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Steady state current rectification in potential clamped nodes of Ranvier (*Xenopus laevis*)

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The membrane potential in single nodes of Ranvier was changed in rectangular pulse steps, while the membrane currents, associated with the potential steps, were measured. Changes were made in the ionic composition of the external and the internal medium. The latter changes were obtained by free diffusion through a cut internode. The steady state currents, described on the basis of potassium and leak permeability, were affected by the solution composition in a characteristic way. Increased inside concentration of sodium and lithium caused a striking rectification of the outward steady state currents at large potential steps. Instantaneous potassium currents in high $[K^+]_o$ at a second potential step to $E \approx -80$ mV were not affected by $[Na^+]_i$. Neither $[Na^+]_o$ nor $[K^+]_i$ affected the potential at which this rectification appeared. Increased $[K^+]_o$ shifted the region for rectification along the potential axis in positive direction. These findings form strict limitations for satisfactory models describing the mechanism for the steady state current in myelinated nerve.

INTRODUCTION

The myelinated nerve fibre shows during step polarizations a sequence of permeability changes. A positive potential step thus causes: (*a*) an initial transient increase in sodium permeability (P_{Na}); (*b*) a delayed increase in potassium permeability (P_K) and (*c*) a still more delayed minor permeability increase (P_p) for an unidentified current carrier. The net membrane current is the sum of the ionic currents through these permeability mechanisms and in addition a leak current and a capacity current. The sodium current (I_{Na}) and the potassium current (I_K) can be relatively satisfactorily described on the basis of the membrane permeability for these ions and the electrochemical driving force as approximated by the constant field equation (Goldman 1943; Hodgkin & Katz 1949*a*; Frankenhaeuser 1960, 1963; Frankenhaeuser & Huxley 1964). At large positive potential steps of long duration the current–potential curve deviates strikingly from the relation described by the constant field equation for constant permeability (Frankenhaeuser 1962). This rectification varies from fibre to fibre. Bergman (1970) observed that fibres, after massive trains of potential steps of an amplitude chosen to cause a large sodium current, show a change of equilibrium potential for the initial current and that these fibres then show a reduction of the delayed current. Bergman's main conclusion is that sodium during the pulse train accumulates near the inner surface of the membrane and competes with potassium for the permeable site but does not transport current through the site. The outward flow of potassium would thus be blocked by the outward flow of sodium. He suggests 'a one-to-one competition of Na and K-ions for the same membrane pathway when both ions tend to flow in the outward direction'.

Bezanilla & Armstrong (1972) have made measurements in potential-clamp experiments on the perfused squid giant nerve fibre and describe a similar rectification caused by $[Na^+]_i$, $[Li^+]_i$ and $[Cs^+]_i$.

In the present investigation a further study was made of the rectification of the delayed currents in the myelinated nerve fibre at large potential steps. Changes were made of the external ionic composition and of the ionic composition of the axis cylinder. The latter was effected by diffusion through a cut end of the fibre under investigation as described by Koppenhöfer & Vogel (1969). It will be shown that the delayed currents were limited to lower maximum amplitudes as the internal concentration of sodium or of lithium was increased. In comparison to this striking effect, external application of these ions had no effect on the described type of rectification. An increase of the internal potassium concentration was without effect on the rectification, while an external application of potassium counteracted the rectification caused by an increase in internal sodium concentration. An internal increase of [K$^+$] increased the delayed currents at all positive potential steps. A clear dichotomy was thus seen between the effects of the electrical potential and the concentration as driving forces. Calcium applied to the inside of the fibre caused a shift of the permeability parameters along the potential axis but had no effect on the rectification properties.

Table 1. Composition of the test solutions

(The amounts given in mM. The cations were all added as chloride salt. Externally applied solutions designated A and internally applied designated E. pH = 7.2.)

solution	Na$^+$	K$^+$	Li$^+$	Rb$^+$	Ca^{2+}	tris
external						
I A	112	2.5	—	—	2.0	2.5
II A	—	114.5	—	—	2.0	2.5
III A	112	114.5	—	—	2.0	2.5
internal						
I E	114.5	2.5	—	—	—	2.5
II E	—	117.5	—	—	—	2.5
III E	112	117.5	—	—	—	2.5
IV E	60	117.5	—	—	—	2.5
V E	60	60	—	—	—	2.5
VI E	60	60	—	—	—	60
VII E	—	117.5	60	—	—	2.5
VIII E	—	117.5	—	117.5	—	2.5
IX E	—	117.5	—	—	10	2.5
X E	—	117.5	—	—	25	2.5
XI E	—	117.5	—	—	50	2.5

Methods

The experiments were carried out on dissected isolated nerve fibres from the sciatic nerve of *Xenopus laevis*. The membrane potential of a single node of Ranvier (N$_0$) in the dissected fibre was changed in steps of required amplitudes by the aid of a feed-back system as earlier described (Frankenhaeuser 1957; Dodge & Frankenhaeuser 1958; Århem, Frankenhaeuser & Moore 1973). The nerve fibre was cut off at the internodes on both sides of node N$_0$. The internode connecting the inside of the node to the output of the feed-back amplifier system was cut so that 0.5–1 mm of it was intact with the node. This procedure was undertaken in order to make it possible to change the inside ionic concentrations in a reasonably short time. The other internode was cut to a length of about 1 mm.

The magnitude of the membrane current was calculated from the potential change between the amplifier output (which is the potential at the cut end) and ground (which is the potential

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to which the inside of the node is held at a constant potential by the feed-back system). The resistance of the axis cylinder between the node and the cut end of the fibre thus enters the calculation of the current scale.

Potential clamp runs were taken with a number of different solutions outside the node under investigation and also with a number of different solutions in the solution pool with the cut end of the internode forming the return circuit for the membrane current, thus changing the internal ionic concentrations. A large number of solutions with different compositions were used. This is the case for the externally applied solutions (named A) as well as for the 'internal' solutions (named E). Table 1 gives a summary of the essential compositions of these solutions.

Nomenclature

Membrane potential is defined as the inside potential minus the outside potential. Outward membrane current is consequently defined as positive. Reference to the solution pools in the recording cell and to the potentials of these pools is made through the letters A–E in concordance with the system originally adopted (Frankenhaeuser 1957; Dodge & Frankenhaeuser 1958). A is thus (see figure 1*d*) the pool where the node under observation is located. B is the grounded pool. The pool with the end of the internode through which membrane potential is measured is named C. The other internode, the membrane current flows through this, has its end in pool E, while the axis cylinder at the observed node is named D. Since the potential difference U_{DB} is held constant by the feed-back, and the current I_{DC} is held ≈ 0 it is evident that changes in membrane current can be calculated from changes in the potential difference U_{ED} or U_{EB} and the impedance Z_{ED} (see Dodge & Frankenhaeuser 1959).

RESULTS

The dissected fibre was mounted in the measuring cell with three petroleum jelly seals (AB, BC and EA, figure 1*d*) at the corresponding perspex partitions isolating four pools (A–E) with Ringer's solution from each other (see Dodge & Frankenhaeuser 1958). The solution in the two end pools (E and C) was replaced by solution IIE which contains 117.5 mM-KCl. The fibre was then cut off in the two end pools. A length of about 1 mm internode was as a rule left intact with the node on the potential measuring side in pool C while the cut in pool E was made about $\frac{1}{2}$ mm from the node. The calomel electrodes were connected through KCl bridges to the pools. The amplifiers were balanced and turned on.

Potential clamp runs were then taken in which the membrane potential was changed in test steps. The membrane current associated with the steps was recorded as traces superimposed to families of curves on photographic film (figure 1*a*). Membrane potential was as a rule held at -90 mV between the test steps. Runs were taken with the ordinary Ringer's solution (IA) in pool A (i.e. as solution external to the node) and with solution IIA which is isotonic KCl solution containing 2 mM-$CaCl_2$ (figure 1*a* and *b*). Clamp runs with these solutions were used as reference runs. The effect of the solution switch was 90% complete in less than 10 s.

The solution in pool E was then changed to one of the test solutions. The effects of these changes in solution composition were to some extent complex:

(1) Resistivity of the test solution affected the relation between membrane current and potential U_{EB} which was used for measurement of membrane current. Application of a

solution with low resistivity was thus associated with a decrease in recorded current signal. The effect started at the time of solution change and progressed for a considerable time.

(2) After a delay more specific effects appeared. Application of solutions containing Na^+ thus caused a striking decrease of the delayed current at large potential steps (figure 1*c*).

Both these effects were slowly progressing and clearly depended on diffusion along the internode. They reached a steady state value more rapidly when the section of intact internode ED was short. To facilitate the treatment of the experimental results a calculation was made of how the concentration changes along the axis cylinder after application of a solution. This calculation was made on the basis of free diffusion with equation (2.45) given by Crank (1956) for diffusion in a semi-infinite medium. Figure 2 presents a family of such calculations. Active transport and absorption in the axis cylinder were neglected. The passive ionic fluxes across the node were also neglected, they were assumed to be small compared to the flux along the axis cylinder from the cut end. The possible effect of a sealing off of the cut end by flow of myelin was neglected because a scaling factor inversely proportional to the length of intact internode ED gave a reasonable agreement for the peak sodium currents measured with several lengths of intact internode. A further approximation was made in assuming a fibre essentially

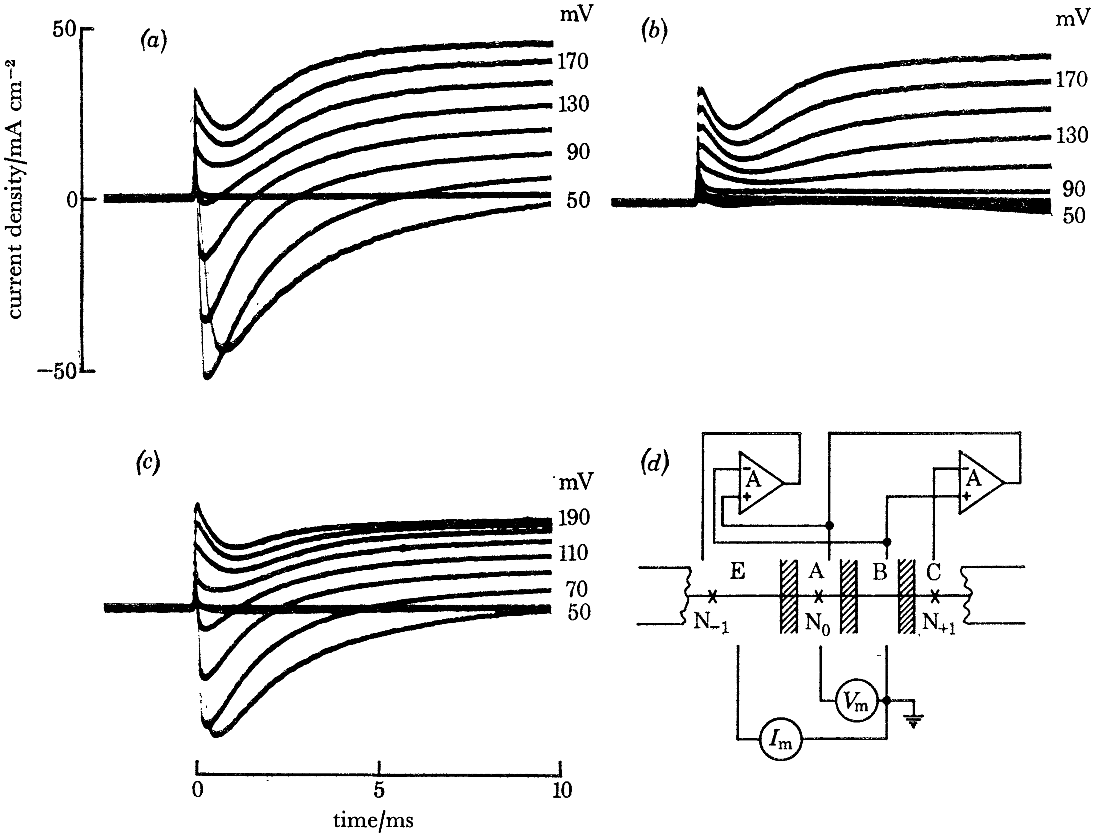

FIGURE 1. Membrane currents associated with rectangular pulse steps of indicated amplitudes. (*a*) External solution IA and internal IIE. (*b*) External solution IIA and internal IIE. (*c*) External solution IA and internal IIIE. Records taken about 15 min after change of solution in pool E. Internode length about 1 mm. Temperature 12 °C. Current scale applies for run (*a*) and (*b*). Current scale for (*c*) not corrected for the difference in resistivity of solution IIIE compared to IIE. (*d*) Schematic diagram of the feed-back amplifier system. Solution pools named A, B, C, E. Node under investigation N_0.

intact in pool C. These simplifications will clearly introduce some errors, but the curves seemed useful as approximations.

A solution with 112 mM NaCl and 117.5 mM KCl (solution III E) was applied to the cut end of the fibre. The records in figure 1*c* were taken about 15 min after this solution change. A comparison between the currents in figure 1*c* with the currents at corresponding potential steps in figure 1*a* together with a plot of the peak initial currents (figure 3, plots *c* and *a* respectively) indicates that (*a*) the initial currents as well as the delayed currents were smaller after application of solution III E, (*b*) the sodium equilibrium potential (U_{Na}) decreased, (*c*) the delayed currents at very large potential steps were much closer together with solution

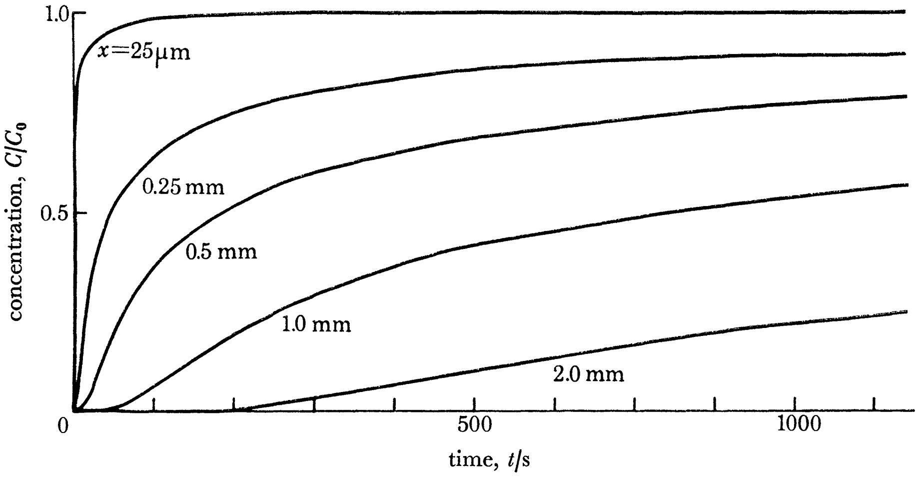

FIGURE 2. Dependence of concentration (C/C_0) on distance (x) and time (t) as calculated by the equation 2.45 in Crank (1956): $C/C_0 = \operatorname{erfc} x/2\sqrt{(Dt)}$. D, diffusion constant, taken for the calculation as 1.5×10^{-5} cm²/s.

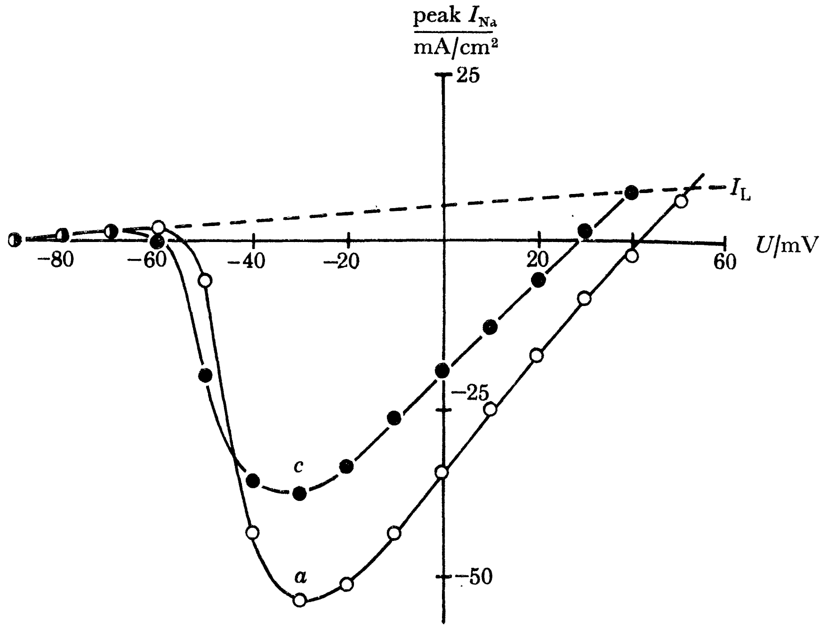

FIGURE 3. Effect of internal [Na⁺] on peak I_{Na}. Curve *a* and *c* plotted from runs (*a*) and (*c*) respectively in figure 1. I_{L}, leak current. Current scale applies to curve *a*; not corrected for curve *c*.

IIIE, (*d*) the increase of internal sodium concentration was associated with a small negative shift of the sodium permeability curve along the potential axis.

The measured shift for the sodium equilibrium potential was in this experiment as well as in other experiments in reasonable agreement with the expected shift calculated from (1) the Nernst equation and (2) the diffusion equation (figure 2). This finding indicates that sodium moves freely in the axis cylinder from the cut end. The amplitude of the initial current was consistent with the amplitude expected when the change in the internal sodium concentration was accounted for by the constant field equation and after the appropriate correction of the current scale due to the change in axis cylinder resistance. This correction was made on the basis of the resistivities of the solutions and by the diffusion equation, figure 2. The delayed steady state currents at small potential steps were not much affected as seen after the correction of the current scale (figure 4). At large potential steps the currents were, however, limited to a maximum amplitude beyond which an increase in potential step was without an effect on the current amplitude. Other experiments showed that the delayed current was limited to a lower value as the internal sodium concentration increased, or, in other words, that the rectification appeared at lower or more negative potential values as $[Na^+]_i$ increased.

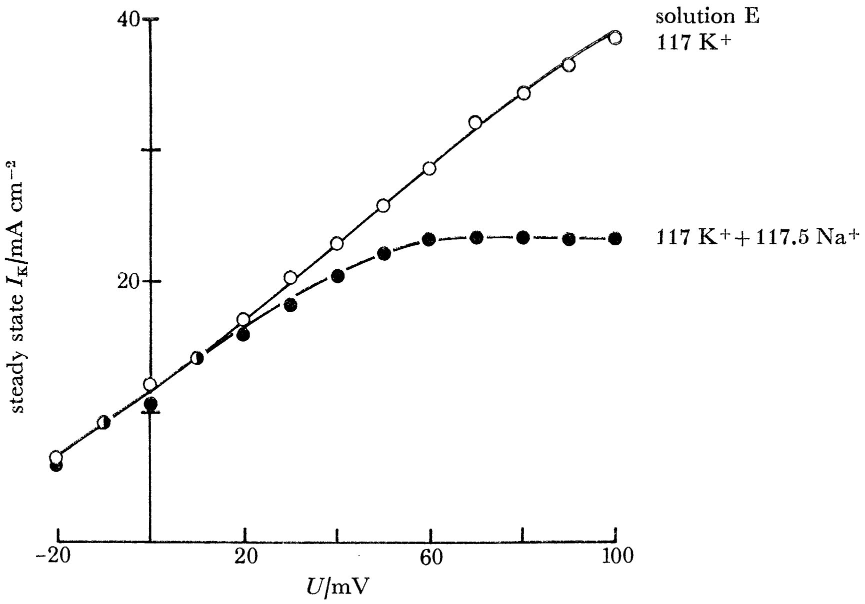

FIGURE 4. Effect of $[Na^+]_i$ on steady state I_K. External solution IA. Temperature 12 °C. Current scale corrected for changed resistance R_{ED}.

Application of lithium to the inside of the fibre caused a similar rectification as sodium. Neither potassium, rubidium nor tris HCl (tris-(hydroxymethyl)-aminomethane HCl) had any effect similar to the rectification caused by sodium and lithium. Tests were also made with hyperosmotic solutions. Tonicity *per se* had very little effect on the rectification.

An increase in inside potassium concentration was associated with an increase in potassium current as the increase reached the node. This was clearly seen in experiments where the resistivity of the solutions E was held relatively constant through corrections with tris HCl. This current increase was predicted by the constant field equation assuming permeability to be unaffected by the concentration. Experiments were now made in which the inside sodium concentration was increased (solution VI E). A further solution change was then made (solution IV E). These two solutions had the same [Na$^+$] while the [K$^+$] was larger in the latter solution. The delayed currents at small potential steps increased. The rectification appeared at the same potential range with the two solutions and the maximum limiting current was larger in the solution with high potassium (figure 5). This experiment indicates for us a completely unexpected dichotomy between the effects as driving forces of an increase in potential and an increase in concentration.

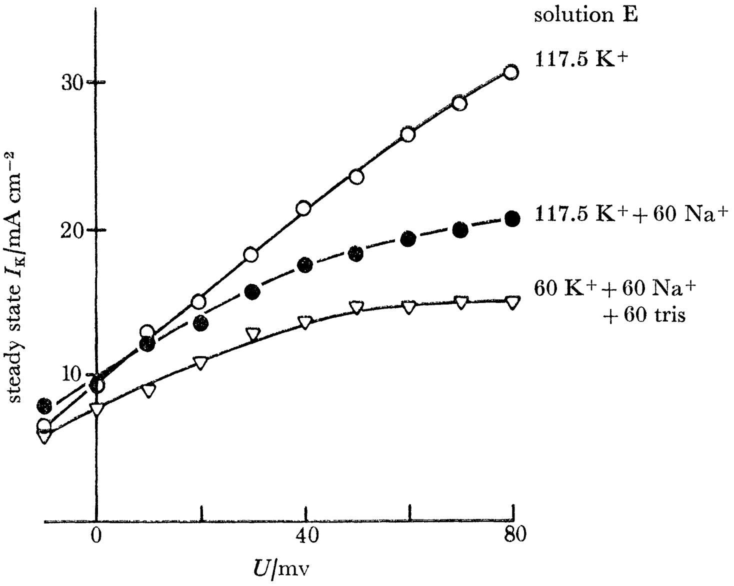

FIGURE 5. Effect of increased [K$^+$]$_i$ on steady state currents. External solution I A. Current scale applies to data with solution II E (unfilled circles). To correct currents for changes in resistivity multiply measured currents marked with filled circles with 1.4 and triangles with 1.2. Temperature 12 °C.

Experiments were further carried out in which changes were made in the composition of the external solution when the internal sodium concentration was so high that the rectification was clearly seen. These experiments indicated that changes in the external sodium concentration were without effects on the rectification. An increase in external potassium concentration was on the other hand associated with a striking shift towards more positive potential values of the potential range where rectification started. The effect of the increase in external [K$^+$] was rapid, i.e. 90 % complete in less than 10 s. This is a much too short time for appreciable changes in the internal ionic composition caused by fluxes through the node to the axis cylinder.

The rectification shifted in positive direction (figure 6) also when KCl was added to the ordinary Ringer's solution (solution III A compared with solution I A). The concentration of potassium in the external solution had thus a dominating effect on the rectification while sodium had it in the axoplasm.

The effect of external sodium concentration on the potassium permeability at lower potential steps, earlier noted by several investigators (Hodgkin & Huxley 1952; Frankenhaeuser 1962; Brismar 1973), was verified also in these experiments, i.e. the potassium permeability was somewhat smaller in low [Na$^+$]$_o$.

Measurements were also made of the effects of changes in the internal calcium concentration (solution IX E, X E and XI E). These experiments showed that the curve relating sodium permeability to membrane potential was shifted along the potential axis in negative direction for an increase in $[Ca^{2+}]_i$, i.e. this shift is in opposite direction to the shift caused by changes in the external $[Ca^{2+}]$. The effect was reversible when solution in pool E was replaced by iso-osmotic KCl solution (solution IE). The high calcium solutions did not cause rectification of the delayed current. These experiments were seriously complicated because the ionic membrane currents (I_{Na}, I_K and I_L) seemed to increase due to the high $[Ca^{2+}]$ (figure 7). Microscopic inspection of the fibres revealed striking constrictions at the sites of seal EA and AB. The

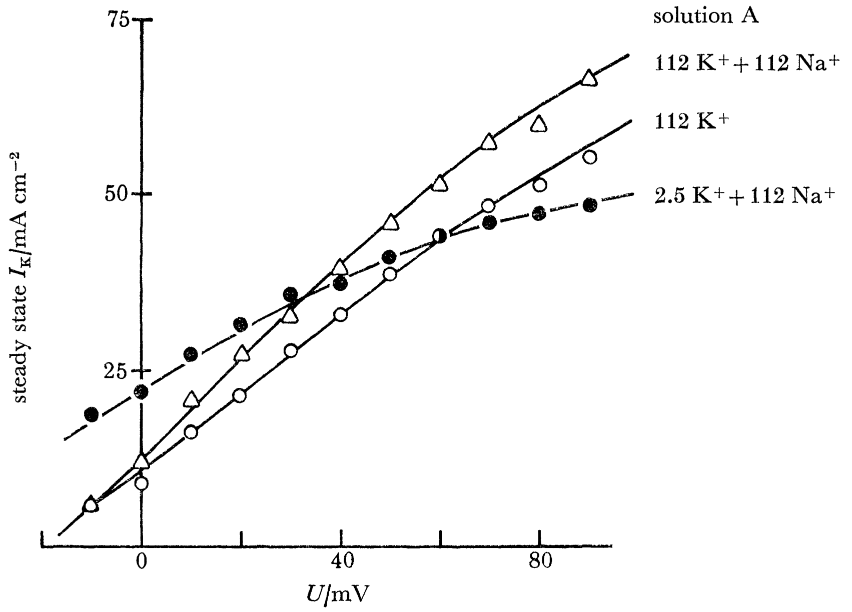

FIGURE 6. Effect of increased $[Na^+]_o$ and $[K^+]_o$. Measurements about 15 min after change to solution IIIE in pool E. Current scale calculated for solution IIE. Temperature 20 °C.

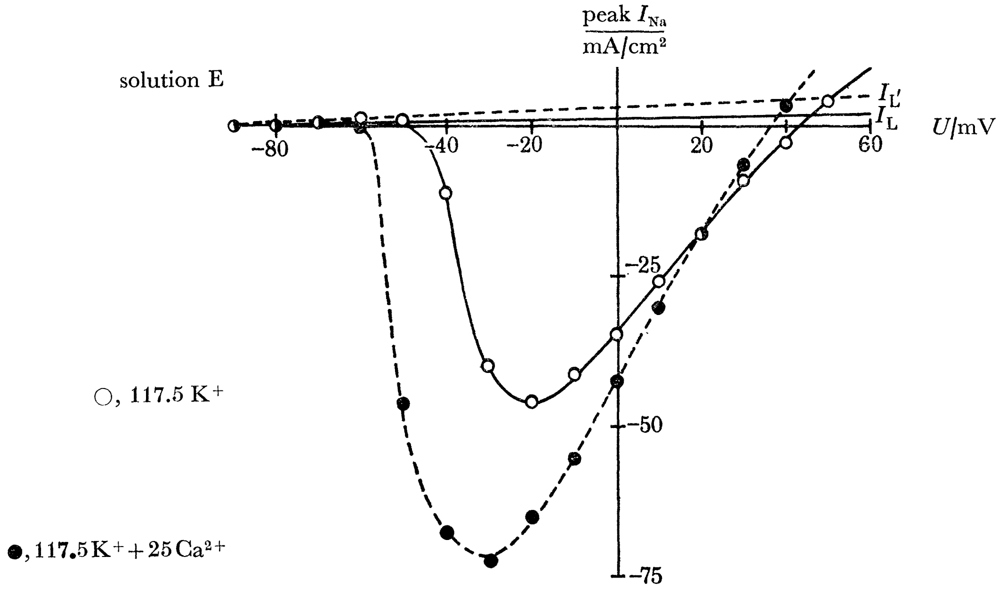

FIGURE 7. Effect of $[Ca^{2+}]_i$ on peak I_{Na}. External solution IA. Leak current, I_L, and circles for measurements with solution IIE; I_L' and filled circles for measurements with solution XE. Measurements taken 25 min after change of solution in pool E. Current scale calculated for solution IIE. Temperature 15.5 °C.

apparent increase in current amplitude was thus caused by these constrictions increasing the resistance R_{ED}. No quantitative measurement could be made of the amount of Ca^{2+} which reached the node.

Discussion

Earlier investigations (Frankenhaeuser 1962) have revealed that the outward potassium currents may be limited at large potential steps. This rectification is not predicted by the constant field equation. Bergman (1970) found that a long train of pulses, with an amplitude selected to cause a large sodium current, elicits this type of rectification. He also observed that the sodium equilibrium potential decreases after such a train.

In the present investigation the main interest was directed towards the rectification at large positive step polarizations. The ionic composition of the axis cylinder was changed through free diffusion through a cut end of the fibre. In addition to the changes of the internal concentrations clamp runs were taken with a number of external solutions.

An increase of the internal $[\mathrm{Na}^+]$ was associated with potassium currents limited to lower values at large potential steps when the internal $[\mathrm{Na}^+]$ increased. This is in agreement with corresponding findings on the squid giant fibre (Bezanilla & Armstrong 1972). The shift of the rectification was graded with the $[\mathrm{Na}^+]_{\mathrm{i}}$. This finding is fully consistent with Bergman's finding that the delayed currents at large potential steps decreased hand in hand with the described changes in sodium currents in this potential range. The time course of the change in sodium equilibrium potential showed a satisfactory agreement with the assumption that sodium moves in free diffusion in the axis cylinder. This is in full agreement with measurements on the squid giant axon (Hodgkin & Keynes 1956). A further indication for free ionic movement from pool E to the axis cylinder is the finding that calculation of R_{ED} as being proportional to the length of intact internode ED gave consistent amplitudes for the currents. The layer of myelin formed at the cut end of a fibre (Lubinska 1956; Johnson, Smith & Lock 1969) is thus low impedance and does not form a major diffusion barrier.

Bergman (1970) measured a quite large shift of the sodium equilibrium potential due to a massive train of potential steps. He suggests that sodium may accumulate in a limited space near the excitable membrane. No suggestion is, however, made of which structure possibly could be the diffusion barrier forming the limit to the space. Further experimental evidence seems to be required in order to obtain conclusive evidence for or against such a possible diffusion barrier on the inside of the node.

The present experiments indicated that the potential range where rectification appears was clearly and consistently affected by the external potassium concentration but not by the internal. An increase in $[\mathrm{K}^+]_{\mathrm{o}}$ thus shifted the bend of the I–U curve in positive direction. This effect was rapid. An effect through a change of the internal concentration is therefore excluded. A change of the internal concentration did affect the outward currents. It did also affect the currents at the potential region where the amplitude of the delayed current was independent of an increase in electrical driving force. A fibre with low $[\mathrm{Na}^+]_{\mathrm{i}}$ shows the rectification at a potential range close to that at which the membrane breaks through. With high $[\mathrm{Na}^+]_{\mathrm{i}}$ the potential range is within the range of the action potential.

The rectification cannot be described as an inactivation of the potassium mechanism because no decrease of permeability was seen when permeability was calculated through the instantaneous currents at repolarizations. No corresponding phenomena were seen in these inward potassium tails, they were neither limited when they had amplitudes larger than the limited delayed outward currents with high $[Na^+]_i$.

Lithium had an effect which was similar to that of sodium, while rubidium had an effect like potassium. Tris and calcium had no effect on the rectification. Calcium increased the apparent membrane currents irreversibly. This effect appeared evidently by liquefying the axis cylinder so that the seals caused constrictions of the fibre. That calcium liquefies the axis cylinder of the squid fibre is well known (Hodgkin & Katz 1949*b*).

Sodium, potassium, lithium and rubidium caused a minor shift of the P_{Na}–U curve along the potential axis. External increase in positive direction (Mozhayeva & Naumov 1970; Brismar 1973) while internal increase in negative. An internal increase in $[Ca^{2+}]$ was associated with a fairly large shift in negative direction.

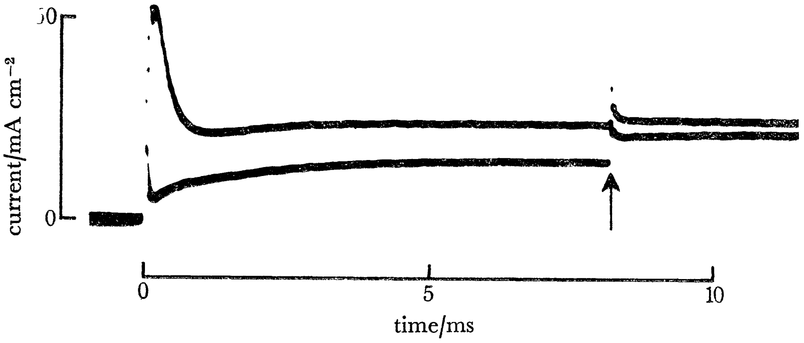

FIGURE 8. Two traces of membrane currents associated with double potential steps. For lower trace first step to +20 mV and second to +60 mV. For upper trace first step to +80 mV and second to +120 mV. Second step starts at arrow. Temperature 12 °C.

At present it seems difficult to specify the mechanism by which these ions affect the potassium permeability mechanism. That the present findings form limitations for a satisfactory permeability model seems evident. The rectifying properties here defined are affected by sodium from the inside of the membrane and by potassium from the outside in a reverse direction. The membrane impedance measured with a long as well as with a short potential step (figure 8) was found to be (in the high impedance region) even higher than predicted by a potential independent leak conductance. This finding is of interest in conjunction with the finding that the identification of the current carrier for the leak current is far from satisfactory (see Århem *et al.* 1973). Sodium and potassium are thus not only current carriers, they affect the permeability properties of the membrane in a rather specific way. At present it seems premature to speculate about the mechanism of these effects, the findings must, however, be accounted for in any satisfactory model for the permeability mechanism.

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