Characterization of the Reverse Na/Ca Exchange in Squid Axons and Its Modulation by Ca; and ATP

Ca; dependent Na;/Ca; and Na;/Na; Exchange Modes

REINALDO DIPOLO and LUIS BEAUGÉ

From the Instituto Venezolano de Investigaciones Científicas (IVIC), Centro de Biofisica y Bioquímica, Caracas 1020A, Venezuela, and the División de Biofísica, Instituto de Investigación Médica M. y M. Ferreyra, 5000 Cordoba, Argentina

ABSTRACT We have used dialyzed squid axons to characterize the ouabain- and bumetanide-insensitive Na efflux components and their relation to the operation of the Na/Ca exchange mechanism. In axons dialyzed with solutions containing nearly physiological concentrations of K, Na, and Mg, three components of the Na efflux can be distinguished: Ca; activated, Ca; dependent Na efflux ("reverse" Na/Ca exchange); Ca; activated, Na;/Ca; dependent Na efflux; and Ca; independent, ATP activated, Na;/Ca; dependent Na efflux. We have studied the effects of internal alkalinization, Mg; Ca; and the ATP analogue [γ-thio]ATP (ATPγS) on the different components of the Na efflux. The results show the following: (a) internal alkalinization activates both Ca; and Na;/Ca; dependent Na efflux components provided that Ca; is present; (b) Mg; inhibits both the Ca; activated, Ca; and Na;/Ca; dependent Na efflux components; (c) Ca; inhibits the Na;/Ca; dependent component by competition for a common site; (d) ATPγS activates both Na;/Ca; and Ca; dependent Na efflux components only in the presence of Ca; and (e) ATP activates the Na;/Na; and Na;/Ca; exchanges, causing a 10-fold increase in the affinity of the reverse Na/Ca exchange toward Ca; In the absence of Ca; ATP stimulates an Na;/Ca; dependent Na efflux that is not affected either by internal alkalinization or high Ca; The ATP analogue does not activate the Ca; independent Na;/Na; exchange system. These experiments demonstrate that the Ca; activated Na;/Na; exchange is a mode of operation of the Na/Ca exchange mechanism that substantially contributes to Na movement during the activation of the Na/Ca antiporter. The experimental evidence obtained on the Ca; independent Na;/Na; exchange component shows that this system is not part of the Na/Ca exchange.

INTRODUCTION

The Na/Ca exchange mechanism is generally considered to be a carrier-mediated transport system in which the movement of Ca ions is coupled to reciprocal

Address reprint requests to Dr. Reinaldo DiPolo, Centro de Biofisica y Bioquímica, IVIC, Apartado 21827, Caracas 1020A, Venezuela.

J. Gen. Physiol. © The Rockefeller University Press · 0022-1295/87/10/0505/21 $2.00 505
movement of Na ions. Furthermore, it is thought that, depending on the magnitude and direction of the electrochemical Na gradient, the Na/Ca exchange mechanism can induce net movements of Ca ions in or out of the cell (Mullins, 1977). Recent experiments in dialyzed squid axons have demonstrated that Ca entry through the \( \text{Ca}_o/\text{Na}_i \) exchange mechanism ("reverse" Na/Ca exchange) requires not only the presence of \( \text{Na}_i \) and \( \text{Ca}_o \) in order to operate, but also micromolar amounts of \( \text{Ca}_i \) (DiPolo, 1979; DiPolo and Beaugé, 1986). (In this article, \( \text{Ca}_i \) refers to the intracellular ionized calcium concentration.) This asymmetry in the activation of the exchange system by \( \text{Ca}_i \) could have important physiological implications, since the levels of \( \text{Ca}_i \) modulate the influx of Ca through the antiporter mechanism (positive feedback). This regulatory effect of \( \text{Ca}_i \) has recently been implicated in the inhibition of Ca influx via \( \text{Na}_i/\text{Ca}_o \) exchange induced by the Ca indicator quin2 (Allen and Baker, 1985), and it could also account for the \( \text{Ca}_i \) requirement of the outward current generated by the Na/Ca exchange in ventricular cells (Kimura et al., 1986).

Previous evidence obtained in dialyzed squid axons indicates that \( \text{Ca}_i \) activates not only a \( \text{Ca}_o \)-dependent Na efflux component (\( \text{Na}_i/\text{Ca}_o \) exchange), but also a sizable \( \text{Na}_o \)-dependent Na efflux component (\( \text{Na}_i/\text{Na}_o \) exchange) (DiPolo and Beaugé, 1986). When the possible modes of operation of the Na/Ca exchange system are studied, a further complication arises from the fact that ATP is able not only to activate these two components, but also to promote an \( \text{Na}_o \)-dependent Na efflux in the complete absence of \( \text{Ca}_i \) (Beaugé and DiPolo, 1981; DiPolo and Beaugé, 1986). Whether these ouabain-insensitive components of the Na efflux are modes of operation of the Na/Ca exchange system remains an open question. An analysis of the possible modes of operation of the exchange system and their magnitude under different experimental conditions is of critical importance when determining the number of Na ions exchanged for Ca (stoichiometry) during the operation of the exchange system.

In the present work, we have used different experimental procedures that are known to affect the Na/Ca exchange mechanism in squid axons, in order to characterize kinetically the ouabain-insensitive Na efflux components and to explore whether they are indeed modes of operation of the Na/Ca exchange system. Our results provide conclusive evidence that \( \text{Ca}_i \) and ATP activate not only the "reverse" \( \text{Na}_i/\text{Ca}_o \) exchange but also a sizable \( \text{Na}_i/\text{Na}_o \) exchange, which occurs during the turnover of this mechanism. Their similarities with respect to activation by internal alkalinization, Mg inhibition, and competition for a common external site are indicative of their similar origin. Interestingly, the Na/Ca exchange component activated by ATP in the absence of \( \text{Ca}_i \) (Beaugé and DiPolo, 1981) appears to be a different mechanism operating in parallel with the Na/Ca exchange.

**MATERIALS AND METHODS**

The experiments were carried out with live specimens of *Loligo pealei* at the Marine Biological Laboratory, Woods Hole, MA, and with the tropical squid *Loligo plei* at the Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela. After careful cleaning, an axon was mounted in a modified chamber for dialyzing and voltage-clamping the axons (DiPolo et al., 1985). An important modification is that the axon is not
cannulated; instead, its ends lie on pedestals and slits are opened in them for the insertion of the electrode and the dialysis capillary. The cut ends of the axon are separated from the central pool by two air gaps (DiPolo et al., 1985). Dialysis capillaries were from hollow regenerated cellulose fibers with a nominal molecular weight cutoff of 9,000 (150 µm o.d., 141 µm i.d., Spectrum, Los Angeles, CA). The dialysis capillary contained a 75-µm platinized platinum wire (5% iridium). In most of the experiments, the axons were predialyzed for 1 h with an isotope-free standard dialysis medium containing no ATP and a given nominal concentration of ionized Ca. Since the axons in the present work do not need to be voltage-clamped during flux measurements, most of the axons were voltage-clamped for a short time (5-10 min) during the predialysis period and their leakage currents were measured with a 20-mV depolarizing pulse.

Solutions

Dialysis medium. The standard dialysis solution had the following composition (millimolar): 310 K, 40 (L. plei) or 50 (L. pealei) Na, 4 Mg, in excess with respect to the ATP concentration, 30 Tris, 98 Cl, 310 aspartate, 1-3 EGTA, and 330 glycine, pH 7.3 (17.5°C). The osmolarity was adjusted to 998 mosmol/kg water. Removal of Na or Mg was compensated with equimolar amounts of Tris. In the experiments designed to measure the reversal of the Na/Ca exchange, Na was increased to a saturating value of 100 mM. The estimation of the ionized Ca was based on a dissociation constant of 0.15 µM for CaEGTA (0.3 ionic strength; DiPolo et al., 1976) and 1.4 mM for CaATP (De Weer, P., personal communication). The estimation of the ionized Mg was based on a dissociation constant of 0.7 mM for MgATP (De Weer, P., personal communication) and 30 mM for MgEGTA (DiPolo et al., 1976). ATP (vanadium-free) was obtained from Sigma Chemical Co. (St. Louis, MO). Phosphoarginine at a concentration of 5 mM was usually added to the ATP-containing solution. Adenosine-5'-(3-thiophosphate) (ATP3,S) was purchased from Boehringer Mannheim GmbH, Federal Republic of Germany. Na orthovanadate (from Fisher Scientific Co., Pittsburgh, PA) was prepared as a 100 mM solution.

Artificial seawater. The standard artificial seawater had the following composition (millimolar): 10 K, 440 Na, 10 Ca, 50 Mg, 10 Tris, 590 Cl, and 0.1 EGTA, pH 7.6 (17.5°C). The osmolarity was 1,000 mosmol/kg water. Removal of Na, Ca, or Mg ions was compensated with equimolar amounts of Tris. All external solutions contained 1 mM cyanide and 300 nM tetrodotoxin (TTX). In most of the experiments, 5 × 10⁻⁴ M ouabain and 10 µM bumetanide (a gift of Dr. J. Russell) were added to the external medium to block the Na/K pump and the Na/K/Cl cotransport.

All reagents used were of analytical grade. Radioactive solutions were made by adding solid [⁴⁰Ca]CaCl₂ (15-30 mCi/mg; New England Nuclear, Boston, MA) or [²⁰Na]NaCl (641 mCi/mg). Radioactive samples collected at 4-min periods were mixed with 5 ml of scintillation liquid and counted in a liquid scintillation counter. Double-label experiments were carried out by dialyzing the axons with an internal medium containing ⁴⁰Na and ⁴⁵Ca. The collected radioactive samples were first counted for ⁴⁰Na in a gamma counter. Na efflux values were corrected for radioactive decay. After complete decay of the ⁴⁰Na activity, samples were counted for ⁴⁵Ca activity in a liquid scintillation counter.

Results

Effect of Ionized Ca, and ATP, on the Ouabain- and Bumetanide-insensitive Na Efflux

Reversal of the Na/Ca exchange in dialyzed squid axons is best studied by measuring the Ca₂⁺-dependent Na efflux. Measuring the efflux of Na instead of
the influx of Ca to investigate the reverse mode of the Na/Ca exchange (Na₉/Caₒ exchange) has the advantage that any catalytic effect of Caᵢ on the reversal of the Na/Ca exchange can be unambiguously ascribed to Naᵢ/Caₒ and not to Caᵢ/Caₒ exchange. Fig. 1 shows the effect of Caᵢ and ATP on the Caₒ- and Naₒ-dependent Na efflux components. The Na/K pump and the Na/K/Cl cotransport were inhibited by ouabain (5 × 10⁻⁴ M) and bumetanide (10⁻⁵ M), respectively. When the internal medium contained nominally zero Ca (3 mM total EGTA)

![Diagram](image)

**Figure 1.** The effect of ionized Caᵢ and ATP on the ouabain- and bumetanide-insensitive Na efflux. All concentrations are in millimolar except Caᵢ, which is in micromolar. The arrows indicate changes in the internal medium. Different symbols are used to indicate different external solutions. All external solutions contained TTX, cyanide, ouabain, and bumetanide. Axon diameter, 550 μm.

and no ATP, the efflux of Na was rather small (<1 pmol·cm⁻²·s⁻¹) and insensitive to changes in Naₒ. Increasing Caᵢ to 80 μM caused an increase in the Na efflux that was completely sensitive to Caₒ, since removal of Caₒ in the absence of Naₒ brought the efflux back to the baseline. The long latency between the addition of 80 μM Ca and the onset of the rise in the Na efflux is the consequence of the slow rise in Caᵢ, owing to washout of free EGTAᵢ. Addition of Naₒ in the absence of Caₒ increased the Na efflux to the same level as that observed with Caₒ alone. This confirms previous findings that, in the absence of ATP and in the presence
of Ca\textsubscript{a}, the Ca\textsubscript{o} and Na\textsubscript{o}-dependent components of the Na efflux are about the same in magnitude (DiPolo and Beaugé, 1986). Under the above experimental conditions (full Na\textsubscript{o}, no Ca\textsubscript{o}), addition of ATP further increased the Na\textsubscript{o}-sensitive Na efflux. In this regard, it is of interest that the Ca\textsubscript{o}-dependent Na efflux component was also increased by ATP. The increase in the Ca\textsubscript{o}-dependent Na efflux induced by ATP was small compared with that of the Na\textsubscript{o}-dependent component. Nevertheless, in a total of six different experiments, an ATP-stimulated, Ca\textsubscript{o}-dependent Na efflux was always observed when ATP was added in the presence of Ca\textsubscript{a} and in the absence of Na\textsubscript{o}. These data support the idea that both Ca\textsubscript{a} and ATP modulate the reversal of the Na/Ca exchange (see Fig. 11). Although the Ca\textsubscript{o}-dependent Na efflux component observed in the presence of Ca\textsubscript{a} (with or without ATP) is clearly a part of the Na/Ca exchange mechanism (reverse mode), it is unclear whether the Na\textsubscript{o}-dependent Na efflux component observed in the presence of Ca\textsubscript{a} is also a mode of operation of the Na/Ca exchange (Na/Na exchange mode) or a different parallel system. This is also true for the Na\textsubscript{o}-dependent Na efflux component stimulated by ATP. The results presented in the following sections deal mainly with the analysis of these Na efflux components and their relation to the Na/Ca antiporter.

**Effect of Internal Alkalinization on the Na\textsubscript{o}- and Ca\textsubscript{o}-dependent Na Efflux**

In the presence of Ca\textsubscript{a} and in the absence of ATP. We have previously demonstrated (DiPolo and Beaugé, 1984) that the forward Na/Ca exchange (Na\textsubscript{o}-dependent Ca efflux) in squid axons is affected by intracellular ligands other than the transported ions (Na and Ca). One such ligand that has profound effects on the forward Na/Ca exchange is the ion H\textsuperscript{+}. Internal alkalinization (from pH 7.3 to 8.5) increases the Na\textsubscript{o}-dependent Ca efflux by a factor of 3 (DiPolo and Beaugé, 1982). In principle, it would be expected that Na ions exiting through the Na/Ca exchange mechanism, whether as Na\textsubscript{o}/Ca\textsubscript{o} or Na\textsubscript{o}/Na\textsubscript{a} exchange, would exhibit a dependence on pH.

In the experiment illustrated in Fig. 2, the axon was dialyzed with an internal ATP-free medium that was buffered at pH 7.3 and contained 80 \(\mu\)M Ca\textsubscript{a}. Under these conditions, the efflux of Na reached a steady value of \(9\ pmol\cdot cm^{-2}\cdot s^{-1}\). Increasing the pH to 8.5 elicited an increase in the efflux to \(\sim 22\ pmol\cdot cm^{-2}\cdot s^{-1}\). Subsequent removal of Na\textsubscript{o} and Ca\textsubscript{a} caused the efflux to drop to "leak" values. Returning the Na\textsubscript{o} to 440 mM in the absence of Ca\textsubscript{a} increased the Na efflux to a value similar to that obtained in artificial seawater. In order to measure the Ca\textsubscript{o}-dependent component in the same experiment, a new baseline ("leak") was obtained in the absence of Na\textsubscript{o} and Ca\textsubscript{a}. Addition of 10 mM Ca\textsubscript{a} increased the Na efflux to \(\sim 9\ pmol\cdot cm^{-2}\cdot s^{-1}\). Finally, the addition of Na\textsubscript{o} increased the Na efflux to a value identical to that found originally in an artificial seawater. The results of this and similar experiments show that internal alkalinization causes an increase in both the Na\textsubscript{o}- and Ca\textsubscript{o}-dependent Na efflux components.

Most of the pH effects on Na efflux take place on the Na\textsubscript{o}-dependent rather than the Ca\textsubscript{o}-dependent component. In the experiment of Fig. 3, both Ca and Na effluxes were measured simultaneously by dialyzing an axon with a standard
internal medium containing both $^{45}\text{Ca}$ and $^{24}\text{Na}$ (see Materials and Methods). This procedure allows the determination of the Na$_o$-dependent Ca efflux, Ca$_o$-dependent Ca efflux, Ca$_o$-dependent Na efflux, and Na$_o$-dependent Na efflux components. At pH 7.3 and in the presence of 80 µM Ca$_i$, the efflux of Ca and Na reached values of ~1.6 and 8 pmol·cm$^{-2}$·s$^{-1}$, respectively.

Removal of both Na$_o$ and Ca$_o$ dropped both fluxes to "leak" values. When Ca$_o$ was added in the absence of Na$_o$, a Ca$_o$-dependent Na efflux of ~6 pmol·cm$^{-2}$·s$^{-1}$ was obtained with little (120 fmol·cm$^{-2}$·s$^{-1}$) activation of the Ca$_o$-dependent Ca efflux.

![Figure 2](image)

**Figure 2.** The effect of internal alkalinization on the Na$_o$- and Ca$_o$-dependent components of the Na efflux in the presence of Ca$_i$. The arrow indicates the change in the internal medium from pH 7.3 to 8.5. Note that the sum of the Na$_o$- and Ca$_o$-dependent Na efflux components is greater than the level of the efflux in artificial seawater. Unless otherwise stated, all concentrations are in millimolar. Axon diameter, 490 µm.

Ca efflux. Under these conditions, raising pH$_i$ to 8.5 caused an increase in the Ca$_o$-dependent component of the Na efflux, with no activation of the Ca$_o$-dependent Ca efflux. Readmission of Na ions to the external medium caused a large increase in both the Na$_o$-dependent Na efflux and the Na$_o$-dependent Ca efflux. If the Na$_o$-dependent Na efflux component is indeed part of the Na/Ca exchange system (see Discussion), this experiment implies that during the operation of the forward Na/Ca exchange, there is a large Na/Na exchange taking place. At the end of the experiment, Ca$_o$ was increased up to 50 mM; this point will be discussed later.
In the presence of Ca\textsubscript{i} and ATP. In a second series of experiments, we measured the effect of increasing pH\textsubscript{i} on the Na\textsubscript{i}/Ca\textsubscript{o} and the Na\textsubscript{i}/Na\textsubscript{o} exchange components in axons containing both Ca\textsubscript{i} and ATP. Ouabain and bumetanide were present in the external medium during the experiment. Fig. 4 illustrates one such experiment. After 1 h of predialysis to remove the ATP, the efflux of Na reached a steady value of 5 pmol·cm\textsuperscript{-2}·s\textsuperscript{-1}. When 2 mM ATP was introduced via the dialysis medium, a net increment of 13 pmol·cm\textsuperscript{-2}·s\textsuperscript{-1} in the Na efflux was obtained. Clearly, most of this increment is on the Na\textsubscript{o}-dependent component, as can be seen upon removal of Na\textsubscript{o}. Increasing pH\textsubscript{i} from 7.3 to 8.5 activated the Na efflux (8 pmol·cm\textsuperscript{-2}·s\textsuperscript{-1} beyond the level activated by ATP). Again, most of the activation caused by raising pH\textsubscript{i} is on the Na\textsubscript{o}-dependent component. Since in the presence of 2 mM ATP the nucleotide effect is completely saturated (see Fig. 11), the extra increment in the Na\textsubscript{o}- and Ca\textsubscript{o}-dependent Na efflux components implies that internal alkalinization does not simply mimic the ATP effect.

In the absence of Ca\textsubscript{i}, with or without ATP. It is already known, and confirmed...
in the experiment of Fig. 1, that no Na/Ca exchange occurs in the absence of Ca (DiPolo, 1979; DiPolo and Beaugé, 1986). If the Na-dependent Na efflux is an operational mode of the Na/Ca exchange system, then its activation by internal alkalinization should also depend on Ca. Fig. 5A shows that in an axon dialyzed without either Ca (2 mM free EGTA) or ATP and bathed in artificial seawater, internal alkalinization caused a very small activation in the Na efflux (~0.5 pmol·cm⁻²·s⁻¹ as compared with that in the presence of Ca, 13 pmol·cm⁻²·s⁻¹; see Fig. 2). Addition of 2 mM ATP in the absence of Ca caused an increase in the efflux of Na that was dependent on the presence of Na. Fig. 5B shows an experiment similar to that in A but carried out at pH 7.3 instead of 8.5. The magnitude of the ATP-stimulated, Na-dependent Na efflux in the absence of Ca was the same at pH 7.3 or 8.5, which suggests an Na/Na exchange system different from that found in the presence of Ca. In three other axons, no significant effect of internal alkalinization was found on the ATP-activated, Ca-independent, Na-dependent Na efflux component.
Effect of Ca\textsubscript{o} on the Na\textsubscript{o}-dependent Na Efflux

In the presence of Ca\textsubscript{i}, Fig. 6 shows that the Ca\textsubscript{i}-activated, Na\textsubscript{o}-dependent Na efflux was greatly reduced by raising the external Ca to 50 mM. This was also evident in the double-label experiment (see Fig. 3), which showed that raising Ca\textsubscript{o} from 10 to 50 mM caused a drop in the Na efflux. Although no kinetic data exist from these experiments that would imply that inhibition by Ca\textsubscript{o} is competitive, such inhibition is in line with the notion that Na\textsubscript{o} and Ca\textsubscript{i} ions compete for a cation-binding site on the exchange carrier (Baker et al., 1969; Blaustein and...
Russell, 1975; DiPolo and Beaugé, 1986; Reeves, 1986); this suggests a common mechanism for the Na\(_i\)/Na\(_o\) and Na\(_i\)/Ca\(_o\) exchanges.

In the absence of Ca\(_{\text{aq}}\). In the experiment of Fig. 5B, the effect of increasing Ca\(_{\text{aq}}\) was explored in an axon dialyzed without Ca\(_i\). After the Na efflux had reached a steady state level of \(\sim 1\) pmol\cdot cm\(^{-2}\) s\(^{-1}\) in the nominal absence of Ca\(_i\), addition of ATP (in the presence of Na\(_o\) and in the absence of Ca\(_o\)) caused the efflux to increase to \(\sim 8\) pmol\cdot cm\(^{-2}\) s\(^{-1}\). Increasing Ca\(_o\) to 50 mM had no effect on the level of Na efflux, in contrast to experiments performed in the presence of Ca\(_i\) (see Figs. 3 and 6).

**Effect of Mg\(_i\) on the Na\(_i\)- and Ca\(_o\)-dependent Na Efflux**

It has previously been shown (DiPolo and Beaugé, 1984) that Mg\(_i\) is an inhibitor of the Na\(_i\)-dependent Ca efflux in squid axons. At physiological levels of Mg\(_i\) (2–3 mM), the forward Na/Ca exchange is inhibited by 50%. The experiment of Fig. 7 shows that removal of Mg\(_i\) from the dialysis medium caused an increase in the Na efflux from a steady level of 4.6 pmol\cdot cm\(^{-2}\) s\(^{-1}\) to a level of 10.5 pmol\cdot cm\(^{-2}\) s\(^{-1}\) in the presence of both Na\(_o\) and Ca\(_o\). The Na\(_i\)- and Ca\(_o\)-dependent components of the Na efflux in the absence of Mg\(_i\) were measured by adding Na\(_i\) in the absence of Ca\(_o\) and vice versa. Clearly, the Na\(_i\)-dependent component of the Na efflux in the absence of Mg\(_i\) ions ("leak subtracted") was greater than
the Cao-dependent one. Furthermore, the sum of the Na<sub>i</sub>- and Cao-dependent components (13 pmol·cm<sup>-2</sup>·s<sup>-1</sup>) was greater than the magnitude of the Na efflux in the presence of both Na<sub>i</sub> and Cao (~9.7 pmol·cm<sup>-2</sup>·s<sup>-1</sup>), which is in line with the hypothesis that the Na<sub>i</sub>- and Cao-dependent components of the Na efflux share the same transport system.

**Effect of ATPγS on the Na<sub>i</sub>- and Cao-dependent Na Efflux**

The preceding experiments show that in the absence of Cao, ATP activates an Na<sub>i</sub>-dependent Na efflux that appears to be different from the Na<sub>i</sub>-dependent Na efflux component activated by Cao. The former is neither activated by internal alkalization nor inhibited by Cao (see Fig. 5). Another point in favor of this hypothesis comes from experiments using the ATP analogue ATPγS. In the experiment of Fig. 8A, Na efflux was measured in an axon dialyzed with an internal medium lacking both Ca and ATP and bathed in artificial seawater containing no ouabain or bumetanide. Under these conditions, the efflux of Na reached a steady value of ~1.3 pmol·cm<sup>-2</sup>·s<sup>-1</sup>. The addition of 1 mM of the ATP analogue to the internal dialysis medium had no effect on the Na efflux level. Raising ionized Cao to 40 μM caused an activation of the Na efflux to a steady state value of ~13 pmol·cm<sup>-2</sup>·s<sup>-1</sup>. This activation was totally dependent
Figure 8.
The presence of Na\textsubscript{o} and Ca\textsubscript{o} again, as in the case for the activation of the Na efflux by ATP in the presence of Ca\textsubscript{i}, a large proportion of the ATP-stimulated flux corresponded to the Na\textsubscript{r}-dependent component. An interesting observation is that neither the Na/K pump nor any other transport system, including Na/K/Cl co-transport and Na/Mg exchange, appeared to be activated by ATP\textsubscript{yS}. It should be mentioned that no activation by the analogue was observed in the absence of Mg\textsubscript{i} (results not shown). Fig. 8B shows an experiment in which ATP\textsubscript{yS} was added to an axon already containing 40 \textmu M Ca\textsubscript{i}. Clearly, the analogue induced a large increase in the Na\textsubscript{r}-dependent Na efflux component.

**Dependence of Na\textsubscript{r}- and Ca\textsubscript{r}-dependent Na Efflux on Na\textsubscript{o} and Ca\textsubscript{o}**

The Na\textsubscript{o} and Ca\textsubscript{o} dependence of the Na efflux is examined in Fig. 9. In these experiments, axons were dialyzed from the beginning with an internal medium containing saturating concentrations of both Ca\textsubscript{i} (100 \textmu M) and ATP (2 \textmu M) and bathed in an external medium containing no Na\textsubscript{o} or Ca\textsubscript{o}. In three different axons, steady state Na effluxes were measured at different values of Na\textsubscript{o} (in the absence of Ca\textsubscript{o}). Na\textsubscript{o} ions activated the Na efflux with relatively low affinity ($K_a$ = 125 mM). Since no clear saturation was found with 440 mM Na\textsubscript{o}, an apparent affinity of 125 mM should be taken as a lower limit. We measured the steady state Na efflux in four axons at different values of Ca\textsubscript{i} and in the absence of Na\textsubscript{o} (Fig. 9B). Ca\textsubscript{i} ions activated the reversal of the exchange with a $K_a$ of 5 mM.

**ATP Dependence of Na\textsubscript{r}-dependent Na Efflux**

Fig. 10 summarizes the results of several experiments in which the activation by ATP on the Na\textsubscript{r}-dependent Na efflux was determined in the presence of Ca\textsubscript{i}. In all these experiments, the axons were predialyzed for ~1 h without ATP before testing a given nucleotide concentration. Phosphoarginine (5 mM) was added to buffer the concentration of ATP in the axoplasm (Brinley and Mullins, 1968). The $K_a$ for the ATP activation is ~120 \textmu M. Interestingly, this value is close to the activation of the Na\textsubscript{r}-dependent Ca efflux by ATP (DiPolo and Beaugé, 1979). As is the case for the ATP-stimulated, Na\textsubscript{r}-dependent Ca efflux (DiPolo and Beaugé, 1984), both Na\textsubscript{r}- and Ca\textsubscript{r}-dependent Na effluxes require Mg ions for the nucleotide effect (results not shown).

**Ca\textsubscript{i} Dependence of Na\textsubscript{r}- and Ca\textsubscript{r}-dependent Na Effluxes**

Inasmuch as the reversal mode of the Na/Ca exchange (Na\textsubscript{i}/Ca\textsubscript{o} exchange) requires the presence of micromolar amounts of Ca\textsubscript{i} for activation (DiPolo and

---

**FIGURE 8.** (opposite) (A) The effect of ATP\textsubscript{yS} on the Na\textsubscript{r}- and Ca\textsubscript{r}-dependent Na efflux components in the absence and presence of Ca\textsubscript{i}. (B) Effect of ATP\textsubscript{yS} on the Na\textsubscript{r}-dependent Na efflux in an axon containing Ca\textsubscript{i}. In these experiments, neither ouabain nor bumetanide was added to the external medium. The arrows indicate changes in the composition of the dialysis fluid. Different symbols correspond to different external solutions. All concentrations are in millimolar except Ca\textsubscript{i}, which is in micromolar. Note the lack of activation of the ATP analogue when no Ca\textsubscript{i} is included in the dialysis medium.
Beaugé, 1986), it is important to determine the dependence of the Na\textsubscript{o}- and Ca\textsubscript{i}-dependent Na efflux components on Ca\textsubscript{i}. In the experiments of Fig. 11, A and B, the axons were predialyzed with nominally zero Ca\textsubscript{i} (2–3 mM total EGTA) before the addition of different concentrations of Ca to the dialysis medium.

![Graph A](image)

**Figure 9.** (A) Dependence of Na\textsubscript{o}-dependent Na efflux on Na\textsubscript{o}. (B) Dependence of Ca\textsubscript{i}-dependent Na efflux on Ca\textsubscript{i}. In all these experiments, the axons were dialyzed from the beginning with an internal medium containing saturating concentrations of Ca\textsubscript{i} and ATP. Each symbol represents a different axon. The Na\textsubscript{o}-dependent component was determined at different concentrations of Na\textsubscript{o} in the absence of Ca\textsubscript{i}. The Ca\textsubscript{i}-dependent component was determined at different concentrations of Ca\textsubscript{i} in the absence of Na\textsubscript{o}. The mean temperature in these experiments was 17.5°C. The steady state values of the Na efflux at 440 mM Na\textsubscript{o} and 20 mM Ca\textsubscript{i} were taken as 100% activation of the Na\textsubscript{o}- and Ca\textsubscript{i}-dependent components, respectively. In all these experiments, the external medium contained TTX, cyanide, ouabain, and bumetanide.

The data represent the steady state Na efflux at different internal ionized Ca concentrations. The Ca\textsubscript{i}-dependent component was measured in the absence of Na\textsubscript{o}. Similarly, the Na\textsubscript{o}-dependent Na efflux component was determined in the absence of Ca\textsubscript{i}. In the absence of ATP, Ca\textsubscript{i} activated the Ca\textsubscript{i}-dependent Na efflux, with an apparent $K_{1/2}$ of 15 μM. In the presence of 2 mM ATP, the
apparent $K_v$ was reduced to 1.8 $\mu$M. Since in the presence of ATP the Na$_o$-dependent Na efflux component that occurs through the Na/Ca exchange system is complicated by the presence of an ATP-activated, Ca$_i$-independent, Na$_o$-dependent Na efflux component (see Discussion), its dependence on Ca$_i$ was determined in the absence of ATP. Fig. 11B shows that ionized Ca$_i$ activated the Na$_o$-dependent Na efflux along a sigmoidal curve, with an apparent $K_v$ of 8 $\mu$M.

**DISCUSSION**

The results of the present study confirm and extend earlier work (DiPolo, 1979; DiPolo and Beaugé, 1986) showing that the level of ionized Ca$_i$ modulates the velocity of the Na/Ca exchange working in the reverse mode. In axons treated with ouabain and bumetanide, Ca$_i$ activates both Ca$_o$- and Na$_o$-dependent Na efflux components. Internal ATP stimulates both Ca$_i$-activated Na$_o$- and Ca$_o$-dependent Na efflux components through the Na/Ca exchange system. The nucleotide also induces a Ca$_i$-independent, Na$_o$-dependent Na efflux component.

**Ca$_i$-activated Na$_o$/Na$_o$ and Na$_o$/Ca$_o$ Exchange**

Although an Na$_o$-dependent Na efflux has been demonstrated in cardiac membrane vesicles exhibiting several properties similar to the Na/Ca exchange system operating in an Na/Na exchange mode (Reeves and Sutko, 1979), Na/Na exchange as part of the Na/Ca exchange mechanism has not yet been demonstrated in an intact preparation. In squid axons, the efflux of Na in the presence of ouabain is inhibited at high Na$_o$ and activated at low Na$_o$. This finding has been interpreted as a competition between Na$_o$ and Ca$_o$ ions and an activation
of the Na\textsubscript{i}/Ca\textsubscript{o} exchange by a monovalent cation (Baker et al., 1969). Although these experiments may suggest that the ouabain-insensitive Na/Na exchange does not exist in squid axons or that it occurs at a slower rate than the Na/Ca exchange, no systematic studies were carried out on the Na\textsubscript{o}-dependent Na efflux component when the Na/K pump was fully inhibited by ouabain and the Na/Ca exchange was completely activated with high Ca\textsubscript{i}.

![Graph A](image1.png)

**Figure 11.** (A) The effect of Ca\textsubscript{i} on the Ca\textsubscript{o}-dependent Na efflux in the absence and in the presence of ATP. (B) The effect of Ca\textsubscript{i} on the Na\textsubscript{o}-dependent Na efflux in the absence of ATP. Each symbol represents a single axon. In these experiments, the axons were predialyzed without Ca\textsubscript{i} and with or without ATP before the addition of the radioactive medium. The ordinate represents the percent activation of the Ca\textsubscript{o} (A) or Na\textsubscript{o}-dependent Na efflux (B) when taken as 100\% the steady state Na efflux obtained at 200 \mu M Ca\textsubscript{i}. The ionized Ca in the dialysis medium was controlled by varying the ratio (CaEGTA/free EGTA) at a constant total EGTA of 2 mM. The apparent dissociation constants for the CaEGTA and CaATP complexes were chosen as 0.15 \mu M and 1.4 mM, respectively (see Methods for references). Half-maximal activation for the Ca\textsubscript{o}-dependent component was obtained at 15 \mu M in the absence of ATP and at 1.8 \mu M in the presence of ATP (2 mM). Half-maximal activation for the Na\textsubscript{o}-dependent component was obtained at 9 \mu M in the absence of ATP.
Effects of pH, Mg, and Ca. One of the major findings reported here is that the Na$_o$-dependent Na efflux component activated by Ca$_i$ corresponds to a mode of operation of the Na/Ca exchange. Several arguments favor a common origin of the Ca$_i$-activated, Na$_o$-dependent Na efflux and the Na/Ca exchange mechanism. (a) Internal alkalinization, which is known to increase the forward Na/Ca exchange, also activates the Na$_o$ and Ca$_o$-dependent Na efflux components. (b) The sum of the Na$_o$-dependent and Ca$_o$-dependent components is always greater than the Na efflux in the presence of both Na$_o$ and Ca$_o$ ions, whether at pH 7.3 or 8.5; this suggests that both components are a manifestation of the same exchange system. (c) Internal alkalinization fails to activate the Na efflux in the absence of Ca$_i$, which indicates that the pH effect is on the Ca$_i$-activated Na/Ca exchange component. (d) Ca$_i$-activated, Na$_o$-dependent Na efflux can be largely inhibited by raising Ca$_o$, in line with the hypothesis that Na and Ca compete for a common external site on the exchanger. (e) Mg inhibits the Na$_o$-dependent Na efflux as well as the Na$_o$-dependent Ca efflux (see Fig. 7 and DiPolo and Beaugé, 1984). The activation of the Na efflux by removal of Mg is preferentially on the Na$_o$-dependent component, a result qualitatively similar to the effect of internal alkalinization. (f) The kinetics of activation of the Na$_o$-dependent Na efflux by Na$_o$ ($K_v = 140$ mM) are in agreement with the activation of the Na$_o$-dependent Ca efflux by Na$_o$ (DiPolo, 1974; Blaustein, 1977). (g) Finally, in the absence of ATP, both the Na$_o$- and Ca$_o$-dependent components are activated by Ca$_i$ with a low apparent affinity (half-maximal activation in the micromolar range) (see Fig. 11, A and B).

Effect of ATP on the kinetics of activation. Experiments investigating the ATP dependence of the forward Na/Ca exchange show that the nucleotide stimulates the Na$_o$-dependent Ca efflux with low affinity ($K_v \approx 200$ μM; DiPolo and Beaugé, 1979). This also appears to be the case for the activation of the Na$_o$-dependent Na efflux through the Na/Ca exchange system. In the presence of saturating concentrations of Na$_o$, Ca$_o$, and Ca$_i$, ATP stimulates the efflux of Na with low affinity ($K_v \approx 130$ μM). It could be thought that the effect of ATP is somehow related to the activating effect of internal alkalinization since both treatments affect the Na$_o$-dependent component more than the Ca$_o$-dependent one. However, the experiment of Fig. 4 shows that even at saturating concentrations of ATP, internal alkalinization still induces an increase in the Na$_o$-dependent Na efflux; this suggests that there are different mechanisms for the activation of the Na/Ca exchange by ATP and by the removal of H$_i$ ions.

From studies of the Na$_o$-dependent Ca efflux in injected (Baker and Glitsch, 1973) and dialyzed squid axons (DiPolo, 1974; Blaustein, 1977), it has been possible to demonstrate that ATP markedly increases the affinity of the Na/Ca exchange system toward Ca$_o$ and Ca$_i$. One of the interesting findings of the present work is that ATP affects the reverse mode of the exchange system by markedly reducing the Ca$_i$ for half-maximal activation of the Ca$_o$-dependent Na efflux component (see Fig. 11A).

The effect of ATP$_{2S}$. The evidence accumulated in squid axons that only hydrolyzable ATP analogues activate the Na/Ca exchange (nonhydrolyzable ATP analogues inhibit it; DiPolo, 1977), and that Mg ions are strictly required
for the ATP effect (DiPolo, 1977; DiPolo and Beaugé, 1984), suggest that the effect of ATP involves a phosphorylation of the Na/Ca exchanger. The experiments reported here with the analogue ATPγS are of interest because this analogue is known to act as a substrate of kinases but not of ATPases (Gratecos and Fischer, 1974; Cassidy et al., 1979). The finding that ATPγS does not promote any Na efflux component in the absence of Ca₂⁺ ions (see Fig. 8A) is in agreement with recent evidence obtained in cardiac sarcolemma vesicles (Caroni and Carafoli, 1983), which suggested a phosphorylation of the Na/Ca exchanger by ATP mediated by a Ca₂⁺-dependent protein kinase. Since in the experiments with ATPγS, ouabain and bumetanide were not added to the external solutions and Mg₂⁺ was always present, one can conclude that the analogue is unable to activate the Na/K pump, the Na/K/Cl co-transport, or the Na/Mg₂⁺ exchange. As in the case of ATP, the analogue preferentially activates the Na/Na exchange mode of operation. Results not shown (DiPolo, R., and L. Beaugé, unpublished results) indicate that ATPγS substantially increases the forward (Na⁺-dependent Ca efflux) Na/Ca exchange without affecting the Ca pump component of the Ca efflux, an indication of a remarkable selectivity of the ATP analogue in activating only the Na and Ca fluxes through the Na/Ca exchange system.

Ca₂⁺-independent, ATP-activated Na⁺/Na⁺ Exchange

The results presented here confirm the early finding that in squid axons there is a Ca₂⁺-independent, ATP-activated Na⁺/Na⁺ exchange (Beaugé and DiPolo, 1981). This Na⁺/Na⁺ exchange has been related to a glycoside-poisoned Na pump (Brinley and Mullins, 1968), and although some evidence exists that this might be the case (Beaugé and DiPolo, 1981), at present it is not clear that this component is really induced by the glycoside. On the basis of theoretical considerations, it is also possible that the Ca₂⁺-independent Na⁺/Na⁺ exchange component is an operational mode of the Na/Ca exchange system (DiPolo and Beaugé, 1986). However, the experimental data presented in this work strongly argue against this possibility and show that it might represent a system different from the Na/Ca exchange. Four main arguments favor this proposal. First, the magnitude of the Ca₂⁺-independent, Na⁺-dependent Na efflux (6.5 pmol·cm⁻²·s⁻¹, average of four axons) is unaffected by internal alkalinization (see Fig. 5, A and B), in marked contrast to the Ca₂⁺-activated Na⁺/Na⁺ exchange system (see Fig. 4). Second, Ca₀ up to 50 mM does not inhibit the level of the Na efflux, which suggests that Na and Ca do not compete for a common external site. Third, this component is not modified by the removal of K⁺ or K⁺ (Beaugé and DiPolo, 1982), an experimental treatment that is known to affect the Na/Ca exchange mechanism (DiPolo and Rojas, 1984). A final important argument is that the ATP analogue ATPγS is unable to activate this component (see Fig. 8A); this demonstrates a specificity of ATP in activating the Ca₂⁺-independent Na⁺/Na⁺ exchange compared with the Ca₂⁺-dependent Na⁺/Na⁺ exchange, which can be activated either by ATP or ATPγS. Unfortunately, no difference in the apparent affinity for ATP exists between these two Na⁺/Na⁺ exchange components (see Fig. 3A of Beaugé and DiPolo, 1981). It is worthwhile to mention that if the Ca₂⁺-independent Na⁺/Na⁺ exchange is an induced ion flux through a glycoside-poisoned Na pump, it would not exist under physiological conditions.
Implications of Ca\textsuperscript{2+}-activated Na\textsubscript{i}/Ca\textsubscript{o} and Na\textsubscript{i}/Na\textsubscript{o} Exchange Systems

As reported here (see also DiPolo and Beaugé, 1986), the dependence of every mode of operation of the Na/Ca exchange system on Ca\textsubscript{i} (the "essential activator") should be taken into account in future kinetic models of the Na/Ca exchange. This is particularly important for calculating net Ca movements from traditional symmetric models (Mullins, 1977; Wong and Bassingthwaighte, 1981; Johnson and Kootsey, 1985) since the electrochemical ionic gradients of Na and Ca exclusively will not predict Ca entry (or Na exit) in an asymmetric system. The presence of a Ca\textsubscript{i}-activated Na\textsubscript{i}/Na\textsubscript{o} exchange as part of the Na/Ca exchange mechanism should also be considered when calculating the stoichiometry of the exchange process from unidirectional Na and Ca isotope flux measurements. Otherwise, in the presence of Na ions at both sides of the membrane, the number of Na ions exchanged for Ca will be overestimated by the presence of the Ca\textsubscript{i}-activated Na/Na exchange component. A point that is worth stressing is that the Na/Na exchange mode of operation of the Na/Ca exchange will occur under physiological conditions whenever the exchanger is activated by a rise in the ionized Ca\textsubscript{i}. This is in contrast to the case of the Ca/Ca exchange mode, which is only significant in the absence of Na\textsubscript{o} and in the presence of other external alkali metal ions (Blaustein, 1977).

The physiological consequences of the effect of Ca\textsubscript{i} as an essential activator of the Na/Ca exchange are under investigation. Nevertheless, it is consistent with earlier experiments reported by Baker (1972) and Baker and McNaughton (1976) showing that injection of EGTA into squid axons inhibits the Ca\textsubscript{i}-dependent Na efflux. The requirement for Ca\textsubscript{i}, but not a direct pharmacological effect of EGTA (DiPolo, 1979), would certainly explain the inhibition of the "reverse" Na/Ca exchange observed in squid axons injected with the Ca chelating agent quin2, as well as the presence in the same preparation of a Ca\textsubscript{i}-activated outward current generated during the operation of the Na\textsubscript{i}/Ca\textsubscript{o} exchange (DiPolo et al., 1987). This interpretation also agrees with the observation of an outward current caused by the Na/Ca exchange in Na-loaded myocytes, which disappears in the absence of Ca\textsubscript{i} ions (Kimura et al., 1986). Recent experiments with membrane vesicles from squid optic nerves have shown that in vesicles loaded with different CaEGTA concentrations, the rate of Ca uptake in exchange for Na is dependent on micromolar quantities of Ca present inside the vesicles, in agreement with an asymmetric Na/Ca exchange system (Condrescu et al., 1987). Finally, if the dependence of the reverse mode on Ca\textsubscript{i} is a generalized property of the exchanger, then Ca entry via voltage-dependent Ca channels (increase in Ca\textsubscript{i}) could modulate the activity of the exchanger. In support of this possibility is the fact that an increase in Ca\textsubscript{i} from 0.1 to 0.6 \textmu M led to a 10-fold increase in Ca entry through the Na/Ca exchange system induced by membrane depolarization (DiPolo et al., 1982).

We wish to thank Madalina Condrescu for helpful discussion. We are grateful to Miss Lilian Palacios for her secretarial help. We also thank the IVIC squid supply team at Mochima, Edo Sucre, and IVIC, Caracas, and the director and staff of the Marine Biological Laboratory, Woods Hole, MA, for the facilities put at our disposal.
This work was supported by grants from the National Science Foundation (RNS-8500595), CONICIT (S1-1954), Fundación Polar, Venezuela, and CONICET Argentina.

Original version received 20 October 1986 and accepted version received 13 April 1987.

REFERENCES

Allen, T. J. A., and P. F. Baker. 1985. Intracellular Ca indicator Quin-2 inhibits Ca$^{2+}$ inflow via Na/Ca$_e$ exchange in squid axon. Nature. 316:755–756.

Baker, P. F. 1972. Transport and metabolism of calcium ions in nerve. Progress in Biophysics and Molecular Biology. 24:177–223.

Baker, P. F., M. P. Blaustein, A. L. Hodgkin, and R. A. Steinhart. 1969. The influence of calcium of sodium efflux in squid axons. Journal of Physiology. 200:431–458.

Baker, P. F., and H. G. Glitsch. 1973. Does metabolic energy participate directly in the Na-dependent extrusion of Ca ions from squid axons? Journal of Physiology. 235:44P–46P.

Baker, P. F., and P. A. McNaughton. 1976. Kinetics and energetics of Ca efflux from intact squid giant axons. Journal of Physiology. 276:103–144.

Beaugé, L. A., and R. DiPolo. 1981. An ATP-dependent sodium-sodium exchange in strophanthinid poisoned dialyzed squid giant axons. Journal of Physiology. 315:447–460.

Blaustein, M. P. 1977. Effect of internal and external cations and of ATP on sodium-calcium exchange in squid axons. Biophysical Journal. 20:79–100.

Blaustein, M. P., and J. M. Russell. 1975. Sodium-calcium and calcium-calcium exchange in internally dialyzed squid giant axons. Journal of Membrane Biology. 22:285–312.

Brinley, F. J., and L. J. Mullins. 1968. Sodium fluxes in internally dialyzed squid axons. Journal of General Physiology. 52:181–211.

Caroni, P., and E. Carafoli. 1983. The regulation of the Na$^+$-Ca$^{2+}$ exchanger of heart sarcolemma. European Journal of Biochemistry. 132:451–460.

Cassidy, P., P. E. Hoar, and W. G. Kerrick. 1979. Irreversible thiophosphorylation and activation of tension in functionally skinned rabbit ileum strip by $35$ATP$_7$S. Journal of Biological Chemistry. 254:11148–11153.

Condrescu, M., A. Gerardi, and R. DiPolo. 1987. Demonstration of Na-Ca exchange asymmetry in squid optic nerve vesicles. Biophysical Journal. 51:10a. (Abstr.)

DiPolo, R. 1974. The effect of ATP on Ca efflux in dialyzed squid axons. Journal of General Physiology. 64:503–517.

DiPolo, R. 1977. Characterization of the ATP-dependent calcium efflux in dialyzed squid axons. Journal of General Physiology. 69:795–814.

DiPolo, R. 1979. Ca influx in internally dialyzed squid axons. Journal of General Physiology. 73:91–115.

DiPolo, R., and L. Beaugé. 1979. Physiological role of ATP-driven Ca pump in squid axons. Nature. 278:271–273.

DiPolo, R., and L. Beaugé. 1982. The effect of pH on Ca$^{2+}$ extrusion mechanisms in dialyzed squid axons. Biochimica et Biophysica Acta. 688:227–245.

DiPolo, R., and L. Beaugé. 1984. Interactions of physiological ligands with the Ca pump and the Na/Ca exchange in squid axons. Journal of General Physiology. 84:895–914.

DiPolo, R., and L. Beaugé. 1986. Reverse Na-Ca exchange requires internal Ca and/or ATP in squid axons. Biochimica et Biophysica Acta. 854:298–306.

DiPolo, R., F. Bezanilla, C. Caputo, and H. Rojas. 1985. Voltage dependence of the Na/Ca exchange in voltage-clamped dialyzed squid axons. Journal of General Physiology. 86:457–478.
DiPolo, R., C. Caputo, and F. Bezanilla. 1987. Ca\(^{2+}\) requirement for the outward membrane current associated with the reversal of the Na/Ca exchange. *Biophysical Journal.* 51:386a. (Abstr.)

DiPolo, R., J. Requena, F. J. Brinley, L. J. Mullins, A. Scarpa, and T. Tiffert. 1976. Ionized calcium concentration in squid axons. *Journal of General Physiology.* 67:433–467.

DiPolo, R., and H. Rojas. 1984. Effect of internal and external K\(^+\) on Na\(^+-\)Ca\(^{2+}\) exchange in dialyzed squid axons under voltage clamp conditions. *Biochimica et Biophysica Acta.* 776:313–316.

DiPolo, R., H. Rojas, and L. A. Beaugé. 1982. Ca entry at rest and during prolonged depolarization in dialyzed squid axons. *Cell Calcium.* 3:19–41.

Gratecos, D., and E. Fischer. 1974. Adenosine 5'-O(3-thio-triphosphate) in the control of phosphorylase activity. *Biochemical and Biophysical Research Communications.* 58:960–967.

Johnson, E., and J. M. Kootsey. 1985. A minimum mechanism for Na\(^+-\)Ca\(^{2+}\) exchange: net and unidirectional Ca\(^{2+}\) fluxes as functions of ion composition and membrane potential. *Journal of Membrane Biology.* 86:167–187.

Kimura, J., A. Noma, and H. Irisawa. 1986. Na-Ca exchange current in mammalian heart cells. *Nature.* 319:596–597.

Mullins, L. J. 1977. A mechanism of Na/Ca exchange. *Journal of General Physiology.* 70:681–696.

Reeves, J. P., and J. L. Sutko. 1979. Na-Na exchange in cardiac membrane vesicles. *Federation Proceedings.* 38:1199. (Abstr.)

Reeves, J. P. 1986. The sarcolemma sodium-calcium exchange system. *Current Topics in Membranes and Transport.* 25:77–127.

Russell, J. M. 1979. Chloride and sodium influx. A coupled uptake mechanism in the squid giant axon. *Journal of General Physiology.* 73:801–818.

Wong, A. Y., and J. Bassingthwaighte. 1981. The kinetics of Na-Ca exchange in excitable tissue. *Mathematical Biosciences.* 53:275–310.