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A STUDY OF THE AFFERENT DISCHARGE PRODUCED BY COOLING A MAMMALIAN MUSCLE SPINDLE

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During experiments on muscle spindles we found that if the temperature of an isolated mammalian muscle fell a few degrees below body temperature an afferent discharge suddenly appeared. This response resembles that of a cold receptor (Hensel & Zotterman, 1951*a*). Other receptors respond to cooling although this is not their primary function, e.g. pressure receptors (Dodt & Zotterman, 1952) and the lateral-line organ of the Japanese sea-eel (Katsuki, Yoshimo & Chen, 1950).

With an isolated muscle it is possible to alter the ionic environment, to apply stretch and to polarize the ending electrically. We have used these procedures to study the mechanism by which cooling produces afferent nerve impulses.

METHODS

In general the experimental techniques were those described in the preceding paper (Lippold, Nicholls & Redfearn, 1960).

Preparation. The isolated tenuissimus muscle of the cat or kitten was used in a temperature-controlled bath containing 15 ml. of Krebs's solution (mM: NaCl 115, KCl 4·6, $NaHCO_3$ 24·1, $CaCl_2$ 2·46, $MgSO_4$ 1·15, KH_2PO_4 1·15, glucose 8·85); 95% O_2 and 5% CO_2 was bubbled through it. Temperature was measured by a thermometer of low thermal capacity or a thermocouple next to the muscle. The temperature of the bath could be altered at rates up to 0·2° C/sec by changing the temperature of water flowing through an outer jacket. Recordings were made from single muscle spindle afferents with the nerve on non-polarizable electrodes in a layer of liquid paraffin.

Whenever the ionic concentration of the Krebs's solution was to be altered, kitten muscles were used. In these thin muscles diffusion is rapid and there is no evidence that the spindles behave differently from those in the adult cat.

Electronically generated square pulses served either to polarize the nerve terminals or to stimulate the muscle for the purpose of identifying the type of endings. The action potentials were photographed on moving film; in some experiments, since the discharge was regular, it was possible to measure frequency by counting the number of spikes during a 10 sec period by electronic methods.

Experiments in vivo. Experiments were also carried out on muscles in the anaesthetized cat; either the whole animal or the limb alone was cooled. Recordings were made from

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single dorsal-root afferent fibres, the ventral roots having been cut previously (Lippold, Redfearn & Vučo, 1958). The tendon of tibialis anterior or gastrocnemius was freed completely from its insertion but the skin over the muscle was not disturbed. The temperature of the muscle and the rectal temperature were measured with thermometers.

Conduction velocity was measured by using three electrodes, separated by known distances and connected to separate amplifying channels. Measurements were made from the rising phase of action potentials initiated by stretch.

Receptor potentials were recorded from kitten muscle by the method of Katz (1950), using the same criteria for distinguishing between receptor potentials and movement artifacts.

RESULTS

Isolated muscle

When activity from the whole nerve trunk of the isolated tenuissimus was recorded at 37° C, action potentials were present only when the muscle was stretched. There was no activity when the muscle was relaxed. On cooling to about 32° C a single-fibre discharge appeared, followed by others as cooling proceeded, until about six or seven were firing independently. If the muscle was now stretched, the frequency of these potentials increased, and in addition two or three larger spikes appeared. The activity remained at approximately the same frequency while the temperature was lowered to about 28° C, below which there was a gradual decline in frequency.

The characteristics of the response to cold were studied in single afferent fibres from muscle spindles. When the muscle was cooled slowly (less than 1° C/min) the discharge began abruptly at about 32° C. Its frequency was at first approximately 10/sec, gradually rose to a maximum of 15/sec at 28° C, and then declined (Fig. 1). It fell to zero at about 15° C.

At any one temperature the frequency of impulses was constant, and so regular that the action potentials could be synchronized for several minutes with the time base on the oscilloscope. Slow rewarming produced the same frequencies at given temperatures and the discharge stopped again at the temperature at which it had begun.

Rapid changes in temperature caused slightly higher rates of firing, but the final rate depended only on the final temperature and not on the rapidity with which it was reached. When the preparation was rapidly rewarmed from 20° C (1° C every 10 sec), although the frequency was higher throughout the temperature range, the temperature at which the discharge stopped was the same as before (Fig. 2).

In all the experiments it was possible to record the normal response to stretch of the muscle; when the regular cold response was present, stretching produced a sharp increase in rate followed by adaptation (Fig. 3).

On relaxation there was a pause of one or two impulses before the cold response was resumed. This occurred during the phase of hyperpolarization

('off-response') of the receptor potential (Fig. 3) (Lippold *et al.* 1960). It was clear that these were afferents from muscle spindles, since a twitch elicited by direct stimulation of the muscle interrupted the firing due to stretch. In some preparations stronger stimulation produced a burst of impulses presumably due to intrafusal contraction (Fig. 4) (Hunt & Kuffler, 1951*a*, *b*; Matthews, 1933).

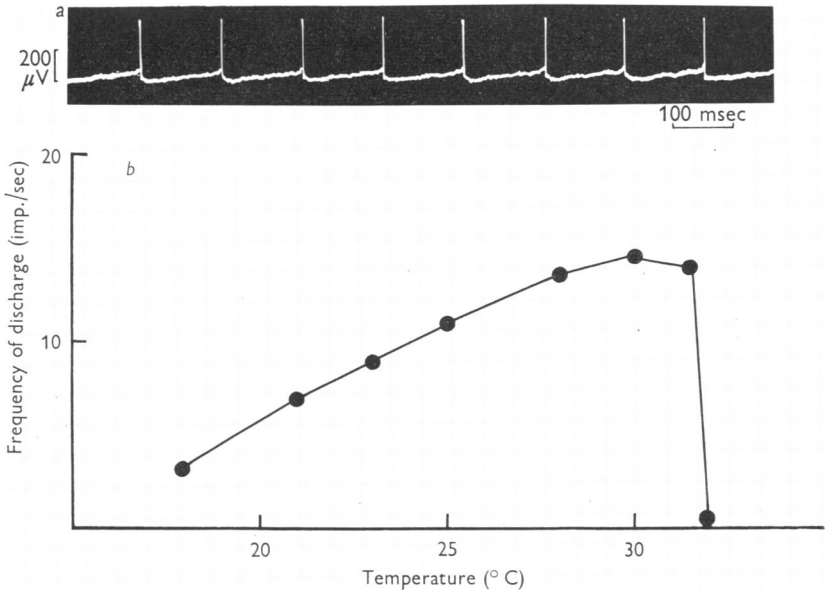

Fig. 1. (*a*) Record of the discharge at 30° C in a single muscle spindle. The electrode was close to the nerve ending and receptor potentials precede each spike. (*b*) The relationship between the frequency of discharge and temperature.

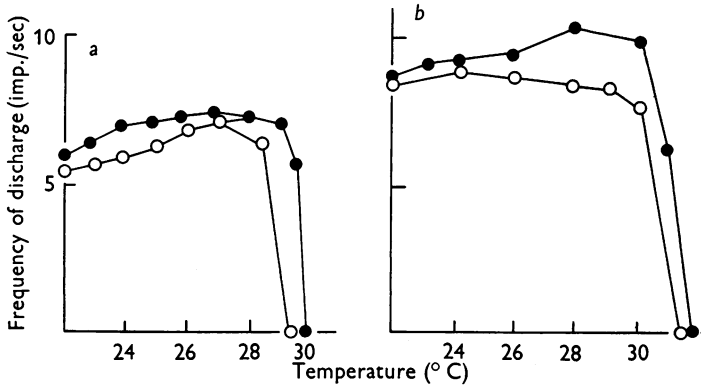

Fig. 2. The relationship between frequency of discharge and temperature, (*a*) during slow cooling and rewarming (1° C/min) and (*b*) during rapid cooling and rapid rewarming (1° C/10 sec). ○, cooling; ●, rewarming.

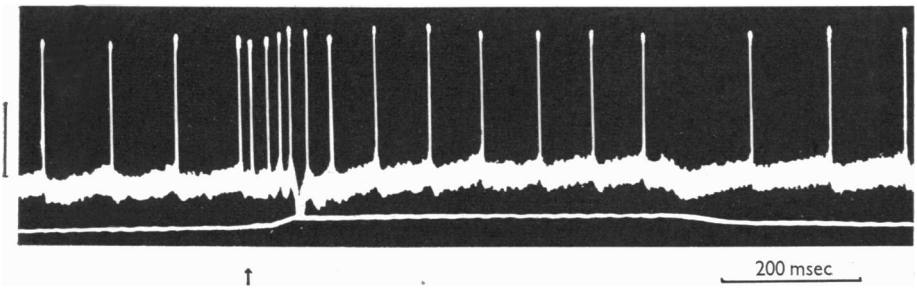

Fig. 3. Record of the response to stretch in a cooled muscle (28° C), upper trace; in lower trace stretch is upwards. With the muscle relaxed there is a steady discharge; stretch gives rise to an increase in frequency followed by adaptation. There is a small receptor potential visible before each spike and a transient hyperpolarization ('off-effect') on release. Onset of stretch at arrow. Voltage calibration 200 μV.

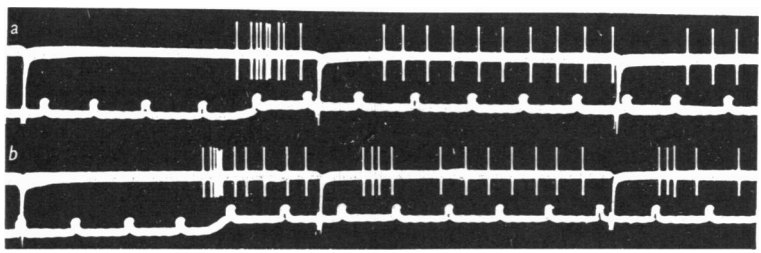

Fig. 4. The effect of electrical stimulation of the whole muscle on the discharge of a spindle when it is stretched (lower beam). (*a*) Weak stimulus (downward stimulus artifact) produces twitch of extrafusal fibres and a silent period in the discharge due to stretch. (*b*) A stronger stimulus introduces a burst in the silent period due to contraction of intrafusal fibres. In both cases the stimulus produces no discharge in the relaxed muscle. Time trace 100 msec.

Experiments on undissected muscle with intact circulation

The effect of cold on spindle discharge was demonstrated in the anaesthetized cat. After the appropriate ventral roots had been cut, there was no activity from relaxed muscle spindles at 37° C. On cooling the whole cat, or the leg alone, the discharge appeared at about 32° C, with the typical frequency of approximately 10/sec (Fig. 5). On rewarming the animal the firing stopped. Stretching the muscle produced a slowly-adapting discharge.

Large and small afferent fibres from tenuissimus

Not all muscle spindles in the isolated tenuissimus became active when cooled. Usually a third of the muscle afferent fibres from which we recorded were inactive unless stretched. This estimate was formed during

the dissection of 70 muscles, while the nerves were being divided. It was confirmed in five muscles in which all afferent fibres were examined; 1 in 3 afferents were found not to fire when cooled although they responded normally to stretch.

The afferent fibres from the endings which did not respond to cold invariably gave the largest action potentials. These spikes were 1½–3 times larger than those conducted from cold-sensitive endings. This was true not only in the dissected single afferents but also in the whole nerve trunk, where it was reasonable to suppose that spike size was proportional to fibre diameter. This was confirmed in an experiment in which the conduction velocities of large and small spikes were measured in a nerve

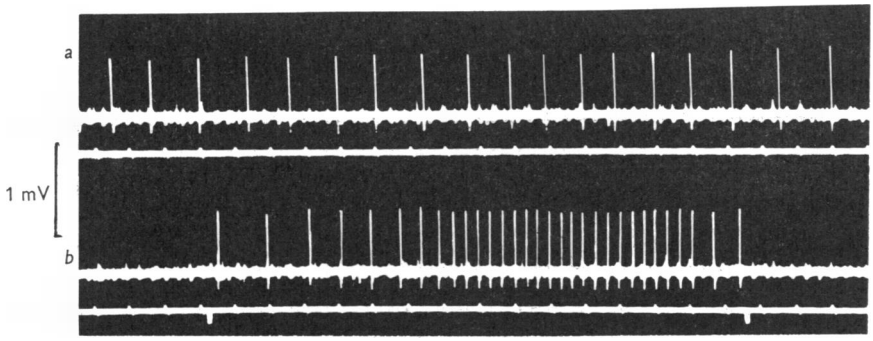

Fig. 5. Cat, 4·2 kg anaesthetized with sodium pentobarbital 40 mg/kg intraperitoneal. Action potentials recorded from single dorsal root filament from gastrocnemius. Ventral roots cut. (*a*) Whole animal cooled, rectal temperature 32° C, muscle completely relaxed. There is a regular discharge. (*b*) Rectal temperature 38° C. Muscle relaxed, no action potentials until, at first downward mark on lower trace, muscle gradually stretched. At second downward mark, muscle released. Time trace 100 msec.

trunk. The spike height was directly proportional to conduction velocity and only those fibres with the largest spikes and the greatest conduction velocity showed no response to cold. Since conduction velocity is proportional to fibre diameter, it follows that there is a cold response only in sensory endings connected to the afferent fibres of smaller diameter. There were also fibres giving spikes of intermediate size which would fire only when the temperature was changed rapidly, although they responded to stretching.

Apart from the spike size and the conduction velocity in the afferent fibre, no difference was found between the nerve endings with and without a cold response.

A few fibres, giving small spikes, have been found to respond to warming as well as to cooling. These fibres began to fire at about 39° C and stopped

when the temperature reached 46° C. Their response to cold and to stretch was the same as in other fibres of similar size (cf. Dodt & Zotterman, 1952).

Mechanical factors

The regular discharge at low temperatures was never affected by curare $5{\cdot}0 \times 10^{-6}$ (w/v), a concentration sufficient to cause complete neuromuscular block. With this concentration, stimulation of the nerve resulted in neither an accelerated discharge nor a silent period (cf. Buchtal & Jahn, 1957).

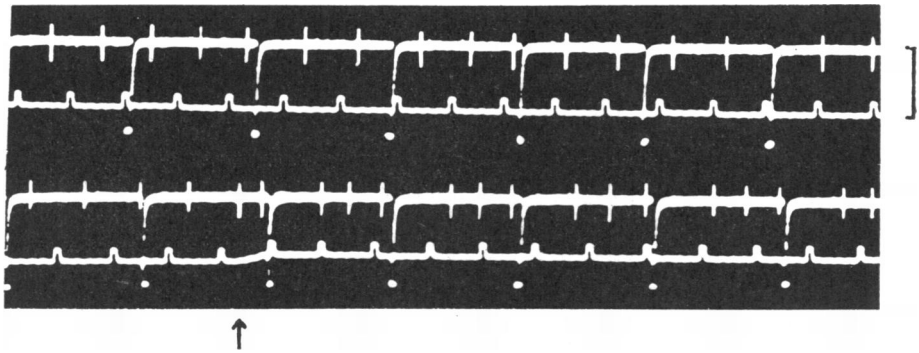

Fig. 6. Effect of direct electrical stimulation of the muscle (dots), upper record during cold discharge, lower record during cold discharge when stretch is applied at arrow (upward movement of lower beam). Stimulation produced a silent period in the stretch response (after arrow) but it had no effect on the cold discharge. Stimulus strength same throughout. Temperature 27° C. Voltage calibration 200 μV. Upper beam, action potentials from single fibre and stimulus artifacts; lower beam, time trace (100 msec).

The discharge was not slowed when the muscle was relaxed as completely as possible; to reduce tension on the spindle to a minimum the muscle, free at both ends, was allowed to rest on the bottom of the bath so that its weight did not stretch it. Even attempts to shorten the spindle by manipulation with needles did not slow the discharge.

A muscle twitch elicited by direct stimulation and contracting the extrafusal fibres was also used to shorten the spindle (Fig. 6). In the completely relaxed muscle this was without effect on the firing rate, which suggested that the cold response was not due to a contracture in series with the spindle. Slightly stretching the muscle caused an increase in frequency and this increase was abolished during the twitch (Fig. 6).

Potassium

There is a quantitative relationship between K^+ concentration and membrane potential (Adrian, 1956), and Katz (1950) has shown that the frequency of firing is proportional to the depolarization at the nerve

terminal. Consequently, a reduction in K^+ which would increase the resting potential should decrease the rate of firing in the cold if it were due to depolarization. The potassium concentration of the Krebs's solution was therefore altered in order to cause changes in the resting potential.

After about 40 min in K^+-free Krebs's solution, the firing began at a lower temperature than usual, and although the temperature–frequency curve was of the characteristic shape, the frequency throughout was reduced. The response to stretch was still present. Increased K^+ concentration raised the rate of firing and extended the temperature range over which it occurred. The concentration of K^+ used was insufficient to stimu-

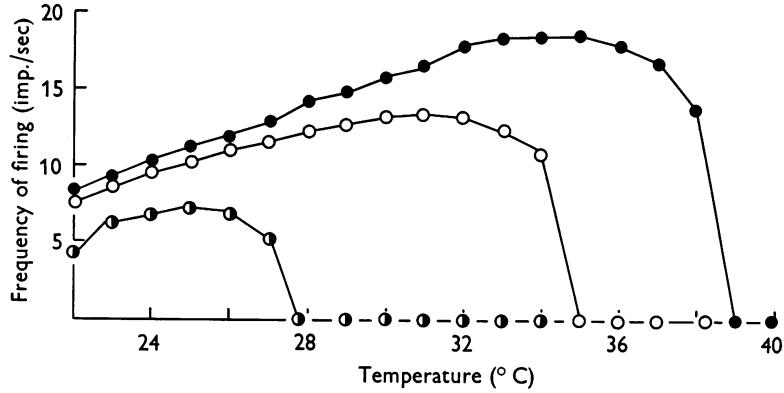

Fig. 7. Frequency of firing plotted against temperature (abscissa) of a single spindle in different K^+ concentrations: ●, 8·6 mM K^+; ○, 5·75 mM K^+; ◑, K^+-free Krebs's solution.

late the nerves directly, to produce muscle contraction, or to interfere with the stretch response. If the nerve was pinched within the muscle all firing was abolished. The effects of both increased and decreased K^+ concentration were reversible on soaking the muscle in normal Krebs's solution for 30–45 min (Fig. 7).

We have shown previously that the responses to cold were found in endings innervated by fibres of small diameter. When using high K^+ concentrations we often observed characteristic cold discharges in the larger fibres which normally were silent unless stretched.

Polarization of nerve terminals

An increase in frequency of the discharge due to cold was brought about by depolarization of the nerve terminals with direct current. Conversely a decrease or cessation occurred when the current was reversed and the terminal hyperpolarized. This alteration in frequency was directly

proportional to the current flow (Fig. 8). Stringent precautions (cf. Lippold *et al.* 1960) were taken to ensure that the nerve terminals alone were being excited.

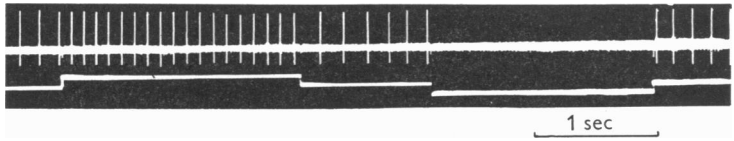

Fig. 8. Polarization of nerve terminals. The lower trace is the current flowing through the preparation; upward deflexion, spindle —ve. The cold discharge (upper trace) is accelerated by depolarizing the spindle and stopped by hyperpolarizing it.

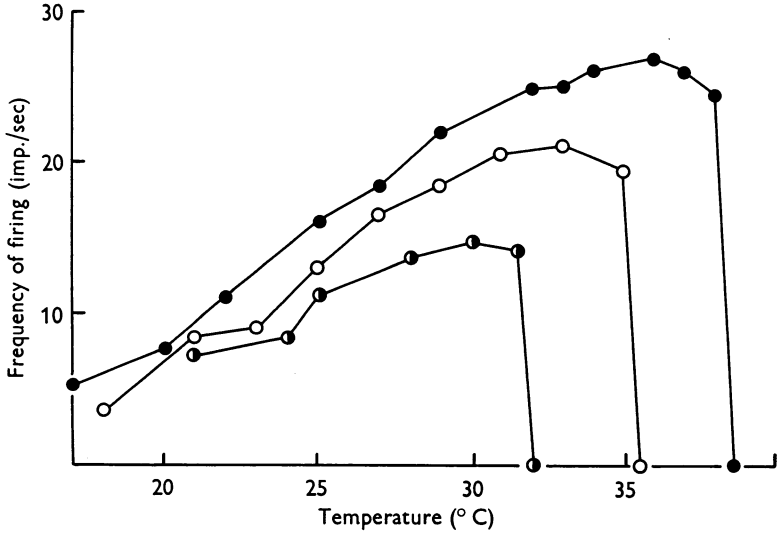

Fig. 9. The frequency of firing plotted against temperature of a single spindle at different muscle lengths, where ◑ is at 95 % of the resting length in the body, ○ is at 110 % and ● is at 131 %.

Alteration in resting length

Another way of depolarizing the nerve terminals is to keep the muscle slightly stretched but not sufficiently to evoke a maintained stretch response. This produced the same changes in firing-rate and temperature–frequency curves as did increased K^+ concentrations (Fig. 9). In all these procedures, pinching the nerve at its entry to the muscle abolished impulses due to cold, stretch and polarization.

Measurements of threshold

The threshold to stretch is reduced at the lower temperatures. We measured the threshold at different temperatures in two ways: one involved a step-wise stretch of the muscle, the other a sinusoidal displace-

ment of varying magnitude but of fixed frequency (Lippold *et al.* 1960). In each case threshold was defined as the minimum displacement producing a single action potential. These determinations of threshold were carried out on fibres of large diameter which did not fire as a result of cooling, because a cold response masked threshold effects. The threshold in both types of experiment progressively fell as the temperature was lowered and reached a minimum value at about 30° C (Fig. 10), which is about the temperature at which the cold response is greatest.

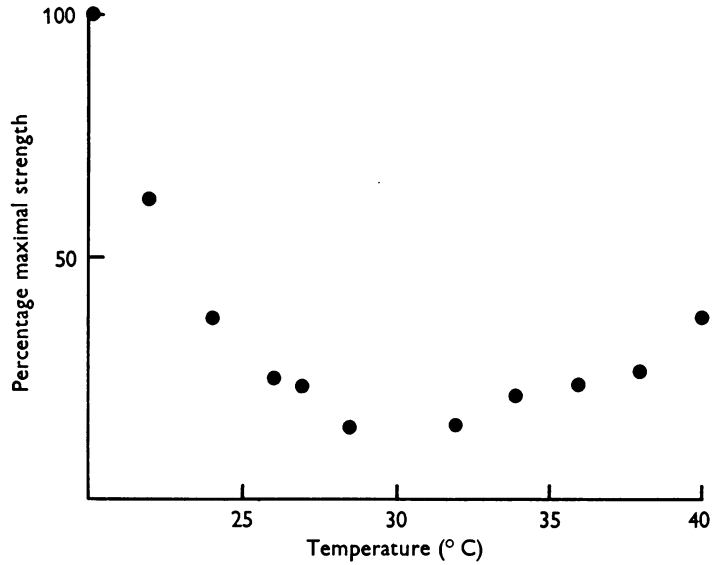

Fig. 10. Measurements of threshold to stretch at different temperatures. Threshold is measured with sinusoidal displacements in a single spindle which had no cold response (see text). Ordinate, % maximum stretch.

The above experiments were designed to measure the threshold of stimulation for a single spike; it is also possible to measure a different threshold—the minimum stretch required for a discharge lasting 10 sec or more. This threshold fell as the temperature was lowered and reached a minimum at 26° C.

A similar relation between threshold and temperature was found in fibres which did have a cold response. In this case it was necessary to measure the minimum stretch required to produce an increase in frequency. The threshold declined as the temperature fell from 37° C to about 28° C, and then rose steeply as the temperature was lowered still further, although the cold discharge was present. Quantitative measurement was difficult, but there was no obvious difference between the large and the small fibres in this respect.

The sensitivity of muscle spindles to a given stretch was measured at different temperatures in most experiments. A stretch at 37° C, which would evoke a high-frequency burst of impulses (300/sec), would produce at 20° C a just detectable increase in frequency of the firing due to the cold discharge. This was true in all experiments and a similar effect occurred in large fibres from spindles without the cold response.

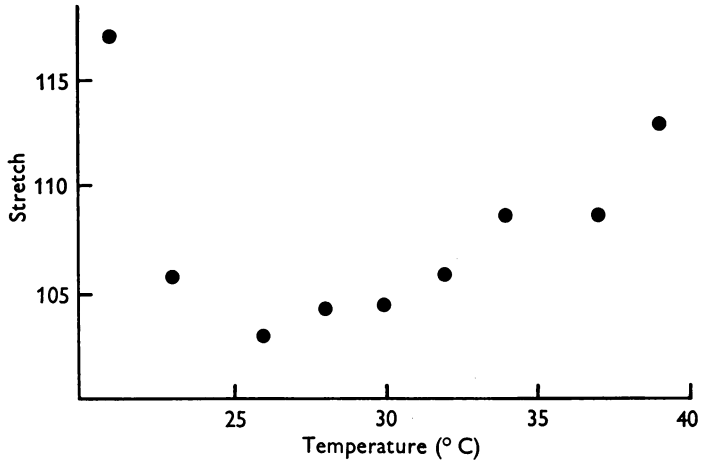

Fig. 11. Measurement of threshold to stretch sufficient for a maintained discharge of more than 10 sec at different temperatures. Stretch expressed as % over resting length. Fibre without cold response (see text).

The effect of calcium

Calcium is known to increase the stability of the membrane in nerve and muscle. Increasing the Ca^{2+} concentration in the Krebs's solution from 2·46 to 3·69 mM completely stopped the response to cold while the stretch response could still be elicited. The sensitivity of the receptors varied inversely with the Ca^{2+} concentration, but no precise measurements of threshold were made.

Reducing the Ca^{2+} concentration to 1·23 mM initially increased the sensitivity of the spindle to a given stretch (increasing the frequency and duration of the burst of impulses). Then, after approximately 30 min, the temperature and frequency range of the cold response was increased (Fig. 12).

Diminished Ca^{2+}, like increased K^+, made spindles innervated by large fibres, which had no cold response in normal Krebs's solution, develop one.

Receptor potentials

Receptor potentials were recorded by the method described by Lippold *et al.* (1960) at the beginning and end of the cold response. Figure 13

shows a record of the cold response at 32° C which was recorded close to the nerve ending. The receptor potential can be seen building up before each action potential. The discharge was just about to stop as a result of rewarming but in the interval where one spike has dropped out no abortive spike can be seen. The receptor potential is still present but is not large enough to generate a spike and there are no large oscillatory potentials.

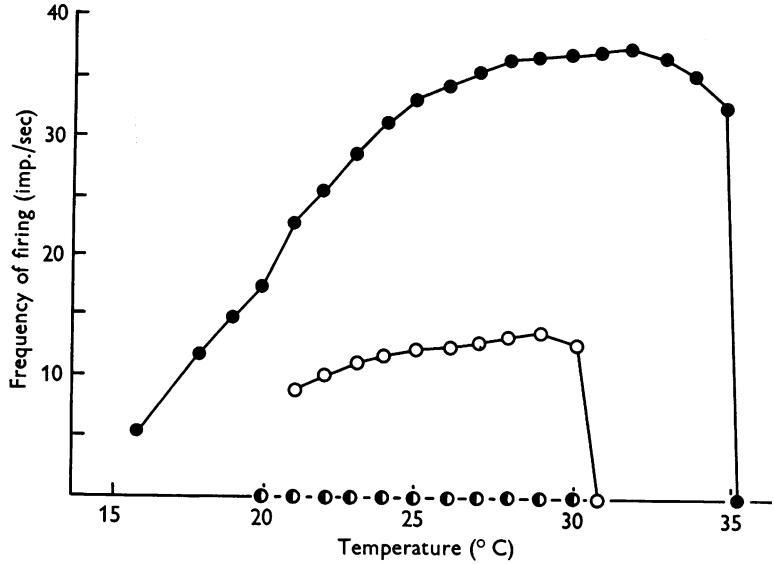

Fig. 12. The effect of calcium concentration on the frequency of firing at different temperatures; ○ in normal Krebs's solution containing 2·46 mM-$CaCl_2$; ◐ in 3·69 mM concentration; ● in 1·23 mM concentration.

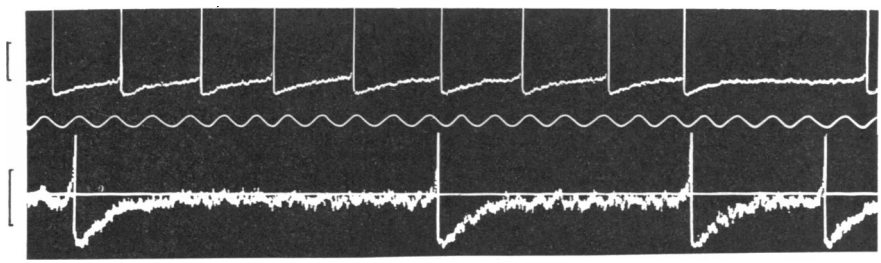

Fig. 13. Action potentials recorded close to nerve entry from a single spindle at 32° C when the cold response is about to stop on rewarming. Both records show action potentials dropped out. The receptor potentials are recorded but show no oscillations or abortive spikes during these gaps. Lower record gain increased 3× and sweep speed 2×. Voltage calibration, upper trace 200 μV, lower trace 100 μV. Time marker, upper trace, 50 c/s.

DISCUSSION

It is clear from our results that muscle spindles in the mammal fire spontaneously, in the absence of stretch, when they are brought to temperatures below 32° C. It might be expected that spindles would give rise to a brief discharge on rapid cooling, for Bernhard & Granit (1945) have described certain nerve fibres which respond to large, quick temperature changes by initiating several spikes. Muscle spindles, however, fire with a regular, non-adapting discharge, of frequency dependent upon the temperature and not its rate of change. The cold response of muscle spindles does therefore seem to be a specific effect of low temperature.

There are two possible explanations of this cold response in muscle spindles. One is that at low temperatures the physical properties of the muscle or the immediate surroundings of the nerve terminal are altered in such a way that a constant deformation of the sensitive membrane is produced. We have experimental evidence that this hypothesis is unlikely. All attempts to shorten further the relaxed muscle spindle, either by direct manipulation or by extrafusal twitches, fail to stop the cold discharge. In addition, the discharge is regular, of comparable frequency in different experiments and has a characteristic temperature–frequency curve which is similar to the discharge of a specific cold receptor (Hensel & Zotterman, 1951*a*). Cold cannot produce its effect in a specific cold receptor by a mechanical deformation of the sensory endings because these are not sensitive to mechanical stimulation (Dodt & Zotterman, 1952).

The second hypothesis which could be invoked to explain the cold response is that low temperatures alter the properties of the nerve terminal membrane. In the first place it is possible that the normal relation between membrane potential and firing rate, found by Katz (1950) to be linear, is altered or has a different slope in the cold. If this factor was solely responsible, it would be necessary to suppose that firing in the cooled terminal now occurred at the resting potential level. In other words there must be a reduction to zero in the threshold for firing, as a result of cooling. This would be similar to the spontaneous discharge observed when nerves are immersed in Ca^{2+}-free media. One might therefore expect that the sensitivity to a given depolarization or stretch would be greater on cooling.

In fact the converse is true. In our experiments a degree of stretch of the muscle which would evoke a high-frequency burst of action potentials at body temperature would only give a just detectable increase in firing rate at 20° C (when the cold discharge was present). However, in Ca^{2+}-free media at body temperature the spindles are firing spontaneously and their

sensitivity to stretch is increased. It is therefore unlikely that changes in the threshold for firing and in the sensitivity of the terminals can explain the production of the cold discharge.

A more attractive explanation is that low temperatures act by depolarizing the membrane of the nerve endings. All those factors which are known to depolarize membranes increase the discharge yet do not alter the general form of the relation with temperature. Furthermore, the same factors produce the characteristic discharge in spindles which otherwise do not have one. Conversely, hyperpolarizing the membrane reduces or abolishes the discharge. With present techniques it is not possible to demonstrate directly a depolarization due to cold; the nerve terminals are too small to enable intracellular recordings to be made.

It is interesting to observe that it is only in the case of recordings made from smaller fibres that this cold response can be obtained. This may reflect some functional or anatomical difference in the endings connected to the small fibre. Hensel & Zotterman (1951*b*) also found that pressure receptors with axons below about 10 μ in diameter would give a brief discharge when they were rapidly cooled, whereas those with axons of diameter greater than this did not do so. Douglas, Ritchie & Straub (1959) found that cooling caused a discharge in mammalian C fibres (as distinct from their endings) which depended upon their diameter. In the muscle spindle, however, it is the nerve ending which responds to cold, since separating the nerve from its receptor abolishes the discharge.

Since the cold discharge occurs in the anaesthetized but otherwise intact animal, it is relevant to consider its physiological implications. We do not at present know whether a continuous low-grade discharge of about 10–15/sec from more than half of the stretch receptors in a cooled limb would have any reflex or central effects.

SUMMARY

1. Spindles in the completely relaxed tenuissimus muscle of the cat gave a regular afferent discharge at temperatures below about 32° C, while still having a normal response to stretch.

2. Two-thirds of afferent fibres from stretch receptors had this property; those without the response invariably had the largest axons. In high K^+ or in low Ca^{2+} concentrations the large fibres developed a response to cold.

3. Factors which caused depolarization of the nerve ending (increased K^+ concentration, stretch, d.c. polarization) accelerated the discharge. Hyperpolarization (d.c. polarization, or low K^+ concentration) reduced or abolished the activity. On the other hand, full relaxation of the muscle or twitches of the extrafusal fibres did not affect the discharge.

4. The threshold to stretch also fell in the cold. This would be expected if depolarization of the membrane were occurring or its excitability were increased.

5. This response to cold is similar to that found in specific cold receptors.

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