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Reverse Na-Ca exchange requires internal Ca and/or ATP in squid axons

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Classical Na-Ca exchange models are based on a symmetric carrier system where Na and Ca competing from the same site, can produce net movement of the other against its electrochemical gradient. We have explored this symmetric assumption by studying the Ca_o and Na_o-dependent Na efflux in dialyzed squid axons in which proper control of both external and internal medium was achieved. The results show: (1) In axons dialyzed without Ca_i and ATP, Ca_o-dependent Na efflux cannot be detected even in the absence of Na_o. Under these conditions, the level of Na efflux (1 pmol · cm^{-2} · s^{-1}) is close to that predicted by an electrical 'leak'. (2) In axons dialyzed with Ca_i (100 μM) and without ATP, Na efflux measured in 440 mM Na_o, is about 4–5 pmol · cm^{-2} · s^{-1} and rather insensitive to Ca_o between 0 and 10 mM. However, in the absence of Na_o, a Ca_o-dependent Na efflux is observed similar in magnitude to that found in the presence of external Na. (3) In the presence of both Ca_i and ATP, Na efflux into artificial sea-water (440 mM Na, 10 mM Ca) is 18 pmol · cm^{-2} · s^{-1}. In the absence of Na_o the efflux of Na is 7.5 pmol · cm^{-2} · s^{-1}. In the absence of both Na_o and Ca_o the efflux is close to 'leak'. With full Na_o but no Ca_o, the Na efflux average 12.6 pmol · cm^{-2} · s^{-1}. These results indicate a marked asymmetry in the modus operandi of the Na-Ca exchange system with respect to Ca_i and ATP. These two substrates are required from the *cis* side to promote Ca_o-dependent Na efflux (reversal Na-Ca exchange).

Introduction

The reverse mode of the Na-Ca exchange mechanism (Na_i-Ca_o exchange) has been proposed as an important source of intracellular Ca in the contraction of cardiac muscle fibers [1,2]. Evidences have been presented in squid axons, cardiac muscle cells and other preparations that a reduction in the electrochemical Na gradient (decrease in Na_o, rise in Na_i, membrane depolarization) causes an increase in the reversal cycle of the Na-Ca exchange, resulting in a net Ca entry. The current models for the operation of the Na-Ca exchange carrier are based on the assumption that a high degree of coupling and symmetry exists with respect to the ions being transported, Na and Ca [2–4]. Na is required at the opposite (*trans*) membrane side with respect to Ca, to bring about Ca translocation and vice-versa; and Na at the same side of the membrane as Ca (*cis*) competitively inhibits Ca movement. Although a great deal of information has been obtained from cardiac membrane vesicles and other membrane preparations exhibiting Na-Ca exchange [5–7], the question of whether the system is indeed symmetric has been difficult to assert, mainly as the result of inhomogeneities in the vesicular population with respect to sidedness (inside-out and right-side-out vesicles). Squid axons under internal dialysis conditions appear to be an ideal preparation to analyze this problem since proper control of both faces of the membrane can be readily achieved [8].

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Previous evidences in dialyzed squid axons suggest that the Na-Ca exchange system may not be symmetric. In fact, in this preparation the Na_i-dependent Ca influx depends not only on the concentration of Na_i but also on the level of internal ionized Ca [9,10]. In this work, we have extended this observation by studying the properties of the Ca_o-dependent Na efflux as the manifestation of the reverse Na-Ca exchange. This approach has the advantage over Ca influx measurements that the effects of Ca_i^{2+} and ATP can be unambiguously ascribed to Na_i-Ca_o exchange, and not to an eventual Ca_i-Ca_o exchange.

The results reported here show that in the absence of Ca_i^{2+} and ATP, no reversal of the Na-Ca exchange can be detected even in the complete absence of external Na, a condition that should induce a large Na_i-Ca_o exchange in a symmetric system. An additional interesting finding is the existence of a ouabain-insensitive Na_o-dependent Na efflux in the absence of ATP that requires internal Ca, probably reflecting Na-Na exchange movement through the Na/Ca carrier mechanism. In the presence of Ca_i and without ATP, Na and Ca ions seem to compete for an external binding site on the carrier. The levels of Na efflux in the presence of Na_o (no Ca_o) or Ca_o (no Na_o) are similar to that in the presence of both Na_o and Ca_o. In axons containing Ca_i and ATP, Na efflux depends on Na_o and Ca_o as well. However, Na efflux is greater in the presence of Na_o (no Ca_o) than in the presence of Ca_o (no Na_o) and total efflux into Na_o- and Ca_o-containing medium is always greater than in the presence of either Na_o or Ca_o alone. A preliminary report has been presented elsewhere [11].

Methods

The experiments were carried out in two squid species: *Loligo plei* at the Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela, and *Loligo pealei* at the Marine Biological Laboratory in Woods Hole, MA, USA. The dissecting procedure and dialysis technique are described in detail elsewhere [8,9].

Solutions. The standard dialysis solution had the following composition (mM): K^+, 310; Na^+, 100; Mg^{2+}, 4 in excess to the ATP concentration; $Tris^+$, 30; Cl^-, 140; aspartate, 310; EGTA, 3; glycine was used to adjust the osmolarity to 1000 mosM. The pH was 7.3 (18°C). The artificial sea-water contained: K^+, 10; Na^+, 440; Mg^{2+}, 50; Ca^{2+}, 10; $Tris^+$, 10; Cl^-, 580; EDTA, 0.1. The removal of Na and Ca was compensated with equiosmolar amounts of Tris and Mg, respectively. Ca-free sea-water contained 0.5 mM EGTA. All external solutions had cyanide (1 mM), tetrodotoxin (300 nM) and ouabain (100 μM). The nominal ionized Ca concentrations are based on a CaEGTA dissociation constant of 0.15 μM [12]. ATP (vanadium-free was obtained from Sigma as Tris salt, neutralized with Tris base and stored at -20°C as 250 mM solution. Phosphoarginine was from Sigma as the Na salt; it was neutralized with HCl and stored as a 400 mM solution at -20°C.

All reagents were of analytical grade. Radioactive solutions were made by adding solid $^{22}NaCl$ (150 μCi per ml) directly to the internal dialysis medium. Radioactive samples containing 4 ml of external medium were mixed with 5 ml scintillation solution and counted in a liquid scintillation counter for a long enough time to give a standard error of about 1%.

Results

Effect of Ca_i^{2+} and ATP on Ca_o- and Na_o-dependent Na efflux

Fig. 1 shows an experiment in which an axon was immersed in artificial sea-water containing cyanide, tetrodotoxin and ouabain and dialyzed with a solution lacking both Ca_i and ATP. Under these conditions, the efflux of Na reaches a steady value close to 1.1 pmol $\cdot$ cm^{-2} $\cdot$ s^{-1} (this level of flux reflects passive 'leak' [8,13,14]). Removal of Na_o in the presence of 10 mM Ca_o failed to induce any change in Na efflux. When the internal medium was changed to one containing 100 μM Ca^{2+}, Na efflux increased about 5 pmol $\cdot$ cm^{-2} $\cdot$ s^{-1}. This increase was totally dependent on Ca_o since its removal brought the efflux to the same 'leak' value. In the second part of the experiment, ATP was added in the presence of internal Ca and in the absence of Na_o, this caused a small but distinct (about 1.28 pmol $\cdot$ cm^{-2} $\cdot$ s^{-1}) increase in Na efflux. Addition of Na_o resulted in a large increment in Na efflux (up to 16 pmol $\cdot$ cm^{-2} $\cdot$ s^{-1}).

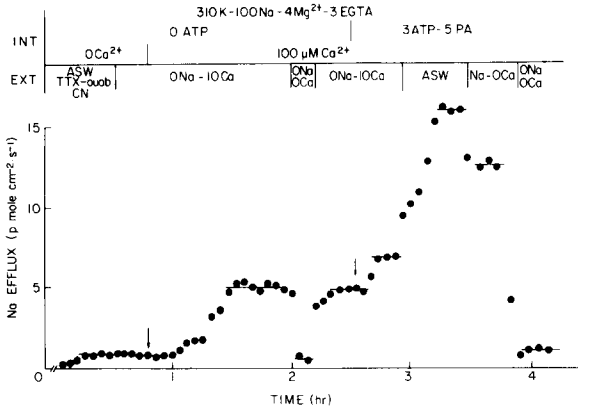


Fig. 1. The effects of internal Ca and ATP on the Ca_o- and Na_o-dependent Na efflux. Ordinate: Na efflux in pmol·cm^{-2}·s^{-1}. Abscissa: time in hours. Unless otherwise states, all concentrations are in millimolar. Temperature, 18°C. Axon diameter, 490 μm. ASW, artificial sea-water, CN, cyanide; ouab, ouabain; TTX, tetrodotoxin; PA, phosphoarginine.

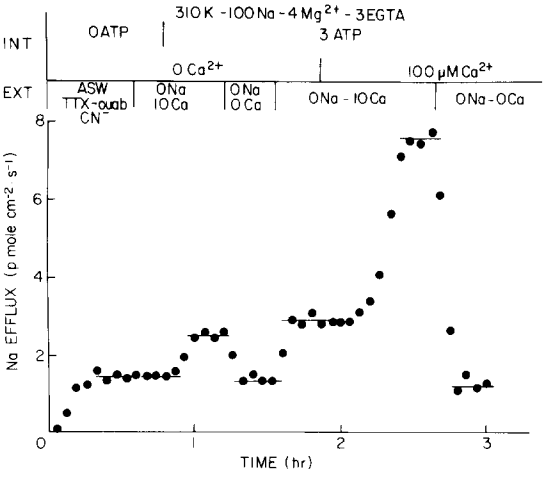


Fig. 2. The effect of ATP on the Ca_o-dependent Na efflux in the absence and presence of internal Ca. Ordinate: Na efflux in pmol·cm^{-2}·s^{-1}. Abscissa: time in hours. The axon was predialyzed for 1 h prior to the introduction of the radioactive dialysis fluid. Abbreviations, see Fig. 1 legend.

Removal of Ca_o in the presence of Na_o decreased the efflux to 12.7 pmol·cm^{-2}·s^{-1} and subsequent removal of Na_o brings the efflux back to the leak levels (1.1 pmol·cm^{-2}·s^{-1}). The fact that the magnitude of the Na efflux in the presence of Ca_i^{2+} and ATP is about 6.8 pmol·cm^{-2}·s^{-1} in 0 Na_o/10 mM Ca_o, 12.7 pmol·cm^{-2}·s^{-1} in 440 mM Na_o/0 Ca_o and only 16 pmol·cm^{-2}·s^{-1} in the presence of both Na_o and Ca_o, suggests that with ATP and Ca_i some competitive interaction exists between Na and Ca for external sites on the carrier.

Fig. 2 shows the effect of ATP on Na efflux in an axon dialyzed with nominal zero Ca_i^{2+} (total free EGTA: 3 mM). Similar to the experiment in Fig. 1, no Ca_o-dependent Na efflux was observed in the absence of Ca_i^{2+} and ATP, even in 0 Na_o/10 mM Ca_o. ATP in the absence of Ca_i^{2+} caused a small (about 1.2 pmol·cm^{-2}·s^{-1}) increase in Na efflux which was abolished by the removal of Ca_o. Raising the Ca_i to 100 μM Ca_i with the axon in Na-free 10 mM Ca_o, resulted in a much larger increase in the efflux which was totally dependent on the presence of external Ca. The conclusion emerging from these experiments is that either ATP or Ca_i can promote a Ca_o-dependent Na efflux in the absence of Na_o. The stimulation by ATP is smaller than that of Ca_i.

Activation of Na efflux by Ca_o and Na_o at constant Ca_i^{2+} in the presence and absence of ATP

Fig. 3 shows an experiment in which the effect of external Ca (in the presence of external Na) on Na efflux was explored in an axon dialyzed with

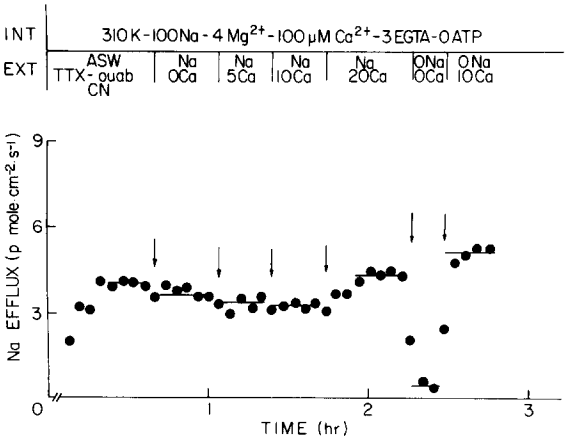


Fig. 3. The effect of external Ca on the Na efflux in an axon bathed in full (440 mM) sodium. Ordinate: Na efflux in pmol·cm^{-2}·s^{-1}. Abscissa: time in hours. The arrows indicate the changes in the external Ca concentration. Notice that the level of Na efflux in full Na_o/0 Ca_o; 10 mM Ca_o/0 Na_o and full Na/20 Ca_o are about the same. Axon diameter 450 μm. Abbreviations, see Fig. 1. legend.

Ca_i and without ATP. Under these conditions, Na efflux reached a steady value of 4 pmol · $cm^{-2} \cdot s^{-1}$ in artificial sea-water. Removal of Ca_o caused no effect on the levels of the efflux. Moreover, addition of 5, 10 or 20 mM Ca_o produces no significant changes in the steady Na efflux. Interestingly, removal of both Ca_o and Na_o brought the efflux of Na to less than 1 pmol · $cm^{-2} \cdot s^{-1}$; subsequent addition of 10 mM Ca_o activated the Na efflux to a value close to that found in full Na_o/10 mM Ca_o. The fact that the level of Na efflux in 0 Na_o/10 mM Ca_o is about the same as in 440 mM Na_o/0 Ca_o suggests that the carrier mechanism can perform Na_o-Na_i and/or Na_i-Ca_o exchange and that Na and Ca ions compete for an external binding site(s).

The experiment of Fig. 4A was designed to explore the activation of Na efflux by external Na in the absence of external Ca in an axon dialyzed with 100 μM Ca_i and without ATP. The steady-state level of Na efflux reached in artificial sea-water was not modified by the removal of Ca_o, but subsequent removal of Na_o brought the efflux to less than 1 pmol · $cm^{-2} \cdot s^{-1}$. Additions of increasing amounts of Na_o progressively stimulated Na efflux. Fig. 4B shows the effect of several Ca_o concentrations in the absence of external Na on the Na efflux in an axon dialyzed with 100 μM Ca_i and without ATP. In artificial sea-water the efflux of Na reached a steady value of 6 pmol · $cm^{-2} \cdot s^{-1}$. Upon removal of both external Na and Ca the efflux dropped to about 0.2 pmol · $cm^{-2} \cdot s^{-1}$: addition of Ca_o progressively stimulates Na efflux to a value of about 6 pmol · $cm^{-2} \cdot s^{-1}$ in 0 Na_o/10 mM Ca_o. It should be noted that the value of the efflux in artificial sea-water (440 mM Na_o/10 mM Ca_o) is the same as in 0 Na_o/10 mM Ca_o or in full Na/0 Ca_o. This is a strong indication that the system can work in Na-Na or Na-Ca exchange modalities (see also Fig. 3).

In the experiments described so far, the activation of Na efflux by different Ca_o was carried out in the presence of Ca_i and without ATP. Fig. 5 shows the activating effect of Ca_o on Na efflux in an axon containing both Ca_i and ATP. In the absence of Na_o, external Ca stimulated Na efflux reaching saturation at about 15 mM Ca_o. Contrary to what happened without ATP, in the presence of the nucleotide addition of Na_o caused a further increase in Na efflux up to 20 pmol · cm^{-2}

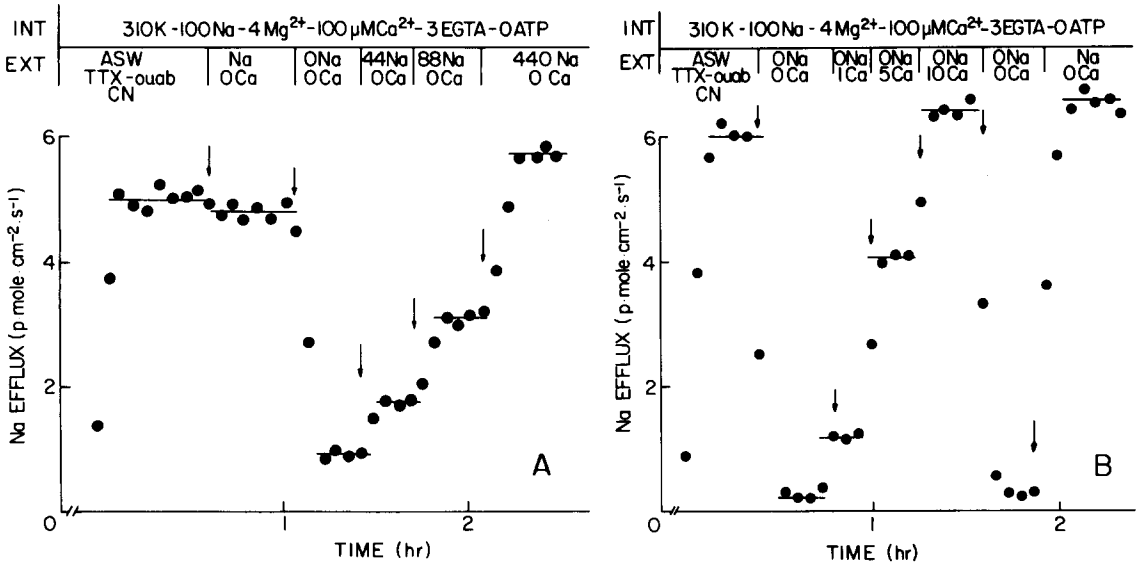


Fig. 4. Na-Na and Ca-Na exchange in an axon dialyzed with Ca_i and without ATP. (A) Activation of the Na efflux (in the absence of Ca_o) by external soium. (B) Activation of the Na efflux by Ca_o (in the absence of Na_o). Ordinate: Na efflux in pmol · $cm^{-2} \cdot s^{-1}$. Abscissa: time in hours. All concentrations are in millimolar except the internal Ca which is in micromolar. Abbreviations, see Fig. 1 legend.

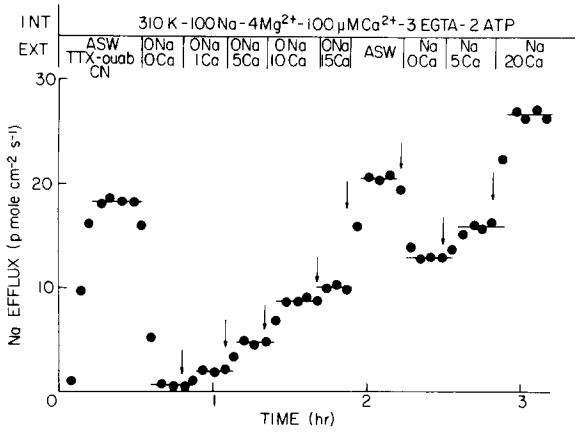


Fig 5. The effect of external Ca on Na efflux in an axon dialyzed with both internal Ca and ATP. Ordinate: Na efflux in pmol·cm^{-2}·s^{-1}. Abscissa: time in hours. The arrows indicate the addition of external Ca. Axon diameter 600 μm. Abbreviations, see Fig. 1 legend.

·s^{-1}. In the second part of the experiment, the activation of Na efflux by different Ca_o was carried out in the presence of Na_o. Two points are worth mentioning: first, the level of Na efflux in artificial sea-water (full Na_o/10 mM Ca_o) is greater than that in 0 Na_o/10 mM Ca_o; and second, in the presence of Na_o, Na efflux can be activated by external Ca_o. These results markedly contrast with those obtained in axons dialyzed without ATP (see Figs. 3, 4A and B).

Fig. 6A and B summarizes the results of several experiments in which the Ca_o-dependent Na efflux was measured under different experimental conditions. In Fig. 6A activation by Ca_o was carried out in axons containing both Ca_i and ATP, in the presence and absence of Na_o. Clearly, the sensitivity of the Na efflux to Ca_o increases in the absence of Na_o. This result strongly suggests a competitive type of interaction between Na_o and Ca_o for external sites. Fig. 6B shows the results of several experiments in which the Ca_o-dependent Na efflux was measured in the presence of external Na. It is clear, that only in the presence of both Ca_i and ATP there is a net increment in Na efflux induced by Ca_o (presence of Na_o).

Table I summarizes the results of several experiments on the effect of Ca_i^{2+} and ATP on the Ca_o-dependent and Na_o-dependent Na efflux. Three points are worth mentioning: first, in the absence of Ca_i and ATP no activation of the Na efflux is found in Na_o-, Ca_o- or Na_o plus Ca_o-containing media. Second, in the absence of ATP

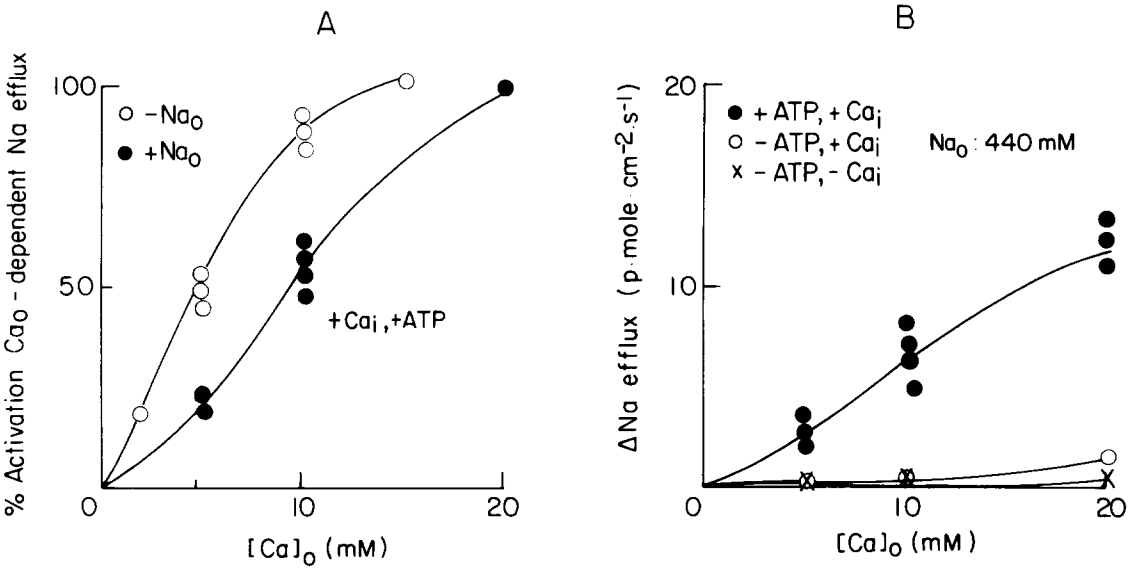


Fig. 6. (A) The activation of Na efflux by external Ca with and without Na_o in axons containing both Ca_i and ATP. Ordinate: percent activation of the Ca_o-dependent Na efflux. Abscissa: external Ca in millimolar. Each point corresponds to a different axon. Open circles: Na efflux in the absence of external Na. Closed circles: Na efflux in the presence of external Na. Mean temperature: 18.0°C.

TABLE I

THE EFFECT OF Ca_i^{2+} AND ATP ON THE Ca_o-DEPENDENT AND Na_o-DEPENDENT Na EFFLUX

The level of Na efflux in 0 Na_o/0 Ca_o (leak) was subtracted in each experimental condition. The ionized Ca_i was 100 μM; Na_i, 100 mM; ATP, 3 mM; Na_o, 440 mM and Ca_o, 10 mM. All external solutions contained 1 mM cyanide, 10^{-4} M ouabain and 300 nM tetrodotoxin. The numbers in parentheses represent different experiments.

Conditions	Na efflux (pmol·cm^{-2}·s^{-1})			
	0 Na_o 0 Ca_o	Na_o 0 Ca_o	0 Na_o Ca_o	Na Ca_o
−ATP, −Ca_i	0 (16)	0 (10)	0 (9)	0 (1)
−ATP, +Ca_i	0 (10)	5.0±0.6 (5)	4.5±0.9 (5)	5.5±0.7 (8)
+ATP, +Ca_i	0 (12)	12.6±1.2 (8)	7.5±1.0 (6)	18.1±1.3 (6)

internal Ca promotes Ca_o-Na_i and Na_o-Na_i exchanges. In the presence of Na_o plus Ca_o, the level of Na efflux is practically the same as that found in 440 mM Na_o Ca-free or Na-free 10 mM Ca_o. Third, in the presence of ATP and Ca_i, Ca_o-Na_i (no Na_o) and Na_o-Na_i (no Ca_o) exchanges are further increased. In this case the total level of the Na efflux in artificial sea-water is always smaller than the sum of the Na_o- and Ca_o-dependent components.

Discussion

Reversal Na-Ca exchange mechanism requires Ca_i and or ATP

Previous work in squid axons had clarified some fundamental properties of the forward Na-Ca exchange (Na_o-dependent Ca efflux), including: (i) *cis* activation by Ca_i^{2+} ($K_{1/2} = 10$ μM, [15]); *cis* inhibition by Na_i ($K_i = 30$ mM, [16]). (ii) *trans* activation by Na_o (in the absence and in the presence of Ca_o [17]), *trans* activation by Ca_o (in the absence or presence of Na_o, [3]). (iii) *cis* activation by ATP [18]. For a symmetrical Na-Ca countertransport model system [2,3] one would expect comparable properties for the reverse of the Na-Ca exchange. As pointed out in Introduction, early studies on dialyzed squid axons had suggested that the Na_i-dependent Ca influx is highly dependent on the levels of internal ionized Ca and ATP [9,10]. In this work we have used the Ca_o-dependent Na efflux as a manifestation of the backward reaction of the Na-Ca exchange carrier.

Our results indicate that the mechanism responsible for the translocation of Na and Ca ions across the excitable membrane of squid axons is not symmetric. In the presence of the two transported ions at opposite faces of the membrane: sodium on the inside and calcium on the outside, countertransport (reverse mode) only occurs in the presence of Ca_i^{2+} and/or ATP. This happens even when the electrochemical Na gradient has been purposely reversed (absence of external Na) to favor Ca entry and Na exit (see Figs. 1 and 2). This markedly contrasts with the forward mode of the Na-Ca exchange in which only Na_o and Ca_i ions are required to induce Na_o-dependent Ca efflux [2,3,15]. The results of experiments of Figs. 1 and 2 indicate that Ca_i is able to activate a much larger Ca_o-dependent Na efflux than ATP; on the other hand Ca_i and ATP together cause a much larger effect on Na efflux than the summation of each one added alone. Recently, Rasgado-Flores et al. [19] reported that the Ca_o-dependent Na efflux in barnacle muscle fibers is not apparently modified by the levels of ATP and Ca_i, suggesting that the Na-Ca exchange is symmetric. In their perfusion experiments, the intracellular ionized Ca was controlled to 0.1 μM with the Ca buffer EGTA, and the ATP was reduced by addition of apyrase. However, due to the large size and to the peculiar geometry (presence of membrane invaginations or clefts) of these muscle fibers, it is not unlikely that the level of internal ionized Ca beneath the membrane might be higher than expected, especially if the external Ca concentration used was 10 mM. Another possible explanation is that the two preparations behave differently.

The mechanism by which internal ionized Ca stimulates the reverse Na-Ca exchange is unknown. One possibility is that the binding of calcium to an internal site of the carrier induces an external site which can then be occupied either by Ca or Na ions, resulting in Na_i being transported (Ca_o-Na_i, or Na_o-Na_i exchange). In this model, internal Ca may act as an activator without actually being translocated. An argument in favor of this hypothesis is that the $K_{1/2}$ for the activation of the reversal by Ca_i is 0.6 μM [10] as compared to about 10 μM for the forward Na-Ca exchange

[15]. This may suggest two different types of sites for Ca_i: one for transport and one regulatory (activation). Another possibility is that the carrier can work in the reverse mode only after a complete forward Na-Ca exchange cycle has taken place; that is, after Ca is translocated in exchange for external Na, the external site is then used in the reverse mode. However, against this view is the fact that ATP without Ca_i is able to promote Ca_o-dependent Na efflux (Fig. 2). Although of smaller magnitude than that activated by Ca_i, this may indicate different operational configurations of the carrier depending on the substrates (Na_i, Ca_i and ATP) present on the cytoplasmic side of the cell.

Activation of the Na efflux by Ca_o and Na_o in the presence of Ca_i and in the absence of ATP

The results of experiments such as those shown in Figs. 3–5, indicate that the reversal of the Na-Ca exchange occurs in the presence of Ca_i and in the virtual absence of ATP. One striking characteristic is that in the presence of external Na (440 mM), Ca_o has little effect on Na efflux in ATP-depleted axons (see Fig. 3). Nevertheless, the fact that the levels of Na efflux are practically the same in 440 mM Na_o (no Ca_o), 10 mM Ca_o (no Na_o) or in 440 mM Na_o and 10 mM Ca_o, suggests that external sodium and calcium compete for an external binding site. It is conceivable that at the Na and Ca concentrations used in these experiments the external sites are near saturation and therefore in the presence of both Na and Ca the system may simultaneously perform Ca_o-Na_i and Na_o-Na_i exchange. The presence of a Na-Na exchange through the Na-Ca carrier appears as an important conclusion from these experiments. In favor of this view is the strict requirement for internal ionized Ca. This is further supported by the fact that the Na_o-dependent and Ca_o-dependent Na effluxes are not additive in the absence of ATP. The similar levels of Na efflux in Na_o- or Ca_o-containing medium, may indicate that the Na_o-loaded carrier has a turnover not very different from that loaded with Ca_o. Alternatively, the data can be accounted for if Na_o drastically reduces the affinity for the carrier site for Ca_o in the absence of ATP; in this case only Na-Na exchange would take place up to 10 mM Ca_o.

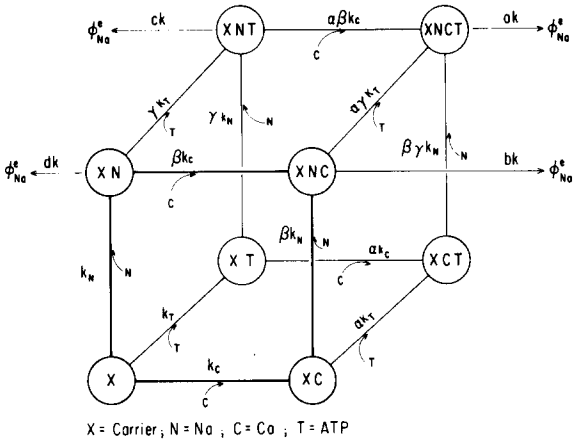
Activation of Na efflux by Ca_o and Na_o in the presence of Ca_i and ATP

Although ATP is not essential for the reversal of the Na-Ca exchange (in the presence of Ca_i) to occur, this nucleotide induces profound modifications on the behavior of Na efflux in ouabain-treated axons: (a) In artificial sea-water (440 mM Na_o/10 mM Ca_o), the level of the Na efflux in the presence of ATP is greater than in its absence. Moreover, under these conditions the level of the efflux is always larger than that in the presence of Na_o or Ca_o alone. A single carrier system with two separate external sites (for Na and Ca) could explain the data (presence of ATP) if the turnover of the carrier loaded with Na_o plus Ca_o is higher than when loaded with Na_o or Ca_o alone. (b) External Na and Ca contribute in different proportions to the total level of the sodium efflux. Na_o-Na_i predominates over Ca_o-Na_i exchange. (c) Only in the presence of ATP it is possible to obtain a significant increment in the Ca_o-dependent Na efflux in Na_o-containing medium (up to 20 mM Ca_o).

An important point to be resolved deals with the interrelationship, if any, between Na_i-Na_o and Na_i-Ca_o exchange in axons containing normal levels of ATP. Beaugé and DiPolo [14] have shown the existence of an ATP-dependent Na-Na exchange in strophantidin poisoned squid axons which does not appear to require Ca_i; whether this component of Na efflux occurs through the glycoside-poisoned Na^+ pump or belongs to another mechanism (Na-Ca exchange) remains an open question. Nevertheless, the fact that ATP is able to activate a Ca_o-dependent Na efflux in the virtual absence of Ca_i suggests that part if not all of the Na-Na exchange observed in the presence of the nucleotide belongs to the Na-Ca exchange system.

Possible mode of operation for the reversal of the Na-Ca countertransport system

The simplest kinetic model (intracellular binding only) which takes into account several observations discussed above is diagrammed in Scheme I. The carrier (X) is assumed to be in rapid random equilibrium with the three internal ligands: Na, Ca and ATP. Of all possible configurations, only those in which the carrier binds Ca_i or ATP can induce Na efflux through the Na-Ca exchange mecha-



Scheme I. Schematic model for the ligand requirements of the Ca_o-dependent Na efflux (reverse Na-Ca exchange). See text for explanation.

nism. In the absence of Ca_i and ATP, no reversal of the exchange can occur. The rate constants for Na translocation via the different complexes are: *a* (XNaCaATP), *b* (XNaCa), *c* (XNaATP) and *d* (XNa); according to the results outlined above $a > b > c > d = 0$. The equilibrium constants K_N, K_C and K_T refer to the interactions of Na, Ca and ATP with the empty carrier. The factors α, β and γ correspond to the modification of these equilibrium constants when another ligand is already bound to the translocator. Finally, the model considers only one site for each ligand involved which, at least for Na ions, might be an oversimplification (see Ref. 21). In the diagram of Scheme I, $N = [Na_i]$; $C = [Ca_i]$; $T = [ATP]$. Since $d = 0$ (no Na translocation for the XNa configuration), Na efflux vanished for the particular case: $Ca_i^{2+} = 0$; ATP = 0. Clearly, the system is markedly asymmetric. It is interesting to point out that in the scheme, ATP is shown as if it were bound to the carrier. This is probably not the case, since only in the presence of Mg_i, ATP and hydrolyzable ATP analogues can induce stimulation of Na-Ca exchange [13,18,20]. This suggests that the transporter, or other structures closely associated to it are being phosphorylated by ATP. Whether this *trans*-activatory effect is mediated by regulatory proteins (protein kinases and phosphatases) is not known.

Since the levels of ATP found in a normal cell are not likely to change widely, one cannot consider this nucleotide as a real modulator of the exchange under physiological conditions. On the other hand, the fact that the $K_{1/2}$ for Ca_i stimulation is in the submicromolar range [10] strongly suggests a modulatory effect of internal Ca on Ca entry. Although the physiological relevance of this finding is unknown, if the observed dependence of the reverse Na-Ca exchange on Ca_i occurs also in other excitable preparations such as cardiac muscle, then Ca entry via voltage-dependent Ca channels (increase in Ca_i^{2+}) could contribute to further activate Ca entry via Na-Ca exchange. An argument which favors this possibility is the fact that an increase in Ca_i from 0.1 to 0.6 μM produces a 10-fold increase in the extra Ca entry induced by a membrane depolarization (high external potassium [10]).

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