Magnesium Efflux in Dialyzed Squid Axons

L. J. MULLINS, F. J. BRINLEY, JR., S. G. SPANGLER, and R. F. ABERCROMBIE

From the Department of Biophysics, University of Maryland School of Medicine, Baltimore, Maryland 21201, and the Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205. Dr. Spangler's present address is the Department of Physiology and Biophysics, University of Alabama Medical Center, Birmingham, Alabama 35294.

ABSTRACT The efflux of Mg^{++} from squid axons subject to internal solute control by dialysis is a function of ionized $[Mg]_i$, $[Na]_i$, $[ATP]_i$, and $[Na]_o$. The efflux of Mg^{++} from an axon with physiological concentrations of ATP, Na, and Mg inside into seawater is of the order of 2–4 pmol/cm²s but this efflux is strongly inhibited by increases in $[Na]_i$, by decreases in $[ATP]_i$, or by decreases in $[Na]_o$. The efflux of Mg^{++} is largely independent of $[Mg]_i$ when ATP is at physiological levels, but in the absence of ATP reaches half the value of Mg efflux in the presence of ATP when $[Mg]_i$ is about 4 mM and $[Na]_i$ 40 mM. Half-maximum responses to ATP occur at about 350 μM ATP into seawater with Na either present or absent. The Mg efflux mechanism has many similarities to the Ca efflux system in squid axons especially with respect to the effects of ATP, Na_o, and Na_i on the flux. The concentrations of free Mg and Ca in axoplasm differ, however, by a factor of 10^5 while the observed fluxes differ by a factor of 10^2.

INTRODUCTION

Intracellular Mg has diverse roles as a cofactor in promoting enzymatic reactions, perhaps the most important one from the standpoint of transport studies being the formation of MgATP, a substrate for Na/K transport. Total analytical Mg in squid axons is about 7–10 mM (Baker and Crawford, 1972; De Weer, 1976) of which roughly 2–3 mmol/kg axoplasm is ionized (Brinley and Scarpa, 1975). While seawater contains of the order of 50 mM Mg, a substantial fraction of this is complexed with sulfate[1] that is present and 25 mM is a rough estimate of the free, ionized Mg. Given the fact that both the electrical and chemical potentials for Mg^{++} are such as to favor Mg entry, the relatively low intracellular [Mg] can be maintained only by some energy-requiring transport process.

Previous studies of Mg efflux from injected axons have established that Mg efflux into seawater is from 1 pmol/cm²s (Baker and Crawford, 1972) to 3.5 pmol/cm²s (De Weer, 1976), that 75–80% of the Mg efflux disappears when the axon is bathed in choline seawater, and that either CN seawater or apyrase injections reduce Mg efflux to 15% or 5%, respectively, of control values.

[1] The dissociation constant of $MgSO_4$ is 6.3×10^{-3} (Harned and Owen, 1950). With total Mg^{++} in seawater of 50 mM and $SO_4^{=}$ of 30 mM, this works out to 25 mM free Mg when allowance is made for 10 mM Ca and a similar dissociation constant for $CaSO_4$.

Measurements of Mg influx (Baker and Crawford, 1972) in intact axons showed that this was 0.6 pmol/cm²s from Na seawater containing 55 mM Mg, and that this value was increased about 10-fold in Li seawater although influx was insensitive to CN poisoning.

The work to be reported involved the measurement of Mg efflux by use of the internal dialysis technique in squid axons (Brinley and Mullins, 1967) since this method allows control of internal dialyzable solutes as well as external ions. In axons injected with ^{28}Mg it is not possible to vary $[Na]_i$ or $[ATP]_i$ over wide ranges nor can $[Mg]_i$ be readily controlled. Therefore, data presently available in the literature are limited to showing the effects of changes in $[Na]_o$ and $[Mg]_o$, and of inhibitors that decrease $[ATP]_i$ by a variable amount. The data to be presented show the following new findings: that $[Na]_i$ has a large effect on Mg efflux and that changes in $[Mg]_i$ result in ATP-dependent and ATP-independent fluxes. It has also been possible to specify an [ATP] that half-activates Mg efflux. The main conclusions from this work are that Mg efflux is a complex function of $[Na]_o$, $[ATP]_i$, $[Mg]_i$, and $[Na]_i$. Decreases of external Na or increases of internal Na lower Mg efflux. While increases in ATP appear to promote Mg transport, Mg efflux in the absence of ATP can reach a value equal to that given in the presence of ATP if Na_i is low and Mg_i is high.

MATERIALS AND METHODS

Axons

Material was obtained from the hindmost axon of the stellar ganglion of live specimens of *Loligo pealei*. The axon was dissected, cleaned, and maintained in artificial seawater with 3 mM Ca.

Internal Dialysis

Axons were mounted in a dialysis chamber and an internal dialysis capillary was run through the axon as described by Brinley and Mullins (1967) with the modification that plastic dialysis capillaries (Fabric Research Laboratories, Dedham, Mass.) rather than glass ones were used throughout. In addition to the usual tests with phenol red for porosity, on occasion further testing was required to be certain that plastic capillaries readily passed divalent cations such as Ca and Mg.

Solutions

The composition of seawater used in the experiments to be reported is given in Table I. The main modifications of seawater were to eliminate $SO_4^=$ in order to obviate the uncertainty of $[Mg]_o$ and to standardize on an $[Mg]_o$ of 25 mM. The pH of seawater was checked with a glass electrode and adjusted to 7.8 when necessary; the osmolarity of the solutions used was standardized at 900 mosmol/liter with a Wescor dewpoint osmometer (Wescor, Inc., Logan, Utah). Internal dialysis solutions had the composition shown in Table I. The pH was adjusted to 7.3. A standard value for internal osmolarity of 810 mosmol/liter was adopted mainly because of the earlier observation that survival of dialyzed axons appears to be promoted by lower internal osmotic pressures.

ATP

ATP was added either as the K salt, which reduced free, ionized Mg because of the formation of MgATP, or as MgATP. Since the ATP was usually added to dialysis

solutions already containing several millimolar Mg, the addition of MgATP produced relatively little change in Mg_i. For example, addition of 5 mM MgATP to a dialysate containing 5 mM Mg raised the internal Mg_i by only about 0.6 mM, or 10%, with De Weer's (unpublished) value of 0.6 mM for the dissociation constant of MgATP. Both methods were used in the study of substrate addition but in fact it was judged easier to correct for specific activity changes so that MgATP was the more frequent form by which ATP was added to solutions. Solutions of K_2ATP and MgATP were made approximately 300 mM with respect to ATP and adjusted to pH 7.3; these stock solutions were analyzed for total ATP content spectrophotometrically via the hexokinase reaction coupled to NADP reduction. When Mg efflux vs. $[Mg]_i$ was studied, the stated [Mg] was always that in addition to the Mg that could be expected to be complexed to ATP.

$[Ca]_i$

Internal dialysis solutions as well as seawater had 0.1 mM EGTA (as the K salt for internal solutions and as the Na salt for seawater). The purpose of this ingredient was to complex heavy metals that might be present as reagent contaminants in seawater. For internal dialysis solutions, EGTA had the additional purpose of removing reagent contaminations

TABLE I

COMPOSITION OF SOLUTIONS USED

Solution	Na^+	Choline$^+$	K^+	Mg^{++}	Ca^{++}	Cl^-	EGTA$^-$	Iseth$^-$	TES$^-$	Glycine
						mM				
Na seawater*	425	–	10	25	3	490	0.1	–	5	–
Choline seawater	–	425	10	25	3	490	0.1	–	5	–
Internal dialysis solution Na=0	–	–	330	–	–	–	0.1	325‡	5	160

* CN seawater was made by the addition of 2 mM NaCN (neutralized to pH 7.8) to Na seawater followed by adjustment of pH if necessary.

‡ Na isethionate was substituted for K isethionate to prepare [Na]=40 mM or [Na]=80 mM solutions. The [Mg] of internal dialysis solutions varied from 5 to 20 mM; it involved replacement of K isethionate by $MgCl_2$ in isosmotic amounts.

of Ca. In particular, K isethionate solutions can have a [Ca] of the order of 50 μM and the EGTA could therefore fix $[Ca]_i$ at levels of 0.1 μM or below.

Measurement of ^{28}Mg

The seawater flowing through the dialysis chamber at 1 ml/min was kept at 15°C by a thermostat and was collected every 2 min in test tubes moved by a fraction collector. The seawater so collected was added to 10 ml of liquid scintillation counting fluid and counted in a three-channel liquid scintillation counter. All counts were decay corrected.

RESULTS

In order to examine the effect of ATP on Mg efflux from a dialyzed axon, the usual procedure is to apply CN seawater and dialyze with a medium that is free of ATP; when ATP has reached low levels and efflux is steady, then a change is made to a medium containing ATP and the effect of this concentration evaluated. A preliminary experiment along these lines is shown in Fig. 1 where an axon was dialyzed with a medium containing ^{28}Mg but no ATP. At the start of the dialysis, the specific activity of Mg in the fiber is zero and the [ATP] is about 3 mM; as the dialysis proceeds, ^{28}Mg specific activity rises and [ATP] falls. Since the time constant for the fall of [ATP] is of the order of 5 min, then 15 min after

the start of the dialysis there is $1/e^3$ or 0.05 of the initial concentration of ATP still present. This is 0.15 mM, a concentration that can lead to substantial activation of Mg efflux and indeed the figure shows that there is a rise in Mg efflux followed by a fall as [ATP] reaches low values. After a baseline in the absence of ATP, the introduction of this substance yields an increase in Mg efflux to a transient level of about 6 pmol/cm²s and a steady value of 3 pmol/cm²s. The removal of ATP brings the efflux back to the level formerly observed in the absence of ATP.

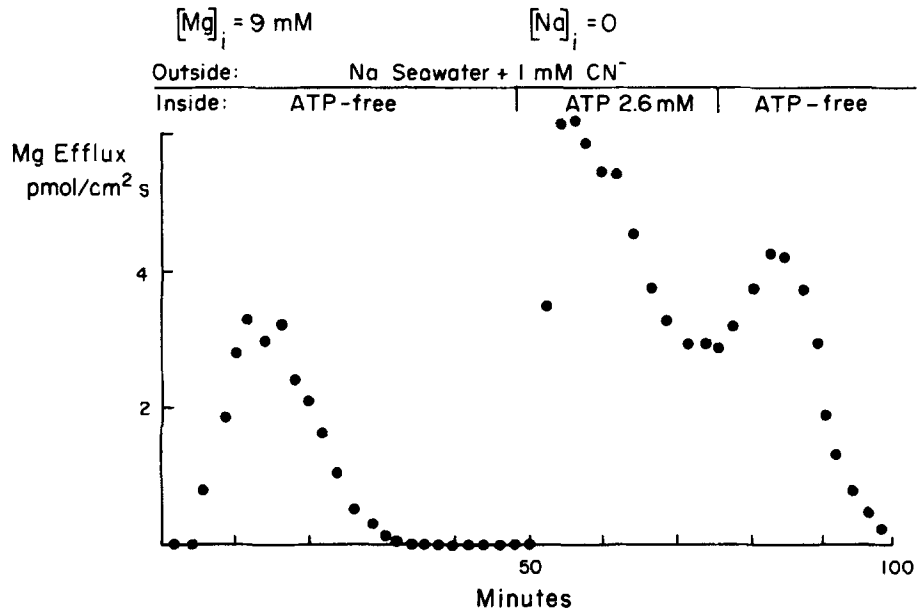

FIGURE 1. This shows the washing in of ^{28}Mg and the simultaneous washing out of ATP by dialysis (0–50 min) followed by the response of the axon to ATP. The initial apparent rise in ^{28}Mg efflux (0–15 min) is produced by the equilibration of Mg in the axon with ^{28}Mg. The subsequent fall is the decline of ^{28}Mg efflux as ATP washes out. A similar transient at 52 min results from the readjustment of ^{28}Mg specific activity as ATP is introduced. The base line in the absence of ATP is unusually low.

Injected Axon

It seemed useful to compare the behavior of the axon shown in Fig. 1 with that of an axon injected with ^{28}Mg. An axon was injected with sufficient counts so that the $[Mg]_i$ was raised by about 1 mM, and the efflux from such a preparation was 1.7 pmol/cm²s (axon 051375-B1); a transfer of this axon to choline seawater led to a fall in Mg efflux to 1.1 pmol/cm²s and a return to Na seawater containing 2 mM CN led to a fall in Mg efflux to 0.44 pmol/cm²s after a delay of about 1 h. This amounts to an efflux in choline of 65% of that in Na seawater and a Mg efflux 26% of that of control in Na seawater when the axon is fully poisoned with CN. The absolute magnitude of the Mg efflux is very much less than that shown in

Fig. 1, but this axon was dialyzed with $Na_i = 0$ while the injected axon described above probably had 40–50 mM $[Na]_i$. It will be shown later that Mg efflux is critically dependent on $[Na]_i$.

Efflux in Choline Seawater

Since injected axons are known to show a decrease in Mg efflux in Na-free seawater, it seemed important to show that dialyzed axons also underwent this change in Mg efflux. Table II lists all the axons studied and it shows that a mean value of 67% is obtained for the efflux into choline seawater compared with Na seawater. This value appears to be independent of whether ATP was present inside the axon, or of whether $[Mg]_i$ was at a high or low value. It is substantially higher than the value (25%) reported for injected axons in choline seawater; possibly because our internal dialysis solutions have omitted substances such as glutamate and aspartate that complex Mg, or because external Mg has been set at 25 mM rather than 55 mM.

Dependence of Mg Efflux on $[ATP]_i$

A systematic investigation of the effect of ATP on Mg efflux requires that values for $[Mg]_i$ and $[Na]_i$ be selected and held constant while [ATP] is varied. We have used 4 mM for Mg (free) and 40 mM as representative of $[Na]_i$ in fresh axons. An experiment of the sort used to evaluate the concentration dependence of Mg efflux on ATP is shown in Fig. 2 for a range of [ATP] from 0.1 to 3 mM ATP. In this experiment, the axon was dialyzed for 30 min in CN seawater without ^{28}Mg to remove most of the ATP, hence the transient rise in Mg efflux noted in Fig. 1 is not present. Each measurement at a particular [ATP] was taken both in Na seawater and in choline seawater in order to evaluate the contribution of $[Na]_o$ to the Mg efflux. The combined data from this experiment and another similarly designed are shown in Fig. 3. Mg efflux is shown relative to the value given at 0.5 mM ATP since the absolute values of the effluxes differed somewhat in the two axons. Although there are not enough points on the curves at high ATP to be certain that Mg efflux is saturating, the $[ATP]_i$ that gives half the value of Mg efflux at 3 mM ATP is 0.35 mM whether the efflux is measured in Na- or choline-seawater.

Dependence of Mg Efflux on $[Mg]_i$

If internal [ATP] is held constant and $[Mg]_i$ is varied, then the results shown in Fig. 4 are obtained. Over a range of $[Mg]_i$ that goes from the physiological to a concentration that is more than three times this value, Mg efflux is constant at a value of about 2 pmol/cm^{2}s when ATP is 3 mM and $[Na]_i$ is 40 mM. If, on the other hand, the axon is dialyzed to make it ATP free, then the efflux of Mg at around 3 mM is about 5% of the value with ATP but this efflux increases with $[Mg]_i$ to about 100% of the ATP value when $[Mg]_i$ is 16 mM. Operationally, one can define the role of ATP as one of increasing the affinity of Mg for transport.

Dependence of Mg Efflux on $[Na]_i$

If internal Mg is held at a high and saturating value (10.4 mM) and [ATP] at 3 mM, while $[Na]_i$ is varied, then the results shown in Fig. 5 are obtained.

Although 50 min were allowed for the dialysis with Na-free solution, the efflux curve shows no sign of leveling off at a steady value for efflux, and tabular data (see Table II) suggest that under truly Na-free conditions Mg efflux with ATP is likely to be 3–5 pmol/cm²s rather than the value of 2.5 shown. The introduction

TABLE II

MAGNESIUM EFFLUX FROM SQUID AXONS (15°C)

Axon reference	Diameter	$[Mg]_i$	$[Na]_i$	$[ATP]_i$	Mg efflux		Efflux remaining in ChSW
					NaSW	ChSW	
	μm	mM	mM	mM	pmol/cm²s		%
050775-A1	550	13.0	80	0	0.2		
050775-B1	515	6.5	40	0	0.1		
		6.5	40	4	0.61		
051375-A2	600	20.6	80	0	0.81	0.81	100
		20.6	0	0	2.71		
		20.6	0	4	4.35		
050675-B1	585	6.3	40	0	0.80	0.61	76
		12.4	40	0	1.44	0.83	58
		16.3	40	0	1.85		
051375-B3	636	10.4	0	3	2.50		
		10.4	40	3	1.40		
		10.4	80	3	0.60		
051976-3	600	4.0	40	0.2	2.50	1.90	75
		4.0	40	0.5	3.00	2.00	67
		4.0	40	3.0	4.40	3.00	68
051976-2	600	4.0	40	0		0.20	
051976-1	800	4.0	40	0	0.3	0.2	67
				0.1	0.6	0.3	50
				0.5	1.3	0.8	62
				3.0	2.6	1.6	62
051276-1	625	2.75	40	0	0.15		
		5.5	40	0	0.90		
		11.0	40	0	1.10		
		2.75	40	3	1.75		
		5.5	40	3	1.75		
		11.0	40	3	1.75		
051176-1	530	5.5	40	0	0.8		
		5.5	40	3	1.6	1.0	63
051176-2	600	5.5	40	0	0.7		
		5.5	40	3	1.4	0.9	64
Mean							67

of 40 mM Na in the internal dialysis medium produces a prompt decrease in Mg efflux to 1.25 pmol/cm²s and the further change to 80 mM Na reduces the efflux to about 0.5 pmol/cm²s. The experiment shows quite conclusively that increases in $[Na]_i$ have a large effect on the magnitude of Mg efflux. Information on other axons examined at two or more values of $[Na]_i$ is shown in Table II, from which it can be concluded that: (*a*) increases in Na_i inhibit Mg efflux in the presence of ATP; and (*b*) increases in Na_i also inhibit Mg efflux in the absence of ATP. The

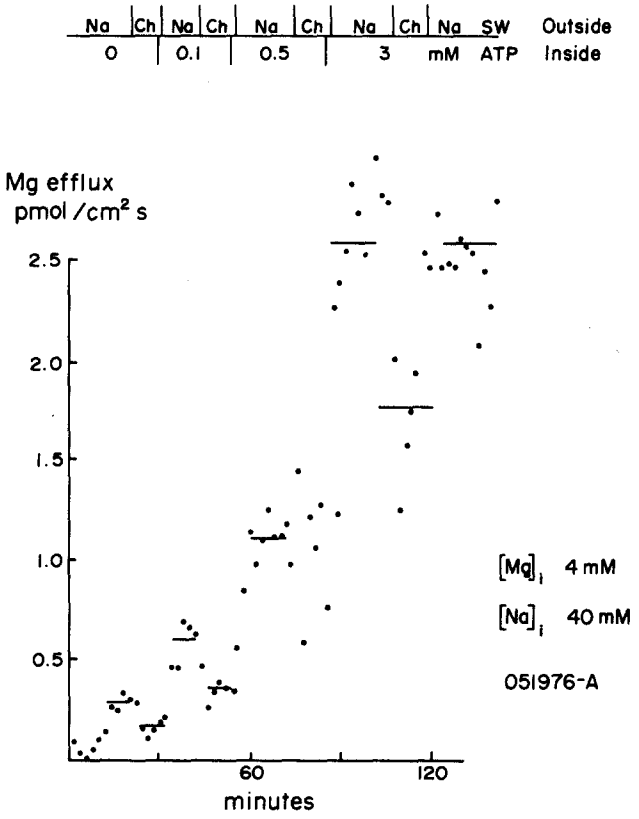

FIGURE 2. The response of the Mg efflux of an axon to three concentrations of ATP is shown for Na seawater (Na) and for choline seawater (Ch). Both seawaters contained 2 mM CN and the axon was dialyzed with ATP-free solution in CN seawater for 0.5 h before the beginning of efflux measurement.

data from Table II relative to the effect of $[Na]_i$ on Mg efflux show that: (*a*) in the absence of ATP, Mg efflux approaches or equals that in the presence of ATP if Mg_i is high enough; and (*b*) this effect occurs at all values of $[Na]_i$ studied. Note that in the absence of ATP, Mg efflux increases in a virtually linear manner with increases in $[Mg]_i$, while from Fig. 4 it is clear that with ATP, Mg efflux is substantially independent of [ATP] from 3 to 11 mM Mg. When $[Na]_i$ is nominally zero, the Mg efflux rises steeply with $[Mg]_i$ while when $[Na]_i$ is 80 mM, the concentration-efflux curve has a very small slope. This sort of information is

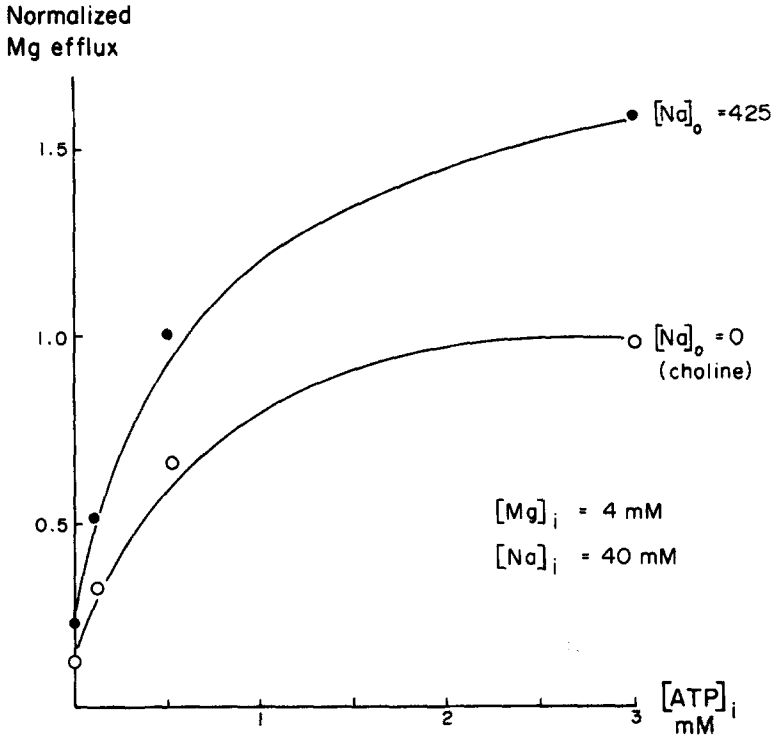

FIGURE 3. Data from Fig. 2 have been averaged with those of another axon (051976-3) to show the mean response of Mg efflux to changes in $[ATP]_i$. The value 1.0 on the ordinate represents an efflux of 2–3 pmol/cm²s.

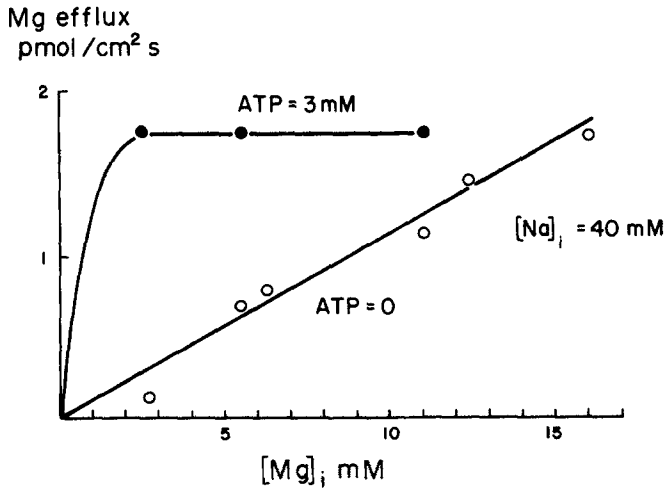

FIGURE 4. Mg efflux is plotted as a function of $[Mg]_i$ in the absence of ATP (open circles) and in the presence of ATP (solid circles). The efflux in the presence and absence of ATP was determined on the same axon (051276A) and additional points in the absence of ATP from a second axon (050675-B1) are also included.

important in establishing that the efflux of Mg in the absence of ATP is not some sort of passive leak of the membrane for Mg since there is no reason to expect that such a leak would be sensitive to $[Na]_i$.

Data from Table II for axons examined at two or more different $[Na]_i$ are shown in Fig. 6 where the Mg efflux in $[Na]_i = 0$ is set at 100% both for an axon

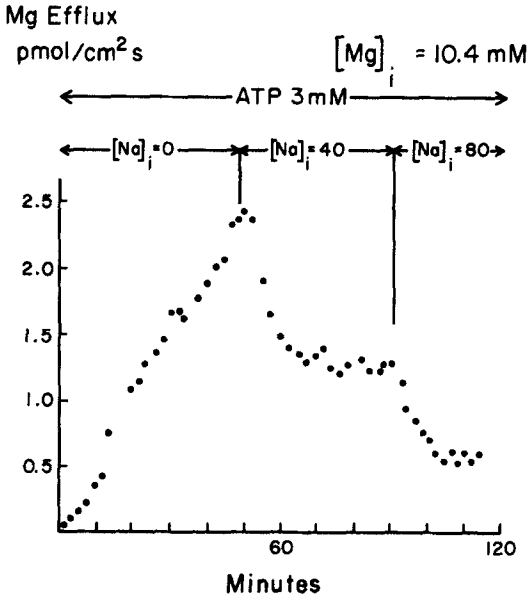

FIGURE 5. Mg efflux into Na seawater is shown as a function of time for an axon dialyzed with ATP throughout and with $[Na]_i$ changed from 0 to 40 to 80 mM.

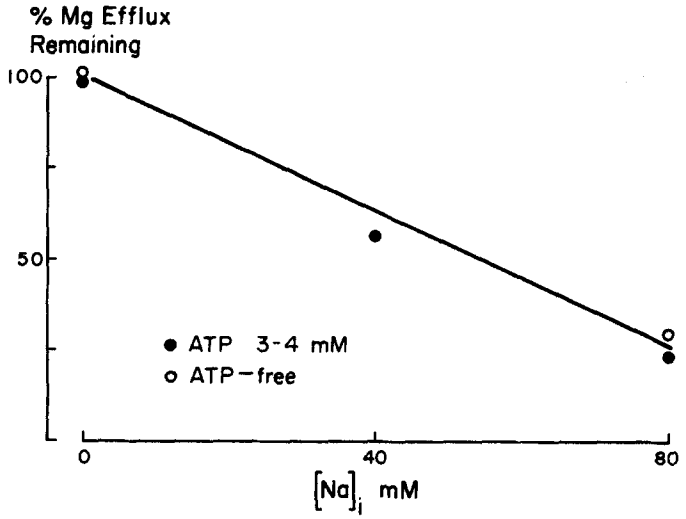

FIGURE 6. This shows the effect of changes in $[Na]_i$ on Mg efflux in the presence or absence of ATP_i.

dialyzed with ATP and for one dialyzed under ATP-free conditions. It seems clear that the effect of $[Na]_i$ under both conditions is the same and that with Na_i = 80 mM, Mg efflux is about 30% of the value under Na_i-free conditions.

DISCUSSION

The work of De Weer (1976) has clearly established the following points: Mg efflux from squid axons is dependent on $[ATP]_i$ and Na_o, but is insensitive to large increases in $[ADP]_i$; increases in $[Mg]_o$ in the range of 0–100 mM inhibit Mg efflux, thus suggesting that Mg:Mg exchange is not a component of Mg efflux. A final point is that in the absence of both Na_o^+ and Mg_o^{++}, Mg efflux was virtually normal in the presence of Ca_o but increased at least 10-fold when Ca_o was removed. This increase could be reversed by La^{+++}.

The experimental data for dialyzed axons presented earlier clearly demonstrate that Mg efflux from a squid axon is a function of $[Mg]_i$, $[ATP]_i$, $[Na]_i$, and $[Na]_o$. The interaction between these variables is likely to be complex and no really complete kinetic description of the way these parameters affect Mg efflux is possible at the present time. On a purely operational basis, the effects of all the above-mentioned variables are similar to their effects on Ca efflux (Brinley et al., 1975). Additionally, Baker and Crawford (1972) have shown that Mg influx is increased about eightfold by changing from Na to choline seawater; again an effect virtually identical with that observed with Ca influx.

There are, however, substantial differences between the Ca and Mg efflux mechanisms. Brinley et al. (1975) have shown that going from Mg_i = 0 mM to Mg_i = 4 mM had no effect on Ca efflux; this finding suggests that the efflux mechanisms are quite separate. Another finding is that of De Weer (1976) that depolarization of the membrane with external K had no effect on Mg efflux, while Mullins and Brinley (1975) showed that Ca efflux was quite sensitive to membrane potential, increasing with hyperpolarization and decreasing with depolarization.

These differences in the behavior of Mg efflux and Ca efflux may reflect mainly the differences in energy requirements for transport rather than any intrinsic difference in the manner by which transport is effected, as the following considerations suggest.

Table III summarizes the following: intracellular Ca and Mg ionized concentrations differ by a factor of 10^5 while their fluxes differ only by a factor of 50–100 in an axon with an assumed normal value of Na_i, Ca_i, Mg_i, and ATP_i. The energy requirements for Mg efflux are such that the coupled entry of 2 Na for the exit of 1 Mg would be possible. This would be, therefore, an electroneutral exchange. For Ca, an entry of 4 Na per Ca extruded would be necessary, hence the system is electrogenic in a sense opposite to that for the Na/K pump. The highest rates of Mg efflux (Na_i = 0, ATP = 3 mM) are of the order of 5 pmol/cm^2s; these would require the coupled entry of 10 pmol/cm^2s of Na. For physiological $[Na]_i$, Mg efflux is about half this maximum value and coupled Na influx would be reduced proportionately, i.e. about 5 pmol/cm^2s. Brinley and Mullins (1968) have observed a ouabain-insensitive but ATP-dependent Na entry of 10–15 pmol/cm^2s of Na upon the addition of ATP to an axon containing no

Na_i but with Mg_i at a concentration of 4 mM. Therefore, there is reason for believing that an appropriate Na influx is activated simultaneously with the Mg efflux reported in this paper. Such a finding does not necessarily mean that ATP is a substrate for the pump transporting Mg outward; indeed the tabulation listed above shows that Mg transport could be energized adequately by the Na gradient and that the role of ATP might be essentially that of a catalyst, i.e. increasing the affinity of the transport system either for Mg at the inside of the membrane or for Na at the outside of the membrane, or both. A finding that makes the role of ATP appear to be that of a catalyst rather than a substrate is that Mg efflux can reach physiological levels in the absence of ATP if (*a*) Na_i is reduced, or (*b*) Mg_i is increased. Another similarity between Mg and Ca effluxes is that the [ATP] giving about half-maximal divalent cation efflux is 0.35 mM in both cases. By comparison, the Na/K exchange system in the squid axon has a value of 0.025 mM or a value 10 times lower (Brinley and Mullins, 1968).

TABLE III

A COMPARISON OF Ca AND Mg FLUXES IN SQUID AXONS

	Mg^{++}	Ca^{++}
Physiological internal ionized concentration (mM)	3*	3×10^{-5}‡
External ion concentration (mM)	30	3
Equilibrium potential for ion (mV)	+29	+145
Electrochemical potential gradient $zF(E_{ion} - E_m)/F$ (mV)	+178	+410
Electrochemical potential gradient/F for Na^+ (mV)	+120	+120
Minimum coupling ratio Na^+/X^{++}	2	4
Divalent cation efflux (pmol/cm²s)	2	0.03§
ATP concentration for half-maximum efflux (mM)	0.35	0.35‡

* De Weer, 1976; Brinley and Scarpa, 1975.
‡ DiPolo et al., 1976.
§ Brinley et al., 1975.

The observation of Baker and Crawford (1972) that Mg influx was increased 10-fold in Li seawater also suggests that the Na:Mg exchange mechanism can run backward as can the Na:Ca exchange. The lack of sensitivity of Mg influx to CN which they observed may not indicate an ATP independence of Mg influx, because one of the first actions of CN is to increase $[Mg]_i$ by releasing it from the complex MgATP. This change in $[Mg]_i$ might then lead to Mg:Mg exchange for part of the Mg influx.

The data of De Weer (1976) showing the effect of $[Mg]_o$ on Mg efflux can be interpreted not only as a competition of Na and Mg at the outside of the membrane but also as a result of the change in the E_{Mg} brought about by changes in $[Mg]_o$. The direction of the change in Mg efflux is such that lowering $[Mg]_o$ lowers E_{Mg} and hence the work required in pumping. Such considerations are not valid in a metabolically driven pump that operates independent of load. They are valid for a coupled Na:Mg pump where the flux depends on the difference in electrochemical gradients of the ions involved.

A final point concerns the Mg efflux that persists in choline seawater. If, as the

data reported suggest, Mg efflux depends on the electrochemical gradient for Na across the membrane, one may ask how any Mg efflux is present when this gradient is reversed. One possibility is that the Mg efflux into choline seawater is Mg:Mg exchange. This, however, seems unlikely since the effect of Mg-free seawater, even in ATP-free axons, is to increase Mg efflux (De Weer, 1976; Baker and Crawford, 1972). Another possibility is that the system responsible for moving Na inward in exchange for Mg moving outward has a selectivity such that choline$^+$ can replace Na^+ to some extent. While one does not usually think of a carrier having such a wide-ranging acceptance of ions, Beaugé and Mullins (1976) have described a system in the squid axon poisoned with ouabain that moves Na^+ and choline$^+$ inward with equal ease in exchange for internal Na.

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REFERENCES

BAKER, P. F., and A. C. CRAWFORD. 1972. Mobility and transport of magnesium in squid giant axons. *J. Physiol. (Lond.).* **227**:855–874.

BEAUGÉ, L. A., and L. J. MULLINS. 1976. Strophanthidin-induced sodium efflux. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **194**:279–284.

BRINLEY, F. J., JR., and L. J. MULLINS. 1967. Sodium extrusion by internally dialyzed squid axons. *J. Gen. Physiol.* **50**:2303–2331.

BRINLEY, F. J., JR., and L. J. MULLINS. 1968. Sodium fluxes in internally dialyzed squid axons. *J. Gen. Physiol.* **52**:181–211.

BRINLEY, F. J., JR., and A. SCARPA. 1975. Ionized magnesium concentration in axoplasm of dialyzed squid axons. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **50**:82–85.

BRINLEY, F. J., JR., S. G. SPANGLER, and L. J. MULLINS. 1975. Calcium and EDTA fluxes in dialyzed squid axons. *J. Gen. Physiol.* **66**:223–250.

DE WEER, P. 1976. Axoplasmic free Mg levels and Mg extrusion from squid axons. *J. Gen. Physiol.* **68**:159–178.

DIPOLO, R., J. REQUENA, F. J. BRINLEY, JR., L. J. MULLINS, A. SCARPA, and T. TIFFERT. 1976. Ionized calcium concentrations in squid axons. *J. Gen. Physiol.* **67**:433–467.

HARNED, H. S., and B. B. OWEN. 1950. The Physical Chemistry of Electrolytic Solutions. Reinhold Publishing Corp., New York. 147.

MULLINS, L. J., and F. J. BRINLEY, JR. 1975. The sensitivity of calcium efflux from squid axons to changes in membrane potential. *J. Gen. Physiol.* **65**:135–152.