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INACTIVATION OF THE SODIUM-CARRYING MECHANISM IN MYELINATED NERVE FIBRES OF *XENOPUS LAEVIS*

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A previous paper (Frankenhaeuser, 1963) described the time and voltage dependence of a slow secondary increase in sodium permeability (P_p) in the myelinated nerve fibre. The late sodium current (I_p) had not yet been analysed when the inactivation of the sodium-carrying mechanism was described (Frankenhaeuser, 1959, 1960). The possibility that this late current might to some extent affect the measured time course of the initial sodium currents was therefore investigated. It was found that the error introduced was negligibly small. Another possible complication also recently came to light, when it was found that the rate constant β_n is affected by the previous history of the fibre and is therefore not entirely independent of time (Frankenhaeuser, 1963). Previous checks had indicated that the rate constants α_m, β_m and α_h were only slightly time-dependent, and any error due to this effect was therefore considered negligible. However, a similar check had not previously been made on β_h. When β_n was found to be time-dependent, it was decided to determine in some new experiments whether β_h also had this property. The previous separation of the sodium currents from the total current was made by measuring the tails of the sodium currents at repolarizations. Recently it has been found (see Frankenhaeuser & Moore, 1963) that hypertonic solutions do not seriously affect the ionic concentrations inside the fibre or the permeability changes, if they are applied for short times only (< 10 sec). Current separations could therefore be obtained experimentally by using such solutions. In this way, it was found that a conditioning cathodal step preceding a test step clearly increased the initial rate of inactivation during the test step, and hence that β_h was dependent on the previous history of the fibre.

METHODS

The membrane potential of single myelinated nerve fibres from *Xenopus laevis* was changed in rectangular pulse steps with the aid of a feed-back amplifier technique (Dodge & Frankenhaeuser, 1958, 1959).

The solutions used had the following compositions (in mg ions/l.):

	Solution I (Ringer's)	Solution II	Solution III
Na	114·5	2·5	114·5
K	2·5	114·5	114·5
Ca	2·0	2·0	2·0
Cl	118·5	118·5	233·0
HCO_3	2·5	2·5	2·5

RESULTS

The previous determinations of the time course of the inactivation of the sodium mechanism during a cathodal step were made from records of instantaneous currents at repolarizations from the cathodal step. This required a large number of repolarizations at different times after the onset of the cathodal steps. The separations of currents, when made from records of the membrane currents for cathodal steps in two different external sodium concentrations, gave an apparent reversal of the sodium current at long times and were therefore unreliable. This difficulty was resolved when it was found that $[Na]_o$ and choline chloride had some effect on the potassium permeability (see Frankenhaeuser, 1962).

Frankenhaeuser & Moore (1963) found that the membrane currents were not markedly affected when non-isotonic solutions were applied for short times (< 10 sec). This suggested the possibility of obtaining a more reliable separation of the sodium current from the net current by comparing two voltage clamp records taken close to the potassium-equilibrium potential, one in a solution containing high [K] and high [Na] (solution III) and the other in a solution containing high [K] and low [Na] (solution II). The advantage of this method is that V_K is about $+70$ mV, which is a potential at which m_∞ is close to unity and the driving force for sodium $(V - V_{Na})$ is fairly large in solution III and small in solution II. The potassium current, on the other hand, is small and rather independent of P_K in both solutions, since $V - V_K \simeq 0$. Figure 1 shows four pairs of such records, A, C, E and G taken in solution III, and B, D, F and H in solution II. The records were taken in the order A, B, C, D, E, F, G, and H, the photographic superposition being made during the experiment. I_{Na} was then calculated in the usual way (see Hodgkin & Huxley, 1952) and β_h determined. These determinations of β_h agreed with the earlier values (Fig. 3 in Frankenhaeuser, 1960).

The next step was to determine whether β_h was dependent on the previous history of the fibre. Separation of I_{Na} from the total membrane current was made as described above. Two potential steps were applied, an initial conditioning step to various potentials and a second, test step to 70 or 80 mV. β_h was then determined at the test step. The test step was applied at a time when I_{Na} was decaying but was still large enough for the

measurements. It was found that the initial τ_h was shorter when the conditioning step was larger (Figs. 2 and 3). τ_h was, therefore, clearly affected by the conditioning pulse. It was thus evident that the previous quantitative description of the inactivation was inadequate in not accounting for this behaviour. This description was made on the basis that inactivation is determined by two rate constants which depend on potential but are independent of time. Figures 2 and 3 clearly indicate, however, that β_h depends on the previous history of the fibre.

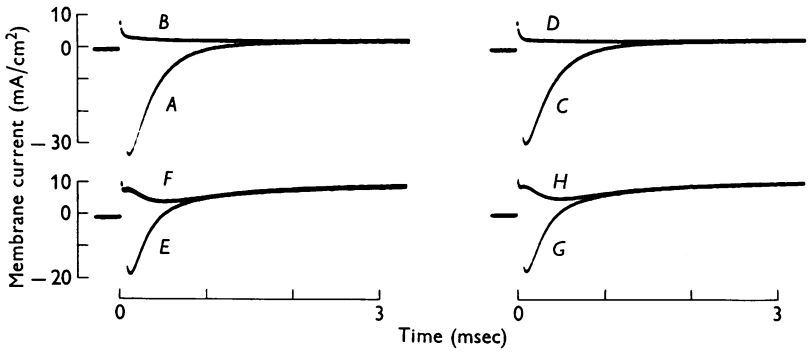

Fig. 1. Membrane currents associated with a potential step of 75 mV in *A–D* and 85 mV in *E–H*. The fibre was in a solution with high [Na] and high [K] (solution III) in *A*, *C*, *E* and *G* and in a solution with low [Na] and high [K] (solution II) in *B*, *D*, *F* and *H*. Solution changes were made rapidly. Records were taken in alphabetical order. Temperature 20° C.

It would be of some interest to obtain a quantitative description of how β_h depends on time and on previous potential. Experiments were done with this end in view. The relations were, however, too complicated for the limited resolution of the technique, since the initial time course of the inactivation at the test step depended on both the potential and duration of the conditioning step. The initial value of β_h at 70 mV was increased by a factor of about 2 when a conditioning step of 100 mV and 0·3 msec duration preceded the test step, compared to the value obtained without a conditioning step. β_h then decreased smoothly towards its value without a conditioning step.

It is clear that for the computation of action potentials the described insufficiency of the empirical equations will introduce some errors. At the peak of an action potential the values for β_h previously obtained from single step measurements should be sufficiently accurate, but these values of β_h will clearly be too low for the major part of the falling phase of the action potential, due to the previous history of the fibre. The previous values should also be reasonable in the region 0–20 mV; this region is of primary importance in determining the excitability. The S-shaped curve

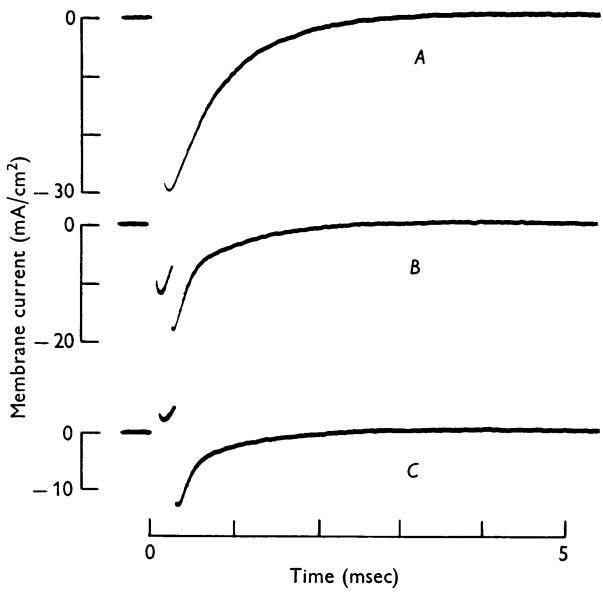

Fig. 2. Membrane currents associated with a test potential step of 70 mV with the fibre in solution III. In *B* and *C* the test step was preceded by a conditioning step of 90 and 120 mV respectively of 0·29 msec duration. Note that the sodium current decayed initially more quickly when the test step was preceded by a conditioning step. Temperature 20° C.

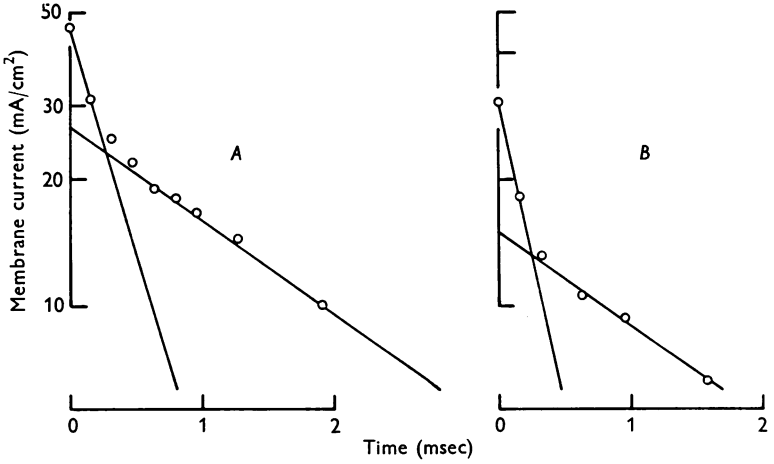

Fig. 3. Semilogarithmic plot of sodium current at a test step of 60 mV preceded by a conditioning step of 80 mV in *A* and 100 mV in *B*. Duration of conditioning step 0·46 msec. Separation of sodium current from net current was made from records taken in solution II and solution III. Temperature 20° C.

in Fig. 4 has been somewhat arbitrarily drawn to account for the effect described. The experimental points marked with circles and the interrupted line are the values previously obtained. The points marked with squares were obtained in the present measurements with a single step and the triangles indicate the initial values obtained after a larger preceding conditioning pulse.

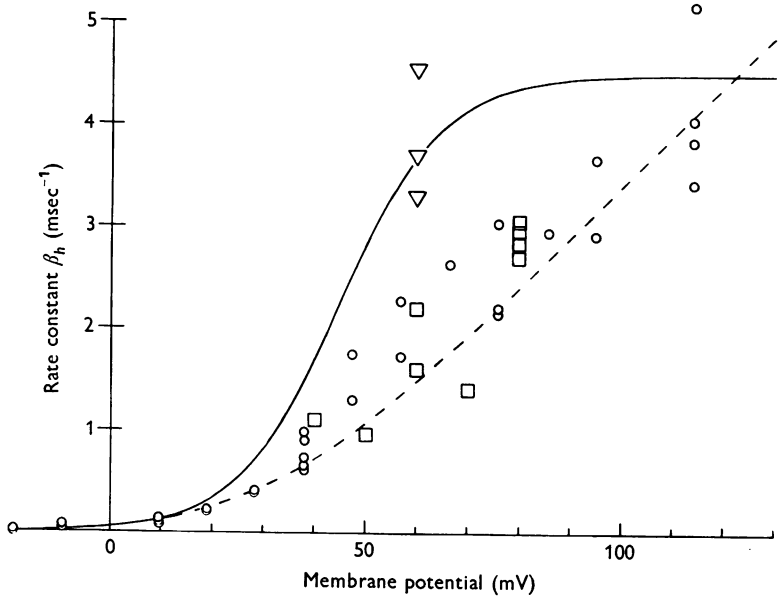

Fig. 4. Rate constant β_h plotted against membrane potential. Circles and interrupted line from the previous measurements (Frankenhaeuser, 1960). Squares indicate the present measurements without a conditioning step and triangles indicate the initial β_h after a conditioning step. The continuous line is drawn as $\beta_h = 4{\cdot}5/\{1+\exp[(45-V)/10]\}$ and indicates the direction of correction required due to the effect that the previous history of the fibre has on β_h in an action potential.

DISCUSSION

The Hodgkin–Huxley analysis of the squid nerve membrane currents indicates that these currents are mainly carried by sodium and potassium, and that these ions move passively, their electro-chemical gradients acting as driving forces. The available voltage clamp data on the myelinated nerve fibre (*Xenopus laevis*) is fully consistent with this view. The quantitative description of the squid fibre currents is based on the assumption that the permeability changes can be described with voltage-dependent, time-independent rate constants (αs and βs). The squid fibre data were successfully treated on this basis, and so were most of the myelinated fibre data.

However, in the previous paper (Frankenhaeuser, 1963) it was noted

that β_n is somewhat dependent on the past history of the fibre, and the present investigation indicates that β_h is also time dependent. These findings clearly show that the description of the permeability changes in terms of the time-independent rate constants is an erroneous description. However, the Hodgkin–Huxley treatment is a comparably simple one and adequately describes most of the experimental findings. The resolution obtained with the voltage clamp technique on the myelinated nerve fibre was sufficient to reveal deviations from this treatment, but it is doubtful whether complete data can be obtained at present which would permit a more accurate formal description of the permeability changes. It therefore seemed reasonable to keep the formal treatment unchanged and to point out the deviations from it. These deviations must be kept in mind, especially when a hypothesis for the permeability mechanism is considered.

For computations of action potentials it is evident that β_h will take higher values during the falling phase of the action potential than those which are obtained from voltage clamp experiments with a single pulse.

While it seems impossible, at present, to obtain a fully satisfactory formal description of β_h, a tentative equation (see legend for Fig. 4) is given, mainly to indicate the direction in which corrections required by the present findings must be applied. The earlier values at potentials of about 120 mV were left uncorrected while those at lower potentials were corrected. A strict quantitative correction was not possible.

Dodge (1961) obtained a reasonable agreement between the duration of a computed and a recorded action potential for the fibre from *Rana pipiens*. This indicates that β_h is rather time-independent in the fibres of *R. pipiens*, in contrast to the situation for *Xenopus laevis*.

SUMMARY

Voltage clamp experiments on myelinated nerve fibres from *Xenopus laevis* were made, and it was found that:

1. Separation of sodium currents from total current could be made by comparing two current traces taken with a potential step to V_K, one in a solution with high [K] and low [Na], the second in a solution with high [K] and high [Na] (hypertonic solution).

2. The inactivation rate constants at a single step were measured and were found to agree with measurements made previously.

3. β_h was affected by a conditioning step and was therefore not entirely independent of time.

4. A tentative equation is given to indicate how the values of β_h may be approximately corrected for computations.

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