

# Interactions of Physiological Ligands with the Ca Pump and Na/Ca Exchange in Squid Axons

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**ABSTRACT** We have studied the interaction of physiological ligands other than  $\text{Na}_i$  and  $\text{Ca}_i$  with the Ca pump and Na/Ca exchange in internally dialyzed squid axons. The results show the following. (a) Internal  $\text{Mg}_i^{2+}$  is an inhibitor of the  $\text{Na}_o$ -dependent Ca efflux. At physiological  $\text{Mg}_i^{2+}$  (4 mM), the inhibition amounts to ~50%. The inhibition is partial and noncompetitive with  $\text{Ca}_i$ , and is not affected by  $\text{Na}_i$  or ATP. The ATP-dependent uncoupled efflux is unaffected by  $\text{Mg}_i$  up to 20 mM. Both components of the Ca efflux require  $\text{Mg}_i^{2+}$  for their activation by ATP. (b) At constant membrane potential,  $K_i$  is an important cofactor for the uncoupled Ca efflux. (c) Orthophosphate ( $\text{P}_i$ ) activates the  $\text{Na}_o$ -dependent Ca efflux without affecting the uncoupled component. Activation by  $\text{P}_i$  occurs only in the presence of Mg-ATP or hydrolyzable ATP analogues.  $\text{P}_i$  under physiological conditions has no effect on the uncoupled component; nevertheless, at alkaline pH, it inhibits the Ca pump, probably by product inhibition. (d) ADP is a potent inhibitor of the uncoupled Ca efflux. The  $\text{Na}_o$ -dependent component is inhibited by ADP only at much higher ADP concentrations. These results indicate that (a) depending on the concentration of  $\text{Ca}_i^{2+}$ ,  $\text{Na}_i^+$ ,  $\text{Mg}_i^{2+}$ , and  $\text{P}_i$ , the Na/Ca carrier can operate under a low- or high-rate regime; (b) the interactions of  $\text{Mg}_i^{2+}$ ,  $\text{P}_i$ ,  $\text{Na}_i^+$ , and ATP with the carrier are not interdependent; (c) the effect of  $\text{P}_i$  on the carrier-mediated Ca efflux resembles the stimulation of the  $\text{Na}_o$ -dependent Ca efflux by internal vanadate; (d) the ligand effects on the uncoupled Ca efflux are of the type seen in the Ca pump in red cells and the sarcoplasmic reticulum.

## INTRODUCTION

In squid axons, extrusion of Ca ions is accomplished by two different Ca transport mechanisms: a high-affinity, low-capacity system driven by ATP (the Ca pump), and a low-affinity, high-capacity system driven by the electrochemical Na gradient (Na/Ca exchange) (DiPolo, 1978; DiPolo and Beaugé, 1979). The concerted

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action of two parallel Ca transport systems in the regulation of  $[Ca^{2+}]_i$ ; does not seem to be a unique property of squid axons. It has been proposed recently for several other excitable preparations (Caroni and Carafoli, 1980; Kurzinger et al., 1980).

The experimental information available on these two systems indicates that they may be affected in a rather different and complex manner by several intra- and extracellular compounds including  $Na^+$ ,  $K^+$ ,  $H^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $P_i$ , ATP, and ADP (Blaustein, 1977; Brinley et al., 1975; Baker, 1978; DiPolo, 1977; DiPolo and Beaugé, 1980; Requena, 1978*b*). However, the lack of detailed information about these multiple ligand interactions has rendered somewhat difficult the dissection and analysis of these two components.

In principle, the characterization of these interactions should be greatly simplified in dialyzed axons, where suitable control of intra- and extracellular variables can be achieved. In fact, experiments carried out in dialyzed squid axons on the dependence of Ca efflux on  $Na_o$  and  $Ca_o$  (forward exchange mode) (Blaustein, 1974; Mullins, 1977; DiPolo, 1979), and of Ca influx on internal  $Na_i$  and  $Ca_i$  (backward exchange mode) (Mullins, 1977; DiPolo, 1979) have led to the actual functional scheme for Na/Ca exchange. Although these models can satisfactorily explain certain kinetic properties of the Na/Ca exchange carrier system, they only describe the interactions of the transport mechanism with the ions being transported ( $Na^+$  and  $Ca^{2+}$ ).

More complete schemes for the functioning of both Na/Ca exchange and the Ca pump should necessarily include their reactions with those ligands normally present in the cell, which, although not transported by the transport system, may influence the rate of Ca transport. Furthermore, the knowledge of these ligand interactions may help to interpret recent kinetic data on Ca transport in isolated membrane preparations in which the experimental medium may contain ions other than Na and Ca.

In this paper, we report the effect of  $Mg^{2+}$ ,  $K^+$ , inorganic phosphate ( $P_i$ ), ATP, and ADP on the forward modes of the Ca pump (uncoupled Ca efflux) and Na/Ca exchange ( $Na_o$ -dependent Ca efflux). Particular effort has been made in determining whether the interaction of these ligands with the Ca transport mechanisms is affected by the natural ionic substrates  $Na_i^+$  and  $Ca_i^{2+}$ . Our results indicate that Mg ions are absolutely required for the activation of both the uncoupled and the ATP-stimulated,  $Na_o$ -dependent Ca efflux components. In addition, we have found that internal  $Mg^{2+}$  is a good inhibitor of Na/Ca exchange. The inhibition is partial and noncompetitive with  $Ca_i^{2+}$ .

$P_i$  activates the  $Na_o$ -dependent Ca efflux. This effect is observed only in the presence of ATP. The interactions of  $Mg_i^{2+}$ ,  $P_i$ , and ATP are apparently not affected by changes in  $Na_i$  and  $Ca_i^{2+}$ , which suggests that they act on sites different from those of the translocating ions ( $Na^+$  and  $Ca^{2+}$ ). The ATP-dependent uncoupled Ca pump was found to be strongly inhibited by ADP and not affected by high levels of  $Mg_i^{2+}$  or  $P_i$ . Nevertheless, under certain conditions (external alkaline pH),  $P_i$  inhibits the uncoupled efflux (perhaps via product inhibition). Finally, internal K appears to be an important cofactor for the operation of the ATP-driven component.

## METHODS

The experiments reported herein were performed with live specimens of *Loligo pealei* at the Marine Biological Laboratory, Woods Hole, MA, and with the tropical squid *Loligo plei* at Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela. Immediately after decapitation, the hindmost giant axon was dissected from the mantle in seawater. After careful cleaning, the axon was mounted horizontally in a standard dialysis chamber (Brinley and Mullins, 1967).

*Solutions*

**DIALYSIS MEDIUM** The standard dialysis solution had the following composition (mM): 310 K<sup>+</sup>, 35–40 (*L. plei*) or 60 (*L. pealei*) Na<sup>+</sup>, 4 Mg<sup>2+</sup>, in excess of the ATP concentration, 30 Tris<sup>+</sup>, 98 Cl<sup>-</sup>, 310 aspartate, 1 or 2 EGTA, 330 glycine, pH 7.3 (18–19°C). Removal of Na<sup>+</sup>, K<sup>+</sup>, or Mg<sup>2+</sup> was compensated for with equiosmolar amounts of Tris<sup>+</sup>. The osmolarity of all solutions was determined using a commercial psychrometer (Wescor Inc., Logan, UT) and adjusted to 990 mosmol. All internal solutions contained 10 µg/ml oligomycin. The estimation of the ionized Ca concentration was based on a dissociation constant of 0.15 µM for Ca-EGTA (0.3 ionic strength, pH 7.3; DiPolo et al., 1976, 1983), and 1.4 mM for Ca-ATP (P. De Weer, personal communication). The estimation of the ionized Mg concentration was based on a dissociation constant of 0.7 mM for Mg-ATP (P. De Weer, personal communication) and 30 mM for Mg-EGTA (DiPolo et al., 1976). ATP (vanadium-free) was obtained from Sigma Chemical Co. (St. Louis, MO) as Tris salt, neutralized with Tris-OH, and stored at -20°C as a 250-mM solution. Phosphoarginine (PA), at a concentration of 5 mM, was usually added to the solutions containing ATP. This procedure minimizes variations in the ATP level caused by its hydrolysis by the axoplasmic ATPases (Brinley and Mullins, 1967). Na orthovanadate (from Fisher Scientific Co., Pittsburgh, PA) was prepared as a 100-mM solution.

**ARTIFICIAL SEAWATER (ASW)** The standard ASW had the following composition (mM): 10 K<sup>+</sup>, 440 Na<sup>+</sup>, 50 Mg<sup>2+</sup>, 10 Ca<sup>2+</sup>, 580 Cl<sup>-</sup>, 0.1 EDTA, pH 7.6 (18–19°C). Removal of Na<sup>+</sup>, Ca<sup>2+</sup>, or Mg<sup>2+</sup> was compensated for with equiosmolar amounts of Tris<sup>+</sup>. Changes in K<sup>+</sup> concentrations were made by simultaneous addition or removal of similar amounts of NaCl. The osmolarity was 1,000 mosmol. All external solutions contained 1 mM cyanide (CN<sup>-</sup>).

All reagents used were analytical grade. Radioactive solutions were made by adding solid [<sup>45</sup>Ca]CaCl<sub>2</sub> (15–30 mCi/mg; New England Nuclear, Boston, MA) directly to the internal solutions. [<sup>14</sup>C]EGTA was obtained from New England Nuclear (9.7 mCi/mmol). Radioactive samples contained in 4 ml ASW were mixed with 5 ml of scintillation solution and counted in a liquid scintillation counter for times long enough to give a standard error of ~1%.

*Experimental Procedure*

**DIALYSIS TECHNIQUE** The general dialysis technique for efflux measurements has already been described in detail (Brinley and Mullins, 1967; DiPolo, 1977). In the present work, porous glass capillaries were used to dialyze axons from *L. pealei* (mean diameter, 550 µm). For axons of smaller diameter, such as those of *L. plei* (mean diameter, 400 µm), control of axoplasmic constituents including ATP could be efficiently achieved with porous cellulose acetate plastic capillaries. The flow rate through the porous capillaries was 1–2 µl/min. The flow of the external solution was 1 ml/min. The collection periods lasted for 4 min. No significant differences in the Ca transport properties were found between the two squid species used in this work. Unless otherwise indicated, each experimental finding was reproduced at least once.

**CA EFFLUX AND THE "LEAK" OF CA-EGTA** Measurements of Ca efflux at submicromolar  $\text{Ca}_i^{2+}$  concentrations can be considered reliable only if a suitable buffer agent is included in the dialysis solution. A previous study (Brinley et al., 1975) indicated that the measured Ca efflux not only represents  $\text{Ca}^{2+}$  transferred across the cell membrane, but also Ca leaking out of the fiber complexed with the chelating agent. In an axon dialyzed with submicromolar  $[\text{Ca}^{2+}]_i$ , most of the total (radioactive plus cold) Ca present in the internal medium is bound to the buffer agent (EGTA) and only a small fraction is ionized. Therefore, knowledge of the Ca-EGTA "leak" is important for establishing the lower detection limit of  $\text{Ca}^{2+}$  efflux. Moreover, it is also important to know whether experimental manipulations (different ionic conditions, etc.) may alter the Ca-EGTA leak. In order to do so, we used  $^{14}\text{C}$ EGTA to measure the leak of Ca-EGTA.

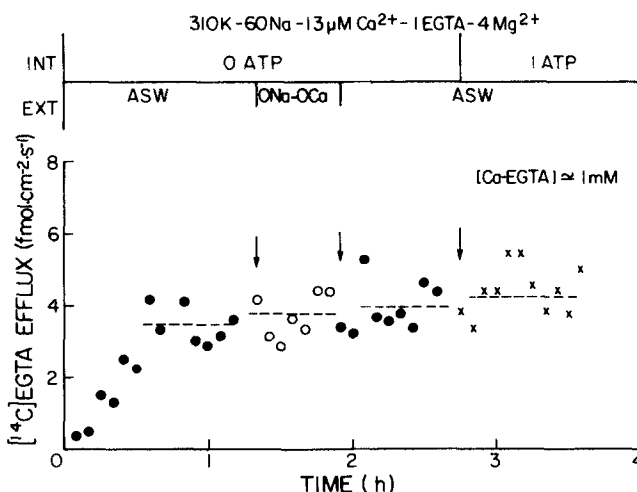


FIGURE 1. Effect of internal and external solutions upon the flux of  $^{14}\text{C}$ Ca-EGTA. The axon was dialyzed with a standard solution containing 1 mM Ca and 1 mM EGTA. Closed circles: efflux into ASW; open circles: efflux in 0  $\text{Na}_o$ , 0  $\text{Ca}_o$ ; crosses: efflux in the presence of 1 mM ATP. All solute concentrations are in millimolar. Axon diameter, 390  $\mu\text{M}$ ; temperature, 21  $^\circ\text{C}$ .

Fig. 1 illustrates an experiment in which an axon was dialyzed with standard dialysis solution containing 1 mM total Ca and 1 mM total EGTA. The calculated ionized Ca and free EGTA were 13 and 11  $\mu\text{M}$ , respectively. Once a steady state EGTA efflux of  $\sim 3$   $\text{fmol}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$  was reached, external Na and Ca were replaced by Tris and Mg ions, respectively. This treatment did not affect the level of flux. Similarly, addition of ATP to the dialysis medium had no effect on  $^{14}\text{C}$ EGTA efflux. In six different experiments, the mean efflux value for the leak of EGTA per millimolar Ca-EGTA (mean  $\pm$  SEM) was  $5.6 \pm 1.2$   $\text{fmol}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ . This value should be taken as the lower limit of  $\text{Ca}^{2+}$  efflux that could be detected per millimolar Ca-EGTA. The magnitude of the leak of chelated Ca-EGTA is almost five times smaller than that reported for EDTA in squid axons (Brinley et al., 1975). A possible explanation for this difference may reside in the larger molecular weight of EGTA over that of EDTA.

## RESULTS

In dialyzed axons, four components of Ca efflux can be distinguished: (a) it is dependent on external Na ( $\text{Na}_o$ -dependent Ca efflux or forward Na/Ca ex-

change); (b) it has ATP-dependent uncoupled efflux (Ca efflux observed in the absence of  $\text{Na}_o$  and  $\text{Ca}_o$ : forward Ca pump); (c) it is activated by external Ca ( $\text{Ca}_o$ -activated Ca efflux, presumably Ca/Ca exchange); and (d) there is leak of Ca-EGTA. The results presented here deal mainly with the effects of different ligands on the two major components of the Ca efflux, the uncoupled and the  $\text{Na}_o$ -dependent. The magnitudes of the different components of the Ca efflux reported in this paper at different internal ionized Ca concentrations are in agreement with those reported previously (DiPolo and Beaugé, 1979, 1980).

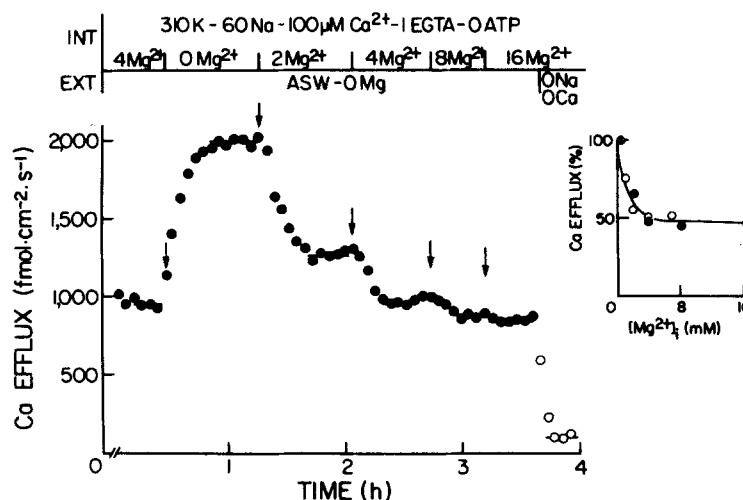


FIGURE 2. Effect of  $\text{Mg}_i^{2+}$  on  $\text{Na}_o$ -dependent Ca efflux in an axon dialyzed without ATP. The axon was bathed in ASW without Mg and predialyzed for 1 h. The arrows indicate the changes in internal dialysis solutions. Closed circles: Ca efflux in the presence of  $\text{Na}_o$  and  $\text{Ca}_o$ . Axon diameter,  $540 \mu\text{m}$ . Inset: inhibition of Ca efflux, normalized to that observed with  $0 \text{Mg}_i^{2+}$  as a function of the  $\text{Mg}_i^{2+}$  concentration. Open symbols correspond to two axons dialyzed with  $0.7 \mu\text{M Ca}^{2+}$ ; filled circles are values from three different axons dialyzed with  $100$  or  $200 \mu\text{M Ca}^{2+}$ . Axon 110583B.

#### *Effects of Internal Mg*

**NA/CA EXCHANGE** Fig. 2 shows the effect of  $[\text{Mg}^{2+}]_i$  on the magnitude of the forward mode of Na/Ca exchange in the absence of ATP. In this type of experiment, Mg was removed from the seawater to assure complete control of  $\text{Mg}_i$  at the inner surface of the membrane. Removal of  $\text{Mg}^{2+}$  from the dialysis medium causes an important increase in the Ca efflux, which is reversed by progressive additions of  $\text{Mg}_i^{2+}$ . The observed inhibitory effect of  $\text{Mg}_i^{2+}$  seems to saturate between 4 and 8 mM (see the inset of Fig. 2). An interesting additional finding is that  $\text{Mg}_i$  inhibition is only partial, since a further increase to 16 mM has no effect on the steady level of the Ca efflux. In other experiments, a similar partial inhibition by  $[\text{Mg}^{2+}]_i$  was observed even at  $[\text{Mg}^{2+}]_i$  as high as 20 mM. The fraction of Ca efflux that remains in the presence of high  $[\text{Mg}^{2+}]_i$  is not a

nonspecific Ca leak, since, as shown at the end of the experiments, removal of  $\text{Na}_o$  and  $\text{Ca}_o$  reduced the efflux of Ca to the expected Ca-EGTA leak level. The experiment illustrated in Fig. 3 shows that the fractional inhibition of the  $\text{Na}_o$ -dependent Ca efflux caused by 4 mM internal  $\text{Mg}^{2+}$  is the same at 0.7 and 200  $\mu\text{M}$  internal  $\text{Ca}^{2+}$  concentration. This and the results of other experiments, which are summarized in the inset of Fig. 2, show that the apparent affinity for  $\text{Mg}_i^{2+}$  as inhibitor is not appreciably changed by varying  $\text{Ca}_i^{2+}$  between 0.7 and 200  $\mu\text{M}$ . This suggests that under these conditions,  $\text{Mg}_i^{2+}$  behaves as a noncompetitive inhibitor.

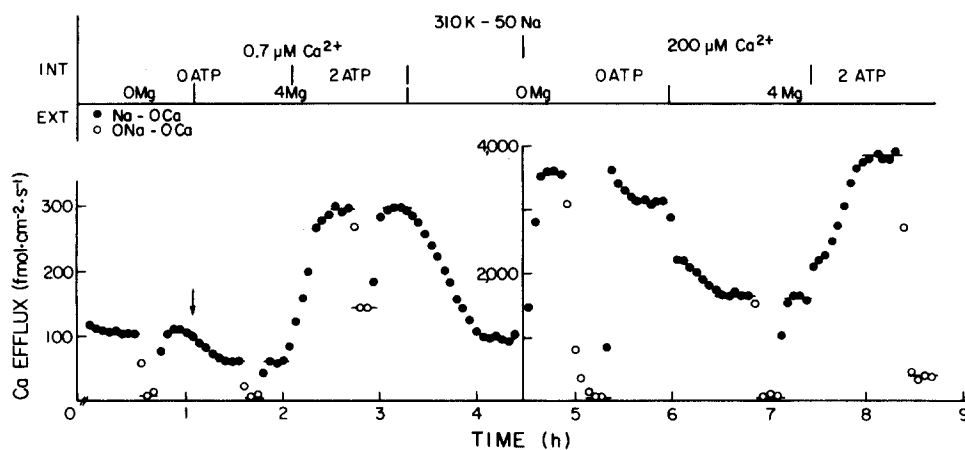


FIGURE 3. Effect of internal ionized Mg on the  $\text{Na}_o$ -dependent Ca efflux at two different  $\text{Ca}_i^{2+}$  concentrations. Notice the change in scale in the ordinate when internal Ca was changed from 0.7 to 200  $\mu\text{M}$ . Closed circles: Ca efflux in the presence of  $\text{Na}_o$ ; open circles: Ca efflux in the absence of  $\text{Na}_o$  and  $\text{Ca}_o$ . Unless otherwise stated, all concentrations are expressed in millimolar. Axon diameter, 500  $\mu\text{m}$ . Axon 090583A.

Since Na/Ca exchange can be activated by ATP (DiPolo, 1974; DiPolo and Beaugé, 1979), the effect of internal  $\text{Mg}^{2+}$  on this mode of Ca efflux was also studied in the presence of the nucleotide. The need for  $\text{Mg}_i^{2+}$  in ATP activation of Ca efflux was already demonstrated in dialyzed squid axons (DiPolo, 1977). However, since in those experiments  $\text{Ca}_i^{2+}$  was buffered near the physiological range (0.06–0.1  $\mu\text{M}$ ), where most of the Ca efflux is uncoupled ( $\geq 80\%$ ; DiPolo and Beaugé, 1979), no information could be obtained about whether  $\text{Mg}_i^{2+}$  was indeed necessary for ATP stimulation of the  $\text{Na}_o$ -dependent Ca efflux. Fig. 4 illustrates an experiment designed to answer this question. In this case, care had to be taken to avoid the presence of internal  $\text{Mg}^{2+}$  near the membrane prior to the addition of ATP. This was accomplished by predialyzing the axon with 1 mM free EDTA for 1 h and by removing the  $\text{Mg}_o$ . As was expected, at high  $[\text{Ca}_i^{2+}]_i$  there is a marked sensitivity of the Ca efflux to  $\text{Na}_o$  even in the complete absence of ATP. In addition, it is clear that the Tris salt of ATP (in the absence of  $\text{Mg}_i$ ) is not able to activate the  $\text{Na}_o$ -dependent extrusion of Ca. Only when

Mg<sub>i</sub><sup>2+</sup> is introduced into the dialysis solution is a substantial activation observed. It could be argued that the increase in Ca efflux following addition of Mg is the result of a rise in [Ca<sup>2+</sup>]<sub>i</sub> caused by the binding of Mg to EGTA. From the known dissociation constants of Ca-EGTA, Mg-EGTA, Ca-ATP, and Mg-ATP (De Weer and Lowe, 1973; P. De Weer, personal communication; DiPolo et al., 1976, 1983), it is possible to calculate the simultaneous equilibrium concentrations of the different complexes and free ligands. The estimated increment in [Ca<sup>2+</sup>]<sub>i</sub> is too small (<10%) to explain the increase in Ca efflux induced by Mg in the presence of ATP.

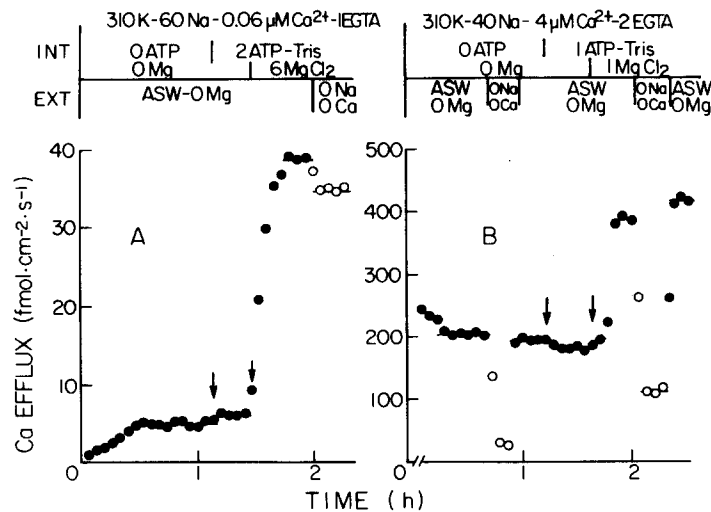


FIGURE 4. Effect of Tris-ATP and Mg-ATP on the uncoupled and Na<sub>o</sub>-dependent Ca efflux components. (A) Axon dialyzed with 0.06 μM Ca to activate only the uncoupled component. (B) Axon dialyzed with 4 μM Ca<sup>2+</sup> to activate the Na<sub>o</sub>-dependent Ca efflux. To completely remove internal Mg, the axons were pre-dialyzed for 1 h with 1 mM EDTA in the absence of external Mg. All concentrations are in millimolar except the [Ca<sup>2+</sup>]<sub>i</sub>, which are in micromolar. Closed circles: Ca efflux in ASW containing no Mg; open circles: Ca efflux into 0 Na<sub>o</sub>, 10 Ca<sub>o</sub>, and 0 Mg<sub>o</sub>.

Fig. 5 shows the effects of Mg<sub>i</sub><sup>2+</sup> on the Na<sub>o</sub>-dependent Ca efflux in the presence of ATP. In this case, the internal ionized Ca was purposely increased to 100 μM in order to saturate Ca extrusion (DiPolo and Beaugé, 1980). With 1 mM Mg<sub>i</sub><sup>2+</sup> and 0.5 mM ATP, Ca efflux reaches a steady level of 200 fmol·cm<sup>-2</sup>·s<sup>-1</sup>, which upon removal of Na<sub>o</sub> is reduced to 300 fmol·cm<sup>-2</sup>·s<sup>-1</sup> (this remainder is pump flux). In the presence of full Na<sub>o</sub>, increasing Mg<sub>i</sub><sup>2+</sup> to 4 mM results in a 40% inhibition of the Na<sub>o</sub>-dependent Ca efflux. This and similar experiments (not shown) show that the fractional inhibition by Mg<sub>i</sub><sup>2+</sup> of Na<sub>o</sub>-dependent Ca efflux is not significantly different in the presence or absence of ATP. Regarding the ATP effect, it should be noted (see Fig. 3 above) that the magnitude of the Na<sub>o</sub>-dependent component in the presence of ATP and Mg<sub>i</sub><sup>2+</sup> is greater than that

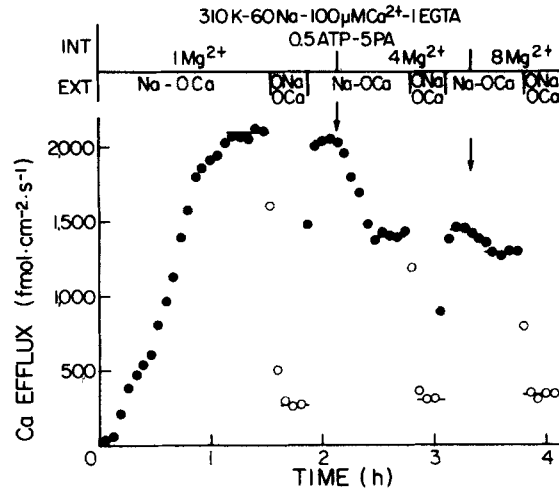


FIGURE 5. Effect of  $Mg_i^{2+}$  on both components of the Ca efflux in the presence of ATP. Closed circles: Ca efflux in the presence of full  $Na_o$ , 0  $Ca_o$ ; open circles: Ca efflux into 0  $Na_o$ , 0  $Ca_o$ . The arrows indicate the addition of internal  $Mg_i^{2+}$ . Observe that the magnitude of the uncoupled Ca efflux (Ca efflux in the absence of  $Na_o$  and  $Ca_o$ ) is not affected by internal Mg. Axon diameter, 600  $\mu m$ . Axon 130583A.

in their absence; this shows that ATP can activate the  $Na_o$ -dependent component beyond the level observed at 0  $Mg_i$ , which suggests that ATP stimulation of  $Na/Ca$  exchange cannot be ascribed to a release of  $Mg_i$  inhibition.

It has been proposed (Requena, 1978b) that inhibition by  $Mg_i^{2+}$  of  $Na_o$ -dependent Ca efflux is somehow counteracted by  $Na_i^+$ . In the experiments described above, the effects of internal Mg were explored at constant  $Na_i$ . In what follows, the influence of  $Na_i$  on  $Mg_i$  inhibition was examined. Fig. 6 shows

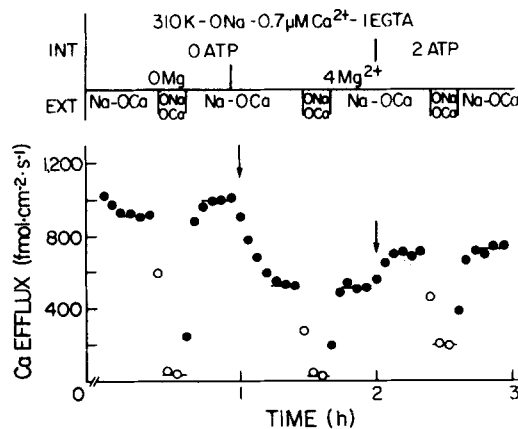


FIGURE 6. Effect of  $Mg_i^{2+}$  on the  $Na_o$ -dependent Ca efflux in the absence of  $Na_i$ . Closed circles: Ca efflux in the presence of full  $Na_o$ , 0  $Ca_o$ ; open circles: Ca efflux into 0  $Na_o$ , 0  $Ca_o$ . All concentrations are in millimolar, except  $Ca^{2+}$ , which is in micromolar.



one of these experiments, in which an axon was dialyzed from the beginning without  $Mg_i^{2+}$ ,  $Na_i^+$ , or ATP and with  $0.7 \mu M Ca^{2+}$ . Under these conditions, all Ca efflux (leak subtracted) is  $Na_o$ -dependent. The addition of  $4 mM Mg_i^{2+}$  resulted in an inhibition of Ca efflux similar (50%) to that seen in axons dialyzed with internal Na (Figs. 2 and 3). Other experiments with axons containing 30–40 mM  $Na_i$  gave identical results (not shown). In addition, Fig. 6 shows that in the absence of  $Na_i$ , ATP has no effect on  $Na_o$ -dependent Ca efflux since removal of external Na produced a drop in the Ca efflux similar to that observed in the absence of the nucleotide (see also DiPolo, 1976).

**CA PUMP** The experiment of Fig. 4 confirms the absolute requirement for  $Mg_i^{2+}$  of the ATP-activated uncoupled Ca efflux (DiPolo and Beaugé, 1979). Interestingly enough, the ATP-dependent uncoupled Ca efflux remains unaffected when  $Mg_i^{2+}$  is increased to 8 (see Fig. 5) or 14 mM (experiment not shown), which indicates that up to the concentrations investigated, high Mg does not inhibit the Ca pump. Furthermore, in other experiments in which  $Mg_i^{2+}$  was reduced to 0.4 mM, no change in the magnitude of the uncoupled efflux was observed. This indicates that low concentrations of  $Mg_i^{2+}$  are sufficient to maximally activate the Ca pump. A similar conclusion has been reached for the Mg activation of the (Ca + Mg)-ATPase from squid nerve membranes (Beaugé et al., 1981).

#### *Effects of Internal Na on Na/Ca Exchange and the Ca Pump*

Although  $Na_i$  has no apparent effect on the magnitude of the uncoupled Ca efflux (DiPolo and Beaugé, 1979), it is well known that decreasing  $Na_i$  increases the  $Na_o$ -dependent Ca efflux. In addition, ATP in the absence of  $Na_i$  is no longer able to activate the  $Na_o$ -dependent component (DiPolo, 1976; see also Fig. 6). This effect has been explained on the basis that ATP antagonizes  $Na_i$  inhibition of  $Na_o/Ca_i$  exchange by reducing the affinity of the carrier toward  $Na_i$  (Requena, 1978a). A question that has not been explored is whether  $Na_i$  modifies the apparent ATP affinity of both components. We have determined the ATP dependency of the two components of the Ca efflux at different  $Na_i$  concentrations. For the case of the uncoupled Ca efflux, its magnitude was determined in solutions lacking  $Na_o$  and  $Ca_o$  and containing low  $[Ca^{2+}]_i$ . In the case of Na/Ca exchange, only the component of the Ca efflux activated by ATP and dependent on  $Na_o$  was taken into account. To increase the exchange,  $[Ca^{2+}]_i$  was raised to 0.7 or  $4 \mu M$ . In the range of 5–65 mM  $Na_i$  for the Ca pump component or 20–80 mM for Na/Ca exchange,  $Na_i$  had no effect on the  $K_{app}^{ATP}$  for the two processes. The  $K_{app}^{ATP}$  values obtained in the present experiments were 35 and 290  $\mu M$  for the Ca pump and Na/Ca exchange, respectively. These values are not different from those previously reported (DiPolo and Beaugé, 1979). It is worth pointing out that large variations in the  $[Ca^{2+}]_i$  (0.1–1  $\mu M$ ) were also without effect on the  $K_{app}^{ATP}$  of both Ca efflux components.

#### *Effect of Internal $K^+$ on the Ca Pump*

In injected axons, there is considerable evidence indicating that neither external  $K^+$  nor membrane potential has any detectable effect on the uncoupled Ca efflux (Baker, 1978). Internal dialysis allows us to explore whether K ions from the

inside can modify this Ca transport system. One of these experiments is illustrated in Fig. 7A. At a  $[Ca^{2+}]_i$  of  $0.08 \mu M$ , Ca efflux reaches a steady level of  $36 \text{ fmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  in the presence of ATP and  $K_i^+$ . The removal of  $Na_o$  and  $Ca_o$  causes only a small (11%) decrease in the efflux, which is consistent with the reduced size of the  $Na_o$ -dependent component under nearly physiological conditions. Substitution of all internal  $K^+$  by  $Tris^+$  causes a decrease in Ca efflux to  $\sim 14 \text{ fmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ , and subsequent removal of  $Na_i^+$  causes a further drop in the efflux level to  $10 \text{ fmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ . These effects are totally reversible.

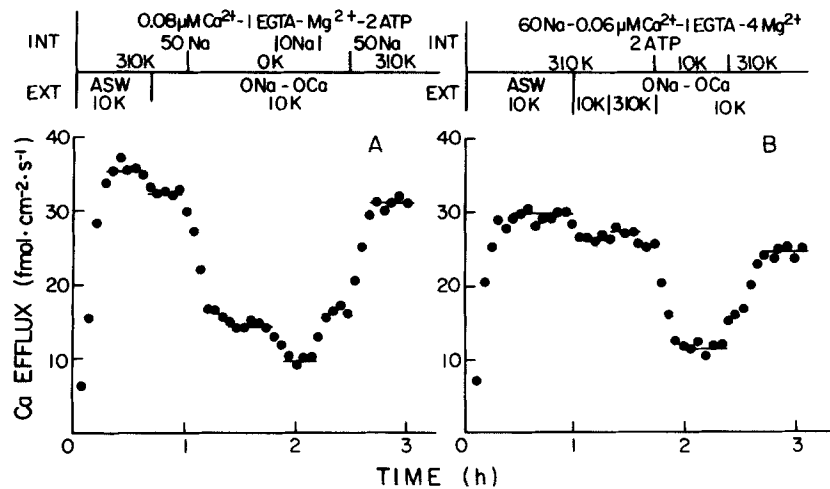


FIGURE 7. Effect of  $K_i$  and  $K_o$  on the uncoupled Ca efflux. In *A*,  $K_i$  is first removed from the dialysis medium, followed by removal of  $Na_o$ . In *B*, the effect of 310 mM  $K_o$  on the uncoupled Ca efflux was explored, followed by the reduction of  $K_i$  to 10 mM in the presence of 10 mM  $K_o$ . Notice that a large depolarization induced by high  $K_o$  is without effect on the uncoupled efflux.

Fig. 7B shows an experiment carried out to explore whether the effects of  $K_i^+$  are due to K per se, or to simultaneous changes in membrane potential. In the presence of 310 mM  $K_i$  and 10 mM  $K_o$ , the magnitude of the uncoupled component is  $\sim 26 \text{ fmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ , and an increase in  $K_o$  from 10 to 310 mM causes practically no change in the level of the flux, which indicates that a large membrane depolarization (to  $\sim 0 \text{ mV}$ ) has no effect on the uncoupled efflux of Ca. In the second part of the experiment, the axon was depolarized by about the same magnitude, by decreasing the  $K_i$  from 310 to 10 mM; in this case, a large inhibition (70%; leak subtracted) of the uncoupled Ca efflux was observed. These experiments suggest that K ions acting from the inside activate the uncoupled Ca efflux in a way that is independent of the membrane potential. In experiments not shown, similar results were obtained whether  $K_i$  was substituted for  $Tris^+$  or  $NMG^+$  (*N*-methylglucamine).

#### Effects of ADP

Previous studies in dialyzed axons have shown that high levels of axoplasmic ADP cause inhibition of total Ca efflux (DiPolo, 1976, 1977). However, most of

those experiments were carried out at a high  $[Ca^{2+}]_i$  and in the presence of  $Na_o$ . This makes it difficult to assess whether ADP inhibition was due to an effect on the Ca pump, Na/Ca exchange, or both. Since the adenylate kinase present in the axoplasm catalyzes the reaction  $2ADP = ATP + AMP$ ,  $[ADP]$  was fixed by maintaining a constant  $[AMP] \cdot [ATP] / [ADP]^2$  ratio. This was done by taking advantage of the fact that AMP has no effect on the Ca efflux (DiPolo, 1976). On the other hand, the presence of PA in the dialysis medium assures a high ATP/ADP ratio because of the reaction  $PA + ADP = ATP + \text{arginine}$ . This reaction is known to be catalyzed by the arginine phosphokinase present in the axoplasm (De Weer, 1970).

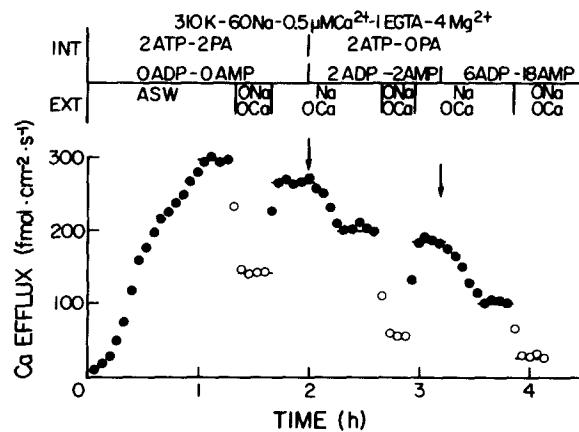


FIGURE 8. Effect of ADP on both the ATP-dependent uncoupled and  $Na_o$ -dependent Ca efflux components. Closed circles: Ca efflux into full  $Na_o$ , 0  $Ca_o$ ; open circles: Ca efflux into 0  $Na_o$ , 0  $Ca_o$ . AMP was added with ADP to keep a constant ADP concentration. Axon diameter, 490  $\mu M$ .

**NA/CA EXCHANGE** The experiment of Fig. 8 was designed to explore the effects of different ADP concentrations on the  $Na_o$ -dependent Ca efflux. In the presence of 0.5  $\mu M$   $Ca^{2+}$  and 2 mM ATP plus 2 mM PA, a significant fraction of the total Ca efflux is  $Na_o$  dependent. Under these conditions, increasing the ADP to 2 mM does not affect the  $Na_o$ -dependent efflux, whereas it is significantly reduced by half when ADP is increased to 6 mM. This effect was totally reversible upon removal of the nucleotide. In addition, it should be stressed that in these experiments, internal ionized Mg was kept constant at 4 mM.

**CA PUMP** The experiment of Fig. 8 has also been used to investigate the effect of ADP on the Ca pump. This can be done by following the behavior of Ca efflux in the absence of external Na and Ca as internal ADP is varied. In the absence of ADP, the magnitude of the uncoupled Ca efflux is  $\sim 140$   $fmol \cdot cm^{-2} \cdot s^{-1}$ ; this is reduced to  $\sim 60$   $fmol \cdot cm^{-2} \cdot s^{-1}$  at 2 mM ADP and further reduced to 30  $fmol \cdot cm^{-2} \cdot s^{-1}$  when ADP is increased to 6 mM. The fact that the Ca pump is more sensitive to ADP inhibition than Na/Ca exchange is also seen in Fig. 9. In this experiment, the axon was dialyzed with 0.1  $\mu M$   $Ca_i^{2+}$  in order to activate mostly the uncoupled component. In the presence of 2 mM ATP and 5 mM PA,

a steady Ca efflux of  $20 \text{ fmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  was obtained after 40 min of dialysis. When 2 mM ADP was added to the internal medium (simultaneously with the removal of PA), a marked inhibition was observed. This inhibition was found to be completely reversible.

#### Effects of $P_i$

**NA/CA EXCHANGE** In the presence of  $0.5 \mu\text{M Ca}_i^{2+}$ , 1 mM ATP, and 60 mM  $\text{Na}_i$  (see Fig. 10), one-half of the Ca efflux is  $\text{Na}_o$  dependent. An unexpected finding was that the addition of 3 mM  $P_i$  in the presence of external Na caused an increase in Ca efflux. Since removal of  $\text{Na}_o$  brings the efflux to the same level

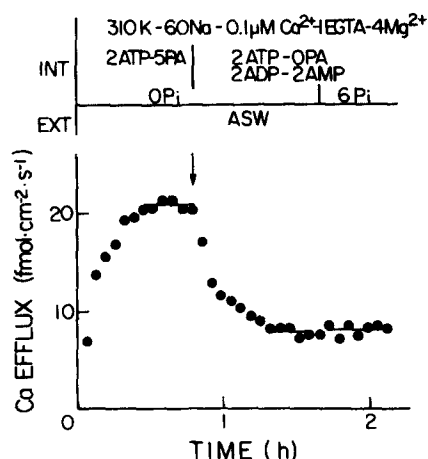


FIGURE 9. Effect of ADP on the ATP-dependent uncoupled Ca efflux. The axon was dialyzed from the beginning with ATP and PA. The addition of ADP was accompanied by removal of PA. Unless otherwise stated, all concentrations are in millimolar. Axon diameter,  $590 \mu\text{m}$ .

as that observed in the absence of  $P_i$ , one can conclude that the activation of Ca efflux by  $P_i$  is exerted only on the  $\text{Na}_o$ -dependent component. In this axon, the effect of  $\text{Ca}_o$  was tested in the absence of  $\text{Na}_o$  and in the presence of 10 mM  $P_i$ . Under these conditions, the increment in Ca efflux induced by external Ca is not significantly different from that reported in the absence of  $P_i$  (DiPolo and Beaugé, 1981). The activation of the  $\text{Na}_o$ -dependent component by  $P_i$  is still observed at high phosphate concentrations, as is shown at the end of the experiment, when  $P_i$  was 20 mM (see inset of Fig. 10).

Since part of the Na/Ca exchange is dependent on internal ATP, it is possible that the stimulating effect of phosphate is somehow related to the nucleotide site. This was investigated in experiments like that shown in Fig. 11. The effect of  $P_i$  was tested first in the presence of a nonhydrolyzable ATP analogue and then in the presence of ATP ( $0.5 \mu\text{M Ca}_i^{2+}$ ). In the absence of both ATP and  $P_i$ , the nonhydrolyzable analogue AMP-PCP had no effect on Ca efflux. Subsequent addition of 5 mM  $P_i$  to the dialysis medium did not modify the steady level of the efflux either. When AMP-PCP and  $P_i$  were simultaneously removed and

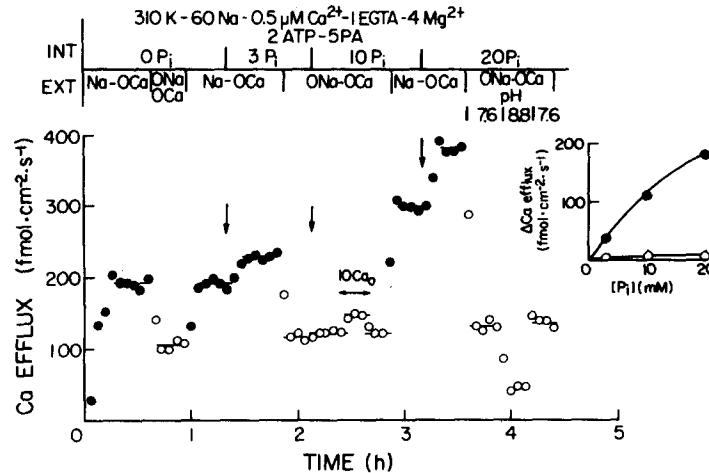


FIGURE 10. Effect of  $P_i$  on the uncoupled and  $Na_o$ -dependent Ca efflux components. Closed circles: Ca efflux into full  $Na_o$ , 0  $Ca_o$ ; open circles: Ca efflux into 0  $Na_o$ , 0  $Ca_o$ . The  $Ca_o$ -dependent Ca efflux component was tested in the presence of 10 mM  $P_i$ . The inset shows the dependence of both the  $Na_o$ -dependent (closed circles) and the ATP-dependent uncoupled Ca efflux (open circles) on the  $[P_i]$  in the dialysis solution. Notice the large inhibition of the uncoupled Ca efflux induced by external alkalization in the presence of  $P_i$ . Axon diameter, 500  $\mu m$ . Axon 190583A.

ATP was added to the internal medium, an increment in Ca efflux to  $\sim 220$   $fmol \cdot cm^{-2} \cdot s^{-1}$  was observed. Under these conditions, 5 mM phosphate in the presence of  $Na_o$  increased Ca efflux to  $300$   $fmol \cdot cm^{-2} \cdot s^{-1}$ . The conclusion that the activation by  $P_i$  of the  $Na_o$ -dependent component takes place only in the presence of ATP seems inescapable.

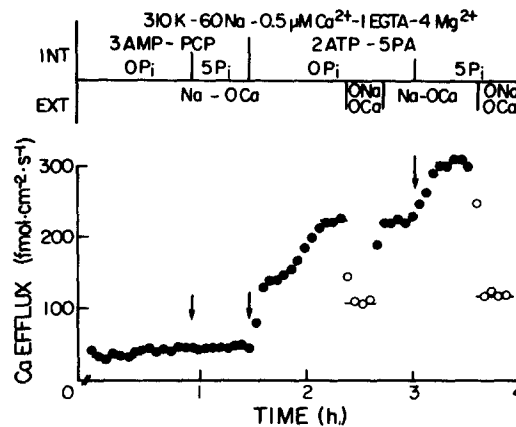


FIGURE 11. Effect of nonhydrolyzable ATP analogue and ATP on the activation of the  $Na_o$ -dependent Ca efflux by  $P_i$ . Closed circles: Ca efflux into full  $Na_o$ , 0  $Ca_o$ ; open circles: Ca efflux into 0  $Na_o$ , 0  $Ca_o$ . Unless otherwise stated, all concentrations are given in millimolar. Axon diameter, 580  $\mu m$ . Axon 250583C.

Since ATP has little effect on the  $\text{Na}_o$ -dependent component in the absence of  $\text{Na}_i$  (see Fig. 4), we decided to explore whether the same applies for  $\text{P}_i$  stimulation of  $\text{Na}/\text{Ca}$  exchange. Fig. 12 shows that in the absence of  $\text{Na}_i$  and in the presence of ATP,  $\text{P}_i$  still produces an increment in the  $\text{Na}_o$ -dependent Ca efflux similar in magnitude to that observed in the presence of  $\text{Na}_i$ . This rules out a possible antagonism between  $\text{P}_i$  and  $\text{Na}_i$ . Interestingly, this experiment also shows that in the presence of 6 mM  $\text{P}_i$ , addition of 2 mM vanadate fails to activate the  $\text{Na}_o$ -dependent Ca efflux (DiPolo and Beaugé, 1981; see Discussion).

**CA PUMP** Figs. 10–12 also illustrate the interaction of  $\text{P}_i$  with the uncoupled Ca efflux component. Under normal external pH (7.6–7.7), increasing  $\text{P}_i$

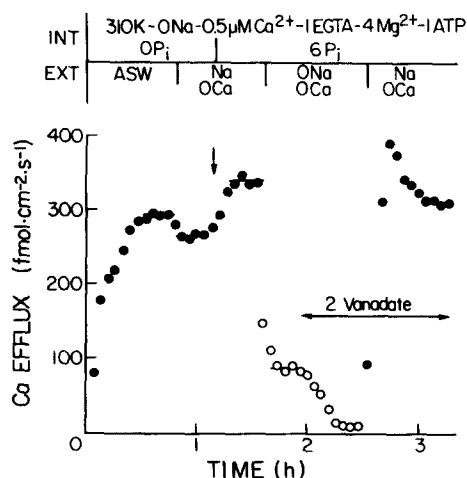


FIGURE 12. Effect of  $\text{P}_i$  on the  $\text{Na}_o$ -dependent Ca efflux in the presence of ATP and in the absence of  $\text{Na}_i$ . Closed circles: Ca efflux into full  $\text{Na}_o$ , 0  $\text{Ca}_o$ ; open circles: Ca efflux into 0  $\text{Na}_o$ , 0  $\text{Ca}_o$ . All concentrations are in millimolar. Axon diameter, 600  $\mu\text{m}$ .

to 20 mM had no effect on the Ca pump. On the other hand, the reported inhibition of the uncoupled component by external alkaline pH (DiPolo and Beaugé, 1982) seemed greatly enhanced by the presence of intracellular  $\text{P}_i$  (see Fig. 10). Although the effect of alkalization in Fig. 10 refers to 20 mM  $\text{P}_i$ , it must be stressed that similar effects were seen at lower  $\text{P}_i$  concentrations (6 and 10 mM).

#### DISCUSSION

The main conclusion drawn from the experiments that have been described is that several physiological intracellular constituents (other than those translocated by the transport mechanisms  $\text{Ca}^{2+}$  and  $\text{Na}^+$ ) do interact with the two transport processes responsible for Ca extrusion. The concentrations of these ligands are not likely to change much under normal circumstances; thus, they are probably not physiological modulators. Nevertheless, their interactions should be taken into account when analyzing the physiological behavior of the Ca pump and  $\text{Na}/$

Ca exchange, as well as in interpreting kinetic data obtained in membrane preparations in which the incubation medium may contain several of these compounds. An important point that emerges from the present results is the marked dissimilarity in the effects of these ligands upon the two transport systems, which confirms their different nature.

### *Mg*

In addition to the involvement of  $Mg^{2+}$  in total and partial reactions of several ion-transporting ATPases, it has recently been recognized that the presence of millimolar quantities of this ion in the cytoplasm indeed influences several  $Ca_i$ -dependent processes (Hess et al., 1982). In squid axons, the effect of  $Mg_i^{2+}$  on Ca efflux has been somewhat controversial. Brinley et al. (1975), working with dialyzed axons, found no effect of  $Mg_i$  on Ca efflux. On the other hand, Requena (1978b) showed that increasing  $Mg_i$  from 0 to 5 mM caused a 10–35% inhibition of the Ca efflux. This effect was reported to be greater at low  $Ca_i^{2+}$ , which suggests competitive interaction between  $Ca_i^{2+}$  and  $Mg_i^{2+}$ .

The results presented here confirm that  $Mg_i^{2+}$  is an inhibitor of the  $Na_o$ -dependent Ca efflux. Actually, the efflux of Ca via the forward Na/Ca exchange at physiological levels of  $Mg_i^{2+}$  is ~50% of that expected in  $Mg_i^{2+}$ -free axons. In addition, this  $Mg_i$  inhibition is only partial and, contrary to the results of Requena (1978b), does not follow competitive behavior toward  $Ca_i^{2+}$ , nor is it affected by the presence or absence of  $Na_i$ . The large inhibition of Na/Ca exchange by millimolar concentrations of  $Mg_i$  contrasts markedly with the lack of effect on the uncoupled efflux. This is in agreement with the absence of any effect of high  $[Mg]_i$  on the (Ca + Mg)-ATPase activity from squid optic nerve membranes (Osses, 1984).

The requirement of internal  $Mg_i$  for the operation of the uncoupled Ca efflux is in line with several other active ion-transport ATPases in which the ATP-Mg complex seems the true substrate of the pumps (see Schatzmann, 1982). The finding that the fraction of the  $Na_o$ -dependent Ca efflux activated by ATP also has a strict requirement for  $Mg_i$  ions suggests that phosphorylation of the Na/Ca exchange carrier may be involved in the ATP effect. Consistent with this idea is the fact that the nonhydrolyzable ATP analogue AMP-PCP is unable to activate Na/Ca exchange either in the presence or in the absence of  $Mg_i^{2+}$ . Since under physiological conditions the internal Mg concentration (3 mM) is not likely to change, one cannot regard this ion as a true regulator. However, when studying the carrier under normal conditions, this effect must be taken into account.

### *Monovalent Cations*

An interesting observation reported in this paper is the large activation of the uncoupled component caused by internal  $K^+$ . This effect cannot be accounted for by changes in membrane potential upon removal of  $K_i$  since a depolarization of a similar magnitude induced by external K is without effect on the uncoupled Ca efflux. A role of K ions as a cofactor for activation of active Ca transport has been reported for the sarcoplasmic reticulum (Duggan, 1977) and human red

blood cells (Rega et al., 1974). The mechanism by which K activates Ca transport is believed to result from an acceleration of the dephosphorylation reaction of the Ca-ATPase (Shigekawa and Pearl, 1976; LaRocca et al., 1981). In squid axons, the mechanism of K activation is probably similar to that postulated in other preparations, as judged from the overall acceleration of Ca-ATPase and ATP-dependent Ca transport in vesicles from squid nerves fibers (Osses, 1984).

From the present experiments, the uncoupled Ca efflux appears rather insensitive to large changes in membrane potential, which contrasts with the reported sensitivity to the potential of the Na<sub>o</sub>-dependent Ca efflux (Mullins and Brinley, 1975). The voltage sensitivity of Na/Ca exchange commonly attributed to an electrogenic Ca transport has rendered difficult the analysis of the effect of K ions per se on Na/Ca exchange. However, recent experiments under voltage-clamp conditions indicate that K ions from the inside strongly activate the Na<sub>o</sub>-dependent Ca efflux (DiPolo and Rojas, 1984).

It is well known that internal Na ions interact with the Na/Ca exchange system, causing inhibition of the Na<sub>o</sub>-dependent Ca efflux (Blaustein, 1977). This inhibition has been explained in terms of an interaction of a single Na ion with a site apparently different from the Ca<sub>i</sub> one (noncompetitive inhibition; Brinley et al., 1975; Requena, 1978a). An interesting property of this interaction of Na<sub>i</sub> with Na/Ca exchange is that it is modulated by ATP (DiPolo, 1976; Requena, 1978a). In fact, in the presence of millimolar ATP concentrations, inhibition of the Na<sub>o</sub>-dependent component by Na<sub>i</sub> is very much reduced (Requena, 1978a). Conversely, in the absence of Na<sub>i</sub>, ATP no longer activates the Na<sub>o</sub>-dependent Ca efflux (DiPolo, 1976). This has been explained on the basis that both compounds compete for the same regulatory site (Requena, 1978a; Mullins, 1977). The results presented here do not favor the idea of competition between Na<sub>i</sub> and ATP, since large variations in Na<sub>i</sub> have no effect on the activation kinetics of the Na<sub>o</sub>-dependent Ca efflux by ATP. An alternative explanation is that the carrier becomes phosphorylated by ATP and that Na<sub>i</sub> ions behave as a cofactor in this step. This is supported by the finding that Mg appears to be an absolute requirement for this ATP effect. On the other hand, Na ions do not seem to interact with the Ca pump. In fact, similar magnitudes of uncoupled Ca efflux and apparent affinities for ATP are obtained in experiments with or without internal Na.

#### *ADP and P<sub>i</sub>*

ADP has no effect in axons from which ATP has been removed by dialysis. However, in the presence of ATP, millimolar concentrations of ADP (2 mM) block an important fraction of the total Ca efflux (DiPolo, 1977). This inhibition was tentatively interpreted in terms of a possible competition between ADP and ATP for the activating nucleotide site of the Na<sub>o</sub>-dependent mechanism. However, the results reported in this paper show that this is not the case, since only the uncoupled component of Ca efflux is inhibited by 2 mM ADP. It is at higher concentrations (6 mM) that ADP also impairs the Na<sub>o</sub>-dependent component. This indicates that ADP interacts with both Ca efflux components, being more effective as an inhibitor of the mechanism activated by ATP with high affinity



(uncoupled Ca pump) than of that stimulated by ATP with low affinity (Na/Ca exchange). At present, the mechanism of inhibition by ADP remains unknown, although it is likely to involve displacement of ATP. Another interesting result for which we have no explanation is that the inhibition of uncoupled Ca efflux by ADP seems to depend on  $[Ca^{2+}]_i$ . In fact, at  $0.1 \mu M Ca_i^{2+}$ , ADP (2 mM) is able to block almost all of the uncoupled Ca efflux (~90%), whereas at  $0.5 \mu M Ca_i^{2+}$ , inhibition amounts to only 50%.

It is known that at a certain step in the cycle the phosphorylated intermediate formed by the interaction of ATP with the Ca pump reacts with water reversibly, releasing the phosphoryl group. Because of the amounts of  $P_i$  present inside the axon (Caldwell, 1960; Baker and Shaw, 1965), it is not unreasonable to think that some rephosphorylation from  $P_i$  could take place under these conditions, leading to product inhibition of Ca transport. However, our results show that  $P_i$  at physiological or even higher concentrations does not affect the operation of the Ca pump. The absence of product inhibition by  $P_i$  under physiological conditions may be explained if the unphosphorylated enzyme binds  $P_i$  with very low affinity. On the other hand, recent experiments in dialyzed squid axons have shown that alkalinization of the external medium decreases the magnitude of the uncoupled Ca efflux (from 20% in the presence of 0 mM  $Ca_o$  to 60% in the presence of 50 mM  $Ca_o$ ; DiPolo and Beaugé, 1981). By analogy with experiments on the sarcoplasmic reticulum Ca pump (Almeida and de Meis, 1977), we interpreted our results as a consequence of an increase in affinity of the external Ca sites (low-affinity sites) for  $Ca_o$  induced by an increase in  $pH_o$ . The experiment of Fig. 10 shows that alkalinization of the external medium in the absence of  $Ca_o$  and in the presence of  $P_i$  causes a large inhibition of the uncoupled Ca efflux. Following the same line of thought, this effect could be explained assuming that at alkaline  $pH_o$ ,  $P_i$  binds to the pump with higher affinity, leading to product inhibition.

The interaction of  $P_i$  with the Na/Ca exchange system can be summarized as follows. (a)  $P_i$  activates the  $Na_o$ -dependent Ca efflux component with low apparent affinity. Although no reliable value has been obtained for the apparent affinity, it is clear that the  $K_{1/2}$  must be  $>10$  mM. (b)  $P_i$  activates the  $Na_o$ -dependent component both in the presence and in the absence of  $Na_i$ , which suggests that it does not act by antagonizing  $Na_i$  inhibition. (c) Activation takes place only in the presence of ATP. The nonhydrolyzable ATP analogue is without effect on the Ca efflux whether  $P_i$  is present or not. Although the mechanism by which  $P_i$  stimulates Na/Ca exchange is unknown, the fact that ATP and Mg are strictly required suggests that  $P_i$  acts on the ATP-stimulated  $Na_o$ -dependent component of Ca efflux. We have previously shown that vanadate is without effect on the  $Na_o$ -dependent Ca efflux in the absence of ATP (DiPolo et al., 1979). However, in the presence of ATP, this compound causes a marked activation of the  $Na_o$ -dependent Ca efflux (DiPolo and Beaugé, 1981). A plausible explanation for vanadate stimulation of Na/Ca exchange is that because of its structure, which is related to that of  $P_i$ , vanadate acts on the same activation site as  $P_i$ . Evidence in favor of this interpretation comes from the fact that vanadate,  $P_i$ , and *p*-nitrophenylphosphate interactions with (Na + K)-ATPases are all of a competitive

type (Beaugé and Berberian, 1983). This is further supported by the experiment of Fig. 12, where in the presence of 6 mM  $P_i$ , the  $Na_o$ -dependent component is not stimulated by vanadate, in contrast with results in axons dialyzed without  $P_i$  (DiPolo and Beaugé, 1981).

In summary, the results presented here show that the two Ca extrusion systems present in squid axons interact quite differently with several intracellular physiological ligands. The effects of Mg, K, ADP, and  $P_i$  on the uncoupled Ca efflux are of the type seen in red blood cells and the sarcoplasmic reticulum Ca pump, which indicates that a similar mechanism of active Ca transport occurs in all these preparations. Of particular interest are the interactions of physiological ligands with Na/Ca exchange. We could visualize the inner side of the Na/Ca antiporter (Fig. 13) as a complex structure interacting with several ligands that

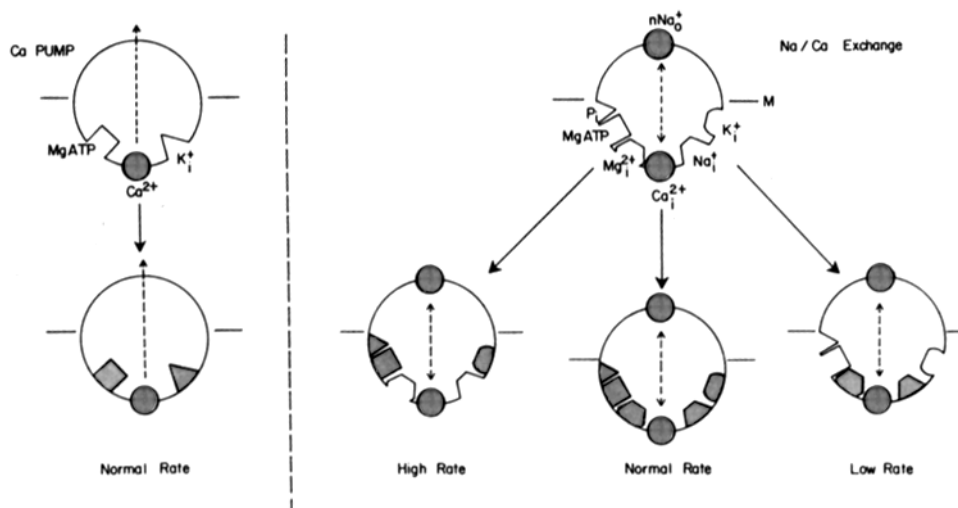


FIGURE 13. Schematic diagram of the ligand interactions with the Ca pump and Na/Ca exchange.

leads to two major states: a low-rate configuration where intracellular  $Na^+$  and  $Mg^{2+}$  are high and  $K^+$ , ATP, and  $P_i$  are low, and a high-rate configuration where intracellular  $Na^+$  and  $Mg^{2+}$  are low and  $K^+$ , ATP, and  $P_i$  are high. Under resting physiological conditions ( $0.1 \mu M Ca_i^{2+}$ ), the Ca pump is working near  $K_m$  in the presence of full  $K_i^+$  and ATP, whereas Na/Ca exchange is barely activated because of its low  $Ca_i^{2+}$  affinity and inhibition by  $Mg_i^{2+}$  and  $Na_i^+$ . At high  $[Ca^{2+}]_i$ , Na/Ca exchange is predominant, being fully activated by Mg-ATP and  $K_i^+$ . In the scheme presented here, ligand interactions take place with the Na/Ca exchange carrier; whether this is the case, or whether interactions actually occur via other molecules (with or without enzymatic activity), remains to be seen.

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