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Magnesium Influx in Dialyzed Squid Axons

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Summary. The influx of magnesium from seawater into squid giant axons has been measured under conditions where internal solute control in the axon was maintained by dialysis. Mg influx is smallest (1 pmol/cm^2 sec) when both Na and ATP have been removed from the axoplasm by dialysis. The addition of 3 mM ATP to the dialysis fluid gives a Mg influx of 2.5 pmol/cm^2 sec while the addition of $[Na]_i$ and $[ATP]_i$ gives 3 pmol/cm^2 sec as a value for Mg influx; this corresponds well with fluxes measured in intact squid giant axons.

The Mg content of squid axons is 6 mmol/kg axoplasm; this is unaffected by soaking axons in Li or Na seawater for periods of up to 100 min.

While measurements of Mg efflux from squid giant axons have been made both in the case where Mg was injected into the axon (Baker & Crawford, 1972; DeWeer, 1976) and in internally dialyzed axons (Mullins *et al.*, 1977), somewhat fewer measurements of Mg influx have been made and none under conditions of internal solute control by dialysis. It is the purpose of this report to present such Mg influx measurements.

The influx of ^{28}Mg into intact squid axons that were subsequently rinsed, had their axoplasm extruded and counted, have been made by Baker and Crawford (1972), who showed that Mg influx from axons bathed in Na seawater had a mean value of 0.62 pmol/cm^2 sec. Mg influx from Li seawater was almost tenfold larger than the flux from Na seawater. A somewhat different method of measuring Mg influx was reported by Rojas and Taylor (1975). These investigators used internal perfusion as a technique for collecting isotopic Mg that entered under conditions where the internal perfusion medium was isotonic KF or 1/2 isotonic KF, 1/2 sucrose. Their values for Mg influx were 0.12 pmol/cm^2 sec, a value almost fivefold lower than the mean observed by Baker and Crawford. The discrepancy between their values and those of Baker and Crawford may be due to the fact that a substantial fraction

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of the normal Mg influx may be an exchange of Mg_o for Na_i. Since their media contained no Na, this mode of Mg influx was impossible.

At constant $[Mg]_i$, measurements have shown that Mg efflux depends on both ATP_i and Na_i; the present measurements have been designed to examine the dependence of Mg influx on these variables. The findings are that both ATP and internal Na enhance Mg influx but that the variability of the data is such that one cannot tell whether ATP or Na_i has the greater effect on Mg influx. Experiments clearly show, however, that maximal Mg influx is obtained when both internal Na and ATP are present in the fiber.

Materials and Methods

The method for making influx measurements using a radioactive isotope has been previously described (Mullins & Brinley, 1969), and the methods there described have been used in the present study.

Experimental Material

The experiments were performed on axons freshly isolated from living specimens of *Loligo pealei* at the Marine Biological Laboratory during the months of May and June, 1977.

Solutions

The composition of the seawater used was Na seawater (all concentrations in mM): [Na], 450; [K], 10; [Mg], 30; [Ca], 3; [Cl], 516. Additions to seawater were NaTES, pH 7.8, 10 mM, and KEGTA 0.1 mM. Li seawater was prepared by substituting Li for the Na in the above formula. Osmotic pressure was adjusted to 1000 mosmol/liter.

Internal dialysis solutions contained (in mM): glycine, 275; K aspartate, 350; KTES, pH 7.3, 10; EGTA, 0.1; $MgCl_2$, 4. The Na concentration of such solutions was adjusted by replacing equivalent amounts of K aspartate by Na aspartate solution so as to make the Na concentration 80–100 mM. ATP was obtained as the Mg salt and was titrated with Tris solutions to a pH of 7.3 and stock solutions of Mg ATP were added to the dialysis fluid as required. These stock solutions ~200 mM were analyzed for [ATP] by the hexokinase reaction. Osmotic pressure was adjusted to 910 mosmol/liter.

Isotopes

^{28}Mg was obtained from Brookhaven National Laboratory as an aqueous solution. This was evaporated to dryness and the isotope taken up in Mg-free seawater with the volume of seawater adjusted so that the resulting isotope plus carrier gave the required concentration (30 mM) of Mg in the isotopic seawater that was to be used. Such radioactive seawater was checked for pH, then introduced into the chamber bathing the axon, and internal dialysis begun. Samples of the dialysis effluent were collected over

periods of 10 min, and since the flow rate of the dialysis solution was of the order of 1 μl/min, the volume of the samples collected was of the order of 10 μl. The samples were added to liquid scintillation-counting fluid and counted in the ^{14}C channel of a Beckman liquid scintillation counter. Radioactive ^{28}Mg seawater was fed into the central slot of the dialysis chamber by a motor-driven syringe at 10 μl/min and withdrawn by the guard syringes.

Analytical

Some axons were rinsed in isotonic sucrose, blotted, the axoplasm extruded, weighed and collected in plastic tubing, and stored in a freezer until analyzed for Mg with a Perkin-Elmer model 305B atomic absorption spectrophotometer.

Results

The measurement of Mg influx is technically more difficult than the corresponding measurement for efflux because any increase in the leakage or nonspecific entry of Mg is accentuated by the electrical and chemical gradients for Mg which tend to drive Mg inward rather than outward. It is to be expected, therefore, that the entry of Mg in the absence of either substrate or internal Na is a somewhat variable quantity. In addition, it would seem reasonable that the trauma necessary to insert a dialysis tube into the axon increases the leakage of Mg somewhat, although again to a variable degree.

Figure 1 shows a measurement of the influx of Mg in an axon that was initially dialyzed for 1 hr with a Na-free, ATP-free dialysis solution. The initial value of influx under such conditions of dialysis was approximately 0.6 pmol/cm^2 sec. The introduction of Na into the dialysis medium while keeping ATP at near zero produced roughly a tripling of Mg influx. The subsequent introduction of ATP as well as internal Na increased influx still further so that the effect of adding both these factors to the internal dialysis medium was to increase Mg influx from a value of 0.6 to 2.6 pmol or an increment of 2 pmol in all.

It is not easy to reverse the addition of ATP to the axoplasm once the axon has been made ATP-free mainly, we believe, because ATP diffuses longitudinally within the axon from the porous region into regions of the axon not under direct solute control. There, ATP acts as a source to feed a region of axoplasm where isotope is being collected and hence influence the local ATP concentration. Since influx measurements are limited to periods not greater than 2–3 hr because the chance of developing a leak increases with time, we have not attempted to reverse ATP effects; however, Fig. 2 shows an axon that had been treated with

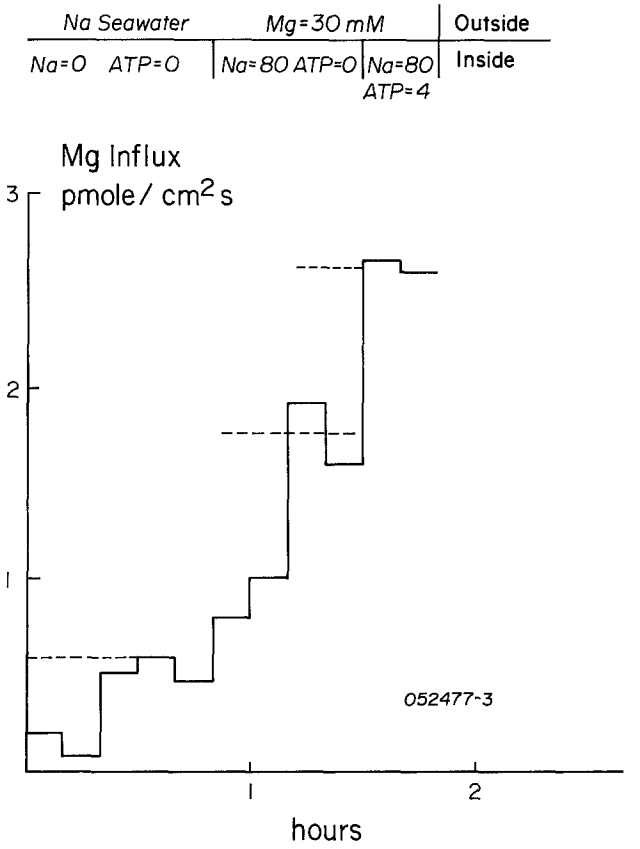

Fig. 1. Magnesium influx is shown as a function of time for an axon in seawater (Na) when $[Na]_i$ and $[ATP]_i$ are changed as indicated at the top of the figure. Note that influx is stable for at least two collecting periods (20 min) in each experimental internal medium

ATP (3 mM) but dialyzed free of internal Na before influx measurements were begun. This axon had a Mg influx of 2 pmol/cm^2 sec; when the dialysis medium contained Na at a concentration of 80 mM, the efflux went up by slightly more than 2 pmol. This change was readily reversed when the $[Na]_i$ was again brought to 0.

Table 1 summarizes measurements of Mg influx into axons dialyzed either free of both internal Na and ATP or of one or the other of these substances, as compared with axons free of both or containing both. This shows that a mean value of 3 pmol is found for axons containing both ATP and Na, as compared with the mean value of 1 pmol for axons where both substances are absent. It appears, therefore, that the ATP-

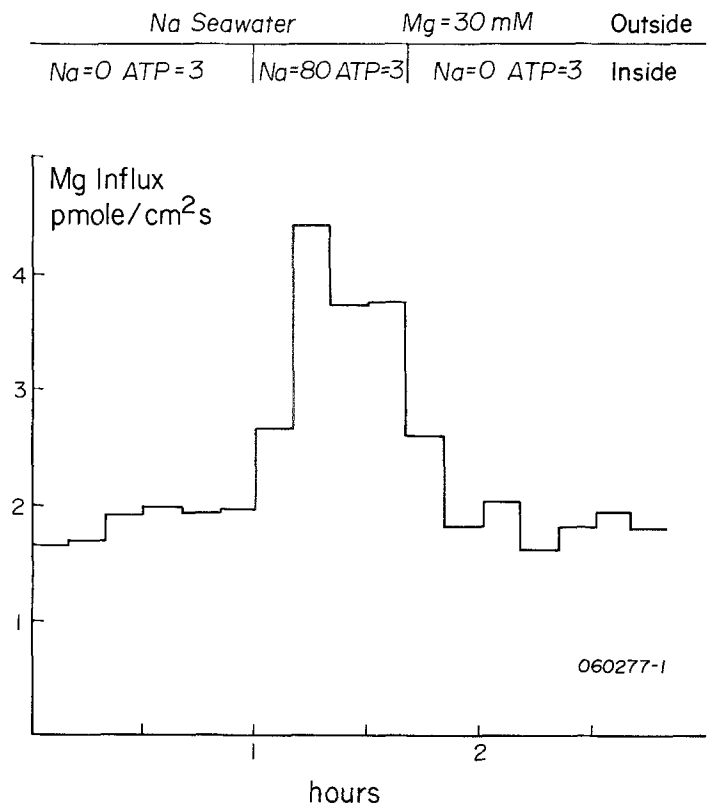

Fig. 2. Magnesium influx is shown as a function of time when $[Na]_i$ is changed from nominally zero to 80 mM and then back to zero

and Na-dependent Mg influx is 2 pmol/cm^2 sec. It agrees reasonably well with the previously published values for Mg efflux from dialyzed squid axons (2–4 pmol/cm^2 sec) and with values obtained by DeWeer (1976) for Mg efflux from injected *Loligo* axons. The mean value for influx is also in reasonable agreement with values that were determined on intact axons as shown in the last two entries of Table 1; these lead also to a mean value for Mg influx of 3 pmol/cm^2 sec. A single value for an influx from Li seawater (15 pmol) also supports the previous observation of Baker and Crawford that Li seawater enhances Mg influx substantially. All of the values for Mg influx that we have measured are substantially larger than those measured by Baker and Crawford, but since Mg influx is so dependent on internal Na, it would be surprising if closer agreement could be obtained.

Table 1. Mg influx in squid axons[a] (17 °C), pmol/cm^2 sec

Dialyzed axons	$[Na]_i$ = 80–100 mM		$[Na]_i$ = 0	
	$[ATP]_i$ = 3–4 mM	$[ATP]_i$ = 0	$[ATP]_i$ = 3–4 mM	$[ATP]_i$ = 0
060277–1	3.7		2.0	
			2.0	
051877–2	2.2			
052477–2	2.4			
052477–3	2.6	1.6		0.5
052577–1			3.0	0.6
060177–2	15[b]			
051177–1	3.7		3.3	2.0
060277–1	3.7		2.0	
060177–1		1.3	2.6	1.0
Mean	3.0		2.5	1.0
Intact axons				
051177–I	3.6			
052577–I	2.5			

[a] (17 °C), pmol/cm^2 sec.
[b] Influx from Li seawater (excluded from mean).

Mg Content of Axons

While both Baker and Crawford (1972) and DeWeer (1976) have shown that the total Mg content of squid axons is about 7–10 mmol/kg axoplasm, there have been no studies of the extent to which the total Mg content of an axon can change in response to altered conditions. Since Li seawater has been shown to both increase Mg influx and decrease Mg efflux, one might expect a change in the Mg content of an axon as a result of Na-free seawater. If Mg influx were 15 pmol and efflux 1 pmol, the axon would gain Mg at a rate of 14 pmol/cm^2 sec or 6.7 mmol/kg in 100 min for a 500 μm axon. Since this value is comparable to the Mg content of axons, it should be easily observable.

Table 2 gives the analytical data of 5 axons, 4 soaked either in seawater or Li seawater for 80–110 min and one fresh axon. It is clear from this table that Li seawater has no effect on Mg content. Since the calculation made above shows that Mg content should have about doubled if Mg net flux were 14 pmol/cm^2 sec, it is clear that the Mg fluxes in either Na or Li seawater were close to being in balance.

Table 2. Analytical magnesium in axons stored in seawater

Axon ref	d (μm)	Treatment	Time (min)	$[Mg]_i$ (mM)
051977–1	400	3 Ca(Na)SW	80	6.3
–2	450	3 Ca(Na)SW	90	4.6
–3	450	3 Ca(Li)SW	101	4.2
–4	425	3 Ca(Li)SW	110	6.4
–5	400	10 Ca(Na)SW	0[a]	7.8
				5.9 Mean

[a] All axons spent about 15 min in seawater.

Discussion

A previous study of Mg efflux (Mullins *et al.*, 1977) established that (i) there was an ATP-dependent Mg efflux into choline seawater, (ii) that Mg efflux in the absence of ATP could reach values obtained in the presence of ATP if $[Mg]_i$ were elevated from about 3 to 15 mM, and (iii) that $[Na]_i$ is strongly inhibitory to Mg efflux with 40 mM being sufficient to reduce Mg efflux to half control values. Taken together, these findings suggest that there are multiple ways of increasing Mg efflux (raising Na_o, decreasing Na_i, increasing ATP, increasing Mg_i). The involvement of Na both inside and outside the fiber in Mg efflux suggests that it is the Na gradient that is used to provide energy for Mg efflux; the involvement of ATP in the control of Mg efflux suggests that it provides a speeding up of the rate of carrier movement, and the fact that it acts when choline is the external cation suggests that the specificity of the carrier for Na is not absolute.

The present findings with respect to Mg influx show that this flux is maximal when both Na and ATP are present in the internal dialysis fluid. There is, however, a substantial increment in Mg influx when Na_i is absent but ATP present in the fiber. Such a finding suggests that K_i (like $choline_o$) may have some finite ability to combine with the Mg carrier and allow the inward movement of Mg. By contrast, adding Na_i in the absence of ATP appears to be less able to promote Mg entry.

For an axon containing Na_i and $[Mg]_i = 4$ mM, the change from ATP-free to ATP-containing media enhanced Mg efflux by about 1.4 pmol (Fig. 4 of Mullins *et al.*, 1977). The present experiments show that the Mg influx would increase by about 1.5 pmol/cm^2 sec under these

circumstances. Thus our data is not persuasive to the view that ATP can promote a net Mg flux, even though it clearly can greatly increase unidirectional Mg fluxes.

Our measurements of Mg content of axons suggest that there is no large net flux of Mg into the fiber even in Li seawater. Since both the dialyzed axon we studied and the results of Baker and Crawford (1972) show that there is a large influx from Li seawater, one must conclude that, while our measured influx was from 80 mM Na_i, the fresh axons used for analysis probably had $Na_i = 40$ mM. If one recalls that Mg efflux is critically dependent on Na_i and the present measurements show a substantial dependence on Na_i, hence it is clear that variation in this parameter can affect the net flux of Mg.

The postulate that Mg fluxes are driven by a coupling to Na fluxes would require that, for example, there be a Na influx of a magnitude r (Mg efflux) where r is the coupling ratio Na/Mg. Since Mg efflux values on the order of 3 pmol have been observed, then an electroneutral carrier would produce a Na influx of 6 pmol. Values of Na influx larger than this (which may indicate a value of $r > 2$) have been observed by Brinley and Mullins (1968) in axons dialyzed with 4 mM Mg_i when Na and ATP are added to the internal dialysis fluid. The corresponding Na movement for Mg influx (an efflux of Na) is somewhat more difficult to observe since an ATP-dependent efflux of Na via the Na pump is likely to overwhelm the Mg-dependent Na flux.

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