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THE ELECTRICAL ACTIVITY OF MAMMALIAN INTRAFUSAL FIBRES

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The function of mammalian spindles has been extensively investigated in recent years. From these studies it is apparent that stimulation of small-diameter ventral root axons increases the discharge rate of spindle afferent fibres. (The motor fibres to the spindle have been variously designated as gamma efferents (Leksell, 1945), small-nerve motor fibres (Kuffler, Hunt & Quilliam, 1951) and fusimotor neurones (Hunt & Paintal, 1958).) The available experimental evidence indicates that the increased sensory discharge during fusimotor activation results from contraction of intrafusal fibres. In fact, Kuffler *et al.* (1951) succeeded in recording a small potential from the muscle surface during selective stimulation of fusimotor neurones. This potential was localized in a small muscle area and these authors concluded that it must have originated from intrafusal muscle fibre activity. However, it could not be decided whether this 'intrafusal potential' resulted from action localized to the intrafusal neuromuscular junction or from activity propagated along the intrafusal fibres.

Activity of muscle fibres of qualitatively different types elicits, in lower vertebrates, either propagated or junctional potentials only. Thus, a 'twitch' and a 'slow' muscle systems have been established in the frog (Kuffler & Vaughan Williams, 1953*a*, *b*). Since mammalian extrafusal fibres are equivalent to the frog's 'twitch' fibres it was of interest to determine whether intrafusal bundles behave like those of the 'slow' system of the frog. It has long been suspected that mammalian intrafusal fibre contraction is slower than that of extrafusal elements, a deduction based upon the sensory discharge pattern evoked during stimulation of fusimotor neurones (Kuffler & Hunt, 1950). Results of the present study suggest that intrafusal fibre activity elicits propagated action potentials during fusimotor stimulation; consequently, intra- and extrafusal fibres are similar. A preliminary report has already been published (Eyzaguirre, 1959).

METHODS

Cats were anaesthetized with sodium pentobarbital. The tenuissimus muscle was excised, together with its own nerve and part of the sciatic (for details see Adrian, 1925). The preparation was mounted in a transparent chamber containing Locke's solution (NaCl 9·0, KCl 0·42, $CaCl_2$ 0·24, and $NaHCO_3$ 0·2 g/l.); dextrose 1·0 g/l. was added immediately before the experiment. A gas mixture containing 95 % O_2 and 5 % CO_2 was bubbled into the saline, which was covered by a layer of paraffin oil. The temperature of the bath was maintained at 37–38° C by means of an insulated nichron loop connected to a battery. Both ends of the muscle were firmly gripped by forceps which were part of a 'stretcher' operated by micrometer screws; this device allowed careful adjustment of the resting tension. In general, the experimental arrangement was similar to one used for cold-blooded preparations (Eyzaguirre & Kuffler, 1955; Eyzaguirre, 1957, 1958) except for the heating element. With the aid of a binocular dissecting microscope and polarized light the preparation was cleaned from connective tissue. The nerve was laid flat on a glass plate and split with sharp dissecting needles in order to isolate single motor and sensory fibres. If the nerve sheath proved too tough the nerve was placed in a solution of papaine or chymotrypsin (1 mg/ml.) and digestion was allowed to proceed for 30–60 min. After that time connective tissue was considerably softer and isolation of single fibres relatively easy. Isolation of fusimotor fibres proved somewhat difficult when the nerve was teased close to the muscle; it was easier to isolate them high up in the sciatic nerve. Normally, fusimotor fibres were identified by their effect upon stimulation on sensory discharge rate in the absence of extrafusal muscular contraction. In a number of experiments fusimotor fibres were stimulated by axon reflex; one nerve branch to the tenuissimus was cut close to the muscle and stimulating electrodes were placed on its proximal end. Nerve action potentials were recorded from small filaments which often contained single sensory fibres. Sometimes it was possible to detect visually a slender filament contracting during fusimotor stimulation. Contraction of such a filament coincided with increased sensory discharges. Intrafusal potentials were recorded from the surface of the muscle immersed in paraffin oil by small (50 μ) platinum-iridium electrodes connected to capacity-coupled amplifiers. High-frequency filters were used in order to decrease amplifier noise. The filtering used did not appreciably change the amplitude or time course of most intrafusal potentials. If the potentials recorded were sufficient in amplitude for ease of measurement the fusimotor fibre was stimulated at 1/sec. However, when the signal: noise ratio was low, about 10 responses were superimposed at a stimulation frequency of 10/sec.

Several drugs were applied either to the whole muscle or locally. For topical application a portion of the muscle was lowered into a small box containing a solution of the substance, while the rest of it remained in oil. Thus, intrafusal potentials could be recorded during drug action. In other experiments 11 % sucrose was applied in similar fashion (Fig. 4*A*).

RESULTS

Location of spindles

With the muscle suspended in oil, a spindle which responded to stimulation of a given fusimotor fibre could be located by determining the longitudinal distribution of the resulting intrafusal potential. In some cases location was aided by seeing the contraction of an intrafusal bundle. If this was not possible one electrode was fixed at one end of the muscle while the other recorded the response to fusimotor stimulation at successive points 1 mm apart along the length of the muscle. At each point the response was

recorded photographically. From these data a map of the longitudinal distribution of the intrafusal potentials was constructed. In most instances intrafusal potentials were recorded with one electrode leading and the other indifferent. An intrafusal bundle is only a short and slender filament surrounded by an encapsulated lymphatic space as well as a mass of 'inert' muscle tissues. Consequently, conditions similar to those in a volume conductor were present in this situation, although the preparation was immersed in oil. In some cases it was clear that the leading electrode picked up the electric potentials of more than one intrafusal bundle (see later).

The amplitude, duration and polarity of the intrafusal potentials varied from one experiment to another. The usual sequence of potential change as the roving and leading electrode approached a spindle region was from a purely positive deflexion to a positive–negative deflexion. When the electrode was further advanced to the active region a positive–negative–positive deflexion was registered. When a positive–negative deflexion was obtained, this sequence of events indicated that such a region acted as a source and then as a sink for current flow and hence was apparently at the end of the spindle. As the electrode was advanced further, the positive–negative–positive potential probably indicated that the region acted as a source, then a sink and then a source for current flow and that presumably was at the centre of the spindle. In some instances negative–positive deflexions were obtained. The latter, however, were difficult to obtain and apparently were produced by superficially located spindles. In other cases only small positive deflexions were registered which did not change appreciably by shifting the recording electrode within an active locus. It is suspected that this type of potential originated from deep-lying spindles.

Figure 1 shows the results of a methodical exploration of a muscle 80 mm long. A single fusimotor fibre was stimulated at 10/sec while one indifferent recording electrode (*Li*) remained stationary at the right end of the muscle (point 80). The other leading electrode (*La*) was moved from point 80 to point 0 (left end of the muscle). Exploration from point 80 to point 45 did not show any intrafusal potentials. At point 45 a small positive deflexion appeared which did not change significantly up to point 35. Between points 33 and 31 a positive–negative deflexion was recorded. At point 31 a triphasic potential appeared and this response was well recorded up to point 29. After point 29 a mainly positive deflexion was recorded (see point 27). At point 26 and toward point 0 another complex appeared, partly superimposed on the previously recorded potential (not illustrated). This last complex apparently originated in another spindle innervated by the same fusimotor fibre. When *La* was kept stationary at any point between points 0 and 45, the recorded potential

did not change when *Li* was moved from point 45 toward the right end of the muscle (point 80). The shaded area in the illustration indicates the probable position and length of the spindle. In other experiments larger intrafusal potentials were recorded, but the sequence of potential changes was similar to that presented in Fig. 1. Figure 2 illustrates such an instance. A 65 mm muscle was explored in millimetre steps and a single fusimotor fibre was stimulated at 1/sec. When both recording electrodes

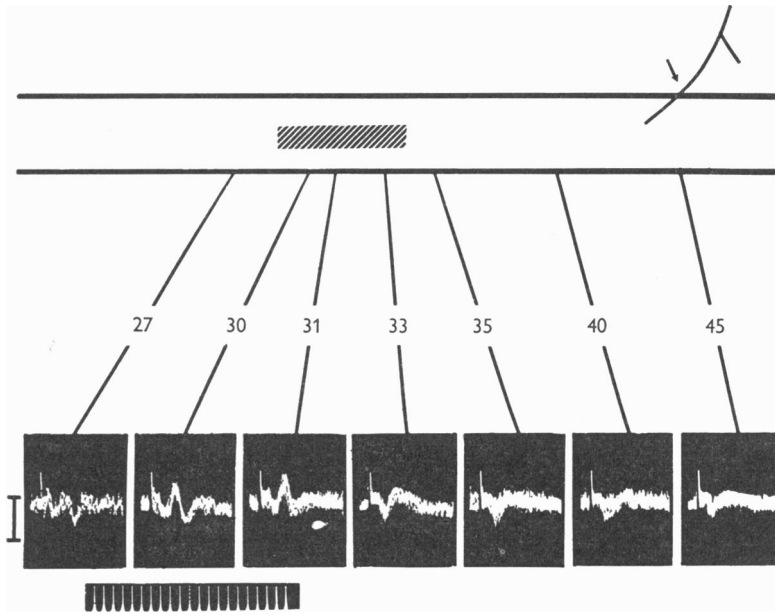

Fig. 1. Electrical exploration of tenuissimus muscle during stimulation of single fusimotor fibre at 10/sec. Muscle was 80 mm long. One electrode (*Li*) placed at an indifferent position (point 80) the other electrode (*La*) explored the surface in millimetre steps. Numbers indicate the position of *La* expressed in millimetres from left end of muscle (point 0). Arrow shows the point of nerve entry into the muscle. Voltage, 20 μV. In all figures except 8*A*, *B*, *D*, *E*, time marker 1 msec.

were placed at any points to the left of point 40 intrafusal potentials were not recorded. *Li* was left fixed at point 40 and *La* was moved past this point toward point 65 (right end of the muscle). At point 55 a small positive–negative deflexion occurred, the negative phase increasing in amplitude up to point 63 where a late positive deflexion appeared, which was more conspicuous at point 65. It is probable that the spindle in this case was located between points 55 and 65 (shaded area).

From these experiments it is concluded that electric exploration of the muscle surface during fusimotor activation allows the location of active intrafusal bundles provided that a single fusimotor fibre is stimulated.

Stimulation of more than one spindle efferent fibre produced intrafusal potentials which were too complex to permit accurate location of the spindle (see below). Further proof that electrical exploration is an adequate means for determining the position of a spindle was provided by experiments in which curare was applied locally. After electrical location of a spindle topical application of curare at this region completely blocked the appearance of the intrafusal potential (p. 179).

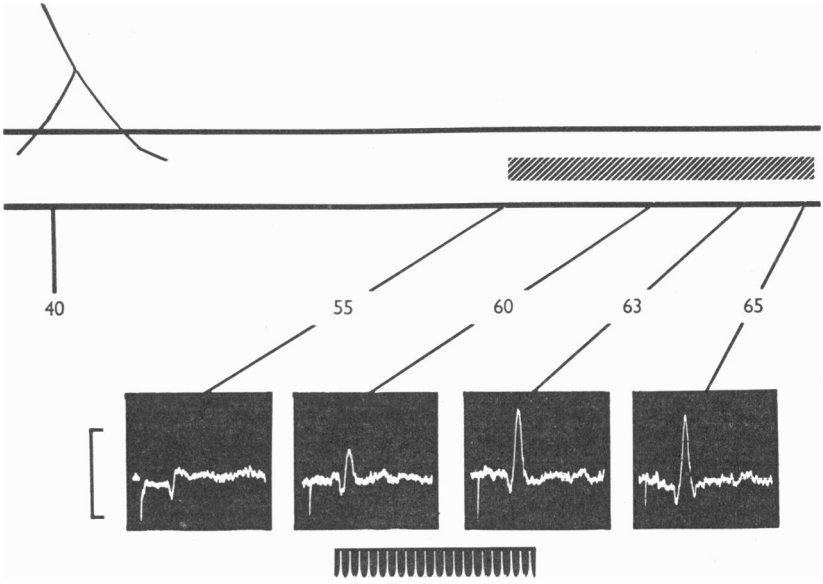

Fig. 2. Exploration of tenuissimus muscle during single fusimotor fibre stimulation at 1/sec. Muscle was 65 mm long. *Li* placed as an indifferent electrode at point 40. Numbers indicate the position of *La* on the surface (see text). Voltage, 20 μV.

The intrafusal potential

It was shown in the previous section that stimulation of a single fusimotor fibre elicited intrafusal potentials which were relatively simple and similar to potentials registered in a volume conductor (Figs. 1, 2). However, stimulation of more than one fusimotor fibre frequently produced complex polyphasic responses. Such potentials are illustrated in Fig. 3. The muscle was immersed in oil and the leading electrode was kept stationary at a given site. The indifferent electrode was placed 35 mm away on an electrically inactive region. At threshold stimulation a single fusimotor fibre was stimulated and the resulting intrafusal potential is shown in Fig. 3*A*. The intensity of stimulation was slowly raised and an additional intrafusal response appeared, partly superimposed on the previous complex (Fig. 3*B*). With stronger stimulating shocks a larger intrafusal potential

appeared near the end of the previous potentials (Fig. 3*C*). Results illustrated in Fig. 3*A*, *B* and *C* show that when several fusimotor fibres are stimulated the resulting intrafusal potential may become complicated, since unitary responses may appear close to and superimposed on each other. Also, the polarity of the potentials recorded by one electrode at a given site may show different characteristics. Thus, the potential illustrated in Fig. 3*A* was formed mainly by a positive deflexion, whereas the complex recorded in Fig. 3*C* was formed by the potentials illustrated in

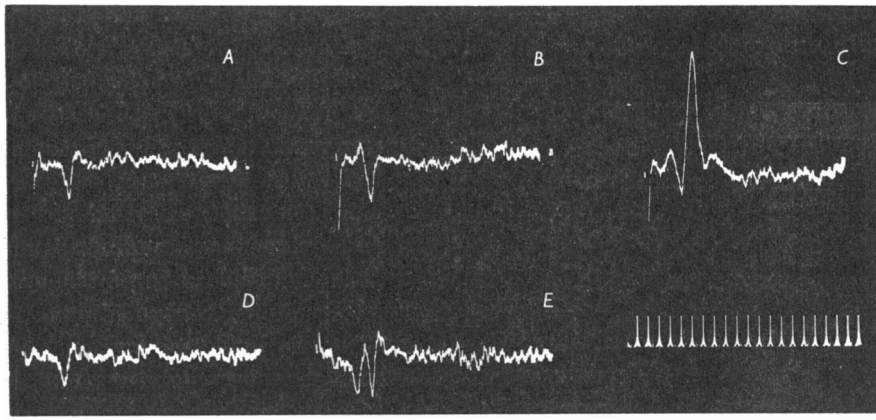

Fig. 3. Intrafusal potentials elicited by stimulation of several fusimotor fibres; leading electrode kept stationary at a given recording site, indifferent electrode 35 mm away. *A*, threshold stimulation evoked an intrafusal potential with prominent positive phase. *B*, activation of an additional fusimotor fibre elicited a smaller potential partly superimposed on previous one. *C*, a larger and mainly negative intrafusal potential produced by additional activation of a third fusimotor fibre. *D* and *E*, taken from different experiments: *D*, intrafusal potential produced by threshold fusimotor stimulation; *E*, additional potential was evoked by activating also another fibre.

Fig. 3*A* and *B* plus a large negative deflexion. In short, at a given recording site the leading electrode may record the sum of potentials of opposite polarity. Compounded intrafusal potentials appearing in close succession were not always the rule. At times, stimulation of more than one fusimotor fibre elicited intrafusal potentials separated by sufficiently long intervals to make them appear as separate entities. This occurrence is illustrated in Fig. 3*D*, *E*. Weak shocks delivered to a nerve filament activated a single fusimotor fibre, and the resulting intrafusal response is illustrated in Fig. 3*D*. Stronger fusimotor shocks produced an additional and slightly larger intrafusal potential after some delay (Fig. 3*E*).

The analysis of intrafusal potentials was difficult when both recording electrodes led off the intrafusal responses from the muscle surface and it

was not known which component of the intrafusal potential was being recorded by one or the other lead. This complication was sometimes unavoidable, even when a single fusimotor fibre was stimulated, since the spindles in the tenuissimus are placed serially (Boyd, 1956) and frequently a single fusimotor fibre may divide and innervate more than one spindle (Hunt & Kuffler, 1951). In order to eliminate this difficulty experiments were designed to produce a localized block under one electrode or between the recording leads. Figure 4 illustrates such a procedure. The muscle was lifted into oil and the spindles were located either by visual observation or by electrical exploration. That portion of the muscle was lowered into a small box and the recording leads were placed at both edges of the groove

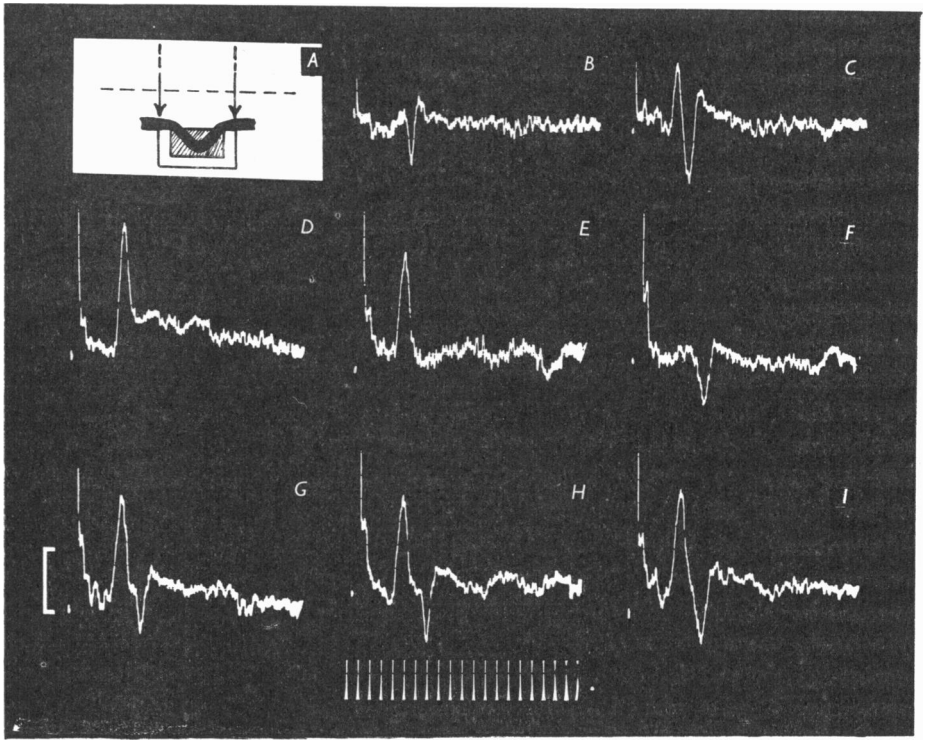

Fig. 4. Complex intrafusal potentials recorded from muscle surface, recording electrodes set 30 mm apart. Two fusimotor fibres stimulated by shocks of different intensities. *A*, schematic diagram of recording conditions (see text). *B*, threshold stimulation elicited one intrafusal potential. *C*, stronger shocks produced an additional intrafusal potential. Between *C* and *D* 11 % sucrose was applied for 10 min between recording electrodes; *D*, larger and monophasic intrafusal potential. *E*, 10 min after *D*. *F*, 10 min later weaker shocks again elicited intrafusal potential previously recorded in *B*. *G–I* illustrate further recovery from sucrose. Voltage, 16·5 μV.

(Fig. 4*A*). During control recording the box was filled with oil. In this particular experiment the recording leads were set 30 mm apart. Intensity of stimulation to the fusimotor fibres was gradually increased. At threshold stimulus strength an intrafusal potential averaging 13·2 μV was recorded. This potential had a shock–peak latency of 4·18 msec $\pm$ 0·07 S.D. (Fig. 4*B*). Stronger stimulation elicited an intrafusal potential of 29·7 μV measured from peak to peak. Shock–peak latency of the upward deflexion was 3·24 msec $\pm$ 0·08 S.D., while the downward deflexion had a latency of 4·17 msec $\pm$ 0·06 S.D. (Fig. 4*C*). It may be noticed that the shock–peak latency of the potential illustrated in Fig. 4*B* and that of the downward deflexion of the potential illustrated in Fig. 4*C* are the same. The following procedure was used to determine which lead recorded the potential in 4*B* and whether the complex illustrated in 4*C* was being recorded by the same lead or if its components resulted from bipolar leading: The groove in the box was filled with a solution of 11 % sucrose and the preparation remained otherwise undisturbed for 10 min. After this period of time the sucrose was removed and the intrafusal responses were recorded in oil as before. The recorded potential became monophasic and larger (32·3 μV) with a latency of 3·5 msec $\pm$ 0·4 S.D. In addition, a rather prominent negative after-potential was detected (Fig. 4*D*). The preparation remained undisturbed for another 10 min and the intrafusal potentials evoked again by fusimotor stimulation at different stimulus strength. Fusimotor stimulation produced *only* an upward deflexion averaging 24·7 μV, with a shock–peak latency of 3·3 msec $\pm$ 0·14 S.D. This is the same response registered earlier during the full sucrose effect although now it had lower amplitude (Fig. 4*E*). A few minutes later it was possible to elicit an intrafusal potential similar to that recorded in Fig. 4*B* by reducing the stimulus strength. Its amplitude was now 16·3 μV and its shock–peak latency was 4·9 msec $\pm$ 0·14 S.D., which is significantly longer than that obtained before sucrose. Slowing down of conduction was obviously produced by sucrose although the site of its action was not determined (Fig. 4*F*). Stronger stimulation now elicited a diphasic potential in which, after the passage of time, the latency of the downward deflexion became progressively shorter until it reached normal values (Fig. 4*G–I*).

It is concluded from the experiment just described that the upward deflexion of the potential illustrated in Fig. 4*C* was recorded by the electrode proximal to the block, since it was not eliminated by sucrose. The intrafusal potential illustrated in Fig. 4*B* and the downward deflexion recorded in 4*C* were registered by the distal lead since they were blocked by sucrose. With regard to the site of origin of these potentials, it is suggested that two intrafusal bundles were involved. One was outside and proximal to the sucrose block, its responses being recorded by the proximal

electrode. The other intrafusal bundle must have been at least partly inside the sucrose solution and its responses were picked up by the distal electrode. This assumption is based on the facts that at least 20 mm of muscle was immersed in sucrose and that the length of the spindles is from 4 to 12 mm (Boyd, 1956). Consequently, the possibility that the recorded intrafusal potentials all originated in one intrafusal bundle could be excluded.

It is of some interest that different intrafusal potentials showed in many instances appreciably different latencies. These potentials had occasionally the same polarity as those illustrated in Fig. 3*D* and *E*, or their polarity could be different (Fig. 3*A*, *B*, *C*; Fig. 4*B*, *C*). In view of the fact that the position of the leading electrode does not appreciably influence the shock–peak latency of an intrafusal potential, as was shown in the preceding section (cf. also Kuffler *et al.* 1951), it is suggested that different latencies could be due to the following factors: (i) potentials originating in different spindles serially located, the differences in latency being accounted for by longer intramuscular nerve distances and (ii) by differences in conduction velocities in motor fibres to the same spindle. Experiments in which a localized sucrose block was employed seem to indicate that the first of the outlined possibilities is more likely to occur.

In several experiments stimulation of a single fusimotor fibre elicited intrafusal potentials in widely separated areas of muscle: intrafusal potentials could be localized to both ends of the muscle while the centre of the preparation remained electrically silent. Also, activation of intrafusal bundles by motor axon reflex (see Methods) was possible. These observations show that a single fusimotor fibre may divide and innervate widely separated muscle regions. Conversely, stimulation of more than one fusimotor fibre produced at times intrafusal responses with the same latency (Eyzaguirre, 1960). This finding probably indicates that a single intrafusal bundle may receive more than one fusimotor fibre. These results agree with previous observations of Hunt & Kuffler (1951).

Recovery of the intrafusal potential

Kuffler *et al.* (1951) reported that the intrafusal potentials do not show facilitation during repeated excitation. Furthermore, they found that if the interval between two successive fusimotor shocks was shortened to about 1 msec, the second intrafusal response was reduced. These points were reinvestigated and somewhat more detailed information is presented in this section.

The intrafusal potentials were recorded during nerve stimulation by paired shocks separated by varying intervals. It was necessary to ensure that only one fusimotor fibre was present in the nerve filament set up for

stimulation; when more than one spindle motor fibre remained, closely spaced, supramaximal shocks could activate more than one fibre, resulting in larger intrafusal potentials. The cathode was placed toward the spindle in order to avoid anodal polarizing effects. As the interval between shocks was decreased to 0·7 msec the response to the second (test) stimulus disappeared in all-or-none fashion. Figure 5 shows amplitude values of the response to the test stimulus expressed as percentage of the potential obtained during the first (conditioning) stimulus. At 0·7 msec interval the response to the test shock appeared, having an amplitude which averaged 98 % of that produced by the conditioning shocks. The second response

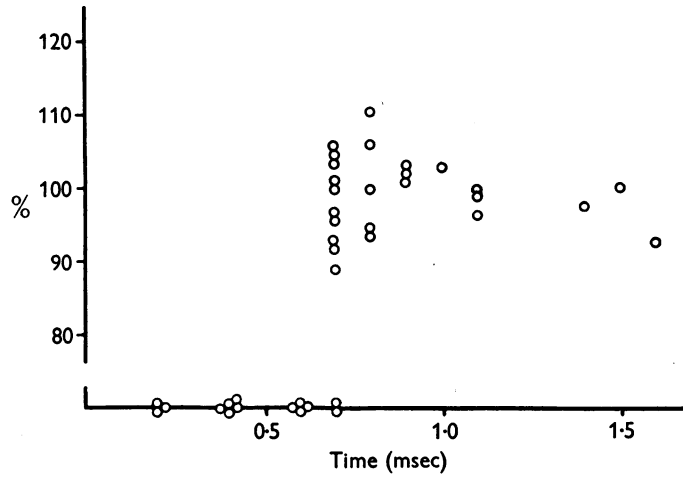

Fig. 5. Two nerve shocks delivered at different intervals. The diagram illustrates the amplitude of the second intrafusal response as compared with the first intrafusal potential. Abscissa, time; ordinate, amplitude of second intrafusal response expressed as percentage of the first potential.

failed to appear in 16·6 % of the cases and no intermediate steps were observed. Another interesting feature is illustrated in Fig. 6. When the intervals between stimuli were greater than 3·4 msec both potentials had the same shock–peak latencies, since all values recorded form a straight line with a slope of 1. At shorter intervals the onset of the second potential was delayed, since the points of the curve abandon the straight line for another with a gentler slope.

Results presented in Figs. 5 and 6 indicate that when two shocks to the fusimotor fibres are delivered in close succession the second intrafusal potential disappears abruptly; at longer intervals its amplitude does not differ significantly from the first one. The longer latency of the responses obtained after the test stimuli was probably due to longer neuromuscular delay caused by partially refractory intrafusal fibres. The latter situation

has been observed when dealing with extrafusal muscle fibres (Kuffler, 1942).

In general, the intrafusal neuromuscular junctions proved to be remarkably resistant to fatigue. When tetanic stimulation at 50–200/sec was maintained for a few seconds the amplitude of the intrafusal potentials did not change. However, if stimulation at these frequencies was maintained for a longer period of time a decline in amplitude of the intrafusal potentials was observed.

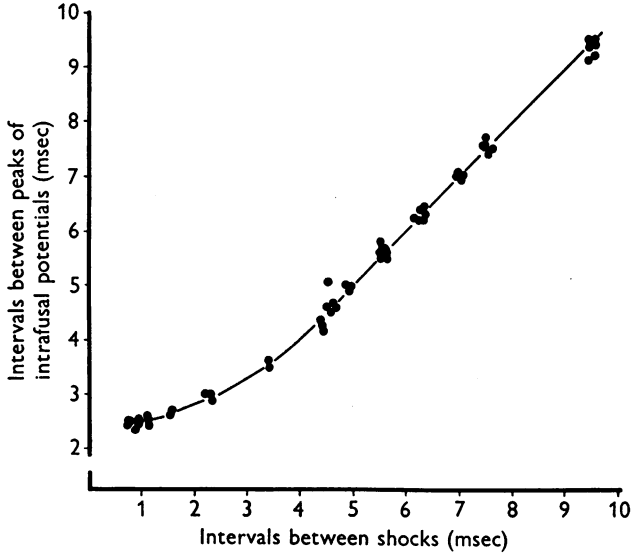

Fig. 6. Delayed onset of the second intrafusal response at short stimulating intervals; two stimulating pulses delivered at decreasing intervals.

Effect of curariform agents and physostigmine on intrafusal junctions

Amphibian intrafusal junctions are more resistant to fatigue and curarization than extrafusal ones. This property has been employed successfully in the study of spindle function without the interference of extrafusal effects (Katz, 1949; Eyzaguirre, 1957, 1958; Henatsch & Schulte, 1958). Experiments were designed to test whether this differential block could also be used in the mammalian preparation. On the whole these attempts were unsuccessful, since the margin between extra- and intrafusal neuromuscular block was too narrow to permit adequate working conditions. Extrafusal block was achieved by adding tubocurarine to the saline in concentrations of from 5 to 6×10^{-7} (w/v). Intrafusal junctions remained unimpaired only for several minutes after extrafusal block. Lower doses of this substance failed to produce block in both types of junctions. Similar results were obtained with Flaxedil (gallamine

triethiodide; American Cyanamid Co.) in concentrations of 3×10^{-6} (w/v), since several trials failed to reveal a clear selective action. Better differential action was obtained with Metubine (dimethyl-tubocurarine iodide; Lilly) in concentrations of from 3 to 5×10^{-7} (w/v). However, this drug had some effect on the intrafusal junctions at these concentrations, since at high frequency motor stimulation the sensory discharge rose rapidly to a peak and declined briefly afterwards while the preparation was still being stimulated. More troublesome was the fact that the differential action of Metubine was unpredictable. Thus, in some experiments a clear selective block was obtained, whereas in others results were doubtful. Larger doses of Metubine clearly blocked intrafusal junctions as well.

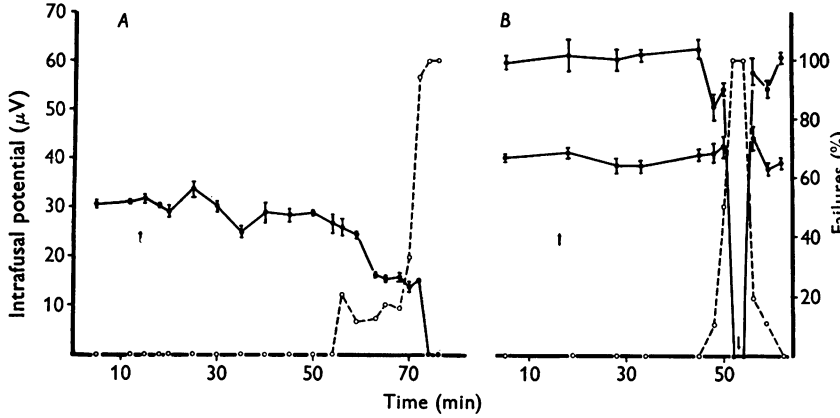

Fig. 7. Effect of curare on intrafusal junctions. *A*, curare applied to the bath ($1{\cdot}2 \times 10^{-6}$(w/v)) at arrow. Solid line (filled circles) shows the amplitude of the intrafusal potentials ± S.E.; broken line connects the points where the percentage of all-or-none failures of transmission was recorded (open circles). *B* illustrates an experiment in which two intrafusal potentials were simultaneously recorded; solid circles amplitude of intrafusal potentials ± S.E.; open circles percentage of all-or-none failures of transmission. Upward arrow, local application of curare 3×10^{-6} (w/v); downward arrow, preparation washed in fresh saline.

In order to test the manner by which curare blocks intrafusal neuromuscular transmission, spindles were located either by visual observation or by electrical exploration. Recording was accomplished in some experiments by keeping most of the muscle in saline while one end was lifted into the oil. Under this condition tubocurarine was added to the bath. However, in most instances the position of the spindle was such that local application was necessary (see Methods). Figure 7*A* illustrates an instance in which tubocurarine $1{\cdot}2 \times 10^{-6}$ (w/v) was applied to the bath. The amplitude of the intrafusal response fluctuated around 28 μV with fusimotor stimulation at 1/sec. Several pictures were taken at each point in order to analyse the potential amplitude statistically. This was found

necessary because of base-line noise. During curarization potential amplitude did not change significantly for 49 min. After this period of time a sudden and significant drop in amplitude was observed. Potentials remained at this new level for 7 min but disappeared abruptly immediately afterwards (solid line). Washing the preparation in fresh saline restored transmission to normal levels (not illustrated). A second curve (broken line) shows the percentage of instances in which all-or-none failure of responses was recorded. A similar situation was present in another experiment in which curare was applied locally in concentrations of 3×10^{-6} (w/v) (Fig. 7*B*). Two distinct intrafusal potentials were recorded simultaneously. The larger one averaged about 60 μV while the other was of the order of 40 μV during fusimotor stimulation at 1/sec. Thirty-two minutes after the drug was applied (at upward arrow) the larger potential suffered a drop in amplitude while the smaller one did not. At this point both potentials failed to appear in 50 % of the cases. Four minutes later all intrafusal responses disappeared abruptly. After that period of time curare was replaced with fresh saline and the responses returned in 60 sec. The larger potential recovered to values intermediate between control responses and the first drop observed before its complete disappearance; the smaller recovered to normal values. The sensory discharges were controlled before and during application of curare in both experiments. Sensory acceleration during motor stimulation to the spindles disappeared during full curarization. It is evident from Fig. 7*A* and *B* that curare blocks intrafusal neuromuscular transmission either in stepwise or all-or-none fashion. Graded blocking effects have not been observed. This is true for any effective dose of the drug, since threshold curarizing doses of from 1 to 2×10^{-6} (w/v) produced similar effects.

In several experiments physostigmine salicylate was applied either to the whole bath or locally in concentrations of from 5×10^{-7} (w/v) to 3×10^{-6} (w/v). This substance did not change the amplitude or time course of the intrafusal potential. However, it occasionally produced intrafusal repetitive responses after single or paired nerve shocks. Figure 8*A–C* illustrates an experiment in which intrafusal repetitive responses were obtained after applying physostigmine 10^{-6} (w/v) for 35 min. Figure 8*D* and *E* shows the disappearance of repetitive responses once the stimulating frequency was increased to 10/sec. This observation means that when the intrafusal neuromuscular preparation is poisoned by the anticholinesterase, changes occur at the intrafusal neuromuscular junction which may fire the intrafusal fibres in an asynchronous manner.

Eccles, Katz & Kuffler (1942) observed repetitive spikes in extrafusal muscle fibres after injecting physostigmine. Part of this effect was due to nerve discharges caused by an action of the drug on the motor nerve

endings. The intrafusal repetitive responses observed in this study could have been produced by a prolonged transmitter action or by asynchronous fusimotor discharges during physostigmine application. No attempt was made to elucidate this point. However, intrafusal repetitive discharges looked as if the intrafusal fibres were firing asynchronously, judging by their shorter time course as compared to that of the evoked intrafusal potential. This fact would indicate that repetitive firing was due to a long-lasting transmitter action. An effect of physostigmine on the motor nerve terminals would, more likely, produce synchronous repetitive discharges.

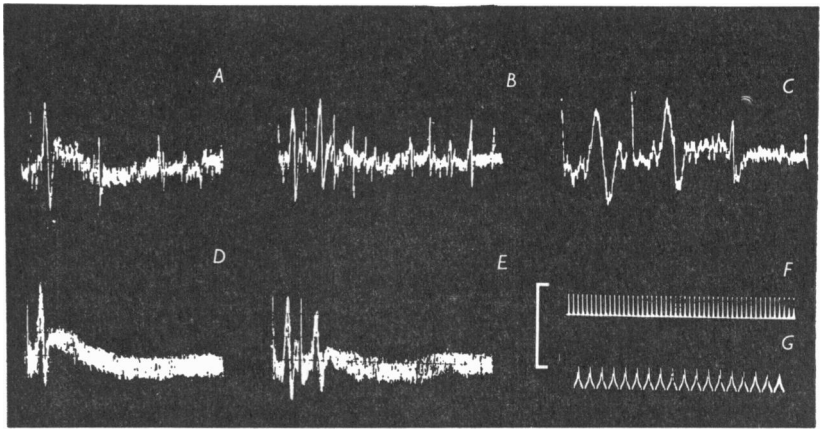

Fig. 8. Effect of physostigmine on intrafusal potentials. Before *A* physostigmine 10^{-6} (w/v) was applied locally for 35 min. *A*, repetitive responses evoked by single fusimotor shock. *B*, effects produced by two fusimotor shocks closely spaced. *C*, same as *B* but faster time base. *D* and *E*, blockage of repetitive responses during stimulation of the fusimotor fibre with one and two pulses respectively at a rate of 10/sec. *E*, voltage 20 μV; *F*, time marker—10 msec for *A*, *B*, *D* and *E*; *G*, 1 msec for *C*.

When intrafusal neuromuscular transmission was blocked by curare, application of physostigmine restored transmission in all-or-none fashion. In fact, in one experiment complete intrafusal neuromuscular block was obtained after local application of curare 10^{-6} (w/v). Physostigmine in concentrations of 10^{-6} (w/v) was then added and transmission was fully restored in 18 min. No transitional amplitude steps were observed in this process.

DISCUSSION

The main task of the present investigation was to learn whether the mammalian intrafusal potentials, first described by Kuffler *et al.* (1951), are propagated events, or whether intrafusal fibres give rise only to junctional

potentials as in the case of the 'slow' muscle system of the frog (Kuffler & Vaughan Williams, 1953*a*; Burke & Ginsborg, 1956). The present experimental evidence shows that the recorded intrafusal potentials are propagated. In fact, the intrafusal potential as herein recorded showed many of the characteristics of propagation in a volume conductor. More conclusive was the fact that curare blocked these responses in stepwise or all-or-none manner. In addition, physostigmine did not alter the amplitude or time course of these responses. These results eliminate the possibility that the recorded intrafusal potentials were exclusively localized at the intrafusal neuromuscular junction. In curarized spindles intrafusal junctional potentials were not recorded with the method employed.

Boyd (1959) has suggested, on the basis of histological evidence, that 'slow' fibres, similar to those found in amphibians, might occur in the mammalian spindle. In the present study no intrafusal responses have been detected which had the characteristics of 'slow' fibres. By analogy with the frog one would expect that mammalian 'slow' intrafusal junctional potentials would be considerably smaller than propagated responses produced by twitch intrafusal fibres. It is possible that the present recording system was not sensitive enough to detect such small potentials. An unequivocal answer should be obtained by recording intracellularly or close to the spindles with extracellular micro-electrodes.

One might recall here embryological studies on the development of mammalian intrafusal bundles (Cuajunco, 1927, 1940). It seems that intrafusal fibres develop from the same elements which eventually produce the ordinary muscle fibres. In the new-born animal the structure of intra- and extrafusal fibres is similar except that intrafusal elements appear more immature. Later on this difference becomes more striking since extrafusal fibres continue to grow while their intrafusal counterparts do not. It is not surprising, therefore, that intrafusal fibres produce in the adult electric responses similar to those obtained from extrafusal fibres. However, the fact that mammalian intrafusal bundles have an independent nerve supply in contrast to other species has yet to be accounted for.

It is interesting to notice the lack of a clear differential blocking action to curariform agents of the mammalian intrafusal neuromuscular junctions. This contrasts with Katz's (1949) original observation in amphibians. He suggested that the differential sensitivity to curare could be due to a higher safety factor because of a motor fibre making contact with a smaller muscle fibre. In mammals the intrafusal fibres are also smaller than extrafusal ones, but a clear differential sensitivity is not obvious. It seems, therefore, that other factors besides a diameter relationship play a part in differential pharmacological sensitivities to curariform agents. At present it is difficult to envisage such factors since the function and submicroscopic

structure of the post-synaptic membrane of intrafusal junctions are to a large extent unknown.

The action of physostigmine, producing repetitive intrafusal potentials, might be correlated with Hunt's results in the intact animal (1952 and private communication). This author showed that injections of physostigmine increased the effectiveness of fusimotor activation on the afferent discharges. This effect was blocked by curare. It is possible that repetitive intrafusal responses, whether produced by a long-lasting transmitter action or by discharges originating in the nerve terminals, might give rise to stronger and longer-lasting intrafusal contractions, as it occurs in extrafusal fibres.

SUMMARY

The electrical activity of mammalian intrafusal fibres was studied in the excised tenuissimus of the cat and the following was found:

1. Intrafusal potentials recorded from the muscle surface during stimulation of single fusimotor fibres showed a potential sequence which is similar to that of propagated potentials recorded from a volume conductor.

2. Methodical exploration of the muscle in millimetre steps permitted the location of 'active' regions during fusimotor activation. Such regions are probably the site of contracting intrafusal bundles.

3. Fusimotor stimulation produced sometimes complex intrafusal potentials. This complexity is due, in part at least, to potentials originating from different spindles. Complex potentials were more frequently obtained if more than one fusimotor fibre was activated.

4. When a single intrafusal fibre was stimulated with two shocks at varying intervals, the second potential disappeared abruptly when the interval was reduced to 0·7 msec. Also, the onset of the second potential was delayed if the intervals between shocks were less than 3·4 msec.

5. Curare in doses of from 5×10^{-7} (w/v) to 3×10^{-6} (w/v) blocked intrafusal potentials either in stepwise or all-or-none fashion. There is no clear differential sensitivity to curare, Flaxedil or Metubine between extra- and intrafusal junctions.

6. Physostigmine in doses of from 5×10^{-7} (w/v) to 3×10^{-6} (w/v) did not change the amplitude or time course of the intrafusal potentials. Occasionally, intrafusal repetitive responses were produced by this drug.

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