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MEMBRANE CURRENTS AT LARGE POSITIVE INTERNAL POTENTIALS IN SINGLE MYELINATED NERVE FIBRES OF *RANA PIPIENS*

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SUMMARY

1. A voltage clamp for single myelinated nerve fibres was developed from commercially available operational amplifiers.

2. Under voltage clamp conditions steady-state currents were measured. The currents reached a maximum value at about $E = +100$ mV. Currents for greater potentials decreased.

3. The preceding polarization markedly affected the magnitude of the delayed current at high cathodal potentials; however, the currents continued to reach maximum values.

4. As predicted by the constant field theory, currents at high cathodal polarizations were essentially independent of the external potassium concentration. Also, these currents were independent of the external calcium concentration.

5. Two pulse voltage clamp experiments showed that instantaneous outward currents did not reach a limiting value. The limiting steady-state current was reached with an exponential time constant of about 1 msec.

INTRODUCTION

The steady-state currents in myelinated nerve are predominantly carried by potassium ions (Frankenhaeuser, 1962*a*) and are well described by the independence principle (Hodgkin & Huxley, 1952) at most potentials. At internal potentials greater than +110 mV the delayed currents show a rectification which in some cases leads to current saturation (Frankenhaeuser, 1962*c*). Some recent theoretical work has indicated that for membranes with mobile ion exchange sites the current should saturate at high potentials (Conti & Eisenman, 1966; Eisenman, Sandblom & Walker, 1967). To determine if such a model would apply to myelinated nerve, experiments were done measuring both the instantaneous and steady-state currents at high potentials.

From these experiments it was concluded that the steady-state membrane current does not saturate but passes through a maximum value which is essentially independent of the external potassium and calcium concentrations. The instantaneous currents, however, were not limited at these cathodal potentials.

METHODS

Single fibres from the frog (*Rana pipiens*) were dissected and mounted in a lucite chamber. Figure 1 illustrates the recording arrangement. Isotonic potassium chloride (solution II, below) replaced the normal Ringer solution in pools *E* and *C* and the single fibre was cut at the first node in pool *E* to provide a low impedance current pathway in the voltage clamp experiments. All experiments were done at 22° C.

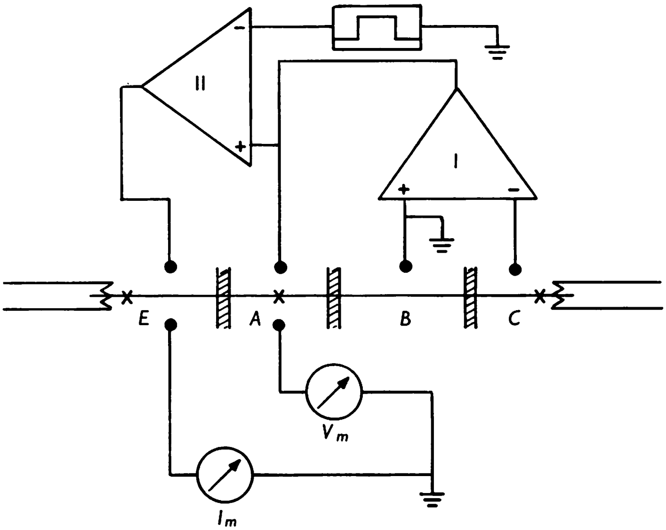

Fig. 1. Diagram of recording system. Xs represent dissected nodes. Pools *E* and *C* contain isotonic KCl (solution II). Pool *B* always contained Ringer solution and pool *A* contained either Ringer or the test solution. The output of amplifier I recorded changes in the membrane potential and amplifier II stabilized the potential for a voltage clamp. Shaded areas are vasoline seals which were 150 μ in thickness. Pool *A* was 200 μ and pool *B* was 250 μ. See text for further details.

The voltage clamp technique used was similar to that described by Dodge & Frankenhaeuser (1958) except that commercially available operational amplifiers were used for feed-back control. In the potentiometric recording system the calomel cells for pools *B* and *C* were connected directly to a Philbrick PP-25A operational amplifier (I). The positive terminal at pool *B* was at ground potential directly instead of being grounded through another calomel electrode. The clamping amplifier (II) used was a Philbrick PP-45-LU.

All inputs and outputs of both amplifiers were directly connected to the calomel electrodes through appropriate potentiometers acting as volume controls. Tektronix 161 stimulators were used for rectangular pulses and the voltage clamp currents were measured on a Tektronix 502A dual beam oscilloscope. Potential control was achieved within 50 μsec.

In all of the figures the current is expressed in terms of V_E, the output of the clamping amplifier which is the potential recorded in pool E. This can be converted into membrane current (I_m) by the relation,

$$I_m = V_E/A_N Z_{ED}, \tag{1}$$

where A_N is the area of the node and Z_{ED} is the impedance from pool E to the centre of the axon at the node in pool A. A reasonable estimate for $A_N Z_{ED}$ with the node cut in pool E is 10–20 Ω cm^2 (Frankenhaeuser, 1962*a*).

Solutions. The solutions used had the following compositions (mM):

	NaCl	KCl	$CaCl_2$	Na_2HPO_4	NaH_2PO_4
I	120	2·5	1·0	1·0	0·4
II	—	120·0	1·0	1·0	0·4
III	45	75·0	1·0	1·0	0·4
IV	95	25·0	1·0	1·0	0·4
V	—	—	41·0	—	—
VI		25·0	32·7	—	—
VII		75·0	15·0	—	—
VIII	120	—	1·0	1·0	0·4

The pH in solutions V, VI and VII was adjusted to 7 by adding small amounts of the sodium phosphate buffer in a total concentration of less than 1 mM.

Nomenclature. Potentials are given as inside potential minus outside potential. Potentials relative to resting membrane potential, E_r, are denoted V, and in absolute units, E, thus $V = E - E_r$. Outward currents are positive. E_r is assumed to be -70 mV.

RESULTS

Steady-state currents in single pulse experiments. In these experiments depolarizing pulses from 100 mV to approximately 200 mV were applied to the node and the corresponding currents measured. A typical experiment is illustrated in Fig. 2 which shows that the steady-state currents recorded reach a maximum value at about $E = +100$ mV. This rectification was always present in good freshly dissected fibres but was not seen in fibres having a high leakage conductance (Frankenhaeuser, 1962*c*).

In Figs. 2 and 3 it is seen that the current apparently reaches a limiting constant value, however, as illustrated in Fig. 4, it was frequently found that rather than a limiting current value a maximum was reached with the current decreasing as the potential increased. This point could not be pursued to potentials above approximately $E = 130$ mV owing to dielectric break-down of the membrane at these potentials. Only fibres with a low leakage conductance showed this decrease in current.

It has been well demonstrated that the potassium current can be inactivated (Ehrenstein & Gilbert, 1966; Frankenhaeuser, 1963) and is thus subject to the preceding polarization of the membrane. Voltage clamp

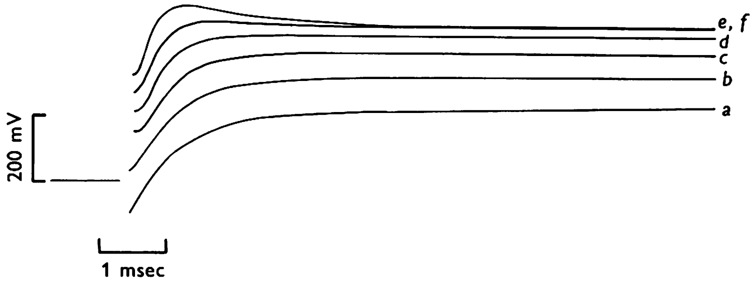

Fig. 2. Steady-state membrane currents. These currents were measured at increasing cathodal step polarizations: *a*, *b*, *c*, *d*, *e* and *f* corresponding to $E = 20$, 40, 60, 80, 100 and 120 mV. Abscissa, time; ordinate, V_E, the output of amplifier II (see Fig. 1) which is proportional to the membrane current. All curves are tracings from the original record. Note that the capacity transient and part of the initial sodium current have been omitted. The voltage step occurs at the break in the horizontal current recording line. Fibre no. 8.

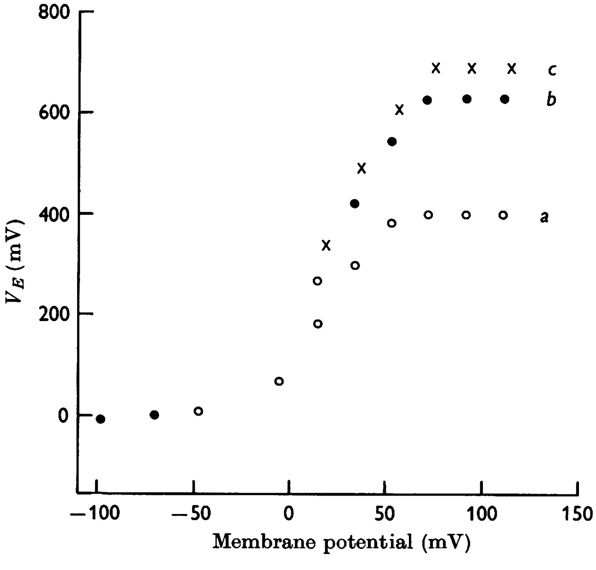

Fig. 3. Steady-state currents for different holding potentials. Steady-state currents similar to those shown in Fig. 2 are plotted versus the membrane potential for different holding potentials as follows: −50 mV, −70 mV and −90 mV corresponding to curves *a*, *b* and *c* respectively. Fibre no. 6.

measurements were made on nodes held at potentials of −70 mV, −90 mV and −50 mV to compare the maximum steady-state currents. As shown in Fig. 3 and Table 1, the steady-state currents are markedly affected by the preceding polarization or holding potential, but the maximum values are reached at about the same potential. Thus, whatever the mechanism of

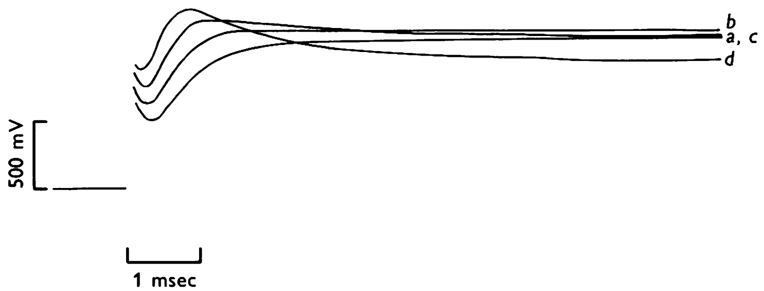

Fig. 4. Steady-state currents measured at increasing cathodal step polarizations. The steps: E = 70, 90, 110 and 130 mV correspond to curves a, b, c and d respectively. Abscissa, time; ordinate, V_E. All curves are tracings from the original record omitting the capacity transient and part of the initial current. Fibre no. 9.

TABLE 1. Maximum steady-state membrane currents (I_m) in high potassium solutions

Fibre reference	Solution reference	$[K]_o$ (mM)	$[Ca]_o$ (mM)	Ratio: $\frac{I_m \text{ in test solution}}{I_m \text{ in Ringer solution}}$ Holding potential (mV) −50	−70	−90	Ratio: $\frac{G_L\dagger \text{ in Ringer solution}}{G_L \text{ in tests solution}}$
5	IV	25	1	—	1·40	1·35	0·83
	III	75	1	0·97*	1·52*	1·53*	0·50
	II	120	1	—	1·02	1·06	0·90
	V	—	41	—	0·89	0·66	2·24
6	III	75	1	1·35	1·28	1·20	0·75
	IV	25	1	—	1·04	—	0·71
	VIII	—	1	—	1·06	—	0·92
	VII	75	15	—	1·17	1·09*	1·00
	VI	25	33	—	0·91	—	1·38
	V	—	41	—	0·73*	—	—
7	III	75	1	1·00	1·10	1·10	—
	III	75	1	—	1·08	—	—
	VI	25	33	—	1·04	—	—
	IV	25	1	—	1·09	—	—
8	IV	25	1	—	0·97	—	—
	VI	25	33	—	1·03	—	—
9	—‡	3	1	—	1·00	1·00	—
	II	120	1	—	1·11	1·06	—
	IV	25	1	0·82	1·00	1·00	—

* No maximum or limiting value was reached.

† The leakage conductance, G_L, was measured under voltage clamp conditions with a 20 mV hyperpolarizing pulse.

‡ NaCl was replaced by tetramethylammonium chloride. All other concentrations were the same as solution I.

this rectification is, it seems to be independent of the magnitude of the steady-state current and appears at similar potentials regardless of the preceding polarization.

Using the constant field theory (Goldman, 1943; Hodgkin & Katz, 1949) these results could be explained by assuming a dependence of the potassium permeability, P_K, on the membrane potential (Frankenhaeuser, 1962*c*; Meissner, 1965). This would account for the negative slope in the current–voltage curve seen in some fibres and also observed in the squid axon (Ehrenstein & Gilbert, 1966).

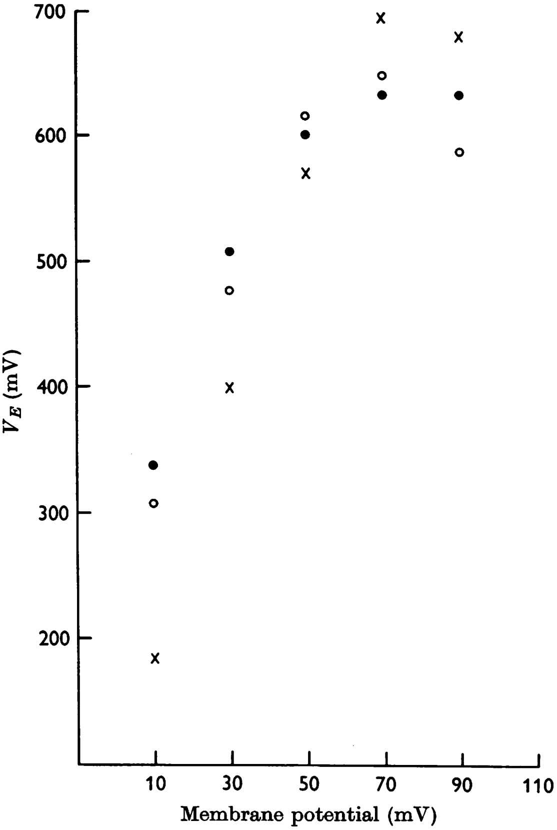

Fig. 5. Plot of steady-state currents in Ringer and high potassium solutions. (●), Ringer solution; (×), high potassium solution (solution III); (○) control in Ringer solution. Abscissa, membrane potential; ordinate, V_E. Fibre no. 7.

If the rectification were due to a decrease in P_K as defined by the constant field theory then the maximum current at high cathodal potentials should be essentially independent of the external potassium concentration, $[K]_0$. To test this hypothesis voltage clamp runs were taken in solutions of varying potassium concentrations. Figure 5 is a plot of the steady-state currents in Ringer solution, 75 mM-KCl (solution III) and a control run in Ringer solution. The holding potential for all three runs was $E = -70$ mV. At depolarizing potentials below $E = 50$ mV the current is clearly less in high potassium compared to Ringer solution; however, at more positive potentials it is of similar magnitude or greater. It should be noted that the steady-state current passed through a maximum in high potassium. Similar results were also obtained for maximum currents in solutions of constant ionic strength containing high calcium concentrations where water movements are presumed to be insignificant (Frankenhaeuser & Moore, 1963).

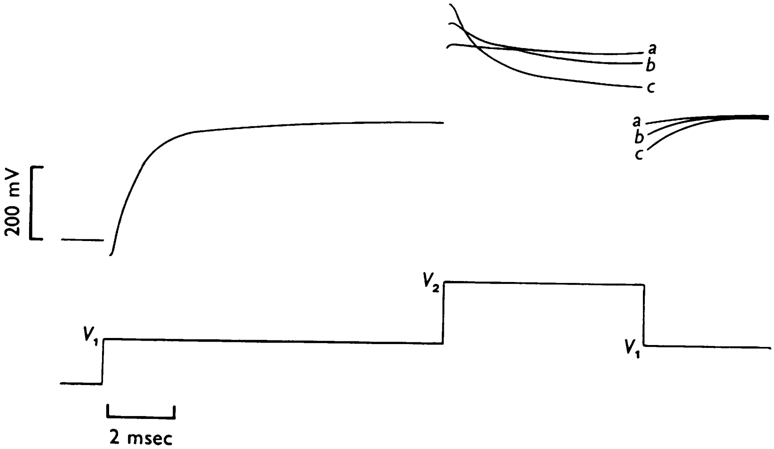

Fig. 6. Two pulse voltage clamp record. Membrane currents from three runs are superimposed for depolarizing pulses, V_1 (E = 30 mV) and V_2 (E = 130, 150 and 170 mV corresponding to curves *a*, *b* and *c* respectively). All curves are tracings from the original record omitting the capacity transient and part of the initial current. The voltage trace is not drawn to scale and only indicates the times at which the potential steps occurred. The holding potential was −70 mV. Fibre no. 11.

Table 1 summarizes these experiments comparing maximum currents obtained for different holding potentials. The larger maximum current often observed in high potassium may be due to an increased leakage current superimposed on the specific potassium current. This would tend to mask the decrease in current at high potentials. It is thus concluded that this rectification of the delayed steady-state currents is independent of the external potassium and calcium concentrations with regard to both the potential at which the maximum current is reached and its value. As in Ringer solution the maximum current in high potassium or calcium is dependent on the preceding polarization.

Two pulse experiments. The finding of a maximum current as discussed above raises the question of a possible limitation or saturation of the current carrying mechanism. To determine whether or not the current carrying capacity was saturated at high potentials the membrane was depolarized to about E = 30 mV (V_1) to bring the potassium permeability to its maximum value (Frankenhaeuser, 1962*c*) and then depolarized to higher potentials (V_2) with short pulses. In Fig. 6 such a two pulse experiment with three superimposed runs illustrates that when the potential changed from V_1 to V_2 the instantaneous current was not limited. The outward current, however, markedly decreased with a time constant of about 1 msec to a steady-state value often below the previous run at a lower potential. Returning the potential to V_1 ($E = +30$ mV) the current is instantaneously below the original steady-state value but comes back to the higher current with a time constant of about 1 msec. If P_K were reduced by the higher potential this result would be expected, owing to a change in membrane conductance during the period at V_2.

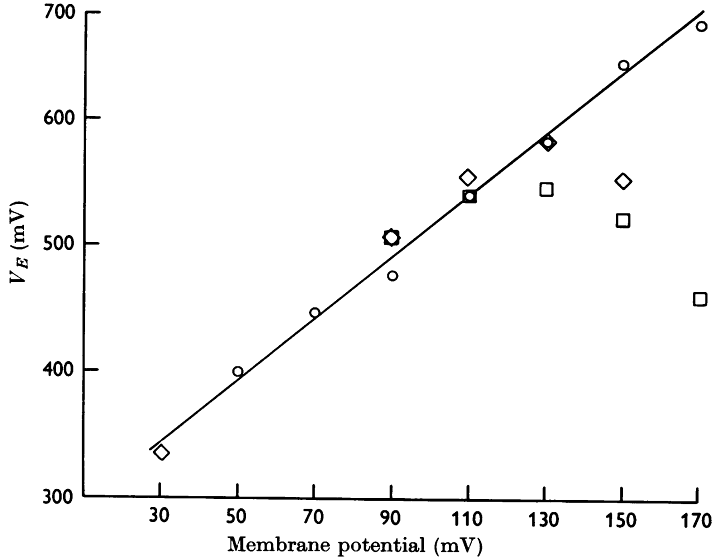

Fig. 7. Steady-state and instantaneous membrane currents. The symbol, ○, represents the instantaneous current at the beginning of the second pulse, V_2 (see Fig. 6); □, the steady-state current during V_2; ◇, the steady-state current for single pulse experiment. In the two pulse experiment $V_1 = 100$ mV ($E = 30$ mV). Fibre no. 11.

With short pulses superimposed on the normal voltage clamp runs it was possible to depolarize to higher potentials than with single pulses and always to observe a marked decrease in the membrane current. In the single pulse experiment it is apparently more difficult to see this current decrease, since the membrane is on the verge of breaking down. Such a dielectric break-down is demonstrated by an abrupt current increase and a higher leakage conductance. After a break-down the membrane generally did not show a current decrease at large cathodal polarizations.

The steady-state currents reached with the single and two pulse experiments are essentially the same. In Fig. 7 the steady-state currents for single pulse and two pulse experiments from the same fibre can be seen to reach a maximum and decrease in a similar manner at high cathodal potentials. On the other hand the instantaneous current versus membrane potential is linear. In view of this finding a likely explanation for the hump in the delayed currents seen in Fig. 4 is that the potassium permeability initially increases during a cathodal polarization but then decreases once the current density becomes relatively high, much the same as observed in the two pulse experiment.

It is therefore concluded that, rather than saturating, the net steady-state specific potassium current is reduced at high cathodal potentials. This result would seem to rule out the mobile ion exchange site as a carrier of the ionic current.

DISCUSSION

It has been shown that using the constant field theory to describe the steady-state currents in normal Ringer solution shows a decrease in P_K at large cathodal steps while P_K determined from instantaneous potassium tails in high $[K]_o$ is significantly higher and reaches a constant value (Frankenhaeuser, 1962*b*, *c*). However, as shown in Fig. 5, the steady-state currents in high $[K]_o$ decrease at high cathodal polarizations.

Depending on the leakage conductance and the clamped potential the results described in this paper show either a limiting delayed current or a maximum current in *Rana pipiens*. Similar results have also been observed in *Xenopus laevis* (B. Frankenhaeuser, personal communication; L. E. Moore, unpublished). It therefore appears that as the outward specific potassium current increases there is a corresponding mechanism operating to decrease the potassium permeability or conductance.

As previously observed in *X. laevis* (Frankenhaeuser, 1962*c*) it was found in *Rana pipiens* that the instantaneous current in high $[K]_o$ on repolarizing from high cathodal potentials to the resting potential did not show the corresponding decrease in current as expected from a lower potassium permeability. However, the instantaneous current after the second pulse in the experiments reported here did show a decreased conductance which changed with a time constant of about 1 msec. Apparently the decrease in potassium conductance is abolished within 50 μsec when the potential is returned to $E = -70$ mV.

If the decreased steady-state current at large cathodal potentials were due to membrane ion depletion then the limiting current density should be constant. However, it was found that for different holding potentials the limiting current varied markedly. Considering that the membrane conductance is determined by a minimum of at least two factors, the mobility and concentration of the ions involved, it is possible to reconcile the membrane ion depletion hypothesis with the data by postulating that the ion mobility in the steady state is determined by the preceding polarization.

I am indebted to Professor Bernhard Frankenhaeuser for stimulating discussions during the initial phases of this work at the Nobel Institute.

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