Sodium Ions as Blocking Agents and Charge Carriers in the Potassium Channel of the Squid Giant Axon

ROBERT J. FRENCH and JAY B. WELLS

From the Laboratory of Biophysics, National Institute of Neurological and Communicative Diseases and Stroke, National Institutes of Health, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT Instantaneous K channel current-voltage (I-V) relations were determined by using internally perfused squid axons. When K was the only internal cation, the I-V relation was linear for outward currents at membrane potentials up to +240 mV inside. With 25-200 mM Na plus 300 mM K in the internal solution, an N-shaped I-V curve was seen. Voltage-dependent blocking of the K channels by Na produces a region of negative slope in the I-V plot (F. Bezanilla and C. M. Armstrong. 1972. *J. Gen. Physiol.* **60:** 588). At higher voltages ($\gtrsim$ 160 mV) we observed a second region of increasing current and a decrease in the fraction of the K conductance blocked by Na. Internal tetraethylammonium (TEA) ions blocked currents over the whole voltage range. In a second series of experiments with K-free, Na-containing internal solutions, the I-V curve turned sharply upward at about +160 mV. The current at high voltages increased with increasing internal Na concentration and was largely blocked by internal TEA. These data suggest that the K channel becomes substantially more permeable to Na at high voltages. This change is apparently responsible for the relief, at high transmembrane voltages, of the blocking effect seen in axons perfused with Na plus K mixtures. Each time a Na ion passed through, vacating the blocking site, the channel would transiently allow K ions to pass through freely.

INTRODUCTION

Only ammonium, rubidium, and thallium ions—other than potassium—have been shown to carry current with relative ease through the K conductance pathway of nerve axons (Binstock and Lecar, 1969; Hille, 1973; Bezanilla and Armstrong, 1972). By contrast, a variety of inorganic and organic ions are able to cause blocking effects on the K currents (see French and Adelman, 1976, for a recent review). Chandler and Meves (1965), using perfused squid axons, showed that replacement of part of the internal potassium by sodium, rubidium, cesium, or choline reduced the steady-state outward K currents below independence principle predictions. Since that time, the blocking action of internal sodium has been confirmed and further described in the work of Bergman (1970), Adelman and Senft (1971), Bezanilla and Armstrong (1972), and Frankenhaeuser and Århem (1975).

Bezanilla and Armstrong (1972) showed that sodium, as well as cesium and

lithium, produced a block of K channel currents that was sufficiently voltage dependent to cause a region of negative slope in the positive quadrant of the current-voltage relation. The block onset was rapid enough to produce the region of negative slope in both the instantaneous and the quasi-steady-state "isochronal" I–V plots. As part of the present study we extended the observations on the blocking action of internal sodium over a considerably wider range of Na concentrations and voltages. The I–V curve became N-shaped, suggesting a relief of the block at high, internally positive transmembrane voltages.

A further series of experiments with K-free, Na-containing internal solution indicated that, at the higher voltages, the K conductance pathway became abnormally permeable to the sodium ions. This raises the question of whether there is a reversible structural distortion of a selectivity filter at the highest field strengths used, or whether some other mechanism is responsible for the increased ease with which Na ions pass through the K channel.

A preliminary report of these results has been presented (French and Wells, 1977).

MATERIALS AND METHODS

Experimental Preparation

The experiments which are described in this paper were carried out on single, cleaned giant axons isolated from the hindmost stellar nerve of the squid *Loligo pealei*. Axons were internally perfused with a variety of solutions, described in detail below, and a low-impedance, axial wire voltage clamp system was used to control membrane potential. Internal and external voltage-sensing electrodes were glass pipets of 80–100-μm tip diameter, filled with 0.5 M KCl in 1–2% agar. The axial wire was fixed concentrically within the perfusion cannula. Instrumentation and methods were based on those described in earlier publications by Adelman and Palti (1969), Binstock et al. (1975), and Adelman and Gilbert (1964).

When initially perfused with the control 300 mM K internal solution and bathed externally in artificial seawater (ASW), the axons used for these experiments had resting potentials at least as negative as −50 mV (inside minus outside). Axons perfused with the phosphate-buffered, fluoride-glutamate mixtures, containing 300 mM K and various Na concentrations, maintained quite stable resting potentials over periods up to several hours. For experiments of this type reported here, the greatest drop in resting potential seen on returning the axon to control solutions at the end of an experiment was 9 mV. Resting potentials also recovered well after perfusion of the axons with K-free solutions for periods of 10–20 min, but prolonged experiments of this type led to greater deterioration.

Axons were routinely set up in ASW and checked with a depolarizing pulse for the ability to generate a membrane action potential. After the voltage clamp was trimmed, the external medium was changed to a tetrodotoxin-containing solution (TTX-ASW) in order to observe K channel currents without the complication of the occurrence of transient Na channel currents.

Solutions

Axons were initially bathed in ASW of the following composition: 430 mM NaCl, 10 mM KCl, 50 mM $MgCl_2$, 10 mM $CaCl_2$, and 10 mM Tris (tris-hydroxymethylaminomethane) buffer. The Tris was added either as preset buffer (Sigma Chemical Co., St. Louis, Mo.), pH 7.0, or as Tris base. In the latter case, the HCl was added to adjust the pH to

7.0 ± 0.1 at a room temperature of about 20°C. This procedure gives a pH of about 7.4 at the chamber temperatures of about 9°C. For the measurement of K channel currents, 0.5 μM tetrodotoxin was routinely included in the solution (TTX-ASW) along with the above constituents.

Internal solutions used in most of these experiments were buffered with 25 mM $HPO_4^=$. The remaining anions in these solutions consisted of a mixture of fluoride and glutamate and pH was adjusted to 7.3–7.4 by addition of small amounts of free glutamic acid. The composition of these solutions is adapted from the standard internal solution used by Narahashi and his co-workers (see Yeh et al., 1976).

Although it would have been preferable to carry out the experiments with solutions of the same ionic strength, the usual impermeant ions used in ion substitution studies on the Na channel—Tris, tetramethylammonium (TMA), and choline—are all quaternary ammonium ions. Because many quaternary ammonium ions block the K channel when present inside the axon (Armstrong, 1971; Chandler and Meves, 1965), we decided to vary the ionic composition of our internal solutions by substituting added ions for equimolar amounts of sucrose.

In Table I we give details of composition of internal solutions used in experiments

TABLE I

COMPOSITION OF INTERNAL SOLUTIONS

Solution	K^+	Na^+	TEA^+	$Tris^+$	Glutamate$^-$	F^-	Cl^-	$HPO_4^=$	Sucrose
Control 300 K	300				200	50		25	505
300 K, xNa	300	x			200	50 + x		25	505 − 2x
300 K, 100 Na, TEA	300	100	40		200	150	40	25	225
150 Na		150			50	50		25	805
450 Na		450			350	50		25	205
450 Na, TEA		450	40		350	50	40	25	125
150 Na, 6 K*	6	150			50	56		25	805
300 KF	300			10		300	10		460
300 K, 50 Tris	300			50	300	50			480

* This solution, used in one experiment, was simply made by adding the appropriate quantity of 3 M KF stock solution to a sample of the 150 Na solution.
All concentrations are in mM.

whose results are considered later in the paper. All chemicals used in preparation of the solutions were of analytical reagent grade if available. Exceptions were as follows: dipotassium phosphate (Matheson Coleman and Bell, ≥99% K_2HPO_4), sodium fluoride (Alfa Products, ultrapure), monosodium glutamate and monopotassium glutamate (Sigma Chemical Co., Sigma grade).

From this point, solutions will be identified in the text by the names assigned in the first column of Table I.

Junction Potential Determinations

In order to correct the membrane potential measurements for diffusion potentials generated at the tips of the voltage measuring electrodes, liquid junction potentials were estimated as follows. Two small beakers, one for external solution and the other for internal solution, were connected by a salt bridge (3 M KCl in 3% agar). Initially, with both voltage-recording pipets in the external solution beaker, the output of the

differential voltage amplifier was set to zero. Then the voltage between two beakers of ASW was checked to ensure that no potential difference was being generated in the salt bridge. Thereafter, the potential difference between the beakers was measured with the internal pipet in one of the perfusion fluids. Since the potentials at the salt bridge-solution interfaces should be dominated by KCl diffusion, the voltage measured in this way gives the sum of liquid junction potentials generated at the tips of the voltage-recording pipets.

The standard deviation for repeated measurements with the same pair of solutions was 1 mV. The only changes in junction potential that were found to be greater than this were correlated with substantial changes in the ionic strength of one of the solutions. Measured junction potentials are presented in Table II. The appropriate values were subtracted from all measured values of transmembrane voltage.

TABLE II

JUNCTION POTENTIALS MEASURED FOR THE VARIOUS INTERNAL SOLUTIONS USED

Solution	Junction potential
Control 300 K	−2.2
300 K, 10 Na	−2.3
300 K, 25 Na	−2.1
300 K, 50 Na	−1.7
300 K, 75 Na	−1.3
300 K, 100 Na	−1.0
300 K, 200 Na	0.0
150 Na	−8.2
450 Na	−1.0
450 Na, TEA	−0.7
300 KF	−3.7
300 K, 50 Tris	−2.2

Determination of Instantaneous and Steady-State I-V Relations

We use the term "steady-state" current to denote current measured at the end of a 10-ms voltage clamp pulse from the holding potential. At this point, under our experimental conditions, the K current and presumably the conductance had attained values that were essentially invariant with time (see Fig. 1, upper frame, end of first pulse). Currents measured in this manner might more strictly be termed "isochronal" according to Bezanilla and Armstrong (1972). However, "instantaneous" currents, as defined below, are isochronal in a similar sense, but on a different time scale, so we adhere to the traditional term, steady state. The magnitude of the steady-state current is in part determined by the amount of periaxonal K accumulation during the pulse (Frankenhaeuser and Hodgkin, 1956; Adelman et al., 1973) but this does not complicate the interpretation of our data.

In order to determine the instantaneous I-V relation, a 120-mV prepulse of 10 ms duration was applied across the membrane. Although a small, time-dependent increase in current is seen after steps to voltages in the range of 120–240 mV (Fig. 1, lower frame), the prepulse is clearly sufficient to activate almost fully the K conductance. The instantaneous current is taken as the value determined from the digital record 100 μs after the step to the new voltage from the prepulse level. The time interval of 100 μs is

sufficiently long that the contamination of the record by the capacitative current should be negligible, and sufficiently short that the number of K channels open should be essentially the same as at the end of the prepulse. K conductance changes are relatively slow and small for the range of voltages of interest. The instantaneous I–V data from

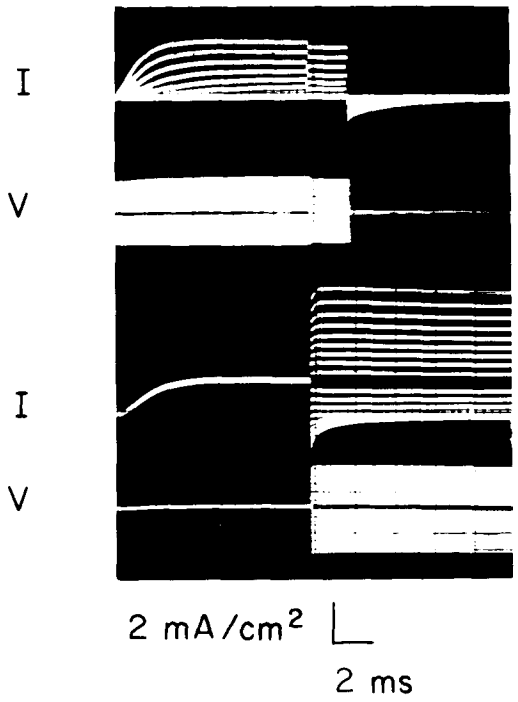

FIGURE 1. A family of current and voltage records from an axon internally perfused with a solution containing 300 mM K^+, 25 mM $HPO_4^=$, 200 mM glutamate. External solution TTX-ASW. Experiment 76.49, temperature 9.0–9.3°C, holding potential −58 mV. *Upper records,* depolarizing and hyperpolarizing pulse sequences of equal magnitude were applied to measure the steady-state K channel current and leakage. Voltage was stepped up and down from the holding potential to a series of levels separated by 20-mV intervals. In each pair of contiguous pulses, the first pulse is of 10 ms duration, and the second, whose magnitude is 10 mV less than the first, is of 2 ms duration. The second pulse is not used in this presentation. *Lower records,* current and voltage records used to determine the instantaneous I–V relation. There were two contiguous 10-ms pulses for each trace. The first is a 120-mV depolarizing pulse to open the K channels. For the second, the voltage was stepped up or down to a series of levels spaced at 20-mV intervals. The "instantaneous" current was measured from digital records at 100 μs into the second pulse.

our experiments may be considered representative of those K channels that were opened by the prepulse. Throughout the paper, V denotes voltage inside minus outside.

Axons were always clamped at a holding potential near −60 mV for at least 30 s before a pulse program was run. The entire pulse sequence to determine an I–V curve was run under computer control with rest periods of 8–10 s between each pulse pair.

RESULTS

Sodium-Free Internal Solutions

With potassium as the only cation in the internal solution, as in our control 300 K solution, the instantaneous I–V relationship is virtually linear through all positive voltages up to at least V = 240 mV. This greatly simplifies the study of the blocking action of an ion such as sodium. In this respect the choice of phosphate as the buffer was significant. Earlier experiments repeatedly showed a slight flattening in the instantaneous I–V curve at high voltages when Tris-buffered internal solutions were used (Fig. 2, upper). When Tris concentrations in the internal solutions were raised above the normal buffer concentrations a pronounced, voltage-dependent reduction in the K channel currents was seen (Fig. 2, lower). Thus Tris is an unsuitable buffer for studies of this type. We avoided the complication of the presence of internal Tris by using phosphate-buffered internal solutions while obtaining the rest of the data reported in this paper.

Internal Solutions Containing Sodium and Potassium

With an internal solution containing 300 mM K and 200 mM Na, a distinct N-shaped I–V characteristic was observed. This can be seen in the records of Fig. 3. Currents during the prepulse to V = +60 mV superpose. In successive traces, the voltage during the second pulse was decreased from +240 mV to −160 mV by 20-mV steps, except that there is no trace for a +60-mV second pulse. The order in which the current traces was recorded is indicated on the figure. As the second pulse voltage decreased, the current first decreased then increased over a 60–80-mV interval, and finally decreased monotonically throughout the rest of the range.

For outward K channel currents, the current records show some slight, time-dependent increase at voltages that were higher than the prepulse level (left and center frames, Fig. 3). This type of behavior was also observed in the control records of Fig. 1. There is, however, no crossing of the records for outward currents, indicating that the qualitative form of the I–V curves is unchanged, regardless of whether one looks at the instantaneous current or the current at long times. Plotting currents at the end of the 10-ms second pulse would still yield an N-shaped graph.

Qualitatively similar results have been seen for frog node K channel when methylammonium (Hille, 1975) and Cs ions (Palti and Stämpfli, unpublished observations) were introduced internally through a cut in the internodal region.

Comparison of Instantaneous and Steady-State I-V Data

In Fig. 4 we present instantaneous and steady-state I–V plots for a single axon perfused first with the control 300 K solution, then with a 300 K, 100 Na internal solution. For both cases, at voltages between 0 and 100 mV, instantaneous and steady-state currents virtually superpose on one another. Since there is no apparent change in the fraction of the K conductance blocked by Na between 100 μs and 10 ms into the pulse, the onset of the Na-blocking effect must be very rapid with respect to the response time of the voltage clamp. The

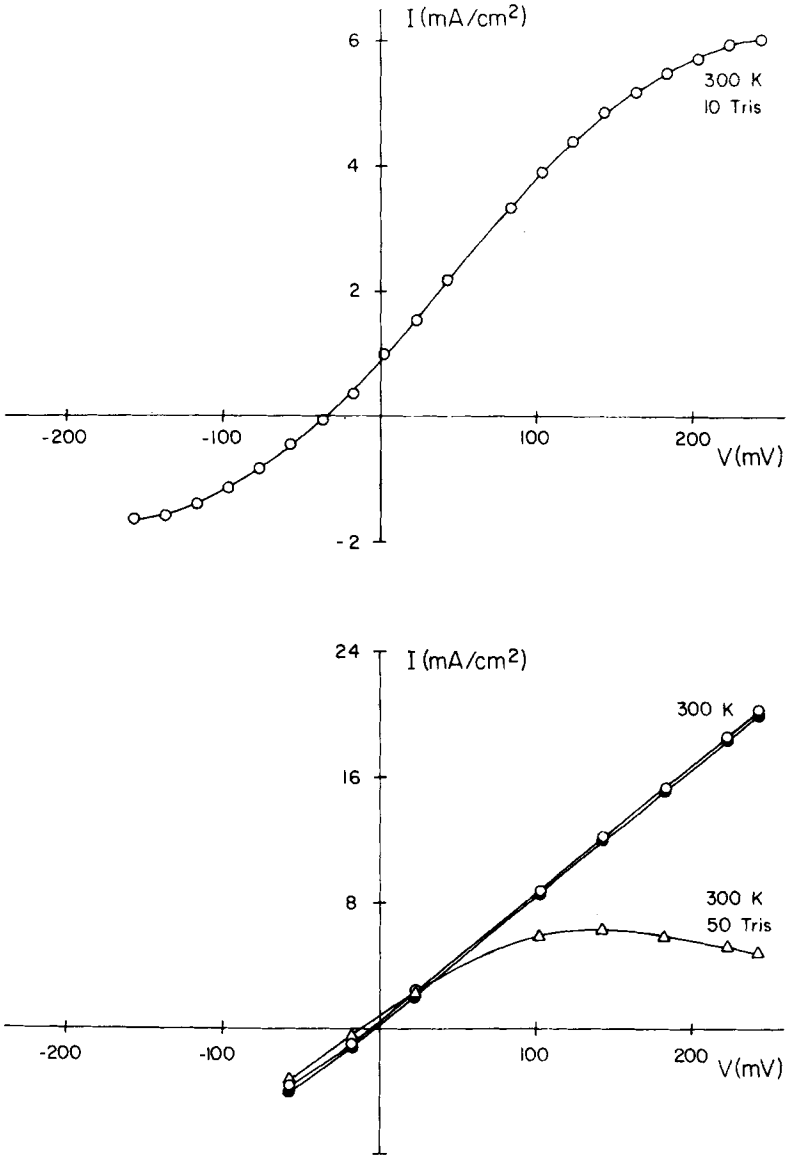

FIGURE 2. Instantaneous I-V relations observed with Tris present inside the axon. Axons bathed externally in TTX-ASW in all cases. Upper, a typical result obtained from an axon perfused with a solution containing 300 mM KF, 10 mM Tris-Cl and 460 mM sucrose. Experiment 76.50, temperature 8.5–8.8°C, holding potential −56 mV. Lower, data taken with 300 mM K^+, 50 mM $Tris^+$ compared to control runs with phosphate-buffered 300 mM K internal solutions (open circles before, closed circles after, exposure to Tris). Except for the buffer (25 mM $HPO_4^=$) in the control solutions, the anions were F^- and $glutamate^-$. Remaining details of solution composition in Materials and Methods. Experiment 76.72, temperature 8.8–9.0°C, holding potential −58 mV.

blocking effect appears to have reached a steady state by the time of recording of the instantaneous currents.

Below 0 mV, the instantaneous and steady-state I-V data differ due to the influence of the normal voltage-dependent activation of the K conductance. In this region, the K conductance is a steep function of voltage causing the steady state I-V plot to curve upwards as voltage increases. From the slight time dependence of currents at high voltages shown in Figs. 1 and 3, there appears to be some increase in conductance over several milliseconds at voltages over 100 mV. This effect is small.

Effect of Varying Internal Sodium Concentration

By perfusing axons with internal solutions containing 300 mM K and Na

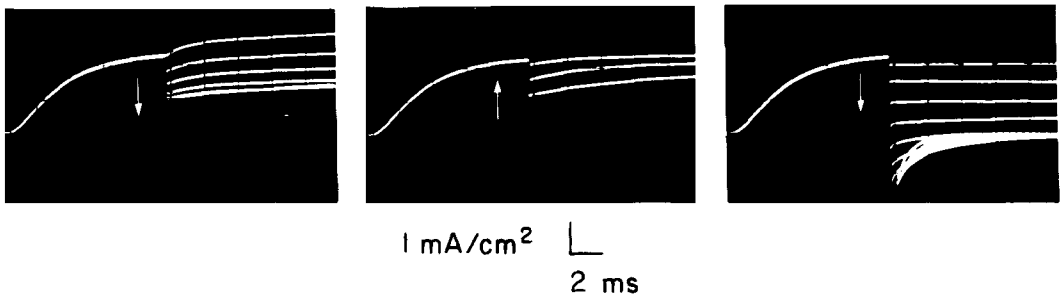

FIGURE 3. Voltage clamp current records from an axon internally perfused with a solution containing 300 mM K^+, 200 mM Na^+, 250 mM F^-, 200 mM glutamate$^-$, 25 mM $HPO_4^=$, and 105 mM sucrose. The arrows indicate the sequence of current records as the voltage of the second pulse is stepped downward. Voltages: left frame −240, 220, 200, 180, 160, 140 (traces for V = 160 and 140 superpose); center frame −120, 100, 80; lower frame −40, 20, 0, −20, −40, . . . −140 mV. Note that the current increases as voltage decreases in the middle frame, indicating the region of negative slope in the I-V curve. Experiment 76.54, temperature 9.3–9.4°C, holding potential −60 mV. External solution TTX-ASW.

concentrations between 0 and 200 mM we obtained families of I-V data of the form illustrated in Figs. 5 and 6. In the instantaneous I-V plots of Fig. 5, regions of negative slope are discernible in the graphs for all Na concentrations from 25 mM upward. The negative slope region appears only over a restricted range of voltages—roughly between 80 and 160 mV, although the exact positions of the maxima and minima appear to be slightly dependent on the Na concentration. The segment of negative slope shifted leftward along the voltage axis by a few millivolts as the concentration of Na was increased over the range used.

No substantial shifts in reversal potential were associated with the presence of the internal Na at any of the concentrations used. Bezanilla and Armstrong (1972) did observe negative shifts in the reversal potential when adding Na, Cs, and Li to the internal solution in the presence of the normal internal K concentration. They attributed this shift to a decrease in the periaxonal K accumulation in the presence of the blocking ions. In our experiments a

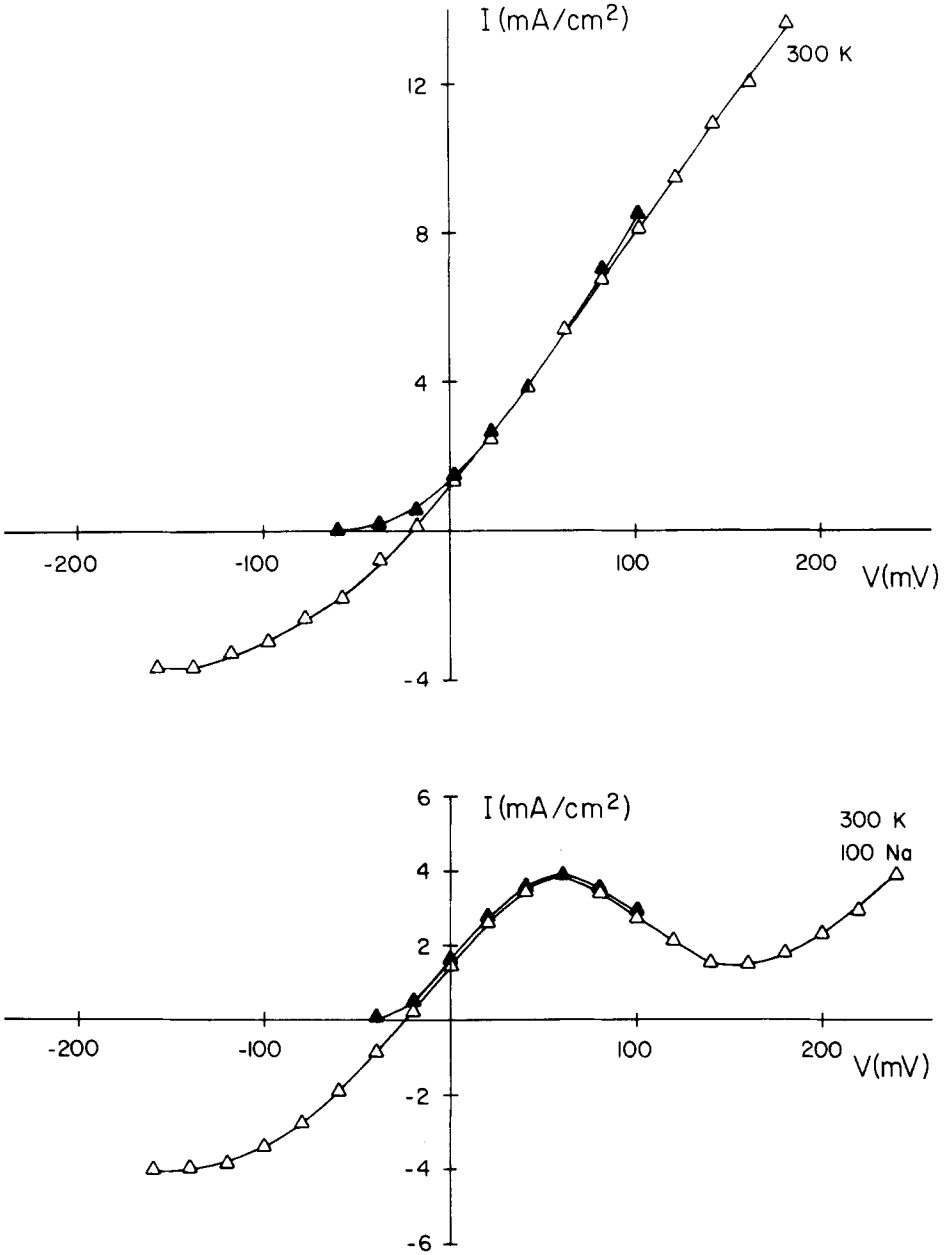

FIGURE 4. Steady-state (filled triangles) and instantaneous (open triangles) I-V plots for an axon perfused with solutions containing the following cations: 300 mM K^+ (upper) and 300 mM K^+ plus 100 mM Na^+ (lower). Further details of solutions in Materials and Methods. Steady-state currents were the values at the end of a 10-ms depolarizing pulse. Instantaneous currents were determined 100-μs after a voltage step which followed a 10-ms depolarizing prepulse used to open the channels. Experiment 76.56, temperature 9.0-9.1°C, holding potentials −58 mV (300 mM K) and −59 mV (300 mM K plus 100 mM Na). External solution TTX-ASW.

somewhat lower prepulse voltage was used—about +60 mV—whereas Bezanilla and Armstrong used values in the neighborhood of +120 mV. At +60 mV the blocking effect is small (see Figs. 5 and 6) and one consequently would expect little difference between the accumulation occurring during the prepulse for the control and for the Na plus K internal solutions. Thus the present results are in no way contradictory to those reported by Bezanilla and Armstrong.

As seen for a single Na concentration in Fig. 4, the family of curves of Fig. 5 indicates that the currents invariably increase with increasing voltage for

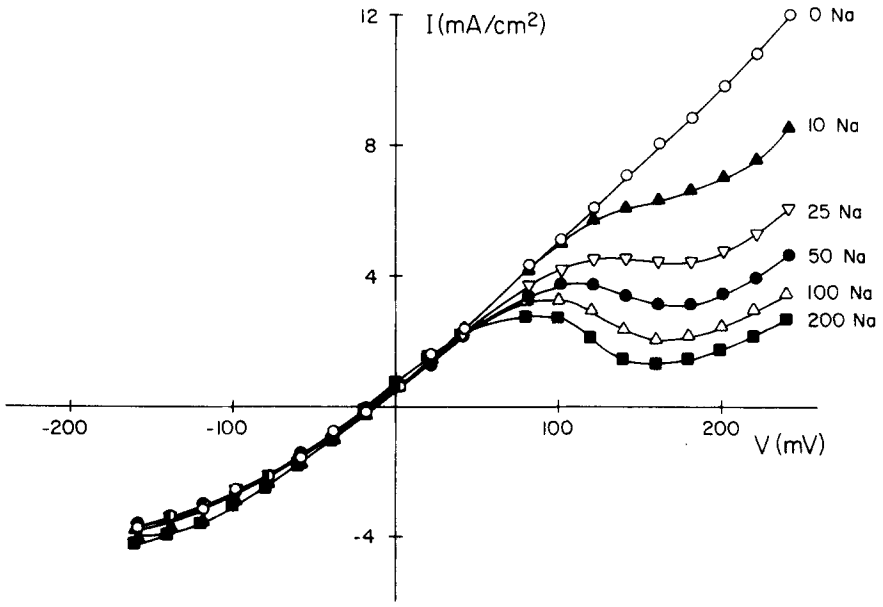

FIGURE 5. Family of instantaneous I–V curves for an axon perfused with internal solutions containing 300 mM K^+ and a series of Na^+ concentrations ranging from 0 to 200 mM, as indicated in the figure. More details of the solutions in Materials and Methods. For an indication of the reversibility of the Na block of the K currents, see Fig. 6. Experiment 76.55, temperature 9.0–9.4°C. Holding potentials: −58 mV (0, 10, and 25 mM Na^+), −59 mV (50 and 100 mM Na^+) and −60 mV (200 mM Na^+). External solution TTX-ASW.

transmembrane potential differences above ~160 mV. That this reflects a genuine reduction in the blocking effect of the internal sodium is emphasized by the plot of relative currents against voltage in Fig. 6. Current data has been expressed as a fraction of that recorded in the initial 300 K control run. For all sodium-containing solutions, the value of the ratio I_{Na+K}/I_K first decreases steeply as voltage increases. At the highest voltages, I_{Na+K}/I_K was always increasing. This is not the result that one would expect for an impermeant blocking ion being driven into the channel by an increasing electrochemical gradient.

The data for the repeat control run plotted in Fig. 6 show clearly the reversibility of the blocking effect and the stability of the axon under the

experimental treatment. For the initial control run, $I_{Na+K/IK} \equiv 1$ by definition. After voltage clamp runs with all of the sodium-containing internal solutions, the repeat control run showed currents within about 10% of the initial values. This was regularly observed, with the final currents usually a little less, but sometimes slightly greater than the initial values.

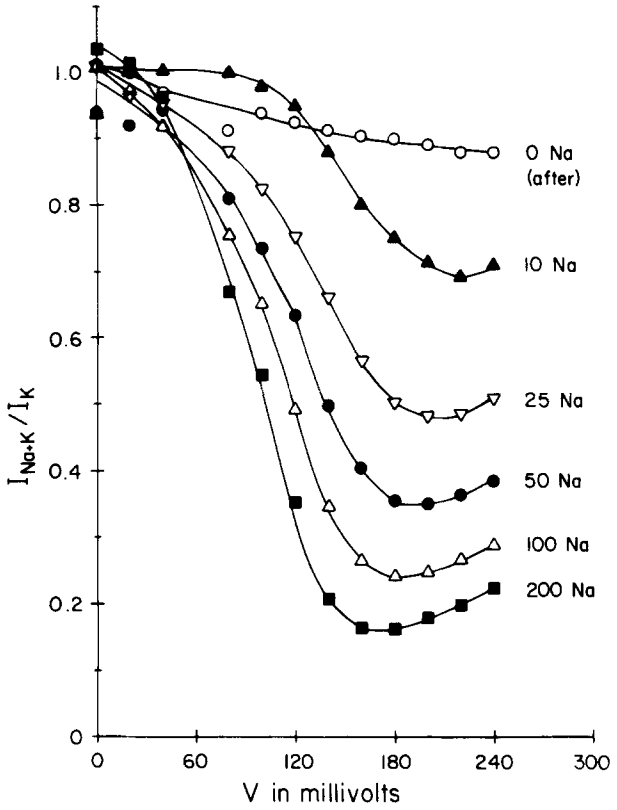

FIGURE 6. Data from the experiment of Fig. 5 plotted as the instantaneous current relative to that observed in the initial O mM Na^+ control run, against transmembrane voltage. The repeat control run was done after perfusion of the axon with the whole series of Na^+-containing solutions. Note that the relative current passes through a minimum and then increases at the high voltages. Experimental conditions as in the legend for Fig. 5.

Currents are TEA Blockable at All Voltages

In Fig. 7 we present two instantaneous I-V plots of data obtained from axons perfused with solutions containing 300 mM K, 100 mM Na, first without and then with 40 mM tetraethylammonium (TEA) chloride. The TEA-Cl, at this concentration, largely blocks the current over the whole range of voltages explored. It is thus unlikely that the upward turn in the I-V curve at high voltages, beyond the normal physiological range, is due to the opening of some nonspecific conductance pathway in the membrane.

I–V Data from Axons with Sodium as the Only Internal Cation

Interpretation of experiments of this type is complicated to some extent by the irreversible loss of the membrane K conductance when axons are perfused with Na-containing, K-free solutions (Chandler and Meves, 1970; Bezanilla and Armstrong, 1972). Current traces recorded under voltage clamp in one such experiment are shown in Fig. 8. In this case currents recorded during perfusion with the control 300 K solution were reduced, after perfusion with the K-free solutions, to somewhat less than half the values seen initially. After the initial control run, the axon was perfused successively with two solutions containing

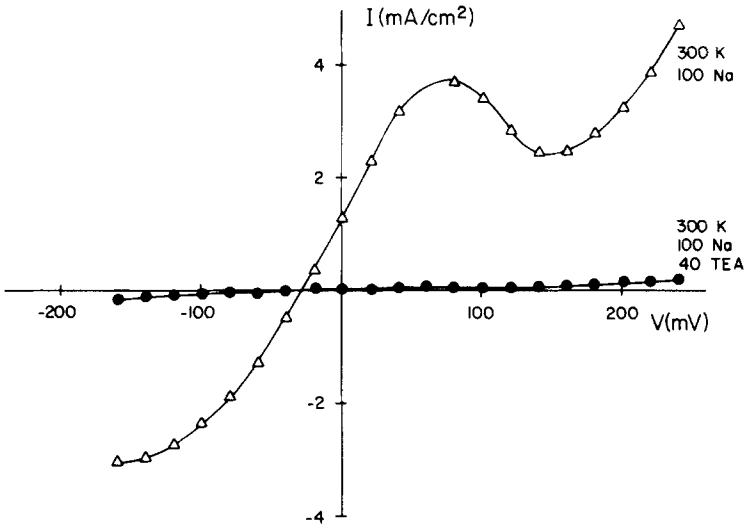

FIGURE 7. Instantaneous I–V relations from an axon internally perfused with solutions containing 300 mM K^+ and 100 mM Na^+, with (filled circles) and without (open triangles) internal tetraethylammonium (TEA^+) ions to block K channel currents. Experiment 76.54, temperature 9.2–9.3°C, holding potential −59 mV. External solution TTX-ASW. Detailed solution compositions in Materials and Methods.

450 mM Na. The first of these contained, in addition, 40 mM TEA (Fig. 8, center left). After thoroughly flushing out the TEA, there was a substantial increase in observed currents, particularly at high positive voltages (Fig. 8, center right). Instantaneous I–V plots from these data are shown in Fig. 9. In the absence of TEA, inward tail currents were observed when second pulse voltage was negative (Fig. 8, center right). These have the appearance of normal K channel tail currents, suggesting that the K conductance has been normally activated during the prepulse even though there was little or no observable outward current at that time. Furthermore, these currents were blocked by TEA as may be seen from both Figs. 8 and 9. The inward tail currents are much smaller than those normally observed, but this is to be expected for the following reasons. First, there is only 10 mM K in the TTX-ASW bathing the axon. Secondly, since the internal solution was K-free there

could be no periaxonal K accumulation during the prepulse. Thus it appears that the remaining functional K channels have opened normally, allowing some outward TEA-blockable current carried by Na at highly positive voltages. Normal, transient inward currents carried by K ions appear to be seen as the channels close at negative voltages.

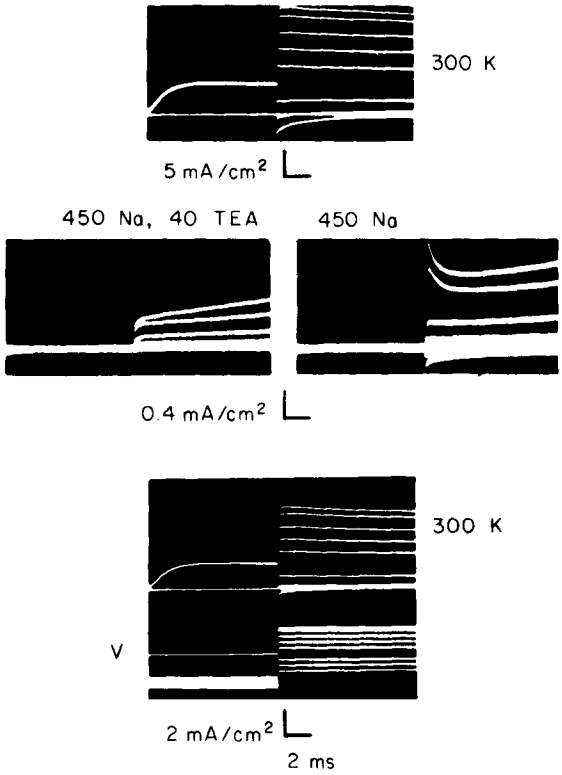

FIGURE 8. Voltage clamp current records from an axon perfused internally with control (300 mM K^+) and K^+-free solutions. The voltage pulse program is shown only in the lowest frame and includes a series of hyperpolarizing pulses to check leakage (merged traces below the prepulse), plus a series of pulse pairs in which there is a fixed depolarizing pulse to open the K channels, followed by voltage steps to a number of levels to determine the instantaneous I-V relation (see Fig. 9). The order in which the records were taken was 300 mM K^+ (uppermost), 450 mM Na^+ plus 40 mM TEA^+ (center left), 450 mM Na^+ (center right), and finally, 300 mM K^+ (lowest). Note the three different current scales. Experiment 76.73, temperature 8.7-9.2°C. Holding potentials: −58 mV (300 mM K^+) and −59 mV (both K-free solutions). External solution TTX-ASW. Times of perfusion with K-free solutions: 18 min (450 Na^+, 40 TEA^+) and 8 min (450 Na^+).

The records of Fig. 8, center right, suggest some conductance inactivation over the 1st ms or 2 of the second pulse. It is unlikely that the currents pass through the Na channel for the following reasons: (*a*) the fast inactivation of the Na channel should be complete by the end of the 10-ms prepulse to +60 mV and (*b*) the currents occur in the presence of TTX and are reduced by TEA.

It is highly significant that we find a sharp upward turn in the instantaneous I-V curve at V ≃150 mV for the 450 Na solution. This is roughly the same voltage at which the negative slope region of the I-V curve ends for axons perfused with K plus Na mixtures. For the 450 Na case, the slope of the I-V curve at V = 200 mV was about 16 times greater than at V = 50 mV (average of four experiments). The data show that, at these voltages, the membrane allows Na to pass more freely via a TEA-blockable pathway. That pathway is K selective since the currents, even though increasing sharply at these voltages with K-free solutions, are still much lower than for the K plus Na solutions (compare, for example, Figs. 7 and 9).

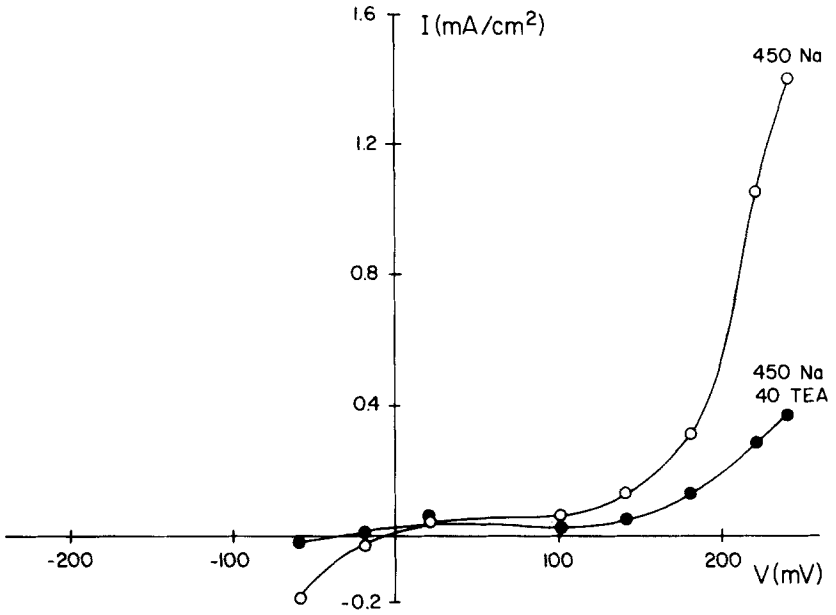

FIGURE 9. Instantaneous I-V data from an axon perfused with Na^+-containing, K^+-free internal solutions, both with and without TEA^+ ions to block K channel currents. Data correspond to the two central frames of Fig. 8. Experimental details may be found in the legend of that figure.

Outward Currents Dependent on Internal Na Concentration

Another series of experiments strengthens the suggestion that the outward currents at high voltages are carried by Na ions. Data in Table III show that instantaneous current at V = 220 mV increased when internal Na concentration was increased in K-free internal solutions. In order to allow for comparison of data from a number of axons, we present the results for currents with K-free internal solutions normalized in terms of the mean of the currents, recorded before and after, with the 300 K control solution. Actual values of the control currents are also presented to show irreversible effects of perfusion with the K-free solutions.

Two control experiments were done to eliminate the possibility that the

outward currents, observed during perfusion of axons with "K-free" internal solutions, might have been carried by K ions that entered the axon from the external solution. The resting potential with the K-free internal solutions was in the vicinity of zero, so it seemed possible that there might be significant K entry before the axon was clamped to the holding potential. In one experiment the membrane voltage was clamped to the holding level of about −60 mV before the solution change, then the instantaneous current was recorded first for the highest voltage. This, for practical purposes, eliminated the possibility that significant K entry could take place through open K channels before the recording of the first instantaneous current point. The current was larger than

TABLE III

OUTWARD CURRENT* MEASURED AT V = 220 mV WITH K-FREE INTERNAL SOLUTIONS

	$I_{600\,K}$		$2I_{Na}/(I_{K\,before} + I_{K\,after})$			
Exp. no.	Before	After	150 Na	150 Na + 6 K	450 Na	450 Na + 40 TEA
76.69	16.7	4.4			0.100	
	4.4	2.0				0.016
76.70	16.2	5.9	0.064			
	5.9	2.9			0.193	
76.71	16.1	11.2				0.015
76.73	17.2	10.4				0.020
	10.4	5.0			0.134	
76.74	11.5	6.3	0.062			
	6.3	4.9		0.070		
76.75	15.6	7.4			0.092	
Means			0.063	0.070	0.130	0.017

* Currents determined at about 100 μs after a 120-mV, 10-ms depolarizing prepulse. Holding potential was −52 mV for 150 Na solutions, −59 mV for 450 Na solutions. Time of exposure to K-free internal solutions was about 10–20 min.

seen with the standard procedure in which the membrane potential was allowed to attain its resting value during the solution change. The opposite result would have been observed had currents recorded with the usual procedure been due to K contamination. Possibly, the irreversible destruction of the K conductance, with Na as the sole internal cation, begins only after the channels are opened by membrane depolarization. Also, some long-term inactivation might have resulted from the normal procedure in which the K-free solutions were allowed to depolarize the membrane.

Results from the second control experiment, in which 6 mM KF was introduced into a 150 Na internal solution, are included in Table III. Little change in the normalized current resulted from this action, and we conclude that the outward current observed with K-free, Na-containing perfusion solution was, indeed, carried primarily by the outward movement of Na ions.

DISCUSSION

Previously published estimates of the ratio, P_{Na}/P_K, of sodium permeability to potassium permeability in the K channel of nerve axons indicate that the K channel is highly selective for K over Na in the physiological voltage range. Hille (1973) reported, for frog node, that P_{Na}/P_K was <0.01, on the basis of reversal potential measurements. From the undershoot of the action potential, Baker et al. (1962) estimated P_{Na}/P_K to be 0.03. This may be considered as an upper limit to the permeability ratio for the K channel, since any Na conductance not completely inactivated would bias the value upward.

A somewhat higher value ($P_{Na}/P_K \leq 0.06$) was obtained by Bezanilla and Armstrong (1972) for squid axon perfused with 300 mM NaF based on measurements of current passing through the K channel at V = 100 mV. While one should be extremely wary of comparing permeabilities derived from reversal potential and current measurements (see the discussion of rate theory models by Hille, 1975) it is interesting, in the light of our own results, to note that the highest value previously estimated for P_{Na}/P_K was also obtained at a rather high positive voltage. In the range of voltages encompassed by an action potential, there does not appear to be any good experimental evidence for appreciable movement of sodium through the K channel.

We concur with Bezanilla and Armstrong (1972) that, for moderately positive voltages, Na ions enter the K channels from the inner mouth and block them to the passage of K ions. At voltages greater than about 160 mV our results suggest that Na ions not only enter the K channels, but can pass through with relative ease. We summarize the salient points of evidence below.

(*a*) With sodium as the only internal cation there is a sharp turn upward in the instantaneous I-V relation at about the same voltage as that at which the negative slope region ends for axons perfused with Na + K mixtures.

(*b*) With both Na + K mixtures and all-Na internal solutions, current is substantially blocked by internal TEA ions through the whole range of voltages observed.

(*c*) In the high-voltage range (>160 mV) the conductance still shows a selective preference for K ions. Currents for 300 K, 100 Na inside are three to five times greater than for 450 Na inside.

(*d*) With all-Na internal solutions the outward current magnitude at high voltages is positively correlated with the Na concentration.

(*e*) Inward current tails are seen on repolarization for axons perfused with all Na internal solutions, suggesting that at least some of the K channels are opening and closing normally.

Evidently, the K channels are less selective at high voltages. It is interesting that the increased permeability to Na ions appears without the abolition of the blocking effect of TEA ions. Also, with 300 K, 50 Tris inside the axon, we observed voltage-dependent blocking but no upturn in the I-V curve (Fig. 2, lower), as seen with the K plus Na mixtures. Perhaps the electrical energy provided at high field strengths is sufficient to dehydrate a sodium ion beyond some crucial state and allow it to pass through the K channel. The covalently bonded side groups of TEA and Tris are not likely to be stripped off in a

similar manner. An alternative mechanism, in which high fields directly distort the structure of the channel rather than affecting the hydration state attained by the permeant ion, is also conceivable.

Our results add an entry to the list of observations which imply that the selectivity of the ionic channels of the axon membrane cannot be considered invariant. Studies of permeability ratios determined from reversal potential measurements also lead to this conclusion. Cahalan and Begenisich (1976) demonstrated that the P_K/P_{Na} ratio for the Na channel of squid is dependent on the internal concentration of K and other ions. Ebert and Goldman (1976) extended these observations to *Myxicola* axons. Although it is not possible entirely to exclude varying concentrations as a contributing factor, Seyama and Narahashi (1977) have reported a small voltage dependence of the permeability ratios for the early transient current of squid axon.

We thank Dr. G. S. Oxford for suggestions about the composition and preparation of the internal solutions, and Drs. Y. Palti and R. Stämpfli for allowing us to refer to their unpublished data. We are grateful to Drs. W. J. Adelman, Jr. and J. Shoukimas for critical comments during the course of the work, and also to Dr. Shoukimas for assistance with some of the experiments. Vivian Gainer and Ruthanne Theran provided invaluable technical support, and Hazel Fermino gave secretarial help beyond the call of duty.

Received for publication 22 April 1977.

REFERENCES

ADELMAN, W. J., JR., and D. L. GILBERT. 1964. Internally perfused squid giant axons studied under voltage clamp conditions. I. Method. *J. Cell. Comp. Physiol.* **64**:423–428.

ADELMAN, W. J., JR., and Y. PALTI. 1969. The influence of external potassium on the inactivation of sodium currents in the giant axon of the squid, *Loligo pealei*. *J. Gen. Physiol.* **53**:685–703.

ADELMAN, W. J., JR., Y. PALTI, and J. P. SENFT. 1973. Potassium ion accumulation in a periaxonal space and its effect on the measurement of membrane potassium ion conductance. *J. Membr. Biol.* **13**:387–410.

ADELMAN, W. J., JR., and J. P. SENFT. 1971. In voltage clamped squid axon internal sodium inhibits potassium ion membrane currents. *Fed. Proc.* **30**:665. (Abstr.).

ARMSTRONG, C. M. 1971. Interaction of tetraethylammonium ion derivatives with potassium channels of giant axons. *J. Gen. Physiol.* **58**:413–437.

BAKER, P. F., A. L. HODGKIN, and T. I. SHAW. 1962. The effects of changes in internal ionic concentrations on the electrical properties of perfused giant axons. *J. Physiol. (Lond.)*. **164**:355–374.

BERGMAN, C. 1970. Increase in sodium concentration near the inner surface of nodal membrane. *Pfluegers Arch. Eur. J. Physiol.* **317**:287–302.

BEZANILLA, F., and C. M. ARMSTRONG. 1972. Negative conductance caused by the entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* **60**:588–608.

BINSTOCK, L., W. J. ADELMAN, J. P. SENFT, and H. LECAR. 1975. Determination of the resistance in series with the membranes of giant axons. *J. Membr. Biol.* **21**:25–47.

BINSTOCK, L., and H. LECAR. 1969. Ammonium ion currents in the squid giant axon. *J. Gen. Physiol.* **53**:342–361.

CAHALAN, M., and T. BEGENISICH. 1976. Sodium channel selectivity. Dependence on internal permeant ion concentration. *J. Gen. Physiol.* **68**:111–125.

CHANDLER, W. K., and H. MEVES. 1965. Voltage clamp experiments on internally perfused giant axons. *J. Physiol. (Lond.)*. **180**:788–820.

CHANDLER, W. K., and H. MEVES. 1970. Sodium and potassium currents in squid axons perfused with fluoride solutions. *J. Physiol. (Lond.)*. **211**:623–652.

EBERT, G. A., and L. GOLDMAN. 1976. The permeability of the sodium channel in *Myxicola* to alkali cations. *J. Gen. Physiol.* **68**:327–340.

FRANKENHAEUSER, B., and P. ÅRHEM. 1975. Steady state current rectification in potential clamped nodes of Ranvier (*Xenopus laevis*). *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **270**:515–525.

FRANKENHAEUSER, B., and A. L. HODGKIN. 1956. The after-effects of impulses in the giant nerve fibres of *Loligo*. *J. Physiol. (Lond.)*. **131**:341–376.

FRENCH, R. J., and W. J. ADELMAN, JR. 1976. Competition, saturation and inhibition—ionic interactions shown by membrane ionic currents in nerve, muscle and bilayer systems. *Curr. Top. Membr. Transp.* **8**:161–207.

FRENCH, R. J., and J. B. WELLS. 1977. Interactions between internal sodium ions and the potassium channel of the squid giant axon. *Biophys. J.* **17**(2, Pt. 2):46 a. (Abstr.).

HILLE, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. *J. Gen. Physiol.* **61**:669–686.

HILLE, B. 1975. Ionic selectivity of Na and K channels of nerve membranes. *In* Membranes—a series of advances. Vol. 3. G. Eisenman, editor. Marcel Dekker, Inc., New York. 255–323.

SEYAMA, I., and T. NARAHASHI. 1977. Voltage dependence of cation permeability ratios in squid axon membranes. *Biophys. J.* **17**(2, Pt. 2):207a. (Abstr.).

YEH, J. Z., G. S. OXFORD, C. H. WU, and T. NARAHASHI. 1976. Dynamics of aminopyridine block of potassium channels in squid axon membrane. *J. Gen. Physiol.* **68**:519–535.