TIME COURSE
OF THE SODIUM INFLUX IN SQUID GIANT AXON DURING
A SINGLE VOLTAGE CLAMP PULSE

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SUMMARY

1. Sodium influx measurements were carried out on internally perfused squid giant axons under conditions of membrane potential control.

2. The ratio 'measured extra sodium influx/calculated ionic influx from the inward current record' is close to unity and is independent of the duration of the rectangular pulse of membrane potential.

3. The variation of the sodium permeability with time during the voltage clamp pulse is obtained by subtracting the sodium influx obtained from the 'tail' current record from the measured extra sodium influx.

4. Evidence was obtained indicating that the measured change in sodium permeability during a sudden decrease of the membrane potential from its resting level involves two processes, namely, a transient increase in sodium permeability (activation), and a decrease in sodium permeability (inactivation).

5. The extra influx of sodium during a voltage clamp pulse is not decreased by raising the internal sodium concentration from 0 to 100 mM, supporting the validity of the independence principle.

INTRODUCTION

In a previous paper, we have reported that the inward current associated with a sudden decrease of the membrane potential from its resting level could be completely accounted for by a net inward movement of sodium ions (Atwater, Bezanilla & Rojas, 1969). This result can be considered as a direct proof that an increase in sodium permeability takes place during this period of depolarization. However, our measurements

did not provide information about the time course of this increase in sodium permeability. The experiments described in the present paper were designed to resolve the time course of the sodium permeability and thus can be considered as a continuation of the above mentioned work.

Hodgkin & Huxley (1952b) studied the effects of restoring the membrane potential after a brief period of depolarization. The sudden return to the resting potential was associated with a 'tail' of inward ionic current which rapidly declined to zero. One important point which emerged from this result is that the process underlying the increase in sodium permeability is reversible. Hodgkin & Huxley (1952b) showed that the 'tail' current disappears after replacement of the external sodium ions by choline ions. That this 'tail' current is in fact carried by sodium ions is shown by the observation, presented and discussed here, that the ratio 'measured extra influx of sodium/net ionic influx' is close to unity, independent of the duration of the depolarization. Here the net ionic influx is represented by the area under the inward current record during the pulse plus the area under the 'tail' current record.

The method used to resolve the time course of the permeability change to sodium ions during the early inward current is based on the above observation. The inward current was separated from the outward current by blocking the latter with tetraethylammonium (TEA; Armstrong & Binstock, 1965; Armstrong, 1966). Then the inward current was integrated to give the ionic flux in two parts, (i) during the period of depolarization and (ii) during the 'tail'. As the transport number for sodium was found in this work to be very close to one, the corresponding flux during the 'tail' was subtracted from the measured extra sodium influx, thus giving the sodium flux during the pulse. This flux as a function of pulse duration gives the temporal course of the sodium permeability change during the period of depolarization.

METHODS

Giant axon fibres from the squid *Dosidicus gigas* were used in this work. The methods which were used have been described previously (Atwater *et al.* 1969).

As a general procedure, the fibres were bathed in potassium-free artificial sea water (K-free ASW: 430 mM-NaCl, 50 mM-MgCl$_2$, 10 mM-CaCl$_2$, 5 mM-Tris-Cl at pH 7·3) and were internally perfused with potassium fluoride solution (550 mM-KF, 5 mM-TEA, 5 mM-Tris-Cl at pH 7·3) for about 30 min. The perfusion rate was monitored during this time. If it showed a tendency to decrease or was smaller than 0·1 cm³/min, the axon was discarded. The external K-free ASW was replaced by [^{22}Na]-ASW (composition as above) only when the perfusion rate did not show any tendency to decrease. However, the perfusion technique used during this work always leaves a layer of axoplasm of variable thickness (from 50 μm for a 700 μm axon to 200 μm for a 1000 μm axon). In many instances the outlet cannula was clogged by small

fragments of axoplasm which had become detached from this layer after the experiment was initiated and the [^{22}Na]ASW had been placed in the axon chamber. Increasing the hydrostatic pressure utilized to drive the perfusion fluid to obtain an increase in perfusion rate always resulted in great variations of the resting influx of sodium. For this reason the hydrostatic pressure was kept constant by placing the perfusion solution reservoir about 50 cm above the axon chamber. When the perfusion rate decreased, the time needed to return to the basal influx after a voltage clamp run increased to more than 5 min. Thus, it was difficult to obtain more than two runs in one experiment. All these experiments were carried out with TEA in the internal solution. TEA blocks the delayed outward currents, thus facilitating a more accurate measurement of the early inward currents.

In a previous paper (Atwater *et al.* 1969) we described the results of experiments in which sodium influxes were measured during rest and during voltage clamping. In those experiments the duration of each rectangular pulse of membrane potential was constant (*ca.* 3 msec) and only the magnitude was changed. In this communication we describe the extra sodium influxes when the magnitude of the pulse is kept constant (*ca.* 50 mV, an absolute membrane potential of about − 10 mV) but the duration is varied between 9·2 and 0·15 msec.

Experimental errors. Two basic measurements are involved in our experiments: (i) the sodium influxes during rest and during voltage clamping and (ii) the area under the current record, which we used to calculate the corresponding ionic flux. In the first, several errors are introduced. The inflow of ^{22}Na can be accurately measured, as the counting error is less than 1 %. However, the determination of the specific activity involves a greater error. Due to slight evaporation during the long-lasting experiments, the concentration of the [^{22}Na]ASW changed from one experiment to another and it had to be determined regularly. The concentrations of sodium and magnesium were measured by flame photometry (Atomic Absorption Flame Photometer Unicam, Model SP–900) and distilled water was added to the [^{22}Na]ASW when it was considered necessary. As the [^{22}Na]ASW was very radioactive (10^8 c.p.m./cm^3), only very small samples were analysed (1 μl.) Several measurements of the concentration of sodium for equally small samples of non-radioactive ASW showed us that such a determination may contain up to a 5 % error. As the counting error introduced in the determination of the radioactivity is less than 1 %, we consider the total error in the determination of the specific activity to be less than 5 %.

Another error introduced into the flux measurement is the estimation of the area of the axon membrane which is incorporated into the exchange of sodium ions. During rest, we can only assume that the entire portion of axon bathing in solution is permeable to sodium ions. However, during voltage clamping, the area which is involved in the exchange of ionic currents can be estimated by measuring the longitudinal distribution of membrane currents with external differential electrodes. The current density was fairly constant along the fibre axis. Towards the extremes, in the last mm still in ASW, the current abruptly decreased. This means that 2 mm of the 25 mm of fibre bathing in ASW (1 mm at each end) were not participating efficiently in the ionic exchange. Assuming some error in the measurement of the diameter we can set an upper limit to our estimate of the error in the determination of the area at 10 %. Thus, the influx contains an error smaller than 15 %, as determined by applying the total derivative rule to the expression: (c.p.m./sec)/{(c.p.m./p-mole).(cm^2)}.

The errors in measuring the currents are essentially two: the separation of the capacitative transient from the ionic currents at the beginning and at the end of the pulse and the estimation of the leakage currents during the pulse (see Fig. 1; in the second current record, the two areas to be measured have been outlined with dotted lines). The electrode system used to measure the current, $I_m(t)$, consisted of a pair of

external platinum plates coated with platinum black. These were electrically connected together and then connected to earth through 10 Ω (Atwater *et al.* 1969). With this system of electrodes, compensation for the series resistance was not used and, in some experiments, the capacitative transient caused by the sudden change in membrane potential lasted for as long as 75 μsec. Such long-lasting transients make it very difficult to resolve the ionic current in the current record. This problem was resolved in part by estimating the time course and the duration of the capacitative transient from the current record taken during a 50 mV hyperpolarizing pulse.

A considerable uncertainty may have been introduced when estimating the leakage current for a 50 mV depolarizing pulse. Here again, a good time resolution of the ionic currents was required. In some experiments leakage currents were measured near the reversal potential (Rojas, Taylor, Atwater & Bezanilla, 1969). A linear leakage I–V curve was assumed. From this I–V curve the leakage for a 50 mV depolarizing pulse was estimated.

During a voltage clamp period each rectangular pulse of membrane potential was applied ten times per second for at least 2 min. We have seen in previous experiments that such frequency of pulsing does not affect the electrical properties of the membrane (Atwater *et al.* 1969). However, in some experiments the recorded currents changed in an irregular way with time. It was necessary, therefore, to take several photographs (at least one every 30 sec) during each clamp period and use the average value of the area under all these current records.

Summarizing, we consider that the error in the calculated flux (calculated from the current records) is of the order of 15 %. However, the ratio 'measured extra influx of sodium/ionic flux represented by the area under the current record' contains a considerably smaller error. This is true because the largest error (that in the measurement of the area of the axon membrane incorporated in the sodium exchange) is a factor in both numerator and denominator, and thus cancels out.

RESULTS

Sodium influxes during voltage clamp as a function of pulse duration

Figure 1 illustrates part of an experimental protocol. This is a plot of the sodium influx in p-mole/cm² sec as a function of time in sec. Each column represents the average influx determined during an interval of approximately 100 sec. After the resting influx reached a steady level (about 20 p-mole/cm² sec) the measured resting potential was clamped and rectangular pulses of membrane potential of variable duration and 50 mV (an absolute membrane potential of −5 mV during the pulse) were applied at a frequency of 10 sec⁻¹. It can be seen that the net effect of repetitively applying rectangular pulses of membrane potential is to increase the rate at which sodium enters the fibre. The starting time of each voltage clamp period is shown in the Figure as 'On' and the end of the pulse period as 'Off'. The flux record shows a delay of about 250 sec in returning to the previous resting influx. This is presumably due, in part, to the time lag in the dead space of the outlet cannula. The increase in sodium influx during the clamp period is expressed in terms of the area under the columns above the assumed resting influx level (the dashed line in Fig. 1 was drawn as a

continuation of the resting influx before the clamp period). These areas are given in p-mole/cm² by the numbers in parentheses in Fig. 1. The recorded currents during each of the consecutive runs are given in the upper part of this Figure, above the corresponding extra sodium influx.

Table 1 summarizes the results obtained with eight different fibres. Columns (*a*) and (*b*) give the resting potential and the resting influx before the voltage clamp run. Column (*c*) gives the duration of the pulse, t_1. Column (*d*), gives the computed net inward ionic movement during the pulse, corresponding to the early inward current; column (*e*) shows the
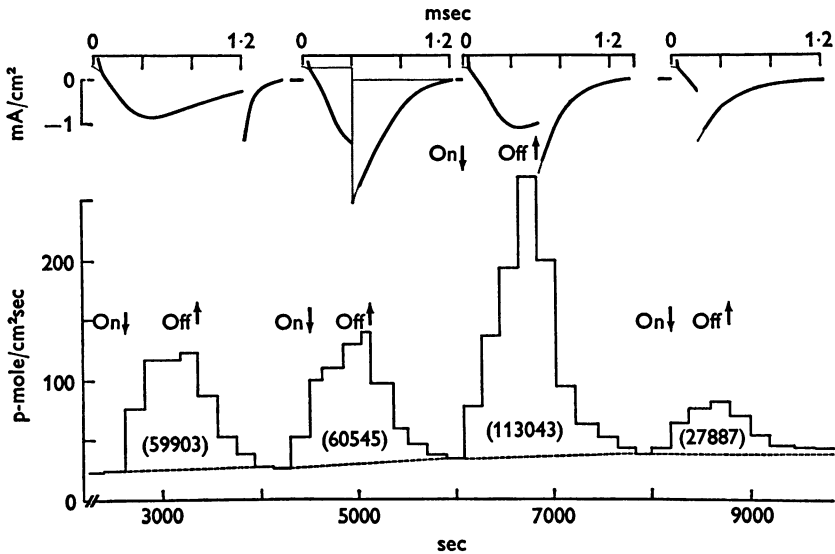

Fig. 1. Protocol of one experiment: recorded membrane currents and measured sodium influx during rest and during voltage clamping.

Each column represents the average influx (p-mole/cm² sec) determined during intervals of approximately 100 sec. Application of the pulse started only a few seconds after the initiation of the membrane potential control (On) and ended simultaneously with the turning off of the clamp (Off). The numbers in parenthese represents the area under the column and above the dashed line which was drawn as a continuation of the resting influx. Every 30 sec pictures of the ionic currents, measured under membrane potential control, were taken. The inserts in the upper part of this Figure represent one of the records of the membrane currents during each of the four runs shown (the first run, for a similar pulse duration as the last run, has been omitted).

The third run in this Figure shows a much larger sodium influx (113043 p-mole/cm²) because the frequency of application of the pulses was 20 sec^{-1} instead of 10 sec^{-1} as for the other pulses.

The fibre was bathing in K-free ASW with 4 p-mole/c.p.m$_{22_{Na}}$ and internally perfused with potassium fluoride plus TEA. Temperature (measured at the end of the last run) 11·5° C.

TABLE 1. A comparison between inward ionic current during a voltage clamp pulse and extra sodium influx

Expt.	(a) Resting potential mV	(b) Resting influx $\frac{\text{p-mole}}{\text{cm}^2\,\text{sec}}$	(c) t_1 (msec)	(d) $\frac{1}{F}\int_0^{t_1} i\,dt$	(e) $\frac{1}{F}\int_{t_1}^{t_i} i\,dt$	(f) (d)+(e) $\frac{\text{p-mole}}{\text{cm}^2\,\text{pulse}}$	(g) $\int_0^{t_i} m_{\text{Na}}\,dt$	(h) (g)/(f)	Temp. °C
TN-53	−53·0	12·3	4·6	13·00	0·16	13·16	17·70	1·34	12
TN-54									
A	−51·0	20·0	0·63	6·22	3·68	9·90	9·35	0·94	—
B	−53·0	14·0	0·30	2·95	13·10	16·05	14·02	0·87	—
C	−51·0	32·5	0·62	6·61	3·10	9·71	8·20	0·84	14
TN-55									
A	−55·0	36·0	0·20	0·45	1·13	1·58	1·50	0·95	—
B	−53·5	29·2	0·39	1·80	1·88	3·66	5·70	1·56	—
C	−56·5	47·5	0·69	3·80	2·01	5·81	5·30	0·91	—
D	−56·0	43·3	0·84	6·57	0·96	7·53	8·25	1·09	—
E	−56·5	39·0	1·23	9·20	0·68	9·88	8·30	0·84	—
F	−55·0	41·5	2·00	11·13	0·37	11·50	8·35	0·73	—
G	−53·0	44·6	0·20	0·17	0·77	0·94	1·25	1·33	13
TN-58	−55·5	46·7	0·22	0·32	0·97	1·29	1·90	1·47	13
TN-59	−58·0	18·6	0·20	0·28	0·81	1·09	1·30	1·19	11
B	−57·5	17·2	0·15	0·11	0·37	0·48	0·65	1·36	11
TN-60									
A	−55·0	24·5	0·17	0·50	2·00	2·50	1·87	0·75	—
B	−54·5	23·3	1·20	9·50	0·84	10·34	11·10	1·07	—
C	−58·3	26·8	0·40	3·36	7·72	11·05	10·05	0·91	—
D	−56·0	34·5	0·62	5·80	3·80	9·60	10·20	1·06	—
E	−52·0	38·2	0·22	0·39	2·50	2·89	2·45	0·85	11·5
TN-64	−58·5	22·0	9·2	19·00	0·30	19·30	19·50	1·01	10·0
TN-67	−57·2	17·0	6·0	27·00	0·30	27·30	28·00	1·02	9·8
Av. ± S.E.	−55·0 ± 2·3	29·9 ± 2·5	—	—	—	—	—	1·04 ± 0·07	—

computed current after the pulse, corresponding to the tail current; and (*f*) gives the addition of these two values. Column (*g*) gives the measured extra influx per pulse (i.e. the area under the flux curve divided by the total number of pulses during the period in which the fibre was under clamp and pulsed repetitively). The ratio of these two last values, (*g*)/(*f*), is given in column (*h*). It is convenient here to indicate that the extra sodium influx is a function of time,

$$m_{\mathrm{Na}}^{i} = m_{\mathrm{Na}}^{i}(t) \qquad (1)$$

with dimensions of p-mole/cm² sec. Presumably, during the application of one depolarizing rectangular pulse of membrane potential this extra influx increases rapidly with a time constant of the order of a fraction of a msec and decreases again with a time constant of the order of 1 msec. The numbers in the column (*g*) represent the average over many pulses of the integrated extra sodium influx. That is,

$$\overline{\int_0^{t_2} m_{\mathrm{Na}}^{i}(t)\,\mathrm{d}t} = \sum_{k=1}^{n} \left[\int_0^{t_2} [m_{\mathrm{Na}}^{i}(t)]_k\,\mathrm{d}t \right] \Big/ n, \qquad (2)$$

where t_2 represents the minimum time necessary for the permeability change caused by the pulse to be completed, and n is the total number of pulses during the clamping period. Because of the integration, the units used in column (*g*) are p-mole/cm² pulse. Thus, these numbers do not represent a rate of change of the sodium influx but the average of the total change as given by eqn. (2). The ratios, (*g*)/(*f*), in column (*h*) represent the transport number of sodium during the inward currents. This number is close to one and is independent of the duration of the pulses.

One instructive way to examine the values in columns (*g*) and (*f*) in Table 1 is to plot the extra sodium influx caused by a pulse (column (*g*)) as a function of the inward ionic movement (column (*f*)). This plot is shown in Fig. 2. The calculated regression line is also shown in the figure. One would expect that those points corresponding to the short duration pulses (about 0·2 msec) would be closer to the origin and that those points corresponding to the long duration pulses (about 5 msec) would be displaced along the line. However, the experimental points are not seen distributed in order of pulse duration. This lack of a sequence along the line as a function of pulse duration most likely reflects the variable condition of the fibres used. In two experiments (TN-64 and TN-67) the extra sodium influxes obtained with depolarizing pulses of 50 mV are 19·5 and 28·0 p-mole/cm² pulse (the duration of the pulse was 9·2 msec for TN-64 and 6·0 for TN-67). As the transient change of ionic conductance is practically completed after 3 msec (temperature of the ASW about 10° C) the

variation in extra influx of sodium must reflect the variation in condition of the fibres. However, these values are within the range found before (Table 1, Atwater *et al.* 1969).
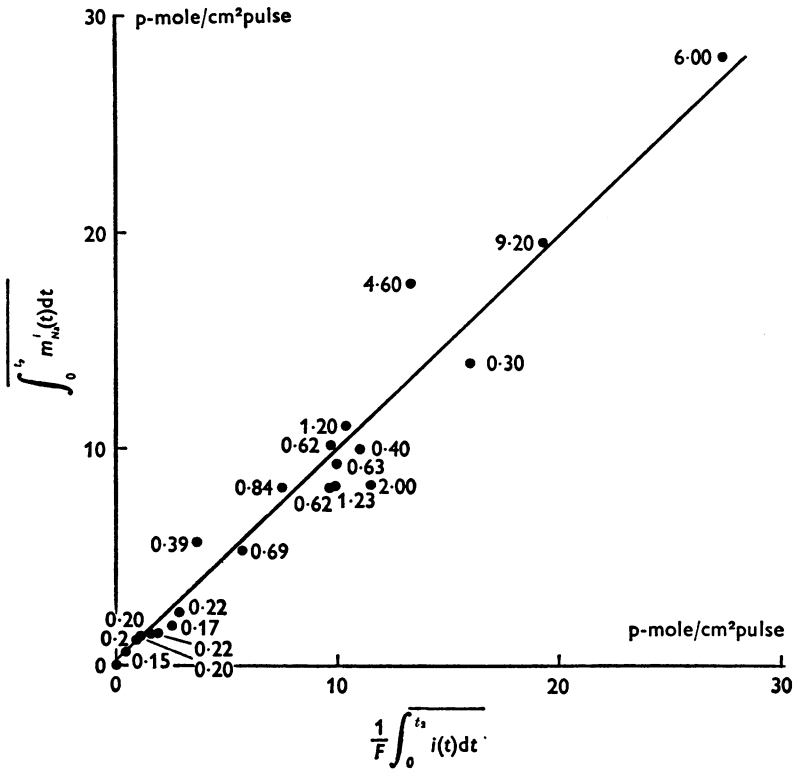

Fig. 2. Measured averaged extra influx of sodium,

$$\int_0^{t_2} m_{Na}^i(t)\,dt,$$

as a function of the net extra flux of ions obtained from membrane current density

$$\left(\frac{1}{F}\int_0^{t_2} i(t)\,dt\right).$$

The duration of each pulse is given next to the corresponding point. Equation of the regression line $(y = ax+b)$ drawn is

$$\int_0^{t_2} m_{Na}^i(t)\,dt = (0\cdot96 \pm 0\cdot05)\,\frac{1}{F}\int_0^{t_2} i(t)\,dt + (0\cdot39 \pm 0\cdot53),$$

where the first parenthesis represents average $a \pm$ s.e.$_a$ and the second parenthesis $b \pm$ s.e.$_b$.

Temporal course of the permeability change to sodium ions

The fact that the transport number for sodium ions is very close to one only when the ratio includes the 'tail' current can be taken as direct evidence that the 'tail' current is also carried by sodium ions. It now seems legitimate to subtract from the measured sodium influx the corresponding sodium influx due to the 'tail' current

$$\int_0^{t_2} \overline{m_{Na}^i(t)}\,dt - \frac{1}{F}\int_{t_1}^{t_2} \overline{i(t)}\,dt = \int_0^{t_1} \overline{m_{Na}^i(t)}\,dt \tag{3}$$

We thus obtain the sodium influx caused by the inward current during the pulse. Notice that t_1 is a variable here so that eqn. (3) represents the integrated flux as a function of time. Figure 3 shows the results obtained with the data corresponding to Expt. TN-55 for which eight runs were available. Figure 3A shows the current record obtained with a 2 msec depolarizing pulse of 50 mV during the last run in experiment TN-55. Figure 3B shows eight determinations of the difference, i.e. total extra sodium influx minus the influx corresponding to the tail current for the same experiment, as given by eqn. (3). Each of these points represents the average over all the pulses delivered during the period of clamping of the instantaneous extra influx of sodium during the pulse integrated over the time interval $(0, t_1)$. That is, each point gives a value of

$$\int_0^{t_1} \overline{m_{Na}^i(t)}\,dt.$$

(The derivative of the curve drawn through these points should be proportional to the ionic current.) The dashed curve represents the integral

$$\frac{1}{F}\int_0^t i(t)\,dt,$$

which is a function of time. The integrated curve compares well with the eight experimental points. As can be seen, the small discrepancy is well within the limits of our experimental errors (15 % for both the points and the curve). Therefore this result indicates that the early inward current is in fact proportional to the change in sodium permeability as was suggested by Hodgkin & Huxley (1952a, b).

Extra sodium influx with or without internal sodium

One of the aims of the present work was to resolve the time course of the permeability change to sodium ions. We have tried to provide direct evidence that the permeability change to sodium ions involves a sodium activation and sodium inactivation. The concept that the permeability

change to sodium ions is a second order process emerged from the classical separation of the ionic currents proposed by Hodgkin & Huxley (1952 *a*, *b*). However, the method used to separate the ionic currents is based on the assumption that the chance that any individual ion crosses the membrane in a specified interval of time is independent of the other ions present in
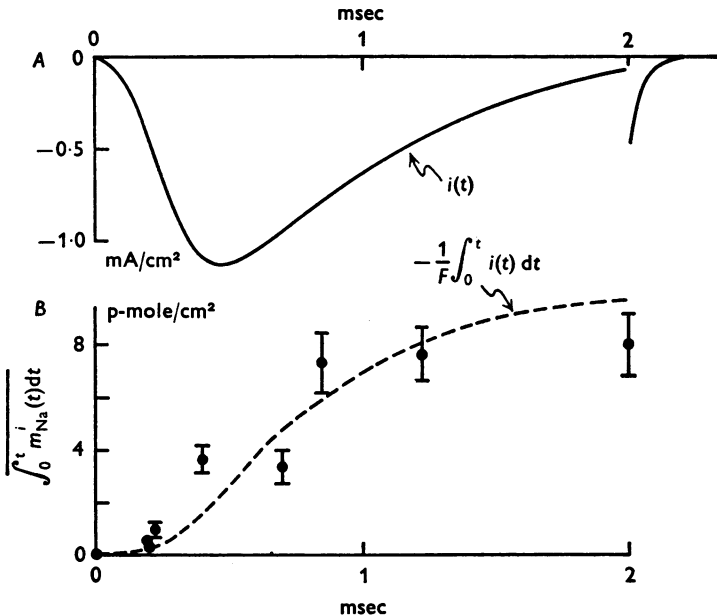

Fig. 3. Extra influx of sodium during the pulse as a function of the pulse duration for experiment TN-55.

A. Membrane current record obtained during the last run in experiment TN-55 corrected for the capacitative transient. This curve was integrated to give

$$\frac{1}{F}\int_0^t i(t)\,dt,$$

which is plotted in part *B*.

B. Each point represents the measured extra influx minus

$$\frac{1}{F}\int_{t_1}^{t_2} i(t)\,dt$$

for the 'tail' current.

the system. Accordingly, the extra influx of sodium should conform to the following expression

$$m^i_{Na} = k a^o_{Na}, \tag{4}$$

where k depends on the condition of the membrane and on the potential difference across it and a^o_{Na} is the chemical activity of sodium outside the fibre. The extra influx of sodium should be independent of the internal sodium concentration provided that the internal sodium does not alter the

membrane (k in the above equation). For high internal sodium concentrations, k is seriously affected (Chandler & Meves, 1966; Adelman & Senft, 1966; Bezanilla, Rojas & Taylor, 1969).

Our test consisted of a comparison of the extra sodium influx in the same fibre when the internal concentration of sodium was raised from 0 to 100 mM. The experimental procedure was essentially the same as described in the preceding sections except that (i) a 50 mV hyperpolarizing prepulse
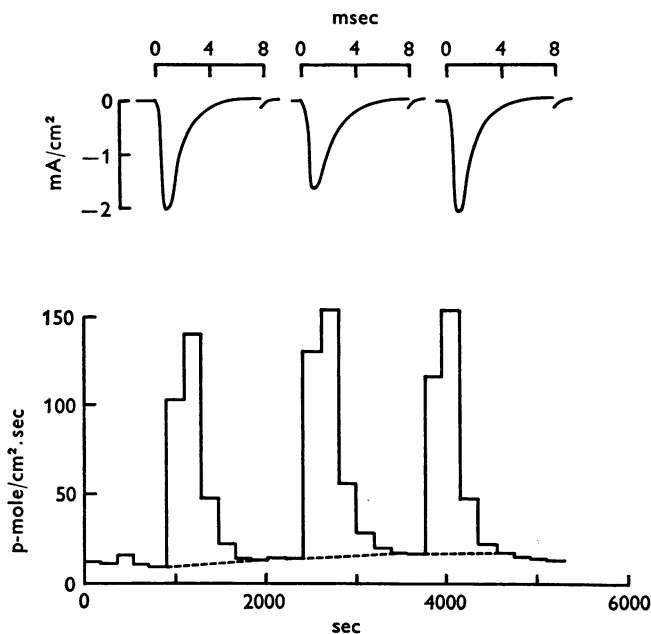

Fig. 4. Effects of internal sodium on the extra influx of sodium during a voltage clamp pulse.
    This Figure has been prepared as Fig. 1. For further explanations see legend for Fig. 1. Expt. TN-64. Temperature of the external ASW was measured at the end of the last run and was equal to 10° C.

(of an absolute membrane potential of about -105 mV) of 10 msec duration was applied and (ii) each fibre was used for three experimental runs, the first internally perfusing with 550 mM-KF (as before), the second perfusing with 450 mM-KF + 100 mM-NaF and the third with the first solution again. The extra influx of sodium was measured as usual. The hyperpolarizing prepulse by itself did not change the influx of sodium (as determined in a separate experiment) and helped us to avoid variations in the current. Only two experiments were performed. Figure 4 shows the results obtained in one of these experiments (Expt. TN-64). From each of these two experiments the following data were obtained (Table 2): resting

potential and resting influx (columns a and b respectively), extra influx of sodium (c), calculated ionic influx from the current record (d).

Each axon was used for three runs. Run B corresponds to the experimental run with 100 mM internal sodium. The extra influx during this run is slightly greater in both axons than the extra influx either before (run A) or after (run C) without sodium in the perfusing solution. The magnitude

TABLE 2. A comparison between the extra sodium influx during a voltage clamp pulse with or without sodium in the perfusing solution

	(a)	(b)	(c) $\int_0^{t_2} m^i_{Na} dt$	(d) $\dfrac{1}{F}\int_0^{t_2} i\,dt$	(e)	(f)
Experiment	(mV)	(p-mole/ cm² sec)	(p-mole/cm² pulse)		(c)/(d)	° C
TN-64						
A	−58·0	26	15·7	15·0	1·04	—
B	−56·5	33	22·1	14·4	1·53	—
C	−58·0	24	19·5	18·8	1·03	10 \
TN-67						
A	−60	10	26·3	28·4	0·93	—
B	−58	14	29·6	26·6	1·11	—
C	−58·5	17	26·2	27·4	0·92	9·8

Fibres were internally perfused with 550 mM-KF, 5 mM-TEA, 5 mM-Tris-Cl during runs A and C. For run B this solution was replaced by 450 mM-KF, 100 mM-NaF, 5 mM-TEA, 5 mM Tris-Cl. A 10 msec duration 50 mV hyperpolarizing conditioning prepulse was applied before the test depolarizing pulse of 40 mV.

of the errors involved in these extra influx determinations is about 5 % for comparisons in the same axon. Thus, the extra influx of sodium during a depolarizing pulse of membrane potential does not significantly change if sodium is present in the internal solution (at least for concentrations between 0 and 100 mM). The currents, however, are considerably decreased, as indicated by the transport numbers given under column (e) in Table 2. Therefore, during run B there was an extra efflux of sodium. For Expt. TN-64 (run B): (c) − (d) = 22·1−14·4 = 7·7 p-mole/cm² pulse and for Expt. TN-67, the same calculation gives 3 p-mole/cm² pulse.

DISCUSSION

The experiments presented in this paper were designed to resolve the time course of the sodium permeability change during a voltage clamp pulse. Voltage-clamp techniques and radioactive tracer analysis have been previously employed by Mullins, Adelman & Sjodin (1962) on unperfused squid axons. They showed evidence that the early *outward* current is carried by sodium. However, accurate determination of inward sodium

flux could not be performed until the advent of the internally perfused giant axons (Atwater *et al.* 1969). To achieve our objective of correlating the time course of the sodium permeability with the time course of the transient current it was necessary to separate inward from outward currents in the current records. This was done by internally perfusing with tetraethylammonium, the ion which specifically blocks the latter currents (Armstrong & Binstock, 1965; Armstrong, 1966). The elimination of the delayed outward current with 4 mM-TEA does not appreciably affect the kinetics of the early inward current. That these currents are independent of each other has been indirectly shown in various ways (Armstrong, 1968; Hille, 1967). The experiments reported in this paper indicate that both the early inward current and the tail of inward current are carried by sodium ions. (For the early outward currents see Mullins *et al.* 1962.) Thus, by subtracting the extra influx of sodium represented by the area under the tail current record from the measured extra sodium influx, the flux during the pulse was obtained. This flux as a function of time (pulse duration) immediately gave the temporal course of the change in permeability to sodium ions.

We have analysed several of the most important sources of experimental errors in the Methods section. It was concluded that the extra influx measurement involves an error of the order of 15 % and that the measurement of the ionic influx corresponding to the current records involves an additional error of the order of 15 %. At least one error cancels out when computing the ratio of these two quantities, the error introduced by the measurement of the area of the axon fibre (about 10 %). Table 1 gives a standard error for the transport number of the order of 8 %.

The regression function relating these two quantities, flux and current, is a more relevant analysis. Thus, for pulses of constant duration (*ca.* 3 msec) but different magnitude (Table 1, Atwater *et al.* 1969)

$$\overline{\int_0^{t_2} m\,i_{\text{Na}}\,dt} = (1\!\cdot\!12 \pm 0\!\cdot\!06)\quad \overline{\frac{1}{F}\int_0^{t_1} i\,dt} - (0\!\cdot\!36 \pm 0\!\cdot\!60)$$

and for pulses of constant magnitude (*ca.* 50 mV) but different duration (Table 1 of this paper)

$$\overline{\int_0^{t_2} m\,i_{\text{Na}}\,dt} = (0\!\cdot\!96 \pm 0\!\cdot\!05)\quad \overline{\frac{1}{F}\int_0^{t_2} i\,dt} + (0\!\cdot\!39 \pm 0\!\cdot\!53)$$

with a correlation coefficient $r = 0\!\cdot\!98$ for both functions. (Numbers in parentheses represent the regression slope ± S.E. and the regression position coefficient ± S.E.) The linear relationship between

$$\overline{\int_0^{t_2} m\,i_{\text{Na}}\,dt} \quad \text{and} \quad \overline{\frac{1}{F}\int_0^{t_2} i\,dt}$$

in both cases is significant. This means that even in the presence of systematic errors in the measurements, the conclusion most affected would have been that about the transport number. However, the transport number was found to be close to unity for any given voltage clamp pulse with the high statistical significance shown by the regression coefficients. Thus, the resolution of the variation in time of the sodium permeability during a voltage clamp pulse as presented here is accurate.

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