Dynamic Asymmetries in the Squid Axon Membrane

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INTRODUCTION

In 1952 Hodgkin and Huxley were able to separate the membrane currents which occur in response to step changes in membrane potential, upon voltage clamping the squid giant axon, into two major current components: an initial transient component mainly carried under normal conditions by sodium ions and a delayed component mainly carried by potassium ions. Tetrodotoxin (TTX) specifically blocks the initial current mechanism when applied externally (Narahashi et al., 1964; Nakamura et al., 1965). However, internal application of TTX at the same concentration does not block the sodium current. At higher internal concentrations, it becomes effective (Narahashi et al., 1966). On the other hand, in the squid axon, tetraethylammonium ion (TEA) blocks the delayed current when applied internally (Tasaki and Hagiwara, 1957; Armstrong and Binstock, 1965; Armstrong, 1966). Whereas cesium ion is able to block the delayed outward steady-state current when applied internally (Chandler and Meves, 1965; Adelman and Senft, 1966), cesium ion has no effect on this current when applied externally (Pickard et al., 1964).

The object of this paper is to examine in some detail interactions among the five readily available group IA metal cations in contributing to and/or inhibiting membrane conductances when the concentrations of these ions are varied externally.

METHODS

Giant axons from the hindmost stellar nerve of the squid *Loligo pealei* were carefully cleaned of surrounding fibers and were placed in the voltage clamp cell. In this cell the axons were voltage clamped by the point control system of Cole and Moore (1960). Membrane potential and current measurements have been described previously (Adelman et al., 1965 *a*, *b*). Table I lists the composition of the various artificial sea water (ASW) solutions used in the experiments.

After an axon had been mounted in the voltage clamp cell and the membrane resting and action potentials had been measured in ASW, the external solution was changed to a sea water in which a fraction of the sodium chloride was replaced by Tris chloride. The axon was then voltage clamped and a series of membrane currents in response to a variety of test voltage pulses was obtained. The resting and action potentials were measured upon unclamping, and the axon was then externally perfused with a similar solution in which the Tris chloride was replaced by an equal concentration of either Li, Na, K, Rb, or Cs chloride. Membrane currents

and potentials were measured again. After this, the axon was exposed to the Tris chloride containing sea water, and another series of recordings were made. This procedure was repeated for substitutions of as many of the five test cations as was consistent with the viability of the axon. For each of the group IA cation substitutions a Tris control was run both before and after. For each axon tested the sequence of cation replacements for Tris was varied. Two basic types of test solutions were used: (*a*) 340 mM Na, 100 mM Tris or group IA cation sea water; (*b*) 240 mM Na, 200 mM Tris or group IA cation sea water. Temperature was held constant at 4° ± 1°C.

TABLE I

COMPOSITION OF SOLUTIONS

Solution	Concentration					
	Cl^-	Na^+	K^+	$Tris^+$	Ca^{++}	Mg^{++}
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
ASW	570	440	10		10	50
340 Na, 100 Tris SW	570	340	10	100	10	50
340 Na, 100 cation SW	100 mM of Li^+, Na^+, K^+, Rb^+, or Cs^+ replaces 100 mM of $Tris^+$					
240 Na, 200 Tris SW	570	240	10	200	10	50
240 Na, 200 cation SW	200 mM of Li^+, Na^+, K^+, Rb^+, or Cs^+ replaces 200 mM of $Tris^+$					

Solutions were adjusted to pH 7.4 by addition of about 1 mM Tris buffer for solutions not containing Tris chloride. For solutions with 100 or 200 mM Tris chloride the pH was adjusted to 7.4 by adjusting the Tris chloride:Tris base ratio and checking pH before use with a glass electrode.

TABLE II

CHANGE IN THE RESTING POTENTIAL, E_{RP}, UPON REPLACING $TRIS^+$ WITH INDICATED CATION

$$\Delta E_{RP} = E_{RP_{Test}} - [(E_{RP_{Tris,before}} + E_{RP_{Tris,after}})/2]$$

External solution	Axon	ΔE_{RP} with:				
		Li	Na	Cs	Rb	K
		mv	*mv*	*mv*	*mv*	*mv*
340 Na, 100 cation SW	66-33	0.8	1.0	6.2	33.5	36.0
	66-36	0.5	0.7	5.8	29.5	32.5
	66-37	1.0	1.5	6.8	36.0	44.0
	66-39	1.0	1.2	6.8	36.0	42.0
	Average	0.8	1.1	6.4	33.5	38.9
240 Na, 200 cation SW	66-41	—	3.0	12.5	42.3	53.5
	66-42	6.0	—	16.5	53.0	71.0
	66-43	1.7	3.0	10.0	44.0	55.5
	66-44	3.0	4.0	14.0	51.0	60.0
	66-45	3.0	4.0	13.5	49.0	60.0
	Average	3.4	3.5	13.3	47.9	60.0

RESULTS

Effects of Group IA Cations on the Resting Potential

Changing the external solution from ASW to 340 Na, 100 Tris SW produced an increase in the resting potential of about 1 mv. Upon substituting Li, Na, K, Rb, or Cs for the Tris in the sea water, a decrease in the resting potential was observed. Average values were as follows: Li, 0.8 mv; Na, 1.1 mv; Cs, 6.4 mv; Rb, 33.5 mv;

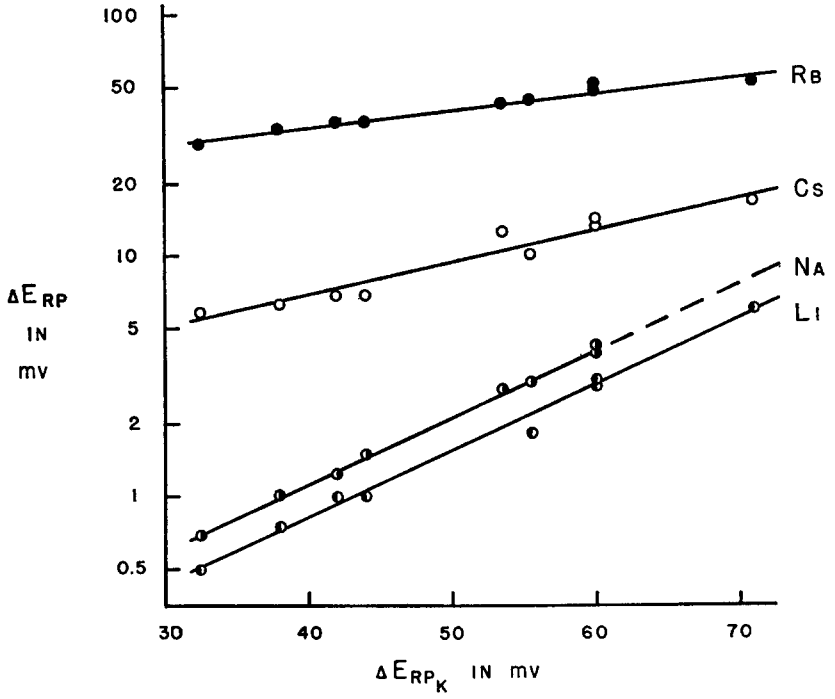

FIGURE 1. Change in resting potential, ΔE_{RP}, obtained upon substituting Rb^+, Cs^+, Na^+, Li^+, for $Tris^+$ in sodium-containing artificial sea water, plotted semilogarithmically against the change in resting potential, ΔE_{RP_K}, obtained upon K^+ substitution. Points to the left of $\Delta E_{RP_K} = 50$ mv are for substituting the listed cations for 100 mM $Tris^+$; points to the right are for substituting the listed cations for 200 mM $Tris^+$. Data plotted from Table II.

and K, 38.9 mv. These averages and the individual values for four axons are given in Table II. Changing the external solution from ASW to 240 Na, 200 Tris SW produced an increase in the resting potential of about 3 5 mv. As may be seen in Table II, the average decreases in the resting potential of five axons upon substituting each of the five group IA cations for the Tris were the following: Li, 3.4 mv; Na, 3.5 mv; Cs, 13.3 mv; Rb, 47.9 mv; and K, 60.0 mv. We have assumed that $Tris^+$ is a nonpermeant cation. Actually, the best we can say definitely is that $Tris^+$ is less permeant than Li^+.

As these studies were performed on intact axons and as one should expect that the

internal ionic concentrations of such axons are not constant, the variations among the individual values obtained for any one species concentration change are not unexpected. One way of expressing these data is shown in Fig. 1. The change in resting potential for Rb, Cs, Na, and Li substitutions is plotted semilogarithmically against the change in resting potential obtained upon substituting K for Tris. Axons which showed the greatest change for a given change in potassium concentration had the highest initial resting potential values. Notice in Fig. 1 that the slopes for Li and Na are steeper than those for Rb and Cs. This implies that axons showing smaller changes in resting potential for a given change in external K concentration had higher selectivity ratios for potassium to lithium or sodium than axons which

TABLE III

RATIOS OF MAXIMUM VALUES OF THE PEAK INITIAL TRANSIENT CURRENT IN SEA WATER CONTAINING SODIUM PLUS INDICATED CATION TO MAXIMUM VALUES IN SEA WATER CONTAINING EQUIVALENT CONCENTRATIONS OF SODIUM AND TRIS

External solution	Axon	Ratio with:				
		Li	Na	Cs	Rb	K
340 Na, 100 cation SW	66-33	1.45	1.43	0.96	0.86	0.71
	66-34	1.32	—	—	0.68	—
	66-36	1.41	1.42	0.84	0.38	—
	66-37	1.49	1.45	0.83	0.73	0.54
	Average	1.42	1.43	0.88	0.66	0.62
240 Na, 200 cations SW	66-41	2.00	2.18	1.03	0.76	0.52
	66-42	2.29	2.44	0.99	—	0.23
	Average	2.15	2.31	1.01	0.76	0.36

Values of ratios given were determined as follows:

$$I_{Na+test}/[(I_{Na+Tris,before} + I_{Na+Tris,after})/2].$$

In all cases the holding potential, *EH*, was between −80 and −88 mv, and the pulse potential, *EP*, at which the maximum peak initial current occurred was between −15 and −30 mv.

depolarized greatly in external potassium. Though correlations of changes in resting potential with either the initial resting potential or the resting potential in the control Tris solutions were not so dramatic as the correlation shown in Fig. 1, a definite trend was seen. Though this is not conclusive evidence it would seem that the selectivity ratios for the contribution of group IA cations to the resting potential may be membrane voltage dependent.

Effects of Group IA Cations on the Initial Transient Membrane Conductance in the Voltage Clamp

For each exposure of an axon to a group IA cation test solution the membrane was voltage clamped and the membrane currents in response to voltage pulses were com-

pared with membrane current values obtained both before and after in the control Tris sea water. Generally, the initial transient currents and the apparent equilibrium potentials for these early currents were profoundly influenced by the group IA cations. Increasing the external sodium and lithium concentrations increased both the amplitude of the initial current at any depolarized membrane potential and the value of the apparent equilibrium potential for this current component. However,

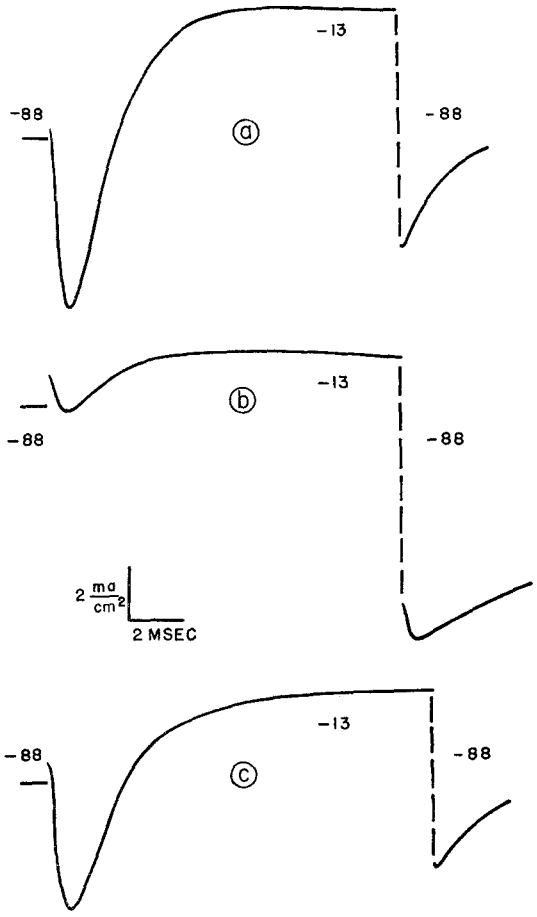

FIGURE 2. Typical records illustrating the effect of external K^+ on membrane currents obtained in the voltage clamp upon a step change in membrane potential from a holding potential of −88 mv to a pulse potential of −13 mv and a step return to −88 mv. *a* and *c* before and after in 240 mM Na, 200 mM Tris SW; *b*, in 240 mM Na, 200 mM K SW. Axon 66–41. See text.

completely unexpectedly, external K, Rb, and Cs decreased both the amplitude of the early current and the value of the apparent equilibrium potential. Table III summarizes the effects of the cation substitutions on the maximum values of the peak initial transient currents. Fig. 2 shows a typical set of membrane current recordings pertaining to the inhibition of the early current by the presence of external potassium. Fig. 2 *a* and *c* are the before and after records obtained in 240 mM Na, 200 mM Tris SW, and Fig. 2 *b* is the comparable voltage clamp membrane current recording obtained in 240 mM Na, 200 mM K SW.

Fig. 3 illustrates the current-voltage relations for this axon's response to voltage

clamp pulses for the peak values of the initial transient current, the steady-state values of the delayed current, and the maximum values of the current tails obtained upon repolarizing the membrane after the pulse. Leakage current estimates from initial step currents are indicated, but are not plotted as such. The steady-state and the tail current values indicate a normal response to external potassium concentra-

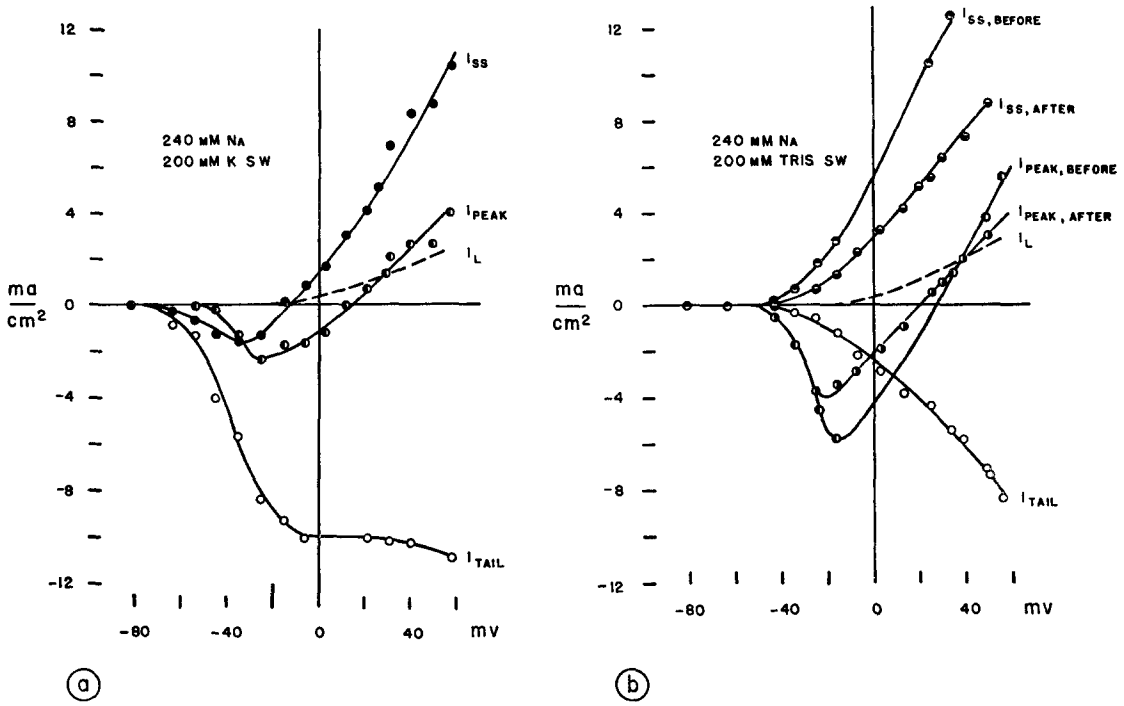

FIGURE 3. Current-voltage relations plotting membrane current densities against voltage-clamped pulse potential obtained upon changing the external solution from 240 mM Na, 200 mM Tris SW to 240 mM Na, 200 mM K SW. *a*, during exposure to external K; *b*, before and after controls in Tris SW. I_{ss}, steady-state values of delayed current; I_{peak}, peak values of early transient current; I_{tail}, peak values of inward-current tail following repolarization from pulse potential to −88 mv; I_l, leakage current estimates from initial step currents. Inward current densities are negative. In *b*, I_{tail} does not differentiate "before" and "after" values, as these were not significantly different. Axon 66-41.

tion, but the early current is decreased by at least a factor of 2. The apparent equilibrium potential is decreased 10.5 mv. Table IV summarizes the effects of external group IA cations on the apparent equilibrium potential for the two test solutions used. Notice that the order of inhibition for this null potential is K > Rb > Cs. This order is the same as that of the inhibitory effect of these cations on the peak current amplitudes. That this effect is due to an inhibition of sodium permeance by external K, Rb, and Cs and not due to a higher permeance of Tris than of these ions in the initial conductance channel is indicated by our complete inability to demonstrate

TABLE IV

CHANGE IN THE APPARENT EQUILIBRIUM POTENTIAL ΔE_{eq}, FOR THE INITIAL TRANSIENT CURRENT UPON SUBSTITUTING INDICATED CATION FOR $TRIS^+$

External solution	Axon	ΔE_{eq} with: Li	Na	Cs	Rb	K
		mv	*mv*	*mv*	*mv*	*mv*
340 Na, 100 cation SW	66-33	+5.5	+8.0	−3.5	−5.0	−7.0
	66-34	+10.5	—	—	−4.5	—
	66-36	+9.0	+7.5	0	−3.5	−9.5
	66-38	—	+4.5	−2.0	−8.0	−5.5
	66-39	—	+7.0	−3.5	−5.5	—
	Average	+8.3	+6.5	−3.0	−5.3	−7.3
240 Na, 200 cation SW	66-41	+8.5	+11.0	−7.5	−14.0	−10.5
	66-42	+8.5	+12.5	−6.0	−7.0	−18.5
	66-43	+14.5	+15.0	−7.5	−9.5	−12.0
	66-44	+16.0	+13.5	−5.5	−5.5	−9.5
	66-45	+14.0	+12.0	−5.5	−10.5	−14.0
	Average	+12.3	+12.8	−6.4	−9.5	−12.9

Values of ΔE_{eq} were determined as follows:

$$\Delta E_{eq} = E_{eqNa+Test} - [(E_{eqNa+Tris,before} + E_{eqNa+Tris,after})/2].$$

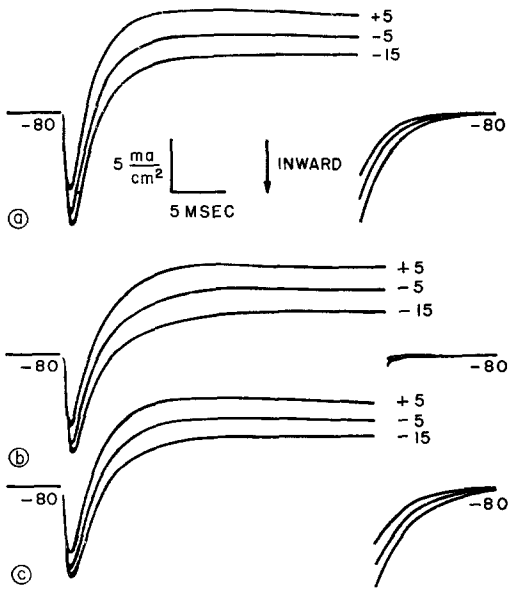

FIGURE 4. Typical records illustrating the effect of external Cs^+ on membrane currents obtained in the voltage clamp upon step changes in membrane potential from a holding potential of −80 mv to pulse potentials of +5, −5, and −15 mv. *a* and *c*, before and after in 340 mM Na, 100 mM Tris SW; *b*, in 340 mM Na, 100 mM Cs SW. Axon 66-33.

any inward current upon voltage clamping axons externally perfused with 440 mM Tris SW solution.

Effects of Group IA Cations on the Current Tails Occurring upon Repolarization of the Membrane Following a Depolarizing Pulse in the Voltage Clamp

In the course of our group IA cation studies on membrane currents in the voltage clamp we noticed that whenever Cs ion was present externally, the inward-current

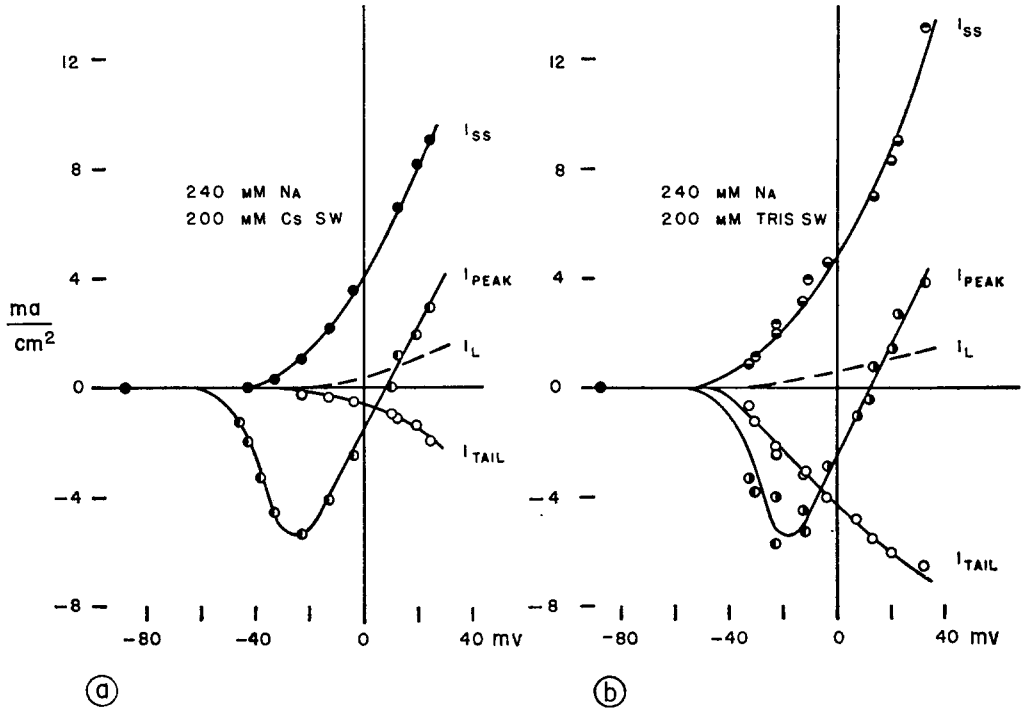

FIGURE 5. Current-voltage relations obtained upon changing the external solution from 240 mM Na, 200 mM Tris SW to 240 mM Na, 200 mM Cs SW. *a*, during exposure to external Cs; *b*, before and after controls in Tris SW. Values are plotted as in Fig. 3. Holding potential, −88 mv. Axon 66-42.

tails associated with repolarization following a large depolarizing pulse were greatly reduced compared to those obtained under similar voltage clamp conditions when any of the other group IA cations were present externally. Both the magnitudes of these tail currents and the time constants of their decline were decreased with respect to values obtained in Tris-containing sea water solutions. Fig. 4 shows typical recordings of membrane currents obtained in the voltage clamp illustrating the external cesium ion effect. Fig. 4 *a* and *c* are the before and after records obtained in 340 mM Na, 100 mM Tris SW, and Fig 4 *b* is the comparable voltage clamp membrane-

current obtained in 340 mM Na, 100 mM Cs SW. Notice that whereas the presence of external cesium ion has little or no effect on the outward steady-state currents, the inward current is greatly reduced in amplitude and returns to the base line at a much faster rate. This figure should be compared with Fig. 2, which demonstrates, as was indicated by Frankenhaeuser and Hodgkin (1956), that increased external

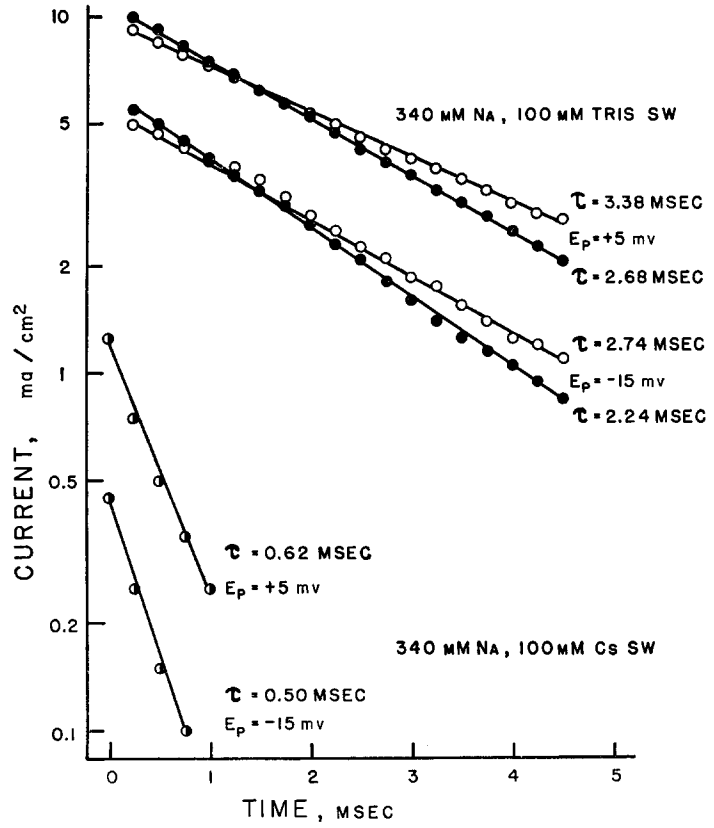

FIGURE 6. Semilogarithmic plots of the decay of the exponential portion of current tails following repolarization from pulse potentials (E_P) of +5 and −15 mv to the holding potential of −80 mv. Closed circles, before in 340 mM Na, 100 mM Tris SW; half-filled circles, during exposure to 340 mM Na, 100 mM Cs SW; open circles, after in 340 mM Na, 100 mM Tris SW. Data taken from records shown in Fig. 4. Axon 66-33.

potassium ion concentration both increases the initial amplitude of the tail current and decreases its rate of decline. Fig. 5 illustrates the current-voltage relations for a typical axon exposed to 240 mM Na, 200 mM Cs SW (*a*) compared with the current-voltage relations obtained before and after in 240 mM Na, 200 mM Tris SW (*b*). In Fig. 5 notice that whereas external Cs ion has almost no effect on the outward steady-state current, the values for the tail current peak are reduced to a mirror image of the leakage current estimate. Figs. 6 and 7 illustrate the effects of external cesium ion

on the time constant of decline of the exponential portion of the current tails obtained for 100 mM (Fig. 6) and 200 mM (Fig. 7) Cs concentrations. Table V gives values of the time constant of decay of the exponential portion of inward current tails following a depolarizing pulse of 6.5 msec duration in 240 mM Na, 200 mM Tris SW, and in SW solutions in which the Tris was replaced by each of the five group IA cations. Both external K and external Rb increased the time constant. Peak values of the inward tail currents were also increased. External potassium ion increased the current peak more than rubidium. Neither external sodium nor external lithium had any significant effect on the time constant. Both these ions, however, increased the

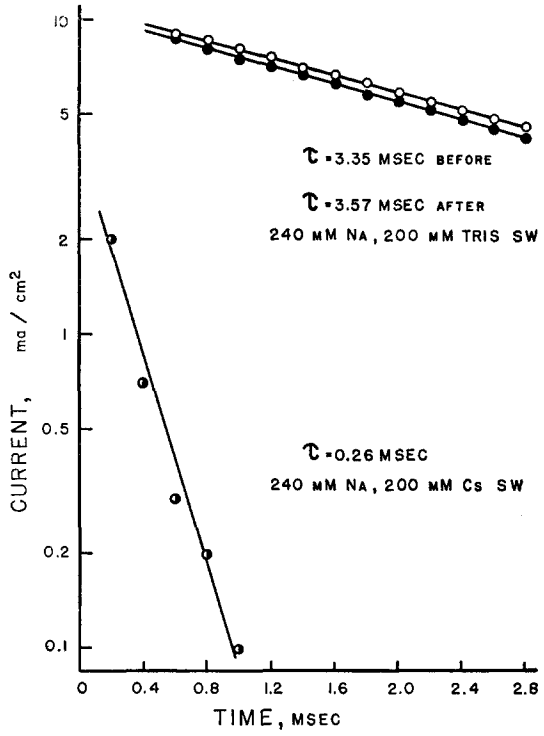

FIGURE 7. Semilogarithmic plots of the decay of the exponential portion of current tails following repolarization from a pulse potential of +30 mv to the holding potential of −80 mv. Points plotted as in Fig. 6. Pulse duration, 28 msec. Axon 66–41.

magnitude of the peak tail current slightly. It is apparent that potassium ion is the major charge carrier for this current, as was suggested by Frankenhaeuser and Hodgkin (1956), and that external Cs^+ inhibits this current.

DISCUSSION

Our results suggest that group IA metal cations have different actions on the different phenomena associated with the electrical behavior of the squid axon membrane, and that these actions depend to a large extent on whether the ions in question are acting on the inner or the outer surface of the membrane. Resting potential changes seen with external group IA substitutions follow Eisenman's glass electrode cationic specificity isotherm IV (K > Rb > Cs > Na > Li), which he has correlated with

a similar cation permeance ratio existing at the resting state for the "biological isotherm" of the squid axon (Eisenman, 1962, 1965; Eisenman and Conti, 1965). This isotherm shows a decrease in P_{Li}/P_K with lowered resting potentials (see Fig. 4 in Eisenman, 1963) of the sort shown in our Fig. 1.

The resting external cation selectivity order is quite different from the Li, Na selectivity order for the early transient conductance. K, Rb, and Cs are external

TABLE V

VALUES OF THE TIME CONSTANT OF EXPONENTIAL DECAY OF CURRENT TAILS UPON REPOLARIZING THE MEMBRANE TO THE HOLDING POTENTIAL FOLLOWING A 6.5-MSEC DEPOLARIZING PULSE* IN 240 Na, 200 TRIS SW AND 240 Na, 200 CATION SW, AS INDICATED IN THE HEADINGS

Axon	Tris		Time constant with:				
	Before	After	Li	Na	Cs	Rb	K
	msec		*msec*	*msec*	*msec*	*msec*	*msec*
66-43	1.97	1.81		1.78			
	1.81	1.92	1.66				
	1.92	1.91					11.42
	1.91	2.52			0.21		
	2.52	2.03				4.02	
66-44	2.00	2.03			0.36		
	2.03	1.86		1.65			
	1.57	1.72				5.77	
	2.01	2.12	2.29				
	2.12	1.28					14.60
66-45	1.61	1.25	1.60				
	1.25	3.17					10.20
	3.13	2.78		3.20			
	2.64	2.54				5.85	
	2.54	2.66			0.22		
Average	2.12‡		1.85	2.21	0.26	5.25	12.07

* In these experiments the holding potential was held between −80 and −84 mv, and the pulse potential was between +28 and +48 mv and was kept constant for any one series of tests.
‡ This average is based on 22 separate measurements: note that a number of the "after" controls are the "before" controls for the succeeding cation test solution.

inhibitors of the initial conductance. This inhibitory order is based on both early current amplitude and equilibrium potential measurements. These same ions in the same descending sequence are charge carriers through the early conductance when perfused internally in the squid axon (Chandler and Meves, 1965). Our finding of an external inhibitory cation order suggests a membrane asymmetry associated with the early conductance. Ion species interactions, inhibitions, or cross-couplings have already been demonstrated for the delayed conductance. Internal application of Cs

or Rb inhibits the delayed conductance in the perfused squid axon (Chandler and Meves, 1965; Adelman and Senft, 1966). In addition, asymmetrical ionic action on the inner and outer membrane surfaces seems likely when one considers that a lower blocking dose of tetrodotoxin is required externally than is required internally in the squid axon (Narahashi et al., 1966). Therefore, we suggest that the site controlling the initial conductance is externally located and that the cation associations with this site are not independent of each other.

Armstrong (1966) has demonstrated that the internal blocking action of TEA^+ is overcome by an inward movement of potassium ions from a potassium-rich external medium. We have demonstrated that the inward movement of potassium ions is blocked by external cesium ions presumably moving inward through the delayed conductance channel sufficiently to block the channel to potassium. Armstrong (1966, see Fig. 5 and Table II) states that "as [TEA^+] . . . (inside) increases, the rate of decline of the current tails following positive steps is progressively faster, and the minimum value of the current is generally smaller." This description fits the effects of external Cs^+ exactly. Neither external Cs^+ nor TEA^+ blocks outward current flow through the delayed conductance channel, which again implies a membrane asymmetry. Therefore,we suggest that the site controlling this conductance is located at the inner membrane surface and that its ion interactions are of a different order from those for the early conductance.

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