

# Coupling and Selectivity of Sodium and Potassium Transport in Squid Giant Axons

R. A. SJODIN and L. A. BEAUGÉ

From the Department of Biophysics, University of Maryland School of Medicine, Baltimore, Maryland 21201, and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543

## INTRODUCTION

Our purpose in this paper is to present some experimental findings that have a bearing on the transport of sodium and potassium across the membrane of the squid giant axon. As in a variety of cellular types that actively transport sodium ions, a marked drop in the efflux of sodium ions from squid giant axons occurs when potassium ions are removed from the external solution (Caldwell et al., 1960). As both sodium exit and potassium entry are susceptible to inhibition by application of cyanide and other metabolic inhibitors, the implication is that some form of coupling exists between K influx and Na efflux. Caldwell et al. (1960) observed that injection of arginine phosphate into cyanide-poisoned axons restored the coupling between K entry and Na exit.

In the experiments described here, an attempt was made to vary the inside concentration of sodium ions in squid giant axons and the outside concentration of potassium and potassium-like ions ( $Rb^+$  and  $Cs^+$ ) in order to study the influence of such variations on the coupled transport of sodium and potassium ions.

## METHODS

The methods employed are described in detail by Sjödin and Beaugé (1967). The main method applied was the microinjection of  $^{24}Na$  ions for the study of sodium efflux. The cell and micro-syringe used for injection of  $^{24}Na$  were of the type described by Hodgkin and Keynes (1956). Experiments performed to determine potassium influx were all accomplished by the extruded-axoplasm technique to avoid inclusion of  $^{42}K$  entrained in the Schwann cell (Caldwell and Keynes, 1960; Sjödin and Mullins, 1967). The temperatures at which the experiments were performed ranged between 12.5°C and 20°C and are stated in the tables or text.

The following external solution formulations were used:

Solution	pH	[ $Na^+$ ]	[ $K^+$ ]	[ $Rb^+$ ]	[ $Cs^+$ ]	[ $Ca^{++}$ ]	[ $Mg^{++}$ ]	[ $Cl^-$ ]	[ $SO_4^{=}$ ]	[ $HCO_3^-$ ]
						<i>mmoles/liter</i>				
Normal sea water	7.9	423	10	—	—	9.3	48.5	497	25.5	2.2
K-free sea water	7.9	433	—	—	—	9.3	48.5	497	25.5	2.2
Rb sea water	7.9	423	—	10	—	9.3	48.5	497	25.5	2.2
Cs sea water	7.9	423	—	—	10	9.3	48.5	497	25.5	2.2
30 mM K sea water	7.9	403	30	—	—	9.3	48.5	497	25.5	2.2
50 mM Cs sea water	7.9	383	—	—	50	9.3	48.5	497	25.5	2.2

## RESULTS

*Coupled Movements of Sodium and Potassium*

Sodium efflux was measured by the  $^{24}\text{Na}$  injection technique when the external sea water contained 10 mM K and when K was removed from the bathing medium. In some cases, 5 mM cyanide inhibition was employed during the final part of the experiment. The results appear in Table I, where the amount of reduction in sodium efflux taking place when K ions are removed from the bathing medium is indicated for each

TABLE I  
THE EFFECT OF EXTERNAL POTASSIUM  
AND CYANIDE ON SODIUM EFFLUX

Axon No.	Temp.	Final [Na]	Sodium efflux			Amount of efflux shutoff		Coupled $\phi_{\text{Na}}^*$	CN $^-$ -sens. $\phi_{\text{Na}}$
			0 mM K	10 mM K	10 mM K cyanide	0 mM K	10 mM K cyanide	CN $^-$ -sens. $\phi_{\text{K}}$	CN $^-$ -sens. $\phi_{\text{K}}$
	$^{\circ}\text{C}$	mmoles/kg	$\mu\text{moles/cm}^2 \text{ sec}$			$\mu\text{moles/cm}^2 \text{ sec}$			
709A	17	67	28	76	23	48	53	2.1	2.3
709B	17	72	22	88	14	66	74	2.9	3.2
712A	17	122	27	60	21	33	39	1.4	1.7
712B	17	108	26	76	16	50	60	2.2	2.6
720A	17	96	35	59	—	24	—	1.04	
721B	12	115	19	30	—	11	—	0.5	
729A	15	115	43	85	—	42	—	1.8	
804B	15	73	32	95	—	63	—	2.7	
805A	17	93	25	76	—	51	—	2.2	
805B	17	83	17	76	—	59	—	2.6	
805C	15	97	25	108	—	83	—	3.6	
806A	15	95	35	79	—	44	—	1.9	
817A	15	137	55	98	9	43	89	1.9	3.9
817B	15	102	55	88	31	33	57	1.4	2.5
Mean	—	98	32	78	19	46	62	2.0	2.7
$\pm$ SE	—	5.3	3.2	5.1	3.1	4.8	6.9	0.2	0.3

\*  $\phi$  signifies flux.

axon. In cases where CN $^-$  was applied, the amount of reduction in sodium efflux is also included. Potassium influx was measured by soaking axons in  $^{42}\text{K}$ -containing sea water and determining the  $^{42}\text{K}$  content of a sample of extruded axoplasm at the termination of the experiment. In several cases, potassium influx was measured in the presence and absence of cyanide, using paired axons from the same squid. All potassium influxes measured are reported in Table II. A comparison of potassium and CN $^-$ -sensitive sodium efflux with CN $^-$ -sensitive potassium influx is made in the last two columns of Table I. The results of the comparison indicate that two to three sodium ions are transported outward for every potassium ion transported inward. This conclusion is in agreement with that of Caldwell et al. (1960), and this portion of our results may be taken as a confirmation of the work of these authors.

The K sensitivity of Na efflux was found to be quite variable, the ratio  $(\text{Na efflux})_{\text{K}^- \text{free}} / (\text{Na efflux})_{10 \text{ mM K}}$  varying between 0.22 and 0.63 from the data in

Table I. Such a variation was also reported by Caldwell et al. (1960), who observed this ratio to vary between 0.2 and 0.5 or more. These authors found the ratio to be larger in axons which had been isolated for long periods. The average value of the ratio in our work (16 observations) was 0.42, which compares with the average value of 0.35 observed by Caldwell et al. A mean value of 0.30 was obtained by Hodgkin and Keynes (1955) for *Sepia* giant axons.

TABLE II  
THE EFFECTS OF CYANIDE AND THE  
INTERNAL SODIUM CONCENTRATION ON POTASSIUM  
INFLUX (TEMPERATURE 17.5°C)

Axon No.	Control*		5 mM CN <sup>-</sup> *		Stimulated in Na sea water		Stimulated in Li sea water		Cyanide-sensitive K influx
	K influx	Final [Na] <sub>i</sub>	K influx	Final [Na] <sub>i</sub>	K influx	Final [Na] <sub>i</sub>	K influx	Final [Na] <sub>i</sub>	
	$\frac{\mu\text{moles}}{\text{cm}^2 \text{ sec}}$	mmoles/kg	$\frac{\mu\text{moles}}{\text{cm}^2 \text{ sec}}$	mmoles/kg	$\frac{\mu\text{moles}}{\text{cm}^2 \text{ sec}}$	mmoles/kg	$\frac{\mu\text{moles}}{\text{cm}^2 \text{ sec}}$	mmoles/kg	
819A,A'	30	80	15	166	—	—	—	—	15
823A,A'	38	86	6	268	—	—	—	—	32
824A,A'	24	94	5	137	—	—	—	—	19
824B,B'	31	92	3	256	—	—	—	—	28
818A	21	106	—	—	—	—	—	—	—
524A,A'	—	—	—	—	32	95†	23	28†	—
524B,B'	—	—	—	—	26	89†	18	43†	—
524C,C'	—	—	—	—	34	87†	19	29†	—
614A,A'	—	—	—	—	43	114	15	20	—
614B,B'	—	—	—	—	33	125	12	23	—
614C,C'	—	—	—	—	40	182	27	22	—
615A,A'	—	—	—	—	32	129	20	24	—
615B,B'	—	—	—	—	46	157	22	33	—
Mean	29	92	7	207	36	122	20	22	23
±SE	2.9	4.4	2.7	32.5	2.3	11.9	1.8	3.4	4.0

\* Experiment lasted 3 hr.

† Stimulated 50 per sec.

An attempt was made to discover possible reasons for the variation in the (K-free/10 mM K) ratio of Na fluxes. No significant correlation could be found between the value of this ratio and the condition of the axon membrane as judged by the magnitudes of the resting and action potentials. Neither was there a correlation between the magnitude of the ratio and the magnitude of the sodium efflux. The possible influence of a variation in the internal sodium concentration was investigated as described in the next section.

*The Influence of Variation in the Internal Sodium Concentration on the Potassium Sensitivity and the Rate of Sodium Transport*

Pairs of axons isolated from the same squid were used to obtain conditions of both high and low internal sodium concentration for a direct comparison of results. One

of a pair of axons from the same squid was electrically stimulated in K-free sea water for 30 min at 50 impulses/sec at 15°C. The other member of the pair was stimulated in an identical manner in a K-free lithium-substituted sea water (all NaCl replaced by LiCl). The increased ionic exchanges resulting during excitation resulted in axons with inside sodium concentrations around 30 mmoles/kg (Li sea water stimulation) and around 200 mmoles/kg (Na sea water stimulation). Axons were then microinjected with  $^{24}\text{Na}$ , and sodium efflux was measured in the presence and absence of external potassium ions as before. Some measurements were made in lithium-substituted sea water to test the influence of external lithium ions. The results appear in Table III, where the (K-free/10 mM K) ratio of Na efflux is presented together with the rate constant for  $^{24}\text{Na}$  loss occurring in 10 mM K Na sea water. The latter figures may be taken as indicative of the intrinsic rate of sodium transport, i.e. the rate of pump operation per gram ion of sodium.

The findings indicate that the K sensitivity of Na efflux, as measured by the (K-free/10 mM K) ratio, may decrease somewhat at very high internal sodium concentrations. The ratios observed for the limited number of low internal sodium cases lie well within the normal range. In addition, any influence of the substitution of lithium for sodium in the external sea water is rather slight. The rate constants for  $^{24}\text{Na}$  loss appear to be about the same for axons with very high sodium contents as for axons with rather low sodium contents. This must mean that sodium efflux is approximately proportional to sodium concentration over a large range in the internal sodium concentration. It is also apparent that a rather large magnitude of sodium efflux disappears upon removal of potassium from the external medium bathing axons with a very high sodium content.

#### *The Dependence of Potassium Influx on the Internal Sodium Concentration*

As shown in Table II, the effect of 5 mM  $\text{CN}^-$  was, on the average, to reduce potassium influx to about 25% of the value observed in the absence of inhibitor. As it is not certain that maximal metabolic inhibition was achieved, the magnitude of the active inward transport of potassium must be at least the amount of influx reduction occasioned by the presence of cyanide. A question arose as to the dependence of this fraction of potassium influx on the sodium concentration inside the axon. If the inward movement of potassium is intimately related to the outward movement of sodium, as seems likely, one would expect the magnitude of inward potassium movement to depend on the rate of sodium pumping and hence on the inside sodium concentration. Paired axons with high and low sodium contents were prepared by the technique described in the previous section.  $\frac{1}{2}$ -hr stimulation periods were employed at a temperature of 18°C. The frequency of stimulation was 100 impulses/sec unless otherwise indicated. As before, potassium influx was measured by the extruded axoplasm technique using  $^{42}\text{K}$  as a tracer. The results of these experiments are included in Table II. It is evident that potassium influx shows a strong dependence on the internal sodium concentration. In some cases potassium influx was almost three times higher in the sodium-rich member of the pair of axons than in the axon with a low sodium content. Comparing the figures on the average shows that an approximately 5- to 6-fold increase in the internal sodium concentration resulted in

an approximate doubling of potassium influx. It is likely that a small fraction of potassium influx is not coupled to sodium efflux in a metabolically dependent manner. This fraction of potassium influx should be subtracted from the total magnitudes observed to obtain the coupled fraction of influx. Although we have no direct way of accomplishing this for each case, it seems reasonable to assume that no great error would result from taking the residual potassium influx remaining during inhibition with cyanide as a measure of the uncoupled portion of inward potassium movement.

TABLE III  
SODIUM-24 RATE CONSTANT RATIOS IN AXONS  
WITH LOW AND HIGH INTERNAL SODIUM CONCENTRATIONS  
IN SODIUM AND LITHIUM SEA WATER

Axon No.	Temp. °C	Final [Na] <sub>i</sub> mmoles/kg	Sodium SW†		Lithium SW 0 K/10 K	10 K Na SW	0 K Na SW	Rate constant in 10 K Na SW min <sup>-1</sup> /1000
			0 K/10 K*	30 K/10 K		10 K Li SW	0 K Li SW	
810A	15	19	0.33	—	0.44	0.98	0.70	4.4
810B	15	17	0.51	—	0.56	1.01	0.92	2.7
811A	15	37	—	—	0.49	1.02	—	3.4
812B	15	30	—	—	0.51	—	—	—
Mean		26	0.42		0.50	1.003	0.81	3.5
±SE		4.7	—		0.03	0.001	—	0.49
813A	15	160	0.62	1.01	—	—	—	3.3
813B	15	205	0.55	1.29	—	—	—	3.4
816A	17	219	0.54	1.00	—	—	—	5.3
Mean		195	0.57	1.10				4.0
±SE		18	0.03	0.09				0.64

\* 0 K/10 K signifies the ratio of the rate constant for <sup>24</sup>Na loss observed in K-free sea water to that observed in 10 mM K sea water. All numbers before K refer to external solution concentrations in mM units.

† The abbreviation SW signifies sea water.

From Table II, the average cyanide-insensitive potassium influx is 7 pmoles/cm<sup>2</sup> sec. Subtracting this amount of flux from the total influxes observed results in somewhat greater percentage increases in the coupled potassium influx when the internal sodium concentration is raised. The rough rule of a doubling of coupled potassium influx for a 5- to 6-fold increase in the inside sodium concentration still seems to hold rather well.

#### *The Effect of the Nature and Concentration of the External Coupling Cation on Sodium Efflux*

In some of the experiments performed, the external potassium concentration was varied to determine the effect on sodium efflux. The effects of varying the external

potassium concentration were such that Na efflux with  $[K]_o = 1$  mM was indistinguishable from Na efflux in K-free sea water;  $[K]_o = 5$  mM was about 75% as effective as  $[K]_o = 10$  mM in eliciting increased Na efflux; and  $[K]_o = 30$  mM was only slightly more effective, on the average, than  $[K]_o = 10$  mM (Table III). It is concluded that the sodium efflux-promoting action of external potassium ions has reached about 90% of saturation at an external potassium concentration of 10 mM

TABLE IV  
THE SELECTIVITY OF CATION-COUPLED SODIUM EFFLUX  
FOR THE SERIES POTASSIUM, RUBIDIUM, AND CESIUM

Axon No.	Temp.	0 K/10 K*	10 Cs/10 K	50 Cs/10 K	10 Rb/10 K
	°C				
714A	17	0.54	—	—	0.96
714B	17	0.49	—	—	0.99
716A	17	—	—	—	1.02
720A	17	0.59	—	—	1.10
721A	17	0.66	—	—	0.74
721C	17	—	—	—	1.09
722A	17	0.41	—	—	0.69
722B	12	0.41	—	—	0.89
805C	15	0.24	—	—	0.84
806A	—	0.38	—	—	0.79
729A	15	0.35	0.55	—	0.92
803A	15	0.46	0.46	—	—
803B	15	0.36	0.50	—	—
804A	15	0.47	0.60	0.74	—
804B	15	0.31	—	0.89	—
805A	17	0.33	—	0.94	—
805B	15	0.22	—	0.68	—
802A	15	0.50	0.62	—	—
Mean		0.42	0.55	0.81	0.91
±SE		0.03	0.03	0.06	0.04

\* The column headings have the same meanings as in Table III. All numbers before K, Rb, and Cs refer to external solution concentrations in mM units.

and that the over-all curve relating sodium extrusion to the external potassium concentration is S-shaped.

In another series of experiments, the action of external rubidium or cesium on sodium efflux was compared with the action of potassium. The procedure followed was always to obtain a base line sodium efflux in potassium-free sea water and then to compare the action of 10 mM Rb or 10 mM Cs on Na efflux with the action of 10 mM K using the same axon. In some cases the 10 mM Rb or 10 mM Cs test preceded the 10 mM K control and in some cases the Rb or Cs test followed the K test. No differences in results were observed as a consequence of alterations in sequence. In all cases an efflux measurement interval in K-free sea water (also Rb- and Cs-free) separated the K from the Rb or Cs tests. All extra sodium effluxes elicited by the

external presence of these cations thus appeared as efflux "square waves" superimposed on a base line efflux in K-, Rb-, and Cs-free sea water.

The results appear in Table IV as ratios of rate constants for  $^{24}\text{Na}$  loss from the same axons. Roughly stated, the results can be summarized by saying that the action of 10 mM Rb does not differ much from the action of 10 mM K, while the action of 10 mM Cs does not differ very much from the action of K-free conditions. The small Na efflux-promoting action of 10 mM Cs is, however, statistically significant. If the ion selectivity of the cation-coupled sodium transport system in the squid giant axon membrane is taken as the relative increment in sodium efflux, over that occurring in K-, Rb-, and Cs-free sea water, that is occasioned by the external presence of a given concentration (10 mM) of the coupling cation, the following relative selectivities are obtained: K:Rb:Cs = 1:0.84:0.22.

The rather low degree of action of external cesium ions on sodium efflux could be due to an unfavorable balance between two actions, one inhibitory, the other enhancing. Should this be the case, raising the external cesium concentration might be expected to increase both actions more or less in pace with little change in net effect on sodium efflux. Some experiments were performed to check this point. Increasing  $[\text{Cs}]_o$  5-fold to 50 mM brought about an approximately 3-fold increase in the extra, cation-stimulated, sodium efflux occurring when  $[\text{Cs}]_o$  was 10 mM. In addition, a 10 mM concentration of external Cs did not interfere with the usual action of 10 mM K. It is concluded that the main action of external cesium is a stimulation of sodium efflux but to a much lower degree than that observed with potassium.

#### DISCUSSION

The two main subjects requiring discussion are the quantitative relations between the coupled sodium and potassium movements observed, and the possible significance of the selectivity sequence found for K, Rb, and Cs.

Though the data in Tables I and II provide support for the notion that two to three sodium ions are actively transported outward for every potassium ion transported inward, agreeing with the work of Caldwell et al. (1960), the possible influence of the sodium concentration inside the axon must be considered. To obtain the flux data in Table I, rate constants observed for  $^{24}\text{Na}$  loss were applied to the final measured sodium concentrations. The average final  $[\text{Na}]_i$  from Table I is 98 mmoles/kg. As this is higher than the average value for fresh axons (about 50 mmoles/kg), it is evident that sodium has been gained by axons during the experiments. This is not surprising, as experiments lasted at least 2 hr and a considerable number of time intervals for Na efflux occurred under K-free conditions, where the rate of sodium pumping is reduced. If an attempt is made to correct the sodium contents to average values for the experimental periods, the average of the next-to-last column of Table I becomes 1.5 instead of 2.0 and the average of the last column becomes 2.0 instead of 2.7. The general conclusion that about two Na ions are transported for every K ion is thus not altered. One must, however, examine the consequences of considering cases of still lower inside sodium concentration. Hodgkin and Keynes (1956) observed that sodium efflux is approximately proportional to the internal sodium con-

centration up to values of 130 mM. Our findings (Table III) indicate that the proportionality rule applies rather well up to values as high as 219 mmoles Na per kg. It should thus be possible to estimate approximately the extent of Na to K coupling at the low and high extremes of inside sodium concentration as well. From the average values presented in Table II, potassium influx is 20 pmoles/cm<sup>2</sup> sec when the internal sodium content is 22 mmoles/kg. Recalling that the best estimate of uncoupled K influx from the cyanide-insensitive residual influx is 7 pmoles/cm<sup>2</sup> sec, the actively coupled inward K transport under these conditions is 13 pmoles/cm<sup>2</sup> sec. To estimate the K-coupled Na efflux under these conditions, the proportionality to concentration rule for Na efflux is applied together with the average value of the fraction  $(\text{Na efflux})_{\text{K-free}}/(\text{Na efflux})_{10 \text{ mM K}}$ . For  $[\text{Na}]_i = 22 \text{ mmoles/kg}$ , total Na efflux (10 mM K) is estimated to be  $(22/98) \times 78 = 18 \text{ pmoles/cm}^2 \text{ sec}$ . The amount of this Na efflux that disappears upon removal of external potassium is equal to  $(1 - 0.42) \times 18$  or 10 pmoles/cm<sup>2</sup> sec. The Na:K coupling ratio under the low-sodium conditions is thus 10/13, which does not differ significantly from 1/1 considering the precision of the values used in the calculation. The same estimate can be made under conditions of a high internal sodium concentration. Potassium influx is 36 pmoles/cm<sup>2</sup> sec when the internal sodium concentration is 122 mmoles/kg (Table II). The actively coupled potassium influx is, accordingly,  $36 - 7 = 29 \text{ pmoles/cm}^2 \text{ sec}$ . Total sodium efflux in 10 mM K under these conditions is estimated to be, on the average,  $(122/98) \times 78 = 97 \text{ pmoles/cm}^2 \text{ sec}$ . The amount of Na efflux that would be expected to disappear upon removal of external potassium under these conditions is  $(1 - 0.42) \times 97 = 56 \text{ pmoles/cm}^2 \text{ sec}$ . The Na:K coupling ratio estimated is thus 56/29, which does not differ significantly from 2/1. Though these calculations must be regarded as rough approximations, in that average values are used and coupling is not reckoned on the same axon, the estimates are probably fairly reliable in view of the rather large number of experiments performed to obtain the average values. Though very accurate measurements of Na:K coupling ratios may have to await Na and K flux measurements on one and the same axon using the internal perfusion or dialysis method (Brinley and Mullins, 1967) and double radioactive labeling techniques, the estimates presented define some limits for the parameters involved and form a basis for comparing future results obtained by perfusion techniques with results observed on essentially intact axons.

The selectivity sequence and ratios we have observed for the actions of K, Rb, and Cs on Na transport in the squid giant axon are of interest in that they may provide a clue as to the possible molecular basis for the coupled Na and K movements. Skou (1960) has proposed a model for active transport in nerve cells, and other cells as well, based on a magnesium-sodium-potassium-activated ATPase which may be located in the membrane. An extensive series of in vitro experiments concerned with the activation by cations of an ATPase isolated from nerve has been performed by Skou (1960). Among the experiments reported are some dealing with the relative abilities of cations in the series K, Rb, and Cs to activate the enzyme in the presence of magnesium and sodium. The relative capabilities for activation are tabulated below and are compared with the selectivities found in our work on transport in the



squid giant axon. Another example of cation selectivity in intact nerve included for comparison is from the work of Baker and Connelly (1966). The numbers reported give the relative effectiveness of K, Rb, and Cs in increasing oxygen uptake by crab nerve.

	Relative selectivity			Reference
	K	Rb	Cs	
1	0.84	0.22		Sjodin and Beaugé (1967) (Stimulation of Na extrusion in squid axons)
1	0.91	0.11		Baker and Connelly (1966) (Relative $K_m$ for stimulation of $O_2$ uptake in crab nerve)
1	0.85	0.23		Skou (1960) (Relative $K_m$ for stimulation of ATPase activity from crab nerve)
1	0.58	0.18		Skou (1960) (Relative concentration needed to obtain $\frac{1}{2}$ the potassium $V_{max}$ of ATPase activity in crab nerve)

As the ATPase that has been extracted from the sheath of the squid giant axon is similar to that from crab nerve studied by Skou, the data suggest that the model proposed by Skou is a possible basis for transport in squid giant axons.

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