Biochimica et Biophysica Acta, 1027 (1990) 79–84
Elsevier

BBAMEM 74935

A calcium pump in plasma membrane vesicles from *Leishmania braziliensis*

Gustavo Benaim and Pedro J. Romero

Centro de Biologia Celular, Facultad de Ciencias, Universidad Central de Venezuela, Caracas (Venezuela)

(Received 23 February 1990)

Key words: ATPase, Ca^{2+}; Plasma membrane; Calcium regulation; Calcium ion transport; Calcium pump; (*Leishmania*)

A subcellular fraction highly enriched in plasma membrane vesicles was prepared from *Leishmania* promastigotes. This fraction showed (Ca^{2+} + Mg^{2+})-ATPase activity. This, however, represented a small fraction (about 25%) of the overall ATPase activity. The Ca^{2+}-ATPase showed general characteristics common to plasma membrane ATPases involved in Ca^{2+} transport. Thus, the Ca^{2+}-ATPase was activated by Ca^{2+} with a high affinity (K_m about 0.7 μM), saturating at about 5 μM Ca^{2+}. Furthermore, it was stimulated by calmodulin (about 70–80% with 5 μg/ml) and almost fully inhibited by trifluoperazine (100 μM). The above vesicles accumulated Ca^{2+} against a concentration gradient and released it after the addition of A23187, as shown independently by $^{45}Ca^{2+}$ and Arsenazo III studies. The transport mechanism showed the same kinetics parameters as described for the enzyme, indicating a single molecular entity. In addition, Ca^{2+}-ATPase activity and Ca^{2+} uptake were completely inhibited by vanadate (20 μM), indicating that an E1-E2 type mechanism is involved. The results clearly demonstrate the presence of a Ca^{2+} pump in the plasma membrane of *Leishmania* which is capable of maintaining a low cytoplasmic Ca^{2+} concentration.

Introduction

There is compelling evidence of the role of Ca^{2+} ions as intracellular messengers in a number of different cellular functions [1,2]. Evidence is accumulating in support of an important role of Ca^{2+} ions in human protozoan parasites, particularly in those from the family *Trypanosomatidae*. Thus, microtubule assembly in *Leishmania* [3], flagellar movements in *Chritidia oncopelti* [4], variant surface antigen release in African trypanosomes [5] and cellular differentiation in *Leishmania* [6] appear to be regulated by Ca^{2+}. In addition, the enzymes responsible for cyclic nucleotide metabolism in these parasites also seem to be regulated by Ca^{2+} [7,8]. Furthermore, the main Ca^{2+} binding protein of eukaryotic cells, calmodulin, has been detected and isolated from American [8,9] and African [10] trypanosomes and from *Leishmania* [11]. It has been shown recently [12–14] that these parasitic protozoa are able to maintain a very low ionic Ca^{2+} concentration in their cytoplasm (about 50 nM). However, little is known about the mechanisms responsible for the maintenance of such low concentrations. The mitochondrion has been shown to be involved in Ca^{2+} regulation by *Leishmania braziliensis* [12], *Leishmania donovani* [14] and *Trypanosoma cruzi* [15,16]. Nevertheless, the low affinity of mitochondrial Ca^{2+} transport cannot account for the decrease of intracellular free calcium to the submicromolar level [2,12].

Intracellular compartments are limited by their storage capacity and, thus, cannot be responsible for long-term homeostasis. This work describes for first time the presence of a Ca^{2+} pump in plasma membrane vesicles from *L. braziliensis*. The kinetic parameters are compatible with a long-term fine tuning of intracellular free Ca^{2+} in these parasites.

Materials and Methods

Organisms. Promastigotes from the Lby strain of *L. braziliensis* [16] were grown in liver infusion-tryptose medium (LIT) at 26 °C, with strong agitation (120 rpm) as described previously [12].

Isolation of plasma membrane vesicles. Exponential phase promastigotes (10^{11} cells) were harvested and washed as described elsewhere [12]. Plasma membrane vesicles were prepared essentially as reported for the isolation of vesicles from *L. mexicana* [18] and *T. cruzi* [19]. Briefly, after a final wash in a medium comprising

Correspondence: G. Benaim, Centro de Biologia Celular, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47114, Caracas, 1041-A (Venezuela).

400 mM manitol, 10 mM KCl, 1 mM magnesium acetate, 1 mM phenylmethylsulfonyl fluoride, soy bean trypsin inhibitor (0.15 mg/ml) and 10 mM Hepes (pH 7.4), the cell pellet was mixed with acid-washed glass beads (75–120 μM in diameter, Sigma) at a ratio of 1 : 4 (wet weight/weight of beads). The cells were disrupted by abrasion in a chilled mortar until 90% disruption was achieved as determined under an optical microscope. This generally took about 7 min. The glass beads, unbroken cells and large cell debris were removed by centrifuging at 1000 × *g* for 15 min at 4° C. The supernatant was sujected to differential centrifugation, first at 16000 × *g* for 30 min at 4° C and then at 105000 × *g* for 1 h at the same temperature. The resulting pellet was resuspended in about 3 ml of a medium comprising (150 mM KCl, 2 mM $MgCl_2$, 0.01 mM $CaCl_2$, 1 mM dithiothreitol and 75 mM Hepes (pH 7.4). The suspension was then gently passed three times through a Dounce homogenizer (AA Arthur Thomas) immersed in an ice-cold water-bath. In accordance with previous results [18,19], this preparation is highly enriched in plasma membrane vesicles, as inferred from its specific binding of ^{125}I-labeled concanavalin A which was 12 to 14-times enriched with respect to the entire homogenate. This was also enriched in 3′-nucleotidase, a marker enzyme for plasma membranes in these parasites [18], and it was devoided of succinate-cytochrome-*c* oxidoreductase activity, thus indicating the absence of mitochondrial contamination (not shown). In addition, the specific activity of Ca^{2+}-ATPase was 13 to 14-times higher with respect to the homogenate, and copurified with the oligomycin-insensitive Mg^{2+}-ATPase, which is also considered to be another marker enzyme for the plasma membrane of these parasites (see below). This preparation was used in all experiments described in the present work. Calmodulin was purified from bovine brain using a phenyl-Sepharose column as described previously [11].

Determination of ATPase activity. Aliquots of vesicles (about 0.3 mg protein per ml) were incubated in a medium comprising 150 mM KCl, 2 mM $MgCl_2$, 2 mM ATP, 1 mM dithiothreitol, 0.001 mM A23187, 75 mM Hepes (pH 7.2), 1 mM EGTA and the appropiate concentrations of $CaCl_2$ to obtain the desired free Ca^{2+} concentration. Total Ca^{2+} was calculated as described previously [20] taking into account the concentration of ATP and $MgCl_2$ in the media and using the dissociation constant of the Ca^{2+}-EGTA complex reported by Schwarzenbach et al. [21]. After 30 min incubation at 28° C, the reaction was arrested by the addition of 8% (final concentration) trichloroacetic acid. The mixture was centrifuged and the supernatant was kept for inorganic phosphate determination. The latter was carried out according to the method of Fiske and SubbaRow [22], modified by the use ferrous sulfate as reducing agent.

Measurement of calcium transport. Transport assays were carried out essentially as previously described for mitochondrial vesicles from *L. braziliensis* [12]. A medium similar to that employed for determination of the enzymatic activity was used to measure Ca^{2+} uptake, according to one of the following methods. (a) Measuring the changes in the absorbance spectrum of Arsenazo III, using an Aminco DW-2a dual wavelength spectrophotometer. The wavelength pair chosen was 675–685 nm to avoid interference with Mg^{2+} present in the assay medium [12,23]. (b) Measuring the radioactivity taken up after incubation in the presence of 2 nmol of $^{45}Ca^{2+}$ per ml (spec. act. of 100 cpm · pmol^{-1}). The assay was started by addition of the membrane fraction and stopped after various times by rapid filtration through nitrocellulose filters (0.45 μm pore diameter, Millipore). Non-specific absorption was corrected for by subtracting the radioactivity trapped in the filters at time zero [12]. Protein was determined according to Lowry et al. [24], using bovine serum albumin as standard.

Results

Ca^{2+}-ATPase activity is operationally defined as the activity observed over the Mg^{2+}-ATPase when Ca^{2+} ions are added to the assay medium [25,26]. As observed in Fig. 1, plasma membrane vesicles from *Leishmania* hydrolyse an appreciable amount of ATP in the absence of Ca^{2+}. This ATPase activity is probably associated with a proton pump which is present in the plasma membrane and is responsible for secondary transport of glucose and amino acids [27,28]. We now show that in the presence of saturating Mg^{2+} con-

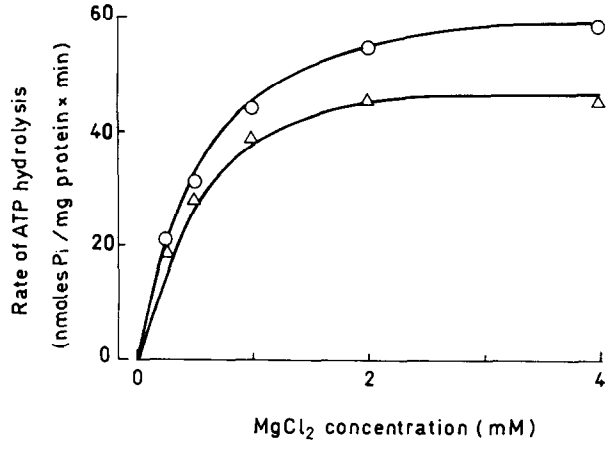

Fig. 1. Effect of Mg^{2+} on Mg^{2+}-ATPase and (Ca^{2+} + Mg^{2+})-ATPase activities of plasma membrane vesicles from *Leishmania*. The assay medium composition was 150 mM KCl, 75 mM Hepes (pH 7.0), 1 mM dithiothreitol, 2 mM ATP, 1 mM EGTA and the indicated $MgCl_2$ concentration. Other conditions were as described under Materials and Methods. △, without Ca^{2+}; ○, with 10 μM free Ca^{2+}.

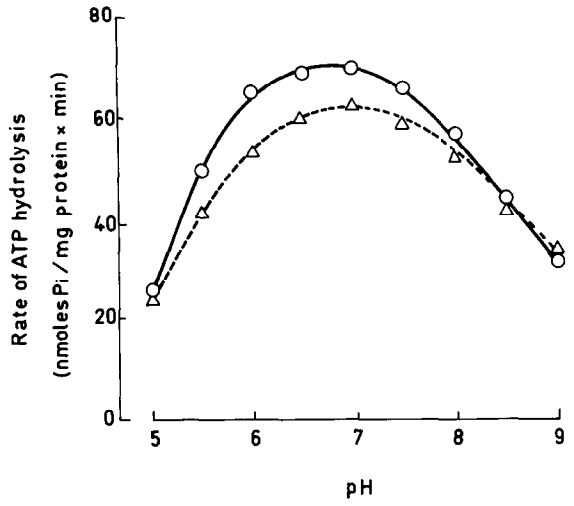

Fig. 2. Effect of pH on Mg^{2+}-ATPase and (Ca^{2+} + Mg^{2+})-ATPase activity of plasma membrane vesicles from *Leishmania*. The assay medium composition and other conditions were as described in Fig. 1, except that the $MgCl_2$ concentration was 2 mM. △, without Ca^{2+}; ○, with 10 μM free Ca^{2+}.

centrations, ATP hydrolysis is further raised by about 20–25% upon addition of micromolar amounts of Ca^{2+}. As in the case of Mg^{2+}-ATPase, maximal Ca^{2+}-ATPase activity is attained when equimolar amounts of MgATP are present in the medium (Fig. 1). This suggests that the complex Mg-ATP is the substrate for the Ca^{2+}-ATPase, as shown for the same enzyme from higher eukaryotic systems [25,26]. The optimal pH of this enzyme is in the physiological range, similar to that of the Mg^{2+}-ATPase (Fig. 2). The Ca^{2+} affinity of the ATPase is very high (Fig. 3), and comparable to other plasma membrane Ca^{2+}-ATPase. Thus, the enzyme becomes saturated with micromolar amounts of Ca^{2+}, reaching a V_{max} of 12 nmol ATP/mg protein per min.

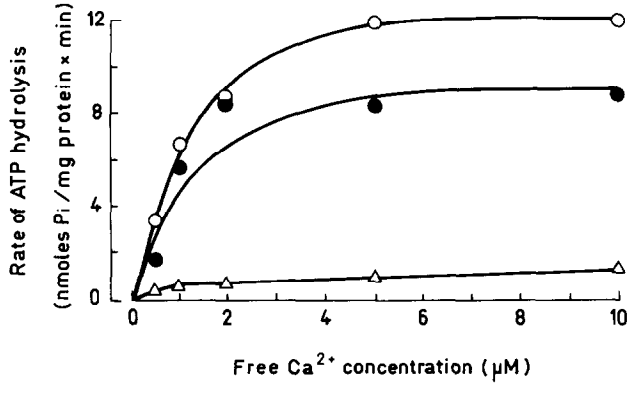

Fig. 3. Activation by Ca^{2+} and inhibition by trifluoperazine. The assay medium composition and other conditions were as described in Fig. 2. Mg^{2+}-ATPase activity was subtracted. Free Ca^{2+} concentrations were as indicates. Vesicles were incubated with trifluoperazine for 5 min prior to start the reaction. ○, without trifluoperazine; ●, with 10 μM trifluoperazine; △, with 100 μM trifluoperazine.

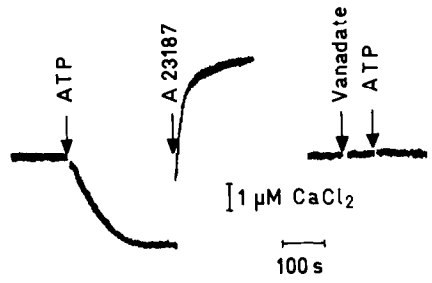

Fig. 4. Ca^{2+} uptake by plasma membrane vesicles from *Leishmania*. Changes in the free Ca^{2+} concentration were followed using Arsenazo III (50 μM) and the wavelength pair 675–685 nm, as described under Materials and Methods. The assay medium composition was as described in Fig. 2, but without EGTA. Arrows indicate successive additions. The A23187 concentration was 5 μM. The vanadate concentration was 20 μM. The vertical bar indicates the increase in absorbance after the addition of 1 μM $CaCl_2$ to the assay medium.

The $K_{m,app}$ is approx. 0.8 μM at optimal Mg^{2+} and ATP concentrations.

When the calmodulin antagonist trifluoperazine (10 μM) is added to the medium, a 30% inhibition of the Ca^{2+}-ATPase activity is elicited (Fig. 3). If the concentration of the drug is raised to 100 μM, the enzyme is almost fully inhibited.

The plasma membrane fraction used in this work is composed of closed vesicles, 0.3 μm in diameter and possessing a high ionic selectivity [18,19]. Here we show that these vesicles are able to accumulate Ca^{2+} against a concentration gradient when ATP is added to the medium. Thus, using Arsenazo III, a rapid Ca^{2+} accumulation was detected which reached steady state after a few minutes (Fig. 4). Ca^{2+}, in fact becomes accumulated within the vesicles as it can be released by A23187. The Ca^{2+} overshoot observed after adding A23187 arises from endogenous Ca^{2+} which was trapped during the fractionation procedure (see Materials and Methods). When vesicles are preincubated with 20 μM vanadate for 5 min before the addition of ATP, Ca^{2+} transport is completely abolished (Fig. 4). The ATPase activity is also inhibited by an identical inhibitor concentration (not shown). However, as reported by others [28], Mg^{2+}-ATPase activity is also completely inhibited.

Under the conditions described in Fig. 4, if the vesicles are loaded with calcium by incubation with ATP, and then 100 mM NaCl is added to the assay medium, no calcium release occurs (not shown). Thus, the presence of a Na^+/Ca^{2+} exchanger in the plasma membrane from these parasites is not supported by these results.

The capability of the vesicles to transport Ca^{2+} against a concentration gradient was tested by a different method. Fig. 5 shows that when $^{45}Ca^{2+}$ is used instead of Arsenazo III, the same time kinetics of Ca^{2+} accumulation is attained. Similarly, the addition of

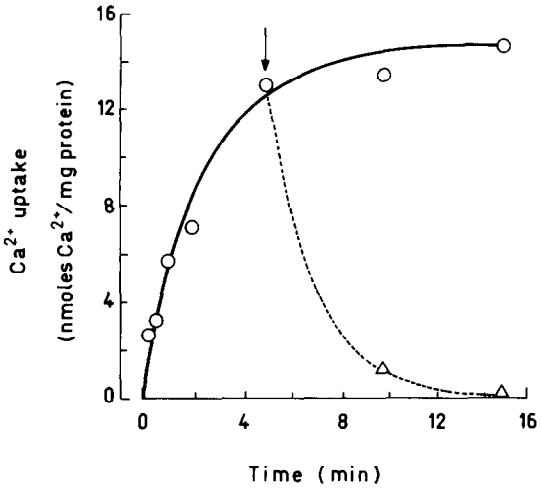

Fig. 5. $^{45}Ca^{2+}$ uptake by plasma membrane vesicles from *Leishmania*. The assay medium composition and other conditions were as described in Fig. 4. Other conditions were as described under Materials and Methods. Each sample contains 2 nmol $^{45}Ca^{2+}$ (100 cpm per pmol). The free Ca^{2+} concentration was adjusted to 10 μM using EGTA [20,21]. The reaction was started by adding 0.5 mM ATP. The arrow indicates the addition of A23187 (5 μM) in a parallel experiment.

A23187 releases Ca^{2+} from the vesicles. The affinity for Ca^{2+} and maximal capacity of the transport system were determined using the above method and were compared to that obtained for the Ca^{2+}-ATPase activity. Fig. 6 shows that the kinetics parameters for Ca^{2+} transport are very similar to those for ATPase. Thus, the $K_{m,app}$ is around 0.7 μM and to V_{max} is 9 nmol $Ca^{2+} \cdot min^{-1} \cdot (mg\ prot)^{-1}$. When vesicles are incubated in the presence of bovine brain calmodulin both activities are stimulated, the maximal velocity being increased by about 70–80% (Table I). Although the degree of stimulation varied among different preparations, it was self-consistent within each preparation. On the other hand, the calmodulin sensitivity was independent of

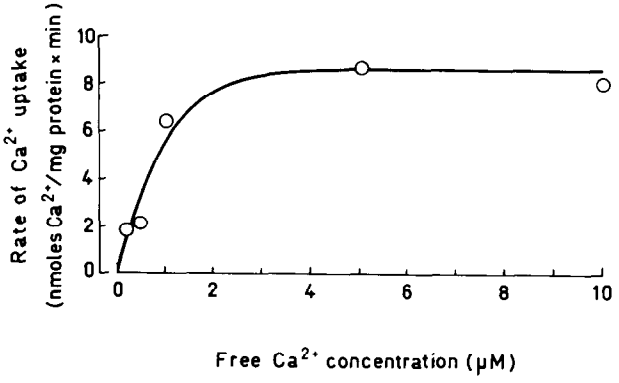

Fig. 6. Effect of Ca^{2+} concentration on the Ca^{2+} transport by plasma membrane vesicles from *Leishmania*. The assay medium composition and other conditions were as described in Fig. 5. The reaction was started by adding 2 mM ATP and arrested after 1 min, as described under Materials and Methods.

TABLE I

Effect of calmodulin on the Ca^{2+}-ATPase activity and Ca^{2+} transport by plasma membrane vesicles from Leishmania

Ca^{2+}-ATPase activity and Ca^{2+} transport were determined as described in the legend to Fig. 3 and 6, respectively. Free Ca^{2+} concentration was 10 μM. Calmodulin, when present, was added to a final concentration of 5 μg per ml. Numbers in parentheses correspond to the stimulation factor for calmodulin (*b/a*). Each value represents the mean ± S.D. deviation of five experiments.

	Control (*a*)	With calmodulin (*b*)	*b/a*
Ca^{2+}-ATPase activity (nmol ATP/mg prot. per min)	11.57 ± 0.58	19.02 ± 1.64	1.65 ± 1.17
Ca^{2+} uptake (nmol Ca^{2+}/mg prot. per min)	9.19 ± 0.43	16.76 ± 2.93	1.85 ± 0.41
Ca^{2+}/ATP stoichiometry	0.80 ± 0.02	0.88 ± 0.15	

whether or not the membrane fraction was previously treated with EDTA during the preparation procedure in order to remove endogenous calmodulin.

Discussion

A number of attempts have been made in the past to show that Ca^{2+}-ATPase is present in the plasma membrane of human protozoan from the family Trypanosomatidae. The difficulties in solving the problem probably arise from the relatively low activity of the Ca^{2+}-ATPase, which is masked by extensive Mg^{2+}-ATPase activity present in the plasma membrane of these parasites [28,29]. The first report on the subject [30], describes an ATPase in *Trypanosoma cruzi* which is stimulated by a rather high Ca^{2+} concentration ($K_{m,app}$ 80 μM). In contrast, another report demonstrates a high affinity Ca^{2+}-ATPase in *T. rhodesiense* [31]. However, Mg^{2+} was ommitted from the assay medium described in these reports. Since Mg^{2+} is a requirement for any plasma membrane transport Ca^{2+}-ATPase [25,26,32], the above enzyme activities cannot be compared with the (Ca^{2+} + Mg^{2+})-ATPase studied in the present work. On the other hand, conflicting evidence has been presented on the presence or absence of a Ca^{2+}-ATPase in the type of parasites described above [33,34]. In addition, none of these controversial reports relate the entity responsible for the Ca^{2+}-ATPase activity to its putative function as a Ca^{2+} transport system. The results presented in this work are the first report of a plasma membrane Ca^{2+} transport system and associated ATPase activity in vesicles from human protozoan parasites. The similarities found between the kinetics

parameters for the ATPase and for the transport activities strongly suggest a common molecular entity. The stoichiometry calculated from this parameters aproaches the 1 : 1 stoichiometry for Ca^{2+} to ATP, as reported for the Ca^{2+} pump from higher eukaryotic cells [35]. The presence of a small fraction of leaky vesicles is likely to account for the small difference found between the V_{max} for transport and that for the Ca^{2+}-ATPase. The general characteristics of the enzyme reported in this study, including both Mg^{2+} and Ca^{2+} requirements, optimal pH and vanadate sensitivity, are essentially identical to those described for Ca^{2+} transport ATPases of higher eukaryotic systems, thus suggesting an E1-E2 type ATPase, which is not different from the plasma membrane Ca^{2+}-ATPase from the host cells [25,26,36]. Although the Ca^{2+}-ATPase from the endoplasmic reticulum shares some general characteristics common to all plasma membrane Ca^{2+}-ATPases, the former is not stimulated by calmodulin [2]. This fact, together with the copurification of the calcium pump with the oligomycin-insensitive Mg^{2+}-ATPase, and the data from other marker enzymes (see above), supports the notion that this Ca^{2+}-ATPase activity arises from plasma membrane and not from endoplasmic reticulum. However, some slight contamination from the latter fraction cannot be ruled out.

The presence of a Na^{+}/Ca^{2+} exchanger, which seems relevant in some mammalian systems [2,37], is not supported by the experiments reported in this work. However, this mechanism seems to be less ubiquitous than the plasma membrane Ca^{2+} pump [2,37]. Since other mechanisms responsible for Ca^{2+} regulation present in these parasites [11,12,14,15] also appear to be similar to those described for higher eukaryotes [2], it can be suggested that intracellular Ca^{2+} homeostasis is well conserved throughout evolution.

An important property of the plasma membrane Ca^{2+} pump from higher eukaryotic cells relates to its activation by calmodulin [38,39]. In the present work, the degree of stimulation obtained by this modulator, albeit been statistically significant, was relatively low and variable depending on the batch of vesicles employed. On the other hand, it is well established that various treatments can substitute for calmodulin in stimulating plasma membrane Ca^{2+}-ATPases. Thus, partial proteolytic degradation [40,41,42], acidic phospholipids [43], enzyme self-association [44] and modification of the water structure surrounding the enzyme [45] can mimic calmodulin. Thus, any of these effects might be partially masking the action of calmodulin on *Leishmania* vesicles. Alternatively, the plasma membrane fraction may not have been totally depleted of endogenous calmodulin, even after pretreatment with EDTA. As shown for other systems, total removal of this protein is not easily achieved [46]. The inhibitory effect of trifluoperazine obtained in this work in the absence of added calmodulin, cannot be taken as evidence for the presence of native calmodulin. Phenothiazine drugs also affect the basal (not calmodulin-stimulated) Ca^{2+}ATPase activity by direct interaction with the enzyme [47,48].

As previously discussed, the cytosolic free Ca^{2+} concentration of intact *Leishmania* cells is about 50 nM [12–14]. Although the mitochondrion might be involved in the transitory removal of large amounts of Ca^{2+} from the cytoplasm [12,14,15], because of its low Ca^{2+} affinity, this transport system is unlikely to regulate this cation to the nanomolar level. Very recently, the endoplasmic reticulum was indicated as a putative Ca^{2+} transport system in these parasites [14,15]. However, the low storage capacity of this system, limited by its size as an intracellular compartment, makes it difficult to asign to this organelle the long-term homeostasis of intracellular Ca^{2+}.

The high affinity of the pump for Ca^{2+} found in this work lends strong support to the view of the plasma membrane Ca^{2+}-ATPase of *Leishmania* as the long-term fine-tuner of the cytoplasmic ionic Ca^{2+} in these cells, as described for higher eukaryotic cells.

Acknowledgement

This work was supported by the Consejo de Desarrollo Científico y Humanistico de la Universidad Central de Venezuela, Grant No. C-03-10-2095-89.

References

1 Rassmusen, H. (1981) Calcium and cAMP as Synarchic Messengers. Wiley Interscience, New York.
2 Carafoli, E. (1987) Annu. Rev. Biochem. 56, 395–433.
3 Dolan, M.T., Reid, C.G. and Voorheis, H.P. (1986) J. Cell. Sci. 80: 123–140.
4 Holwill, M.E.J. and McGregor, J.L. (1976) J. Exp. Biol. 65, 229–242.
5 Voorheis, H.P., Bowles, D.J. and Smith, J.A. (1982) J. Biol. Chem. 257, 2300–2304.
6 Morrow, C.D., Flory-Granger, B. and Krassner, S.M. (1981) Comp. Biochem. Physiol. 69A, 65–72.
7 Voorheis, H.P. and Martin, B.P. (1981) Eur. J. Biochem. 116, 471–477.
8 Tellez-Iñon, M.T., Ulloa, R.M., Torruela, M. and Torres, H.N. (1985) Mol. Biochem. Parasitol. 17, 143–154.
9 Goncalves, M.F., Zingales, B. and Colli, W. (1980) Mol. Biochem. Parasitol. 1, 107–118.
10 Ruben, L., Egwagu, C. and Patton, C.L. (1983) Biochim. Biophys. Acta 758, 104–113.
11 Benaim, G., Szabo, V. and Cornivelli, L. (1987) Acta Cient. Venezol. 38, 289–291.
12 Benaim, G., Bermudez, R. and Urbina, J.A. (1990) Mol. Biochem. Parasitol. 38, 61–68.
13 Cohen, B.E., Benaim, G., Ruiz, M.C. and Michelangeli, F. (1990) FEBS Lett. 259, 286–288.
14 Philosoph, H. and Zilberstein, D. (1989) J. Biol. Chem. 264, 10420–10424.
15 Docampo, R. and Vercesi, A.E. (1989) J. Biol. Chem. 264, 108–111.

16 Docampo, R. and Vercesi, A.E. (1989) Arch. Biochem. Biophys. 272, 122–129.
17 Villalba, E. and Ramirez, J.L. (1982) J. Protozool. 29, 438–441.
18 Cohen, B.E., Ramos, H., Gamargo, M. and Urbina, J.A. (1986) Biochim. Biophys. Acta 260, 57–65.
19 Urbina, J.A., Vivas, J., Ramos, H., Larralde, G., Aguilar, Z. and Avilan, L. (1988) Mol. Biochem. Parasitol. 30, 185–196.
20 Romero, P.J. (1976) J. Membr. Biol. 29, 329–343.
21 Schwarzenbach, G., Senn, H. and Anderegg, G. (1957) Helv. Chim. Acta 40, 1186–1190.
22 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400.
23 Benaim, G., Clark, A. and Carafoli, E. (1986) Cell Calcium 7, 175–186.
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
25 Schatzmann, H.J. (1982) in Membrane Transport of Calcium (E. Carafoli, ed.), pp. 41–108, Academic Press, New York.
26 Rega, A.F. and Garrahan, P. (1986) In The Ca^{2+} pump of Plasma Membranes (A.F. Rega and P.J. Garrahan, eds.), CRC Press.
27 Bonay, P. and Cohen, B.E. (1983) Biochim. Biophys. Acta 731, 222–228.
28 Zilberstein, D. and Dwyer, D.M. (1985) Biochem. J. 256, 13–21.
29 DeSastre, M.B.R. and Stoppani, A.O.M. (1973) FEBS Lett. 31, 137–142.
30 Frash, A.C.C., Segura, E.L., Cazzulo, J.J. and Stoppani, O.M. (1978) Comp. Biochem. Physiol. 60B, 271–275.
31 Mclaughlin, J. (1985) Mol. Biochem. Parasitol. 15, 189–201.
32 Roufogalis, B.D., Akyempon, C.K., Al-Jobore, A. and Minocherhomjee, A.M. (1982) Ann. N.Y. Acad. Sci. 402, 349–367.
33 Bababunmi, E., Emeh, J.K. and Bolaji, O.M. (1982) Ann. N.Y. Acad. Sci. 402, 435–437.
34 Bababunmi, E. (1987) Microbiol. Lett. 36, 71–76.
35 Clark, A. and Carafoli, E. (1983) Cell Calcium 4, 83–88.
36 Carafoli, E., Zurini, M. and Benaim, G. (1985) In Calcium in Biological Systems (R.P. Rubin, G.B. Weiss and J.W. Putney, Jr., eds.), pp. 265–273. Plenum Press, New York.
37 DiPolo, R. and Beaugé, L. (1988) Biochim. Biophys. Acta 947, 549–569.
38 Gopinath, R.M. and Vincenzi, F.F. (1977) Biochem. Biophys. Res. Commun. 77, 1210–1216.
39 Jarret, H.W. and Penniston, J.T. (1977) Biochem. Biophys. Res. Commun. 77, 1210–1216.
40 Sarkadi, B., Enyedi, A. and Gardos, G. (1980) Cell Calcium 1, 287–297.
41 Taverna, R.D. and Hanahan, D.J. (1980) Biochem. Biophys. Res. Commun. 94, 652–659.
42 Benaim, G., Zurini, M. and Carafoli, E. (1984) J. Biol. Chem. 259, 8471–8477.
43 Niggli, V., Adunyah, E.S., Penniston, J.T. and Carafoli, E. (1981) J. Biol. Chem. 256, 395–401.
44 Kosk-Kosicka, D., Bzdega, T. and Wawrzynow, A. (1989) J. Biol. Chem. 264, 19495–19499.
45 Benaim, G. and de Meis, L. (1989) FEBS Lett. 244, 484–486.
46 Klee, C. and Vanaman, T.C. (1982) Adv. Protein. Chem. 34, 213–321.
47 Vincenzi, F.F., Adunyah, E.S., Niggli, V. and Carafoli, E. (1982) Cell Calcium 3, 545–559.
48 Benaim, G. and De Meis, L. (1990) Biochim. Biophys. Acta 1026, 87–92.