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THE ELECTRICAL PROPERTIES OF THE SLOW MUSCLE FIBRE MEMBRANE

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Kuffler & Vaughan Williams (1953*a*, *b*) have shown that the small motor axons in the frog innervate a specific group of fibres, the 'slow' fibres, which constitute a small proportion of many skeletal muscles. The electrical response of these fibres to nerve stimulation is a non-propagated depolarization, the small-nerve junctional potential (s.j.p.), analogous to the end-plate potential of 'fast' (or 'twitch') fibres. But whereas the end-plate potential normally leads to an action potential, the s.j.p. does not. A further distinctive feature of the s.j.p. is that it decays diphasically, i.e. with a terminal phase of hyperpolarization.

Our object was to investigate the electrical properties of the slow fibre membrane as distinct from its response to the neuromuscular transmitter and, in particular, to find out if propagated responses could be produced by direct stimuli. The experiments required the insertion of two microelectrodes into the same fibre, one to stimulate it selectively by passing current through the membrane, and the other to record the potential. It will be shown that the slow fibres are not, as a rule, capable of giving action potentials even in response to strong direct stimuli. The absence of action potentials on nerve stimulation therefore simply reflects the 'inexcitability' of the slow fibre membrane. The diphasic decay of the s.j.p. will be shown to be due to a 'delayed rectification' and not to any special effect of the neuromuscular transmitter.

A preliminary account of the experiments has been published (Burke & Ginsborg, 1955).

METHODS

Slow fibres were impaled with two microelectrodes, one for passing current through the membrane and the other for recording. The superficial fibres of the muscle were explored with the recording electrode until an s.j.p. was obtained in response to nerve stimulation. There was fortunately no need to search for a focus of small-nerve junctions since s.j.p.'s can be recorded over the whole length of a slow fibre (Kuffler & Vaughan Williams, 1953*a*). The current electrode was then inserted, usually within 50μ of the recording electrode.

It was not always possible to insert the second electrode, perhaps because of the small size of the slow fibres. Possibly for the same reason, the second insertion when made close to the first electrode generally caused a large drop in resting potential, sometimes amounting to 10 or 20 mV. Only a small fraction of this drop (1–3 mV) was due to the electrical shunting of the membrane by the current electrode and its external circuit. The phase of hyperpolarization of the s.j.p. was frequently reduced and sometimes abolished after the insertion of the second electrode. The arrangements for polarizing and recording were conventional (see, for example, Fatt & Katz, 1951).

Preparation. The sciatic-iliofibularis preparation was used throughout. In most experiments it was taken from *Rana temporaria*, but during the summer months poor s.j.p.'s were obtained, and preparations from *R. esculenta* (Dutch) were found more satisfactory. These preparations had the additional advantage of a larger number of superficial slow fibres.

Resting potentials. Very small adjustments to the micromanipulator whilst the electrode was in a fibre produced changes in resting potential of up to 10 mV (cf. Kuffler & Vaughan Williams, 1953*a*), and successive insertions into the same fibre gave values which might differ by as much as 20 mV. These inconsistencies were probably due to imperfect sealing of the microelectrode and to changes in its 'tip potential' (see Castillo & Katz, 1955). In spite of this uncertainty, it was clear that the resting potentials of the slow fibres were considerably lower than those of fast fibres: in agreement with Kuffler & Vaughan Williams (1953*a*), they ranged from 35 to 76 mV with a modal value of 60 mV.

Selective stimulation of the small motor nerves. To avoid mechanical artifacts from the response of twitch fibres during nerve stimulation it was necessary to stimulate the small motor axons selectively. The method used was a modification of that introduced by Kuffler & Vaughan Williams (1953*a*). A voltage pulse was applied to the peripheral trunk of spinal nerves 9 and 10 through two platinum electrodes about 3 mm apart; the electrode nearer to the muscle was made positive with respect to the other electrode. The pulse rose to a maximum in a few msec and, to avoid anode break excitation, was made to decay slowly, with a time constant of about 30 msec. If the amplitude of the pulse was suitable, all the motor nerves were stimulated at the cathode, but the large motor axons which innervate the twitch fibres were anodally blocked. The appropriate stimulus strength could readily be determined by varying it, while observing the muscle under a binocular microscope ($\times 30$). With small stimuli, the muscle twitched; as the stimulus was increased, the twitch became smaller and finally ceased. The stimulus could then be increased several-fold before the twitch reappeared as a result of anode break excitation. It seems likely that the stimuli strong enough to block the large motor axons were sufficiently strong to stimulate all the small motor axons, since increasing the stimulus within the lower part of the range in which there was no twitch did not change the size of the s.j.p. In the upper part of this range, the s.j.p. was sometimes reduced, presumably because some of the small motor axons were now being blocked. The method has the advantage that the spinal nerve roots do not need to be dissected, but because of the long stimulating pulse, it cannot be used for repetitive stimulation at a frequency greater than about 10/sec.

The maximum s.j.p.'s were about 30 mV. The maximum hyperpolarizations (following the s.j.p.) were about 10 mV in *R. temporaria* and 15 mV in *R. esculenta*. The usual size of the s.j.p. in both species was 10–20 mV, but there was a striking difference in the ratios of hyperpolarization to depolarization. In *R. temporaria* this rarely exceeded 0·25; in *R. esculenta* it was often close to, and sometimes exceeded, unity (Fig. 1).

Movement artifacts. Movement due to twitches was avoided by the use of selective nerve stimulation, but occasionally a single stimulus produced visible *slow* movement of the tonus bundle, and this could not always be eliminated by stretching the muscle. This movement caused a delayed depolarization following the s.j.p., and usually dislodged the electrode. Smaller irregularities were sometimes superimposed on the s.j.p., without visible movement, but these could easily be discounted. There was no indication that movement distorted any records of the response to applied currents.

Solutions. Except where stated, 'frog' Ringer's solution of the following composition was used: 115 mM-NaCl, 2·0 mM-KCl, 1·8 mM-$CaCl_2$. The experiments were done at room temperature (15–22° C).

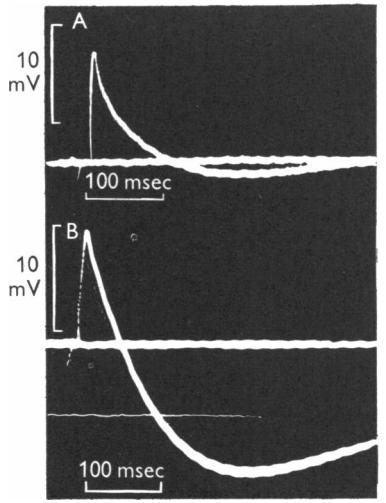

Fig. 1. S.j.p.'s from single slow fibres. A, *Rana temporaria*, B, *R. esculenta*. The s.j.p.'s are approximately equal, but the hyperpolarization in B is larger than that in A and it lasts for a longer period. The resting potential was 60 mV in each case.

RESULTS

Electrotonic depolarization of the membrane

A slow fibre having been identified by the appearance of an s.j.p. in response to nerve stimulation, a second electrode was inserted into the fibre and rectangular pulses of outward current were passed through the membrane. The results of such an experiment are illustrated in Fig. 2. In A, successively stronger currents were applied, and as a result of the strongest, the membrane potential was transiently reduced to zero: in B an even stronger current was applied (lower trace) and the membrane potential was reversed. However, at no level of depolarization was an action potential initiated.

This result was confirmed in all but one of fifty slow fibres. In this fibre, depolarization led to the sequence: local response–action potential, similar to that in other excitable cells, the threshold depolarization being 15 mV: the resting potential was 40 mV and the initial s.j.p. 10 mV. There was no evidence that this fibre was in better condition than the remaining forty-nine 'inexcitable' fibres, many of which had larger resting potentials and s.j.p.'s. The behaviour of this fibre must therefore be regarded as anomalous.

Two other striking features of the records shown in Fig. 2 are:

(i) When the depolarization exceeds 5–10 mV, there is a 'hump' on the catelectrotonic potential. The 'hump' is probably not a local response (in its usual sense) as can be seen from Fig. 3: this shows superimposed tracings of

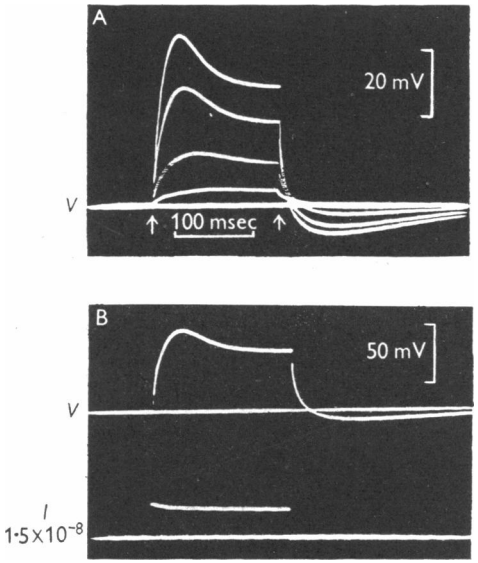

Fig. 2. The response of the slow fibre to 'cathodic' currents. *V*, potential; *I*, current. Resting potential 50 mV. In A the beginning and end of the rectangular current pulses are indicated by the arrows. Four successive traces are shown. The zero potential is at the level of the peak of the uppermost trace. In B (from the same fibre at reduced gain), the zero potential is at the level of the steady depolarization (*R. esculenta*).

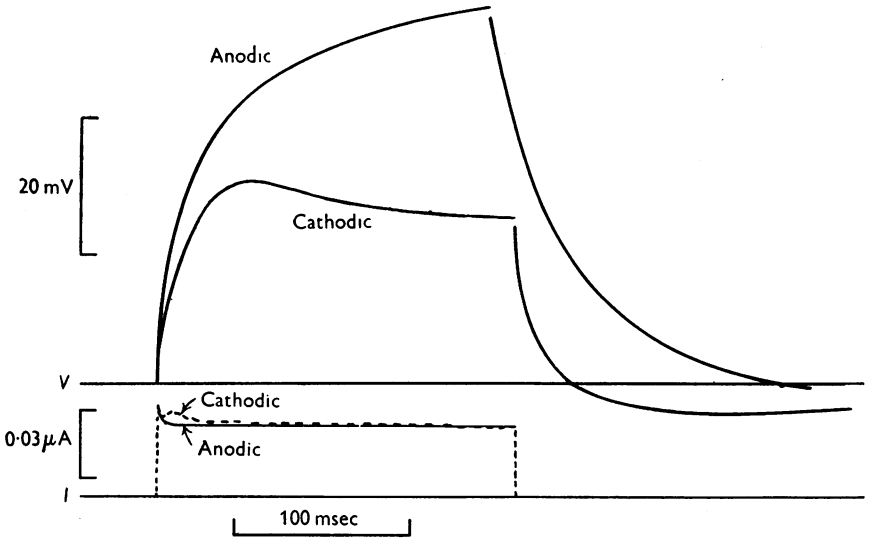

Fig. 3. Superimposed tracings of the electrotonic potentials (*V*) produced by opposite and approximately equal currents (*I*). The tracings are from records shown in Fig. 7 (see text).

the electrotonic potentials (from another fibre) produced by equal and opposite currents. The catelectrotonic potential even during the 'hump' is smaller than the anelectrotonic potential.

(ii) At the end of the current pulse, the depolarization decays with a phase of hyperpolarization. Both these features are probably due to 'delayed rectification', as will be discussed below (p. 594).

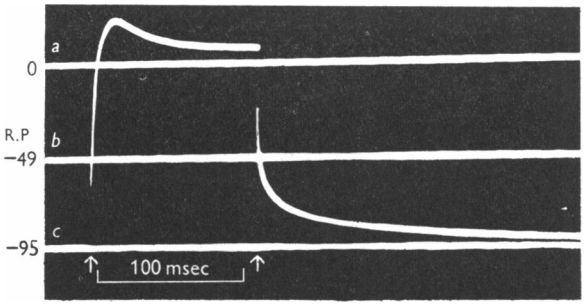

Fig. 4. The effect of preliminary hyperpolarization on the response to 'cathodic' current. (*a*) and (*b*) show the zero level and the resting base line (−49 mV) respectively. A steady 'anodic' current ($0{\cdot}8 \times 10^{-8}$ A) was passed through the membrane, hyperpolarizing it to the level (*c*) (−95 mV). A 'cathodic' pulse (3×10^{-8} A), its beginning and end marked by arrows, was then superimposed on the steady anodic current (*R. esculenta*).

The effect of preliminary hyperpolarizations

It was conceivable that the slow fibres were unable to produce action potentials simply because their low resting potentials (see Methods) caused them to be in a state of cathodal depression. If this was the case, excitability should be restored by a brief period of anodic polarization (see, for example, Lorente de Nó, 1947). The possibility of cathodal depression was therefore tested by raising the resting potential of slow fibres with a steady 'anodic' current before applying a depolarizing pulse (Fig. 4). The results were clearly negative: hyperpolarizations of up to 130 mV followed by depolarizations to the zero potential or beyond failed to produce action potentials. The 'inexcitability' of the slow fibres is therefore not due to their being in a state of cathodal depression.

Comparison between electrotonic depolarization and the s.j.p.

In the light of the present results, the inability of nerve stimulation to produce action potentials in slow fibres (Kuffler & Vaughan Williams, 1953*a*) is an inevitable consequence of the 'inexcitability' of the slow fibre membrane. The phase of hyperpolarization that follows the depolarization caused by nerve stimulation also appears to be due to the electrical properties of the membrane, since electrotonic depolarizations decay in a similar way. This is

brought out clearly in Fig. 5, which shows an s.j.p. and a catelectrotonic potential of the same size, from the same fibre. Both depolarizations are followed by hyperpolarizations similar in duration and magnitude. For a closer comparison of the two time-courses of decay the effect of the different spatial distributions of the depolarizations must be considered.

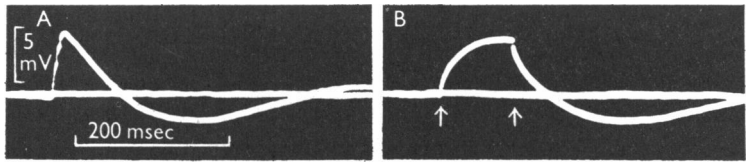

Fig. 5. Comparison of the hyperpolarization following the s.j.p. with that following an equal electrotonic depolarization. The s.j.p. (A) and the electrotonic potential (B) were recorded, from the same fibre after the insertion of both 'voltage' and 'current' electrodes. Resting potential, 50 mV. Arrows mark the beginning and end of the current pulse (*R. esculenta*).

Multiple innervation

Kuffler & Vaughan Williams (1953*a*) found that an s.j.p. could be recorded whenever a slow fibre was impaled and that, with maximal nerve stimulation, there was only a relatively small variation in amplitude and time-course of the s.j.p.'s. From this and other evidence, they concluded that slow fibres were innervated over their whole length. We have obtained additional evidence for this view by recording s.j.p.'s at different points in the same fibre.

Since the slow fibres could not be followed visually, the procedure was as follows. The recording and polarizing electrodes were first inserted into the fibre at the same point; successive insertions along the fibre were then made with the recording electrode, the polarizing electrode being left in place. The appearance of electrotonic potentials when current was applied through the polarizing electrode served as a check that the recording electrode had entered the same fibre.

The results of the two experiments in which this was done are shown in Fig. 6. There was some variation in the s.j.p.'s: over a length of about 2 mm, their amplitudes varied from 7 to 15 mV (Fig. 6A) and 8 to 14 mV (Fig. 6B); their rise times from 11 to 14 msec in A and 6 to 8 msec in B; and their decay times, to $1/e$, from 20 to 37 msec in A and from 21 to 26 msec in B. The smaller s.j.p.'s were not, however, associated with the longer rise and decay times as would be the case if the variations were due to different displacements from a focus of depolarization (see Fatt & Katz, 1951). This suggests that such differences in amplitude as were observed were due to variations in the intensity of the transmitter action along the fibre. A similar situation has been found in crustacean muscle (Fatt & Katz, 1953).

Comparison of decay times

The distributed innervation of the slow fibre requires that the s.j.p. should decay more slowly than a focally produced electrotonic potential. If the membrane resistance were constant (i.e. independent of the membrane potential), and if nerve stimulation produced an *exactly* uniform depolarization of the whole fibre, the s.j.p. would decline to 37% (1/*e*) of its maximum in a

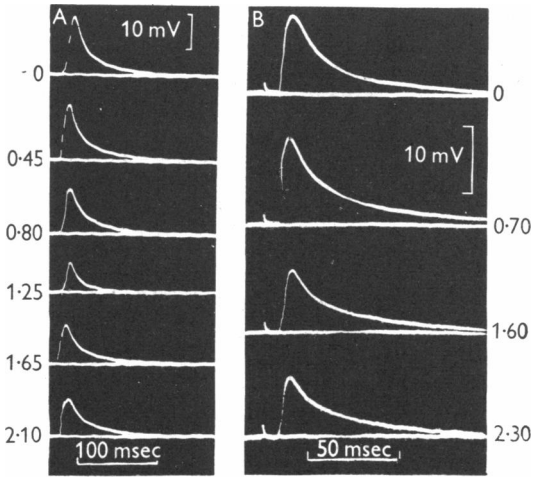

Fig. 6. Spatial distribution of s.j.p.'s in two slow fibres. The numbers attached to the records indicate the displacement in mm of the electrode along the muscle fibre (A, *R. temporaria*; B, *R. esculenta*).

time equal to the time constant of the membrane. The electrotonic potential, however, which falls off with distance from the polarizing electrode, would decay to a smaller fraction, viz. to 16% (erfc 1) in the same time (Hodgkin & Rushton, 1946).

Although the situation is complicated by the departure from spatial uniformity of the s.j.p. and by the 'rectifier property' of the membrane, it is nevertheless of interest to compare the decay time of the s.j.p. to 1/*e* (t_1) with that of an equal catelectrotonic potential to erfc 1 (t_2). (The amplitudes were measured from the level of the peak hyperpolarization, and not from the resting base-line.) In ten fibres, the ratio t_1/t_2 varied between 0·6 and 1·6; the average values of t_1 and t_2 happened to be equal (39 msec).

It seems probable, therefore, that the decay of the s.j.p. is governed by the electrical properties of the membrane, or in other words, that the transmitter action does not outlast the rising phase of the s.j.p. The decay of the s.j.p. is thus analogous to the passive decay of the end-plate potential (Fatt & Katz, 1951); the slower time course of the s.j.p. (as compared with an e.p.p. recorded focally) is due largely to the distributed innervation of the slow fibre.

Rectifying properties of the slow fibre membrane

Fig. 7 shows a series of electrotonic potentials produced by opposite and approximately equal currents. These records (and the tracings in Fig. 3) show that the slow fibre membrane behaves as a rectifier, having a greater resistance to anodic currents than to cathodic currents. The records are analysed in Figs. 8 and 9 from which it is evident that the relation between membrane current and potential is markedly non-linear, the resistance being increased

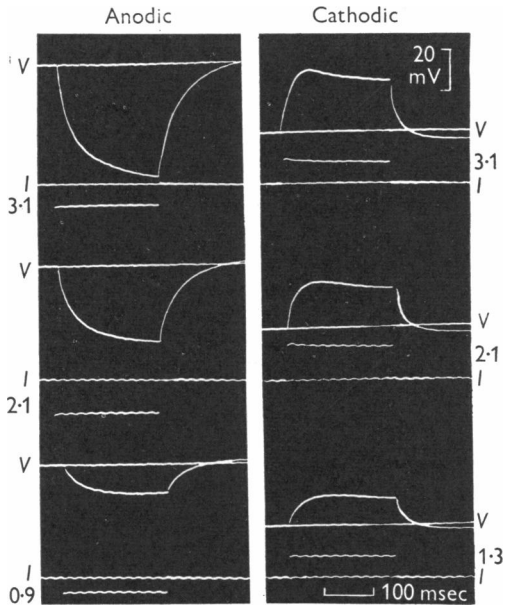

Fig. 7. Rectification by the slow fibre membrane. Electrotonic potentials, V, produced by opposite but approximately equal currents, I. Depolarization upwards. The current strengths are indicated in each record in 10^{-8} A. Resting potential, 57 mV. (*R. temporaria*). (See also Fig. 3.)

by hyperpolarization and decreased by depolarization. The degree of rectification, although rather variable from fibre to fibre, is of the same order of magnitude as that in the squid axon. In the experiment illustrated in Fig. 9, for example, the 'slope resistance' (dV/di) is about four times its resting value at a hyperpolarization of 30 mV, and falls to about one-tenth of its resting value at large depolarizations. Corresponding values for the squid axon, from an example given by Hodgkin, Huxley & Katz (1952), were 3·7 and 0·05.

It seems likely that as in the squid axon (Hodgkin, Huxley & Katz, 1949; Hodgkin & Huxley, 1952), rectification in the slow fibre is due to changes in

permeability of the membrane to potassium ions, and that the rectification occurs with a delay. The phase of hyperpolarization following a catelectrotonic potential or an s.j.p. would then be directly analogous to the positive phase of the action potential in the squid axon (see Hodgkin, 1951). Accordingly, it arises because of 'delayed rectification' and the fact that the resting potential of the slow fibre is smaller than the K equilibrium potential. In support of this view, changes in the external K concentrations were found to have reversible effects on the phase of hyperpolarization: in K-free Ringer's solution, the ratio hyperpolarization/depolarization of the s.j.p. was increased

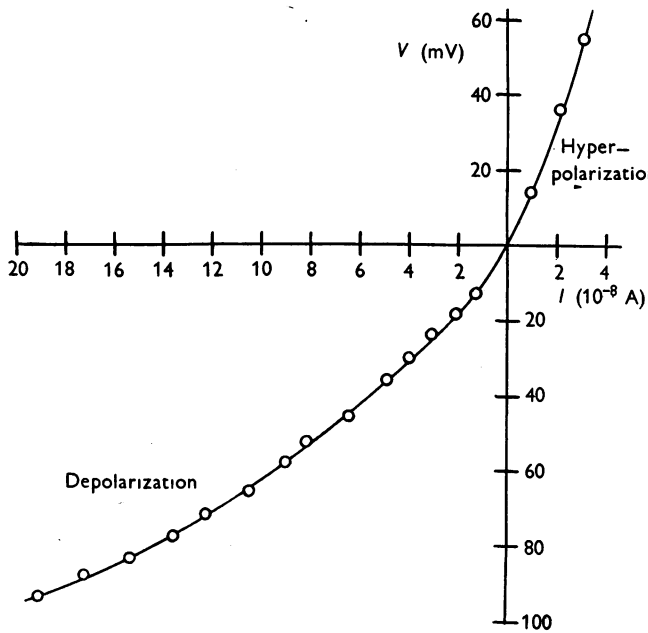

Fig. 8. Voltage/current relation for the fibre illustrated in Fig. 7. Abscissa (I), current strength in 10^{-8} A; ordinate (V), displacement from the resting potential in mV.

by about 10%; in Ringer's solution containing 8 mM-K (four times its normal K concentration) this ratio was reduced to about 40% of its normal value. Furthermore, replacement of four-fifths of the NaCl in the bath solution with 'isotonic sucrose' had no effect on the amplitude of the hyperpolarization, which suggests that neither Na nor Cl ions make any significant contribution to this phase.

The initial 'hump' of the catelectrotonic potentials can also be explained by 'delayed rectification'. In Fig. 2, for example, the depolarization shortly after the beginning of the larger current pulses is greater than its final steady value because the decrease in resistance on depolarization takes an appreciable

time to develop to its full extent. The increase in the size of the 'hump' in successive records and the similar increase in the rate of decay of the electrotonic potential and in the amplitude of the hyperpolarization (see Fig. 2) are due to the enhanced degree of rectification with increasing depolarization.

Alternatively, it might be supposed that there is no delay in the onset of rectification and that the 'hump' is entirely due to a transient increase in Na permeability. This seems improbable because the 'hump' still occurs even when the membrane potential is reversed to 60 mV, a potential likely to be above the Na equilibrium potential. This, however, does not rule out the possibility that some increase in Na permeability occurs during the 'hump'.

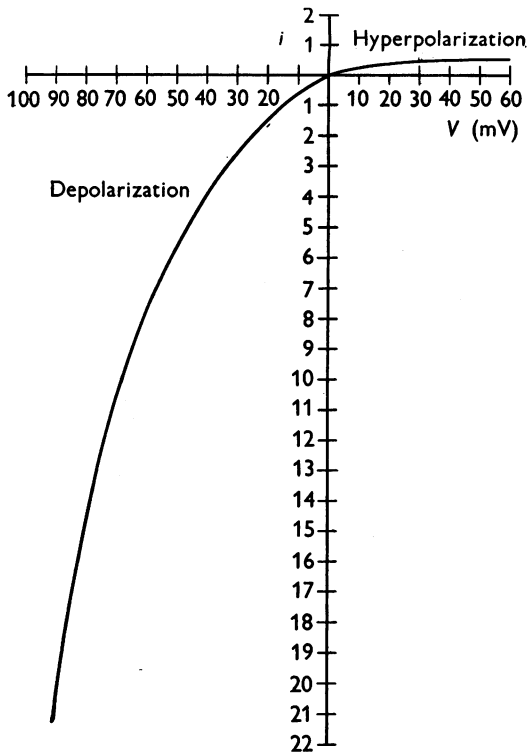

Fig. 9. Relation between membrane current density and membrane potential for the fibre illustrated in Fig. 7. Abscissa (V), displacement from the resting potential in mV; ordinate, (i), current density in arbitrary units (i.e. current per unit area of membrane which would result from a uniform change in potential (V) over the whole length of the fibre). The relation calculated from Fig. 8, was derived from the fact that $i \propto I.dI/dV$, even where the membrane resistance is non-linear (Cole & Curtis, 1941). If the specific resistance of the myoplasm (R_i) and the diameter of the fibre (d) were known, i would be given by

$$\frac{R_i}{\pi^2 d^3} I \frac{dI}{dV}.$$

By way of example, if R_i were 250 Ω cm (as in the fast fibre—Bozler & Cole, 1935; Katz, 1948) and the diameter were 50 μ, each unit of current density would be equivalent to about 5 μA/cm^2.

The time-course of the catelectrotonic potential depends on the rate and degree of rectification and the 'time constant' of the membrane. The initial 'hump' may be absent (e.g. Fig. 5B), although (*a*) the voltage/current relation shows that the membrane is rectifying, and (*b*) the depolarization decays diphasically, which suggests that the rectification is 'delayed'. On some occasions (e.g. in Fig. 7) the anelectrotonic potential decays with a phase of depolarization: this arises in an analogous way to the hyperpolarization following the catelectrotonic potential.

The fact that rectification occurs with a delay is illustrated in a slightly different way in Fig. 10. Cathodic current pulses of different duration have been passed through the membrane, leading to approximately the same depolarizations. (In fact, the shorter depolarizations were made slightly

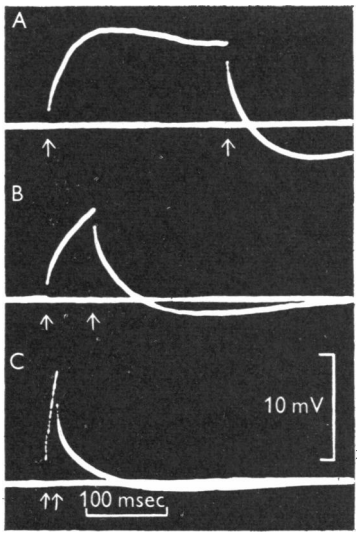

Fig. 10. The effect of the duration of a depolarization on the amplitude of the following hyperpolarization. Resting potential, 46 mV. Cathodic current pulses of increasing strength and decreasing duration were passed through the membrane, leading to approximately the same depolarization at the end of each pulse. The beginning and end of each pulse is marked by arrows. Pulse durations: A, 230 msec, B, 60 msec, C, 16 msec (*R. esculenta*).

larger to compensate for the difference in the spatial distributions of the potential (see Hodgkin & Rushton, 1946)). During the shorter current pulses, as a result of the time lag between resistance changes and potential changes, the rectification did not develop to its full extent. In consequence, the hyperpolarizations in (B) and (C) were smaller than that in (A).

DISCUSSION

The response of the slow fibre to applied currents resembles that of the squid axon when the axon has been made inexcitable by bathing it in 'choline seawater' (Hodgkin *et al.* 1949). Thus the slow fibre membrane is, as a rule, 'inexcitable', and it has the property of 'delayed rectification'. The inexcitability

of the membrane is not an entirely unexpected finding, in view of its response to tetanic stimulation of the motor nerves, described by Kuffler & Vaughan Williams (1953*a*). They found that the membrane could be depolarized by up to half its resting potential (the maximum depolarization that could be obtained by repetitive nerve stimulation) without producing an action potential. It was conceivable, however, that this degree of depolarization was 'subthreshold' or, alternatively, that, as a result of their low resting potentials, slow fibres are in a state of cathodal depression. These possibilities have now been ruled out by the failure of large depolarizations to produce action potentials, even after a preliminary period of hyperpolarization.

Our results also account for the way in which the depolarizations caused by nerve stimulation decay. Thus an s.j.p. decays with apparently the same time-constant as a catelectrotonic potential of the same size, and both depolarizations are followed by hyperpolarizations similar in duration and magnitude. Evidently the decay of the s.j.p. is governed by the 'delayed rectifier' property of the membrane, and is not the result of any special action of the neuromuscular transmitter. The same property accounts for the fact that, with increasing amplitudes, the depolarization due to repetitive nerve stimulation decays more rapidly and with an increasing amplitude of hyperpolarization (Kuffler & Vaughan Williams, 1953*a*).

The fact that the phase of hyperpolarization of the s.j.p. can be altered by changes in the external K concentration and is indifferent to changes in the external NaCl concentration suggests that, as in the squid axon, delayed rectification in the slow fibre is due to changes in permeability to K ions, and that the resting potential is below the K-equilibrium level. The similarity between the degrees of rectification (p. 593) supports the idea that a similar permeability change occurs in both preparations.

SUMMARY

1. The electrical response to applied currents of slow muscle fibres in the frog has been investigated with intracellular electrodes for polarizing and recording.

2. When 'cathodic' currents are passed through the membrane, the fibre can be depolarized to zero potential or beyond without, as a rule, producing action potentials. The failure of nerve stimulation to elicit propagated responses therefore reflects the 'inexcitability' of the membrane.

3. The membrane resistance decreases with depolarization and increases with hyperpolarization. The catelectrotonic potential often initially exceeds its steady level and it decays with a phase of hyperpolarization. This shows that 'delayed rectification' occurs in the slow fibre. The diphasic decay of the s.j.p. is due solely to this property and not to any special effect of the neuromuscular transmitter. The 'delayed rectification' is probably due to changes in permeability to K ions.

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