

***Trypanosoma cruzi* Epimastigotes Express the Ouabain- and Vanadate-Sensitive (Na⁺+K⁺)ATPase Activity**

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The presence of (Na⁺+K⁺)ATPase activity in CL14 clone and NIH NTY strain of *Trypanosoma cruzi* epimastigotes is demonstrated. A Na⁺ plus K⁺ stimulated ATPase activity is found in both strains. The optimal Na⁺/K⁺ ratio is 5:1 and 9:1 in CL14 clone and NIH NTY strain, respectively. In both strains, vanadate completely inhibits the ouabain-sensitive ATPase activity indicating that it belongs to the P-type (E₁/E₂) family of ion-transporting ATPases. The I₅₀ for vanadate is 0.66 ± 0.04 and 0.04 ± 0.02 μM in CL14 clone and NIH NTY strain, respectively. These data indicate that both strains of *T. cruzi* epimastigotes express the ouabain- and vanadate-sensitive (Na⁺+K⁺)ATPase activity. On the other hand, the discrepancy between the parameters analyzed for the inhibitors suggests that they express different isoforms of this enzyme.

Introduction

Trypanosoma cruzi (*T. cruzi*), the etiologic agent of Chagas disease, has a complex life cycle, involving different developmental stages adapted to the conditions imposed by the insect vector and the mammalian host environments (Chagas, 1909; 1916; 1922). Epimastigotes are dividing forms, insect-borne, which differentiate into trypomastigotes. These are non-dividing, circulating forms that enter the cells and initiate infection in vertebrates. Trypomastigotes differentiate into amastigotes, which can divide intracellularly (Chagas, 1922).

Little is known about transport mechanisms involved in intracellular ion homeostasis during the life cycle of the parasite. Among the mechanisms involved in this process, the primary active transport is the most important transport system, since it has high affinity, high ion selectivity and creates

the electrochemical gradient present across the cell membrane. Several ATPases have been described in different parasitic protozoa such as the H⁺-ATPase which has been the principal primary active transport described in *Leishmania donovani* (Zilberstein and Dwyer, 1988; Anderson and Mukkada, 1994), *Leishmania major* (Vieira *et al.*, 1994; 1995) and *T. cruzi* (Benain *et al.*, 1991; 1995). In *Leishmania major* promastigotes the H⁺ gradient produced by this enzyme is involved in the transport of sugars and amino acids (Vieira and Cabantchik, 1995). Another ATPase activity described in the plasma membrane of different trypanosomatids including *T. cruzi* is the Ca²⁺-ATPase (Benain *et al.*, 1991; 1995).

The (Na⁺+K⁺)ATPase is the plasma membrane enzyme that catalyzes the active transport of 3Na⁺ and 2K⁺ across the cell membrane and it is found in almost all eukaryotic cells (Skou, 1957; Nørby *et al.*, 1983). Although this enzyme has been shown in *Leishmania* (Felibert *et al.*, 1995) and *T. brucei* (Mancini *et al.*, 1982), its presence in *T. cruzi* is subject of controversy. Meirelles and De Souza (1984), using a cytochemical method, were not able to identify a (Na⁺+K⁺)ATPase in *T. cruzi* epimastigotes. However, these authors do not exclude the possibility that a very low activity of this enzyme could be present in *T. cruzi* and not detected

Abbreviations: EDTA, (ethylenediaminetetraacetic acid); Hepes, (N-2-hydroxyethylpiperazine N'-2-ethanesulfonic acid); Pi, orthophosphate; Tris, tris(trishydroxymethyl)-aminomethane; ATP, adenosine triphosphate (magnesium salt).

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by the method used. Frasch *et al.* (Frasch *et al.*, 1978) showed that the ATPase activity of *T. cruzi* was insensitive to ouabain, a specific inhibitor for (Na⁺+K⁺)ATPase, in spite of the fact that living epimastigotes of *T. cruzi* are able to concentrate K⁺ and exclude Na⁺ from the medium. However, the authors observed that the Na⁺ and K⁺ gradient across the cell plasma membrane decreased when the metabolism of the parasite was blocked by the simultaneous addition of deoxyglucose and antimycin A, indicating involvement of an active transport. On the other hand, Oz *et al.* (1992) postulated the presence of (Na⁺+K⁺)ATPase in *T. cruzi* epimastigotes based on the fact that ouabain increases the concentration of intracellular Ca²⁺ when added in the extracellular medium, due to an increase in intracellular Na⁺ and a subsequent decrease in the activity of the Na⁺/Ca²⁺ antiporter.

The objective of the present work was to determine the presence of a ouabain-sensitive (Na⁺+K⁺)ATPase activity among other ATPase activities in two different cell types of *T. cruzi* epimastigotes: CL14 clone and NIH NTY strain. It was shown that both cell types expressed the vanadate and ouabain-sensitive, (Na⁺+K⁺)ATPase activity.

Material and Methods

Chemicals

ATP (magnesium salt), ouabain, sodium orthovanadate, Hepes, Tris, sodium azide, oligomycin and sodium deoxycholate were purchased from Sigma Chemical Co (St. Louis, MO). All chemical reagents were of the highest purity available. [³²P]Pi was obtained from the Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brazil). [^γ-³²P]ATP was prepared as described by Maia *et al.* (1983).

Cell cultures

T. cruzi (epimastigotes) from NIH NTY (a gift from Dr. G. Cross, Rockefeller University, NY) and CL strain CL14 clone (a gift from Dr. Egler Chiari, Federal University of Minas Gerais, MG, Brazil), were cultivated in LIT medium (Chiari, 1985), as previously described (Rondinelli *et al.*, 1988).

Cell Preparations

The cells were counted in a hemocytometer and washed four times in 0.5 M Hepes-Tris (pH 7.0) in the absence of Na⁺ and K⁺. The cell lysates were prepared by pre-incubation for 30 minutes in a solution containing: EDTA 1 mM, deoxycholate (DOC) 0.1% (w/v) and sufficient cells to give 6 mg·ml⁻¹ protein (3 × 10⁹ cells·ml⁻¹). After this solubilization step the hydrolytic activity was assayed by addition of cell lysates (0.2 ml; 5% of the total volume of reaction medium) to give a final protein concentration of 0.3 mg·ml⁻¹. The Na⁺ contamination from the sodium deoxycholate and EDTA tetrasodium salt was 0.12 mM and 0.2 mM, respectively.

Measurement of ATPase activity

Except when noted, standard assay medium (0.2 ml) contained: 10 mM MgCl₂, 5 mM [^γ-³²P]ATP, 20 mM Hepes-Tris (pH 7.0), 2 μg·ml⁻¹ oligomycin and requisite amounts of Na⁺ and/or K⁺. In any case, the total concentration of Na⁺ plus K⁺ was always kept at 150 mM in order to maintain constant the ionic strength.

ATPase activity was measured using the method described by Grubmeyer and Penefsky (1981). The reaction was started by the addition of cell lysates (final protein concentration, 0.3 mg·ml⁻¹), and stopped after 30 min by the addition of 2 volumes of activated charcoal in 0.1 N HCl. The [³²P]Pi released was measured in an aliquot of the supernatant obtained after centrifugation of the charcoal suspension for 20 min at 1,500×g in a clinical centrifuge. Spontaneous hydrolysis of [^γ-³²P]ATP was measured in tubes run in parallel in which the enzyme was added after the acid. Protein concentrations were determined using the Bradford method (Bradford, 1958) with bovine serum albumin as a standard.

Statistical analysis

The data were analyzed by two-way analysis of variance (ANOVA), considering as factors the treatments. The magnitude of the differences were verified "a posteriori" by the Bonferroni's test. In all cases the considered level of significance was less than 0.05. Statistical comparisons for each experimental group are shown in the legends of the

Figures and Table. The experiments were made in duplicate using different cell cultures. When the data were expressed as percentage of the control values, the statistical test was applied to the absolute data.

Results

The principal characteristic of the (Na⁺+K⁺)ATPase is its dependence on Na⁺ and K⁺ (Skou, 1957; Nørby *et al.*, 1983). As shown in Table I, the addition of 120 mM Na⁺ and 30 mM K⁺ significantly increase the ATPase activity from 34.4 ± 4.9 and 42.4 ± 4.2 to 51.6 ± 5.5 and 58.2 ± 2.5 nmol Pi x mg⁻¹ x min⁻¹ in CL14 clone and NIH NTY strains, respectively. The effect of K⁺ was completely abolished by 2 mM ouabain, the well known inhibitor of the (Na⁺+K⁺)ATPase (Skou, 1957; Nørby *et al.*, 1983; Blanco *et al.*, 1995; Akera *et al.*, 1985). The K⁺ stimulated ATPase activity (in the presence of the 120 mM Na⁺) was identical to the ouabain-sensitive ATPase activity.

Table I. ATPase activities in *T. cruzi* epimastigotes.

Condition	ATPase activity nmol Pi x mg ⁻¹ x min ⁻¹	
	CL14 clone	NIH NTY
a. Mg ²⁺	34.4 ± 4.9	42.4 ± 4.2
b. Mg ²⁺ + Na ⁺	46.8 ± 4.3	54.6 ± 2.0
c. Mg ²⁺ + Na ⁺ + K ⁺	51.6 ± 5.5*	58.2 ± 2.5*
d. Mg ²⁺ + Na ⁺ + K ⁺ + ouabain	43.5 ± 4.0†	53.7 ± 3.5*
e. (c - b)	4.9 ± 0.1	5.2 ± 1.2
f. (c - d)	4.9 ± 0.8	4.3 ± 0.9
g. (d - b)	-1.5 ± 1.3	0.3 ± 0.7

Before the ATPase assays, the cells were treated with 0.1% (w/v) deoxycholate and 1 mM EDTA for 30 minutes at room temperature (see Material and Methods). All assays were carried out in duplicates in the presence of 10 mM MgCl₂, 5 mM ATP (as magnesium salt), 20 mM Hepes-Tris pH 7.0, 2 µg.ml⁻¹ oligomycin and when indicated 120 mM Na⁺ (as NaCl), 30 mM K⁺ (as KCl) and 2 mM ouabain. The differences were calculated by paired data. The data are expressed as means ± SEM (*n* = 20). *e* is the difference between the ATPase activity in the presence and in the absence of K⁺, both in the presence of Na⁺; *f* is the difference between the ATPase activity in the absence and in the presence of ouabain, both in the presence of Na⁺ and K⁺; *g* is the difference between the ATPase activity in the presence of K⁺ and ouabain and in the absence of K⁺ and ouabain, both in the presence of Na⁺. *Statistically significant when compared to ATPase activity in the presence of Mg²⁺ alone and to that measured in the presence of Mg²⁺ plus Na⁺. †Not statistically significant when compared to ATPase activity in the presence of Mg²⁺ plus Na⁺.

The addition of 2 mM ouabain in the absence of Na⁺ and K⁺ did not change the ATPase activity (data not shown).

In both strains approximately 10% (*p* < 0.05) of the total ATPase activity is inhibited by ouabain in both strains. The maximum inhibition was attained at 1 mM (data not shown). This is a reasonable and expected result, since this parasite expresses other ATPase activities.

Figure 1 shows the relation between the (Na⁺+K⁺)ATPase activity and different concentrations of Na⁺ and K⁺. The total Na⁺ plus K⁺ concentrations were maintained constant at 150 mM. The optimal Na⁺/K⁺ ratio for ATPase activity was

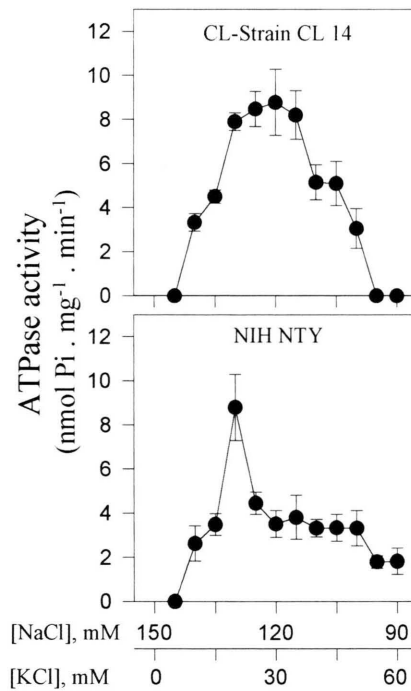


Fig. 1. Effect of different concentrations of Na⁺ and K⁺ on the ATPase activity in *T. cruzi* epimastigotes. ATPase activity was measured as described under Material and Methods. Before the ATPase assays, the cells were treated with 0.1% deoxycholate (w/v) and 1 mM EDTA for 30 minutes at room temperature. All assays were carried out in the presence of 10 mM MgCl₂, 5 mM ATP (as magnesium salt), 20 mM Hepes-Tris pH 7.0, 2 µg.ml⁻¹ oligomycin and indicated Na⁺ (as NaCl) and K⁺ (as KCl) concentrations. The Na⁺ plus K⁺ concentrations were kept constant (150 mM). The data (mean ± SE) correspond to the difference in assays performed in parallel in the absence or presence of 2 mM ouabain. All the experiments were done in duplicates (*n* = 10).

5:1 and 9:1 for the CL14 clone and NIH NTY strains, respectively. The ATPase activity at each experimental point was calculated as the difference between the ATPase activity at different concentrations of Na⁺ and K⁺ plus 10 mM MgCl₂ and that measured in the presence of 150 mM Na⁺ plus 10 mM MgCl₂. The maximal (Na⁺+K⁺)ATPase activity was 8.8 ± 1.6 and 8.7 ± 1.5 nmol Pi·mg⁻¹·min⁻¹ in the CL14 clone and NIH NTY strain, respectively (Fig. 2).

The (Na⁺+K⁺)ATPase is a P-ATPase since it is able to form phosphorylated intermediates during the catalytic cycle (Møller *et al.*, 1996). This class of enzymes is characterized by sensitivity to vanadate inhibition. Fig. 2 shows the dose-response curve for vanadate of the ouabain-sensitive ATPase activity in epimastigotes. In both strains,

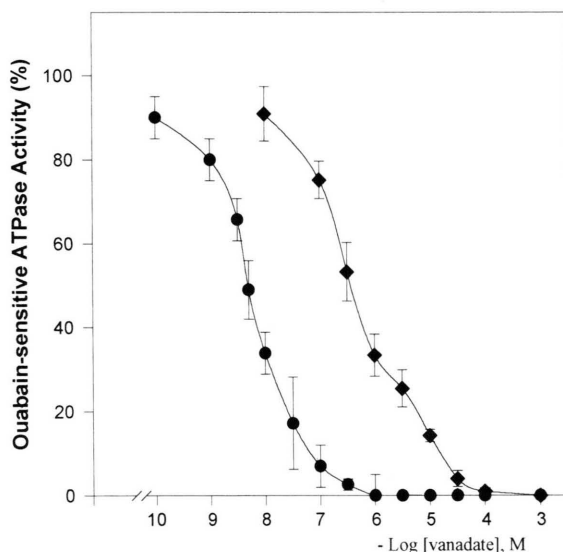


Fig. 2. Vanadate concentration dependence of the ouabain-sensitive ATPase activity in *T. cruzi* epimastigotes. ATPase activity was measured as described under Material and Methods. Before the ATPase assays, the cells were treated with 0.1% deoxycholate (w/v) and 1 mM EDTA for 30 minutes at room temperature. All assays were carried out in the presence of 10 mM Mg²⁺ (as MgCl₂), 5 mM ATP (as magnesium salt), 20 mM HEPES-Tris pH 7.0, 2 µg·ml⁻¹ oligomycin, 120 mM Na⁺ (as NaCl) and 30 mM K⁺ (as KCl). The ouabain-sensitive ATPase activity was calculated as the difference between the ATPase activity in the absence and in the presence of 2 mM ouabain. The vanadate concentration was increased from 10⁻¹⁰ to 10⁻³ M. The data are expressed as percentage of the control. All the experiments were done in triplicates (*n* = 6). Symbols: ● – NIH NTY cells; ◆ – CL14 clone.

the increase in vanadate concentration from 10⁻¹⁰ up to 10⁻⁴ M completely inhibited the ouabain-sensitive ATPase activity. These data indicate that the ouabain-sensitive, (Na⁺+K⁺)ATPase activity expressed by both strains is a P-ATPase type. However, the affinity for vanadate was significantly different among the strains. The concentration of vanadate that promoted half-maximal inhibition (I₅₀) was 0.66 ± 0.20 and 0.04 ± 0.02 µM for CL14 clone and NIH NTY strain, respectively.

Discussion

In this paper we show that CL14 clone and NIH NTY strains express (Na⁺+K⁺)ATPase activity. The characterization of the ATPase activities in *T. cruzi* is a very difficult process due to the presence of a very high Mg²⁺-ATPase activity which could mask the presence of the (Na⁺+K⁺)ATPase with the Fiske & Subarow method (Fiske and Subarow, 1925). The Mg²⁺-ATPase activity in both strains ranged 34–42 nmol Pi·mg⁻¹·min⁻¹ (Table I). Therefore, in this paper we used the more sensitive method in which [γ-³²P]ATP is used as a substrate (Grubmeyer and Pnifsky, 1981). This method has been used to determine low ATPase activities in different cell types, such as the Ca²⁺-ATPase activity of plasma membrane from proximal tubule cells, even in the presence of high Mg²⁺-ATPase activity (Vieyra *et al.*, 1991).

In spite of the Na⁺ and K⁺ gradient present across the plasma membrane no effect of ouabain has been observed neither in this gradient nor in the ATPase activity of *T. cruzi* epimastigotes (Frasch *et al.*, 1978). The data shown in Fig. 1 and Table I clearly indicate the presence of a ouabain-sensitive, (Na⁺+K⁺)ATPase activity in *T. cruzi* epimastigotes. The possibility that the K⁺ stimulation on the ATPase activity in the presence of 120 mM Na⁺ could be catalyzed by an enzyme other than the (Na⁺+K⁺)ATPase is ruled out since the addition of K⁺ alone did not change the ATPase activity (data not shown). Although we have worked with total cell membranes, the (Na⁺+K⁺)ATPase is located only in the plasma membrane (Skou, 1957). Therefore, it can be concluded that the (Na⁺+K⁺)ATPase activity observed in *T. cruzi* (Table I) is also located in the plasma membrane of the parasite. The absence of an inhibitory effect of ouabain reported by others (Meirelles and De

Souza, 1984) could be explained by the presence of a dense extracellular matrix composed by glycoconjugates in *T. cruzi* epimastigotes called glycoinositolphospholipids (GIPL), which could block the effect of ouabain (Felibertt *et al.*, 1995; Prevato *et al.*, 1995). It is noteworthy that the Kinetoplastida parasites have a strong subpellicular microtubule network, which is not easily removed. Treatment with 0.1% deoxycholate seems to be effective in the removal of the GIPL (Carreira *et al.*, 1996) and may be the reason for the inhibitory effect of ouabain observed in the present work.

In most tissues the affinity for ouabain is in the micromolar range, but other preparations such as rat proximal tubule, show a millimolar affinity for this drug (Sweadner, 1989; Tobin and Brody, 1972). The sensitivity of the (Na⁺+K⁺)ATPase for ouabain depends on the isoform expressed by the cells and is also tissue specific (Aker *et al.*, 1985). Recently, Felibertt *et al.* (1995) showed that the I_{50} of the (Na⁺+K⁺)ATPase for ouabain is 0.21 mM in *Leishmania mexicana*. We also observed differences in affinity for ouabain among the strains studied (Fig. 1), but both are in the millimolar range. So we suggest that both strains of *T. cruzi* epimastigotes express the (Na⁺+K⁺)ATPase isoform(s) with low affinity for ouabain.

We observed that the optimal Na⁺/K⁺ ratio is 5:1 and 9:1 for CL14 clone and NIH NTY strains, respectively (Fig. 2). It is well known the competition of K⁺ for the internal Na⁺ binding site in (Na⁺+K⁺)ATPase of the different tissues (Horisberger *et al.*, 1991). So it is possible to postulate that the competition of K⁺ for Na⁺ binding site is higher in NIH NTY strain than CL14 clone and may reflect structural differences in the cation binding sites. Felibertt *et al.* (1995) observed that the optimal Na⁺/K⁺ ratio for (Na⁺+K⁺)ATPase activity in isolated plasma membrane of *Leishmania mexicana* was 6.5/1.

Differences in vanadate sensitivity was also observed (0.66 ± 0.04 and $0.04 \pm 0.02 \mu\text{M}$ in CL14 clone and NIH NTY, respectively). P-ATPases type are also referred to as E₁/E₂-ATPases since they are phosphorylated by ATP or Pi in each main conformational state, respectively (Møller *et al.*, 1996). The vanadate binding in the P-ATPase occurs in E₂ conformation, leading to formation of a transition state complex with the ATPase (Møller *et al.*, 1996). Since the catalytic domain is more hydrophobic in the E₂ conformation (Jorgensen, 1992), the differences observed in the affinity for vanadate in CL14 clone and NIH NTY could be due to differences in water access to the catalytic site. Two vanadate sites have been found in the (Na⁺+K⁺)ATPase isolated from dog kidney: one of high affinity ($I_{50} = 4 \text{ nM}$) and one of low affinity ($I_{50} = 0.5 \mu\text{M}$) (Cantley *et al.*, 1978). It may be that solubilization of (Na⁺+K⁺)ATPase exposes different vanadate sites in each cell type of *T. cruzi* assayed in this work. Finally, we conclude that the gradients of Na⁺ and K⁺ across plasma membrane observed in epimastigote *T. cruzi* (Frasch *et al.*, 1978) are created by the operation of the ouabain-sensitive (Na⁺+K⁺)ATPase found in the present work.

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Anderson A. S. and Mukkada A. J. (1994), Biochemical and immunological characterization of a P-type ATPase from *Leishmania donovani* promastigotes plasma membrane. *Biochim. Biophys. Acta* **1195**, 81–88.

Aker *et al.*, Yuk-Chow N. G., Shieh I. S., Bero E., Brody T. M. and Braselton W. E. (1985), Effects of K⁺ on the interaction between cardiac glycosides and Na⁺, K⁺ – ATPase. *Eur. J. Pharmacol.* **111**, 147–157.

- Benaïm G., Losada S., Gadelha F. R. and Docampo R. (1991), A calmodulin-activated (Ca²⁺+Mg²⁺)ATPase is involved in Ca²⁺ transport by plasma membrane vesicles from *Trypanosoma cruzi*. *Biochem. J.* **280**, 715–720.
- Benaïm G., Moreno S. N. J., Hutchinson G., Cervino V., Hermoso T., Romero P. J., Ruiz F., de Souza W., Docampo R. (1995), Characterization of the plasma-membrane calcium pump from *Trypanosoma cruzi*. *Biochem. J.* **306**, 299–303.
- Blanco G., Sánchez G. and Mercer R. W. (1995), Comparison of the enzymatic properties of the Na, K-ATPase $\alpha 3\beta 1$ and $\alpha 3\beta 2$ isozymes. *Biochemistry* **34**, 9897–9903.
- Bradford M. M. (1958), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal. Biochem.* **72**, 248–254.
- Cantley L. C. Jr., Cantley L. G. and Josephson L. (1978), A characterization of vanadate interactions with (Na, K)ATPase. *J. Biol. Chem.* **253**, 7361–7368.
- Carreira J. C., Jones C., Wait R., Previato J. O. and Mendonça-Previato L. (1996), Structural variation in the glycoinositolphospholipids of different strains of *Trypanosoma cruzi*. *Glycoconj. J.* **13**, 955–966.
- Chagas C. (1909), Nova tripanosomíase humana. Estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen. nsp. agente etiológico de nova morbidade do homem. *Memórias do Instituto Oswaldo Cruz* **1**, 159–218.
- Chagas C. (1916), Tripanosomíase americana. Forma aguda da Moléstia. *Memórias do Instituto Oswaldo Cruz* **8**, 37–52.
- Chagas C. (1922), Descoberta do *Trypanosoma cruzi* e verificação da tripanosomíase americana. *Memórias do Instituto Oswaldo Cruz* **15**, 67–81.
- Chiari E. (1985), Diferenciação do *Trypanosoma cruzi* em cultura. Tese de Doutorado, Universidade Federal de Minas Gerais (UFMG).
- Feliberto P., Bermúdez R., Caryno V., Dawidowicz K., Dagger F., Provérbio T., Marín R. and Benaïm G. (1995), Ouabain-sensitive Na⁺, K⁺-ATPase in the plasma membrane of *Leishmania mexicana*. *Mol. Biochem. Parasitol.* **74**, 179–187.
- Fiske C. H. and SubbaRow Y. (1925), The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**, 375–400.
- Frasch A. C. C., Segura E. L., Cazzulo J. J. and Stoppani A. O. M. (1978), Adenosine triphosphatase activities in *Trypanosoma cruzi*. *Comp. Biochem. Physiol.* **60B**, 271–275.
- Grubmeyer C. and Penefsky H. S. (1981), The presence of two hydrolytic sites on beef heart mitochondrial adenosine triphosphatase. *J. Biol. Chem.* **256**, 3718–3727.
- Horisberger J. D., Lemas V., Kraehenbuhl J. P. and Rosier B. C. (1991), Structure-function relationship of Na,K-ATPase. *Annu. Rev. Physiol.* **53**, 565–584.
- Jørgensen P. L. (1992), Na,K-ATPase, structure and transport mechanism. In: *Molecular aspects of transport proteins*. Elsevier Science Publishers BV, 1–26.
- Maia J. C. C., Gomes S. L. and Juliano M. H. (1983), Preparation of (gamma-³²P)- and (alfa-³²P)-nucleoside triphosphates with high specific activity. In: C. M. Morel (ed.) *Genes and antigens of parasites: a laboratory manual*. Editora Fundação Oswaldo Cruz, Rio de Janeiro, 146–167.
- Mancini P. A., Stricker J. E. and Patton C. L. (1982), Identification and partial characterization of plasma membrane polypeptides of *Trypanosoma brucei*. *Biochim. Biophys. Acta* **688**, 399–410.
- Meirelles M. N. L. and De Souza W. (1984), Localization of a Mg²⁺-activated ATPase in the plasma membrane of *Trypanosoma cruzi*. *J. Protozool.* **31**, 135–140.
- Møller J. V., Juul B. and Le Maire M. (1996), Structural organization, ion transport, and energy transduction of P-type ATPases. *Biochim. Biophys. Acta* **1286**, 1–51.
- Nørby J. G., Klodos I. and Christiansen N. O. (1983), Kinetics of Na-ATPase activity by the Na,K pump. *J. Gen. Physiol.* **82**, 725–759.
- Oz H. S., Wittner M., Tanowitz H. B., Bilezikian J. P., Saxon M. and Morris A. S. (1992), *Trypanosoma cruzi*: Mechanism of intracellular calcium homeostasis. *Exp. Parasitol.* **74**, 390–399.
- Previato J. O., Jones C., Xavier M. T., Wait R., Travassos L. R., Parodi A. J. and Mendonça-Previato L. (1995), Structural characterization of the major glycosylphosphatidylinositol membrane-anchored glycoprotein from epimastigote forms of *Trypanosoma cruzi* Y-strain. *J. Biol. Chem.* **270**, 7241–7250.
- Rondinelli E., Silva R., Carvalho J. F. O., Soares C. M. A., Carvalho E. P. and Castro F. T. (1988), *Trypanosoma cruzi*: an *in vitro* cycle of cell differentiation in axenic culture. *Exp. Parasitol.* **66**, 197–203.
- Skou J. C. (1957), The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta* **23**, 394–401.
- Sweadner K. J. (1989), Isozymes of the Na⁺/K⁺-ATPase. *Biochim. Biophys. Acta* **988**, 185–220.
- Tobin T. and Brody T. M. (1972), Rates of dissociation of enzyme-ouabain complexes and *K_{0.5}* values in (Na⁺+K⁺) adenosine triphosphatase from different species. *Biochem. Pharmacol.* **21**, 1553–1560.
- Vieira L., Lavan A., Dagger F. and Cabantchik Z. I. (1994), The role of anions in pH regulation of *Leishmania major* promastigotes. *J. Biol. Chem.* **269**, 16254–16259.
- Vieira L., Slotki I. and Cabantchik Z. I. (1995), Chloride conductive pathways which support electrogenic H⁺ pumping by *Leishmania major* promastigotes. *J. Biol. Chem.* **270**, 5299–5304.
- Vieira L. and Cabantchik Z. I. (1995), Amino acid uptake and intracellular accumulation in *Leishmania major* promastigotes are largely determined by an H⁺ – pump generated membrane potential. *Mol. Biochem. Parasitol.* **75**, 15–23.
- Vieira A., Caruso-Neves C. and Meyer-Fernandes J. R. (1991), ATP \rightleftharpoons ³²P exchange catalyzed by plasma membrane Ca²⁺-ATPase from kidney proximal tubules. *J. Biol. Chem.* **266**, 10324–10330.
- Zilberstein D. and Dwyer D. (1988), Identification of a surface membrane proton translocating ATPase in promastigotes of the parasitic protozoan *Leishmania donovani*. *Biochem. J.* **256**, 13–21.