The Influence of External Cations
and Membrane Potential on Ca-Activated
Na Efflux in *Myxicola* Giant Axons

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ABSTRACT   In microinjected *Myxicola* giant axons with elevated $[Na]_i$, Na efflux was sensitive to Ca_o under some conditions. In Li seawater, sensitivity to Ca_o was high whereas in Na seawater, sensitivity to Ca_o was observed only upon elevation of $[Ca]_o$ above the normal value. In choline seawater, the sensitivity of Na efflux to Ca_o was less than that observed in Li seawater whereas Mg seawater failed to support any detectable Ca_o-sensitive Na efflux. Addition of Na to Li seawater was inhibitory to Ca_o-sensitive Na efflux, the extent of inhibition increasing with rising values of $[Na]_o$. The presence of 20 mM K in Li seawater resulted in about a threefold increase in the Ca_o-activated Na efflux. Experiments in which the membrane potential, V_m, was varied or held constant when $[K]_o$ was changed showed that the augmentation of Ca-activated Na efflux by K_o was not due to changes in V_m but resulted from a direct action of K on activation by Ca. The same experimental conditions that favored a large component of Ca_o-activated Na efflux also caused a large increase in Ca influx. Measurements of Ca influx in the presence of 20 mM K and comparison with values of Ca-activated Na efflux suggest that the Na:Ca coupling ratio may be altered by increasing external $[K]_o$. Overall, the results suggest that the Ca_o-activated Na efflux in *Myxicola* giant axons requires the presence of an external monovalent cation and that the order of effectiveness at a total monovalent cation concentration of 430 mM is K + Li > Li > Choline > Na.

INTRODUCTION

In *Myxicola* giant axons with elevated $[Na]_i$, Na efflux becomes sensitive to external Ca in Li seawater (Abercrombie and Sjodin, 1977 *a*). Sensitivity of Na efflux to Ca was not observed in normal Na seawater, nor was it observed in Li seawater at normal values of $[Na]_i$. Another feature of this component of Na efflux in *Myxicola* giant axons is that it is not inhibited by ouabain at concentrations that maximally inhibit the Na pump. These properties of the Ca-activated component of Na efflux in *Myxicola* giant axons are at least qualitatively similar to those reported for the Ca-activated Na efflux observed in squid giant axons (Baker et al., 1969). The Ca_o-dependent Na efflux in squid giant axons is accompanied by a Na_i-dependent Ca influx, a fact that strongly suggests the presence of a Na:Ca exchange mechanism.

The purpose of the present work is to study some of the properties of Ca-activated Na efflux in *Myxicola* giant axons in greater detail and to investigate Ca influx under similar conditions to see if a Na:Ca exchange process is also present in this preparation. The main area of the investigation is the sensitivity of Ca-activated Na efflux to the external cations Na, Li, Mg, La, K, and choline. As any action of external K could be due to either a specific effect of K on membrane sites involved in Na:Ca exchange or to a secondary effect via membrane depolarization, it was also necessary to investigate the possible influence of V_m on the Ca-activated component of Na efflux. A preliminary report of this investigation has been made (Abercrombie and Sjodin, 1977*b*).

MATERIALS AND METHODS

Myxicola were obtained from Marine Research Associates, New Brunswick, Canada. Handling of animals, dissection of giant axons, microinjection of ^{22}Na for Na efflux determination, and counting of radioactivity in axoplasm and efflux samples were as previously described (Abercrombie and Sjodin, 1977*a*). Unless otherwise indicated, axons were microinjected with nonradioactive Na to achieve a final $[Na]_i \cong 100$ mM/kg for the reason described previously and in the present text. Also, unless otherwise stated, experiments were performed in the presence of 10^{-4} M ouabain for reasons previously discussed and also to avoid activation of the Na:K pump by K in the experiments performed at elevated $[K]_o$. Efflux samples of ^{22}Na were taken at 3-min intervals. Sample radioactive counts were back-added to the final radioactive counts remaining in the axoplasm at the end of the experiment. The fraction of radioactivity remaining in the axoplasm during the experiment was plotted semilogarithmically against time to obtain rate constants for ^{22}Na efflux. The rate constants remained stable in a solution of constant composition except in cases in which the Ca$_o$-activated Na efflux was occurring at a very high rate as in (K + Li) seawater. In such cases a rate constant that declined at a moderate rate with time in a given solution was often observed (Fig. 6). The reason for the decline is not known. It is not due to axon deterioration, however, inasmuch as the decline was not observed in cases where the Ca$_o$-activated Na efflux was occurring at a lower rate nor was it observed in the K-activated, ouabain-sensitive Na pump fraction of Na efflux. When a declining rate constant occurred, solution changes were always of the type A-B-A where the letters refer to solution composition. In such cases, the rate constant for a given time interval was taken as the average over the interval. The difference in rate constant between solutions A and B was then obtained using the average of the two rate constants in A for the initial and final time intervals.

Ca Influx Measurement

Axons were soaked for 30 min in seawaters of varying composition which also contained ^{45}Ca of known specific activity. At the termination of influx, axoplasm samples were taken and prepared for radioactive counting as previously described. As before, radioactivity was determined using a Beckman low level beta counter (Beckman Instruments, Inc., Fullerton, Calif.). Influx was calculated from a knowledge of radioactivity, the specific activity of Ca in the loading solution, the weight of axoplasm and the radius of the fiber.

Solutions

The experimental seawater formulations used are summarized in Table I. Solutions with higher K concentrations or different concentrations of Ca were made by replacing an

osmotic equivalent of the major cation. Na-free and full Na solutions were mixed to obtain the desired Na concentration. The pH was adjusted to 7.5 and osmolarity monitored at 950 mosM as previously described. Experiments were performed at 11°C.

Membrane Potential Control

In some experiments it was necessary to prevent change in resting potential (V_m) when $[K]_o$ was altered and also to alter V_m with $[K]_o$ held constant. This was accomplished by means of a platinum electrode attached to the microinjection capillary and a DC current source. The experimental arrangement is diagrammed in Fig. 1. The Pt-blacked Pt electrode was positioned after microinjection of ^{22}Na so as to include all of the injected region made visible with low concentration of a dye. The 50 V DC voltage source was used to pass current through a 10^6 Ω resistor providing a constant current controllable by a potentiometer which was adjusted manually. At constant $[K]_o$, V_m could be altered to the desired new value in about 10 s. Current was monitored with a microammeter (not

TABLE I
ARTIFICIAL SEAWATER SOLUTIONS

Solution	CaCl₂	MgCl₂	MgSO₄	NaCl	LiCl	ChoCl	Mannitol	Tris-HEPES	EDTA
					mM				
K-free (Na)	10	25	25	430				5	0.5
K-free (Li)	10	25	25		430			5	0.5
K-free (Cho)	10	25	25			430		5	0.5
K-free (Mg-mannitol)	10	164	25				450	5	0.5

shown). When $[K]_o$ was altered and it was desired to maintain V_m constant, the potentiometer was adjusted to maintain a constant V_m during the ≅ 1-min period required to change solutions and reach stable conditions. The value of V_m was read with an oscilloscope using a high impedance preamplifier to record from an intracellular electrode.

RESULTS

The Effects of Na_o, Li_o, and Ca_o on Na Efflux in the Presence of Ouabain

The effect of variations in $[Ca]_o$ on the Ca_o-sensitive Na efflux was determined in both Li seawater and Na seawater. All experiments were performed with elevated $[Na]_i$ and in the presence of 10^{-4} M ouabain to enhance resolution of flux changes. Measurements were made in Ca-free solutions and in solutions containing different values of $[Ca]_o$. The Ca-sensitive Na efflux is defined as the difference between efflux measured in the presence of a given value of $[Ca]_o$ and that measured in a Ca-free medium. The results are plotted in Fig. 2 as rate constants for ^{22}Na efflux vs. $[Ca]_o$. Actual Na efflux in pmol/cm²·s is proportional to the rate constant via a factor that involves the value of $[Na]_i$ and the diameter for any given axon. As an approximate calibration, a rate constant of 1×10^{-3} min^{-1} is equivalent to an efflux of about 30 pmol/cm²·s for the values of $[Na]_i$ holding in these experiments (see Table III). Lowering $[Na]_i$ reduced sensitivity of Na efflux to Ca_o, and at normal $[Na]_i$ no Ca_o-sensitive Na efflux
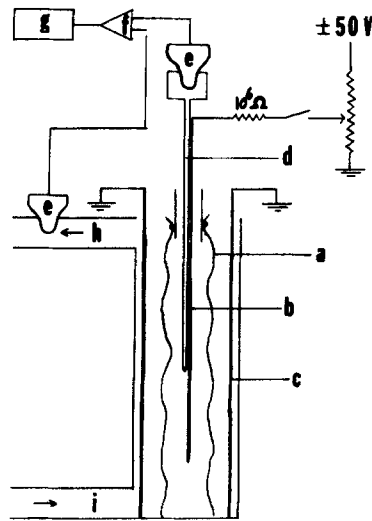
FIGURE 1.   Diagram of experimental arrangement for altering and controlling V_m. (a) *Myxicola* axon membrane, (b) internal current passing electrode, (c) external ground electrode, (d) internal potential measuring capillary, (e) calomel electrodes, (f) differential preamplifier, (g) recorder or oscilloscope, (h) outflow, (i) inflow.
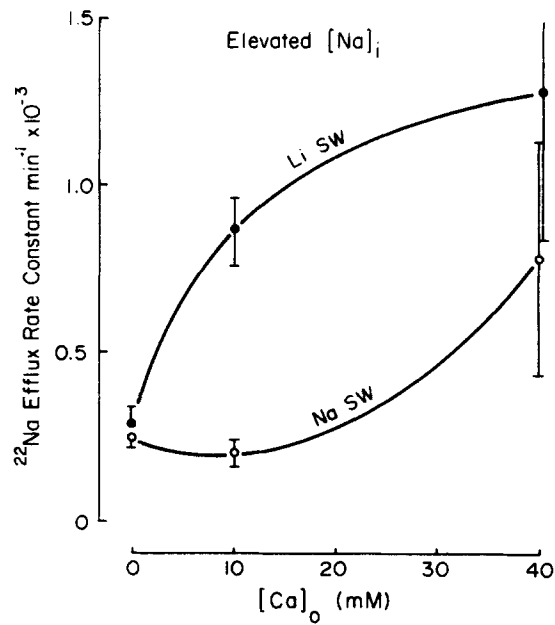

FIGURE 2.   The effect on Na efflux of replacing Na seawater with Li seawater at different values of $[Ca]_o$. All solutions were K-free and contained 10^{-4} M ouabain. The vertical bars represent ± 1 SE for four or more measurements.

was observed even in Li seawater. The results show that increasing $[Ca]_o$ causes an increasing activation of a component of Na efflux that is considerably higher in Li seawater than in Na seawater. At the normal value of $[Ca]_o$ = 10 mM, no Ca_o-sensitive Na efflux was observed in Na seawater in confirmation of our previous work (Abercrombie and Sjodin, 1977 a). The curve in Li seawater seems to approximate simple Michaelis kinetics with a K_m for Ca of $\cong$ 8 mM. The curves would have a partial explanation if the binding of an external Li ion at a membrane site increased the affinity of an external activation site for Ca ions above the value existing in Na seawater.

The Influence of Other Cations

In the next series of experiments, $[Ca]_o$ was varied when Na_o was replaced with substitutes other than Li. The results are illustrated in Fig. 3. In choline
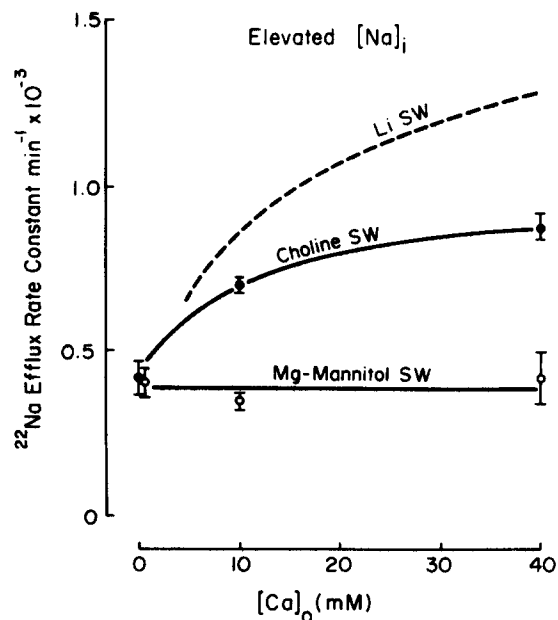

FIGURE 3.   The influence of substituting choline or Mg for Na in seawater on Na efflux in the absence and in the presence of different concentrations of Ca. All solutions were K-free and contained 10^{-4} M ouabain. The vertical bars represent $\pm$ 1 SE for four or more measurements.

seawater, an activation due to Ca_o was clearly apparent, whereas in Mg seawater no activation of Na efflux by Ca_o was observed at any value of $[Ca]_o$ employed. In Fig. 3, the curve obtained in Li seawater is included as a reference. Above $[Ca]_o$ = 10 mM, activation by Ca_o in choline seawater is intermediate between activation in Na and Li seawater until [Ca] = 40 mM, when activations in choline and in Na seawater are about the same. The results indicate that no activation of Na efflux by Ca_o occurs when only divalent cations are present in the external medium. This could signify that monovalent cations are required externally for

activation by Ca_0 to occur, or alternatively, that high concentrations of Mg inhibit the activation due to Ca_0.

The Stimulating Effect of K_0

The influence of external K ions on Ca-activated Na efflux was investigated. Raising $[K]_0$ to a value of 20 mM resulted in about a threefold increase in the Ca-activated component of Na efflux over the value observed in K-free, Li seawater. Raising $[Na]_0$ by substitution for Li resulted in a diminution of Ca-activated Na efflux both in K-free seawater and in seawater with $[K]_0 = 20$ mM. The results are summarized in Fig. 4 where the Ca-activated component of Na efflux is plotted against $[Na]_0$ in the Na − Li seawater mixtures. The influence of external K is most pronounced when $[Na]_0 = 100$ mM. When $[Na]_0 = 200$
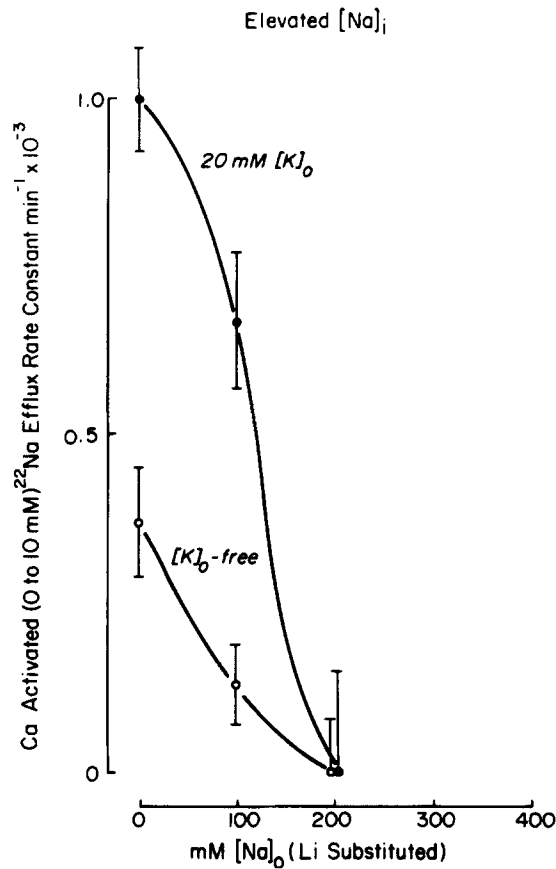

FIGURE 4. The Ca-activated component of Na efflux is plotted against $[Na]_0$ in Na-Li seawater mixtures for K-free media and for media in which $[K]_0 = 20$ mM. The ordinate axis refers to the difference in rate constant for ^{22}Na efflux between that observed in the presence of 10 mM Ca_0 and that observed in Ca-free media. All solutions contained 10^{-4} M ouabain. The vertical bars represent ± 1 SE for four or more measurements.

mM, no activation by Ca_0 occurs either in the presence or absence of K and when $[Na]_0 = 0$, the percentage reduction in Ca-activated Na efflux occurring upon removal of K_0 is less than when $[Na]_0 = 100$ mM. Measurements of Na efflux were also made at various other values of $[K]_0$. The results are shown in Fig. 5. The enhancing effect of K_0 was not complete at 20 mM because a greater Ca-sensitive fraction was observed at $[K]_0 = 50$ mM. Also, it should be observed that at a commonly employed K concentration, $[K]_0 = 10$ mM, K is exerting a
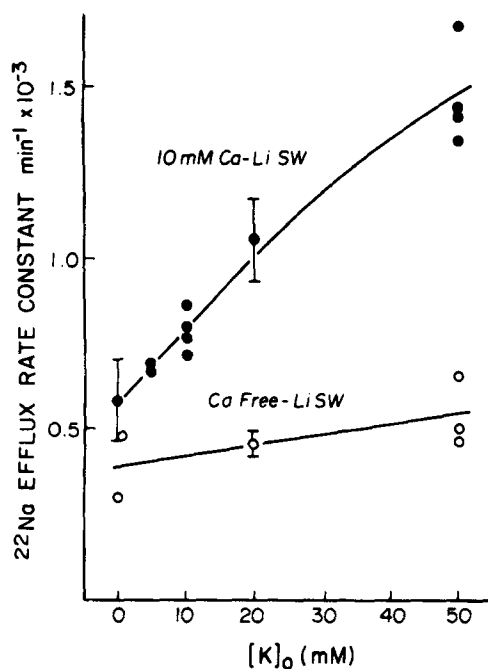

FIGURE 5.   The influence of $[K]_0$ on Na efflux in Li seawater with and without Ca. All solutions contained 10^{-4} M ouabain. All axons were subjected to experimental elevation of $[Na]_i$. The points with vertical bars represent average values $\pm$ 1 SE for more than four measurements.

detectable influence on activation by Ca_0 in Li seawater. The presence of 5 mM La ions completely inhibited the Ca-activated Na efflux occurring in 20 mM K, Li seawater, as illustrated in Fig. 6. The Na efflux was reduced to the value observed in Ca-free 20 mM K, Li seawater, indicating that all of the Ca-activated Na efflux under these conditions is blocked by La.

Similar experiments were performed in choline seawater. Addition of K to choline seawater gave rise to increases in the Ca_0-activated Na efflux as in the case of Li seawater. In contrast with results in Li seawater, however, addition of Na to K-free choline seawater first increased the magnitude of the Ca_0-sensitive Na efflux as $[Na]_0$ was increased up to 100 mM. When $[Na]_0$ was increased to values above 100 mM in choline/Na seawater mixtures, the Ca_0-sensitive Na efflux diminished. The effects of K_0 are thus similar in Li and choline seawaters

whereas the effects of Na_0 differ in that values of $[Na]_o < 100$ mM enhance Ca_o-activated Na efflux in choline seawater but are inhibitory in Li seawater.

Separation of Effects of V_m and $[K]_o$

An interesting question arose regarding the effect of 20 mM K on the Ca-activated Na efflux. The enhancing effect of K_0 could be due to a secondary
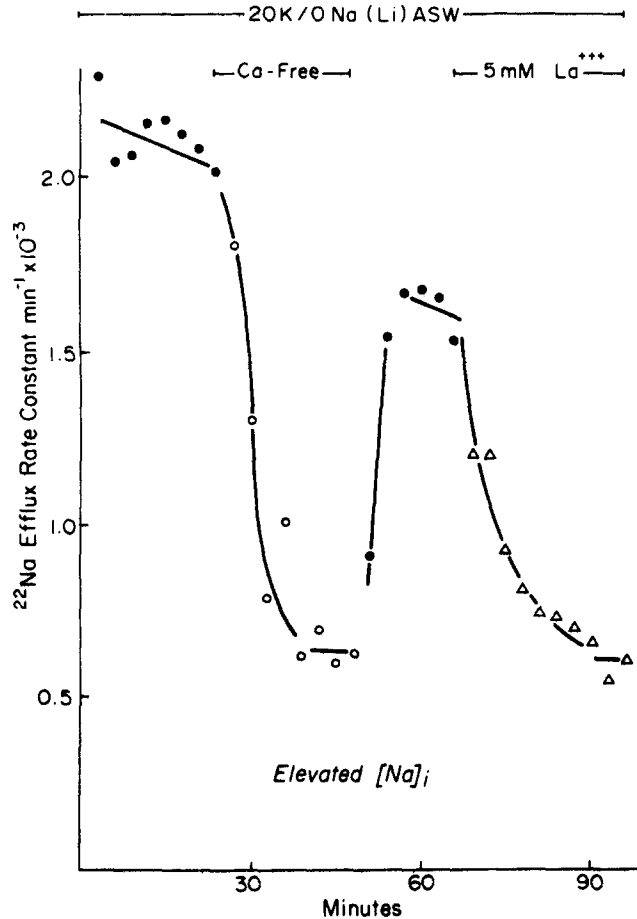

FIGURE 6. The inhibitory action of La ions on Ca-activated Na efflux ($[Ca]_o = 10$ mM) in 20 mM K, Li seawater (ASW = artificial seawater). All solutions contained 10^{-4} M ouabain.

action via membrane depolarization. To check this point, some experiments were performed in which the membrane potential was varied independently of $[K]_o$ by passing electrical currents from a central wire attached to the potential measuring capillary. A typical experimental protocol is illustrated in Fig. 7. First, Na efflux was measured in K-free, Li seawater with 10 mM Ca. Current was then passed to achieve a depolarization of the membrane about equivalent to that produced by $[K]_o = 20$ mM. As is evident from the record, no change in

Na efflux occurred. After returning to the initial membrane potential, the solution was changed to 20 mM $[K]_o$, Li seawater. The membrane depolarized by about the amount that occurred with current flow. Now, however, the depolarization was accompanied by a large increase in Na efflux. Furthermore, a hyperpolarization by current flow during the period of high Na efflux failed to lower the flux. Subsequent removal of Ca_o promptly reduced Na efflux to the value measured before addition of K indicating that all of the increase in Na efflux brought about by K_o was dependent on external Ca. The conclusion from this experiment and others similar to it is that the stimulating action of K_o on
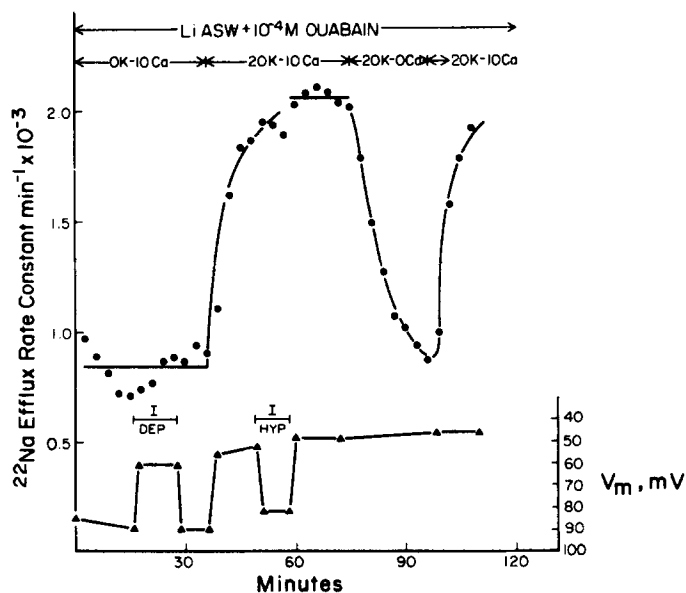

FIGURE 7.   The effects of V_m compared with the effect of $[K]_o$ on Ca-activated Na efflux in Li seawater (ASW). Numbers before constituents refer to mM concentrations. As before, $[Na]_i$ was elevated to around 100 mM by microinjection of Na_2SO_4 before the experiment.

the Ca-activated component of Na efflux is a direct effect on membrane sites at which Ca ions activate Na efflux rather than an indirect effect via the membrane potential. The membrane potential per se, on the other hand, does not appear to have a regulatory effect on the Ca-activated component of Na efflux.

To answer a possible criticism that different conclusions might have been reached had ouabain not been present, similar experiments were performed in the absence of ouabain. The results are shown in Fig. 8. The main difference in the results is that, in the absence of ouabain, membrane depolarization via current flow now has a detectable effect on Na efflux. Depolarizing the membrane by an amount roughly equivalent to that produced by $[K]_o = 10$ mM increased Na efflux in a K-free medium by about 10%. The increase is quite small, however, compared with the increase in Na efflux brought about by $[K]_o = 10$ mM and amounts to only 15% of the K-dependent increase. Furthermore,

the effect of V_m on Na efflux in the absence of ouabain has an adequate explanation that is independent of the action of Ca_o. When a depolarizing current is passed, a net efflux of K ions occurs to carry the current. In a K-free medium with no external Na to inhibit activation of the Na pump by K_o, the outflowing K ions would be expected to have an activating effect on the Na pump in the absence of ouabain but not in the presence of ouabain. Also, the presence of adequate external K should remove or reduce the effect. The results in Fig. 8 show that this prediction is borne out experimentally.

Dependence of Ca Influx on $[Na]_o$

Ca influx was first measured on fresh, normal *Myxicola* giant axons (see Methods). Axons were then microinjected with Na_2SO_4 to achieve elevated
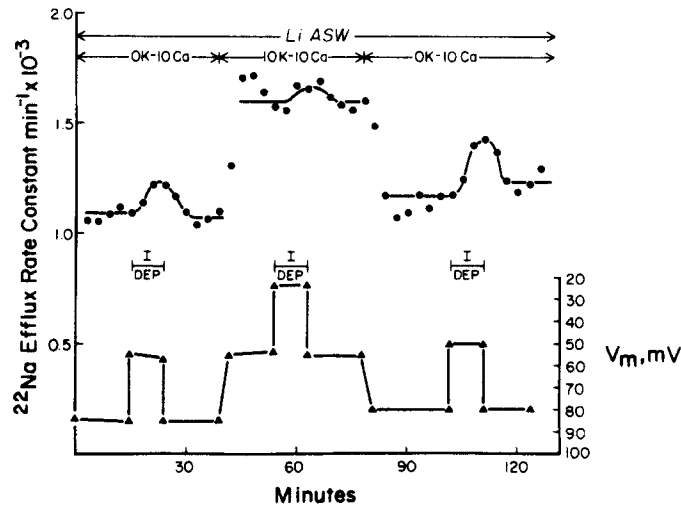

FIGURE 8. The effect of V_m on Na efflux in Li seawater (ASW) in the absence of ouabain.

$[Na]_i$, and Ca influx was measured in different external media. From a knowledge of axon diameter, length of injection path, injection volume, and concentration of the Na_2SO_4 injected, the change in $[Na]_i$ could be calculated for each axon. The normal $[Na]_i$ for fresh axons was taken to be 20 mM, the average between previous results obtained by flame analysis in our laboratory and those reported by Gilbert (1975). The results are summarized in Table II. In Na seawater, a fivefold elevation of $[Na]_i$ produced no significant change in Ca influx. Elevation of $[Na]_i$ and replacement of external Na by Li, however, produced a 3.5-fold increase in Ca influx. The value of Ca influx measured in 20 mM K, Li seawater did not differ significantly from that measured in 0 K, Li seawater. The presence of 5 mM La inhibited Ca influx in Li seawater to values below those measured in Na seawater. The results clearly reveal a component of Ca influx that is sensitive to [Na]. This component of Ca influx is activated by the same conditions that favor a Ca_o-dependent Na efflux and inhibited by the

same conditions that inhibit Ca_o-dependent Na efflux, namely high $[Na]_o$ or La.

From a knowledge of the measured rate constants for Na efflux, the axon diameters, and the values of $[Na]_i$, Na efflux can be calculated in units of pmol/ $cm^2 \cdot s$. The Ca-dependent Na efflux can be determined by subtracting efflux in the absence of Ca_o from efflux in the presence of Ca_o. The average values obtained for two conditions, K-free and $[K]_o$ = 20 mM, are presented in Table III. The flux values reported in Tables II and III can then be used to arrive at estimates of the stoichiometry of the Na:Ca interchanges (see Discussion).

TABLE II

Ca INFLUX FROM 10 mM Ca_o SOLUTIONS

External solution, mM	Observations*	$[Na]_i \pm$ SE	Ca influx $\pm$ SE
	n	*mM/kg*	*pmol/cm²·s*
0 K−Na SW	3	20±5	1.28±0.43
0 K−Na SW	3	97±17	1.57±0.37
0 K−Li SW	3	111±12	5.47±1.52
20 K−Li SW	7	90±5	6.71±1.44
20 K−Li SW+La	5	128±12	0.90±0.19

* Number of experimental observations (Ca influx determinations) on separate axons.

TABLE III

10 mM Ca_o-DEPENDENT Na EFFLUX IN Na-FREE (Li) ASW

$[K]_o$	Observations	Rate constant	Diam	Approx. Na_i	Efflux $\pm$ SE
mM	*n*	*×10⁻³ min⁻¹*	*μ*	*mM/kg*	*pmol/cm²·s*
0	6	0.37±0.08	642±24	114±7	11±2.8
20	4	1.0±0.08	675±32	104±10	29±4.4

DISCUSSION

The results show that elevation of $[Na]_i$ in *Myxicola* giant axons gives rise to a component of Na efflux that is activated by Ca_o in a manner that depends strongly on the cation composition of the external medium. The Ca_o-activated Na efflux is large in Li seawater at normal values of $[Ca]_o$ and is accompanied by an increased influx of Ca that is revealed experimentally in axons with elevated $[Na]_i$ when external Na ions are replaced by Li ions. The results are so similar to those observed in squid giant axons that it seems safe to conclude that the same general mechanism for linked Na:Ca transport is present in *Myxicola* giant as is present in squid axons (Baker et al., 1969). The *Myxicola* giant axon is thus a suitable preparation in which to study Na:Ca exchanges. The ratio of the average Ca_o-dependent Na efflux to the average Na_o-sensitive Ca influx in *Myxicola* giant axons is 2.8 ± 40% from the present data in K-free solutions. The range for this ratio observed in squid giant axons was 3-5 (Baker et al., 1969).

The effects of external cations on the glycoside-insensitive Na efflux in squid giant axons has been investigated (Baker et al., 1969; Beaugé and Mullins, 1976). The cations K and Li had mainly an activating effect on glycoside-treated axons as did Na at concentrations below 200 mM. At higher concentrations, Na was inhibitory. Choline had no activating effect above the value of efflux observed in Na seawater. In Mg seawater, however, Na efflux was below that observed in

either choline or Na seawater. In addition, little or no Ca sensitivity of Na efflux was observed in Mg seawater (Beaugé and Mullins, 1976). Their work shows that one must exercise caution in ascribing effects of external cations in glycoside-treated axons to the Ca-sensitive component of Na efflux, as the cations can induce an increment in Na efflux apart from the action of Ca. The previous results with K ions in squid giant axons especially suffers from this difficulty; it is not clear how much activation is an enhancement of activation by Ca and how much activation is simply a K-induced Na efflux. Also, the role of membrane depolarization at elevated values of $[K]_o$ is unknown in the previous work. In the present work, care has been taken to delineate the Ca_o-sensitive component of Na efflux and to determine the possible role of the membrane potential.

The main conclusions about the effects of external cations on the Ca_o-sensitive Na efflux in *Myxicola* giant axons are that the external monovalent cations tested vary in their ability to support activation by Ca and that the only divalent cation used as a Na replacement, Mg, failed to support any detectable activation by Ca. The action of Na_o is interesting because some evidence for a dual effect on activation by Ca_o is present. When $[Ca]_o$ is raised to 40 mM, external Na clearly supports activation by Ca_o (Fig. 2). When Na is added to Li seawater, however, only decreases in the Ca_o-activated Na efflux occurred. This could be partially due to the reduction in [Li] that occurs in Na-Li seawater mixtures. It is unlikely that this is the sole explanation, however, as in K-free media the Ca_o-activated Na efflux was reduced by two-thirds when $[Na]_o = 100$ mM and $[Li]_o$ was still as high as 330 mM. When Na_o was increased in experiments such as those shown in Fig. 4 using choline instead of Li as the substitute cation, more complex kinetics were observed. At low concentrations, Na_o increased the Ca_o-activated Na efflux whereas at higher concentrations an inhibition occurred in agreement with results reported for squid giant axons (Baker et al., 1969). However, the Ca_o-activated Na effluxes observed in *Myxicola* axons using choline/Na or choline/Na + K seawater mixtures were not as reproducible as those in Li seawater mixtures.

The rather large enhancing effect of K_o on activation of Na efflux by Ca is clearly in addition to any enhancement due to Li alone. Also, the enhancing effect of K_o is independent of its action in depolarizing the membrane. The influence of K_o is interesting because Ca-activated Na efflux was increased with little or no effect on Na_i-dependent Ca influx. Taken at face value, this would indicate that elevating $[K]_o$ alters the stoichiometric ratio of Na to Ca in *Myxicola* giant axons. Calculations indicate that the ratio is increased to a value of 5.6 ± 33% in Li seawater with 20 mM K. Some caution is, however, warranted in this interpretation because of the magnitude of the inherent errors involved and because the axons used for Na efflux and Ca uptake in the two conditions (i.e., 0 mM K [Li] and 20 mM K [Li]) had slightly different concentrations of Na_i (Tables II and III), and Ca fluxes are known to depend strongly on Na_i.

Though a completely satisfactory explanation for these results and the previously discussed results on squid axons does not yet exist, it seems clear that Ca ions act at external membrane sites to activate a portion of Na efflux and

that the ability of Ca ions to activate at these sites depends strongly on external cations. Either the cations affect the affinity for Ca, they control the proportion of the sites that are in a suitable conformation to react with Ca, or they themselves exert a catalytic effect on the translocation step in the presence of Ca_o. A theory for similar results in squid giant axons has been proposed (Baker et al., 1969). Two externally directed kinds of carrier sites are postulated, one that can bind either Ca or two monovalent cations and another that binds only monovalent cations. When the sites that bind either Ca or two monovalent cations are occupied by Ca and the monovalent cation sites are occupied, transport is activated. Inhibitory effects according to this model are ascribed to displacement of Ca from the Ca site by some monovalent cations. None of the present results are inconsistent with this view and most of them could be explained on the basis that the Ca site has a relatively low affinity for Li and choline and a much higher affinity for Na. The action of K ions is less clear according to this model inasmuch as addition of a relatively small concentration of K (20 mM) to a much larger concentration of Li gave about a threefold enhancement of the Ca_o-activated Na efflux. It is possible that the monovalent site is not saturated even when $[Li]_o = 430$ mM and that these sites have a considerably higher affinity for K. It is also possible that the model is incomplete, at least in *Myxicola* giant axons, and that a third carrier site is present that binds K with high affinity to modulate either the affinities of the other sites for ions or the velocity of turnover of the carrier. Further investigation would be required to address these points. Because of the fact that monovalent cations have the potential to both catalyze transport by the carrier and displace Ca from the carrier, it is not possible to assign very precise affinities or rank orders of effectiveness in the absence of much more kinetic data. The most reasonable model-independent statement that can be made is that at a total external monovalent cation concentration of 430 mM, the order of effectiveness in supporting carrier activation by Ca in *Myxicola* giant axons is K + Li > Li > choline > Na.

The lack of an effect of membrane potential alone on Ca-activated Na efflux in *Myxicola* giant axons deserves some comment. It is widely accepted that the Ca-activated Na efflux is a part of a more general mechanism for transporting Ca and Na ions in either direction across excitable cell membranes (Reuter and Seitz, 1968; Baker et al., 1969; Blaustein and Hodgkin, 1969; Brinley et al., 1975; Blaustein and Russell, 1975; Mullins and Brinley, 1975). At low values of $[Na]_i$, the Na:Ca exchange system operates to move Ca out of the cell via the large inward gradient for Na. Mullins and Brinley (1975) found Ca efflux in squid giant axons to have the sort of dependence on V_m that would be expected if changes in V_m acted by altering the electrochemical gradient for Na. For this reason, one might have expected V_m to influence the Ca-activated Na efflux as well, assuming that it is due to essentially the same system operating in a reversed direction. An explanation for the lack of effect of V_m in the present study might be that $[Ca]_i$ was higher than in the experiments on squid axons in which Na_o-dependent Ca efflux was found to be a function of V_m. In squid axons, sensitivity of Ca efflux to V_m was greatly reduced when $[Ca]_i$ was

elevated. Although fresh *Myxicola* axons remained in Li seawater for only 15 min before altering V_m, a significant increase in $[Ca]_i$ could have occurred. Clearly further investigation would be useful in *Myxicola* giant axons in which $[Ca]_i$ could be controlled and altered during the experiment by means of internal dialysis.

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