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INTRACELLULAR AUTOGENETIC AND SYNERGISTIC EFFECTS OF MUSCULAR CONTRACTION ON FLEXOR MOTONEURONES

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SUMMARY

1. Intracellular records have been taken from cat motoneurones innervating flexor muscles of the hind limb. Contractions of the ankle flexors tibialis anterior and extensor digitorum longus were elicited by stimulation of the peripheral end of the cut L 7 ventral root and the reflex effects of these contractions were recorded in silent and repetitively firing motoneurones.

2. Contraction usually produces a hyperpolarizing response inside flexor motoneurones. This hyperpolarization is tension-sensitive in the sense that when, at constant muscle extension, the strength of the contraction is increased, the magnitude of the inhibitory response is augmented.

3. Increasing the resting length of the muscles, while using a stimulus of constant strength to the ventral root, causes this inhibitory response to increase in some cells. More often, however, the hyperpolarization caused by contraction is gradually reduced in duration and/or amplitude as the muscles are extended.

4. Even with the muscles slackened, so that they develop no tension at their ends, contraction usually produces prominent hyperpolarization of the motoneurones.

5. By passing polarizing currents or injecting chloride ions through the intracellular micro-electrode, the hyperpolarizing potentials produced by contraction of the slack and extended muscles are shown to be, at least in part, genuinely post-synaptic inhibitory events.

6. When the neurone is fired repetitively by injected current, the 'silent period' in contraction corresponds to the hyperpolarization of the post-synaptic membrane.

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7. Monosynaptic testing of the flexor motoneurone pool has been used to confirm the essential features of the intracellularly recorded activity.

8. Acutely spinalizing the animal increases the magnitude of the inhibitory responses caused by contraction.

9. Recordings from dorsal root fibres show that Golgi tendon organs of the ankle flexors are very sensitive to contraction and are indeed often activated by the internal forces developed in a contracting slack muscle.

10. A number of muscle spindles of the ankle flexors are activated by stimulation of the ventral root at a strength submaximal or just maximal for the α-motor fibres, despite the simultaneous unloading effect of the contracting extrafusal fibres. This spindle activation, which occurs mainly during the phase of tension development in contraction, is favoured by an increased extension of the muscle. Attempts were made to establish whether it is due to α-motor innervation of the receptors or to some mechanical interaction between the intra- and extrafusal muscle fibres.

11. The possible central and peripheral causes of the changes in motoneurone excitability produced by flexor muscle contraction are discussed. It is suggested that tendon organs of flexor muscles strongly inhibit flexor motoneurones and that α-motor innervation of muscle spindles is likely to play a more prominent role in flexors than in extensor muscles.

INTRODUCTION

The reflex actions of muscle end-organs excited by natural stimuli have only recently been studied with intracellular electrodes in α-motoneurones. A series of papers have dealt with muscle stretch (Granit, Kellerth & Williams, 1964*a*, *b*) and with extensor muscle contraction (Granit, Kellerth & Szumski, 1966*a*) .The autogenetic effects of muscle contraction on flexor motoneurones have not previously been investigated from the intracellular point of view.

Flexor motoneurones are of particular interest since the reflex action of tendon organs has been the subject of some controversy. Laporte & Lloyd (1952), using monosynaptic testing, found that homonymous and synergistic flexor motoneurones were inhibited when group Ib afferents were stimulated. However, recording with intracellular micro-electrodes, Eccles, Eccles & Lundberg (1957*c*) were unable to confirm these findings and reported instead that in flexor motoneurones Ib inhibitions were very rare. Bianconi, Granit & Reis (1964*b*) recorded autogenetic inhibition caused by contraction of flexor muscles and assumed it to be caused jointly by the action of Golgi tendon organs and by the pause in excitatory afferent impulses from muscle spindle primaries. The latter authors also found that the monosynaptic reflex was facilitated during the late phase

of muscle relaxation. They attributed this facilitation to the excitatory action of the spindle secondaries.

The present study extends the work of Granit *et al.* (1964 *b*) and examines the autogenetic and synergistic effects of flexor muscle contraction. By recording from flexor motoneurones with an intracellular micro-electrode it is possible to determine the time course of excitability changes produced by contraction. Moreover, it is possible to differentiate hyperpolarizations caused by true post-synaptic inhibition from those due to the removal of excitatory background activity by passing polarizing currents through the impaling micro-electrode (Coombs, Eccles & Fatt, 1955).

METHODS

Adult cats were anaesthetized with an initial minimum dose of 35 mg/kg of pentobarbitone injected intraperitoneally. Small additional doses were given intravenously from time to time to maintain adequate anaesthesia throughout the experiment. In addition a number of cats were spinalized at the level of cord segment L 2. Artificial respiration (98 % O_2 + 2 % CO_2) was regularly employed and in several cases pneumothorax was performed in order to minimize respiratory movements.

The left hind limb was used for all experiments. The ankle flexor muscles tibialis anterior (TA) and extensor digitorum longus (EDL) were carefully freed from surrounding structures without damaging the blood vessels or nerves supplying them; the insertions of the two muscles were cut and the tendons were attached by stiff hooks to an isometric strain gauge myograph which was mounted on a sliding metal rod provided with a catch making it possible to extend the muscles millimetre by millimetre. Both hind limbs were completely denervated except for the nerves supplying the left TA and EDL muscles. Stimulating electrodes were placed on the central ends of the cut left hamstring, popliteal and common peroneal nerves. The latter nerve also included the intact branches supplying the TA + EDL muscles. After fixation of the animal and its leg in a rigid frame the lumbar cord was exposed and the left ventral roots L 7 and S 1 were cut in the middle. By stimulating the peripheral stumps of the cut ventral roots unwanted contractions around the hip and spine were localized and ultimately abolished after cutting nerves and muscles in these regions. Finally, therefore, the TA and EDL muscles alone responded to the stimulus.

Conventional single-barrelled microcapillaries filled with 2 M-potassium citrate or 3 M potassium chloride (5–10 MΩ) were used for intracellular recording from motoneurones of the L 7 segment. Often the L 6 dorsal root was cut across to give easier access to them. Stimulation of the central end of the cut L 7 ventral root was used for locating the fields leading to the motoneurones. The latter were identified by their monosynaptic responses as being popliteal, hamstring or common peroneal. Only records obtained from hamstring and common peroneal motoneurones will be considered in the present paper. To verify that the motoneurone impaled belonged to or was a synergist to the TA + EDL group, brief stretches were applied to the TA + EDL muscles in order to obtain the early phasic excitatory response mediated by the large spindle afferents (see Fig. 4) which project monosynaptically on homonymous and synergic motoneurones (Eccles, Eccles & Lundberg, 1957*b*). A bridge circuit similar to that of Araki & Otani (1955) was arranged for passing polarizing currents through the recording micro-electrode.

In the experiments dealing with monosynaptic testing the monosynaptic reflex evoked by stimulation of the common peroneal nerve was recorded in the central stump of the cut L 7 ventral root. The control size of the unconditioned monosynaptic reflex was generally kept to around 50 % of its maximum value. The behaviour of different types of stretch receptors

during muscle contraction was studied in the usual way by recording from isolated filaments in the L 7 dorsal root. The identification of the end organs will be discussed in Results.

Zero muscle extension was defined by slowly stretching the muscles and observing at a high myograph sensitivity when the strain gauge picked up the slack. The amount of resting muscle tension was varied from 'slack', i.e. the muscles unhooked from the myograph, to a maximum of 10 mm extension. The peripheral stump of the cut L 7 ventral root was used for stimulation of the alpha and gamma motor fibres supplying the TA and EDL muscles.

Local application of a dilute solution of Xylocaine (Astra) to the muscle nerves was sometimes used to obtain a selective block of the small gamma efferents (Matthews & Rushworth, 1957*a*, *b*). The effect of intravenously administered succinylcholine ('Celocurine-iodide', Vitrum) upon the discharge of stretch receptors was studied on several occasions as a means of differentiating between spindle afferents and tendon organ afferents (Granit, Skoglund & Thesleff, 1953; Verhey & Voorhoeve, 1963). Gallamine triethiodide ('Flaxedil', May and Baker Ltd.) was occasionally used in small doses (1·5–2·0 mg/kg I.V.) to produce a more or less selective block of the extrafusal motor end-plates with a considerable portion of the spindle loop activation still remaining (Granit, Homma & Matthews, 1959; Bianconi, Granit & Reis, 1964*a*, *b*; Granit, Kellerth & Szumski, 1966*b*).

RESULTS

Intracellular recording. In all, forty-four motoneurones were intracellularly investigated, thirty-seven innervating the common peroneal muscle group and seven the hamstring flexors. With respect to the reflex effects produced by contraction of the ankle-flexor muscles no differences were found between these groups of flexor motoneurones and they may therefore be considered jointly in the following presentation.

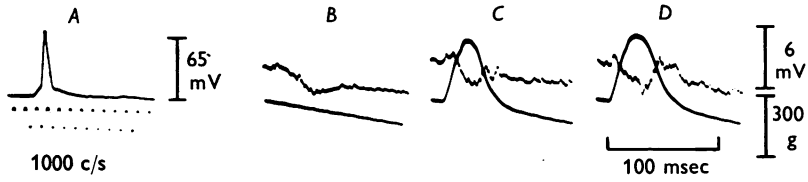

Fig. 1. Common peroneal motoneurone. Monosynaptic spike resulting from stimulation of the common peroneal nerve is shown in *A*. In *B–D* the peripheral stump of the cut ventral root was stimulated at a constant strength giving submaximal contraction of the TA + EDL muscles. The muscles were slack (*B*) or extended by 0 mm (*C*) or 5 mm (*D*). Lower sweep shows muscle tension and upper sweep the intracellularly recorded activity produced by the muscle twitch.

Figure 1 refers to a common peroneal motoneurone whose monosynaptic spike is shown in *A*. In *B*, *C* and *D* of the same figure the peripheral stump of the cut ventral root was stimulated at a strength which produced submaximal contraction of the TA + EDL muscles. In *B*, with the muscles unloaded after being unhooked from the myograph, a twitch gave rise to a hyperpolarizing shift of the post-synaptic membrane potential. As the muscles were extended to 0 mm (Fig. 1*C*) and 5 mm (Fig. 1*D*) the ampli-

tude of this hyperpolarization increased slightly while, on the other hand, its duration was shortened owing to excitatory synaptic activity appearing during both the rising and the falling phase of contractile tension. An early excitation which appeared at a time corresponding to the foot of the contraction (see Fig. 1*C* and *D*) was frequently encountered in the present study. It may at this point be sufficient to restate that the stimulus strength to the ventral root was submaximal for the α-motor fibres and hence no

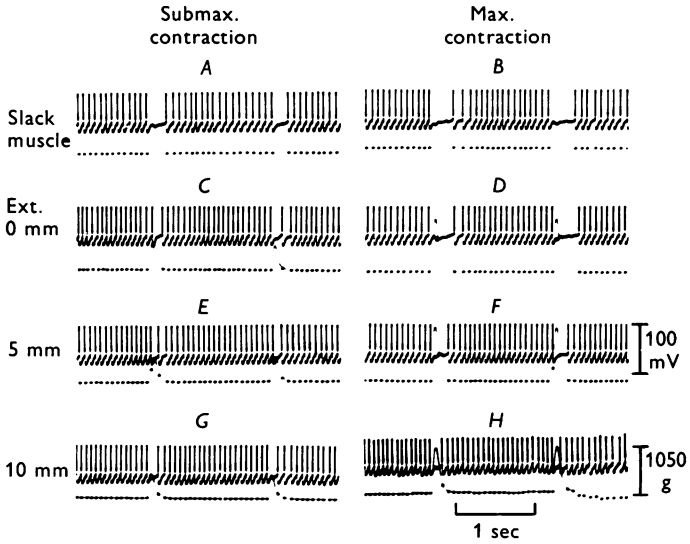

Fig. 2. Hamstring motoneurone. The maintained motoneurone discharge, seen in the upper trace, was caused by depolarizing current injected from the tip of the intracellular micro-electrode. The TA + EDL muscles were kept slack or extended by 0 mm, 5 mm or 10 mm. The contractile tension produced by triple shocks at two different strengths to the peripheral portion of the cut ventral root is illustrated in the lower trace.

significant activation of the smaller γ-motor fibres is likely to have occurred. The late depolarizations which appeared during relaxation were favoured by an increased extension of the muscles and were similar to those seen intracellularly in extensor motoneurones by Granit *et al.* (1966*a*). The inhibitory response produced by contraction of the completely unloaded muscles (Fig. 1*B*) is unexpected. In extensor motoneurones such autogenetic inhibitions produced by contraction of slack muscles were only occasionally observed (Granit *et al.* 1966*a*), but in the present study they were regularly found and, indeed, occurred in forty out of the forty-four flexor motoneurones investigated. Furthermore, of the four cells which showed no such inhibition three were obtained from a cat which in general displayed very poor synaptic inhibitory effects. In Fig. 1 the

amplitude of this post-synaptic hyperpolarization was increased slightly by increased muscle extension, but this was not always found to be the case as will be illustrated by the following experiment.

Figure 2 is from a hamstring motoneurone which was fired repetitively by depolarizing current injected through the impaling micro-electrode. The peripheral end of the cut ventral root was stimulated at two different strengths to produce submaximal and just maximal extrafusal muscle contractions, respectively. The muscle extension was varied between 'slack' and 10 mm as indicated in the figure. In *A* of Fig. 2 the repetitive discharge was powerfully inhibited by twitches of the unloaded TA + EDL

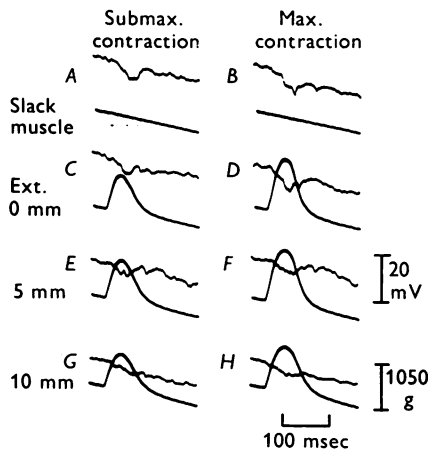

Fig. 3. Same motoneurone and same sequence of experiments as in Fig. 2 but recorded with high gain at a faster time base and without current passing through the intracellular micro-electrode.

muscles, more so when maximal contraction was used. As the extension of the muscles was increased the amount of inhibition, as measured by the duration of the pause of the repetitive discharge, was gradually reduced and became smallest at 10 mm extension (*G* and *H*, respectively). In Fig. 3, which illustrates the very same motoneurone, the sequence of Fig. 2 was repeated at a high gain without passing current through the micropipette. Contraction of the slack muscles as usual caused a prominent hyperpolarization of the post-synaptic membrane (Fig. 3*A* and *B*). When the muscles were extended the amplitude of this hyperpolarization was gradually diminished throughout its time course and became smallest with the largest muscle extension (*G* and *H* of Fig. 3). Thus, the reflex effects of muscle contraction measured in terms of shifts of membrane potential (Fig. 3) corresponded well with the amount of reduction in induced repetitive firing (Fig. 2). On the basis of the present concepts of the behaviour of

different muscle receptors during contraction and their central effects on motoneurones, it may seem rather unexpected that in Figs. 2 and 3 the amount of autogenetic inhibition decreased as the resting tension of the muscles was raised. In the present study, however, this was more often than not found to be the case and it will be even more forcibly illustrated by the following experiment.

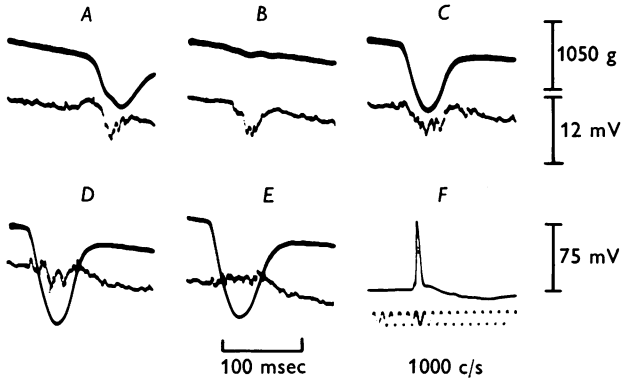

Fig. 4. Common peroneal motoneurone. *A* shows the intracellular effects (lower trace) of a rapid stretch of the TA + EDL muscles (upper trace). In *B–E* the peripheral end of the cut ventral root was stimulated at a constant strength giving submaximal contraction of the TA + EDL muscles. The muscles were slack (*B*) or extended by 0 mm (*C*), 5 mm (*D*) or 10 mm (*E*). (Tension is exceptionally signalled by downward deflexion of myograph trace in this Figure.) *F* illustrates the monosynaptic spike produced by stimulation of the common peroneal nerve.

Figure 4 refers to a common peroneal motoneurone whose monosynaptic spike is shown in *F*. In *A* the intracellular effect of a rapid stretch of the TA + EDL muscles is illustrated. (The stretch was produced by tapping the hook joining the tendons of the muscles with the myograph.) This stretch gave rise to an early excitatory response followed later by a hyperpolarization. (In this figure tension is exceptionally signalled by a downward deflexion of the myograph.) By assuming that the early excitation is caused by the phasic spindle primaries which project monosynaptically on homonymous and synergistic motoneurones (Eccles *et al.* 1957*b*), this response was frequently used as a test to verify that the common peroneal or hamstring motoneurone impaled was a true synergist to the TA + EDL motoneurones. In Fig. 4*B–E* the peripheral end of the cut ventral root was stimulated at a strength which produced only submaximal extrafusal contraction. Hence, it may be concluded that the smaller γ-motor fibres were not significantly activated at this stimulus strength. Figure 4 *B* shows that a twitch of the slack muscles as usual caused a hyperpolarizing shift of the post-synaptic membrane potential. When the muscles were extended,

depolarizing synaptic activity appeared which had a shorter latency than the original hyperpolarization, but which lasted throughout the latter and in this way counteracted and reduced the amount of inhibition. At 10 mm extension (Fig. 4*E*) the hyperpolarization was completely overcome by dominant excitatory activity which now produced a sustained depolarization of the membrane throughout the time course of the contraction, and this despite the fact that no γ-efferent fibres were likely to have been activated by the ventral root stimulus so as to discharge the muscle spindle receptors.

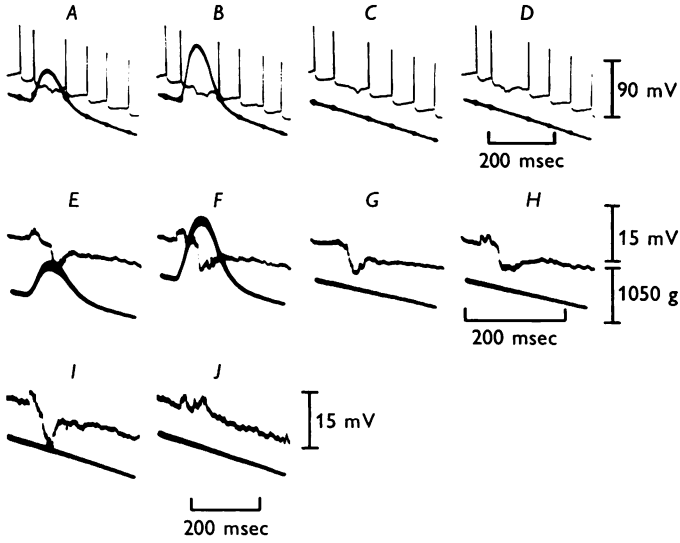

Fig. 5. Common peroneal motoneurone in a spinalized (at cord segment L 2) animal. In *A–D* the cell was fired repetitively by injected depolarizing current. With a muscle extension of 5 mm triple shocks at two different strengths to the ventral root produced submaximal (*A*) and just maximal (*B*) contraction of the TA + EDL muscles. In *C* and *D*, with the muscles slack, the same stimulus strengths to the ventral root were used as in *A* and *B*, respectively. *E–H* correspond directly to *A–D*, respectively, but were recorded at a high gain without current passing through the micro-electrode. The inhibitory potential produced by maximal contraction of the slack muscles (*I*) could be reversed by hyperpolarizing current passed through the micro-electrode (*J*).

In the motoneurones of the ankle extensor muscles (triceps surae) the maximum of the autogenetic inhibition usually occurs during the rising phase or the peak of the muscle twitch (Bianconi *et al.* 1964 *a*; Granit *et al.* 1966 *a*), which would be expected since the inhibitory Golgi tendon organs are maximally activated during this phase of the contractile tension. In the ankle-flexor motoneurones, on the other hand, the maximum of the autogenetic inhibition was generally delayed and more often than not even

occurred after the peak of the contraction (see Figs. 1, 3). The longer latency of the autogenetic flexor inhibitions might have been taken to mean that part of the afferent inflow ascends to supraspinal structures and is then relayed back to the spinal level to inhibit the motoneurones. To test this possibility a number of experiments were performed on cats which had been acutely spinalized at the level of cord segment L 2.

Figure 5 refers to such an experiment on a spinalized cat and shows a common peroneal motoneurone which was fired repetitively by depolarizing current injected through the impaling micropipette (*A–D*). With a muscle extension of 5 mm the peripheral end of the cut ventral root was stimulated at two different strengths, one giving submaximal, the other just maximal extrafusal muscle twitches (*A* and *B*, respectively). In both cases the repetitive discharge was inhibited by the contractions. The muscles were then unhooked from the myograph and the experiments of *A* and *B* repeated in *C* and *D*, respectively. Here also the repetitive activity was inhibited by the twitches, and by approximately the same amount as with 5 mm extension. *E–H* of Fig. 5 correspond directly to *A–D* of the same Figure, respectively, but were recorded at a higher gain and without passing current through the micro-electrode. When comparing *E* with *G* and *F* with *H*, it is seen that with the same stimulus strength to the ventral root the amount of hyperpolarization was not appreciably affected by a change in muscle extension, although at a constant muscle extension the stronger ventral root stimulus in itself produced a prolongation of the inhibition. The only effect obtained by varying the muscle extension is seen in *G* and *E* , where at 5 mm extension (*E*) the submaximal muscle twitch caused an early excitatory response at the foot of the contraction which was not present when the muscles were slack (*G*). Figure 5*I* again shows the sequence of excitation–inhibition produced by a just maximal twitch of the slack muscles. This is to be compared with *J* where hyperpolarizing current was passed through the impaling micro-electrode causing the inhibition to be reversed into a depolarizing response. Thus, the inhibition was genuinely post-synaptic and not merely caused by a removal of background excitation. This test for post-synaptic inhibition was performed on several occasions, both in spinalized and non-spinalized animals, and it always proved to be possible to reduce or reverse the autogenetic hyperpolarizations produced by contraction of the ankle-flexor muscles. In the few experiments where KCl micro-electrodes were used, the same effect could be obtained by intracellular injection of chloride ions from the impaling micro-electrode, demonstrating in another way the post-synaptic character of the autogenetic flexor inhibitions (Coombs *et al.* 1955; Eccles, Eccles & Ito, 1964). The experiment of Fig. 5 also shows that the autogenetic inhibitions caused by flexor muscle contraction are mediated by

spinal segmental pathways since they remain in the spinalized animals. As a matter of fact, the autogenetic flexor inhibitions were generally found to be more powerful in spinalized animals than in cats with intact spinal cords, suggesting a supraspinal control of the interneuronal pathways mediating these inhibitions (see also Fig. 8 of the present study).

Monosynaptic reflex testing. Intracellular recordings of the autogenetic and synergic effects of flexor muscle contraction, as described in the previous section of this paper, were serially obtained from a limited num-

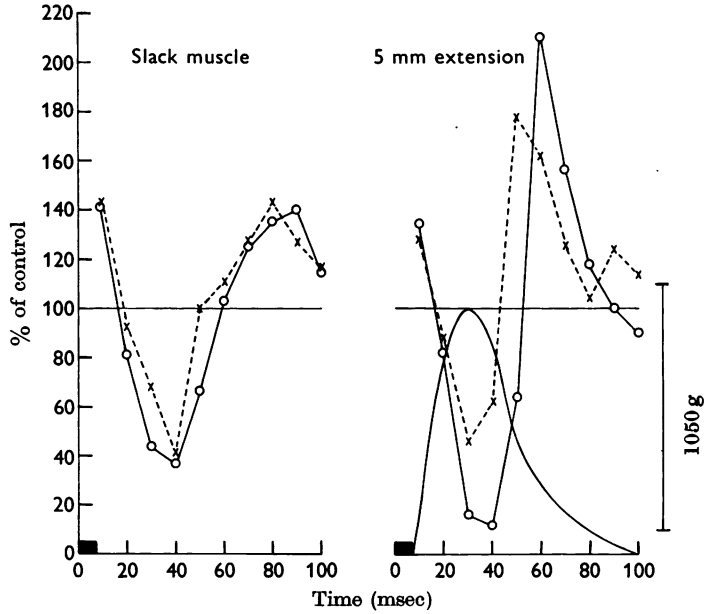

Fig. 6. Time course of the reflex effects of muscle contraction upon the size of the monosynpatic reflex. The conditioning stimulus to the peripheral end of the cut ventral root produced contraction of the TA+EDL muscles. The magnitude of the monosynaptic reflex caused by stimulation of the common peroneal nerve is expressed in per cent of its control amplitude and is plotted against the time between conditioning and test shocks. The heavy line along the horizontal axis indicates the duration of the conditioning stimulus. Each point on the curves represents the mean of ten measurements. The smooth curve to the right shows the time course of the contractile tension. Measurements were made with submaximal contraction (× - - - ×) and just maximal contraction (○——○).

ber of cells in each experiment. Information about the general response characteristics of the motoneurones therefore had to be based on experiments performed on several animals. In this way, however, large cells may be preferentially selected since only penetrations which were stable over extended periods of time could be used. As a check on sampling errors it seemed worth while to extend the work of Bianconi *et al.* (1964*b*) to in-

clude the effects of grading the strength of the contraction and changing the length of the muscle, the idea being to confirm the essential findings of the intracellular study by using the technique of monosynaptic reflex testing (Renshaw, 1940; Lloyd, 1943).

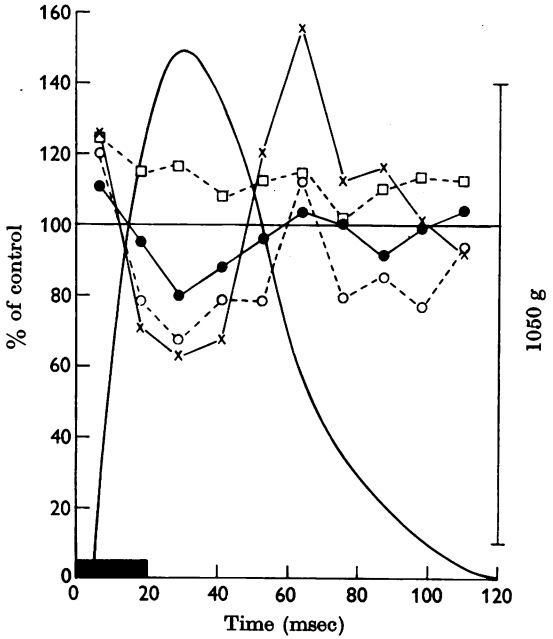

Fig. 7. The effects of muscle contraction on the monosynaptic reflex. The magnitude of the monosynaptic reflex produced by stimulation of the common peroneal nerve is expressed as the percentage of its unconditioned amplitude and is plotted against the time between conditioning and test stimuli. Each point on the curves represents the mean of ten measurements. The duration of the conditioning stimulus to the ventral root is indicated by the heavy line along the time axis. The smooth curve in the figure represents the time course of the contractile tension which was of submaximal strength. Measurements were made at four extensions of the muscles (× —— × = slack muscle, ○ - - - ○ = 0 mm extension, ● —— ● = 5 mm extension, □ - - - □ = 10 mm extension).

The results of one such experiment are shown in Fig. 6. The magnitude of the monosynaptic response recorded in the central end of the cut ventral root is expressed as per cent of the control value and is plotted against the time between the conditioning shock to the peripheral stump of the ventral root and the test shock to the common peroneal nerve. Two different stimulus strengths to the peripheral end of the ventral root were used, one submaximal for the α-motor fibres, the other just maximal. The duration of the conditioning stimulus is indicated by the heavy line along the horizontal axis. Measurements were made with the muscles both completely

slack and extended by 5 mm. The smooth curve to the right in the figure shows the time course of tension when the muscles were extended by 5 mm and the ventral root stimulus was just maximal for contraction. The time course of tension during the submaximal contraction, though of course smaller in amplitude, did not differ appreciably from that of the maximal contraction. Turning first to the sequence of events produced by contraction of the slack muscles it is seen that very early in the contraction, at a time which in the extended muscles corresponds to the beginning of tension development, the monosynaptic reflex was facilitated. Thereafter, there was a rapidly developing depression of the reflex, the peak of which occurred at about 40 msec after the start of the conditioning stimulus. This peak coincided with the onset of muscle relaxation in the extended muscles. Slightly later in the phase of relaxation the depression reverted to

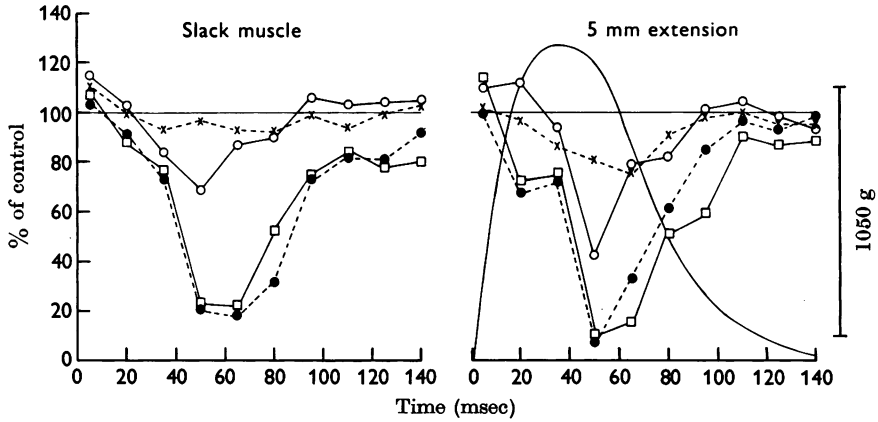

Fig. 8. The effects of muscle contraction on the monosynaptic reflex measured before and 1 hr after spinalization at cord segment L 2. The magnitude of the monosynaptic reflex produced by stimulation of the common peroneal nerve is expressed as the percentage of its unconditioned amplitude and is plotted against the time between conditioning and test stimuli. The tension developed by the TA+EDL muscles during maximal contraction is shown by the smooth curve to the right in the figure. × - - - × and ○——○ indicate measurements made with submaximal and maximal contraction, respectively, before spinalization. ● - - - ● and □ —— □ indicate measurements made with submaximal and maximal contraction, respectively, after spinalization.

a facilitation. Grading the strength of the contraction had little effect on either the depression or the facilitation. With a muscle extension of 5 mm the same sequence of events occurred at approximately the same times. However, the inhibition at the peak of the contraction and the excitation during the phase of falling tension were increased in magnitude. In addition, the magnitudes of the inhibition and excitation were now graded with the strength of the contraction.

The excitability changes in the pool of motoneurones of Fig. 6 are very similar to the sequence of membrane potential shifts which were recorded intracellularly from the neurone illustrated in Fig. 1. The facilitation of the monosynaptic reflex occurring at the foot of the contraction appears intracellularly as an excitatory potential which starts just as the contraction starts. Corresponding with the depression of the reflex there is a hyperpolarizing shift of the post-synaptic membrane potential which peaks right at the onset of the muscle relaxation. Likewise, on the decaying portion of the contraction a depolarizing shift of the membrane potential corresponds with the facilitation of the monosynaptic reflex.

Intracellularly the inhibitory effects were often found to decrease or even disappear with increasing muscle extension (see Figs. 2–4). Figure 7 shows that analogous effects can be reproduced with a pool of activated motoneurones. In the experiment illustrated in this figure the peripheral end of the cut ventral root was stimulated at a constant strength giving only submaximal extrafusal contraction. It is seen that the extent to which the monosynaptic reflex was depressed gradually decreased as the ankle-flexor muscles were extended from slack to 5 mm. At 10 mm extension, ultimately, there was a sustained facilitation of the monosynaptic reflex during the whole time course of the contraction. These results therefore compare very well with the intracellular records of Fig. 4 which were obtained from another cat.

In the experiment of Fig. 8 the time course of the excitability changes were determined before and after complete section of the spinal cord at the level of cord segment L 2. Two stimulus strengths to the ventral root were used, one giving submaximal, the other just maximal contraction of the ankle-flexor muscles. Recordings were made both with the muscles slack and extended by 5 mm. The smooth curve to the right in the figure indicates the time course of tension development during maximal contraction of the extended muscles. Before spinalization the contraction produced a moderate reflex depression which was graded with the muscle extension and the strength of the contraction. Section of the spinal cord caused an increase in both the magnitude and the duration of the depression which had its maximum after the onset of muscle relaxation. It is interesting to note that the afferent inflow produced by submaximal contraction of the slack muscles caused only a negligible inhibition of the monosynaptic reflex before the spinalization. After the spinalization, however, the same afferent input produced a large depression which could be only slightly potentiated by increasing the stimulus strength to the ventral root and by extending the muscles to 5 mm. This suggests that there is a powerful interneuronal 'amplifier' available for the autogenetic flexor inhibition, but which was in this experiment partly suppressed by the influence from

supraspinal structures. Again, it should be noted how well the changes in excitability as measured with monosynaptic testing (Fig. 8) compare with the potential shifts recorded intracellularly from motoneurones of the same spinalized cat (Fig. 5).

Recording from dorsal root fibres. From the results previously described in this paper it becomes clear that some type of muscle receptor which is excited by contraction, even in a slackened muscle, has autogenetic inhibitory actions on a large percentage of the ankle-flexor motoneurones. Both primary and secondary afferents of flexor muscle spindles are supposed mainly to excite flexor motoneurones (Laporte & Lloyd, 1952; Eccles *et al.* 1957*b*; Eccles & Lundberg, 1959; Bianconi *et al.* 1964*b*). Pain or pressure receptors, reported by Paintal (1960) as sometimes being activated by muscle contraction, are part of the system of 'flexor reflex afferents' and should generally cause reflex excitation of the flexor motoneurones (Eccles & Lundberg, 1959; Paintal, 1961). Only Golgi tendon organs are believed to produce autogenetic inhibition in flexors (Laporte & Lloyd, 1952; Bianconi *et al.* 1964*b*), although controversy exists on this point (Eccles *et al.* 1957*c*; Eccles & Lundberg, 1959). The tendon organs being in series with the extrafusal fibres are activated during an extrafusal contraction, but as recorders of muscle tension they should not be fired appreciably by the contraction of a slack muscle.

To determine whether tendon organs are excited by contraction of the slack ankle-flexor muscles and, in addition, to establish some of the response characteristics of the muscle spindles in these flexor muscles, recordings were made from dorsal root fibres. In total, thirty-five units belonging to the tibialis anterior or extensor digitorum longus muscles were isolated, thirty-two of which had conduction velocities greater than 72 m/sec. Unexpectedly, difficulty was encountered in differentiating tendon organs from muscle spindles on the basis of their 'classical' responses to muscle contraction. Acceleration rather than a pause in the receptor discharges during an extrafusal muscle twitch was found to be an unreliable index. Units were identified as being tendon organs only if they were unaffected by intrafusal contractions produced either by intravenous administrations of succinylcholine (Granit *et al.* 1953; Verhey & Voorhoeve, 1963) or by tetanic stimulation of the peripheral stump of the cut ventral root at a strength adequate to excite the small γ-motor fibres. Out of the twenty-six units excited by intrafusal contractions and thus considered to originate in muscle spindles, a total of twenty-one units fired a burst of impulses during the phase of rising tension in response to an extrafusal twitch of the extended muscle. Thirteen spindles were found which fired a burst of impulses during the phase of falling twitch tension in the extended muscle. Both an early burst during rising muscle tension and a

late burst during relaxation was fired by eight of these thirteen spindles in response to an extrafusal contraction. In the slack muscle a ventral root stimulus of α-strength only occasionally caused a spindle to discharge a few impulses.

A total of nine fast-conducting dorsal root afferents were assumed to arise from Golgi tendon organs since they were unaffected by intrafusal contractions. All of those fibres discharged during the phase of rising muscle tension. Contraction of the slack muscle caused eight of these nine tendon organs to discharge one or more spikes. A typical tendon organ is illustrated in Fig. 9. This unit fired three to four spikes during contraction of the slack TA muscle (*A* and *E*). Since the muscle in this situation

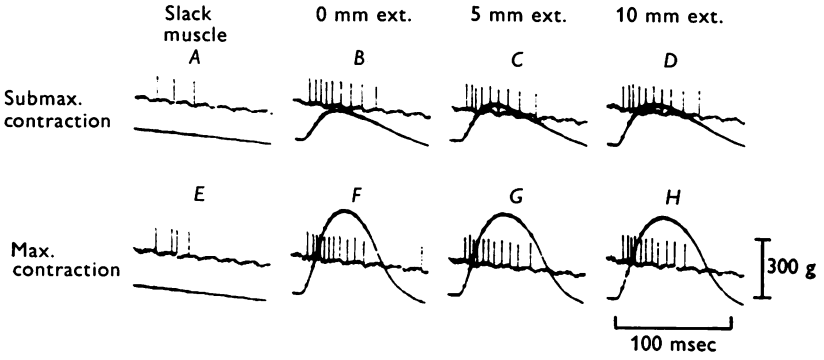

Fig. 9. Response characteristics of a tendon organ from the tibialis anterior muscle. The two sets of records (*A–D* and *E–H*) show the responses produced by submaximal and maximal contractions, respectively, of the tibialis anterior muscle at four extensions. Conduction velocity of spike was 89 m/sec.

developed no tension at its ends, the receptor was activated by the internal forces caused by the contraction. When the muscle was extended from slack to 0 mm (*B* and *F*) the response of the tendon organ greatly increased. Further extension of the muscle did not increase the number of spikes fired during the contraction. Grading the magnitude of the contraction only slightly altered the magnitude of the response. The maximal response of this tendon organ was therefore already obtained when a relatively small load was applied to the muscle. The general pattern of responses to changes in extension and active tension shown by this receptor seemed to be rather typical of the tendon organs of these ankle-flexor muscles. The end organ of Fig. 9 did not fire tonically at any extension, but would fire a spike in response to a twitch producing as little as 2 g of contractile tension at 5 mm extension. With such low thresholds it is perhaps not surprising that tendon organs are excited by the internal forces developed in a contracting slack muscle.

Figure 10 shows a recording made from a small dorsal root filament which contained two units, both of which had conduction velocities well within the range of the group I fibres. The fibre with the larger spike was spontaneously active even with slack muscle and increased its rate of firing as the muscle was extended (Fig. 10*A–D*). Submaximal contraction of the slack muscle produced a burst in the fibre with the smaller spike (*E*).

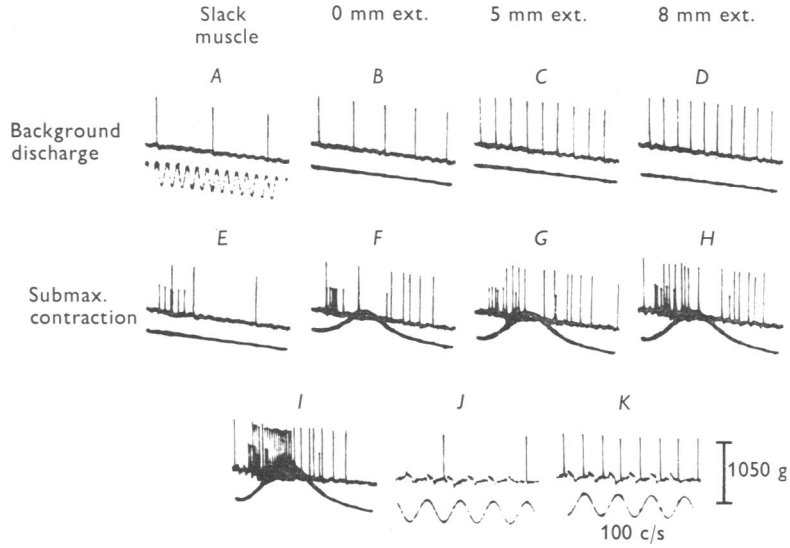

Fig. 10. Muscle spindle afferent (large spike) and tendon organ afferent (small spike) belonging to the EDL muscle. *A–D* shows the responses to passive extension of EDL. *E–H* shows the effects of submaximal contraction of EDL at the four extensions indicated. In I, recorded at 8 mm extension, a supramaximal stimulus to the ventral root excited the fusimotor fibres with a resulting increase in the discharge of the spindle. *J* and *K* show the resting discharge at 0 mm extension before and after an I.V. injection of succinylcholine (0·8 mg/kg), respectively. Time mark in *A* applies to *A–I*. Conduction velocity of large spike 92 m/sec, of small spike 84 m/sec.

When the muscle was extended (*F–H*) the larger unit responded with a gradual increase in firing during both the rising and falling phase of the submaximal contraction. The discharge of the smaller unit early in contraction was not much affected when the muscle was extended. Intrafusal contractions produced either by supramaximal stimulation of the ventral root (*I*) or by intravenous administration of succinylcholine (*K* to be compared with the control record of *J*) selectively discharged only the larger unit, thus identifying it as a spindle and the smaller unit as a tendon organ.

The spindle of Fig. 10 accelerated its discharge during the rising portion of contraction of the extended muscle, although the stimulus strength to

the ventral root was believed to be subthreshold for the γ-motor fibres. Since 80 % of the flexor spindles investigated behaved in this way it was considered to be of interest to determine whether these early spindle excitations reflected the presence of α-motor innervation to the spindles or were due to mechanical pull on the muscle spindles caused by the contracting extrafusal fibres. On several occasions, when a spindle responded with a burst during the rising phase of the submaximal contraction, the ventral root was split up and each subdivision stimulated to determine whether the early spindle response could be reproduced. If several subdivisions were found, each of which gave rise to a spindle burst early in the submaximal contraction, the

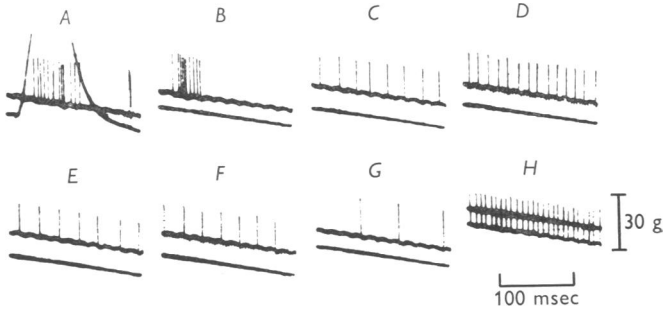

Fig. 11. Muscle afferent (conducting at 97 m/sec) originating in the tibialis anterior muscle. In *A* a small ventral root filament was stimulated at a strength just maximal for its α-motor fibres (notice the high sensitivity of the myograph). *B* is a repetition of *A* but was recorded after the intravenous administration of Flaxedil (2 mg/kg). *C* and *D* show the responses recorded 3 sec after the onset of a maintained ventral root stimulation at α- and γ-strengths, respectively. *E* and *F* are repetitions of *C* and *D*, respectively, but were recorded a few minutes after procaine (0·5 % solution) had been applied to the common peroneal nerve in order to block the γ-motor fibres. *G* is a control record and shows the resting discharge of the receptor without stimulation of the ventral root filament. *H* shows the spontaneous discharge after an intravenous injection of succinylcholine (0·8 mg/kg).

effect was assumed possibly to be mechanical (Hunt & Kuffler, 1951*a*, *b*) although, on the other hand, multiple α-motor innervation of muscle spindles of the ankle-flexors has recently been described (Haase, Meuser & Tan, 1966). If through repeated subdivision it was possible to isolate a small ventral root filament which alone caused a spindle burst early in contraction, tetanic stimulation was employed at various frequencies using a stimulus strength which was submaximal or just maximal for the α-motor fibres of this filament. The idea was the one of Bessou, Emonet-Denand & Laporte (1965), that the spindle would increase its rate of discharge with increasing rates of stimulation above the fusion frequency for extrafusal muscle contraction. In several instances it was possible to do

this, suggesting that mechanical factors were not entirely responsible for the spindle activation early in contraction.

On other occasions a different approach was taken and attempts were made selectively to block the extrafusal motor end-plates by intravenous administration of small amounts of Flaxedil (Granit *et al.* 1959). Figure 11*A* shows the response of a muscle spindle to stimulation of a small ventral root filament at a strength just maximal for the α-motor fibres of this filament (note high myograph sensitivity). The unit continued to fire an early burst even after the extrafusal contraction had been completely blocked by an intravenous injection of 2 mg/kg of Flaxedil (Fig. 11*B*). After Flaxedil, in response to a long-lasting tetanic stimulation of the ventral root filament at the strength which had been just maximal for contraction, the spindle discharge was accelerated (*C*, to be compared with the base discharge of *G*). Supramaximal stimulation of the ventral root increased the firing rate still further (*D*), indicating that at this stimulus strength the γ-motor fibres were activated. While the extrafusal muscle block caused by Flaxedil was still complete, a 0·5 % solution of procaine was applied to the common peroneal nerve in order to block the small γ-efferents (Matthews & Rushworth, 1957*a*, *b*). Some minutes later supramaximal stimulation of the ventral root filament (*F*) produced the same response as just maximal stimulation (*E*) and, furthermore, the effect caused by the latter stimulus strength had not been appreciably affected by the application of procaine (compare *C* and *E*). These results very strongly suggest that the spindle was innervated by motor fibres of large diameter. Finally, in *H* of Fig. 11, an intravenous injection of succinylcholine caused the end organ to discharge tonically at a high frequency (compare with the control discharge of *G*), thus definitely identifying it as a muscle spindle (Granit *et al.* 1953; Verhey & Voorhoeve, 1963). The shift of the myograph base line in *H* was purely accidental and does not indicate a change in muscle tension.

DISCUSSION

In the present study inhibitory potentials were regularly observed in flexor motoneurones when the ankle-flexor muscles were caused to contract by stimulation of the ventral root. These hyperpolarizing potentials, the peak of which usually coincided with the onset of muscle relaxation in a contraction, were shown to be due, at least in part, to genuine postsynaptic inhibitory activity since they could be diminished or reversed into depolarizing potentials either by intracellular injection of chloride ions or by passing hyperpolarizing current through the impaling microelectrode (Coombs *et al.* 1955; Eccles *et al.* 1964).

The source of this flexor inhibition could be either peripheral muscle receptors or recurrent collaterals from adjacent motoneurones which were activated by the afferent volley coming from the contracting muscle. The latter possibility seems unlikely since the inhibitory response to muscle contraction was obtained in motoneurones which showed no sign of receiving recurrent inhibition when such inhibitions were looked for by antidromically stimulating the ventral roots.

Let us therefore consider which muscle receptors could conceivably be inhibiting flexor motoneurones. Spindle primaries, as is well known, make excitatory connexions with homonymous and synergistic motoneurones (Eccles *et al.* 1957*b*; Granit, Phillips, Skoglund & Steg, 1957; Tsukahara & Ohye, 1964). Spindle secondaries, which belong to the group II afferent fibres (Hunt, 1952), generally inhibit extensors and excite flexors (Lloyd, 1943; Laporte & Lloyd, 1952; Bianconi *et al.* 1964 *a*, *b*) although additional effects have also been described (Eccles & Lundberg, 1959; Wilson & Kato, 1965). Furthermore, while we have only studied three muscle spindle afferents which had conduction velocities within the group II range these were not excited by contraction of the slack ankle-flexor muscles, while the inhibitory activity was generally very powerful in this situation. The small group III afferents, which are generally included among the so-called reflex afferents (Lloyd, 1943; Eccles & Lundberg, 1959; Paintal, 1961), mediate pain and pressure information and have been reported not to be excited to any large degree by muscle contraction (Paintal, 1960). The possibility which then remains is that the inhibitory effects originate from the Golgi tendon organs. In the present study an increased strength of the contraction always augmented the inhibitory activity as would be expected if it originated from the tendon organs (Figs. 2, 3, 4, 6, 8). Furthermore, nine out of ten tendon organs were found to be activated by a twitch of the slack ankle-flexor muscles (Figs. 9, 10) which can account for the presence of inhibitory activity under these conditions. It may be necessary to postulate repetitive internuncial activity to explain the potency of the inhibitions caused by the contracting slack muscles (Figs. 3, 4, 6), but the results of Fig. 8 suggest that such an internuncial amplifiying mechanism is available for the flexor inhibitions.

In the present study, when stimulating the ventral root at a constant strength (submaximal or just maximal for the α-motor fibres) the amount of inhibition in response to muscle contraction usually diminished as the initial length of the muscles was increased. This may seem surprising, since if the tendon organs of flexors were inhibitory they would, in analogy with what is found in extensors (Granit *et al.* 1966 *a*), be expected to add more inhibition in response to muscle contraction when the resting tension of the muscles is raised. This unexpected result can be satisfactorily ascribed

to a combination of factors. Firstly, the activity of the flexor tendon organs in response to muscle contraction seems to 'saturate' at a rather modest muscle extension or contractile strength and a further increase in any of these two variables often does not significantly affect the frequency or duration of the discharge of the tendon organs. Secondly, the flexor muscle spindles were frequently found to fire bursts of activity during both the rising and the falling phase of the contraction. The late spindle burst is most probably caused by mechanical pull on the muscle spindles in relaxation and corresponds to the myotatic 'appendage' in extensors (Ballif, Fulton & Liddell, 1925; Granit *et al.* 1966*a*). The spindle burst occurring during the rising phase of contractile tension is favoured by an increased muscle extension and is, at least to some extent, dependent upon activation of large fusimotor fibres (Figs. 10, 11). Intracellularly these spindle bursts will produce depolarizing synaptic activity (Figs. 1, 4, 5). Depending on the synaptic projections and on the duration and frequency of the spindle bursts the effect of increased muscle extension will then be a reduction in the potency of the flexor inhibition (Fig. 3) or even a sustained depolarization which completely obscures the inhibitory activity (Fig. 4).

As mentioned previously, Eccles *et al.* (1957*c*) and Eccles & Lundberg (1959) only rarely found inhibitory post-synaptic potentials in flexor motoneurones in response to stimulation of Ib afferents. Provided that in the ankle flexor muscles the group Ib afferents are synonymous with tendon organ afferents, as is the case with the muscles of the thigh (Eccles, Eccles & Lundberg, 1957*a*), the discrepancy between our results and those of the Canberra group may possibly be due to the tendon organs discharging repetitively in muscle contraction and in this way giving rise to inhibitory effects which may be difficult to reproduce with single shock stimulation of afferent nerves.

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