Biochimica et Biophysica Acta, 433 (1976) 520–530

BBA 77310

ADP-ACTIVATED CALCIUM ION EXCHANGE IN SARCOPLASMIC RETICULUM VESICLES

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(Received November 3rd, 1975)

SUMMARY

(1) Ca^{2+} efflux from rabbit skeletal muscle sarcoplasmic reticulum vesicles pre-loaded with $^{45}Ca^{2+}$ was studied in the presence and in the absence of external Ca^{2+}.

(2) In the absence of Ca^{2+} in the assay media, ADP activates the Ca^{2+} efflux. The increment of Ca^{2+} efflux requires P_i, is coupled to ATP synthesis, and is inhibited by external Ca^{2+} (K_i 0.1–0.2 μM).

(3) When Ca^{2+} is added to the assay media, ADP alone activates the Ca^{2+} efflux, but this is coupled to a Ca^{2+} influx of the same magnitude. It is therefore an exchange of internal for external Ca^{2+} in a 1 : 1 ratio.

(4) The ADP-activated Ca^{2+} exchange requires external Ca^{2+} with an apparent K_m of 0.1–0.2 μM, does not require the addition of P_i or Mg^{2+}, although 3–10 mM $MgCl_2$ activates it. It is not inhibited by the removal of contaminating ATP with hexokinase plus glucose.

(5) It seems likely that Ca^{2+} can be translocated across sarcoplasmic reticulum membrane without the formation of a phosphorylated intermediate.

INTRODUCTION

Sarcoplasmic reticulum vesicles in the presence of ATP, Mg^{2+} and Ca^{2+} actively accumulate calcium inside the vesicles. The transport of 2 Ca^{2+} is coupled with the hydrolysis of 1 ATP molecule [1–4].

When incubated in Ca^{2+}-free medium, vesicles previously loaded with calcium allow a passive efflux of the accumulated calcium. Addition of ADP, P_i and Mg^{2+} activates the Ca^{2+} efflux, and the increment of the Ca^{2+} efflux is coupled to ATP synthesis. In this reversal of the calcium pump, the stoichiometric relationship of about 2 Ca^{2+} : 1 ATP is maintained [5–10].

Calcium uptake and reversal of the calcium pump are mediated by the same membrane-bound (Ca^{2+} + Mg^{2+})-dependent ATPase (EC 3.6.1.3). In both processes

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N,N'*-tetraacetic acid.

the enzyme is phosphorylated either by ATP (during Ca^{2+} uptake) or by P_i (during reversal), forming an acylphosphoprotein (E ~ P) [8, 11–13].

Makinose [14] has shown that ADP can activate a Ca^{2+} exchange in conditions which permit formation of the phosphorylated intermediate E ~ P and hydrolysis of ITP. He proposed a scheme in which the Ca^{2+} exchange would be mediated by this E ~ P. In the present study an exchange of Ca^{2+} is observed in the absence of either ATP or P_i, when no E ~ P formation would be expected. The efflux component of this exchange is compared with the net efflux that occurs during reversal of the calcium pump.

METHODS

Sarcoplasmic reticulum vesicles were prepared from rabbit skeletal muscle as previously described [15].

Loading with calcium phosphate or calcium oxalate

In most of the experiments the calcium efflux was measured using vesicles loaded with calcium phosphate (Figs. 1, 3–6 and Table I). Sarcoplasmic reticulum vesicles at a final protein concentration of 1.9 mg/ml were incubated with 20 mM Tris · maleate (pH 6.5), 1 mM $^{45}CaCl_2$, 5 mM $MgCl_2$, 50 mM potassium phosphate (pH 6.5) and 2 mM ITP. After 9 min of incubation at room temperature the mixture was centrifuged at 25 000 × *g* for 30 min at 0 °C. The pellet was re-suspended in 20 mM Tris · maleate (pH 6.5) to a final protein concentration of 6 to 9 mg/ml. When indicated the pellet was washed once in 20 mM Tris · maleate (pH 6.5) containing 5 mM EDTA to lower Mg^{2+} contamination.

For Figs. 2 and 4 the sarcoplasmic reticulum vesicles were loaded with calcium oxalate, incubating vesicles (3 mg protein/ml) with 20 mM Tris · maleate (pH 6.5), 1 mM $^{45}CaCl_2$, 5 mM $MgCl_2$, 1 mM EGTA, 5 mM potassium oxalate and 2 mM ITP. After 3 min at room temperature aliquots of the mixture were diluted 30 fold in the efflux media.

The mean specific activity of $^{45}CaCl_2$ used was 250 mCi/mol.

Ca^{2+} efflux determinations

Ca^{2+} effluxes were measured in the presence and in the absence of external Ca^{2+}. In the absence of external Ca^{2+} the Ca^{2+} efflux is a net efflux because no influx can take place. In the presence of Ca^{2+} in the assay media the influx must be taken into account.

Net efflux was distinguished from Ca^{2+} exchange in the experiment of Fig. 3. Sarcoplasmic reticulum vesicles preloaded with $^{45}Ca^{2+}$-labelled calcium phosphate were diluted in media containing $^{45}CaCl_2$ with the same specific activity. In this condition, whenever the efflux was coupled to an influx of the same magnitude there would be no increase of radioactivity in the medium. Thus, in this condition only net fluxes can be measured. The Ca^{2+} efflux which appears when the reaction is performed in media containing unlabeled Ca^{2+} and which does not appear under the conditions described above for measurement of net Ca^{2+} efflux is referred to as Ca^{2+} exchange.

To distinguish a Ca^{2+} efflux coupled with a reversal of the calcium pump from

other Ca^{2+} effluxes, it was necessary to demonstrate its dependence on P_i [5, 10] and an ATP synthesis accompanying the Ca^{2+} efflux.

The assay media are described in the figure legends.

When Ca^{2+} concentrations lower than 1 mM were used in the efflux media, the $CaCl_2$ concentration was 1 mM and different EGTA concentrations were used to obtain the desired free Ca^{2+} concentrations. Different association constants for Ca^{2+} and EGTA have been reported [16–18]. In this paper the apparent association constant for pH 6.8 reported by Schwarzenbach et al. [18] was used ($2.07 \cdot 10^6 M^{-1}$). The high $CaCl_2$ concentration was chosen in order to avoid significant back flux of $^{45}Ca^{2+}$ from the medium during the efflux measurements.

The reactions were started by the addition of sarcoplasmic reticulum vesicles previously loaded with $^{45}Ca^{2+}$-labeled calcium phosphate or $^{45}Ca^{2+}$-labeled calcium oxalate and stopped after different incubation intervals at 25 °C by removal of the vesicles using Millipore filters (average pore diameter 0.45 μm) as previously described [19]. The radioactivity of the filtrate was measured in a liquid scintillation counter.

ATP synthesis during Ca^{2+} efflux

The assays were carried out in Ca^{2+}-free or Ca^{2+}-containing media. Ca^{2+}-free media contained 20 mM Tris · maleate (pH 6.7), 15 mM EGTA, 20 mM $MgCl_2$, 1 mM potassium phosphate (pH 6.7), 0.1 mM ADP, 5 mM AMP, 100 mM glucose, 6 units/ml hexokinase (EC 2.7.1.1) and sarcoplasmic reticulum vesicles (0.2 mg protein/ml) loaded with $^{45}Ca^{2+}$-labeled calcium phosphate. The composition of the Ca^{2+}-containing media was 20 mM Tris · maleate (pH 6.8), 0.1 mM $CaCl_2$, 0.1 mM ADP, 3 mM $MgCl_2$ and vesicles (0.2 mg protein/ml) loaded with $^{45}Ca^{2+}$-labeled calcium phosphate. The reaction was stopped by filtration with Millipore filters after 3 min of incubation at 25 °C. To 0.8 ml of the filtrate was added 0.2 ml of a mixture containing 0.5 M Tris · Cl (pH 8.5), 50 mM $MgCl_2$, 250 mM glucose, 5 mM EGTA, 4 units/ml hexokinase and 4 units/ml glucose-6-phosphate dehydrogenase (EC 1.1.1.4.9). For the Ca^{2+}-free media hexokinase, glucose and EGTA were omitted. 10 min after initiating the reaction at room temperature by the addition of 0.025 ml of a 10 mM solution of NADP the optical extinction at 340 nm was measured against a blank carried through the same procedure but in which the sarcoplasmic reticulum vesicles used were empty.

ATP synthesis by contaminating adenylate kinase (EC 2.7.4.3)

The reaction was carried out as described in the legend of Table II and stopped by filtration with Millipore filters immediately after addition of the empty sarcoplasmic reticulum vesicles and after 5 min of incubation. The glucose-6-phosphate formed was measured by mixing 0.8 ml of each filtrate with 0.2 ml of a mixture containing 250 mM Tris · Cl (pH 8.5), 100 mM $MgCl_2$, 100 mM EGTA and 4 units/ml glucose-6-phosphate dehydrogenase. This was followed by the addition of 0.025 ml of a 10 mM solution of NADP. The absorbance at 340 nm was measured against water. The difference in optical extinction between the two times of reaction was used to calculate the amount of ATP synthesized by adenylate kinase.

Chemicals

ADP, ITP, NADP, glucose-6-phosphate dehydrogenase and hexokinase were

obtained from Sigma. $^{45}Ca^{2+}$ from The Radiochemical Centre. All the other reagents were analytical grade.

RESULTS

ADP-activated calcium efflux with and without external Ca^{2+}

Fig. 1 shows a comparison of calcium effluxes in the absence and in the presence of external Ca^{2+}. In Ca^{2+}-free media (Fig. 1A) containing 5 mM P_i, the calcium efflux is markedly activated by the addition of 0.1 mM ADP. In the absence of external P_i, the addition of ADP does not activate the calcium efflux. The small increase in efflux at prolonged incubation intervals may be promoted by the P_i which accompanies the Ca^{2+} into the medium. The requirement for P_i in reversal of the calcium pump had been reported previously for vesicles loaded with calcium oxalate [5, 10]. Fig. 1A shows that external P_i is required even with vesicles loaded with calcium phosphate, suggesting that the pool of P_i inside the vesicles is not sufficient to activate reversal of the calcium pump.

In the presence of 5 mM $CaCl_2$ (Fig. 1B), ADP also activates the calcium efflux, but in this condition external P_i is not required. On the contrary, the addition of 5 mM P_i to the medium decreases the ADP-activated component by 30–50 %. This inhibition may be related to a lowering of the internal Ca^{2+} concentration,

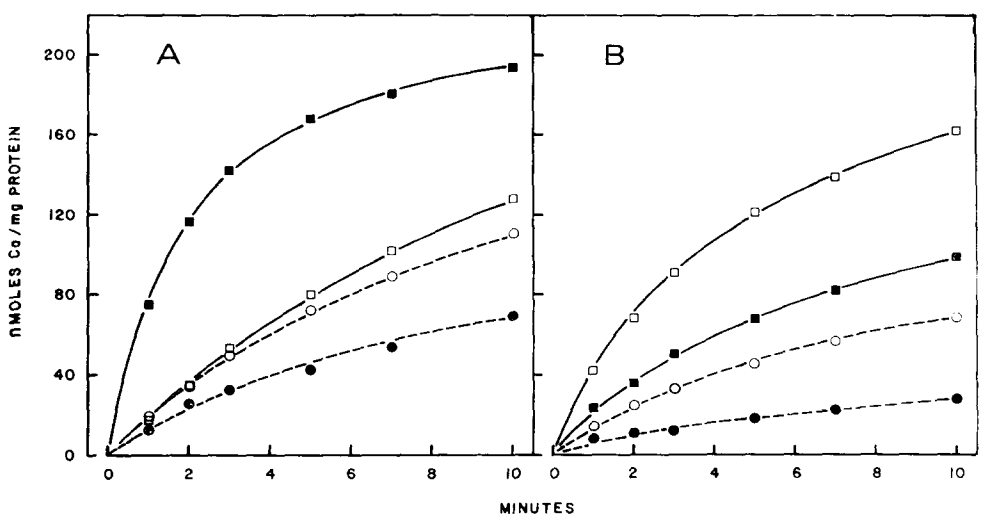

Fig. 1. Calcium efflux in the absence and in the presence of external Ca^{2+}-dependence on external P_i. Sarcoplasmic reticulum vesicles were loaded with $^{45}Ca^{2+}$-labeled calcium phosphate. (A) Calcium efflux in the absence of external Ca^{2+}-pre-loaded vesicles were incubated at 0.2 mg/ml and 25 °C in 20 mM Tris · maleate (pH 6.8), 2 mM EGTA, 3 mM $MgCl_2$, plus: ○, no addition; ●, 5 mM potassium phosphate; □, 0.1 mM ADP; ■, 0.1 mM ADP plus 5 mM potassium phosphate. Total efflux in 10 min (■) corresponds to the release of 55–60 % of the calcium contained in the vesicles. Each point represents the average of 2 experiments. (B) Calcium efflux in the presence of external Ca^{2+}-pre-loaded vesicles were incubated at 0.2 mg/ml and 25 °C in 20 mM Tris · maleate (pH 6.8), 5 mM $CaCl_2$, 5 mM $MgCl_2$ plus: ○, no addition; ●, 5 mM potassium phosphate; □, 0.1 mM ADP; ■, 0.1 mM ADP plus 5 mM potassium phosphate. Total efflux in 10 min (□) corresponds to 30–50 % of the calcium contained in the vesicles. Each point represents the average of 2 experiments.

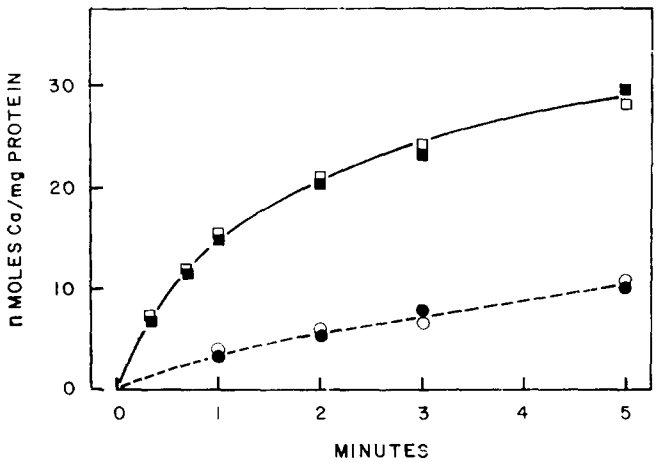

Fig. 2. Calcium efflux from vesicles loaded with $^{45}Ca^{2+}$-labeled calcium oxalate-dependence on P_i. Incubations were performed at 25 °C using 20 mM Tris · maleate (pH 6.8), 5 mM $CaCl_2$, 5 mM $MgCl_2$, 0.1 mg/ml sarcoplasmic reticulum vesicle protein, plus: ○, no addition; ●, 5 mM potassium phosphate; □, 0.1 mM ADP; ■, 0.1 mM ADP plus 5 mM potassium phosphate. Each point represents the average of 4 experiments.

TABLE I

ATP SYNTHESIS IN THE ABSENCE AND PRESENCE OF EXTERNAL Ca^{2+}

Sarcoplasmic vesicles were loaded with $^{45}Ca^{2+}$-labeled calcium phosphate and incubated as described under Methods. ATP synthesis was measured by formation of glucose-6-phosphate while calcium efflux was measured by increase in radioactivity in the assay media. Passive efflux (without ADP) was subtracted. Values represent average ± S.E. (number of experiments).

Additions	ADP-activated Ca^{2+} efflux (nmol Ca^{2+}/mg protein)	ATP synthesized (nmol ATP/mg protein)
15 mM EGTA	159 ± 10 (9)	111 ± 12 (9)
0.1 mM $CaCl_2$	47 ± 7 (4)	0 ± 0 (4)

since P_i also inhibits the efflux measured in the absence of ADP. This conclusion was substantiated in the experiment of Fig. 2 which shows that, using vesicles loaded with calcium oxalate, the addition of P_i to the assay medium does not change the Ca^{2+} efflux either in the presence or in the absence of ADP. Sarcoplasmic reticulum vesicles loaded with calcium oxalate show an ADP-activated Ca^{2+} efflux 2- to 3-fold lower than that observed in vesicles loaded with calcium phosphate (cf. Figs. 1B and 2).

Table I shows that only in the absence of Ca^{2+} in the assay medium was the Ca^{2+} efflux activated by ADP coupled with ATP synthesis.

Net flux determinations

In order to test whether in the presence of Ca^{2+} the ADP-activated calcium efflux is a net efflux or the efflux component of a $Ca^{2+}_{in} \rightleftharpoons Ca^{2+}_{out}$ exchange, net fluxes were measured in the presence of 50 μM $^{45}CaCl_2$ of the same specific activity as

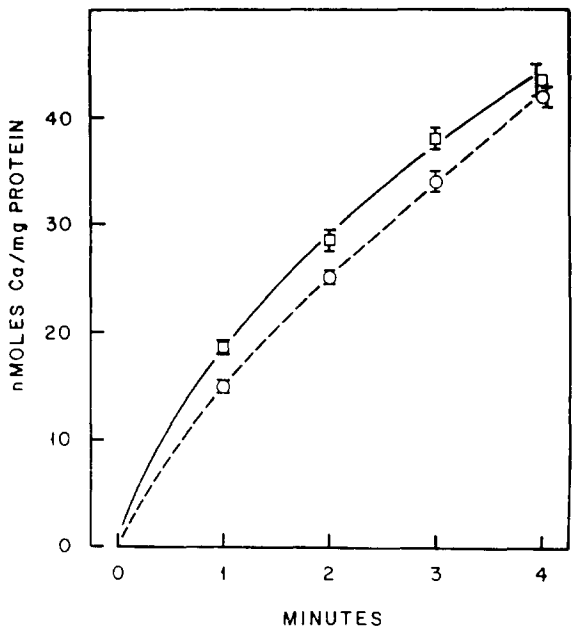

Fig. 3. Net efflux measurements. Sarcoplasmic reticulum vesicles were loaded with $^{45}Ca^{2+}$-labeled calcium phosphate. Incubations were performed at 25 °C in 20 mM Tris · maleate (pH 6.8), 50 μM $^{45}CaCl_2$, 3 mM $MgCl_2$, 0.3 mg/ml sarcoplasmic reticulum vesicle protein, plus: ○, no addition; □, 0.1 mM ADP. Each point represents the average of 12 experiments ±S.E.

that used for loading the vesicles (see Methods). Fig. 3 shows that in the absence of added ADP the amount of Ca^{2+} in the medium increases progressively with incubation time. When the experiments are carried out in the presence of ADP, there is no increase in the net flux. This indicates that the increased efflux promoted by ADP in the presence of unlabeled Ca^{2+} in the medium (Figs. 1B and 2) is coupled to an influx of nearly the same magnitude. Thus in the presence of external Ca^{2+}, ADP induces a Ca^{2+} exchange, rather than a net efflux as described for the reversal of the calcium pump (Fig. 1A).

Effect of external Ca^{2+} concentrations

Advantage was taken of the difference in P_i requirement for Ca^{2+} exchange and for reversal of the calcium pump in order to examine their dependence on external Ca^{2+}. Fig. 4 shows that the P_i-independent Ca^{2+} exchange induced by ADP is activated by external Ca^{2+} with an apparent K_m in the range of 0.1–0.2 μM. Raising the Ca^{2+} concentration from 0.5 μM to 5 mM does not modify the rate of Ca^{2+} exchange. This suggests that the Ca^{2+} concentration gradient does not play a significant role in this exchange.

On the other hand, external Ca^{2+} strongly inhibits the efflux under conditions for reversal of the calcium pump [5]. The apparent K_i for this inhibition is in the range of 0.1–0.2 μM. Vesicles loaded with calcium oxalate were used in these experiments in order to minimize the exchange activated by Ca^{2+} and ADP. The identity of values found for the apparent K_m for Ca^{2+} in Ca^{2+} exchange and the apparent K_i for Ca^{2+}

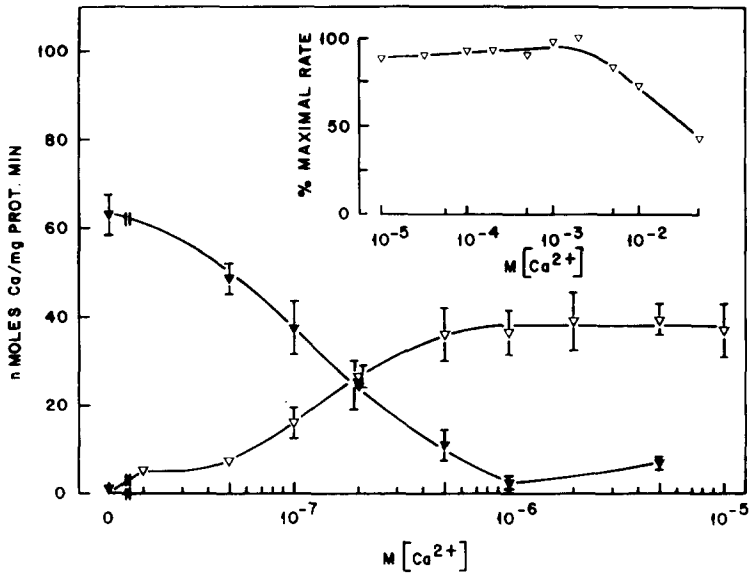

Fig. 4. Dependence of rates of reversal of the calcium pump and Ca^{2+} exchange on external Ca^{2+} concentrations. ▼ Reversal of the calcium pump-incubations were performed for 1 min at 25 °C with 20 mM Tris · maleate (pH 6.8), 5 mM $MgCl_2$, 20 mM potassium phosphate, 0.1 mM ADP and 0.1 mg/ml sarcoplasmic reticulum vesicle protein loaded with $^{45}Ca^{2+}$-labeled calcium oxalate. Efflux without ADP was subtracted. Each point represents the average ± S.E. of 4 experiments. ▽, Ca^{2+} exchange-incubations were performed for 1 min at 25 °C with 20 mM Tris · maleate (pH 6.8), 3 mM $MgCl_2$, 0.1 mM ADP and 0.2 mg/ml sarcoplasmic reticulum vesicle protein loaded with $^{45}Ca^{2+}$-labeled calcium phosphate. Efflux without ADP was subtracted. Each point represents the average ± S.E. of 3 experiments. In the inset the range of Ca^{2+} concentrations in 3 experiments was extended for the Ca^{2+} exchange, normalizing the rate of efflux to the maximal rate (at 2 mM $CaCl_2$). The Ca^{2+} concentrations were adjusted as described under Methods. For 0 Ca^{2+}, 2 mM EGTA was added and $CaCl_2$ was omitted.

in reversal of the calcium pump suggests that external Ca^{2+} is simultaneously inhibiting the Ca^{2+} release during reversal of the Ca^{2+} pump and activating the Ca^{2+} exchange.

Magnesium dependence

A significant rate of Ca^{2+} exchange can be measured in the absence of added Mg^{2+}. This is not abolished when the loaded vesicles are previously washed in a Mg^{2+}-free solution containing 5 mM EDTA, nor is it enhanced by the addition of $MgCl_2$ to the assay media up to a concentration of 0.3 mM (Fig. 5). However, concentrations of $MgCl_2$ from 3 to 10 mM enhance the rate of Ca^{2+} exchange by 75 %. This complex $MgCl_2$ activation profile is rather different from the Mg^{2+} dependence that has been reported for reversal of the calcium pump. In previous reports it has been shown that in the absence of added Mg^{2+}, ADP and P_i do not activate the rate of Ca^{2+} efflux [5, 10].

Role of contaminating ATP

Commercial ADP has been shown to be contaminated with ATP from 1 to 6 % on a molar basis (de Meis, L., unpublished). Our sarcoplasmic reticulum preparation

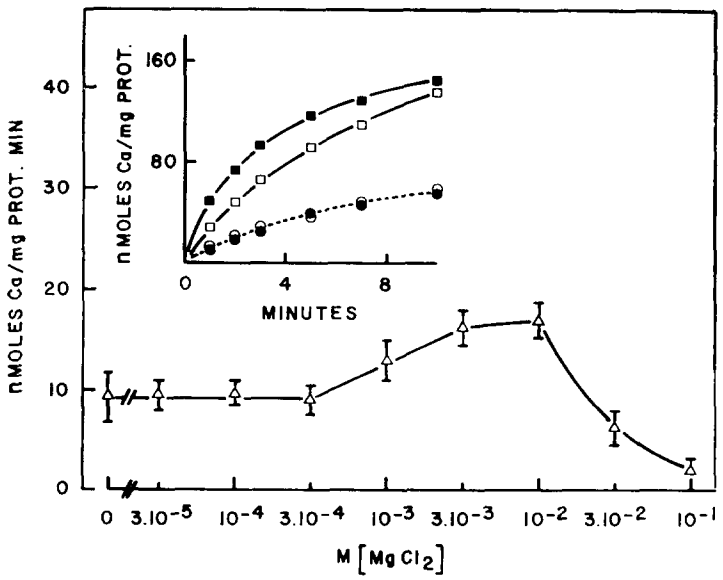

Fig. 5. Magnesium dependence of the Ca^{2+} exchange rate. Sarcoplasmic reticulum vesicles were loaded with $^{45}Ca^{2+}$-labeled calcium phosphate and incubated for 1 min at 0.2 mg protein/ml and 25 C in media containing 20 mM Tris · maleate (pH 6.8), 5 mM $CaCl_2$, 0.1 mM ADP and different $MgCl_2$ concentrations. Efflux without ADP was subtracted. Each point represents the average ± S.E. of 5 experiments. Inset, time course of efflux without ADP and Ca^{2+} exchange with and without $MgCl_2$. Experimental conditions were the same as above, with; ○, no ADP and no added $MgCl_2$; ●, no ADP and 5 mM $MgCl_2$; □, 0.1 mM ADP and no added $MgCl_2$; ■, 0.1 mM ADP and 5 mM $MgCl_2$. Each point represents the average of 2 experiments.

contains traces of adenylate kinase which catalyzes the reaction $2ADP \rightleftharpoons ATP + AMP$ (Table II). Therefore the possibility arises that the small amount of ATP derived from these sources could be hydrolyzed by the Ca^{2+} transport enzyme and

TABLE II

ATP SYNTHESIS BY CONTAMINATING ADENYLATE KINASE

Empty sarcoplasmic reticulum vesicles (0.3 mg/ml) were incubated in 20 mM Tris · maleate (pH 6.9), 5 mM $MgCl_2$, 50 mM glucose and 13 units/ml hexokinase, plus the additions indicated in the table. Glucose-6-phosphate formed from the ATP synthesized by adenylate kinase was determined as described under Methods. Values represent average of 2 experiments (variability of about ±5%). Values below 0.005 μmol ATP/mg protein could not be measured with accuracy.

Additions	ATP synthesis (μmol ATP/mg protein 5 min)	
	Without AMP	AMP 15 mM
1 mM ADP + 2 mM EGTA	0.179	0.008
1 mM ADP + 0.1 mM $CaCl_2$	0.174	—
1 mM ADP + 5 mM $CaCl_2$	0.094	<0.005
0.1 mM ADP + 2 mM EGTA	0.025	<0.005
0.1 mM ADP + 5 mM $CaCl_2$	0.013	<0.005

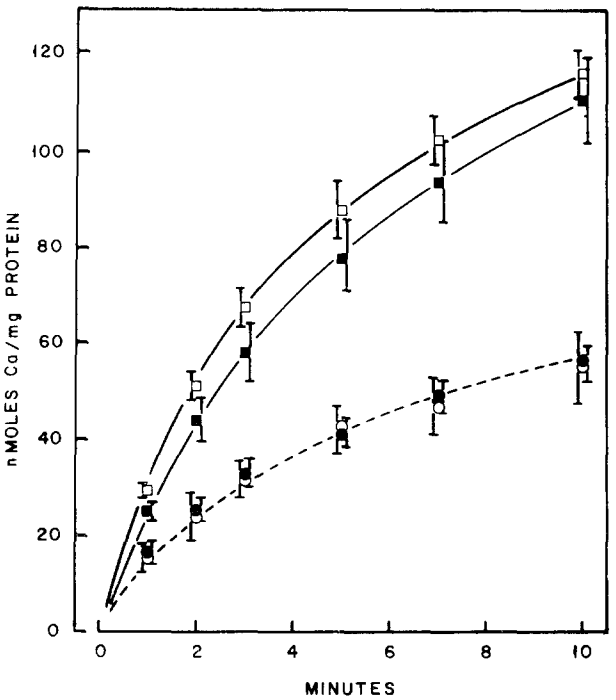

Fig. 6. Dependence of Ca^{2+} exchange on contaminating ATP. Sarcoplasmic reticulum vesicles were loaded with $^{45}Ca^{2+}$-labeled calcium phosphate. The incubation medium composition was 0.2 mg protein/ml, 20 mM Tris · maleate (pH 6.8), 5 mM $CaCl_2$, 5 mM $MgCl_2$ and 13 units/ml hexokinase, plus: ○, no addition, ●, 50 mM glucose, □, 0.1 mM ADP, ■, 0.1 mM ADP and 50 mM glucose. Before the addition of sarcoplasmic reticulum vesicles, the assay medium was preincubated 15 min at 25 °C in order to allow the draining of contaminating ATP into glucose-6-phosphate. Each point represents the average of 5 experiments ± S.E.

induce Ca^{2+} exchange in the presence of ADP. If this were the case, one would expect that the rate of Ca^{2+} exchange would vary if the steady state concentration of ATP in the medium varies. The following experiments help to exclude this possibility:

(1) The rate of Ca^{2+} exchange is not significantly modified when an excess of hexokinase and glucose is included in the assay medium in order to drain off contaminating ATP (Fig. 6). Table II shows that the concentration of hexokinase used is able to prevent the hydrolysis by the vesicles of the ATP formed by adenylate kinase. The Ca^{2+} transport ATPase is inactivated in presence of EGTA, severely inhibited in presence of 5 mM $CaCl_2$ and fully activated in presence of 0.1 mM $CaCl_2$ [20]. The amount of glucose-6-phosphate formed in the presence of 0.1 mM $CaCl_2$ was the same as in the presence of 2 mM EGTA (Table II). This means that the Ca^{2+} transport ATPase is not cleaving the ATP formed by the adenylate kinase.

(2) Large Ca^{2+} concentration inhibit the adenylate kinase (ref. 21 and Table II) by forming the less effective ADP · Ca^{2+} complex. Fig. 4 shows that increasing the Ca^{2+} concentration of the assay medium from 10^{-5} to $5 \cdot 10^{-3}$ M does not modify the rate of Ca^{2+} exchange.

(3) Table II shows that the ATP synthesized by adenylate kinase increases

7 fold when the ADP concentration was raised from 0.1 to 1.0 mM. This is consistent with the reported apparent K_m for ADP. Mg of rabbit muscle adenylate kinase [22]. In four different experiments, using sarcoplasmic reticulum vesicles loaded with calcium phosphate and an incubation medium identical with that described in Fig. 6 but without glucose and hexokinase, no difference in the rate of Ca^{2+} exchange was found when the ADP concentration of the medium was raised from 0.1 to 1.0 mM.

(4) AMP inhibits the synthesis of ATP from ADP catalyzed by adenylate kinase (Table II). In one experiment, using incubation medium and experimental conditions identical to those described in Fig. 2 (0.1 mM ADP) the rate of Ca^{2+} exchange was not modified by the addition of 15 mM AMP to the medium.

DISCUSSION

The data presented show that ADP is able to induce the Ca^{2+} exchange in conditions where phosphorylation of the Ca^{2+}-transport enzyme and ATP hydrolysis are improbable. A possible utilization of contaminating ATP seems to be excluded by the experiments described in connection with Table II and Fig. 6. For the Ca^{2+} efflux associated with reversal of the calcium pump, the enzyme has been shown to be phosphorylated by P_i [8]. This requires the addition of Mg^{2+} to the assay medium. The experiments of Fig. 5 show that more than 50 % of the maximal exchange rate is attained in media without added Mg^{2+}. The data of Figs. 1 and 2 show that P_i is not required for the Ca^{2+} exchange. This is consistent with the finding of Makinose [14] that during the Ca^{2+} exchange which occurs in the presence of ITP there is no incorporation of P_i into the NTP pool.

The high affinity for Ca^{2+} in activating the Ca^{2+} exchange is a strong indication that Ca^{2+} transport enzyme is involved in this exchange, since it is the only known protein of sarcoplasmic reticulum which has such a high affinity for Ca^{2+} [23–26]. Furthermore, the apparent K_m for Ca^{2+} activation of the Ca^{2+} exchange is about the same as that for the $Ca^{2+}+Mg^{2+}$-dependent ATPase activity, Ca^{2+} uptake and membrane phosphorylation by ATP [2, 12, 26–29]. It also coincides with the apparent K_i for Ca^{2+} in reversal of the calcium pump (ref. 5 and Fig. 4), phosphorylation by P_i [30] and $P_i \rightleftharpoons HOH$ exchange [31], provided that corrections are made for the different Ca^{2+}–EGTA association constants used by the different authors.

The data suggest that the binding of ADP to the membrane is enough to allow the translocation of Ca^{2+} through the calcium pump. Makinose [14] has observed that during the Ca^{2+} exchange in the presence of ITP there is a rapid NDP $\rightleftharpoons$ NTP exchange, indicating a participation of enzyme phosphorylated by ITP. The Ca^{2+} exchange through an unphosphorylated enzyme suggested in this paper may be an alternative pathway in the absence of NTP, i.e., the Ca^{2+} transport enzyme could mediate the Ca^{2+} exchange both in the phosphorylated and unphosphorylated forms.

ACKNOWLEDGEMENTS

This investigation was supported in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico by the Banco Nacional de Desenvolvimento

U.F.R.J. Paulo Sérgio Lacerda Beirão is a fellow of the Coordenação do Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors are grateful to Dr. Martha Sorenson for helpful discussion.

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