Some Effects of Calcium Ions on the Action Potential of Single Nodes of Ranvier

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ABSTRACT Action potentials of single frog nerve fibers were recorded with the air-gap method in "low Ca" (0.26 mM) and "high Ca" (4.2 mM) solutions and compared to spikes in normal Ringer's (1.05 mM Ca). On increasing $(Ca)_o$ the action potentials became shorter, the "knee" during the falling phase as well as the threshold for abolition moved to internal potentials more positive, and the spike recovery during the relative refractory period was faster. Outward current pulses applied during an action potential affected its configuration more in low Ca than in high Ca. The onset of the delayed rectification (in the absence of Na) was found faster in high Ca. After-potentials during anelectrotonus declined more rapidly in high Ca than in low Ca. The results are compared primarily with the voltage-clamp analysis of Ca effects on squid axons and satisfactory qualitative agreement is reached.

The important role of calcium ions for proper functioning of excitable tissues has long been recognized and consequently a considerable amount of work has been dedicated to studies of Ca effects (reviewed by Brink, 1954). Major advance was made by Frankenhaeuser and Hodgkin (1957) in their voltage-clamp experiments on squid axons. These authors showed that a far reaching interchangeability exists between external Ca concentration $(Ca)_o$ and membrane potential in their effects on the kinetic and stationary characteristics of the ionic conductances. Frankenhaeuser (1957), in a subsequent paper, tested successfully the applicability of this principle to myelinated nerve fibers and was primarily interested in the reaction of threshold and action potential amplitude to a variation of $(Ca)_o$ over several orders of magnitude including total calcium lack. Distinct changes in the shape of the action potential were reported only with the extreme concentration used. It was, therefore, rather surprising to observe marked effects on the spike configuration with relatively

moderate deviations of $(Ca)_o$ from the standard. Several types of experiments were subsequently performed in order to find out how far this observation agreed with already known Ca effects on the Ranvier node and the squid axon.

METHODS

Single motor fibers were isolated from the sciatic nerve of the European frog, *Rana esculenta*, and were transferred to a chamber which was described in detail by Stämpfli (1959). The node under investigation was placed in a small slit across a thin polyethylene tube (inside diameter 0.6 mm) which was connected to a stopcock with minimal dead space. This arrangement permitted continuous perfusion and fast switching between test solutions. The neighboring nodes and the rest of the nerve trunk were situated in larger side pools containing cocaine-Ringer's solution and were separated from the tube by air gaps. Electrical contact to the pools and the tube was made through KCl-agar bridges by means of Ag-AgCl electrodes. The preparation was stimulated between one side pool and the polyethylene tube (at ground potential). Constant current flow during electrotonus experiments was achieved by a high series resistance. Potential differences were recorded between ground and the other side pool by a negative-capacitance preamplifier in connection with a tektronix 502 oscilloscope. The resistance across the air gap varied, especially with the relative humidity, so that a varying (but large) portion of the "true" potential difference was recorded. Thus the term "zero current condition" has to be understood as a condition in which no current was fed through electrodes to the node under investigation.

Most of the experiments were done at room temperature (21–25°C) but in some cases the test solutions were cooled by passing through coils which were in contact with circulating cooling fluid from a large (20 liter) thermostat. The temperature was measured with a copper-constantan thermocouple a few millimeters downstream from the node and recorded by a Kipp Al 4 galvanometer (the reference junction being in melting ice).

The normal Ringer's solution contained (in mM); NaCl 110.5, KCl 2.5, $CaCl_2$ 1.05, and $NaHCO_3$ 2.4.

High Ca Ringer's solution contained 4.2 mM Ca (4 times normal) and low Ca Ringer's solution, 0.26 mM Ca (one-fourth normal).

RESULTS

Shape of the Action Potential

The most striking effect on the action potential of a change from high Ca solution to low Ca solution is an increase of its duration (Fig. 1). This lengthening is almost entirely due to a slower repolarization. Especially at the low temperature at which Fig. 1 was taken, a distinct concavity of the first falling phase (between crest and "knee") is observed in low Ca Ringer's solution. Furthermore, the potential at which the knee occurs is closer to the resting potential than it is in high Ca. The difference in the action potential amplitude

seen in Fig. 1 has already been described (Frankenhaeuser, 1957). It should be mentioned here that there is a small depolarization of the membrane after a change from low Ca to high Ca (Ulbricht, 1963).

In order to give a more quantitative description of the Ca effect on the repolarization phase a somewhat arbitrary measure of this phase had to be chosen because of the vagueness of the spike termination in the presence of an after-depolarization ("negative" after-potential). A "repolarization time" was defined as the time from the peak of the spike to the point where a tangent to the steepest part of the late falling phase intersects the resting potential. In twenty-three experiments action potentials of single nodes were recorded in the standard solution and in low and high Ca Ringer's solution. The repolarization time (at 20 to 25°C) was found to be in low Ca 118 ± 7 per

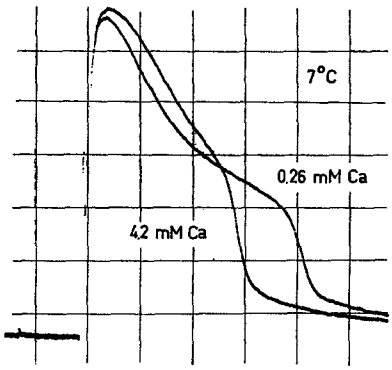

FIGURE 1. Ca effect on shape of action potential. 16 mv per vertical division, 1 msec. per horizontal division. While shutter of camera was open, an action potential was elicited in 4.2 mM Ca, then the solution was switched to 0.26 mM Ca and another stimulus was applied to the node. Interval between the two stimuli was 7 sec. Temperature of both solutions was 7°C.

cent (SE) and in high Ca 71 ± 2 per cent (SE) of that in normal Ringer's solution. It is difficult to measure the "knee potential" accurately because of the rather gradual change in the rate of fall. However, this potential shifted clearly by about 10 to 15 mv towards the resting potential upon transition from high to low Ca solutions.

In some experiments the abolition of the action potential (Tasaki, 1956) was studied by applying a strong hyperpolarizing pulse of short, constant duration during the early falling phase. The intensity of the pulse was such as to give a threshold phenomenon, *i.e.* at the break of the pulse the action potential either continued or was terminated. The membrane potential immediately after the end of the pulse ("abolition threshold") showed a shift as a function of $(Ca)_o$ similar to that of the knee potential.

The effect of Ca on the after-potential was not systematically studied. In

four experiments the time course of the decline of a just subthreshold response was determined. This repolarization was, except for the earliest part, practically exponential and the time constants were 0.27 msec. in high Ca and 0.37 msec. in low Ca (mean values at room temperature).

Electrotonus and Spike Configuration

Frankenhaeuser (1957) has shown that the Ca effect on the action potential amplitude was due to a change in the steady-state relation between the inactivation of the sodium-carrying system and the (conditioning) membrane potential and that the spike height became independent of $(Ca)_o$ when the inactivation of the sodium system was completely removed by anelectrotonus. It was, therefore, of interest to find out whether the Ca-induced changes of

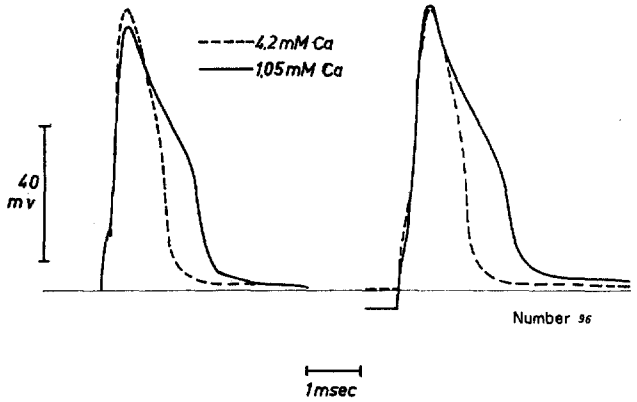

FIGURE 2. Adjustment of action potential height in 1.05 mM Ca to that in 4.2 mM Ca by anelectrotonus prior to stimulation (right-hand tracings) as compared to condition without current flowing (left-hand tracings). In spite of adjusted amplitude the action potential in standard $(Ca)_o$ does not approach the shape in high Ca.

the spike shape could likewise be abolished by a current flow prior to the action potential.

In several experiments the action potential amplitude in normal Ringer's solution was brought to the height in high Ca solution by subjecting the node to a hyperpolarizing current pulse of 20 to 30 msec. duration immediately preceding the brief stimulus (Fig. 2). This figure clearly shows that the spike duration in the lower Ca concentration remains longer than it does in the high Ca solution although the falling phase starts from approximately the same peak potential. The characteristic dependence of the relative spike duration is also maintained if the action potential amplitude in normal and high Ca solutions is adjusted to that in low Ca solution by appropriate depolarizing prepulses. Upon closer inspection of Fig. 2 one finds that the duration of the action potential in normal Ringer's fluid after a hyperpolarizing

pulse is even slightly increased when compared to an action potential in the same solution but without a preceding current flow. Conversely, after a depolarizing prepulse the action potential is not only smaller in amplitude but also shorter.

For moderate hyperpolarizing currents (about 5×10^{-10} a) the shape of the action potential was also studied when the current flow continued during the spike. Again, the duration of the action potentials was prolonged in the Ca concentrations employed (high, normal, and low), and traces of spikes under current flow and those at zero current, when superimposed, did not intersect before the beginning of the after-depolarization.

Although the action potential amplitude is not a very direct measure of the availability of the sodium-carrying system it seems a reasonable conclusion from the experiments just mentioned that the level of inactivation (at the crest of a spike) *per se* cannot be responsible for the Ca-dependent variations of the spike duration. This does not exclude the possibility that Ca exerts its effect through a change of the time constant τ_h of the inactivation process. Without applying the voltage-clamp technique a direct determination of τ_h cannot be accomplished. In order to obtain some crude measure of τ_h, however, it seemed worth while to study the recovery of the spike amplitude during the relative refractory period as a function of $(Ca)_o$ with the implication that this reflects, at least to a great extent, the time course of recovery from inactivation.

Refractory Period

With increasing delay after an action potential brief stimuli were applied to the relatively refractory membrane and the amplitudes of the resulting all-or-none responses were plotted as a function of time from the "end" of the repolarizing phase (Fig. 3). This figure demonstrates the quicker recovery of the spike height with increasing $(Ca)_o$.

As a convenient measure the time from the end of the first spike to the crest of a relatively refractory action potential of 85 per cent the final amplitude was determined in ten experiments and found to be 2.5 ± 0.3 msec. (SE) in normal Ringer's solution for temperatures between 20 and 25°C. In high Ca the time was 74 ± 3 per cent (SE) and in low Ca 112 ± 3 per cent (SE) of the value in normal Ringer's solution.

In order to obtain a time constant of the spike recovery the differences between the final action potential amplitude and the amplitudes of the second (or test) spike were plotted on semilog paper *versus* time. Especially for high Ca solutions this plot showed two slopes: a steeper one for the first part of the recovery period and a less steep one for the rest of this period. Only this latter portion was assumed to be representative for the recovery from inactivation.

The fact that more than one time constant is involved shows that the spike recovery over the whole duration of the relative refractory period is not solely governed by the recovery from inactivation, which should have a simple exponential time course (Hodgkin and Huxley, 1952; Frankenhaeuser, 1960). And indeed, the ionic theory shows that not only the inactivation but also the persistently increased membrane conductance to K ions plays a role during the refractory period. It is obvious that an attempt to separate these factors experimentally can be promising only if the recovery from inactivation and the decline of the K permeability have considerably different time constants. Therefore, τ_h and the time constant for the decline of P_K

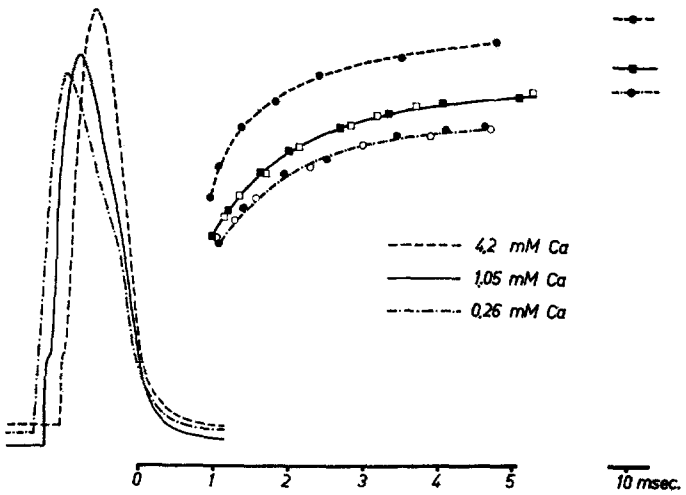

FIGURE 3. Ca effect on spike height during relative refractory period. Tracings of action potentials in 4.2, 1.05, and 0.26 mM Ca superimposed at the end of the spike (definition in text) = 0 msec. Measuring points denote crests of action potentials during relative refractory period; filled symbols refer to measurements taken upon return to the respective solutions from a different $(Ca)_o$. Note that after 10 msec. the recovery is virtually complete only in 4.2 mM Ca, 22°C.

were calculated for a sudden repolarization to the resting potential using the node data from Frankenhaeuser (1962). For this simplified model τ_h appeared to be about 4 times as long as the time constant of the P_K shut-off.

The time constants were plotted *versus* the ratio of the final spike height over the maximum spike height obtainable by hyperpolarization. Such a plot showed a considerable scatter. However, for a given nerve fiber the time constants in high Ca were always shorter than in low Ca. The amplitude ratio was divided by the time constant in analogy to the determination of $\alpha_h = h_\infty/\tau_h$. The mean values of these "α's" were 0.63 msec.$^{-1}$ for high Ca and 0.32 msec.$^{-1}$ for low Ca (four experiments at 24 ± 1°C).

It remains to be decided to what extent these α's are representative of the actual α_h. The value of α_h for $V = 0$ (resting potential), normal Ca and 20°C, calculated from Frankenhaeuser's equations (1960, 1962) is 0.41 msec.$^{-1}$. The higher value of α in high Ca seems to be in agreement with the general concept that the Ca effect simulates a hyperpolarization (Frankenhaeuser and Hodgkin, 1957).

Time constants were also determined for the case of constant current flow during the first spike and the following relative refractory period. An attempt was made to match the spike amplitude in high and low Ca by proper ad-

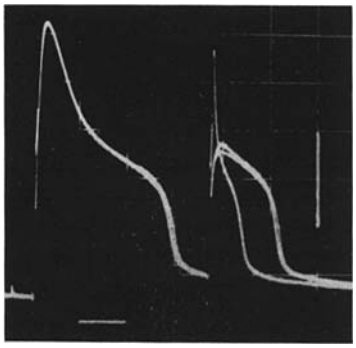

FIGURE 4. Shortening of action potential in early relative refractory period by several times superthreshold stimulus, superimposed on oscilloscope screen with two traces of refractory spikes elicited with just superthreshold stimuli. Horizontal bar = 2.5 msec., vertical bar = 35 mv; 0.26 mM Ca, 7°C.

justment of the electrotonus and to compare the time constants for the attainment of various final spike heights. This type of experiment is limited by the development of a considerable after-depolarization following the first spike under anelectrotonus which causes the test spikes to start from different membrane potentials at a given current flow. In spite of this shortcoming the time constants in high Ca were always found to be shorter than in low Ca for a given final spike amplitude.

In a third series of experiments the recovery of the spike height after a long depolarizing pulse was determined. An outward current pulse of variable strength and 30 msec. duration was followed by a long hyperpolarizing pulse of constant amplitude so as to give a maximum spike height in the steady state. Brief superthreshold stimuli were applied at varying intervals from the break of the cathodal pulse, and the amplitudes of the resulting action potentials were plotted as a function of time. A plot of this kind resembles the equivalent pattern during the relative refractory period with the difference that the membrane is not conditioned by a constant depolarizing "pulse;" *i.e.*, the first action potential, but by a long pulse the intensity of which can be set at will. The time constants found were not completely independent of the level of the preceding depolarization; they were shorter for a stronger depolarization. However, they were always shorter in high Ca than in low Ca for comparable starting levels.

Spikes elicited at various times during the relative refractory period were compared by superimposing the second, faster parts of their repolarizing phase (Tasaki, 1956). For a given $(Ca)_o$ the fit was very good, but a test spike in one $(Ca)_o$ could not be reasonably well fitted to a spike in another $(Ca)_o$.

An interesting phenomenon was observed during the relative refractory

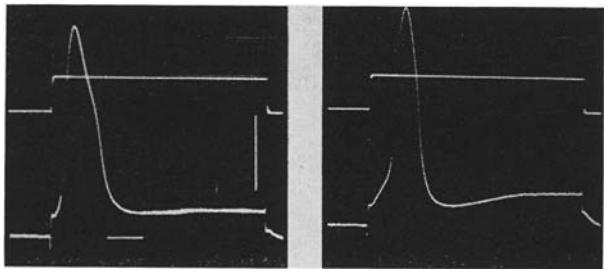

FIGURE 5. After-hyperpolarization by flow of outward current (long lasting stimulus of 0.6×10^{-9} a) during action potential. Left-hand photograph, in 0.26 mM Ca, right-hand photograph, in 4.2 mM Ca with identical calibration: horizontal bar = 1 msec., vertical bar = 35 mv. Upper beam denotes duration of current flow. Note more marked after-potential and longer delay of action potential in 4.2 mM Ca, 22°C.

period in low Ca at low temperature. An all-or-none response during the early refractory period could be considerably shortened if it was elicited by a brief stimulus of several times threshold intensity (Fig. 4).

Further Experiments on Current Flow during the Action Potential

It has long been known that during the flow of a moderate depolarizing current the action potential of a frog nerve fiber shows an underswing at the end of its falling phase, an after-hyperpolarization. In a recent detailed study on single frog nerve fibers Meves (1960) explained this after-hyperpolarization as being due to the still decreased membrane resistance across which the current causes a smaller voltage drop than in the steady state. In order to study the effect of Ca on this phenomenon nodes of Ranvier were stimulated with a current pulse which lasted several times the spike duration. As shown in Fig. 5 the after-hyperpolarization for a given current pulse was more marked in high Ca than in low Ca. The maximum of the after-potential occurred sooner after the spike in high Ca than it did in low Ca, and this difference was greater than the difference of the repolarization time. The voltage drop towards the end of the current pulse was larger in high Ca than in low Ca. This does not necessarily mean that the steady-state slope conductance of the membrane, dI/dV, was smaller in the high Ca solution for this potential region since it is known that the delayed rectification sets in at a greater depolarization as $(Ca)_o$ is increased (Frankenhaeuser, 1957; see also Fig. 6 below).

After-potentials following an action potential during anelectrotonus were also studied at different $(Ca)_o$. A constant hyperpolarizing current of the order of 5×10^{-10} a was applied to the node. In the steady state it caused a slightly smaller voltage drop at the membrane in low Ca as compared to that in high Ca (91 per cent, mean value of eleven measurements). Action po-

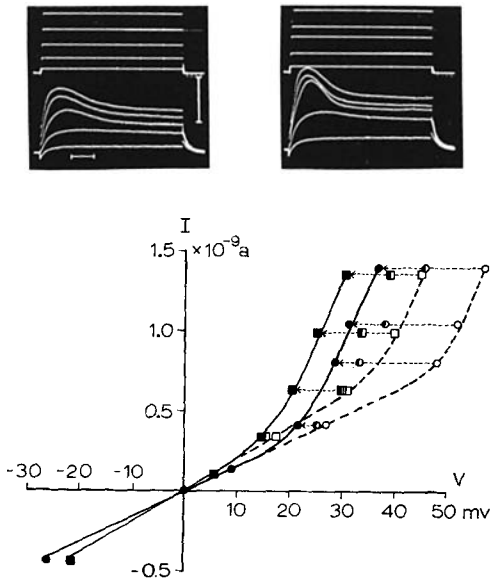

FIGURE 6. Temporal development of delayed rectification in Na-free solutions (choline Ringer's solution). Original oscilloscope traces on the left, in 0.26 mM Ca, on the right, in 4.2 mM Ca. Calibration for both photographs, horizontal bar = 1 msec., vertical bar = 1.0×10^{-9} a (upper beam) or 35 mv (lower beam). Upper traces are constant current pulses of about 5 msec. duration and lower traces the potential changes caused by the flow of these currents.

In the lower diagram the changes in potential difference across the membrane are plotted in relation to the currents producing them. Steady-state values are indicated by the filled symbols; the values at the peaks of the transients, by the open symbols; and the values corresponding to 1 msec. after the peaks, by the half-filled symbols. Squares refer to 0.26 mM Ca, circles to 4.2 mM Ca. The horizontal dashed lines (with arrows) indicate the temporal sequence during the transition from the peak to the steady state. Points for inward currents are not shown in the oscilloscope tracings. 24°C.

tentials elicited during the current flow were followed by a large after-depolarization the time course of which could not be fitted to a simple exponential. Therefore, a qualitative comparison was made by superimposing the traces at the final membrane potential under current flow and at the point where the falling phase intersected the normal resting potential ($I = 0$). The decline of the after-potential in high Ca was always found faster than in low Ca.

The experiments described so far have shown that an increased $(Ca)_o$ en-

hances the speed with which the membrane reaches a steady state after a disturbance. At least in squid axons the importance of K ions for the termination of the action potential is well established. In order to find out whether $(Ca)_o$ affects the dynamics of the K permeability of the nodal membrane, the temporal development of the delayed rectification was studied. It is obvious that this can be done only if the membrane is prevented from undergoing an

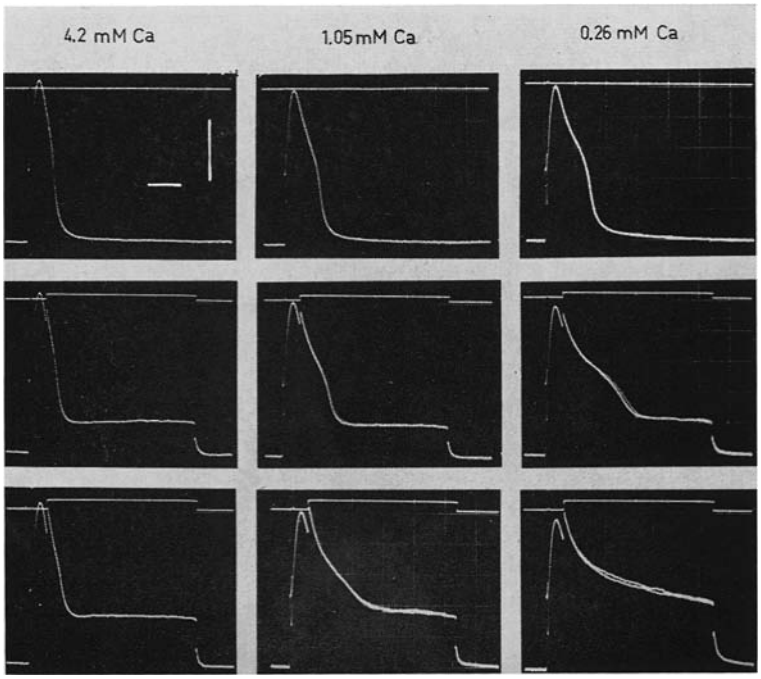

FIGURE 7. Effect of outward current pulses during action potentials in different $(Ca)_o$. Calibration for all photographs in upper left-hand picture, horizontal bar = 1 msec., vertical bar = 35 mv. Upper beam shows timing of current pulse. Left-hand column 4.2 mM Ca, middle column 1.05 mM Ca, and right-hand column 0.26 mM Ca. Upper row without current flow, middle row with current pulse of about 0.8×10^{-9} a, and bottom row with current pulse of about 1.6×10^{-9} a. 24°C.

action potential. Therefore, in the low and in the high Ca solution all the NaCl was substituted by choline chloride.

The node was submitted to depolarizing current pulses of increasing intensity, and the resulting potential changes were recorded (Fig. 6). The original oscilloscope traces showed that at comparable current intensities the membrane resistance decreased faster in high Ca than in low Ca. The graph in Fig. 6, which has been derived from the original records of this figure, gives the voltage-current relation at three different times, at the peak (varying by about 0.5 msec.), 1 msec. thereafter, and immediately before the break of

the current. This graph demonstrates that 1 msec. after peak time the *I-V* curve in high Ca has approached the "final" curve to a greater extent than did the similar curve in low Ca.

Another type of experiment with depolarizing current pulses was performed in the presence of the normal $(Na)_o$. An action potential was triggered by a brief stimulus and 0.5 msec. later (*i.e.* soon after the peak) a strong current

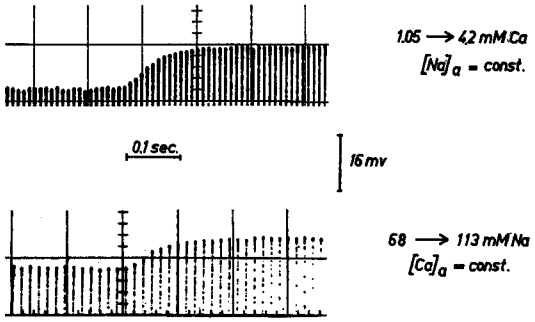

FIGURE 8. Effect of fast change of solutions on action potential amplitude during train of impulses. Only crests of action potentials are shown. Upper part, switch from 1.05 to 4.2 mM Ca at constant $(Na)_o$ = 113 mM. Stimulus interval about 13 msec. Lower part, switch from 68 to 113 mM Na at constant $(Ca)_o$ = 1.05 mM, stimulus interval about 16 msec. Different node. 22 ± 1°C.

pulse of 5 msec. duration was applied to the node. Fig. 7 shows the observed changes of the repolarization in high, normal, and low Ca. Even during the strongest current pulse (bottom row) the membrane potential attained a steady state rather soon in high Ca while in the low Ca solution the decline of the potential was considerably slower and the characteristic phases of the repolarization became indistinguishable.

Finally, an experiment should be mentioned which enabled a rough estimate of the speed with which Ca ions act on the membrane. A preparation which had been used in an extended experiment usually showed a decreased action potential because the sodium-carrying system was more inactivated. When placed in a high Ca solution, however, a marked recovery of the spike height could be observed. The time course of this recovery was followed by recording (at a slow sweep speed) a train of impulses while the node was subjected to a quick change of solutions (technical details in Ulbricht, 1963). The result of such an experiment is illustrated in Fig. 8 in which only the upper portions of the action potentials are shown. The transition from the lower to the increased amplitude was practically complete within 200 msec. This was comparable to the time course of a change of spike height following a switch from 68 to 113 mM Na at constant $(Ca)_o$ and nearly identical flow rates.

The mean velocity of flow in the perfusion tube was about 20 cm/sec. The sharpness of fluid change was estimated by monitoring photoelectrically the exchange of dye solutions at the site of the node. It was found that the paraboloid of the new solution occupied one-half of the inside diameter of the perfusion tube within about 10 msec.

DISCUSSION

The falling phase of an action potential is determined by a complex change of the membrane permeabilities to Na and K ions. A quantitative interpretation would require computations based on a complete voltage-clamp analysis. So far, analytical data at different $(Ca)_o$ have been compiled only for the squid axon by Frankenhaeuser and Hodgkin (1957). Therefore, it seems appropriate to restrict the discussion to a comparison of the limited data from the node with the results of these authors, especially since some voltage-clamp experiments done on lobster axons (Julian, Moore, and Goldman, 1962) and some attempted on frog fibers (Wright and Ooyama, 1962) appear to justify the application of the findings on squid axons to other preparations.

Frankenhaeuser and Hodgkin (1957) found that an increase in $(Ca)_o$ acts on the membrane as if it were hyperpolarized. This comes about by a shift along the voltage axis of the functions which relate the quantities m, h, and n and their time constants to the membrane potential. Since the knee of the nodal spike marks the potential at which the decline in P_{Na} is speeded up by the reversible shutting-off of the activation process, the observed shift in high Ca of the knee towards the peak of the action potential (Fig. 1) suggests a corresponding shift of $\alpha_m(V)$ and $\beta_m(V)$ along the voltage axis. A shift of that kind would also explain the Ca-induced changes of the threshold potential for initiation (Frankenhaeuser, 1957) and abolition of an action potential.

Frankenhaeuser and Hodgkin (1957) reported that reducing $(Ca)_o$ decreases the rate at which inactivation of the sodium conductance is removed under the anode. In frog nerve the observed retardation of spike recovery during the relative refractory period in low $(Ca)_o$ is in good agreement with this finding. Frankenhaeuser (1957) had given evidence that the steady-state inactivation curve for frog fibers is shifted along the voltage axis in the manner described for Purkinje fibers (Weidmann, 1955) and squid axons (Frankenhaeuser and Hodgkin, 1957).

The observed changes in the time constant of spike recovery are clearly compatible with the described shift of the steady-state inactivation curve. The latter must be brought about by a change of α_h and β_h as functions of membrane potential (Hodgkin and Huxley, 1952). In spite of an electrotonic adjustment of the final spike amplitude in high and low Ca to the same, slightly submaximal value (roughly corresponding to h_∞ slightly less than 1), the time constant of recovery remained longer in low Ca than in high Ca. This suggests that $\alpha_h(V)$ and $\beta_h(V)$ undergo in low Ca changes in addition to a shift along the voltage axis. There is a possible relation to the observed flattening

of the steady-state inactivation curve at reduced $(Ca)_o$ in squid (Frankenhaeuser and Hodgkin, 1957).

The development of the delayed rectification, as studied in Na-free solutions (Fig. 6), was found to be faster in high Ca than in low Ca. Frankenhaeuser and Hodgkin (1957), on the other hand, reported that in squid axons the potassium conductance g_K turns on slower and with a greater delay as $(Ca)_o$ is increased. These two findings, however, do not necessarily contradict each other as will be shown by the following discussion.

In the formulation of Hodgkin and Huxley (1952) the increase of g_K upon a sudden depolarization is governed by the time constant τ_n. When τ_n is plotted as a function of the membrane potential the curve is bell-shaped and shows a maximum at a potential which is less negative than the resting potential. According to the general concept of Ca action (Frankenhaeuser and Hodgkin, 1957), an increase in $(Ca)_o$ will shift the τ_n-V curve along the voltage axis so that the maximum will occur at a greater depolarization, while lowering $(Ca)_o$ will shift the curve in the opposite direction. From this it follows that a τ_n curve in low Ca intersects a τ_n curve in high Ca at a potential V' between the maxima of the two curves. For a membrane depolarized with respect to V', τ_n in high Ca will be larger than in low Ca, while for one hyperpolarized with respect to V' the reverse will be true. Frankenhaeuser and Hodgkin (1957) studied the time course of g_K at the sodium equilibrium potential, *i.e.* during a large depolarization; hence they found in a Ca-rich solution a slower rise of g_K, which corresponds to an increased τ_n. In the frog nerve experiments illustrated by Fig. 6 the depolarizations obtained by the flow of constant outward currents were much smaller and probably in the range of potentials at which τ_n in high Ca is expected to be smaller than in low Ca. Although these experiments cannot strictly be compared to those performed with the voltage-clamp technique, the observed faster development of the delayed rectification seems quite compatible with the results of Frankenhaeuser and Hodgkin.

Within the limits of the method used for the node experiments, no evidence could be found which would qualitatively disagree with the squid axon results. The assumption of a similar action of Ca ions in both preparations is supported by the fact that an increased $(Ca)_o$ enhances the spike repolarization in squid axons (Shanes *et al.*, 1959) as it does in frog nerve fibers.

The numerous effects of Ca ions on excitable tissues have been reviewed extensively by Brink (1954). In more recent papers it has been reported that an increase in $(Ca)_o$ up to about 10 mM accelerates the falling phase of frog muscle action potentials (Ishiko and Sato, 1957; Jenerick, 1958). Mammalian ventricular fibers show a shortening of the action potential duration in excess Ca (Hoffman and Suckling, 1956; Distel, 1960). In frog ventricular

fibers the total duration is prolonged by 3 times normal Ca, while the initial repolarization phase is clearly faster (Ware, 1961). Action potentials of Purkinje fibers do not show any marked changes upon transition to 4 times Ca Tyrode solution (Weidmann, 1955) and are prolonged only in Ca-free solutions which contain EDTA (Chang and Schmidt, 1960). Action potentials of lobster giant axons do not change when $(Ca)_o$ is doubled, but in Ca-free solutions a decrease of amplitude and duration and the development of an undershoot are observed (Dalton, 1958). Motor axons of the lobster limb respond to Ca-free solutions with a decreased amplitude and maximum rate of fall of their action potentials and also develop an undershoot (Adelman and Adams, 1959). Thus it has been demonstrated that the Ca effects on the action potential configuration show considerable differences between species and preparations.

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