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FUNCTIONAL ANALYSIS OF GROUP III AFFERENT FIBRES OF MAMMALIAN MUSCLES

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It is generally accepted that the myelinated afferent fibres of some muscle nerves fall into three groups according to the diameter of their nerve fibres (Lloyd, 1943; Lloyd & Chang, 1948; Rexed & Therman, 1948; Hagbarth & Wohlfart, 1952), a grouping also borne out by electrophysiological studies (Hunt, 1954). The fibres of Group I (12–20 μ) arise from muscle spindles and Golgi tendon organs (Hunt & Kuffler, 1951; Hunt, 1954). Those of Group II (4–12 μ) also arise from muscle spindles (Merton, 1953; Hunt, 1954) but not from tendon organs (Hunt, 1954). Further, recent evidence indicates that the Group II fibres probably arise from the secondary endings of the muscle spindle (Cooper, 1959).

So far nothing is known about the endings of Group III afferent fibres (1–4 μ), although there is considerable information about the distribution of fibre diameters within this group in different muscle nerves (Lloyd & Chang, 1948; Rexed & Therman, 1948; Hagbarth & Wohlfart, 1952). As will become clear from the present paper, this gap in our knowledge of the sensory innervation of muscles was apparently due to the difficulty in stimulating some of the Group III endings and also to certain experimental procedures followed by previous workers. The experiments to be described in this paper were therefore, at first, specifically designed to elucidate the behaviour of these Group III endings, the basic procedure being to isolate a Group III afferent fibre, i.e. one with a conduction velocity below 24 m/sec, and then to determine how its ending could be stimulated. The results of this procedure have revealed that most Group III afferent fibres end in pressure receptors, i.e. receptors that are stimulated by local pressure but not by stretching the muscle or by asphyxia. Hitherto little attention has been paid to these receptors owing to the general belief that there are few of them in muscles (Hagbarth & Wohlfart, 1952). However, the recent

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histological studies of Barker (1959), showing that there are significant numbers of so-called pressure receptors in the rectus femoris, should serve to focus attention on them.

METHODS

Experiments were carried out on adult cats anaesthetized with chloralose (70–80 mg/kg) after preliminary induction with ether. The arrangements for fixing the left hind limb, stimulating nerves and recording nerve impulses from afferent fibres were identical with those used previously (Paintal, 1959*a*).

In the earlier experiments filaments were dissected from the lateral gastrocnemius-soleus nerve and the medial gastrocnemius and tibial nerves were cut. Nerve action potentials were recorded monophasically from the peripheral end of the cut filament. When recording impulses from afferent fibres of the tibialis anterior, the superficial peroneal and extensor digitorum longus nerves and the deep peroneal nerve distal to its branch to tibialis anterior were cut. In these experiments the stimulating electrodes were placed under the nerve close to the entry of the nerve into the tibialis anterior muscle. Nerve impulses from tibialis anterior endings were recorded in filaments dissected from the deep peroneal portion of the common peroneal nerve. This proved to be convenient because it provided adequate conducting distance over which to measure the conduction velocities of fibres. Although by this procedure fibres destined for regions other than tibialis anterior, e.g. extensor digitorum longus, were not eliminated, the fact that a particular fibre belonged to the tibialis anterior nerve could always be established by observing the characteristic impulse evoked by stimulating the nerve near its entry into the muscle or by the effects of antidromic stimulation (Paintal, 1959*b*). This was necessary, although the nerves to all regions other than tibialis anterior were cut, because of possible vicarious stimulation of cut nerve fibres. The peroneus longus and extensor digitorum longus muscles were cut from their origins and separated from tibialis anterior muscle for a distance of 2–4 cm to expose the point of entry of the tibialis anterior nerve into the muscle.

Conduction velocities of individual afferent fibres were determined by the methods and criteria described previously (Paintal, 1953). The likely sources of error were also similar to those described in that paper, and as before no arbitrary allowance of 0·1 msec was made for setting-up time at the stimulating electrodes (Blair & Erlanger, 1936).

The conduction velocities of most of the fibres that could be activated by mechanical stimuli were established by criteria mentioned above (i.e. Paintal 1953), but in some such proof could not be obtained. In the latter even the simpler though less reliable criterion of correlating the appearance of a component of the compound action potential with the simultaneous appearance of evidence of antidromic invasion of the sensory ending, as indicated by re-setting of the rhythm of the ending (Iggo, 1958), was of no help, because of the absence of a slowly adapting train of impulses in the fibres examined in the present investigation (see below). In such fibres strong evidence of identity of a fibre with a particular conduction velocity with that yielding the impulses on natural stimulation was obtained as follows: The cat was curarized and the stimulus to the nerve adjusted so that it was just subthreshold for the fibre suspected to be the one activated by natural stimulation (spike at arrow, Fig. 1*A*). With this stimulus strength, just sufficient to produce the compound action potential appearing with a latency of 1–1·5 msec in Fig. 1*A* (but not the spike at arrow), the nerve was stimulated repetitively at about 90/sec and the natural stimulus applied to the muscle, as in Fig. 1*B*. If impulses appeared as in Fig. 1*B* it was clear that they did not belong to one of the fibres in the faster-conducting component. The stimulus was then increased to include the slower fibre under examination and if the impulses did not appear it was concluded that the fibre activated by natural stimulation was identical with that yielding the specific slower component. This test was very convenient

because the potentials of the fibres conducting below 24 m/sec stood out clearly from the faster compound action potential as discrete individual spikes (Fig. 1*A*).

The temperature of the paraffin pool was measured with a mercury thermometer and kept as close to 37° C as possible. This was not difficult because the room temperature at which most of the conduction velocities were determined was about 37° C. The intra-abdominal temperature was used as a measure of deep body temperature. In those instances where the pool temperature deviated from 37° C a temperature correction in the conduction velocity was made by using a Q_{10} of 1·3 for *in situ* conditions, as in the cervical vagus (Paintal, 1953).

The tension of the muscle concerned was measured with a strain gauge (Statham) and d.c. amplifier and recorded on one channel of the oscilloscope.

Flaxedil (May & Baker) or tubocurarine were used to produce neuromuscular block whenever required.

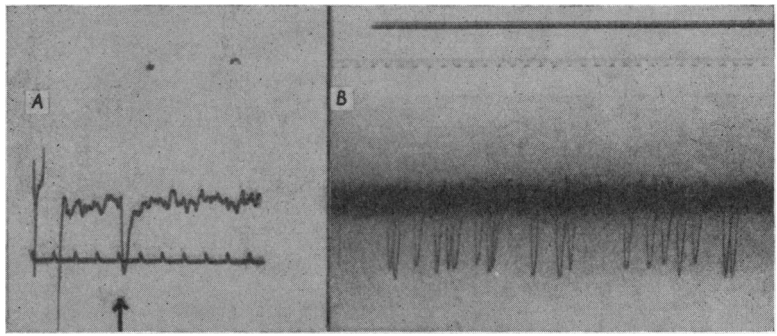

Fig. 1. Records showing how the identity of a particular sensory fibre stimulated by an electrical stimulus with that activated by natural stimulation was demonstrated in some experiments. *A* is a single sweep showing an early fast-conducting compound action potential followed by a single spike in a slowly-conducting fibre (conduction velocity, 14·1 m/sec) at arrow. In *B* the strength of the stimulus was reduced a little so that it did not stimulate the slow fibre but it elicited the entire early compound action potential. The sweep was set 'free running' and this compound action potential was kept out of view so that it did not mask the impulses elicited by pressing the muscle at signal in the continuous record shown in *B*. The spikes in *B* are similar to that at arrow in *A*. When the electrical stimulus was increased to stimulate the slow fibre as well, the naturally evoked impulses shown in *B* did not appear. From above downwards in *B*, signal, time marker 1/10 sec, impulses.

RESULTS

Receptors in lateral gastrocnemius and soleus

Initially, the main aim was to isolate Group III afferent fibres with conduction velocities below 24 msec. While doing so it was necessary to ensure that fibres having an 'early discharge' (see Masland & Wigton, 1940; Lloyd, 1942; Hunt & Kuffler, 1951; Granit, Pompeiano & Waltman, 1959; Cooper, 1959) were not mistaken for slowly-conducting fibres. As is shown in Fig. 2, this was achieved by placing a second stimulus of greater

intensity at a proper interval before the appearance of the slow impulse. If the slow impulse belonged to the 'early discharge' it was delayed or abolished (Fig. 2*A*, *B*) because of antidromic depression of excitability (Matthews, 1933; Paintal, 1959*b*). The second stimulus had to be of greater intensity than the first in order to avoid possible relative refractoriness of the nerve fibres following the first. This procedure also avoided inclusion of impulses arising out of repetition at the stimulating electrodes.

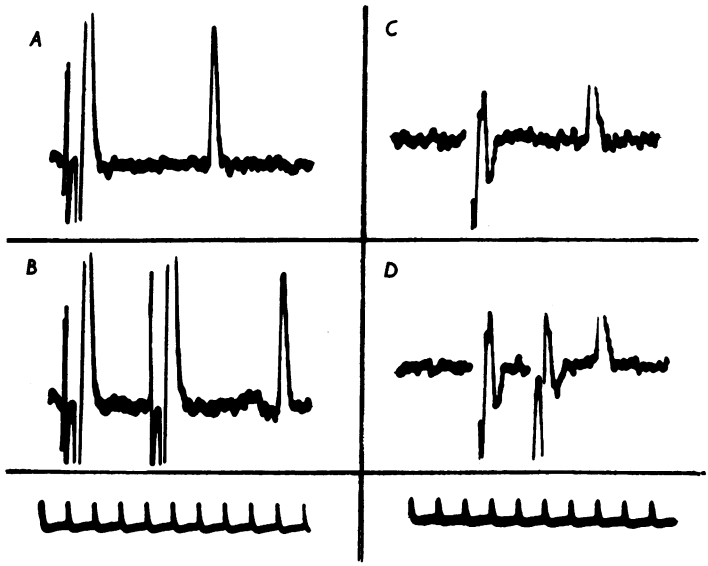

Fig. 2. Sweeps showing how an impulse of an 'early discharge in *A* was distinguished from an impulse belonging to a slowly-conducting fibre (conduction velocity, 10·2 m/sec) in *C*. Application of a second stimulus in *B* delays the 'early discharge' impulse owing to antidromic depression, but leaves the slowly-conducted impulse unaffected in *D*. Time marker, msec.

The fact that any fibre with conduction velocity below 24 m/sec was sensory in function was regarded as established if natural peripheral stimuli such as stretch or pressure initiated impulses in it characteristic of responses of sensory receptors. If impulses could not be initiated then doubt about the sensory identity of the fibre remained *only* if it had a conduction velocity above 15 m/sec, because the lower limit of conduction velocities of efferent fibres (i.e. arising from the ventral roots) is 15 m/sec (Kuffler, Hunt & Quilliam, 1951; Hunt & Paintal, 1958). In this investigation only three such fibres from lateral gastrocenmius and soleus and one from tibialis anterior were met with.

Responses of Group III fibres. Out of 31 Group III afferent fibres isolated from the lateral gastrocnemius-soleus nerve 6 could not be stimulated by any type of mechanical stimulus such as pulling, pressing, squeezing or

prodding the muscle. Two fibres were stimulated by pulling the muscle, the discharge being characteristic of muscle stretch receptors. The response in one of these during muscle twitch indicated that it originated from the muscle spindle, presumably from a secondary ending. The conduction velocities of these two fibres were respectively 18 and 21 m/sec.

The majority of Group III afferent fibres, 23 out of 31, were stimulated by local pressure but not by stretching the muscle (Figs. 3, 8). Most of them did not yield even one impulse on pulling the muscle rapidly. Such

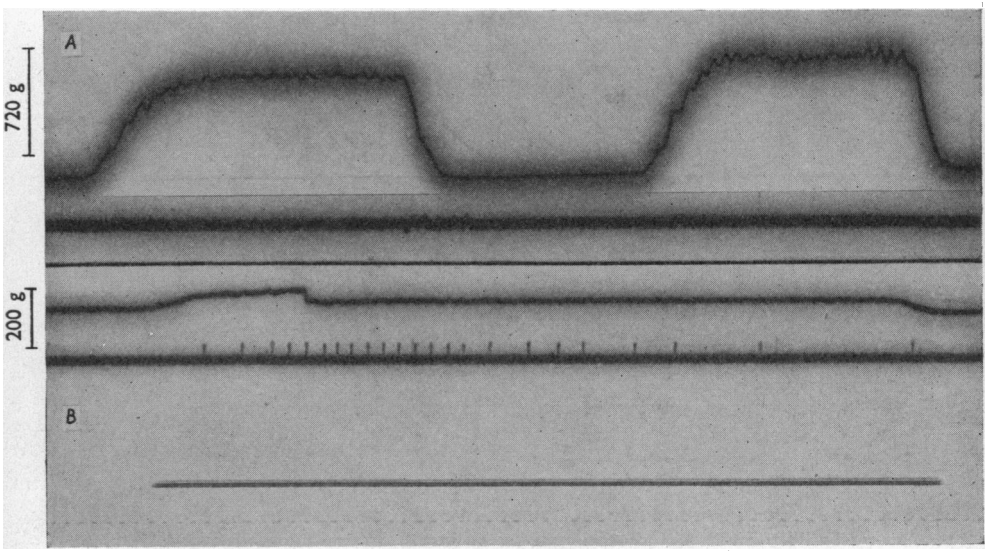

Fig. 3. Responses of a pressure receptor in lateral gastrocnemius-soleus muscle. *A*, shows that stretching the muscle to over 700 g did not stimulate the ending, whereas pressing the muscle in *B* yielded a train of impulses. The rise in muscle tension in *B* is due to pressing the muscle but it does not indicate the amount of pressure applied. From above downwards in *A* and *B*, record of muscle tension, impulses in a filament and in *B*, signal and time marker 1/10 sec. Conduction velocity of afferent fibre was 14·1 m/sec.

endings may therefore be regarded as pressure receptors and will be referred to as such in this paper. In these experiments the muscle was stretched sufficiently to raise the tension by from over 400 to 700 g (Figs. 3, 8); in some fibres this yielded one impulse, in others two or three impulses in some of the trials. From such observations it was clear that the fibres were not connected to stretch receptors, considering that the threshold of most stretch receptors is less than 350 g (Matthews, 1933; Hunt & Kuffler, 1951), although the threshold of some of Matthews's B endings was as high as 700 g.

The response to local pressure varied in different receptors not only quantitatively, but also in the type of mechanical stimulus that stimulated them. Thus while some were stimulated by pressing the appropriate region of the muscle gently with a glass rod, others could be stimulated only by squeezing the muscle between finger and thumb. Again, the direction in which the muscle was pressed or squeezed was important. Also, sometimes the receptors which were stimulated by pressing with a glass rod were poorly stimulated by squeezing the muscle. Owing to such variations it was not feasible to use any single device for applying pressure.

A little less than half the pressure receptors isolated could be stimulated consistently each time local pressure was applied at the proper place. At the other extreme there were receptors that could be stimulated in only 20% of the trials under the most favourable circumstances. The remaining receptors fell in between these two extremes.

The stimulus threshold was difficult to assess in those receptors which could only be stimulated by pressing or squeezing the muscle manually. However, a rough estimate of the pressure exerted was made by noting the pressure recorded by an algometer (Baird & Tatlock) when pressure of the same order was applied to it. The threshold estimated roughly in this way varied from 300 g to 2 kg/cm.^{2} Those receptors that could be stimulated by pressing locally with a blunt point allowed a more precise measurement of the threshold to be made, as direct pressure could be applied by the blunt point of the strain gauge which had an area of about 16 mm^2. The threshold pressure registered by the strain gauge varied in different receptors from 25 to 200 g. The threshold varied somewhat in each trial and also with the direction of application of pressure. The receptors were apparently more easily stimulated if the muscle was not stretched initially, but owing to the variation in the responses mentioned the precise influence of stretch on stimulus threshold was not studied.

Most of the pressure receptors adapted rapidly, the discharge ceasing in about 0·5–2 sec after its onset. About a third of them adapted more slowly (Figs. 3, 8) and there were only a few which showed a truly slowly-adapting type of discharge.

The peak frequency of discharge also varied considerably from one fibre to another, the lowest value being in the range of 2–10 impulses/sec and the highest 100–150 impulses/sec. Such peak frequencies were averaged over 0·1 sec at high frequencies and over 0·5 sec in the low-frequency range. Attempts to raise the peak frequency of the receptors firing at a low frequency were seldom successful. These observations contrast with the responses of stretch receptors, in which the peak frequency may reach 500/sec (Matthews, 1933).

An attempt was made to determine the range of conduction velocities

over which the fibres of pressure receptors were distributed. This was achieved by reversing the sampling procedure, so that fibres in which impulses were aroused by pressing the muscle, but not by stretching it,

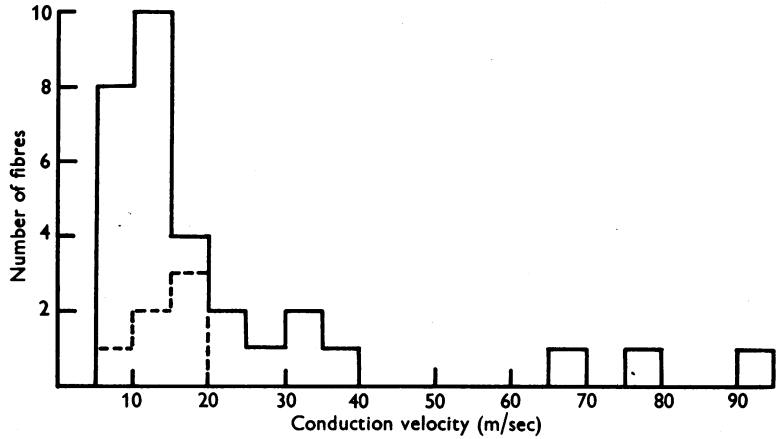

Fig. 4. Frequency distribution of conduction velocities of afferent fibres connected to pressure receptors in lateral gastrocnemius and soleus muscles. Interrupted line, distribution of fibres that could not be stimulated by any type of mechanical stimulus.

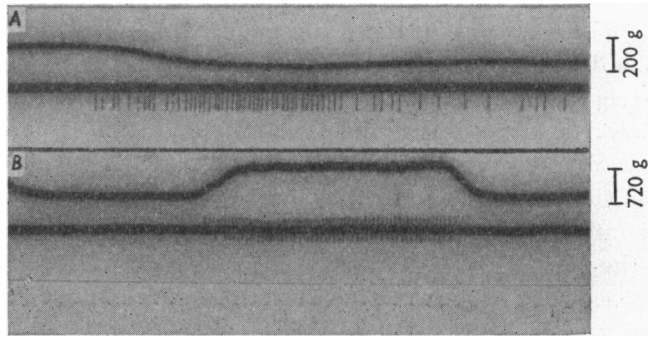

Fig. 5. Response of a pressure receptor of lateral gastrocnemius-soleus connected to a Group I afferent fibre. *A* shows that pressing the muscle locally with the tip of the strain gauge yielded a train of impulses, whereas stretching the muscle from 0 g initial tension to over 700 g did not stimulate the receptor, although it stimulated 2 stretch receptors which were not stimulated by local pressure in *A*. From above downwards in *A* and *B*, record of strain gauge, impulses in fibres; and in *B*, time marker 1/10 sec.

were isolated first and then the conduction velocities of such fibres were determined. The results of these experiments showed that there was a wide range of conduction velocities extending from 6 to 91 m/sec. How-

ever, there are apparently few fast-conducting fibres, because only 4 fibres with conduction velocities of > 55, 66, 76, and 91 m/sec were encountered in the lateral gastrocnemius-soleus nerve. The distribution of the conduction velocities of these fibres have been plotted in Fig. 4. It is clear from these results that pressure receptors with characteristics described above have afferent fibres distributed in all three groups, but the majority of them are in Group III, the peak being in the 10–15 m/sec range. In this

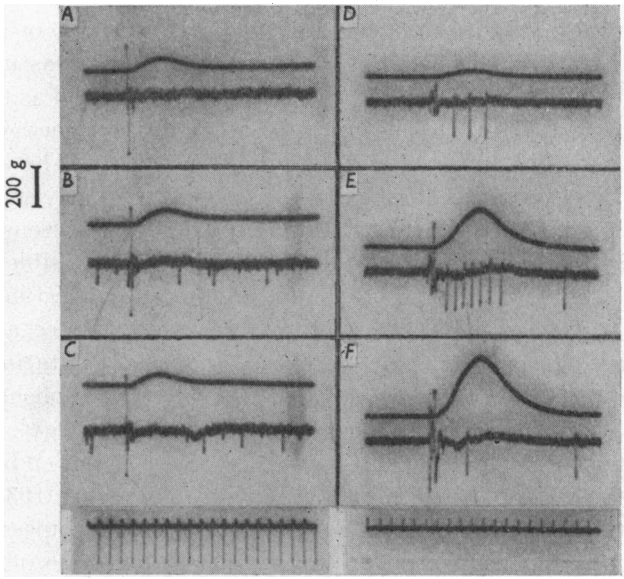

Fig. 6. Effect of muscular contraction on pressure receptors of lateral gastrocnemius-soleus. In *A*, muscle twitch without local pressure had no effect; in *B* and *C* muscle twitch with applied local pressure gave no response during contraction. Conduction velocity of afferent fibre was 33·6 m/sec. In records *D*, *E*, and *F*, which are from another fibre (conduction velocity 23 m/sec), local pressure applied with a glass rod was kept constant but the strength of muscular contraction was increased from *D* to *F*; note optimal contraction in *E*. Time marker, 10 msec. Calibration at left applies only to *A*, *B*, *C*.

series only 4 fibres in the Group II range were encountered, but it is felt that with better sampling procedure perhaps a slightly greater number will be found in Group II. All the pressure receptors had the characteristics of Group III pressure receptors described above, i.e. they were unaffected by stretching the muscle to over 700 g but were stimulated by local pressure. The discharge of the endings with Group I fibres was in most cases slowly-adapting and the threshold seemed lower, these being perhaps the only significant differences from those of Group III (Fig. 5).

Effect of muscle twitch. The effect of muscular contraction was observed

in only 13 out of 24 pressure receptors. This was because neuromuscular block which was often required for identification did not permit similar observations to be made in the remaining receptors. Five out of the 13 examined were unaffected by muscular contraction with or without applied external stretch. The responses of the remaining 8 were rather unimpressive and varied in the same ending (Fig. 7*A*). Five of the latter showed an 'early discharge' somewhat characteristic of that seen in muscle spindles and tendon organs (Hunt & Kuffler, 1951; Granit *et al.* 1959), in which one or more impulses appeared with a latency of 3–6 msec after the stimulus to the nerve. The number of impulses elicited was usually 2 or 3, rarely 6. The peak frequency of this discharge, measured as the reciprocal of the interval between the impulses, was 500–600 /sec as in stretch receptors (Hunt & Kuffler, 1959; Granit *et al.* 1959). The number of impulses in this 'early discharge' did not vary consistently with the tension applied; sometimes it fell with increase of initial tension. These impulses were abolished by curare or Flaxedil. It is possible they were ephaptic in origin, as has been postulated by Granit *et al.* (1959), but one cannot rule out mechanical stimulation with the evidence available at present. One observation that supports ephaptic excitation is that, although the response of one receptor to pressure was abolished by local mechanical injury, its fibre still yielded the 'early discharge'. The 'early discharge' must influence the subsequent response of the ending in view of the known effects of antidromic depression (Matthews, 1933; Paintal, 1959*b*), particularly if the train consists of several impulses. Perhaps these endings would have been excited during some phase of the twitch if the 'early discharge' were not there. Such a discharge was observed in two receptors in which an 'off-effect' type of response was seen in the form of 2–4 impulses after the active phase of contraction (Fig. 7*A*).

Since there was no resting discharge in these receptors it was considered worth while to see what would happen during a twitch if such a steady discharge could be created. Accordingly, the effect of a muscle twitch during application of local pressure was examined, and as expected there was no consistent response. Some receptors showed a pause during contraction, as in Fig. 6*B*, *C*, others a tendon-organ type of discharge (Fig. 6*D*, *E*, *F*). With constant pressure the discharge in one of these increased with increasing strength of contraction up to a point and then declined (Fig. 6*D*, *E*, *F*); this is of course quite unlike the discharge from tendon organs. In this receptor increase in the local pressure applied increased the number of impulses in the train.

Effect of tetanic contraction. The effect of tetanic contraction of muscle at 40–100 /sec was observed on 10 pressure receptors. Six of them were apparently unaffected by this procedure, although the muscle was

tetanized through its nerve at subthreshold strength for the afferent fibre so that its receptor was not depressed antidromically (Paintal, 1959*b*). The remaining 4 were stimulated to varying degrees. In two fibres tetanic stimulation at about 40/sec yielded a discharge of 1–2 impulses/sec in one fibre and about 6/sec in the other; this ceased soon after tetanus. In a third, stimulation at 90/sec produced a discharge of 10 impulses/sec which

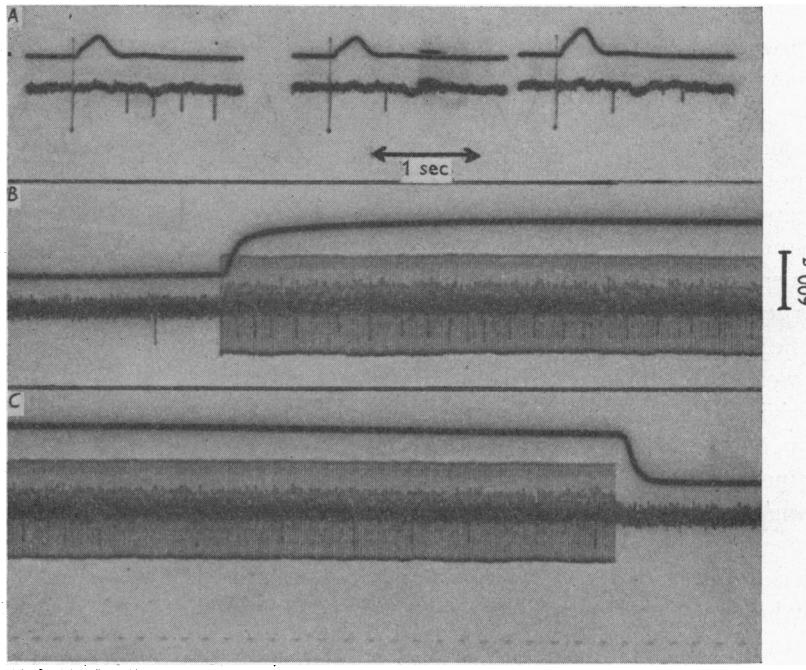

Fig. 7. Effect of muscular contraction on a lateral gastrocnemius-soleus pressure receptor. *A* shows variable responses to single muscle twitches in 3 successive sweeps. *B* and *C*, which are continuous records, show that tetanic muscular contraction stimulated the receptor. Nerve was stimulated at about 90/sec and the muscle was partially curarized. Lowest trace in *C*, time marker 1/10 sec. Conduction velocity of fibre was 11·4 m/sec.

started about 80 msec after the beginning of the tetanus (Fig. 7*B*). This discharge was not maintained throughout the period of repetitive stimulation and it was present during apparently constant tension, and in one trial while the tension was falling. Tetanus at about 40/sec yielded a similar response but the discharge frequency was lower; at 18/sec the receptor was not stimulated. In the fourth receptor a train of impulses at a frequency of about 10/sec appeared after the end of the tetanus and lasted for about 6 sec. The absence of discharge during tetanus in

this fibre was probably due to antidromic bombardment of the ending, as the stimulus used was suprathreshold for the afferent fibre. These results indicate that prolonged muscular contraction as in a tetanus can stimulate some pressure receptors, and that this excitatory action ceases with the end of the tetanus although in certain receptors it may outlast it.

Location of receptors. Only 10 pressure receptors have been located in the muscle; in the case of the others either no attempts were made to locate them or the attempts were unsuccessful. The method of location was quite simple. The region which yielded the maximum response to pressure was at first tentatively recognized as the location of the receptor. This region was then either destroyed or disconnected from the rest of the muscle, and if the response disappeared the tentative conclusion stood. If it did not, the procedure was repeated. To determine whether the receptors were in the soleus or lateral gastrocnemius the muscles were separated from one another and the procedure for location described above followed.

Three of the 10 receptors were located precisely in the musculo-tendinous region of the tendo Achillis; two were located in the fascia covering the flexor digitorum longus muscles. Of the remaining five receptors two were located in the belly of the lateral gastrocnemius muscle (one near its origin) and three in the belly of the soleus. These results indicate that many of the pressure receptors are probably located in the musculo-tendinous region of the muscle. This information may be of value for determining the central effects of these receptors.

Effect of asphyxia. As is well known, occlusion of the circulation or asphyxia has profound excitatory effects on muscle stretch receptors (Matthews, 1933; Paintal, 1959*b*). It was therefore of interest to see if the pressure receptors were similarly affected. Accordingly, the behaviour of pressure receptors was examined after stopping the respiratory pump (followed by cardiac standstill) in curarized animals. Out of 9 pressure receptors on which the effect of asphyxia was tried, only 1 was stimulated and this was connected to a Group I fibre with a conduction velocity of 91 m/sec. All the others, 7 of Group III and 1 of Group II, were not stimulated by asphyxia. The responses of some of the receptors to pressure lasted for as long as 1 hr after stopping the pump but in no case were their responses enhanced.

Effect of NaCl 6 % (*w*/*v*). Since the pressure receptors were not stimulated by any type of stimulus other than pressure, which had to be severe to stimulate some of them, the possibility that they were pain endings was considered, particularly since subjective experience indicates that squeezing a muscle produces pain—this local tenderness being marked after strenuous exercise. Further, Lewis has shown that severe muscular pain is produced by local injection of 6 % NaCl (Lewis, 1942). Accordingly,

the effect of locally injected 6% NaCl on some pressure receptors was noted. For this purpose observations were made exclusively on Group III pressure receptors.

After locating a receptor a No. 12 gauge needle was inserted into the site. This procedure itself evoked a train of impulses (Fig. 8*C*). This was apparently not a pressure effect of the type described above because the pressure exerted by the needle in some cases was much less than that required to stimulate the endings by pressure *per se*. Further, excitation

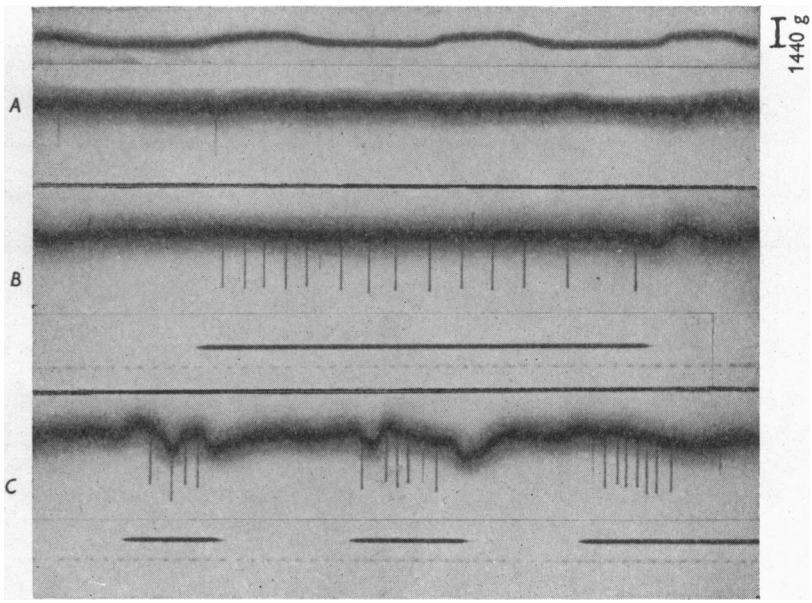

Fig. 8. Responses of a lateral gastrocnemius-soleus pressure receptor. *A* shows that stretching the muscle did not stimulate the receptor, whereas pressing the muscle at signal in *B* stimulated the ending. *C* shows that inward movement of a hypodermic needle inside the muscle at signals stimulated the receptor. Lowest trace in *B* and *C* time marker 1/10 sec. Conduction velocity of fibre was 17·1 m/sec.

occurred only after penetration of the muscle, at which time the pressure exerted by the needle was minimal. It appeared that this excitation was due to the movement or progress of the needle inside the muscle, because small displacements of the needle in and out of the muscle excited the receptor. In fact, in some receptors there was excitation when movement occurred in a particular direction, e.g. inward in the case of the receptor shown in Fig. 8*C*. The excitation by a needle was short-lasting, the discharge rarely exceeding 1 sec and being usually over within a fraction of a second. Occasionally this was followed by infrequent and irregularly occurring impulses with a frequency of less than 1/sec.

With the position of the needle unchanged about 0·5 ml. of 6 % NaCl was injected. In 8 out of 9 cases this yielded a discharge of impulses lasting from 2 to 5 min. Usually the discharge built up gradually as in Fig. 9 to reach peak frequency about 15–50 sec from start of stimulation. The latent period, reckoned from the beginning of injection to the beginning of stimulation, varied in different receptors from 1·3 to 23 sec. The longer latencies were probably due to the needle being at some distance from the endings and the interval therefore represents the time taken for the solutions to diffuse to the ending. The peak frequency was 3–30 impulses/sec.

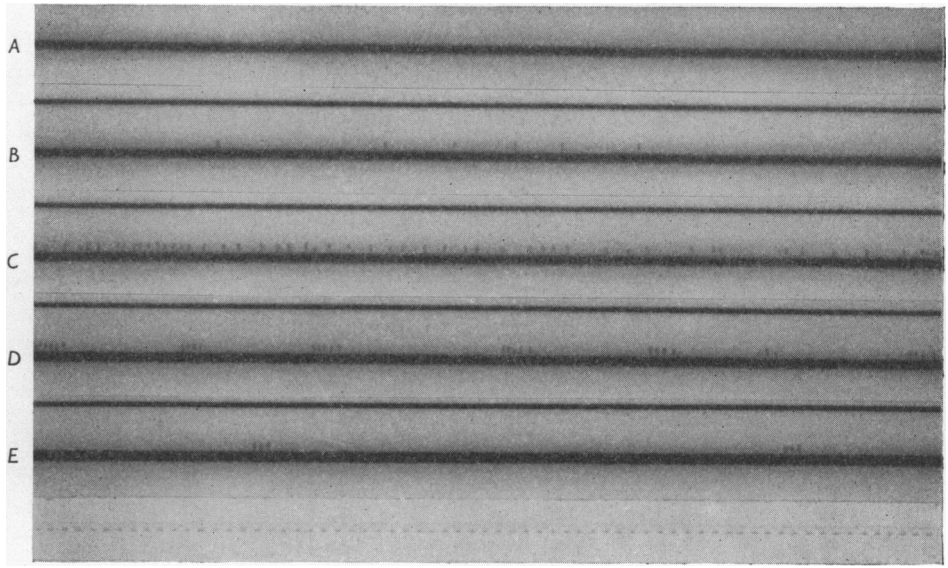

Fig. 9. Effect on a lateral gastrocnemius-soleus pressure receptor of locally injected 0·5 ml. 6 % NaCl. *A* is a record before injection of 6 % NaCl; *B–E* are records respectively at 50, 120, 175, and 260 sec after injecting 6 % NaCl. Lowest trace, time marker 1/10 sec. Conduction velocity of fibre was 14·1 m/sec.

After a period of continuous activity, as in Fig. 9*C*, cyclical discharges appeared in some receptors. This consisted of short trains of impulses lasting about 0·2–2 sec, separated by variable periods of silence (Fig. 9*D*, 10*C*). As time passed the duration of the trains diminished and the periods of silence increased (Fig. 9*D*, *E*).

After the excitation by NaCl had died down it was found that the response of the receptors to pressure was reduced. It was also found consistently that the response to a second injection of NaCl was less than that following the first. The response in Fig. 9 shows the effect of a second injection; the response to the first being much more marked.

The effect of NaCl 0·9 % (w/v) was tried on 4 receptors. This was injected in the same way before injecting 6 % NaCl. It had no excitatory action on 3 receptors that were markedly stimulated by 6 % NaCl. In one case the receptor was clearly stimulated, the discharge lasting for about 4 min (Fig. 10*B*). In this case 6 % NaCl stimulated the ending after a latency of only 1·3 sec (Fig. 10*D*), which is small when compared

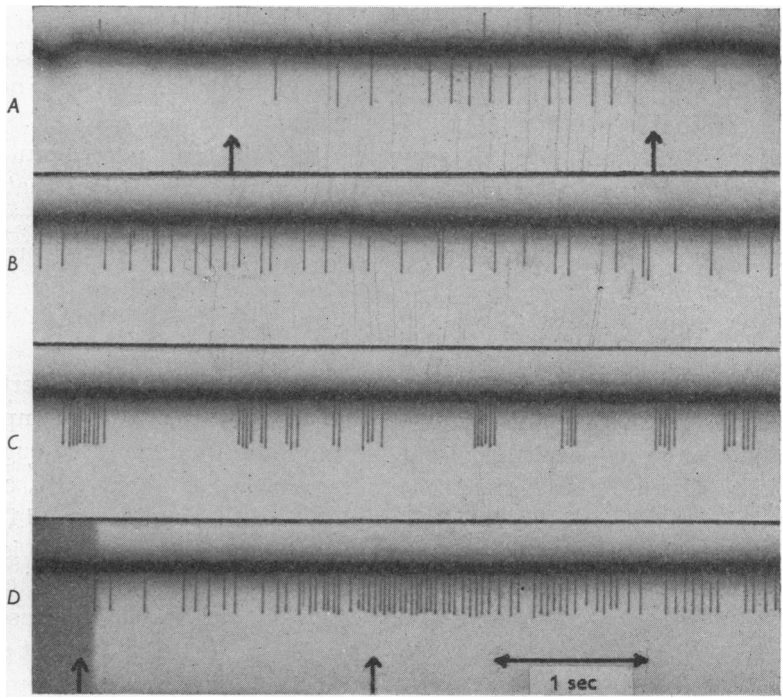

Fig. 10. Responses of a lateral gastrocnemius-soleus pressure receptor. *A* shows the response to local pressure applied to the muscle between arrows. *B* and *C* are records taken respectively at 7 and 30 sec after intramuscular injection of 0·5 ml. 0·9 % NaCl near location of receptor. *D* shows the marked and immediate stimulation of the receptor by injecting 6 % NaCl at the same place; the injection is indicated by arrows. Conduction velocity of fibre was 17·1 m/sec.

to the usual latency of 8–23 sec seen in the other receptors. It is conceivable that in this instance the hypodermic needle was closer to the ending than in the other cases.

The behaviour described above is the kind that would explain some of the subjective experiences of pain following intramuscular injection of 6 % NaCl in man (Lewis, 1942). Probably these pressure receptors are indeed responsible for the sensation of pain from 6 % NaCl, but the possibility that stretch receptors may also be involved has to be kept in mind.

The effect of 6 % NaCl on stretch receptors was therefore tried. For this purpose the whole nerve of either medial gastrocnemius or lateral gastrocnemius-soleus was placed on the recording electrodes and 6 % NaCl injected as before. This did not produce any visible change in the resting discharge although 0·5 ml., which is a very large dose for a cat's gastrocnemius compared to the 0·03 ml. used by Lewis (1942), was injected into several regions of all three muscles in a number of experiments. Occasionally a high-frequency discharge appeared in 1–4 fibres of unknown identity, especially if the solution was injected near the origin of the muscle. Larger doses (1·0 ml.) activated several fibres, but this discharge was only a fraction of that accompanying moderate ventriflexion of the foot. Since ventriflexion of the foot consistently produced a pronounced maintained discharge and since this procedure is quite painless, it is evident that pain following local infiltration of 6 % NaCl is not mediated by stretch receptors. The receptors that qualify for this role so far are therefore the pressure receptors of Group III and possibly also those of Group II.

Receptors in tibialis anterior

The experiments on the receptors of tibialis anterior were undertaken after those on the lateral gastrocnemius and soleus had been completed and the results assessed. Figure 2 of Lloyd & Chang (1948) suggests that in the mixed lateral gastrocnemius-soleus nerve about 16 % of the fibres belong to Group III (18 % in lateral gastrocnemius and 14 % in soleus). With this population of Group III fibres it was possible to isolate usually 1–2, rarely 3, Group III fibres in each experiment. This of course would depend on the skill of the experimenter and the techniques used by him. On the other hand, Fig. 3 of Lloyd & Chang (1948) shows that about 40 % of the afferent fibres in tibialis anterior nerve belong to Group III. It was therefore expected that two to three times the number of Group III fibres isolated per experiment in the lateral gastrocnemius-soleus would be isolated in each experiment on the tibialis anterior.

Altogether four experiments were done on the left tibialis anterior and a total of 25 Group III fibres were isolated, an average of about 6 fibres per experiment (range 5–8 fibres/expt.). These were isolated without particular difficulty and they do not represent the maximum that could be isolated, because usually the experiment was terminated before the entire nerve could be examined. These results therefore confirm the expectations arising from the fibre calibre spectra of 'demotored' muscle nerves (Lloyd & Chang, 1948). More experiments on tibialis anterior were not done because the other results were similar to those obtained in the lateral gastrocnemius-soleus nerve.

The conduction velocities of the fibres isolated ranged from 5·2 to

22 m/sec, the maximum occurring in the 5–10 m/sec range. Relatively few fibres were isolated in the 20–25 m/sec group; this may have been due to some undetected bias for the smaller fibres in the sampling procedure. Assuming that the distance travelled by an impulse in 1 sec is equal to 6×10^6 times the fibre diameter (Hursh, 1939) the mean conduction velocity calculated for the Group III fibres in Fig. 3 of Lloyd & Chang (1948) would be about 15 m/sec. This is a little different from the mean of 11 m/sec found in the present experiments.

No impulses could be aroused in 13 of the 25 Group III fibres isolated by stimulating the muscle mechanically; 2, with conduction velocities of 18 and 22 m/sec, respectively, were connected to typical stretch receptors. Ten were connected to pressure receptors whose responses closely resembled those found in the lateral gastrocnemius and soleus. The range of threshold to pressure was also similar. Sometimes, presumably owing to altered conditions, the receptors which could be stimulated by pressure at one stage of the experiment could not be stimulated at all at another. These receptors represented the border-line behaviour between pressure receptors and inactive Group III afferent fibres. It is, therefore, possible that the 13 Group III fibres labelled as inactive could have been activated by pressure at another stage of the experiment. In this connexion the conditions of the experiment should be kept in mind, i.e. separation of muscles, etc. (see Methods).

An important observation, which indicates that there are certain minor differences in the behaviour of pressure receptors of different muscles, is that in 6 of the 10 receptors stretching the muscle rapidly to a tension of about 700 g yielded 1–10 impulses. This represents a greater excitation by stretch than that which occurred in the receptors of the lateral gastrocnemius and soleus muscles. Figure 11*B* shows a receptor which had the best response to stretch out of all the pressure receptors examined. However, the same receptor at an earlier stage of the experiment discharged only 1–3 impulses during stretching of the same degree.

The response to stretch shown in Fig. 11*B* is the kind which Matthews noted in his 'C' endings, most of which were presumably from peroneus longus muscle (Matthews, 1933). The pressure receptors resemble the 'C' endings further in that their responses during active contraction are variable and that some of them, especially those of lateral gastrocnemius and soleus, are attached to fascia.

Unlike those in the lateral gastrocnemius and soleus, a few receptors discharged impulses irregularly at a frequency of less than 1–2/sec under resting conditions. Such pressure receptors could always be stimulated mechanically.

Nine of the 10 pressure receptors were located within the muscle. Unlike

those in the lateral gastrocnemius and soleus the pressure receptors of tibialis anterior were concentrated near the entry of the nerve into the muscle. Two were located about 5 mm central to the entry of the nerve, 4 were located within 1 cm distal to the entry of the nerve, and the remaining 3 within 3 cm distal to the entry of the nerve. The total length of the muscular portion of the muscle is about 8 cm and the nerve enters it about 1·5 cm from its origin.

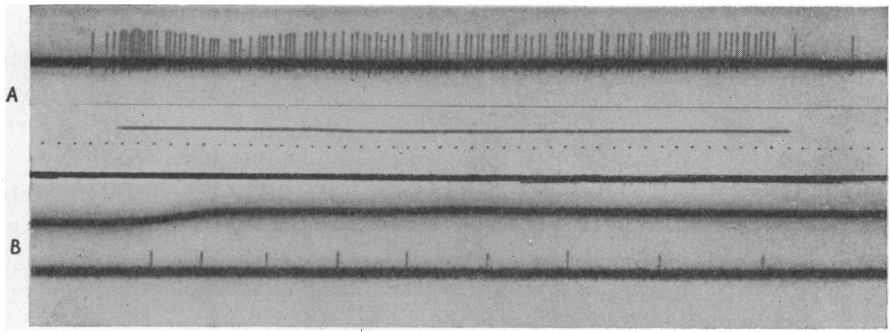

Fig. 11. Responses of a tibialis anterior pressure receptor. *A* shows the response to gentle local pressure. *B* shows the response of the same receptor to stretching the muscle from zero initial tension to 780 g. Lowest trace in *A*, time marker 1/10 sec. Conduction velocity of fibre was 11·1 m/sec.

Electrical threshold of Group III fibres

This was carefully determined in 19 fibres. The threshold was evaluated relative to the threshold of motor fibres; threshold of Group I afferent fibres could not be used as a reference owing to the conditions of the experiments. The threshold ranged from 7 to 44 times the threshold for motor fibres; the average was 24. The duration of the stimulus was kept constant at 0·1 msec. The results indicate that with a stimulus about 20 times threshold for motor fibres about half the total number of Group III fibres would be stimulated.

DISCUSSION

The main conclusion emerging from the experiments is that the majority of Group III fibres in muscles are connected to pressure receptors. Some stretch receptors are also connected to Group III fibres but the number of these is very small. Further, since the results obtained in the lateral gastrocnemius and soleus are similar to those obtained in the tibialis anterior it may be assumed that they apply to other muscles as well.

A great deal of histological study has been devoted to the sensory endings in muscles (for references see Barker, 1948), but there have been

no reports concerning presence of specific structures other than muscle spindles, Golgi tendon organs, and occasional Pacinian corpuscles. There is, therefore, a possibility that some of the Group III fibres merely terminate in naked endings. This is the sort of termination attributed to pain fibres. (Wolff & Wolf, 1951) Recently Barker (1959) has reported a fair number of Pacinian corpuscles in the rectus femoris. It would be interesting to know the size of the nerve fibres supplying these corpuscles.

The Group III fibres probably produce several types of central effects. First, there is a possibility that they mediate the sensation of muscle pain, although there is no actual proof to show that this is so. However, there is some evidence to support this view if it is assumed that the Group III pressure receptors in man respond in the same way as those in cats. First, many of the pressure receptors are stimulated only by strong pressure, e.g. 1–2 kg/cm^2, just that pressure required to produce the pain sensation in man. Secondly, preliminary experiments indicate that the pain produced by squeezing a muscle (e.g. calf muscles) is conducted fast enough for it to be mediated by myelinated afferent fibres, which the Group III are. Finally, the pressure receptors are stimulated markedly by locally injected 6 % NaCl, which produces severe pain in man (Lewis, 1942). The quantities injected into cats (0·5 ml.) might seem large when compared to the quantities required to produce pain in man (0·02–0·03 ml.; Lewis, 1942), but it must be remembered that in the present investigation 6 % NaCl was used to stimulate single pressure receptors which could only be localized in two dimensions but not the third, namely depth. In order to stimulate the endings, therefore, the seemingly large quantity of 0·5 ml. was injected so that it could diffuse to particular pressure receptors in effective concentrations. It is certain that if the solution could be introduced precisely at the receptors only very small amounts would be needed. The above evidence suggests that the Group III pressure receptors may be responsible for some aspects of muscle pain, in which case they could be appropriately referred to as 'pressure–pain' receptors. However, the evidence is still only circumstantial and further experiments on man will be needed to establish the point. In this connexion the role of non-myelinated fibres must be kept in mind because there are endings connected to non-myelinated fibres which are stimulated by strong pressure but not by external stretch (unpublished observations).

According to Lewis the pain accompanying muscle activity during ischaemia is of the same quality and presumably arises from the same source as that produced by squeezing the muscle (Lewis, 1942, p. 41). The results of this investigation do not fit into this scheme, because the pressure receptors are not stimulated by asphyxia and some not even by tetanic contraction during asphyxia. It is possible that there is more than

one pain-conducting mechanism and the fact that there are several types of non-myelinated afferent fibres in muscle (unpublished observations) supports this possibility. At any rate it can be concluded that Group III pressure receptors are probably not responsible for the pain of muscular ischaemia.

Since Group III fibres of muscles facilitate flexor reflexes (Lloyd, 1943; Brock, Eccles & Rall, 1951; Eccles & Lundberg, 1959), it may be concluded that the Group III (and perhaps Group II) pressure receptors of different muscles produce the flexor reflex, especially since the majority of Group III fibres are connected to pressure receptors. This is consistent with their probably nociceptive function outlined above. Recently Eccles & Lundberg (1959) have shown that the interneurones concerned with this pathway are inhibited by higher centres in the brain stem, because the flexor reflex of Group II and Group III fibres, which is insignificant or absent in the decerebrate cat, comes into prominence on dividing the cord in the upper cervical regions. However, the reflex effects of Group III fibres on muscles of the opposite limb (Perl, 1958) suggest that their reflex actions must be complex.

A third possibility is that the pressure receptors are responsible for the increase in ventilation during muscular activity. From the experiments of Iaria, Jalar & Kao (1959) it would appear that the stretch receptors of muscle are not responsible for this increase in minute ventilation on stimulation of a muscle nerve. As shown in this investigation some pressure receptors are stimulated by muscular contraction and if the stimulus strength is high enough the Group III fibres themselves may be stimulated. The possibility that their activity increases ventilation has therefore to be kept in mind. Experiments to test this possibility are in progress.

Finally, the observations of Hunt & Paintal (1958) on the effects of Group III fibres on fusimotor neurones (gamma fibres) must be kept in mind.

The Group II band of afferent fibres can no longer be regarded as a homogeneous group of stretch fibres, owing to the presence of a fair number of fibres connected to pressure receptors (Fig. 4). For the present, the central effects of these receptors may be considered to be the same as those of the Group III ones.

SUMMARY

1. By recording impulses in fibres of known conduction velocity it has been established that the majority of Group III sensory fibres in lateral gastrocnemius, soleus and tibialis anterior muscles terminate in pressure receptors, very few in stretch receptors; in some no impulses could be aroused by mechanical stimuli.

2. The threshold of pressure receptors varied considerably. Most of them adapted rapidly to the stimulus. With a few exceptions they were not stimulated by stretching the muscle. Some yielded a variable number of impulses during a muscle twitch. Tetanic contraction of muscle produced a low-frequency discharge in about half the pressure receptors.

3. Most of the pressure receptors of tibialis anterior were located near the point of entry of the nerve into the muscle. In the lateral gastrocnemius and soleus more were located near the musculo-tendinous region; others in the belly of the muscles.

4. The pressure receptors of lateral gastrocnemius and soleus were connected to afferent fibres with conduction velocities ranging from 6 to 91 m/sec. Most of them were connected to Group III fibres (maximum in the 10–15 m/sec range), some to Group II and very few to Group I fibres. There appear to be more Group III pressure receptors in tibialis anterior than in the lateral gastrocnemius and soleus.

5. Some Group III pressure receptors were stimulated by introducing a hypodermic needle into the muscle at the localized site. Nearly all were stimulated strongly by injecting 0·5 ml. NaCl 6% (w/v) at this site; the duration of stimulation lasted about 2–5 min.

6. It is suggested that the Group III pressure receptors may mediate the sensation of muscle pain produced by squeezing the muscle or injecting 6% NaCl locally (Lewis, 1942); Groups I and II stretch receptors are not responsible for this pain. The Group III pressure receptors are probably not responsible for the pain of muscle ischaemia, because none of them were stimulated following asphyxia and cardiac standstill, some not even by tetanic contraction of muscle during this period.

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REFERENCES

BARKER, D. (1948). The innervation of the muscle spindle. *Quart. J. micr. Sci.* **89**, 143–186.

BARKER, D. (1959). Some results of a quantitative histological investigation of stretch receptors in limb muscles of the cat. *J. Physiol.* **149**, 7–9*P*.

BLAIR, E. A. & ERLANGER, J. (1936). On the process of excitation by brief shocks in axons. *Amer. J. Physiol.* **114**, 309–316.

BROCK, L. G., ECCLES, J. C. & RALL, W. (1951). Experimental investigations on the afferent fibres in muscle nerves. *Proc. Roy. Soc.* B, **138**, 453–475.

COOPER, S. (1959). The secondary ending of muscle spindles. *J. Physiol.* **149**, 27–28*P*.

ECCLES, R. M. & LUNDBERG, A. (1959). Supraspinal control of interneurones mediating spinal reflexes. *J. Physiol.* **147**, 565–584.

GRANIT, R., POMPEIANO, O. & WALTMAN, B. (1959). The early discharge of mammalian muscle spindles at onset of contraction. *J. Physiol.* **147**, 399–418.

HAGBARTH, K. E. & WOHLFART, G. (1952). The number of muscle-spindles in certain muscles in cat in relation to the composition of the muscle nerves. *Acta anat.* **15**, 85–104.

HUNT, C. C. (1954). Relation of function to diameter in afferent fibres of muscle nerves. *J. gen. Physiol.* **38**, 117–131.

HUNT, C. C. & KUFFLER, S. W. (1951). Stretch receptor discharges during muscle contraction. *J. Physiol.* **113**, 298–315.

HUNT, C. C. & PAINTAL, A. S. (1958). Spinal reflex regulation of fusimotor neurones. *J. Physiol.* **143**, 195–212.

HURSH, J. B. (1939). Conduction velocity and diameter of nerve fibres. *Amer. J. Physiol.* **127**, 131–139.

IARIA, C. T., JALAR, U. H. & KAO, F. F. (1959). The peripheral neural mechanism of exercise hyperpnoea. *J. Physiol.* **148**, 49–50*P*.

IGGO, A. (1958). The electrophysiological identification of single nerve fibres, with particular reference to the slowest-conducting vagal afferent fibres in the cat. *J. Physiol.* **142**, 110–126.

KUFFLER, S. W., HUNT, C. C. & QUILLIAM, J. P. (1951). Function of medullated small-nerve fibres in mammalian ventral roots. *J. Neurophysiol.* **14**, 29–54.

LEWIS, T. (1942). *Pain*, p. 3. New York: Macmillan Co.

LLOYD, D. P. C. (1942). Stimulation of peripheral nerve terminations by active muscle. *J. Neurophysiol.* **5**, 153–165.

LLOYD, D. P. C. (1943). Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat. *J. Neurophysiol.* **6**, 293–315.

LLOYD, D. P. C. & CHANG, H. T. (1948). Afferent fibres in muscle nerves. *J. Neurophysiol.* **11**, 199–208.

MASLAND, R. L. & WIGTON, R. S. (1940). Nerve activity accompanying fasciculation produced by prostigmin. *J. Neurophysiol.* **3**, 269–275.

MATTHEWS, B. H. C. (1933). Nerve endings in mammalian muscle. *J. Physiol.* **78**, 1–53.

MERTON, P. A. (1953). Slowly conducting muscle spindle afferents. *Acta physiol. scand.* **29**, 87–88.

PAINTAL, A. S. (1953). The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve. *J. Physiol.* **121**, 341–359.

PAINTAL, A. S. (1959*a*). Intramuscular propagation of sensory impulses. *J. Physiol.* **148**, 240–251.

PAINTAL, A. S. (1959*b*). Facilitation and depression of muscle stretch receptors by repetitive antidromic stimulation, adrenaline and asphyxia. *J. Physiol.* **148**, 252–266.

PERL, E. R. (1958). Crossed reflex effects evoked by activity in myelinated afferent fibres of muscle. *J. Neurophysiol.* **21**, 101–112.

REXED, B. & THERMAN, P. O. (1948). Calibre spectra of motor and sensory nerve fibres to flexor and extensor muscles. *J. Neurophysiol.* **11**, 133–140.

WOLFF, H. G. & WOLF, S. (1951). *Pain*, 1st ed. Revised third printing, p. 15. Springfield: Charles C. Thomas.