cord at the atlanto-occipital membrane under preliminary ether anaesthesia, the brain being anaemically destroyed (Hunt & Kuno, 1959). Respiration was artificially maintained. After laminectomy dorsal roots L5–S2 were cut, leaving the ventral roots intact. All the nervous tissue exposed was covered with paraffin oil equilibrated with O_2 95 + CO_2 5%. The monosynaptic reflexes evoked by stimulation of the dorsal root were simultaneously recorded from the nerves to lateral gastrocnemius (fast pale muscle) and soleus (slow red muscle) which were distally severed. The reflexes were conditioned by antidromic volleys in each of the three motor nerves to triceps surae. As a measure of excitability change, amplitude of the monosynaptic reflex discharge was used. The response was determined by a mean of 15–25 records successively obtained at intervals of 2 or 2·5 sec for a given set of conditions. At the end of the experiments, the centrally cut ventral roots innervating the triceps surae were stimulated, and the volley due to synchronous stimulation of all the motoneurones was recorded from the several muscle nerves under the same conditions as when recording the monosynaptic reflexes. The amplitude of reflex response could then be expressed relative to amplitude of response given by discharge of the total motoneurone pool, as has been done in analysis of reflex fluctuation (Hunt, 1955). In some experiments the area of the monosynaptic reflex was used as a measure of response by means of an electronic integrator with digital output (Hisey & Perl, 1958).

For internal recording from spinal motoneurones almost the same apparatus and technique were used as were described in a previous paper (Hunt & Kuno, 1959). The cats were firmly suspended by a spinous clamp and a pair of pelvic pins. Two pairs of horizontal clamps were also applied to the vertebral column to minimize disturbance by respiratory movements. A transistor d.c. amplifier with a cathode follower input was used for recording internal responses. The stray input capacity was reduced by capacitative feed-back. Micro-electrodes were filled with 1·5 M-Na citrate solution (cf. Boistel & Fatt, 1958) except a few experiments in which 3 M-KCl was used. The junctional potential in the electrode tips may have been considerable. The resting potential obtained by these electrodes, however, was reasonably high. No correction has been made for the junctional potential. Muscle movements were abolished by injection of tubocurarine.

RESULTS

Response of motoneurone populations

Inhibition by axon collaterals. As originally described by Renshaw (1941), response of spinal motoneurones to reflex stimulation may be partially inhibited by activation of neighbouring motoneurones. The distribution of this nhibitory action among motoneurones depends upon their position in the spinal cord rather than on their functional organization (Renshaw, 1941; Eccles, Fatt & Koketsu, 1954; see, however, Brooks & Wilson, 1958). Since the three components of triceps surae, medial gastrocnemius (MG), lateral

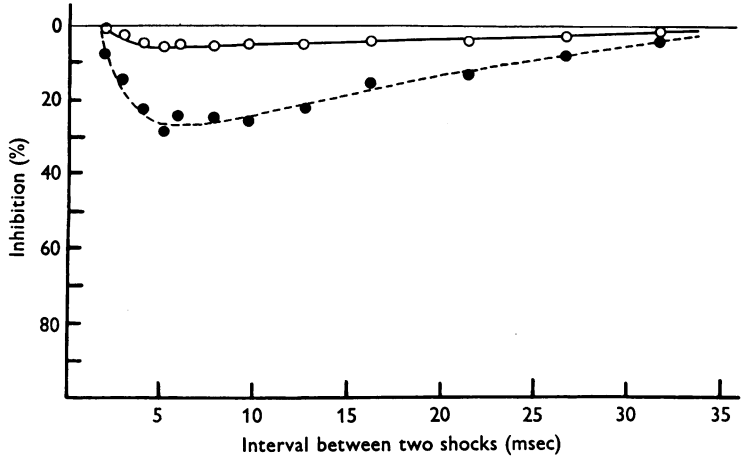

Fig. 1. Inhibition of monosynaptic reflexes simultaneously recorded from nerves to soleus (filled circles) and lateral gastrocnemius (open circles) by an antidromic volley in motor nerve to medial gastrocnemius. Ordinate, inhibition expressed as percentage of each total motoneurone pool size. Abscissa, the interval between the test shock to dorsal root S1 and the conditioning shock. Each point is a mean of 15 responses.

gastrocnemius (LG) and soleus (S), comprise a synergic group, one might expect the antidromic inhibition from one component (for example MG) to be equally distributed to the other two (LG and S). By delivering test shocks to the dorsal roots, the effects of antidromic MG volleys were observed on the monosynaptic reflexes recorded simultaneously from LG and S nerves. Stimulation of the dorsal root inflow was invariably much more effective on S motoneurones than on LG or MG (Fig. 3). In observations on facilitation or inhibition of monosynaptic reflexes it is common experience that the smaller the size of the reflex, the larger is the percentile change. Changes in monosynaptic reflexes resulting from inhibition were therefore compared in relation to total pool size of gastrocnemius and soleus motoneurones by the method indicated above. A typical example is shown in Fig. 1. On the abscissa the

intervals between the conditioning shock to MG motor nerve and the dorsal root test shock are plotted. The strength of inhibitory action was much greater on S motoneurones (filled circles) than on LG motoneurones (open circles). The maximal inhibition produced by MG antidromic impulses was 20·8 ± 9·4 (S.D.) % of the S motoneurone pool and 5·5 ± 1·2 % of the LG motoneurone pool, in six cases in which simultaneous recordings were made. Similarly, the antidromic inhibition from LG to S motoneurones was also stronger (average 18·5 %) than from S to LG motoneurones (average 5·0 %). The antidromic inhibition of MG motoneurones by LG or S motoneurones was also in a range of 4–7 % of the total MG motoneurones pool size. There was, however, little difference in the time course of antidromic inhibition in S, LG or MG motoneurones, the total duration being approximately 40 msec (Fig. 1).

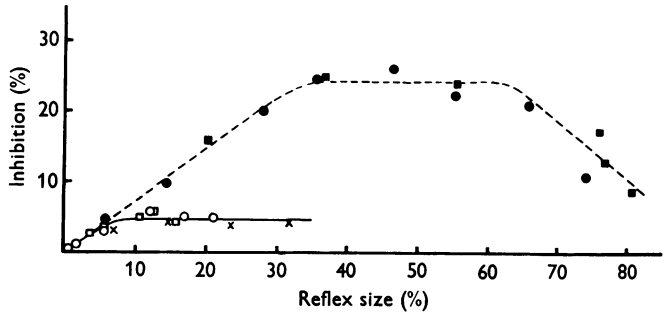

Fig. 2. Relation between size of test reflex and degree of inhibition. The intervals between the test and the conditioning shocks were adjusted to obtain maximal effects, being 5–6 msec. Reflexes were evoked by stimulation of dorsal roots L7 and S1 and recorded from each branch of motor nerves to triceps surae. Ordinate, inhibition as percentage of each total motoneurone pool size. Abscissa, size of test reflex as percentage of size of each total pool. Soleus (filled circles and filled squares in different preparations) and lateral gastrocnemius (open circles and open squares in different preparations) motoneurones were conditioned by medial gastrocnemius antidromic volleys. Medial gastrocnemius (crosses) motoneurones were conditioned by soleus antidromic volleys.

Because of difference in the degree of synaptic excitation in S and LG monosynaptic reflexes, the reflex size used for measurement of antidromic inhibition of S motoneurones was necessarily different from that of LG motoneurones. In order to compare the differences in reflex responses of the same size, the relation between test reflex size and strength of antidromic inhibition from MG to S and LG motoneurones was studied. In Fig. 2 inhibition of S and LG motoneurones is plotted as a function of reflex size, both being expressed as percentage of total pool size. Inhibition increased in parallel with an increase in test reflex size and finally formed a plateau when the reflex size attained about 7 % of the LG pool and 35 % of the S pool. The maximal inhibition produced by MG antidromic volleys, at conditioning-test intervals of 5–6 msec,

was about 5% of the total pool size for LG motoneurones (open circles and squares) and about 25% for S motoneurones (filled circles and squares). The antidromic inhibition from S to MG motoneurones (crosses) showed almost the same behaviour as that to LG motoneurones. The final decrease of inhibitory action in the S reflex, as the latter approached its maximum, is probably due to the strong synaptic drive which may overcome the inhibition by MG antidromic volleys.

The constancy of the antidromic inhibitory effect within a certain limit of synaptic drive (plateaus in Fig. 2) bears similarity to Lloyd & McIntyre's (1955) analysis of individual motoneurones comprising a synthetic pool, in which they found the number of motoneurones in the intermediate zone was

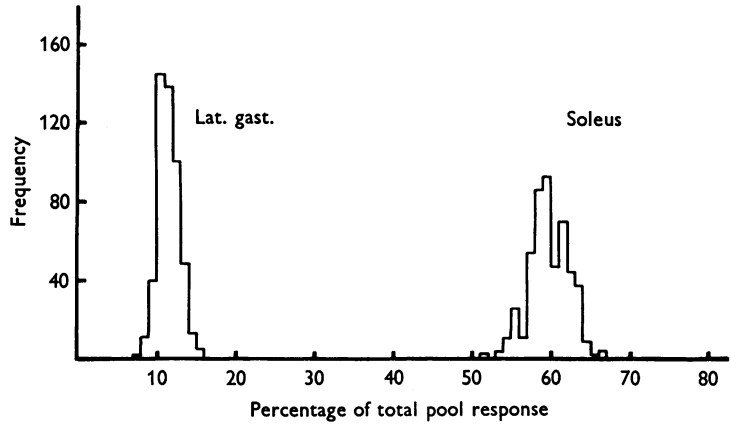

Fig. 3. Frequency distribution of amplitudes of 500 successive responses of monosynaptic reflexes simultaneously recorded from soleus and lateral gastrocnemius nerves following a maximal stimulus to dorsal roots L7 and S1. Ordinate, frequency of occurrence. Abscissa, measured amplitude of responses as percentage of size of each total motoneurone pool. Temperature of paraffin oil covering spinal cord 38·0° C.

nearly the same at different levels of synaptic drive. A similar or an even greater constancy of intermediate zone was also shown by Hunt (1955) in his analysis of excitability fluctuation of monosynaptic reflex response. If the number of motoneurones in the intermediate zone is increased the monosynaptic response would appear subject to more variation; such a reflex system would be more strongly inhibited than one having a smaller intermediate zone. It si therefore assumed that the number of motoneurones subject to inhibition of response is proportional to the number of motoneurones in the intermediate zone. Monosynaptic reflex responses to dorsal root volleys repeated every 2 sec were simultaneously recorded from motor nerves to S and LG muscles. A typical frequency distribution, derived from 500 responses, is illustrated in Fig. 3, reflex amplitudes being expressed relative to total pool size. As described above, the monosynaptic reflex response of S motoneurones

to dorsal root stimulation was much greater than that of LG motoneurones. As a consequence, the mean amplitude amounted to about 59% of the total pool size in the S reflex and about 11% in the LG reflex. As a measure of variation in reflex amplitude the standard deviation was used. In three cases the standard deviations were 1·21, 1·30 and 1·24 for LG reflexes and 2·20, 2·57 and 2·10 for S reflexes; the difference between standard deviations of LG and S reflex response amplitudes was significant. It may be concluded that the number of S motoneurones in the intermediate zone is larger than that of LG motoneurones. This difference in the intermediate zone is, however, too small to account completely for the difference in the antidromic inhibitory effects of the two pools.

Recovery subnormality following antidromic activation. There has been general agreement that spinal motoneurones remain subnormally excitable 100 msec or more following antidromic activation (Brooks, Downman & Eccles, 1950; Lloyd, 1951). Correspondingly a long-lasting hyperpolarization following a motoneurone spike was observed with intracellular electrodes (Brock, Coombs & Eccles, 1952). Recent investigations have shown that soleus motoneurones have, in general, a much more prolonged after-hyperpolarization than gastrocnemius motoneurones (Eccles *et al.* 1957*a*; see also below). It may therefore be reasonable to expect that the duration of depression in excitability following antidromic activation is longer in S motoneurones than in LG motoneurones. This has been found to be the case, as is shown in Fig. 4 (a in *A* and a′ in *B*). The depression was here again expressed relative to the total pool size of S and LG motoneurones. The test shock was given to the dorsal root and the conditioning shock to S or LG motor nerves antidromically. Abscissae give the intervals between the test and the conditioning shocks. The duration of depression was apparently longer in S motoneurones than in LG. The average values derived from five experiments were 165 msec (120–205 msec) and 110 msec (95–130 msec) for S and LG motoneurones respectively. There was also a significant difference in the magnitude of depression between these two motoneurone pools. The mean values were 55 for S motoneurones and 20 for LG motoneurones when expressed as percentage of each total pool size. It should, however, be noted that the depression following antidromic activation as here measured includes the antidromic inhibition from homonymous motoneurones, i.e. axon collateral inhibition from S to S or from LG to LG motoneurones. In order to eliminate this antidromic inhibitory effect and also to estimate the strength of homonymous inhibitory action, the antidromic recovery cycles were obtained before and after injection of dihydro-beta-erythroidine (DHE; Merck) 0·5 mg/kg. As is shown in Fig. 4*A*b and 4*B*b′, the drug effect was restricted to a diminution in the initial part of the depression, little change being seen in the total duration. A subtraction of (b) (filled circles, after injection of DHE) from (a) (open

circles, control) gives the axon collateral inhibitory action from soleus (broken lines a–b). The duration of the inhibition is about 45 msec, which is approximately the same as in the heteronymous antidromic inhibition from MG to S motoneurones. This homonymous antidromic inhibitory action produced an inhibition of S motoneurones amounting to about 16% of the total pool size.

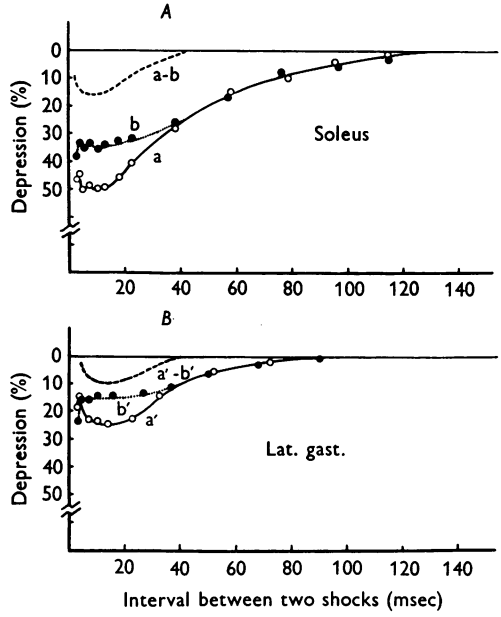

Fig. 4. Effects of dihydro-β-erythroidine on antidromic recovery cycles of soleus (*A*) and lateral gastrocnemius (*B*) motoneurones. Monosynaptic reflexes were evoked by stimulation of dorsal roots L7 and S1 and recorded from motor nerves to soleus and lateral gastrocnemius. Conditioning shocks were given to the same motor nerves central to recording positions. Ordinates, depression following antidromic activation as percentage of size of each total motoneurone pool. Abscissae, intervals between the test and the conditioning shocks. Open circles (a in *A* and a′ in *B*) were obtained before intravenous injection of dihydro-β-erythroidine 0·5 mg/kg; filled circles (b in *A* and b′ in *B*) after the injection; broken lines (a–b in *A* and a′–b′ in *B*) were obtained by subtraction b or b′ from a or a′ respectively.

Although DHE blocks the antidromic inhibitory action, the effect is not complete, and the initial phase of antidromic inhibition may often remain unchanged by administration of DHE (Eccles *et al.* 1954; Brooks & Wilson, 1958). These factors made it difficult to estimate the homonymous inhibitory strength. Nevertheless, one observation on LG motoneurones showed that the homonymous inhibition was obviously stronger than the heteronymous inhibition (a′–b′ in Fig. 4*B*; compare with that in Fig. 1). The difference in the magnitude of depression following antidromic activation between S and LG motoneurones is probably attributable to a difference in the strength of their

homonymous antidromic inhibitory actions. Depression of motoneurones during true post-spike subnormality is, therefore, probably of the same order for both S and LG motoneurones, being 10–15% of each pool size.

Responses of individual motoneurones

General features of spike potentials. Intracellular recordings were made from 17 soleus, 23 lateral gastrocnemius and 42 medial gastrocnemius spinal motoneurones. Representative units are shown in Fig. 5. Figure 5*A* illustrates examples of their spike potentials activated by antidromic stimulation;

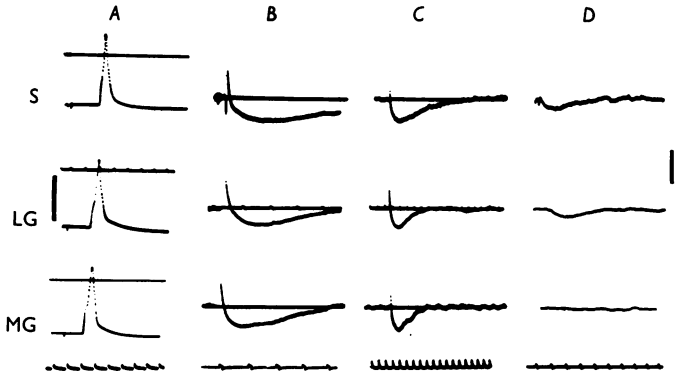

Fig. 5. Intracellular recordings from soleus (S), lateral gastrocnemius (LG) and medial gastrocnemius (MG) motoneurones. *A*. Resting potentials and spike potential activated antidromically; spikes were slightly retouched by dots. *B*. After-hyperpolarization following antidromically evoked spikes with higher amplification. *C*. Same as in *B* but recorded at a slower sweep speed. *D*. Inhibitory hyperpolarization in soleus and lateral gastrocnemius motoneurones produced by antidromic impulses in medial gastrocnemius nerves; no inhibitory potential was detected in this medial gastrocnemius motoneurone by antidromic lateral gastrocnemius volleys. Time, 1 msec for *A* and 10 msec for *B*, *C* and *D*; voltage scale on left side 50 mV for *A* and that on right side 10 mV for *B*, *C* and *D*.

their after-hyperpolarization is shown with greater amplification in *C*. As was shown by Eccles, Eccles & Lundberg (1957*a*, 1958), soleus motoneurones showed, in general, a slower conduction velocity, a slower decline of spike potential and a longer after-hyperpolarization than MG or LG motoneurones. In addition, it was found that the action potentials were significantly different in soleus and gastrocnemius motoneurones. The resting potentials were $66 \pm 3{\cdot}9$ mV (S.D.) and $65 \pm 3{\cdot}4$ mV, and the overshoot potentials $17 \pm 2{\cdot}5$ mV and $22 \pm 3{\cdot}6$ mV for gastrocnemius and soleus motoneurones respectively. The overshoot potential was significantly larger in S motoneurones, while there was no difference in the resting potential.

The conduction velocity of every motoneurone recorded was calculated from the conduction time and distance. A significant correlation was found between the overshoot potential of a motoneurone and the conduction velocity along

its axon. Figure 6*A* illustrates the relation. Only those motoneurones with overshoot potential in excess of 10 mV are included in this figure. It is also evident from this graph that soleus motor fibres (filled circles) have generally a slower conduction velocity than gastrocnemius motor fibres (open circles for MG and crosses for LG). The resting potentials of motoneurones were similarly plotted against their axonal conduction velocities in *B*. Although some correlation appears, its significance is doubtful, the level of significance, *P*, being 0·10.

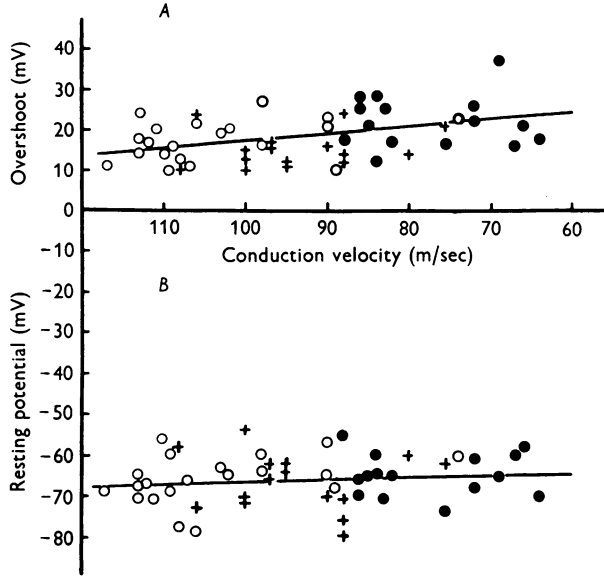

Fig. 6. *A*. Relation between axonal conduction velocity and overshoot potentials of motoneurone spikes evoked antidromically. *B*. Relation between axonal conduction velocity and resting potential of motoneurones. Ordinates, overshoot potentials positive and resting potentials negative. Filled circles for soleus, open circles for medial gastrocnemius and crosses for lateral gastrocnemius motoneurones. Only those motoneurones with overshoot potentials in excess 10 mV are included.

The termination of the declining phase of the spike potential was sometimes difficult to determine, especially in S motoneurones, because it merged gradually into the negative after-potential. However, in nine soleus motoneurones in which the end of the spike was sufficiently conspicuous to allow measurement, the average duration of the falling phase of the spike potential was 0·9 msec as compared with an average of 0·67 msec for G motoneurones. In the maximum rate of decline of the falling phase, however, there was no conspicuous difference between S and G motoneurones (175 and 190 V/sec respectively). Also, there was no significant difference in the maximum rate

of rise of spike potentials between S and G motoneurones (365 and 340 V/sec respectively).

Antidromic inhibitory hyperpolarization. In the response of a motoneurone population, it was found that the inhibitory action produced by antidromic MG volleys was stronger on S motoneurones than on LG motoneurones (see above). The following experiments show the behaviour of individual gastrocnemius and soleus motoneurones to antidromic impulse conduction in neighbouring motoneurones, using the inhibitory hyperpolarization as an index of the degree of inhibition produced. Since inhibitory hyperpolarization is modified by Cl^- ions in a micro-electrode (Coombs, Eccles & Fatt, 1955*b*), all recording utilized electrodes filled with 1·5 M-Na citrate solution.

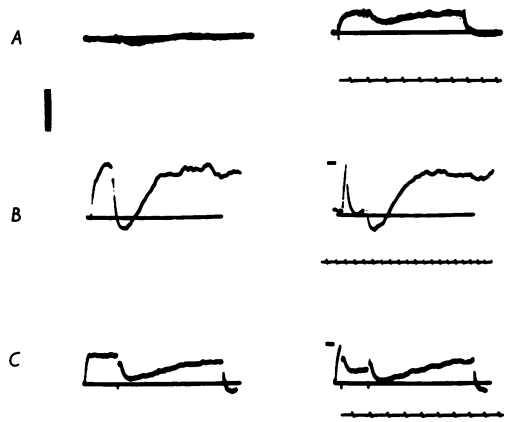

Fig. 7. *A*. Inhibitory hyperpolarization in a lateral gastrocnemius motoneurone produced by antidromic medial gastrocnemius volley; horizontal line gives the original resting potential level. Left record, inhibitory potential at the original resting potential level (69·5 mV); right record, same as the above but under depolarization by extrinsic current. *B*. Another lateral gastrocnemius motoneurone. Left record, after-hyperpolarization following antidromic spike potential with membrane potential depolarized to nearly the critical firing level by extrinsic current; right record, same as the above but with another spike potential which was directly fired by the depolarizing current; small bars show the firing level for the directly evoked spike; for further explanation see in text. *C*. Same as in *B*, but different motoneurone (medial gastrocnemius). Voltage scale, 10 mV; time, 10 msec.

In accordance with observations on population responses of motoneurones, there was little difference in the time course of inhibitory hyperpolarization between soleus and gastrocnemius motoneurones (Fig. 5*D*, S & LG). However, in some gastrocnemius motoneurones the inhibitory hyperpolarization by activation of neighbouring motoneurones was hardly detected (Fig. 5*D*, MG). In order to examine the inhibitory potential in greater detail, two methods were used. The first is shown in Fig. 7*A*. The left trace shows the control response of an LG motoneurone to an MG volley. When this MG antidromic volley was delivered during depolarization produced by an applied

current pulse, the inhibitory potential in the LG motoneurone became more prominent and could easily be detected (right trace). Figure 7*B* shows the second method. Another LG motoneurone was depolarized nearly to the firing level; an antidromic impulse was then evoked by supramaximal stimulation of the LG nerve (left trace). The resultant hyperpolarization includes the post-spike after-hyperpolarization of the motoneurone as well as the inhibitory hyperpolarization produced by homonymous LG motoneurones. In the right trace the hyperpolarization seen immediately after the onset of depolarization followed a spike potential evoked directly by the depolarizing current, while the hyperpolarization which appeared about 25 msec later was produced by the LG antidromic volley. When the peak voltage of after-hyperpolarization of the directly evoked spike was measured from the firing level (shown by a small bar), it was markedly smaller than that in the left trace. This difference indicates the extent of inhibitory hyperpolarization on this motoneurone by homonymous motoneurones. In Fig. 7*C*, a similar procedure was applied to another motoneurone (MG). In contrast to *B*, no appreciable difference can be seen between the hyperpolarization following the directly fired spike (the first spike in right trace) and that after the antidromic volley (left trace). Hence it may be concluded that this motoneurone received little antidromic inhibition from homonymous motoneurones.

By means of the above methods twenty-one gastrocnemius and seven soleus motoneurones were tested. In six of the gastrocnemius motoneurones no inhibitory hyperpolarization was detected. On the other hand all the soleus motoneurones examined showed inhibitory hyperpolarization. Such a detailed examination was actually unnecessary, for all the soleus motoneurones were considerably hyperpolarized by antidromic MG or LG volleys even at the normal resting potential (Fig. 5*D*, S).

The amplitude of inhibitory hyperpolarization may be changed by varying the membrane potential (Coombs *et al.* 1955*b*). Since the resting potentials of motoneurones may differ, the voltages of their inhibitory hyperpolarization cannot simply be compared. However, on changing membrane potential by passage of current, the relation between the membrane potential and the peak amplitude of antidromic inhibitory potential may be studied and compared in different species of motoneurones. Figure 8*A* shows changes in the size of antidromic inhibitory potential produced by heteronymous volleys when the membrane potential is varied by passing current through the cell with a bridge circuit (Araki & Otani, 1955; Hunt & Kuno, 1959). When plotted against membrane potential, the peak voltage of inhibitory potential can be seen to fall roughly along a straight line within the limits of membrane potential (Fig. 8*B*). Three lines for MG, LG and S motoneurones cross through zero at a membrane potential of about -75 mV. There was little difference in this equilibrium potential between gastrocnemius and soleus motoneurones. The

slope of the relation shown in Fig. 8*B* will depend upon the magnitude of the conductance change responsible for the inhibitory hyperpolarization. The greater the conductance change the more nearly would the relation approach a maximal slope of 45 degrees. Although the slope of the line was usually steeper in S motoneurones than in gastrocnemius motoneurones, it varied considerably from cell to cell. For instance, as is shown in Fig. 8*B*, the line of one LG motoneurone was steeper than that of one S motoneurone.

It was usually more difficult to record from S motoneurones for a long period than from gastrocnemius motoneurones. Some S motoneurones deteriorated before completion of a series of observations. The number of observations on the relation between the membrane potential and the antidromic inhibitory potential in S motoneurones was consequently limited. However, it was

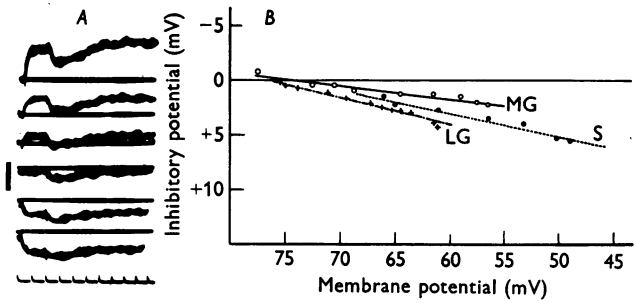

Fig. 8. *A*. Inhibitory hyperpolarization in a soleus motoneurone produced by antidromic medial gastrocnemius volleys; records were of 2–4 superimposed traces. Horizontal lines indicate the original resting potential level, −60 mV, which fluctuated from −59 to −63 mV. The initial displacement of potential shows depolarization (upward) and hyperpolarization (downward) controlled by passing current through the cell. Voltage scale, 10 mV; time, 10 msec. *B*. Plot of the peak amplitude of inhibitory hyperpolarization against membrane potential. Ordinate, inhibitory potential, hyperpolarization positive and depolarization negative. Inhibitory potentials in soleus (filled circles) and lateral gastrocnemius (crosses) were generated by antidromic medial gastrocnemius volleys and that in medial gastrocnemius motoneurone (open circles) by antidromic lateral gastrocnemius volleys.

possible to estimate roughly the slope of the relation by assuming a value for the equilibrium potential and considering the relation linear. For example, a S motoneurone shown in Fig. 5 had an antidromic inhibitory hyperpolarization of 2·7 mV at a resting potential of 59 mV. Assuming that the equilibrium potential for the inhibitory potential is −75 mV for all motoneurones, the slope will be given by 2·7/(75–59).

The values obtained in this manner were plotted against the axonal conduction velocities in Fig. 9*A*. The inhibitory potentials for S (filled circles) and LG (crosses) motoneurones were produced by antidromic stimulation of MG motor nerves, while that of MG motoneurones (open circles) was produced by LG antidromic volleys. This procedure, of course, gives only approximate

values. Nevertheless, a correlation can be seen between the slope obtained in this manner and the axonal conduction velocity. Similar tests were also applied to the after-hyperpolarization. For example, a S motoneurone presented in Fig. 5 showed an after-hyperpolarization of 6·7 mV at a resting potential of 59 mV. Since this value of after-hyperpolarization included an inhibitory hyperpolarization of 2·7 mV (see above), the true after-hyperpolarization was 4·0 mV. Assuming the equilibrium potential for the hyperpolarization to be −80 mV for all motoneurones (see below), the relation

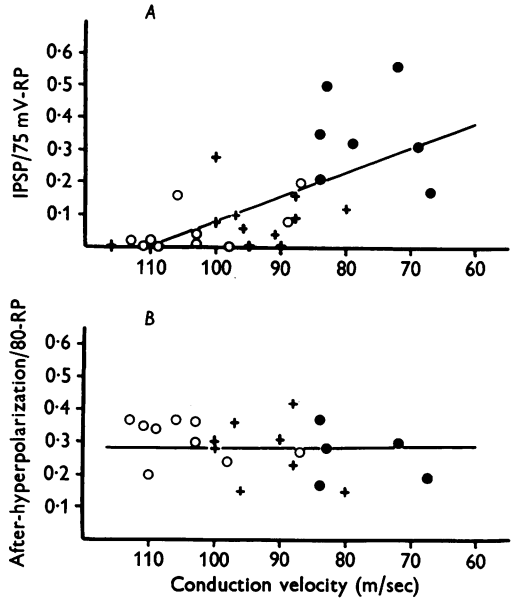

Fig. 9. *A*. Relation between axonal conduction velocity and the slope of the line relating peak amplitude of inhibitory potential to membrane potential. Inhibitory potentials in soleus (filled circles) and lateral gastrocnemius (crosses) motoneurones were generated by antidromic medial gastrocnemius volleys and those in medial gastrocnemius motoneurones (open circles) by antidromic lateral gastrocnemius volleys. Ordinate, calculated fractional displacement of potential toward the equilibrium potential for inhibition; see text. *B*. Relation between axonal conduction velocity and the slope of the line relating peak amplitude of after-hyperpolarization to membrane potential. Filled circles for soleus, open circles for medial gastrocnemius and crosses for lateral gastrocnemius motoneurones. Ordinate gives calculated fractional displacement of potential toward the equilibrium potential for after-hyperpolarization; see text.

between the size of after-hyperpolarization and the membrane potential can similarly be given by 4·0/(80−59). The values obtained were plotted against the axonal conduction velocity in Fig. 9*B*. There was no correlation between them, the mean of the slope being approximately 0·30.

After-hyperpolarization following antidromic activation. After-hyperpolarization following antidromic stimulation of the homonymous muscle nerve

includes the post-spike after-hyperpolarization as well as the hyperpolarization produced by homonymous antidromic inhibition. Since the duration of antidromic inhibition is brief as compared with post-spike hyperpolarization and has a similar time course in soleus and gastrocnemius motoneurones (see above), comparison of the duration of hyperpolarization following homonymous antidromic volleys may be used to indicate differences in after-hyperpolarization among motoneurones. Eccles, Eccles & Lundberg (1957*a*, 1958) separated the post-spike hyperpolarization from total hyperpolarization by a subtraction method. In the present experiments the two components contributing to hyperpolarization following homonymous antidromic volleys have not been separated. As noted, measurements of the duration of after-hyperpolarization would not be appreciably influenced thereby, although measurements of amplitude of the after-hyperpolarization could be altered by the presence of homonymous antidromic inhibition.

As is shown in Fig. 5*C*, the duration of after-hyperpolarization is apparently longer in S motoneurones than in gastrocnemius motoneurones. Average values were 138 msec (95–195 msec) for S motoneurones and 66 msec (40–120 msec) for gastrocnemius motoneurones. These values were considerably shorter than those obtained by Eccles *et al.* (1957*a*, 1958). It should, however, be pointed out that in the present study the duration was measured to the point where the potential passes from hyperpolarization to the initial level. In contrast, Eccles *et al.* (1957*a*, 1958) measured it to the summit of the terminal after-depolarization which follows the after-hyperpolarization.

Not only the total duration, but also the summit time of after-hyperpolarization is longer in S motoneurones (Fig. 5*B*). Eccles *et al.* (1957*a*) found that there was a linear relation between the duration of after-hyperpolarization of a motoneurone and the conduction velocity of its axon. A similar correlation can be seen between the summit time of after-hyperpolarization of a motoneurone and the axonal conduction velocity. This is shown in Fig. 10. The summit time has been measured from the point where the spike potential passes into hyperpolarization (Fig. 10*A*). Clearly, from Fig. 10*B*, the summit time of after-hyperpolarizations of motoneurones showed an inverse relation to the conduction velocities of their axons. Thus motoneurones which had slower conduction velocities showed a hyperpolarization in which the rise to maximum and subsequent decay were both slower, i.e. both phases of the after-hyperpolarization have a slower time course.

In a manner similar to that used in the analysis of inhibitory hyperpolarization, changes in the size of after-hyperpolarization of a LG motoneurone were observed when the membrane was depolarized or hyperpolarized by passage of current (Fig. 11*A*). The maximal amplitude of hyperpolarization is plotted against the membrane potential for MG (open circles), LG (crosses) and S (filled circles) motoneurones (Fig. 11*B*). Such observations by the use of

extrinsic current were limited to a certain range of membrane potential by two factors: block of initiation of motoneurone spike when hyperpolarized over a certain level (about −75 mV) and firing of the motoneurone by passage of current when depolarized beyond the critical level (about −55 mV). Within these limits of the membrane potential, the relation of the size of after-

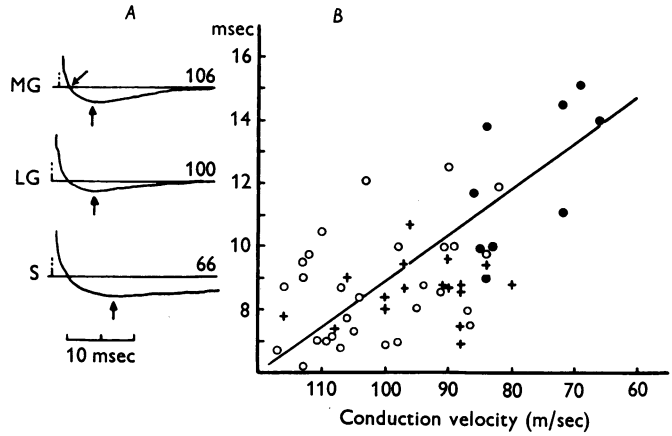

Fig. 10. *A*. Records of after-hyperpolarization of antidromically evoked spike potentials of triceps surae motoneurones. The summit time of after-hyperpolarization was measured from point where spike passes into hyperpolarization (shown by the first arrow in top trace) to the peak shown by up-pointing arrows. The numbers attached on each base line indicate the axonal conduction velocity (m/sec). *B*. The summit time of after-hyperpolarization is plotted as ordinate against the axonal conduction velocity as abscissa for soleus (S, filled circles), lateral gastrocnemius (LG, crosses) and medial gastrocnemius (MG, open circles).

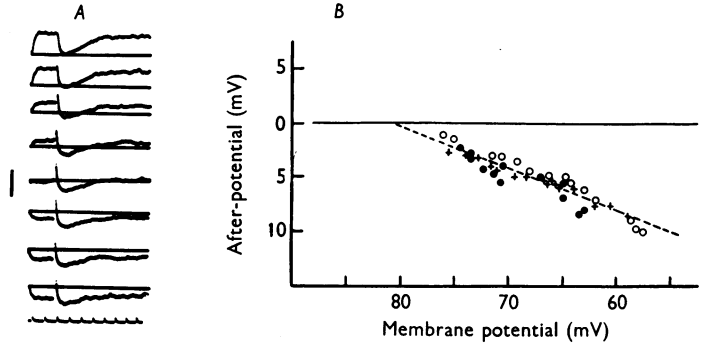

Fig. 11. *A*. After-hyperpolarization of a lateral gastrocnemius motoneurone following antidromic activation, occurring at various levels of membrane potential; horizontal lines indicate the original resting potential level, −71 mV. The initial displacement of potentials from the horizontal lines shows depolarization (upward) and hyperpolarization (downward) controlled by passing current through cell membrane. Voltage scale, 10 mV; time, 10 msec. *B*. Plot of peak amplitude of after-hyperpolarization against membrane potential for soleus (filled circles), lateral gastrocnemius (crosses) and medial gastrocnemius (open circles) motoneurones. Ordinate, hyperpolarization positive and depolarization negative.

hyperpolarization and the membrane potential was approximately linear (Fig. 11*B*). The equilibrium level at which there is no after-potential can be found by extrapolation. Observations on eighteen motoneurones indicated that the equilibrium potential was in the range of from −73 to −89 mV, with a mean of −79 mV. There was little difference in the equilibrium level among those three types of motoneurones. This value, about −80 mV, of the equilibrium potential is lower than the value of about −90 mV obtained by Coombs, Eccles & Fatt (1955*a*). One reason for this discrepancy may be the junctional potential of electrodes which were filled with sodium citrate solution in the present study. This also resulted in a slightly lower value of the resting potential (see above). A similar discrepancy in the equilibrium potentials for antidromic inhibition occurred between the present study and that of Coombs *et al.* (1955*b*) (see above).

Figure 11*B* shows the relation between amplitude of after-hyperpolarization and membrane potential in three motoneurones (from S, LG, and MG) which had the same equilibrium potential. Just as in inhibitory hyperpolarization, the greater the conductance change during the after-hyperpolarization, the steeper the slope of the relation. As would be expected from Fig. 9*B*, there is no obvious difference in the slope between soleus and gastrocnemius motoneurones.The only difference in hyperpolarization between these two groups of motoneurones is that in time course.

DISCUSSION

In the present study two main factors which may be responsible for tonic or phasic behaviour were analysed by antidromic activation of spinal motoneurones; one factor is the after-hyperpolarization and the other is the antidromic inhibitory action. These two factors will first be commented upon.

After-hyperpolarization. It was confirmed that motoneurones supplying red muscles are characterized by a much more prolonged after-hyperpolarization than motoneurones to pale muscles (Eccles *et al.* 1957*a*). The after-hyperpolarization of these motoneurones was longer, both in the initial and decaying phases, than that of motoneurones supplying pale muscles. Consequently the subnormality following antidromic activation of motoneurones innervating red muscles was more prolonged as indicated by monosynaptic test reflexes. When the size of the after-hyperpolarization was modified by changing the membrane potential, extrapolation (e.g. Fig. 11) showed that it should be converted into a depolarization at an equilibrium potential of −80 mV. This equilibrium potential was the same for both types of motoneurones. This suggests that the intracellular concentration of the ion concerned in the after-hyperpolarization process, probably K^+ ions (Coombs *et al.* 1955*a*), may be regarded as the same in both types of motoneurones. The latter is also supported by the finding that the resting potential is nearly the same for both

types of motoneurones. The slope of the linear relation between the peak amplitude of after-hyperpolarization and the membrane potential showed little difference between gastrocnemius and soleus motoneurones, which suggests that the permeability to K^+ ions during after-hyperpolarization is approximately of the same order for these two types of motoneurones. The only difference in after-hyperpolarization was, therefore, that in its time course.

A difference in time course of the post-spike change of permeability to K^+ ions may account for the more prolonged declining phase of the spike in soleus motoneurones as compared with gastrocnemius motoneurones. Repolarization during the falling phase of the impulse is mainly brought about by an increase in K^+ permeability (Hodgkin, 1951). Therefore a slower rise of potassium permeability would result in a more prolonged falling phase of the spike. A slower increase in potassium permeability of soleus motoneurones would also account for the fact that the overshoot during the spike potential is larger than in gastrocnemius motoneurones.

Antidromic inhibition. When the strength of antidromic inhibition was tested by its effect on the size of the monosynaptic reflex, it was found that the inhibitory influence was much stronger on soleus motoneurones than on gastrocnemius motoneurones. A number of triceps surae motoneurones were also examined with intracellular electrodes, recording the inhibitory hyperpolarization in a partially depolarized state as an index of inhibition. In all soleus motoneurones an inhibitory potential was set up by excitation of neighbouring motoneurones. About 28% of gastrocnemius motoneurones showed no evidence for an inhibitory connexion from neighbouring triceps surae motoneurones.

As with the equilibrium potential for after-hyperpolarization and with the resting potential, there was little difference between the equilibrium level for the antidromic inhibitory potential in soleus and gastrocnemius motoneurones. This may further support the view that the ionic distribution in soleus motoneurones is the same as in gastrocnemius motoneurones. However, the slope of the linear relation of the inhibitory potential against the membrane potential was generally steeper in soleus motoneurones. Since the equilibrium potential for antidromic inhibition is the same for all motoneurones, this slope would be proportional to conductance change during inhibitory processes. The greater permeability change during inhibitory processes is plausibly due to the greater density of inhibitory endings on the cell. It is therefore concluded that soleus motoneurones probably receive much denser inhibitory contacts by axon collaterals. This finding bears some similarity to a larger Group I*a* receptiveness of soleus motoneurones (Eccles, Eccles & Lundberg, 1957*b*). In the present investigation soleus motoneurones have shown the greatest responses to dorsal root stimulation (p. 377), and Denny-Brown (1929*a*) found

a lower threshold in soleus for stretch and other postural reflexes. J. B. Preston and D. G. Whitlock (personal communication) have found stronger inhibition of soleus than of gastrocnemius motoneurones by cortical volleys.

General deductions

Further similarities have been found between tonic alpha motoneurones and the motoneurones supplying red muscles in their responses to antidromic inhibitory action (cf. Granit, Pascoe & Steg, 1957). However, in this regard, as in the duration of post-spike hyperpolarization, motoneurones show a correlation with axonal conduction velocity. There appears to be a continuous gradation of these properties, although average values for the generally more slowly conducting soleus motoneurones differ appreciably from average values for gastrocnemius motoneurones. Another difference found in the present study between motoneurones of red and pale muscles was in the overshoot potential of the spike. This was also correlated with axonal conduction velocity. The present experiments do not permit an evaluation of whether motoneurones fall into two distinct categories (Granit *et al.* 1956; Granit, Pascoe & Steg, 1957) tonic and phasic in response to stretch afferent excitation, but their after-hyperpolarization and antidromic inhibition show continuous gradation in their relation to axonal conduction velocity.

It is possible that motoneurones supplying red muscles are developmentally more primitive than those innervating pale muscle, since evidence exists that red muscle is less differentiated than pale muscle (Denny-Brown, 1929*b*). Such less differentiated motoneurones may be characterized by a prolonged time course of potassium permeability following excitation and by relatively dense synaptic contacts, both excitatory or inhibitory, on their cell bodies. As motoneurones differentiate, the potassium permeability following a spike may have a shorter time course and synaptic connexions may become simpler.

SUMMARY

1. Antidromic inhibition by axon collaterals and the depression following antidromic activation of spinal motoneurones innervating red and pale muscles have been studied in acute spinal cats by intracellular recording and monosynaptic test reflexes.

2. In population responses, soleus motoneurones have revealed a much longer subnormality phase following antidromic activation than have gastrocnemius motoneurones. Correspondingly, after-hyperpolarization of soleus motoneurones recorded with intracellular electrodes was in general longer in total duration and summit time.

3. The peak amplitude of after-hyperpolarization and the equilibrium potential for after-hyperpolarization were the same in soleus and gastrocnemius motoneurones.

4. Conduction velocities of motor axons were correlated with the summit time as well as with total duration of after-hyperpolarization of their motoneurones.

5. By antidromic inhibition by axon collaterals of medial gastrocnemius motoneurones, about 25% of the total soleus motoneurone pool was inhibited, but only 5% of the lateral gastrocnemius motoneurones was inhibited. The inhibitory potential in individual cells was also generally larger in soleus motoneurones than in gastrocnemius motoneurones.

6. There was little difference in the equilibrium potential of the inhibitory potential between both types of motoneurones. The slope of the linear relation between the inhibitory potential and the membrane potential was correlated with the conduction velocity of the axon.

7. There was no appreciable difference in resting potential between soleus and gastrocnemius motoneurones, but the spike potential of soleus motoneurones showed a larger overshoot.

8. There was a correlation between the overshoot potential of a motoneurone and the conduction velocity of its axon.

9. The time course of the post-spike change of permeability to K^+ ions and the density of synaptic contacts were mentioned as probable main differences between motoneurones supplying red and pale muscles. Tonic and phasic characteristics were discussed with the conclusion that there is a continuous gradation between two types of motoneurones with respect to after-hyperpolarization and antidromic inhibition rather than a complete separation into two distinct categories.

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