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EFFECT OF MAGNESIUM ON CALCIUM EFFLUX IN DIALYZED SQUID AXON

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Summary

The Ca efflux mechanism located in the axolemma of the tropical squid *Doritheutis plei* is shown to be affected by the concentration of intracellular Mg (Mg_i). The removal of all of the Mg from the experimental preparation causes an increase in Ca efflux. This effect seems to be more pronounced at low levels of internal ionized calcium and high levels of internal Na.

In order to further characterize the Na-Ca counter transport mechanism [1—6], the effect of Mg in axons in which the concentration of electrolytes and ATP was carefully controlled was explored. The effect of Mg_i on Ca efflux has been explored previously by Brinley and co-workers [8] who found that, in the nominal absence of ATP, the Ca efflux system in axons bathed in artificial sea water containing 50 mM Mg was unaffected by changes in the concentration of Mg_i in the range of 0—4 mM, and by DiPolo [10] who showed that Mg_i was a strict requirement for ATP to produce a stimulation of Ca efflux in axons bathed in Mg-free artificial sea water.

Experiments were performed on giant axons from the tropical squid *Doritheutis plei*, which were subjected to intracellular solute control by means of the dialysis technique developed by Brinley and Mullins [7] as modified by DiPolo [11]. The composition of solutions used is given in Table I. Great care was taken in preparing artificial sea water solutions which contained minimal amounts of contaminating cations (such as Ca, Mg and Na). The contamination for solutions described as free of Mg was in the order of 1 μM.

Fig. 1 shows the time course for Ca efflux of an axon bathed in 50 mM Mg_o artificial sea water, dialyzed with a medium containing 300 μM ionized Ca and in which the effect of internal Mg and ATP was tested. Under the

TABLE I

SOLUTIONS

Concentrations of constituents are given in mM.

Constituent	External			Internal*			
	Sea water	30 mM Ca, 50 mM Mg	30 mM Ca, no Mg	Na, 5 mM Mg	Ch 5 mM Mg	Na, no Mg	Ch no Mg
K	10	10	10	340	340	340	340
Na	440	420	420	100	0	100	0
Mg	50	50	0	5	5	0	0
Ca	10	30	30	**	**	**	**
Choline	—	—	75	0	100	0	100
Tris	10	10	10	—	—	—	—
Cl	575	565	575	110	110	110	110
CN	1	1	1	—	—	—	—
EDTA	0.1	0.1	0.1	—	—	—	—
EGTA	—	—	—	1.0	1.0	1.0	1.0
Aspartate	—	—	—	330	330	330	330
Glycine	—	—	—	300	300	300	300
HEPES***	—	—	—	10	10	10	10
pH	7.8	7.8	7.8	7.3	7.3	7.3	7.3
mosM/g per kg	1010	1010	1010	990	990	990	990

*Internal media were poisoned with 2 μg/ml of oligomycin and carbonyl cyanide *p*-trifluoromethoxy-phenylhydrazone.
**Adjusted to give the desired level of ionized Ca using a CaEGTA dissociation constant of 0.15 μM [12].
***HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

condition of nominally no Na_i, 5 mM Mg_i and no ATP a Ca efflux level of 6.3 pmol·cm^{-2}·s^{-1} was observed. The addition of 100 mM Na_i in exchange for 100 mM choline reduced this level to 1.7 pmol·cm^{-2}·s^{-1}. This drop in Ca efflux is due to the inhibitory action of internal Na [6,8], which is very marked in the absence of ATP_i [13]. The removal of all of the internal Mg, i.e. the condition of "virtual" absence of Mg_i, increased the Ca efflux level to 2.3 pmol·cm^{-2}·s^{-1}. Under this condition the addition of 2 mM of the Tris salt of ATP to the dialysis medium raised the Ca efflux level to 4.8 pmol·cm^{-2}·s^{-1} in spite of the fact that nominally there was no Mg_i. The addition of 5 mM Mg to the internal media further raised the Ca efflux level to 6.0 pmol·cm^{-2}·s^{-1}, producing the maximal stimulation of the Ca efflux mechanism to be observed under the experimental conditions of 100 mM Na_i and (Tris)ATP. It is obvious, that under the condition of no Mg_i and 2 mM (Tris) ATP there must have been some Mg at the inner side of the axolemma since the Ca efflux was partially activated by Mg-free ATP. The Mg responsible for this stimulation of the Ca transport must have come from the external media, which in the present experiment contained 50 mM Mg_o. If the figure of 300 μM ATP is taken for the apparent activation constant of ATP on Ca efflux [9], a concentration of Mg at the transport site of some 500 μM must have existed in order to account for the observed 3/4 partial activation of the Ca efflux mechanism by (Tris) ATP when there was no Mg_i. This value, although large, is not unreasonable, given the known permeability of Mg in squid axon [3] and the efficiency [7] of the dialysis technique to control solute concentration. It is fair to conclude, therefore, that in order to guarantee an absolute absence of Mg at the transport site for Ca all of the external Mg and of the internal Mg needs to be removed.

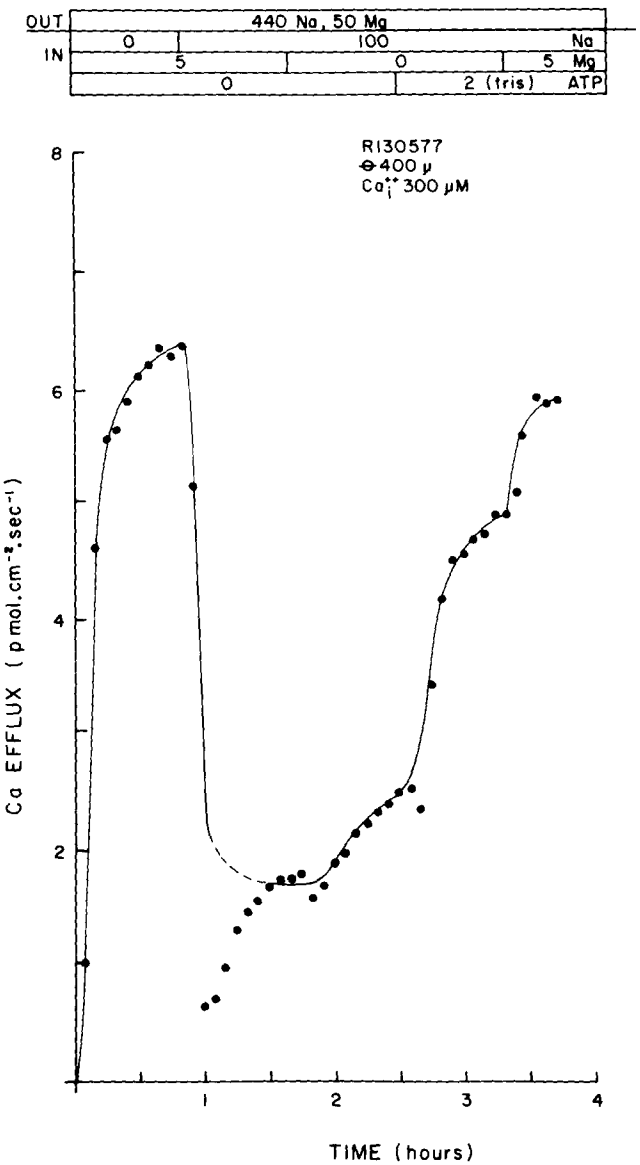

Fig.2. The time course of Ca efflux in the absolute or relative absence of internal Mg at two concentrations of internal Na in an axon dialyzed with an ATP-free media. *T*, 18°C. Ordinate, Ca efflux in pmol·cm^{-2}·s^{-1}; abscissa, time in hours.

The experiment of Fig. 2 begins with the dialysis of an axon with no Na_i, 5 mM Mg_i and 50 mM Mg_o and a high level of ionized Ca_i. Removal of all of the external Mg does not have a significant effect on the Ca efflux level. However, the elimination of all of the Mg from the internal media, that is, the condition of "absolute" absence of Mg, produced an increment in the Ca efflux level which went from 9.3 to 10.5 pmol·cm^{-2}·s^{-1}. In this absence of Mg_i, 100 mM internal Na was added to the dialysis fluid causing the Ca efflux level to drop down to 6.8 pmol·cm^{-2}·s^{-1}. At this point,

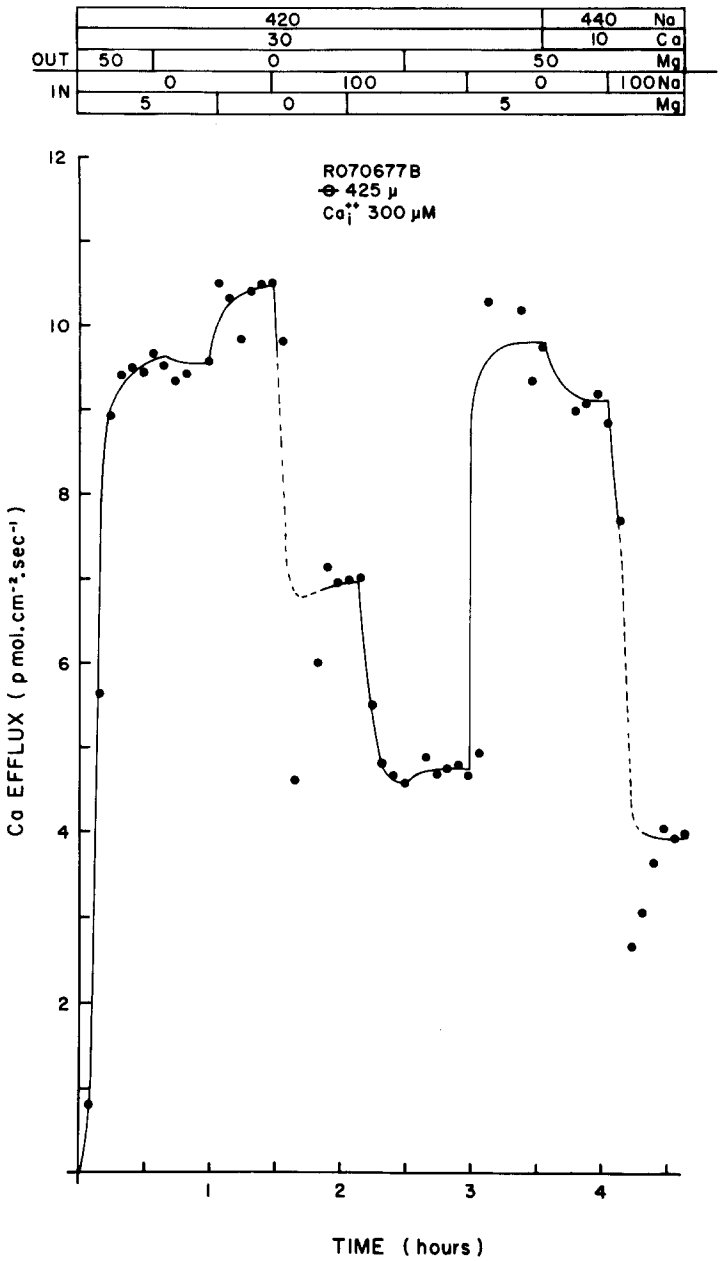

Fig.1. The time course of Ca efflux in the absence and in the presence of internal Mg and ATP at two concentrations of internal Na in a dialyzed squid axon. *T*, 18°C. Ordinate, Ca efflux in pmol·cm^{-2}·s^{-1}; abscissa, time in hours.

5 mM Mg_i were introduced into the dialysis media which caused a further reduction in the Ca efflux level to 4.5 pmol·cm^{-2}·s^{-1}. Reintroduction of 50 mM Mg_o produced an almost insignificant reduction of Ca efflux. In the last part of the experiment some typical features [4,13] of the Ca efflux mechanism can be seen again. Removal of all of the internal Na returned

TABLE II

EFFECT OF Mg ON Ca EFFLUX

Axon reference	Ca_i^{2+} (μM)	Ca efflux (pmol·cm^{-2}·s^{-1})							
		No ATP						(Tris) ATP	
		No Na_i			100 mM Na_i			100 mM Na_i	
		50 mM Mg_o, no Mg_i	5 mM Mg_i	No Mg_o, no Mg_i	50 mM Mg_o, no Mg_i	5 mM Mg_i	No Mg_o, no Mg_i	50 mM Mg_o, no Mg_i	5 mM Mg_i
R 070677 B	300	—	9.3	10.5		4.5	6.9	—	—
R 130577	300	—	6.5	—	2.3	1.7	—	6.0	4.8
R 120477 A	0.25	0.270	0.140	—	0.040	0.026	—	—	—
R 080277 C	0.10	0.042	0.036	—	—	—	—	—	—

the Ca efflux near its initial level of 10 pmol·cm^{-2}·s^{-1}. Withdrawal of 20 mM Ca from the external medium which had been kept 30 mM Ca_o during most of the experiment, produced a 15% drop in the Ca efflux level while the addition of 100 mM internal Na at the end of the experiment produced a typical 60% inhibition of Ca efflux.

Table II lists Ca efflux levels observed in the presence of internal Mg, in its virtual absence (50 mM Mg_o, no Mg_i) and in its absolute absence (no Mg_o or Mg_i) for the condition of nominally no Na_i, 100 mM Na_i and 100 mM Na_i + ATP. The ionized axoplasmic Ca concentration used in the experiments reported ranged from 0.1 μM to 300 μM. In all cases listed, it is clear that the total or partial removal of internal Mg produced an increase in the Ca efflux values. It should be noted that the data presented here have not been corrected for changes in the ionized level of Ca due to liberation of the small fraction of the ethyleneglycol bis(β-aminoethylether)-*N*,*N*'-tetraacetic acid (EGTA) normally complexed with Mg [8]. This correction will tend to make the observed increment in Ca efflux, caused by the removal of internal Mg even larger, since the liberation of EGTA would reduce the ionized Ca level present in the axoplasm which, in turn, should manifest in a decrement in the Ca efflux to be calculated.

Although no attempt can be made at this stage to assert quantitatively the extent of the effect of Mg_i on Ca efflux, a qualitative analysis of the data presented in Table II, however, indicates that the reduction in Ca efflux produced by Mg_i is more marked in axon in which the transport mechanism is inhibited by the presence of internal Na and in those having a relatively very low level of ionized axoplasmic Ca. These two facts suggest that internal Mg is capable of interacting and/or substituting for Ca at the inner site of the transport system.

Finally, in spite of metabolical poisoning and dialysis, the possibility of assigning to micromolar quantities of ATP, thought to remain at the transport site, the role of supporter to the Ca level observed in the absence of internal Na, can be ruled out when the observation made herein, that Mg is not necessary to maintain the high level of Ca efflux observed at zero Na_i, is related to the finding of DiPolo [10] who showed that Mg is a strict requirement for ATP to stimulate that Na_i-inhibited Ca efflux mechanism. In other words, the observed increment in Ca efflux caused by the removal of Na_i, interpreted to be a purely inhibitory phenomena of Na_i [13], can not be explained in terms of the appearance of an ATP-activated fraction of Ca efflux, as a consequence of the removal of Na_i, produced by a shift in the affinity constant for ATP of the transport mechanism towards smaller concentrations of ATP. Since in the limit of zero Na_i, ATP has been shown to have no effect on Ca efflux [10,13] and Mg_i not to be necessary to maintain Ca efflux.

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