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THE EFFECT OF CALCIUM ON THE MYELINATED NERVE FIBRE

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In experiments with the voltage clamp technique it was found that in the squid giant fibre the external calcium concentration $[Ca]_0$ determined the membrane potential at which the specific changes in sodium and potassium permeability occurred (Frankenhaeuser & Hodgkin, 1957). The sodium and potassium conductance-membrane potential curves were shifted about 15 mV for a fivefold change in $[Ca]_0$ without an appreciable change in maximum amplitudes; the shift was in such a direction that lowering $[Ca]_0$ reduced the depolarization required to bring about a given increase in conductance. When the membrane was repolarized at the peak of sodium current the return to resting sodium conductance was found to be quicker in high $[Ca]_0$ than in low. Further, it was shown that the inactivation curve was shifted so that the sodium-carrying system was more inactivated in low $[Ca]_0$ than in high.

There is not sufficient information on the effect of calcium to judge whether or not calcium plays a similar role in the frog nerve fibre. Some findings seem to be generally accepted: (*a*) calcium raises the threshold; (*b*) decalcification brings about a low threshold, slow accommodation and repetitive firing. A full account of these and related observations is given in Brink's (1954) review.

Since the actions of Na and K on the spike height and resting potential of a myelinated fibre are similar to the action of these ions on cephalopod axons (Huxley & Stämpfli, 1951*b*), it seems reasonable to assume that changes in sodium and potassium permeability constitute the basis for changes in membrane current in both cases. On the other hand, Tasaki and his collaborators (Tasaki & Frank, 1955; Tasaki & Freygang, 1955; Tasaki, 1956) have recently rather indirectly arrived at the conclusion that the frog nerve does not show permeability changes similar to those in the squid fibre.

If specific permeability changes, similar to those in the squid fibre (Hodgkin & Huxley, 1952) are the basis of electrical activity in the frog nerve, and if calcium has a similar effect on these permeabilities in both nerves, then it

would follow that: (1) the threshold would change with $[Ca]_0$, (2) anode break excitation would appear in low $[Ca]_0$, (3) the action potential amplitude would be smaller in low $[Ca]_0$, and (4) the fibre might be inexcitable in a calcium-free solution.

In the present investigation the membrane potential at a single node of Ranvier of an isolated fibre was recorded while the [Ca] in the external solution was varied. Thresholds and maximum amplitudes of the action potential were measured. The membrane potential was recorded with a technique based on negative series feedback (Frankenhaeuser, 1957). In a number of experiments the longitudinal current was recorded instead of the membrane potential.

TECHNIQUE

Preparation. Isolated fibres in the frog sciatic nerve were used (*Rana esculenta*). The fibres were mounted in a partitioned Perspex cell and sealed with petroleum jelly at appropriate places for stimulation and recording (see Frankenhaeuser, 1957).

Solutions. The Ringer's solution used had the following composition:

	g/l.	mM
NaCl	6·47	110·5
KCl	0·186	2·5
$CaCl_2$	0·120	1·08
$NaHCO_3$	0·200	2·4

Note that this solution contains less calcium than frog plasma, which has 2 mM-Ca (Fenn, Cobb, Hegnauer & Marsh, 1934). Cocaine Ringer's solution was applied to that part of the fibre which had to be inactive when the longitudinal current from one node was recorded. When the membrane action potential at a node was recorded cocainization was as a rule not necessary.

Solutions of varying [Ca] were applied to the node under observation. The osmotic pressure of these solutions was kept constant by changing the NaCl as required. The solutions with high [Ca] thus contained somewhat less NaCl than solutions with low [Ca].

Stimulation and recording. A number of experiments were done in which the longitudinal current produced by a single node was recorded. The isolated fibre was mounted in a Perspex cell with two petroleum jelly seals (Fig. 1A). The node (N_0) under observation was between the seals. The one seal limited the stimulating current in the external fluid. The other seal provided a high external recording resistance. Both seals prevented the solution in the middle pool from mixing with the solution in the other pools. Cocaine Ringer's solution was kept in the two side pools in order to prevent activity in nodes other than N_0. The longitudinal current was recorded with a d.c. amplifier. Cathode followers were used at the input.

Rectangular pulse stimulators were used; in some experiments a stimulator which produced a slow linear rise of applied potential was also employed.

The longitudinal currents at threshold depolarization and at maximum activity were measured on enlarged photographic traces. The aim of the experiments was to obtain figures (in mV) for the membrane action potential and for the membrane potential at threshold in solutions with varied [Ca]. The technique does not allow such measurements without some assumptions. It was thus assumed that the action potential amplitude was 116 mV in the solution with 1·08 mM-Ca (Huxley & Stämpfli, 1951*a*). Another difficulty was that the longitudinal current for an applied rectangular pulse is peaked, owing to the cable structure of the fibre, whereas the membrane potential rises more or less exponentially to the final value. This introduces some uncertainty in the calculation of the membrane potential from the records of longitudinal currents. These difficulties do not appear when the membrane potential is recorded.

The membrane potentials at threshold stimulus and at the peak of the action potential were

measured on records of membrane action potentials obtained with a recording system using a strong negative feedback (Fig. 1 B). This technique has previously been fully described (Frankenhaeuser, 1957). Here all that need be said is that changes in membrane potential were recorded without any appreciable attenuation. Thresholds, polarization and action potential amplitudes were therefore measured directly without any assumptions about attenuation or distortion at the recording site. The node under observation was sealed with petroleum jelly on either side, so that any solution applied to this node did not mix with those at the other nodes. The test solutions were applied only to the node under observation.

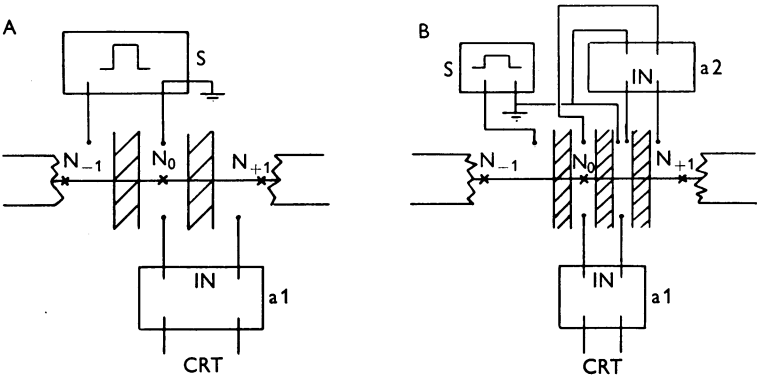

Fig. 1. Arrangements for stimulation and recording. N_0, node under observation. N_{-1} and N_{+1}, neighbouring nodes. S, rectangular pulse stimulator. a1, d.c. amplifier, connected to a cathode-ray tube. Shaded regions indicate petroleum jelly seals. A: arrangement for recording longitudinal current from node N_0, whole fibre, except part between the seals, cocainized. B: arrangement for recording membrane potential at node N_0. a2, feedback amplifier as described previously. Changes in membrane potential appear at the output of the feedback amplifier, these are recorded with amplifier a1.

RESULTS

Threshold and external calcium concentration

The changes in membrane potential were recorded when a threshold stimulus was applied to the isolated node under observation. The threshold was measured as the difference between the potential at rest and the potential at which the rate of rise in membrane potential due to regeneration was 20 V/sec. The threshold could equally well be defined as the lowest cathodal polarization at which a subthreshold activity is visible, or as the critical cathodal polarization necessary to give rise to a full-grown action potential (see Tasaki, 1956). A measurement of just-visible subthreshold activity gives a somewhat lower value for the threshold than the two other methods, which give nearly the same value.

The thresholds were measured in solutions containing 0·04–27 mM-Ca, and plotted against the logarithm of the [Ca] in the external fluid (Figs. 2, 3). It is evident that there is not a rectilinear relation between the threshold and the logarithm of the $[Ca]_0$, the slope of the curve being less in low $[Ca]_0$. In other experiments where the calcium concentration was increased in smaller steps

(twofold instead of fivefold) there was an indication of a linear relation over the range of about 1–16 mM-Ca.

The resting potential was not determined in these experiments. Small shifts in membrane potential were sometimes noted when the solutions were changed. These shifts were not regular, and were less than about 4 mV, even when the calcium concentration was changed 25-fold (cf. Stämpfli & Nishie, 1956). All measurements have been made relative to the resting membrane potential in the solutions in question, so these small shifts in resting potential have been neglected. In low $[Ca]_0$ there was, however, a complication which had to be considered: the fibre showed an anode-break excitation, suggesting

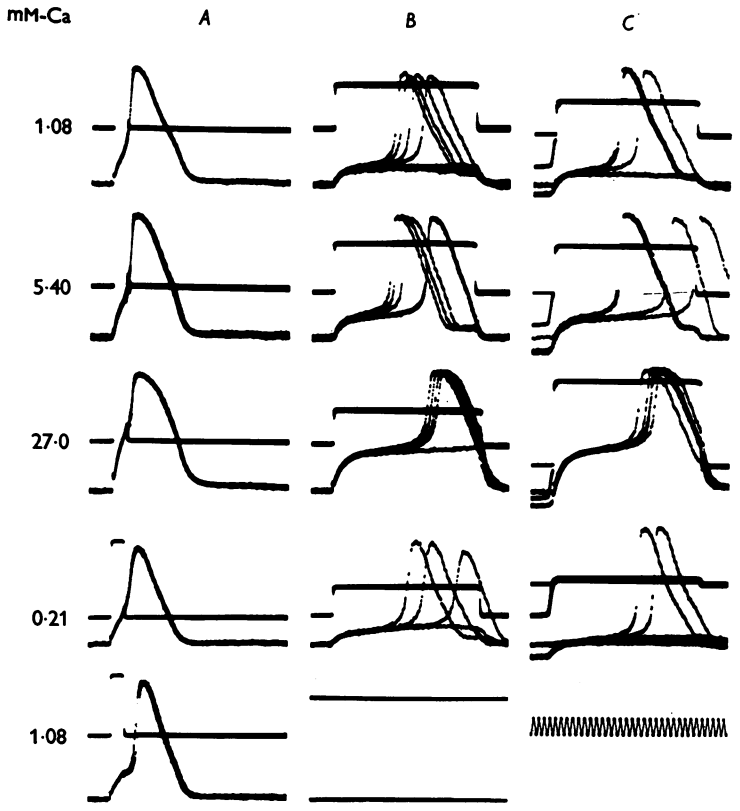

Fig. 2. Membrane action potentials from an isolated node of Ranvier at different external calcium concentrations. The action potential amplitude was measured on records similar to those in column *A*, where a short, well-suprathreshold stimulus was applied. Thresholds were measured on records similar to column *B*, where a long-lasting just-threshold stimulus was applied, or to column *C* when the stimulating pulse was preceded by anodal polarization. One or two sweeps were taken without anodal polarization to indicate the level of the resting potential before the anodal polarization was applied. Stimulating pulse indicated on upper beam (this beam has no relation to zero resting potential). Calibration lines indicate 100 mV, time 10 msec.

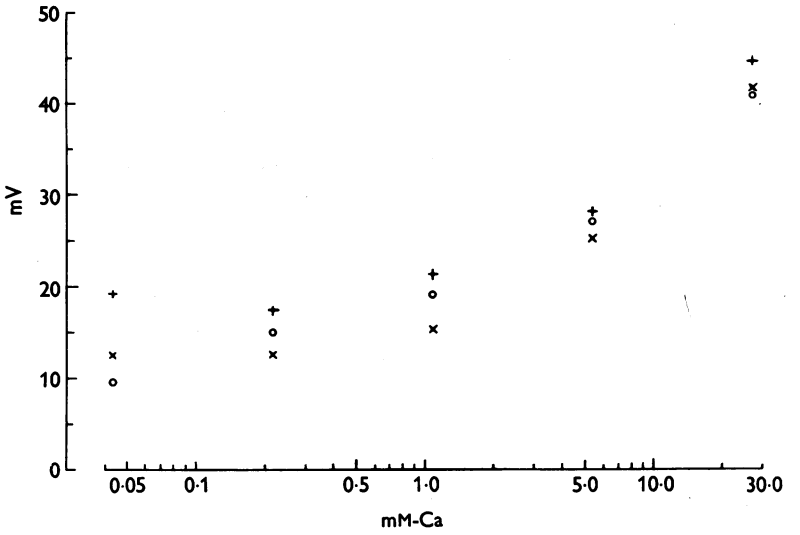

Fig. 3. Threshold membrane potential in three isolated fibres plotted against calcium concentration in the external medium. Abscissae, logarithmic. Thresholds were measured on records similar to column *B* in Fig. 2. Solutions made up with A.R. grade salts and distilled water, and kept in Pyrex bottles (not silicone treated) in this and other experiments, except when otherwise stated. Resting potential shown as zero, cathodal polarization shown upwards.

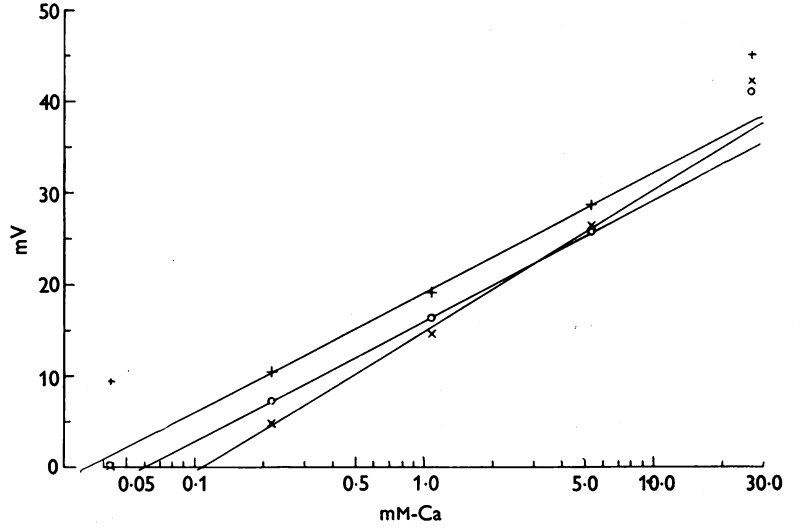

Fig. 4. Threshold plotted against $[Ca]_0$ when test pulse is preceded by anodal polarization. Measured on records like column *C* in Fig. 2. The same fibres as in Fig. 3. Lines are drawn with a slope of 9·2, 9·2 and 10·8 mV for a fivefold change in $[Ca]_0$.

that there was some permanent inactivation of the sodium-carrying system (Hodgkin, 1951). A fibre in a solution with 1·08 mM-Ca or more did not show anode-break excitation as long as the fibre was not seriously run down (cf. Hodgkin, 1951; Frankenhaeuser & Widén, 1956). Frankenhaeuser & Hodgkin (1957) found that in the squid fibre the sodium-carrying system was more inactivated in low $[Ca]_0$ than in high. It therefore seemed reasonable to ascribe at least part of the deviation from a linear relation to inactivation in low $[Ca]_0$. In the squid fibre, inactivation of the sodium-carrying system was removed by anodal polarization. In order to find out how far an increase in threshold in low $[Ca]_0$ was due to decreased regeneration (as a result of inactivation), threshold measurements were made when an anodal polarization preceded the cathodal test pulse. The threshold was measured as in the preceding experiments from the level of the resting potential (Fig. 2). Fig. 4 shows that in solutions with 1·08 mM-Ca or more the threshold was almost unaffected by a preceding anodal polarization (10–40 mV, lasting about 10 msec). However, in low $[Ca]_0$ the threshold was clearly lower when the test pulse was preceded by anodal polarization. In this case there was a linear relation between the threshold and log $[Ca]_0$ over a wide range, although the threshold in the lowest $[Ca]_0$ was still somewhat higher than the value extrapolated from this linear relation Evidence will, however, be given (p. 253) that at least part of this deviation was due to calcium contamination of the solutions. In these experiments the threshold changed 9–11 mV for a fivefold change in $[Ca]_0$. Including five other experiments, the change was 6·5–12 mV, with a mean of 9·3 mV.

Action potential amplitude

The action potential amplitudes were also measured on the records, being taken as the potential difference between the resting potential and the peak of the action potential. How the action potential amplitude depended on the $[Ca]_0$ is shown in Fig. 5. In the range of low $[Ca]_0$ the action potential was clearly smaller. When an anodal polarization was applied before the test pulse then the amplitude was independent of the $[Ca]_0$ (Fig. 6). The amplitude was somewhat lower in 27 mM-Ca, as expected from the decreased $[Na]_0$ in this solution. A stepwise increase of the anodal polarization in the low $[Ca]_0$ increased the action potential amplitude to the size it had in high $[Ca]_0$, and a further increase in polarization had no additional effect. The amount of polarization was therefore not critical. It should, however, be pointed out that in successively lower $[Ca]_0$'s stronger polarizations were necessary in order to make the action potential reach the limiting value.

Inactivation of the sodium-carrying system decreases the amplitude of the action potential. Anodal polarization removes the inactivation and hence restores the size of the action potential. It should be noted that the fibre

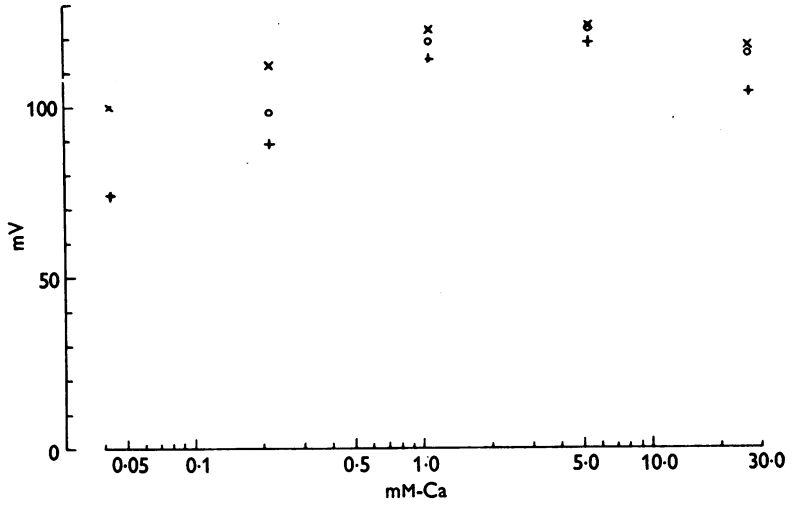

Fig. 5. Peak amplitude of membrane action potential plotted against $[Ca]_0$. From the same three experiments as Fig. 3.

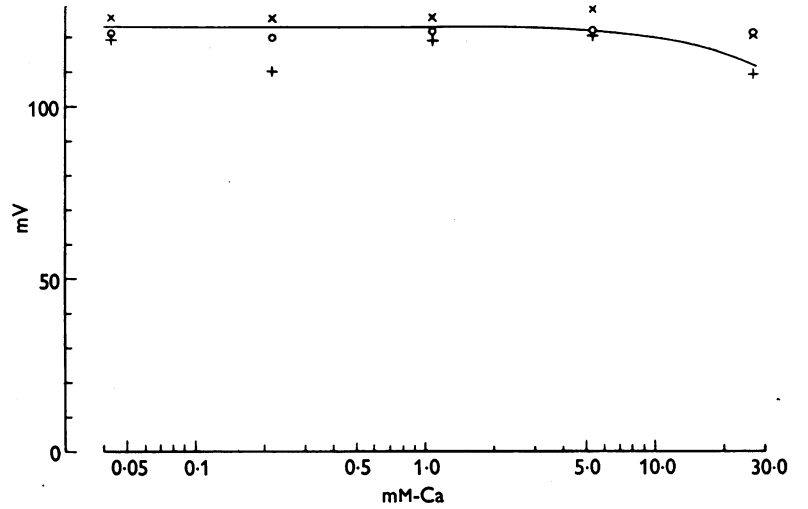

Fig. 6. Peak amplitude of membrane action potential plotted against $[Ca]_0$. The test pulse was preceded by anodal polarization. From the same three experiments as Fig. 3. The line is drawn through 123 mV at 1 mM-Ca, and shows how V_{Na} changes with the composition of the solutions.

might be somewhat inactivated without a marked drop in action potential height. The frog fibres were evidently partly inactivated in low $[Ca]_0$. In the squid fibre the sodium-carrying system was also more inactivated in low $[Ca]_0$ than in high (Frankenhaeuser & Hodgkin, 1957). The findings are thus so far fully consistent with the view that calcium has the same influence on the myelinated nerve fibre as it has on the squid fibre.

Inactivation

The experiments just described show that the fibre was inactivated at its resting potential in low $[Ca]_0$ but not in a solution containing 1·08 mM-Ca or more, in as far as the inactivation can be judged by the relatively crude method of measuring the action potential amplitude. From these experiments it cannot be decided whether the level of membrane potential at which inactivation begins is shifted further in the direction of lower membrane potentials in the high $[Ca]_0$. In order to obtain some information on this point the following experiment was made. The longitudinal current produced by the stimulated node was recorded (other nodes were cocainized).

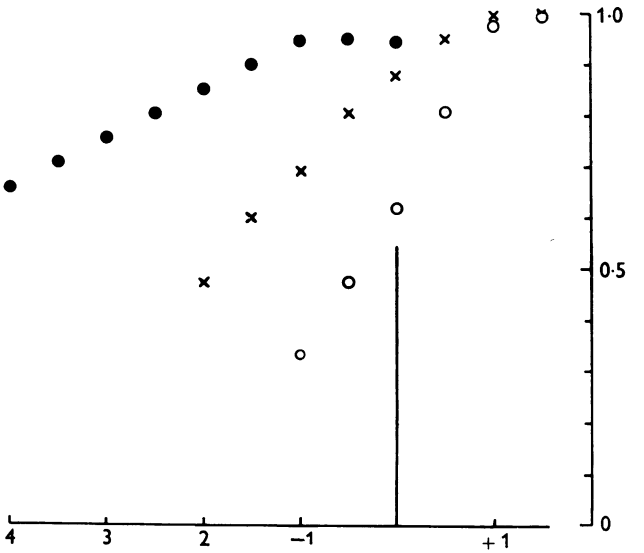

Fig. 7. Peak amplitude of longitudinal current plotted against conditioning potential applied across a seal at a neighbouring internode. Conditioning potential given in units of the rheobasic potential in the solution with 1·08 mM-Ca. Cathodal polarization as negative values. Calcium concentrations: ×, 1·08 mM; ●, 8·6 mM; ○, 0·27 mM.

The node was conditioned with a slowly increasing cathodal polarizing current with a slope much smaller than the critical slope. The node was stimulated with a short test pulse superimposed at different times on the slowly increasing current. The amplitude of the longitudinal current was recorded at a number of depolarizations in the solutions with different $[Ca]_0$. It was found that larger depolarizations were necessary to decrease the amplitude of the longitudinal current if the $[Ca]_0$ is increased (Fig. 7). The fibres were thus partly inactivated already at resting membrane potential in the low $[Ca]_0$, and more so the lower the $[Ca]_0$. In high $[Ca]_0$ the fibres were not markedly inactivated at resting potential and the higher the $[Ca]_0$, the larger the depolarizations needed for the inactivation to occur (cf. Weidmann, 1955).

Inactivation (as measured here) in the myelinated nerve is first detectable at a membrane potential which is relatively close to the threshold (1·08 mM-Ca or more) (e.g. Frankenhaeuser, 1952). This complicates an analysis of the inactivation with the ordinary rectangular conditioning and test pulse technique, since the fibre fires a regenerative impulse already at conditioning pulses which by no means fully inactivate the fibre. To avoid this complication a slowly increasing conditioning current was used. The regenerative response was thus avoided but the time course of inactivation could not be investigated. Further observations with the technique of recording the membrane potential were not made since it seemed that this would not give much more information on the subject.

Summarizing, it may be said that the myelinated fibre at corresponding membrane potentials was more inactivated in low $[Ca]_0$ than in high. The question then arises whether the fibre is fully inactivated in a calcium-free solution or not.

Failure of spike in calcium-free solution

The longitudinal current was recorded from the stimulated node, other nodes were cocainized. A supposedly 'calcium-free' solution, made up from A.R. NaCl, KCl and $NaHCO_3$ and twice-glass-distilled water, was applied to the node under observation. The node was stimulated and the longitudinal current was found to be somewhat smaller than in a solution with 0·067 mM-Ca. The question then arose as to whether there were still traces of calcium in the solution, or whether the fibre actually was excitable in a calcium-free solution. Similar 'calcium-free' solutions with some sodium citrate or sodium fluoride were applied in other experiments. The result was very much the same as in the experiment just mentioned, the fibre still responding with a longitudinal current, diminished but appreciable. The next step was to avoid any calcium contamination as far as possible. The precautions taken were: (1) Johnson and Matthey's 'Specpure' NaCl was used; (2) A.R. KCl and $NaHCO_3$ were used; (3) the distilled water was passed through an ion-exchange resin; (4) all the glassware (Pyrex) was silicone-treated and very carefully cleaned; (5) contamination by touching pipettes, etc., was avoided as far as possible. When this solution was applied, the fibre became inexcitable within a minute, the time taken to change the solution (Fig. 8). The effect was regular and well reversible many times on each preparation. To a fibre which was blocked in the solution the less thoroughly prepared 'calcium-free' solution was applied. The fibre immediately became excitable. It was further found that a trace of $CaCl_2$ added to the solution (0·01 mM) was sufficient to make the fibre excitable. The possibility remained that the resin introduced into the solution some surface-active substance which caused the block. Some rather special assumptions about the action of this substance would be required: (1) the substance would be active only in the absence of calcium, the calcium in the A.R. salts being sufficient to prevent their action (see record *B*); (2) the action of the substance is required to be similar to the action of low [Ca], since the linear relation between threshold and log [Ca] (in the polarized

fibre) holds without much deviation to about 0·01 mM-Ca when the solutions are made with the precautions mentioned. In Fig. 4, where no special precautions were taken, the deviation is markedly earlier. The presence of a surface-active substance derived from the resin is therefore a rather remote possibility, even if it cannot be fully excluded. The frog fibre was thus inexcitable in a calcium-free solution. Small traces of calcium were sufficient to maintain or restore excitability.

Delayed rectification

So far the experiments have been designed to deal with the effect of calcium on the changes in threshold and the amplitude of the action potential. Now the influence of calcium on the delayed rectification will be considered. A linearly increasing voltage, with a slope much smaller than the critical one, was applied across a seal at one internode and the longitudinal current at the next

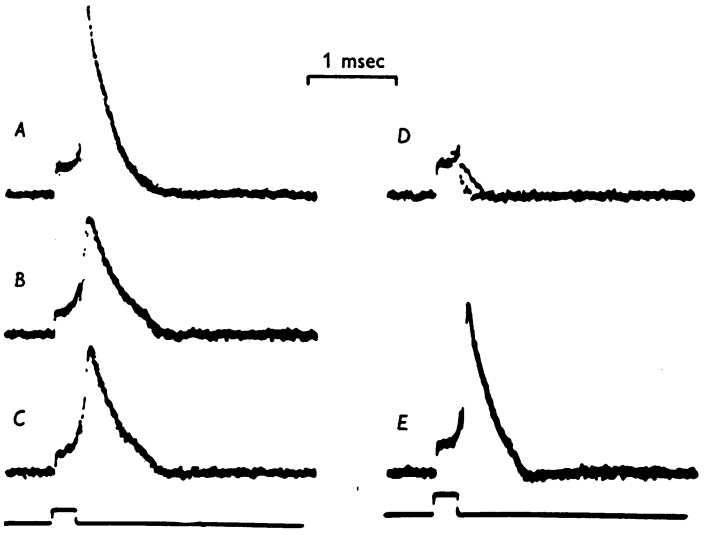

Fig. 8. Longitudinal currents produced by one 'active' node, other nodes cocainized. The external solutions were: *A*, Ringer's solution with 1·08 mM-Ca, made up from A.R. grade substances and distilled water; *B*, 'calcium-free' Ringer's solution made up from A.R. grade substances and distilled water passed through an ion-exchange resin; *C*, 'calcium-free' Ringer's solution made up from A. R. grade substances and distilled water; *D*, 'calcium-free' Ringer's solution made up from 'Specpure' NaCl, A.R. grade KCl and $NaHCO_3$ and distilled water passed through an ion-exchange resin; *E* as in *A*. The order of solution change was *A*, *B*, *C*, *D* and *E*. All records were taken immediately after the solutions were changed, 4–6 traces superimposed. Stimuli well above threshold, increase in stimulus did not increase the small response seen in *D*. Note that the only difference in solutions used in *B* and *D* is that in *B* A.R. grade NaCl was used and in *D* 'Specpure' NaCl. Known amounts of calcium were added to the solution used in *D*, and the longitudinal currents in these solutions were compared with *B* in order to obtain a value for the apparent calcium concentration in the solution used in *B*. This value was about 0·02 mM-Ca. Maximum calcium impurity specified on the A.R. grade NaCl used would account for 0·008 mM-Ca and 0·014 mM-Mg. The agreement in order of size is considered satisfactory. The amount of calcium chloride (or substances having a similar action) in solution *B* would be about 2 mg/l.

internode was recorded, while all the nodes except the one between the seals were cocainized. The recorded current would increase linearly if the nerve fibre were equivalent to a cable with ohmic and capacitive components. For anodal currents this was approximately the case, as it was for cathodal currents when the node between the seals was also cocainized. When the node between the seals was fully excitable, the recorded current rose with a steeper slope at applied potentials lower than the rheobasic value; at higher external potentials the slope became smaller (Fig. 9). This suggests that when the node is depolarized its conductance increases so that there is proportionately less current in the region beyond the node. An increase in sodium conductance would only tend to make the slope steeper at large depolarizations. An increase in potassium conductance at rheobasic or larger potentials would decrease the slope as in the experiments. It should be pointed out that the slope for small cathodal currents was the same as the slope for anodal currents. Further,

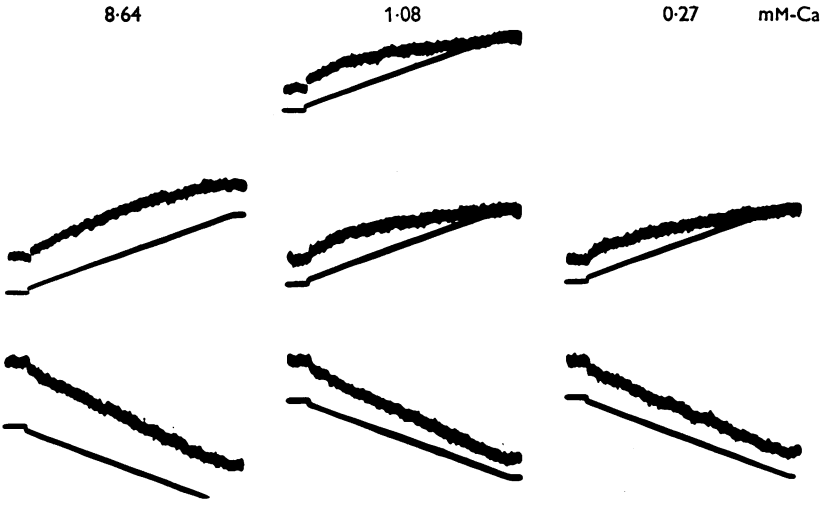

Fig. 9. Longitudinal current (upper trace) recorded across a seal at one internode, associated with a linearly increasing potential (lower trace) applied across a seal at a neighbouring internode. The node between seals fully excitable, other nodes cocainized. Depolarization of node N_0 between seals deflexion upwards. Maximal external potential about three times the rheobase in solution 1·08 mM-Ca. Calcium concentration as marked.

it might be mentioned that the amount of rectification (ratio of slope at low potentials to slope at large potentials) was not strictly independent of the slope of the applied potential and that the rectification was larger when larger external potentials were used. Too large external potentials could not be used, since they damaged the anodally polarized node (N_{-1}) when the external potential exceeded the rheobasic potential by about 10 times. All this rather seriously limits investigations of the rectification by the present technique. Some points were, however, quite clear: (1) the rectification started when the external potential approached rheobasic value, and (2) the node depolarized somewhat above threshold level had a resistance, measured in this way, which was some 7–10 times lower than that of the node at resting potential or when hyperpolarized.

Experiments of the same type were done with the node under observation kept in solutions with different $[Ca]_0$ (Fig. 9). The slope of the recorded current was the same at small cathodal polarizations in solutions from 1·08 mM-Ca upwards and also at anodal polarizations. The level at which the slope decreased was shifted approximately as much as the rheobase towards larger depolarizations when the $[Ca]_0$ was increased. The curves at larger depolarizations had much the same shape in all the solutions.

Summarizing, it may be said that: (1) the myelinated fibre showed a rectification, of the type of 'delayed rectification' in the invertebrate nerve fibre, which began near threshold membrane potential; (2) the membrane resistance at high membrane potentials was nearly the same in different external [Ca]'s; (3) the level at which 'delayed rectification' began was shifted about 5–10 mV for a fivefold change in external [Ca] in the direction towards lower membrane potentials when the $[Ca]_0$ was increased; (4) the resolution of the method was limited, partly for the reasons given above, partly because the signal amplitude was relatively small compared with random noise in the amplifier.

Changes in the form of the action potential

It should be noted that there are some typical changes in the shape of the action potential associated with the external [Ca]. These changes were not very distinct in $[Ca]_0$'s near 1·08 mM, but they were more clear with the extreme concentrations used. In the highest $[Ca]_0$ the rising phase of the action potential was somewhat less steep, the peak more rounded and the falling phase began rather slowly (see Fig. 2). A preceding anodal polarization changed the shape and amplitude very little. In very low $[Ca]_0$ the rising phase was also less steep than in 1·08 mM, the peak of the action potential was sharp and the beginning of the falling phase was steep, but before the resting potential was reached a plateau occurred. A weak anodal pulse applied at the time of the plateau brought about a regenerative response so that the resting potential level was reached more quickly, and a weak cathodal pulse again prolonged the plateau markedly (cf. Tasaki, 1956). A preceding anodal polarization had rather striking effects on the action potential in low $[Ca]_0$. The rising phase was much steeper and the amplitude of the action potential was inincreased towards the limiting value of the size of the action potential in high $[Ca]_0$.

It should further be pointed out that the effects of calcium were quickly reversible. The fibres, however, do 'run down' somewhat when they are kept in low $[Ca]_0$ for long periods, e.g. an hour or more.

When only one node was kept in low $[Ca]_0$ the isolated fibre rarely fired spontaneously. In solutions with less Ca than about 0·2 mM the fibres regularly responded with an action potential at an anode break. Threshold conditions for this effect were not investigated in further detail. In higher $[Ca]_0$'s ($\geqslant 1{\cdot}08$) this was never seen except in cases where the fibre was obviously damaged.

Experiments on desheathed nerve and nerve with intact connective tissue sheath

Most of the findings described above for single fibres have been confirmed with experiments on desheathed nerve. Threshold measurements were made, and the thresholds changed qualitatively as described. Relative values only were obtained, and some assumptions were required to get the slope of the threshold change (cf. Brink, 1951). Some idea about the amplitude of the

action potential in low $[Ca]_0$ was obtained from measurements of the potentials necessary to produce an anode block. In 'calcium-free' solutions, made up with the precautions mentioned on p. 253, the nerve did not block fully and a small action potential was still obtained. The decrease in action potential might have occurred in several ways, for example: (1) some fibres may have become refractory, as a result of spontaneous firing, (2) the action potentials of individual fibres may have decreased, (3) some fibres may have been blocked by inactivation. Spontaneous firing decreased in the 'Ca-free' solution, compared to low $[Ca]_0$, but never ceased completely. The amplitude of the action potential in the individual fibres must have been much reduced since anode block appeared at very low external potentials (about 10–20 mV across a 0·7 mm long stimulating gap for complete block).

Anode-break excitation appeared regularly in low $[Ca]_0$. All the changes were nearly complete after the fluid change, which took one or two minutes.

The calcium solutions were also applied to nerves which had an intact connective tissue sheath. These preparations behaved quite differently. The threshold changed only slowly when a test solution was applied. The change in threshold was half complete in about half an hour, when no branch of the nerve was cut in the vicinity of the stimulating site.

DISCUSSION

In the voltage clamp experiments on the squid fibre (Frankenhaeuser & Hodgkin, 1957) it was found that a fivefold change of the external calcium concentration had the effect that the level of the membrane potential at which the specific changes in sodium and potassium conductance appeared was shifted about 15 mV. On the basis of this, threshold changes of the same order of magnitude would be expected. The shift of threshold would, however, be affected by some other complications. The driving force for sodium is decreased at threshold in very high calcium concentrations, since some sodium is replaced by calcium, and the membrane potential at which change in sodium conductance appears is shifted. This results in decreased regeneration and increased threshold. In low calcium the amount of inactivation increases and this tends to increase the threshold potential unless the fibre is anodally polarized.

Summarizing the results it was found that: (1) the action potential amplitude was smaller in solutions with very low $[Ca]_0$; (2) the action potential amplitude in low $[Ca]_0$ approached the amplitude in high $[Ca]_0$ when the fibre was anodally polarized before the test pulse was applied; (3) larger applied cathodal potentials were needed the higher the $[Ca]_0$ in order to inactivate the fibre so much that the action potential was decreased; (4) the relation between the threshold and the logarithm of the external [Ca] was nearly linear (slope about 9 mV/fivefold change in $[Ca]_0$) over a wide range of [Ca], provided that

the fibre was previously anodally polarized so that the whole sodium-carrying system was available for the regenerative response; (5) the potential level at which rectification appeared was shifted approximately as the threshold when the external [Ca] was changed; (6) the fibres discharged an impulse when in low $[Ca]_0$ at the break of an anodal polarization; (7) the fibre became inexcitable within a minute when a calcium-free medium was applied (for precautions against calcium contamination see p. 253), this effect being quickly reversible.

From these results it is concluded that some calcium in the external fluid is necessary for maintained excitability in the myelinated nerve fibre. The inexcitability in a calcium-free medium described above should be distinguished from the well-known slow 'running down' which the nerve shows in a solution with low calcium concentration. An isolated fibre showed a relatively large action potential when it was washed five hours in a solution containing about 0·01 mM-Ca or in a 'calcium-free' solution made up from A.R. sodium chloride. In such solutions the fibres did slowly run down to some extent. This effect was, however, not well reversible. When the high-grade calcium-free solution (see p. 253) was applied, inexcitability appeared within a minute (time taken for changing the solution), this effect being equally quickly reversible if the calcium-free solution was applied for a relatively short time (5–15 min). If applied much longer, then the fibre did 'run down' and the full recovery was slow. These results are all consistent with the idea that external calcium ions are essential for conduction. In other preparations the evidence is less clear. Cephalopod axons conduct impulses for some time in supposedly calcium-free solutions (5–20 min in *Loligo*, longer in *Sepia*), and when finally blocked quite high concentrations of calcium, 2–4 mM, are needed to restore conduction (Frankenhaeuser, Hodgkin & Keynes, unpublished). Possible explanations of this result are: (1) calcium in the external solution is not immediately necessary for impulse generation; (2) even if there is no calcium in the solution outside the fibre, there may be traces of calcium immediately outside the active membrane, possibly derived from the Schwann cell or from the connective tissue. The experiments on the myelinated fibre seem clearer on this point since here calcium is immediately necessary for impulse generation. It was not possible to find out exactly at what calcium concentration excitability reappeared in the fibre, since the concentrations are so small that it was impossible to check well enough unwanted contaminations.

Since only very small traces of calcium are needed to maintain conduction, the present finding that the fibre quickly loses its excitability in a genuinely calcium-free Ringer's solution is not inconsistent with the finding that a nerve conducts impulses for a considerable time when in an 'ordinary' Ringer's solution without calcium.

A point which requires further investigation is that the fibres did not

become inexcitable when they were in a solution with low $[Ca]_0$ to which some sodium citrate was added.

Calcium plays an important role in the mechanism that determines the specific conductance changes in the nerve membrane. The present findings on the myelinated frog fibre are in good agreement with the findings obtained with the voltage clamp technique on the squid fibre. It therefore seems reasonable to assume that the mechanism for the calcium action is essentially the same in these two rather different types of nerve fibres.

SUMMARY

1. Longitudinal currents or membrane action potentials were recorded in isolated myelinated frog nerve fibres in different external calcium concentrations.

2. The action potential amplitude was smaller when $[Ca]_0$ was low.

3. The effect of anodal polarization was to make the action potential amplitude independent of $[Ca]_0$.

4. The threshold increased with the $[Ca]_0$. The relation between the threshold and the log $[Ca]_0$ was nearly linear, with a slope of about 9 mV/fivefold change in $[Ca]_0$, when the fibre was anodally polarized before the test pulse was applied.

5. The fibre was more inactivated in low $[Ca]_0$ than in high.

6. The potential level at which rectification appeared was shifted approximately as the threshold when the $[Ca]_0$ was changed.

7. The fibre was excited at the break of an anodal polarization when the $[Ca]_0$ was low.

8. The fibre became inexcitable within a minute when a calcium-free medium was applied provided that precautions were taken to avoid calcium contamination. The effect was quickly reversible.

9. Small traces of calcium ($< 0{\cdot}01$ mM) were sufficient to maintain or restore excitability.

10. It is concluded that calcium is immediately necessary for impulse activity.

11. The results are discussed in relation to the results obtained on the squid giant fibre with the voltage clamp technique.

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