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Ecto-ATPase activity on the surface of *Trypanosoma cruzi* and its possible role in the parasite–host cell interaction

Abstract This study describes the possible role of Mg^{2+}-dependent ecto-ATPase activity on the *Trypanosoma cruzi*–host cell interaction. Mg^{2+}-dependent ecto-ATPase activity is observed on the cell body and flagellar membranes of the parasite and is about 20 times greater in trypomastigotes, as compared with epimastigotes. Suramin (a competitive antagonist of P2 receptors) and the impermeant agent 4,4′-diisothiocy-anostylbene 2′,2′-disulfonic acid (DIDS), both inhibitors of ecto-ATPases, strongly inhibited ATPase activity and the adhesion and internalization of both evolutive forms by mouse resident macrophages. Suramin inhibited the growth of epimastigotes, suggesting a direct participation of ecto-ATPase activity in this process. To overcome the presence of suramin in the culture medium during the time of growth, Mg^{2+} ecto-ATPase activity was enhanced 4-fold, as compared with control parasites. The over-expression in enzyme activity was followed by a dramatic increase in the adhesion of epimastigotes to resident macrophages above the level observed for non-treated parasites.

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Introduction

Trypanosoma cruzi is the etiological agent of Chagas′ disease, a chronic debilitating disease highly prevalent in Latin America, with no immunoprophylactic agents available. The life cycle of *T. cruzi* takes place in vertebrate and invertebrate hosts. Transmission to vertebrates is carried out by insects belonging to the Reduviidae family, through feces contaminated with an infective stage of the parasite, called the metacyclic trypomastigote. In the vertebrate host, *T. cruzi* is an obligate intracellular parasite that, to complete its life cycle in different nucleated cell types, must gain access to the cytoplasm of the host cell. The invasion process can be divided into three phases: parasite attachment to the plasma membrane of the host cell, internalization via the formation of a host cell-derived parasitophorous vacuole and disruption of the vacuolar membrane so that the parasite reaches the cytoplasm and replicates into amastigote forms (De Souza 1984). Attachment of the parasite is an energy-dependent process that also requires the presence of specific surface molecules (Schenkman et al. 1991a, 1991b, 1991c). Besides being an energy-dependent process, internalization of *T. cruzi* is dependent on the presence of specific glycoproteins (Abuin et al. 1989; Ouaissi et al. 1990; Yoshida et al. 1990; Ortega-Barria and Pereira 1991), mucins (Acosta-Serrano et al, 2001), protein kinase activities (Vieira et al. 1994; Yoshida et al. 2000) and a transient increase in the Ca^{2+} concentration in the parasite and host cells (Moreno et al. 1994; Burleigh and Andrews 1998).

Internalization of *T. cruzi* also depends on enzymes whose active sites face the external medium rather than the cytoplasm (Souto-Padrón et al. 1990; Meir-elles et al. 1992; Colli 1993; Schenkman and Eichinger

1993; Schenkman et al. 1994; Scharfstein et al. 2000). The activities of these enzymes, referred to as ecto-enzymes, can be measured using intact cells (Furuya et al. 1998; Meyer-Fernandes et al. 2000). Some examples are *trans*-sialidase (Colli 1993; Ming et al. 1993; Vieira et al. 1994; Burleigh and Andrews 1998; Yoshida et al. 2000), cysteine proteinase (Souto-Padrón et al. 1990; Meirelles et al. 1992; Colli 1993; Schenkman and Eichinger 1993; Moreno et al. 1994; Scharfstein et al. 2000) and the protein tyrosine phosphatase (Zhong et al. 1998) of *T. cruzi*. Different functions in host-cell infection have been attributed to these enzymes. More recently, considerable progress was achieved in the study of cell membrane ecto-ATPases (Plesner 1995; Dombrowski et al. 1998). They hydrolyze extracellular nucleoside tri-and/or diphosphates such as ATP which, in contrast to the established view, can be found in significant concentrations outside the cells (Dombrowski et al. 1998). Extracellular ATP mediates diverse effects by interacting with P2 receptors (purine, pyrimidine receptors) and several hypothesis about putative ecto-ATPase functions have been proposed, such as: (1) regulation of P2 receptors, neurotransmission and signal transduction (Dubyak and El-Moatassim 1993), (2) involvement in cellular adhesion and cancer metastasis (Aurivillius et al. 1990; Dzhandzhugazyan and Bock 1993; Dzhandzhugazyan et al. 1998), (3) modulation of apoptosis (Zheng et al. 1991) and (4) regulation of NO (Yagi et al. 1994) and the production of reactive oxygen radicals (Nakanishi et al. 1991). Filippini et al. (1990) showed that ATP can kill different cells, with the exception of those that express a high level of ATP-breakdown activity on their surfaces.

Ecto-ATPase activities were identified in different cells and tissues from different animals (Plesner 1995) and on the cell surface of protozoan parasites from the genera *Toxoplasma* (Nakaar et al. 1998), *Leishmania* (Meyer-Fernandes et al. 1997; Berrêdo-Pinho et al. 2001; Peres-Sampaio et al. 2001), *Entamoeba* (Barros et al. 2000), *Trichomonas* (Jesus et al. 2002) and *Crithidia* (Lemos et al. 2002). In some of these parasites, the ecto-ATPase activity is associated with virulence and the evasion of parasites from the host defense mechanisms (Barros et al. 2000; Berrêdo-Pinho et al. 2001).

In this paper, we present evidence for a correlation between the extracellular ATP hydrolysis catalyzed by an ecto-ATPase and the adhesion and internalization of *T. cruzi* by resident macrophages.

Materials and methods

Reagents

All reagents were purchased from E. Merck (Darmstadt, Germany) or Sigma Chemical Co. (St Louis, MO). $[\gamma^{32}P]$ ATP was prepared as described by Glynn and Chappell (1964). Deionized water (MilliQ system of resins; Millipore Corp., Belford, Mass.) was used in the preparation of all solutions.

Parasites

The Y strain of *Trypanosoma cruzi* was used throughout this study. It was isolated from an acute case of Chagas' disease (Silva and Nussenzweig 1953) and exhibited an in vivo tropism for mouse macrophages. Epimastigote forms were maintained at 28 °C in LIT medium (Camargo 1964) supplemented with 10% fetal calf serum (FCS) and used on cultivation days 1–3. Parasites were collected by centrifugation 1,000 g for 10 min and washed once with 10 mM phosphate-buffered saline (PBS). For the isolation of trypomastigote forms, LLC-MK 2 cells were infected with tissue culture trypomastigotes. After 5–6 days, the supernatant was collected, centrifuged at 500 g for 5 min and allowed to stand at 37 °C for 30 min. During this period, the trypomastigotes in the pellet moved into the supernatant medium which was then collected and centrifuged at 1,000 g for 10 min. Cellular viability was assessed, before and after incubation, by motility and trypan blue dye exclusion (Dutra et al. 2001).

DIDS, suramin and ATP treatments

Epimastigotes were grown for 1–7 days in the absence or presence of 500 µM suramin. About 1×10^6 cell/ml were incubated per tube and the cellular growth was estimated daily by counting parasites in a Neubauer chamber.

Epimastigote and trypomastigote forms were submitted to treatment with 500 µM DIDS or suramin for 1 h before interaction with resident macrophages. Trypomastigote–host cell interaction was also analyzed in the presence of 50, 100 and 200 µM ATP. Cellular viability was assessed, before and after incubation, by motility and trypan blue exclusion (Dutra et al. 2001).

Macrophages

Peritoneal macrophages from normal 6- to 8-week-old male Swiss mice were collected in Hanks' balanced salt solution and plated onto glass coverslips in 24-well tissue culture plates (Falcon; Becton Dickinson Labware, New Jersey, N.J.). The cells were allowed to adhere for 30 min at 37 °C in a 5% CO$_2$ atmosphere, after which the non-adhering cells were removed and fresh culture medium (RPMI 1640 plus 10% FCS) was added. Adhered cells were then incubated overnight under the same conditions as above before the interaction assays.

Ecto-ATPase activity measurements

Intact parasites were incubated for 1 h at 30 °C in 500 µl of a mixture containing, unless otherwise specified, 116 mM NaCl, 5.4 mM KCl, 5.5 mM D-glucose, 50 mM Hepes-Tris buffer (pH 7.2), 5 mM ATP and 1.0×10^8 cells/ml in the absence or presence of 5 mM MgCl$_2$. Mg^{2+}-dependent ecto-ATPase activity was calculated from the total activity, measured in the presence of 5 mM MgCl$_2$, minus the basal activity, measured in the absence of MgCl$_2$. ATPase activity was determined by measuring the hydrolysis of $[\gamma^{-32}P]$ATP (10^4 Bq/nmol ATP; Lemos et al. 2000). The experiments were started by the addition of living cells and terminated by the addition of 1 ml of a cold mixture containing 200 mg charcoal in 1 M HCl. The tubes were then centrifuged at 1,500 g for 10 min at 4 °C. Aliquots (500 µl) of the supernatants containing the released inorganic phosphate ($^{32}P_i$) were transferred to scintillation vials containing 9 ml of scintillation fluid (2 g PPO in 1 l of toluene). ATPase activity was calculated by

subtracting the non-specific ATP hydrolysis measured in the absence of cells. ATP hydrolysis was linear with time under the assay conditions used and was proportional to cell numbers. Using other nucleotides, the hydrolytic activities measured under the same conditions described above were assayed spectrophotometrically by measuring the release of P_i from the nucleotides (Lowry and Lopes 1946). The values obtained for ATPase activities measured using both methods (spectrophotometric, radioactive) were exactly the same.

Phosphatase measurements

In addition to the measurements of ecto-ATPase activity, ecto-p-nitrophenylphosphatase activity was determined in the same medium as that for ATP hydrolysis, except that ATP was replaced by 5 mM p-nitrophenylphosphate. The reaction was determined spectrophotometrically at 425 nm, using a molar extinction coefficient of $\epsilon = 14.3 \times 10^3$ (Meyer-Fernandes et al. 1999).

Parasite–macrophage interaction

Parasites and macrophages were left in contact at 37 °C in a parasite–macrophage ratio of 20:1 in RPMI 1640 medium. A set of experiments was simultaneously carried out at 4 °C in order to discriminate the adhesion and internalization phases. Coverslips were collected after 30 min at 4 °C or 1 h at 37 °C, rinsed in PBS, fixed in Bouin's fixative and stained with Giemsa. The percentages of infected macrophages or those containing adhered parasites were determined by counting 1,000–2,000 cells on duplicate or triplicate coverslips of each preparation; and each experiment was repeated at least three times. The adhesion indices were determined by multiplying the percentage of macrophages with adhered parasites by the mean number of parasites per cell. The endocytic indices were determined by multiplying the percentage of infected cells by the mean number of parasites per cell. In each experiment, the endocytic indices obtained were normalized by considering the value obtained for the control as 100. The results obtained in experiments in which the parasites were treated with suramin, DIDS or ATP were expressed as a percentage in relation to the controls.

Scanning electron microscopy

For scanning electron microscopy, macrophages were plated onto glass coverslips and cultivated overnight, as already described. After interaction with parasites, coverslips were washed twice with Ringer's solution and fixed with a solution containing 2.5% glutaraldehyde and 4% formaldehyde in 100 mM cacodylate buffer (pH 7.2) for 1 h at room temperature. They were then washed with cacodylate buffer and post-fixed with 1% OsO_4 in 100 mM cacodylate buffer for 10 min, dehydrated in ethanol, critical-point-dried in CO_2, covered with a layer of gold and observed in a JEOL JSM-5310 scanning electron microscope.

Transmission electron microscopy

For cytochemical detection of Mg^{2+} ecto-ATPase activity, control and suramin-treated trypomastigotes were fixed in 1% glutaraldehyde in 100 mM cacodylate buffer (pH 7.2) with 100 mM sucrose for 10 min at room temperature. After that, cells were washed twice in 100 mM cacodylate buffer and twice in Tris-maleate buffer (pH 7.2). Wachstein and Meisel medium (Wachstein and Meisel 1957) with a little modification was used for the detection of Mg^{2+}-activated ATPase. The medium had the following composition: 50 mM Tris-maleate buffer (pH 7.2), 2 mM

adenosine-5′-triphosphate, 100 mM $MgSO_4$, 100 mM sucrose and 3 mM $CeCl_3$. This medium was filtered before use. Cells were incubated for 1 h at 37 °C, after which they were rinsed twice in 50 mM Tris-maleate buffer and twice in 100 mM cacodylate buffer (pH 7.2). Parasites were fixed again in 2.5% glutaraldehyde in 100 mM cacodylate buffer and post-fixed in 1% osmium tetroxide in 100 mM cacodylate buffer for 1 h at room temperature. Cells were then rinsed in 100 mM cacodylate buffer, dehydrated in acetone and embedded in Polybed 812. Thin sections obtained with a Reichert Ultracut S were observed unstained, using a ZEISS 900 transmission electron microscope operating at 80 kV.

Statistical analysis

All experiments were performed in triplicates, with similar results obtained in at least three separate cell suspensions. Statistical significance was determined by Student's t-test. Significance was considered as $P < 0.05$.

Results

Suramin inhibits Mg^{2+} ecto-ATPase activity

Living epimastigote and trypomastigote forms of *Trypanosoma cruzi* (10^8 cells) were able to hydrolyze ATP at a rate of 27.9 ± 2.6 nmol P_i/h and 596.4 ± 61.7 nmol P_i/h, respectively (Fig. 1). Mg^{2+}-dependent ecto-ATPase activity on the surface of trypomastigote forms was about 20 times higher, when compared with epimastigote forms (Fig. 1). Incubation of parasites for 1 h in the presence of 500 μM suramin promoted a reduction of about 80% in the Mg^{2+}-dependent ecto-ATPase activity of epimastigotes and trypomastigotes (Fig. 1). In the cytochemical analysis of
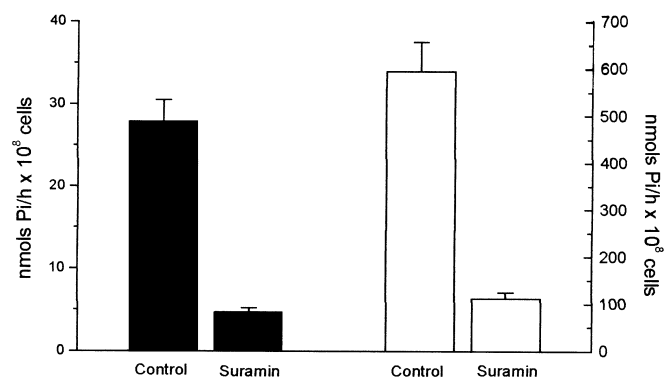

Fig. 1 Biochemical detection of Mg^{2+}-dependent ecto-ATPase activity in *Trypanosoma cruzi*. Epimastigotes (*black columns*) or trypomastigotes (*white columns*) at 1×10^8 cells/ml were incubated for 1 h in a reaction medium containing 10 mM Hepes (pH 7.2), 116 mM NaCl, 5.4 mM KCl, 5.5 mM D-glucose, 5 mM Tris-ATP (with [γ-^{32}P]ATP at 10^4 Bq/nmol ATP) and 5 mM $MgCl_2$ in the absence or presence of 500 μM suramin. Data are means $\pm$ SE of three determinations with different cell suspensions. *Pi* Inorganic phosphate
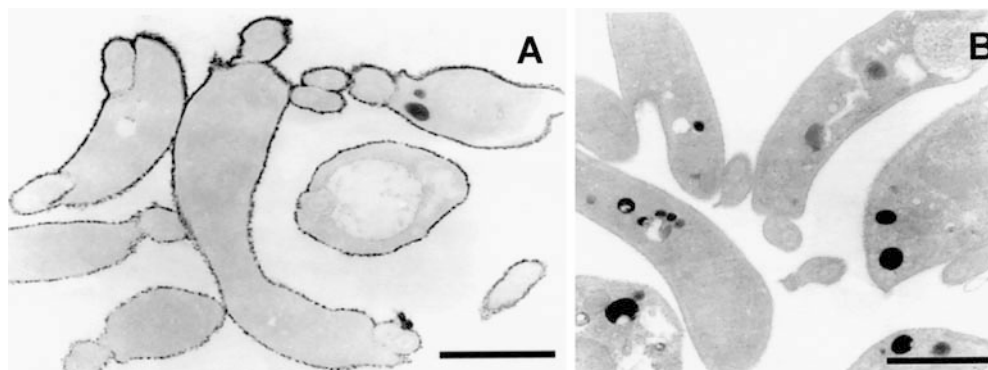
Fig. 2A,B Cytochemical detection of Mg^{2+}-dependent ecto-ATPase activity in *T. cruzi*. **A** General aspect of trypomastigote forms, showing the presence of reaction product indicative of the Mg^{2+}-dependent ecto-ATPase activity, in association with the flagellar and cell-body membranes. **B** No reaction product was seen when parasites were previously treated with 500 μ*M* suramin for 1 h. *FP* Flagellar pocket, *K* kinetoplast. *Bar* 1 μm

Mg^{2+}-dependent ecto-ATPase and parasite–host cell interaction

When the interaction of epimastigotes with macrophages was performed at 4 °C, the parasites only adhered to the macrophage surface (Table 1). Adhesion of epimastigotes and trypomastigotes to macrophages was inhibited about 63% and 45%, respectively, when parasites were treated with DIDS and about 54% and 66%, respectively, when epimastigotes and trypomastigotes were treated with suramin. DIDS and suramin did not change the mean number of adhered epimastigotes and trypomastigotes per macrophage. The internalization of parasites made during 1 h at 37 °C also changed when the parasites were pre-incubated in the presence of the drugs. There was an accentuated decrease, of about 50%

the control trypomastigotes, the reaction product appeared as an electron-dense layer homogeneously distributed throughout the plasma and flagellar membranes (Fig. 2A). However, analysis by transmission electron microscope showed a substantial variability in the intensity of labeling among trypomastigotes of the same sample (data not shown). A faint or no reaction was observed after suramin treatment (Fig. 2B).

Table 1 Effect of 4,4′-diisothiocyanostylbene 2′,2′-disulfonic acid (DIDS) and suramin on the adhesion and internalization of epimastigote and trypomastigote forms by resident peritoneal macrophages

Parameter	Treatment	Epimastigotes (% change)	Trypomastigotes (% change)
Binding			
Percentage of cells adhering to macrophages[a]	DIDS	−63.4	−45.0
	Suramin	−54.7	−66.6
Mean number of adhered parasites[b]	DIDS	−4.5	−4.7
	Suramin	−10.7	−1.8
Adhesion index	DIDS	−61.8	−40.0
	Suramin	−60.9	−67.7
Internalization			
Percentage of infected macrophages	DIDS	−49.8	−38.0
	Suramin	−35.5	−48.2
Mean number of parasites per infected macrophage	DIDS	+2.5	−7.0
	Suramin	+2.72	0
Endocytic index	DIDS	−48.5	−43.5
	Suramin	−36.1	−48.2

[a] Percentage of macrophages containing adhered parasites
[b] Mean number of adhered parasites per macrophage containing parasites

Table 2 Effect of ATP on the trypomastigote–macrophage interaction

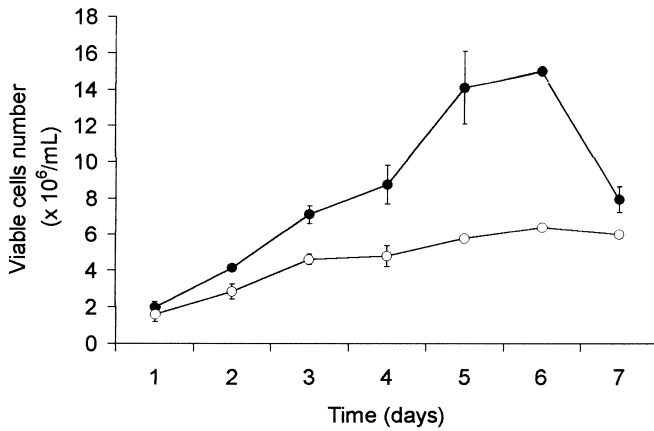| ATP concentration (μ*M*)	Treatment	Infected macrophages (%)	Change (%)	Mean (±SD) of trypomastigotes per infected macrophage
50	No	15.56 ± 1.27		1.26 ± 0.07
	Yes	16.56 ± 1.44	+6.0	1.16 ± 0.04
100	No	15.56 ± 1.27		1.26 ± 0.07
	Yes	20.18 ± 3.33	+29.7	1.21 ± 0.17
200	No	15.56 ± 1.27		1.26 ± 0.07
	Yes	20.95 ± 1.36	+34.6	1.23 ± 0.11

Fig. 3 Suramin effect on epimastigote growth, shown by the growth curve of epimastigote forms of *T. cruzi* cultivated in LIT medium (Camargo 1964) for 7 days at 28 °C in the absence (*black circles*) or presence (*white circles*) of 500 μM suramin. Values shown are means of determinations in triplicate from three different experiments. *mL* Milliliters

Fig. 4 Effect of long-term treatment with suramin on Mg^{2+}-dependent ecto-ATPase activity. Epimastigotes (at 1×10^8 cells/ml) were grown for 24, 48 and 72 h in LIT medium supplemented with 10% fetal calf serum (FCS) in the absence (*black columns*) or presence (*white columns*) of 500 μM suramin and then incubated for 1 h at 28 °C in the reaction medium described in Fig. 1

and 38% for DIDS and about 35% and 48% for suramin, in the infected macrophages with epimastigote and trypomastigote forms, respectively. No significant changes were observed in the mean number of parasites per infected macrophage (Table 1). The addition of suramin during the trypomastigote–macrophage interaction decreased adhesion and internalization at the same rate as described for pre-treated trypomastigotes. The interaction between trypomastigote forms and resident macrophages in the presence of ATP was also analyzed. As shown in Table 2, ATP in concentrations above 100 μM significantly increased both the percentage of infected macrophages and the endocytic index. No significant differences were observed in the mean number of trypomastigotes per infected macrophage (Table 2).

Suramin treatment and parasite growth

Epimastigote growth was inhibited by suramin and a reduction of 70% was observed on day 5 of culture (Fig. 3). Suramin did not induce metacyclogenesis. The number of trypomastigote forms after 7 days of growth in LIT medium supplemented or not with 500 μM suramin did not differ (data not shown). The cellular integrity and viability of epimastigotes during growth was evaluated by analysis of cell motility and trypan blue dye exclusion. They were not affected by any of the conditions used in the assays.

Modulation of Mg^{2+}-dependent ecto-ATPase activity by suramin

After 24 h of cultivation, the ATPase activity was about 126 nmol P_i/h for 10^8 cells and, after 48 h and 72 h, the
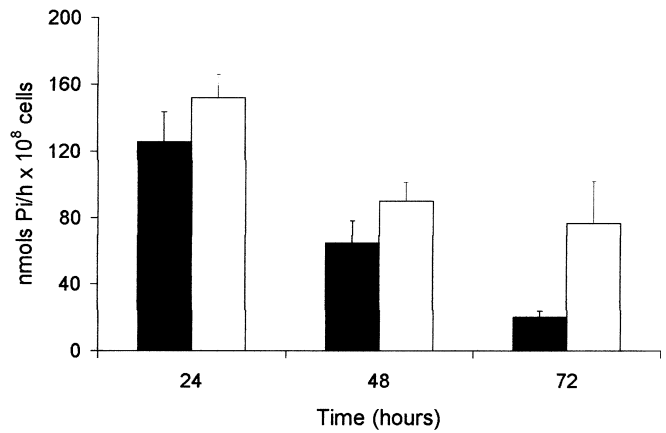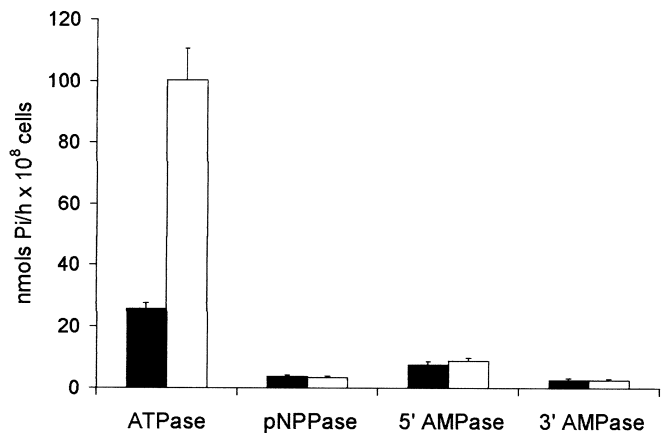

Fig. 5 Effect of long-term treatment with suramin on the activity of distinct ecto-enzymes of *T. cruzi*. Epimastigotes (at 1×10^8 cells/ml) were grown for 72 h in LIT medium supplemented with 10% FCS in the absence (*black columns*) or presence (*white columns*) of 500 μM suramin and then incubated for 1 h at 28 °C in distinct incubation media for the detection of Mg^{2+}-dependent ecto-ATPase, ecto-phosphatase (*pNPPase*), 5′-nucleotidase (*5′AMPase*) and 3′-nucleotidase (*3′AMPase*). The values represent means of at least three independent experiments performed in triplicate

enzymatic activity had a significant reduction (about 48%, 80%, respectively; Fig. 4). Epimastigote forms cultivated in LIT medium containing 500 μM suramin showed an increase in Mg^{2+} ATPase activity, when compared with epimastigotes grown in the absence of suramin during the same period of time (Fig. 4). The ATPase activity of parasites grown in the presence of the drug also decreased with the time of cultivation. This decrease, however, was less accentuated when compared with control cells (Fig. 4). Epimastigotes (10^8 cells) grown with suramin during 72 h hydrolyzed ATP at
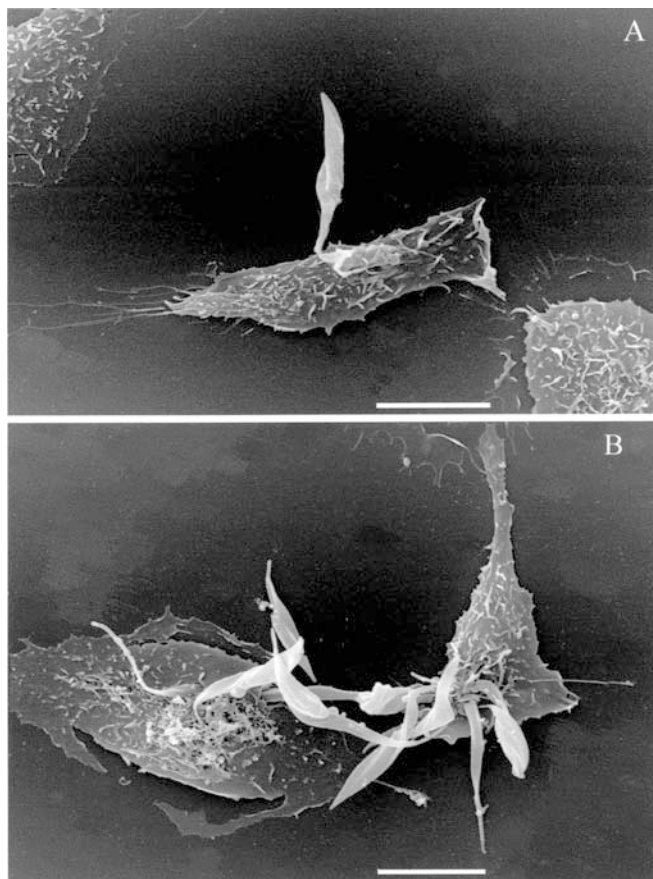
Fig. 6A,B Scanning electron images of the interaction between epimastigotes and mouse resident macrophages. Epimastigotes grown in LIT medium for 72 h in the absence (**A**) or presence (**B**) of 500 µM suramin were incubated for 1 h at 37 °C with macrophages and processed for scanning electron microscopy. Suramin induced a significant increase in the number of adhered epimastigotes per macrophage. *Bar* 10 µm

an inhibition of the Mg^{2+}-dependent ecto-ATPase activity similar to that obtained for control cells (data not shown). Figures 4 and 5 show that epimastigotes grown with suramin during 72 h present a Mg^{2+}-dependent ecto-ATPase activity more than three times higher than control cells, while no differences were observed in the other phosphomonoesterase activities (Fig. 5).

Enhancement of Mg^{2+}-dependent ecto-ATPase activity and parasite adhesion

Scanning electron microscopic analysis of the epimastigote–macrophage interaction process showed an accentuated increase in the number of adhered epimastigotes grown in the presence of suramin during 72 h adhered to macrophages. Frequently, more than 4 parasites were observed adhered to the surface of resident macrophages when the interaction was carried out for 1 h at 37 °C. (Fig. 6). There was also a significant increase in the percentage of macrophages with attached suramin-treated parasites, as compared with control cells (Table 3). The increase was about 62% when the interaction was made during 30 min at 4 °C and was 375% during 1 h at 37 °C. At 4 °C, there was a slightly increase in the mean number of adhered parasites per macrophage. However, this number was significantly greater when interaction was made at 37 °C (Fig. 6). The binding indices for suramin-treated parasites at 4 °C and 37 °C were respectively 66% and 560% greater than that obtained for control cells submitted to the same experimental conditions (Table 3).

Discussion

During the past two decades, considerable progress has been achieved in the study of ectonucleotidases in general (Zimmermann 1996) and ecto-ATPases in particular (Plesner 1995; Dombrowski et al. 1998). This progress is related to the finding that, in contrast to the established view, nucleotides can be found in significant concentrations outside cells (Gordon 1986;

100.3 ± 10.4 nmol P_i/h (in contrast with control cells which hydrolyzed ATP at 25.9 ± 1.9 nmol P_i/h), which represents only 25% of the ATPase activity obtained with suramin-treated parasites. Growth in the presence of suramin increased the Mg^{2+}-dependent ecto-ATPase activity and did not select a resistant population of epimastigotes. A further treatment with suramin caused

Table 3 Growth of epimastigote forms during 72 h in the presence of 500 µM suramin increases parasite adhesion to resident macrophages. Macrophage number (*MN*) was evaluated by determining the percentage of macrophages containing attached parasites after 30 min interaction at 4 °C or 1 h interaction at 37 °C, by direct counting of Giemsa-stained coverslips in triplicate. *Adhered parasites* represents the mean number of adhered epimastigotes per macrophage. Binding index (*BI*) was determined by multiplying the number of macrophages containing adhered parasites by the mean number of parasites per macrophage

Conditions	Growth with suramin	MN	MN change (%)	Adhered parasites	BI	BI change (%)
4 °C for 30 min	No	6.87 ± 2.7		1.12 ± 0.14	7.69 ± 2.89	
	Yes	11.13 ± 2.9	+ 62	1.15 ± 0.05	12.75 ± 2.81	+ 66
37 °C for 1 h	No	5.95 ± 2.42		1.39 ± 0.13	8.17 ± 2.78	
	Yes	28.3 ± 4.1	+ 375	2.0 ± 0.14	54.06 ± 11.8	+ 561

Dombrowski et al. 1998). Nucleotides released to the extracellular medium may exert their effects on other cells in the vicinity of the secretion site and modulate biological processes, by binding to specific cell-surface receptors (Dombrowski et al. 1998). The role of Mg^{2+}-dependent ecto ATPase activity on the surface of parasites is still the subject of speculation. In some parasites, such enzymatic activity is considered an important extracellular signal in the regulation of virulence (Barros et al. 2000; Berrêdo-Pinho et al. 2001; Peres-Sampaio et al. 2001; Jesus et al. 2002). In *Toxoplasma gondii* the secreted E-type ATPase (NTPase) may play a role in initiating parasite egress, since treatment of infected host cells with dithiols (e.g. dithiotheitol) activates the ATPase activity and stimulates a mass parasite exodus, associated with a depletion of host-cell ATP levels (Silverman et al. 1998; Carruthers 1999). In *T. gondii*, two isoforms of an E-type ATPase were isolated. The avirulent strains expressed only the NTPase 1 isoform, which is a true apyrase, whereas the virulent strains expressed NTPase 1 and the NTPase 3 isoform, which cleaves predominantly nucleotide triphosphates (Bermudes et al. 1994). Recently, it was shown that the invasive ameba *Entamoeba histolytica* has a much higher Mg^{2+}-dependent ecto-ATP diphosphohydrolase activity than non-invasive *E. histolytica* and the free-living ameba *E. moshkovskii* (Barros et al. 2000). This ameba E-type ATPase is stimulated more than 2-fold by D-galactose (Barros et al. 2000), an important molecule involved with *E. histolytica* adhesion (Radvin et al. 1980). So, it was proposed this enzyme may be a pathogenesis marker for this cell (Barros et al. 2000). In *Leishmania amazonensis*, virulent promastigotes showed a Mg^{2+}-dependent ecto-ATPase activity 2-fold higher than that observed in avirulent promastigotes (Berrêdo-Pinho et al. 2001). In the present study, we observed that, albeit present in all trypomastigote forms, the amount of electron-dense precipitate indicative of Mg^{2+}-dependent ecto-ATPase activity varied among distinct individuals in the same population. The lack of homogeneity in the distribution of a particular surface molecule on individuals of the same isolate or clone is characteristic of *Trypanosoma cruzi* (De Souza 1989) and can reflect differences in infectivity, as shown by Pereira et al. (1996).

The ecto-ATPase activity can be inhibited by the impermeant agent DIDS (Knowles 1988; Plesner 1995; Barbacci et al. 1996; Meyer-Fernandes et al. 1997) and by suramin, a polyanionic compound with known anti-parasitic activities that has been shown to be a P2 receptor-antagonist (Chen and Lin 1997). Suramin has also been found to interfere with the mitogenic signaling of P2 receptors to MAP kinases (Neary et al. 1998), to interfere with the binding of growth factors to their receptors at the cell surface (Middaugh et al. 1992) and to inhibit nuclear enzymes and other intracellular enzymatic systems (Voogd et al. 1993; Eisenberger and Reyno 1994).

The Mg^{2+}-dependent ecto-ATPase of *T. cruzi* is inhibited by suramin. This inhibition is dose-dependent and is expressed by an accentuated reduction in the ATP hydrolysis rate and a significant reduction in the electron-dense reaction product observed on the cell surface of epimastigote and trypomastigote forms (Fig. 2). Suramin also inhibits the growth of epimastigote forms without affecting their cell viability or metacyclogenesis rate. Since epimastigote growth in vitro is serum-dependent (O'Daly et al. 1987) and is not enhanced in the presence of exogenous ATP or adenosine (data not shown), we suggest that suramin exerts its growth-inhibitory effects in *T. cruzi* by preventing the binding of growth factors on the surface of epimastigote forms, as described previously for tumor cell lines (Hosang 1985; Abdiu et al. 1999), inducing a receptor-shedding (Galvani et al. 1995) or interfering with the uptake of host low-density lipoprotein through receptor-mediated endocytosis, as described for African trypanosomes (Vansterkenburg et al. 1993).

Interestingly, the growth of epimastigotes in the presence of suramin induces an increase in Mg^{2+}-dependent ecto-ATPase activity not followed by an increase in other ecto-enzymatic activities, such as 3′ and 5′ nucleotidase and ecto-phosphatase. This fact discards the possibility that enhancement of Mg^{2+}-dependent ecto-ATPase caused by suramin could be caused by subversion of the exocytic traffic of molecules, as described in macrophages (Pesanti 1978). The accumulation of physiologically active molecules on the cell surface by suramin was previously described in glioma cell lines, resulting in an increase in cellular attachment (Hinek et al. 1999). Epimastigotes over-expressing ecto-ATPase activity are not suramin-resistant, since their enzymatic activity is inhibited by a subsequent suramin treatment.

The idea that ecto-ATPase activity could be related to virulence seems to be also applicable to *T. cruzi*. In this study, we show that trypomastigotes, evolutive forms infective to mammalian hosts, were up to 20 times more efficient than non-infective epimastigotes in hydrolyzing ATP (Fig. 1). Recently, the association of ecto-ATPase activity with adhesion molecules raised the possibility that extracellular nucleotides may play important roles in regulating cell adhesion (Stout et al. 1995; Kirley 1997; Meyer-Fernandes 2002). As is well known, the attachment capacity of parasites is a prerequisite for entry into host cells, in the case of intracellular parasites, or for triggering the cytotoxicity mechanism of extracellular parasites, as described in *E. histolytica* and *Trichomonas vaginalis* (Espinosa-Cantellano and Martínez-Palomo 2000).

Another important finding is that the addition of up to 200 μM ATP to the interaction medium increased by about 30% the number of macrophages infected by trypomastigote forms. Taking account of the fact that

ATP can be released by different kinds of cells, such as neutrophils and endothelial cells (Fredholm 1997), together with the role of these cells in the pathogenesis of Chagas' disease (Chen et al. 2001; Petkova et al. 2001), we suggest that Mg^{2+}-dependent ecto-ATPase activity on *T. cruzi* could stimulate the adherence of parasites to host cells and protect them from neutrophil attack, by releasing adenosine to inhibit superoxide production.

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