# Sodium–proton exchange stimulates $Ca^{2+}$ release from acidocalcisomes of *Trypanosoma brucei*

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Acidocalcisomes are acidic vacuoles present in trypanosomatids that contain a considerable fraction of intracellular $Ca^{2+}$ [Vercesi, Moreno and Docampo (1994) Biochem. J. **304**, 227–233; Scott, Moreno and Docampo (1995) Biochem. J. **310**, 789–794; Docampo, Scott, Vercesi and Moreno (1995) Biochem. J. **310**, 1005–1012]. The data presented here indicate that $Na^+$ stimulates $Ca^{2+}$ release from the acidocalcisomes of digitonin-permeabilized *Trypanosoma brucei* procyclic trypomastigotes in a dose-dependent fashion, this effect being enhanced by increasing pH of the medium from 7.0 to 7.8. The hypothesis that this $Na^+$ effect was mediated by alkalinization of the acidocalcisomes via a $Na^+/H^+$ antiporter was supported by experiments showing that $Na^+$ promotes release of Acridine Orange previously accumulated in these vacuoles. This putative antiporter did not transport $Li^+$ and was not sensitive to the amiloride analogue 5-(*N*-ethyl-*N*-isopropyl)amiloride. Addition of the $Na^+/H^+$ ionophore monensin to intact cells loaded with fura 2, in the nominal absence of extracellular $Ca^{2+}$ to preclude $Ca^{2+}$ entry, was followed by an increase in cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$), which was more accentuated in the presence of extracellular $Na^+$. An increase in intracellular pH ($pH_i$) of BCECF-loaded cells was detected after addition of monensin in the presence of extracellular $Na^+$, whereas a dramatic decrease in $pH_i$ was detected in its absence, thus indicating the presence of a significant amount of releasable protons in the acidic compartments. These results are consistent with the presence of a $Na^+/H^+$ antiporter in the acidocalcisomes that could be involved in the regulation of $pH_i$ and $[Ca^{2+}]_i$ in these parasites.

## INTRODUCTION

Although in plants the existence of $Na^+/H^+$ antiporters in the vacuole membrane is well documented [1], in animal cells reports on activities in subcellular organelles other than mitochondria [2,3] are very scattered [1]. Chromaffin granules, the secretory vesicles from bovine adrenal medulla, have a $Na^+/H^+$ antiport activity [4]. This is an electroneutral antiporter with a $K_m$ for $Na^+$ of 20–30 mM. Amiloride is a competitive inhibitor, but with a low affinity ($K_i$ 0.26 mM). An amiloride-insensitive antiporter has also been described in renal endocytic membranes [5].

In previous papers [6–8] we provided evidence that trypanosomatids possess acidic vacuoles that we termed acidocalcisomes, where, by analogy to the yeast vacuole [9], calcium is accumulated via a vanadate-sensitive $Ca^{2+}$-ATPase. The acidic pH of this compartment is generated and sustained by a bafilomycin $A_1$-sensitive $H^+$-ATPase and seems to be important for $Ca^{2+}$ retention, since alkalinization induced by nigericin, $NH_4Cl$ or bafilomycin $A_1$ treatment is followed by $Ca^{2+}$ release [6–8]. The mechanism by which $Ca^{2+}$ is released from the acidic compartment following these treatments is unclear. It is apparently not due to the $Ca^{2+}$-ATPase activity, as nigericin effects release from permeabilized cells in the absence of ATP and the presence of the ATPase inhibitor orthovanadate [6]. This may suggest that, in addition to the $Ca^{2+}$-ATPase, these vacuoles also contain a $Ca^{2+}/nH^+$ antiporter that mediates $Ca^{2+}$ release.

In this paper we provide evidence that $Na^+$ stimulates $Ca^{2+}$ release from the acidocalcisomes of *Trypanosoma brucei* procyclic trypomastigotes by alkalinization of the vacuoles via a $Na^+/H^+$ antiporter, followed by $Ca^{2+}/nH^+$ exchange.

## MATERIALS AND METHODS

### Culture methods

*T. brucei* procyclic forms (ILTar 1 or MITat 1.4 procyclics) were grown at 28 °C in medium SDM-79 [10] supplemented with haemin (7.5 mg/l) and 10% heat-inactivated foetal-calf serum. Then 2–3 days after inoculation, cells were collected by centrifugation and washed twice in a buffer containing 116 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose and 50 mM Hepes, pH 7.4. Cell concentrations were determined with a Neubauer chamber. The protein concentration was determined by the biuret assay [11] in the presence of 0.2% deoxycholate.

### Chemicals

ATP, arsenazo III, EGTA, nigericin, antimycin A, oligomycin, monensin and digitonin were purchased from Sigma. Bafilomycin $A_1$ was obtained from Dr. K. Altendorf (University of Osnabrück, Osnabrück, Germany). Acridine Orange, 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxyl]-2-(2′-amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid acetoxymethyl ester (fura 2/AM), 2′,7′-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein acetoxymethyl ester (BCECF/AM) and 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) were from Molecular Probes, Eugene, OR, U.S.A. All other reagents were of analytical grade.

### Determination of $Ca^{2+}$ movements

Variations in free $Ca^{2+}$ concentrations were monitored by measuring the changes in the absorbance spectrum of arsenazo III

Abbreviations used: $[Ca^{2+}]_i$, intracellular $Ca^{2+}$ concentration; $pH_i$, intracellular pH; fura 2, 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxyl]-2-(2′-amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid; BCECF, 2′,7′-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein; AM, acetoxymethyl ester; EIPA, 5-(*N*-ethyl-*N*-isopropyl)amiloride.

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[12], by using a SLM Aminco DW2000 spectrophotometer at the wavelength pair 675/685 nm. No free-radical formation from arsenazo III occurred under the conditions used [13–15]. The concentrations of the ionic species and complexes at equilibrium were calculated by employing an iterative computer program as described previously [16]. Each experiment was repeated at least three times with different cell preparations, and the Figures show representative experiments.

### Proton-pump activity

Acidification of digitonin-permeabilized cells was monitored by measuring the changes in the absorbance spectrum of Acridine Orange [17], by using a SLM Aminco DW2000 spectrophotometer at the wavelength pair 493/530 nm. Each experiment was repeated at least three times with different cell preparations, and the Figures show representative experiments.

### Fluorescence measurements

Fura 2 and BCECF determinations were performed essentially as described previously [7]. After harvesting, cells were washed twice in buffer A, which contained 116 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM D-glucose and 50 mM Hepes at pH 7.4 [7]. Cells were resuspended to a final density of $1 \times 10^9$/ml in loading buffer, which consisted of buffer A plus 1.5% sucrose, and 6 $\mu$M fura 2/AM or 9 $\mu$M BCECF/AM. The suspensions were incubated for 30 min in a 30 °C water bath with mild agitation. Subsequently, the cells were washed twice with ice-cold buffer A to remove extracellular dye. Cells were resuspended to a final density of $1 \times 10^9$ cells/ml in buffer A and were kept in ice [7]. For fluorescence measurements, $5 \times 10^7$ cells/ml (final density) were suspended in a cuvette containing buffer A or buffer A in which 116 mM choline chloride was substituted for NaCl, placed in a thermostatically regulated (30 °C) Hitachi F-2000 spectrofluorimeter. Excitation was at 340 and 380 nm, and emission was measured at 510 nm. The fura 2 fluorescence response to intracellular calcium concentration ($[Ca^{2+}]_i$)was calibrated from the ratio of 340/380 nm fluorescence values after subtraction of the background fluorescence of the cells at 340 and 380 nm as described previously [7]. $[Ca^{2+}]_i$ was calculated by titration with different concentrations of Ca-EGTA buffers [6–8]. Concentrations of the ionic species and complexes at equilibrium were calculated by using an iterative computer program as described previously [16]. For intracellular pH ($pH_i$) measurements, the fluorescence ratio, with wavelengths for excitation set at 505/440 nm and for emission at 530 nm, were recorded and translated into pH values on the basis of the ratios obtained at various pH values [18]. Other experimental conditions and calibrations were as described previously [18]. Representative traces from experiments conducted on three separate cell preparations are shown in the Figures.

## RESULTS

### $Na^+$ stimulates $Ca^{2+}$ release from permeabilized cells

We have previously shown that nigericin is able to release endogenous $Ca^{2+}$ from the acidic compartment (acidocalcisome) of permeabilized *T. brucei* [7]. In order to investigate if $Na^+$ had any effect on $Ca^{2+}$ release from acidocalcisomes, *T. brucei* procyclic trypomastigotes were permeabilized first with digitonin in $Na^+$-free medium, in the presence of mitochondrial inhibitors and the absence of ATP to prevent $Ca^{2+}$ accumulation [7]. The experiment of Figure 1(A) (trace a) shows that little change in $Ca^{2+}$ concentration in the medium was observed until nigericin was added. A total amount of 30 nmol of $Ca^{2+}$/mg of cell protein was released by nigericin. This indicates that vacuole alkalinization favours $Ca^{2+}$ release. When the KCl medium was replaced by a medium containing 130 mM NaCl (Figure 1A, trace c), cell permeabilization was followed by a continuous increase in $Ca^{2+}$ in the medium. The subsequent addition of nigericin released an additional amount of the cation that made the total concentration of released $Ca^{2+}$ similar to that previously obtained with nigericin in the KCl medium (Figure 1A, trace a). This suggests that in both experiments the total $Ca^{2+}$ released was present in the same compartment, the acidocalcisome. Figure 1(A) (traces a–c) show that the $Ca^{2+}$-releasing effect of $Na^+$ was concentration-dependent. These experiments suggest the existence of either a $Ca^{2+}/nNa^+$ exchanger or a $Na^+/H^+$ antiporter together with a $Ca^{2+}/nH^+$ exchanger in the vacuolar membrane. Since $Ca^{2+}$ release is favoured by alkalinization of the vacuoles [7,8] and can take place in a $Na^+$-free medium when alkalinization is imposed by nigericin (Figure 1A, trace a), the existence of a $Ca^{2+}/nH^+$ antiporter to mediate $Ca^{2+}$ efflux from these vacuoles is more likely than a $Ca^{2+}/nNa^+$ exchanger. Alkalinization of the vacuoles via an exchange between external $Na^+$ and internal $H^+$ would facilitate $Ca^{2+}$ release via a $Ca^{2+}/nH^+$ exchanger. If this is so, then the $Na^+$ effect would be enhanced thermodynamically by alkalinization of the external medium, which would increase the driving force imposed by the opposite $H^+$ and $Na^+$ gradients. The experiments depicted in Figure 1(B) confirm this; the rate of $Na^+$-stimulated $Ca^{2+}$ release was increased progressively by increasing the pH of the medium from 7.0 to 7.8. It is, however, important to note that at pH 7.0, at which the $Na^+/H^+$ exchange should still be favourable taking into account the more acidic intravacuolar pH, little $Ca^{2+}$ release was detected. This suggests that the putative $Na^+/H^+$ antiporter is less active at the cytosolic

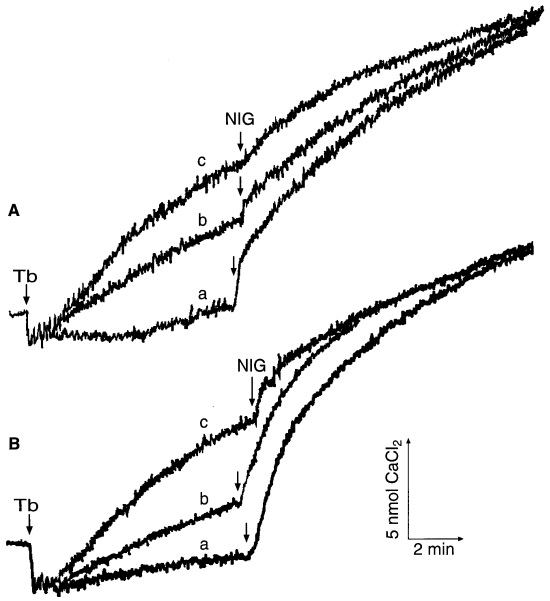


**Figure 1 Effect of NaCl concentrations (A) and pH (B) on endogenous $Ca^{2+}$ release from permeabilized cells**

(**A**) The cells (Tb, 0.36 mg of protein/ml) were added to the medium [130 mM KCl (trace a), 65 mM KCl/65 mM NaCl (trace b), or 130 mM NaCl (trace c), plus 1 mM $MgSO_4$, and 10 mM Hepes, pH 7.8] containing 2 $\mu$g/ml oligomycin, 2 $\mu$M antimycin A, 28 $\mu$M digitonin and 40 $\mu$M arsenazo III. Nigericin (NIG; 1 $\mu$M) was added where indicated. (**B**) Conditions as in (**A**), trace c (buffer containing 130 mM NaCl), except that the pH was 7.0 (trace a), 7.35 (trace b) or 7.8 (trace c).

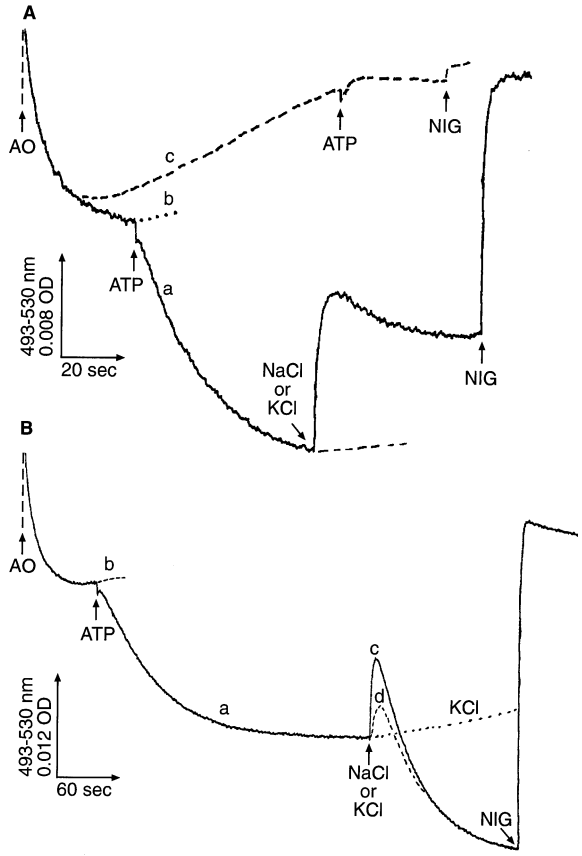


**Figure 2 ATP-driven Acridine Orange accumulation by permeabilized cells**

(**A**) Trace (a), the cells (0.36 mg of protein/ml) were incubated in a reaction medium containing 130 mM KCl, 1 mM $MgSO_4$, 1 mM EGTA, 10 mM Hepes, pH 7.8, 2 $\mu$g/ml oligomycin, 1 $\mu$g/ml antimycin A and 28 $\mu$M digitonin. Acridine Orange (AO, 1.0 $\mu$M) was added where indicated (1 min after the cells). ATP (0.5 mM), 45 mM NaCl, 45 mM KCl (trace a, dashed line), and 1 $\mu$M nigericin (NIG) were added where indicated. Trace (b), the same as in (a), but without ATP addition. Trace (c), the same as in trace (a), but the buffer contained 130 mM NaCl instead of KCl. (**B**) Trace a, the same as in (**A**) trace (a), but the pH of the buffer was 7.0. Trace (b) is without ATP addition, trace (c) is after addition of 45 mM NaCl, and trace (d) is after addition of 30 mM NaCl. Dotted line is after addition of 45 mM KCl instead of NaCl. OD = *A*.

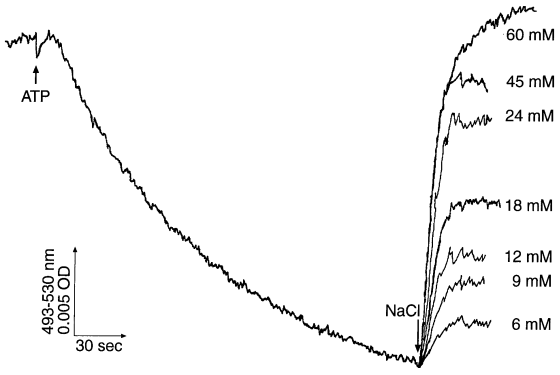


**Figure 3 Effect of NaCl on Acridine Orange release by permeabilized cells**

Conditions were as in Figure 2(**A**), trace (a). ATP (0.5 mM) and NaCl (6, 9, 18, 24, 45 or 60 mM) were added where indicated.

pH of procyclics [7], as is the case with the antiporter present in the plasma membrane of other cell types [19].

### $Na^+$ induces alkalinization of acidocalcisomes

The hypothesis that the acidocalcisomes possess a $Na^+/H^+$ antiporter was further examined by using Acridine Orange, whose distribution between two compartments varies with the pH gradient; its accumulation increases and its absorbance decreases when the compartment becomes more acidic [17]. In this regard, Figure 2(A) (trace a) shows that, in agreement with previous results [6], ATP drives Acridine Orange accumulation by the acidocalcisomes in KCl medium (pH 7.8). In NaCl medium (dashed trace, c) this was not observed, because the $Na^+$-induced alkalinization of the vacuoles via the proposed $Na^+/H^+$ antiporter overcomes the acidification caused by the $H^+$-ATPase. When gluconate replaced chloride as the anion in the buffer preparation, no ATP-driven Acridine Orange uptake was detected (results not shown), in agreement with the postulated role of $Cl^-$ in the stimulation of $H^+$ uptake by vacuolar $H^+$-ATPases [19,20]. A partial release of Acridine Orange, under the conditions of Figure 2(A) (trace a), could be induced by the addition of 45 mM NaCl (continuous line) but not 45 mM KCl (dashed line), thus indicating that the effect was not due to changes in the osmotic pressure and that $Na^+$ was the active cation. Interestingly, when NaCl was added to permeabilized cells suspended in KCl medium at pH 7.0 (Figure 2B), but not at pH 7.8 (Figure 2A), the partial release of Acridine Orange was only transient, and was followed by uptake of an additional amount of the dye. This is compatible with a futile cycling of $H^+$ across the vacuolar membrane caused by the simultaneous operation of the $H^+$-ATPase and the putative $Na^+/H^+$ antiporter, pumping $H^+$ in and out respectively. Because $Cl^-$ facilitates ATP-dependent vacuolar acidification, probably by moving into the vacuoles to serve as a charge-compensating anion [20], this would result in net accumulation of NaCl, which in turn would draw in water and result in swelling of the vacuole and accumulation of an extra amount of Acridine Orange. Another possible explanation is that at pH 7.8 (Figure 2A) the capacity of the $H^+$-ATPase to maintain the acidic pH of the vacuole may be overcome by both the faster operation of the $Na^+/H^+$ antiporter at high pH (Figure 1B) and the larger driving force of the $H^+$ gradient, whereas at pH 7.0 the $Na^+/H^+$ antiporter is less active (Figure 1B, trace a).

As shown in Figure 3, both the extent and the rate of Acridine Orange release induced by $Na^+$ were concentration-dependent, the rate attaining saturation at concentrations close to 45 mM $Na^+$, as judged by the similar rates of Acridine Orange release in the range 45–60 mM NaCl. However, the data presented in Figure 3 do not allow for a $K_m$ determination, because the rates of Acridine Orange release seem to be largely underestimated, mainly at low $Na^+$ concentrations, due to the simultaneous operation of the $H^+$-ATPase. This putative antiporter was very specific for $Na^+$ and did not transport $Li^+$, since addition of a similar concentrations of LiCl (45 mM) did not release Acridine Orange (results not shown). In addition, Figure 4 shows that it was not inhibited by the amiloride analogue EIPA, which, in a dose-dependent manner, either decreased the rate of ATP-driven Acridine Orange accumulation (Figure 4, traces b and d) or induced release of the previously accumulated dye (Figure 4, trace c). Addition of NaCl as well as EIPA was followed by Acridine Orange release at rates similar to those obtained in the control experiments (trace a). The dashed line (trace e) shows the effect of NaCl addition after bafilomycin $A_1$. The pattern obtained after addition of 40 $\mu$M EIPA (trace c) was similar to that obtained after addition of the $H^+$-ATPase inhibitor [21]

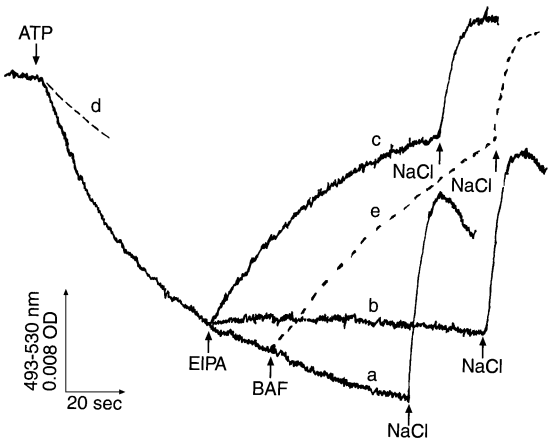


**Figure 4 Effect of EIPA and bafilomycin $A_1$ on Acridine Orange release from permeabilized cells by NaCl**

Conditions were as in Figure 2(**A**), trace (a). ATP (0.5 mM), EIPA (20 $\mu$M, trace b; 40 $\mu$M, trace c), bafilomycin $A_1$ (BAF, 1 $\mu$M), or NaCl (45 mM) were added where indicated. In trace (d) EIPA (40 $\mu$M) was present in the initial mixture.

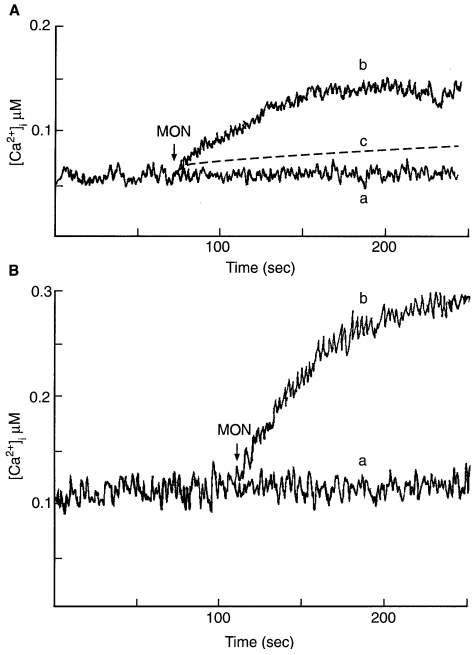


**Figure 5 Effect of monensin on $[Ca^{2+}]_i$**

Procyclic trypomastigotes were loaded with fura 2/AM as described in the Materials and methods section and suspended in buffer containing 116 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 50 mM Hepes, pH 7.4, and either 1 mM EGTA (**A**) or 1 mM $CaCl_2$ (**B**). Monensin (MON, 2 $\mu$g/ml; traces b and c) was added where indicated. Trace (c) was obtained with cells suspended in the same buffer described above, but containing 116 mM choline chloride instead of NaCl.

bafilomycin $A_1$ (1 $\mu$M, trace e). Although it has been proposed [22] that EIPA has an uncoupling property, its possible effect on the $H^+$-ATPase cannot be ruled out on the basis of these experiments.

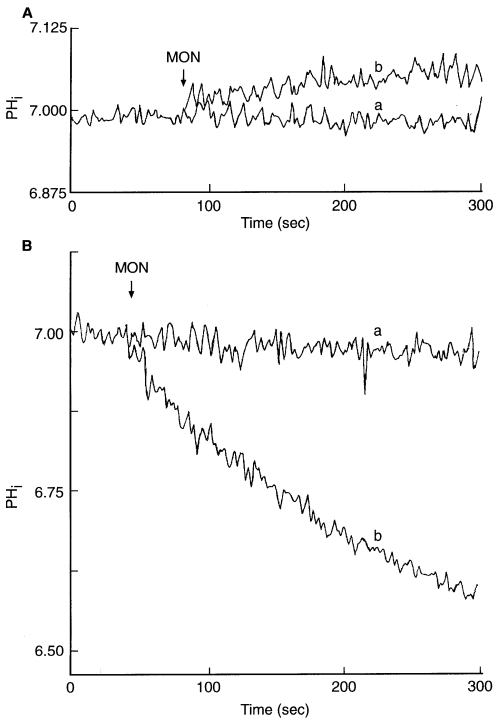


**Figure 6 Effect of monensin on $pH_i$**

Procyclic trypomastigotes were loaded with BCECF/AM as described in the Materials and methods section and suspended in buffer containing 116 mM NaCl (**A**) or 116 mM choline chloride (**B**), 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 50 mM Hepes, pH 7.4, and 1 mM EGTA. Monensin (MON, 2 $\mu$g/ml; traces b) was added where indicated.

### Changes in $[Ca^{2+}]_i$ and $pH_i$ in intact cells produced by the $Na^+/H^+$ ionophore monensin

Addition of the $Na^+/H^+$ ionophore monensin resulted in changes in $[Ca^{2+}]_i$ and $pH_i$ in fura 2- and BCECF-loaded cells respectively. Figure 5 (traces a) shows that fura 2 fluorescence remained unchanged during the time of observation. Addition of monensin (traces b) was promptly followed by a significant increase in $[Ca^{2+}]_i$ from about 100 to 300 nM when the cells were suspended in medium containing 1 mM $CaCl_2$ (Figure 5B), or from 50 to 150 nM when the cells were suspended in $Ca^{2+}$-free medium (Figure 5A). When these experiments were repeated in medium without $Na^+$ (replacing NaCl by choline chloride in the buffer) and in the absence of extracellular $Ca^{2+}$ (with addition of 1 mM EGTA) to avoid $Ca^{2+}$ entry and to detect only $Ca^{2+}$ release from intracellular stores, a small but reproducible increase in $[Ca^{2+}]_i$ was detected upon addition of monensin (Figure 5A, trace c), whereas fura 2 fluorescence remained unchanged in the absence of monensin (results not shown). These results indicate that cytosolic $Na^+$ was being exchanged with vacuolar $H^+$, then allowing $Ca^{2+}$ release by the $Ca^{2+}/nH^+$ exchanger. To confirm this hypothesis, we measured changes in $pH_i$ under similar conditions (with and without extracellular $Na^+$). Basal $pH_i$ remained unchanged at about pH 7.0 [7] during the time of observation at either condition (Figure 6, traces a). Addition of monensin to BCECF-loaded cells in the presence of extracellular $Na^+$ (Figure 6A, trace b) caused a small increase in $pH_i$. However, when monensin was added to cells suspended in a buffer without $Na^+$, a dramatic decrease in $pH_i$ was detected (Figure 6B, trace b). These results indicate that, when extracellular $Na^+$ was present (Figure 6A), monensin exchanged $Na^+$ and $H^+$ at the

plasma-membrane and at the vacuolar-membrane level, and the exchange at the plasma membrane predominated, resulting in alkalinization of the cytosol. When extracellular $Na^+$ was absent, monensin could only exchange $Na^+$ and $H^+$ at the vacuolar-membrane level, and the amount of $Na^+$ present in the cytosol was able to release sufficient $H^+$ from the acidic vacuolar compartment to acidify the cytosol. Taken together, these results indicate that alkalinization of the vacuoles releases $Ca^{2+}$, and that the amount of release depends on degree of alkalinization and confirm the presence of a $Ca^{2+}/nH^+$ exchanger in the vacuolar membrane.

## DISCUSSION

Taking into consideration the experimental results obtained in this and previous papers [6,7], we propose that, in addition to the $Ca^{2+}$ and $H^+$-ATPases, the *T. brucei* acidocalcisome membrane possesses a $Na^+/H^+$ and a $Ca^{2+}/nH^+$ antiporter (see the scheme in Figure 7). Existence of the $Ca^{2+}$- and $H^+$-ATPases is supported by vanadate- and bafilomycin-$A_1$-sensitivities of $Ca^{2+}$ accumulation or acidification of the vacuoles, respectively [6,7]. Vacuolar alkalinization by $Na^+$, as observed in Figures 2 and 3 of the present work, strongly suggests the presence of a $Na^+/H^+$ antiporter. Because $Ca^{2+}$ efflux via the $Ca^{2+}$-ATPase is very unlikely, since this reaction would require ADP and $P_i$, and would be strongly coupled to ATP synthesis, the stimulation of $Ca^{2+}$ release by alkalinization of the vacuoles (Figures 1 and 5A, traces b and c) strongly favours the hypothesis that this release occurs via a $Ca^{2+}/nH^+$ antiporter. According to this scheme, and among many other possibilities, this integrated transport system could play important roles in the regulation of both cytosolic $Ca^{2+}$ concentration and $pH_i$. The low activity of the $Na^+/H^+$ antiporter at physiological pH is consistent with a role of this transporter in the regulation of cytosolic pH; activation of this antiporter may protect the cells against alkaline pH by releasing $H^+$ from the acidic vacuoles. In this regard, it is known that in many micro-organisms relatively small increases in $pH_i$ halt cell division and activate the expression of different genes [1]. This role in $pH_i$ regulation is consistent with the rapid $H^+$ release that occurs in permeabilized cells after addition of even low $Na^+$ concentrations (6–18 mM, Figure 3), and with the considerable acidification that could occur in intact cells when intravacuolar protons are released (Figure 6B). On the other hand, an increase in the cytosolic $Ca^{2+}$ concentration evoked by the activation of the putative $Na^+/H^+$ antiporter present in the acidocalcisome membrane may be an important event related to signal-transduction mechanisms in these parasites. Release of $Ca^{2+}$ by this mechanism appears to be slow and to require high $Na^+$ concentrations in permeabilized cells (Figure 1). However, since $Na^+/H^+$ exchangers are known to be regulated in many cells by diverse signalling mechanisms, such as activation of protein kinases A and C [23], activation of this process in intact cells could be involved in a sustained elevation of $[Ca^{2+}]_i$ through its release from acidocalcisomes. In agreement with this hypothesis, it has been reported [24] that activation of the plasma-membrane $Na^+/H^+$ exchanger is a prerequisite for $Ca^{2+}$ mobilization in human platelets, in this case through $Ca^{2+}$ influx from the extracellular medium.

In plant cells, sequestering $Na^+$ within the vacuole (that also accumulates $Ca^{2+}$) plays a major role in $Na^+$ tolerance [1]. Although vacuolar storage of various ions in yeast has been reported, there is no report yet of $Na^+$ transport [1] or of the involvement of $Na^+$ in $Ca^{2+}$ release in either plant cells or yeast. The operation of a vacuolar $Na^+/H^+$ antiport has been reported in isolated tonoplast vesicles of various plants, including glycophytes and halophytes [25–28] and in isolated vacuoles of *Beta vulgaris* [29–31] and *Catharantus roseus* [32]. The transport of $Na^+$ from the cytosol, via the tonoplast antiporter, and its accumulation in the vacuole of halophytes and salt-tolerant glycophytes is an important mechanism for averting the damaging effects of $Na^+$ on key biochemical processes in the cytosol [1]. In certain plants the operation of the $Na^+/H^+$ antiporter at the cytoplasmic membrane excretes $Na^+$ from the cells, and thus acts synergistically with the tonoplast antiporter in maintaining the cytoplasmic $Na^+$ concentration [1,26].

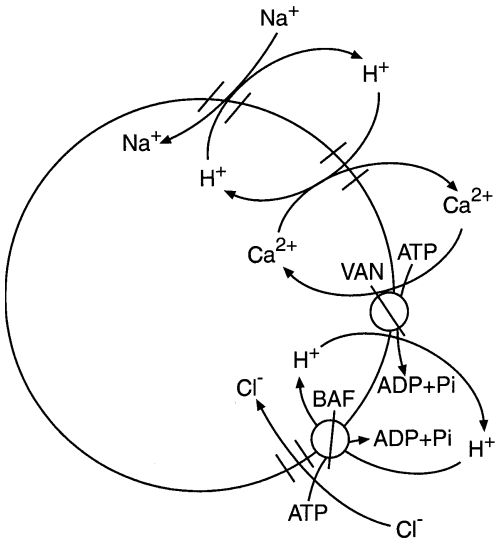


**Figure 7 Model for $Ca^{2+}$ release and $Ca^{2+}$ uptake into acidocalcisomes**

$Ca^{2+}$ uptake occurs in exchange for $H^+$ by a reaction catalysed by a vacuoler $Ca^{2+}$-ATPase, which is inhibited by vanadate. A $H^+$ gradient is established by a bafilomycin $A_1$-sensitive vacuolar $H^+$-ATPase present in the same organelle and associated with $Cl^-$ transport. $Ca^{2+}$ release occurs in exchange for $H^+$ and is favoured by sodium–proton exchange.

There are several reports of the presence of $Ca^{2+}$ in acidic vacuoles [33–39]. The presence of a $H^+$-countertransporting $Ca^{2+}$-ATPase involved in $Ca^{2+}$ sequestration that resides in acidic vacuoles has been described in *Dictyostelium discoideum* [33,34]. Calcium storage in acidic compartments has been reported in some mammalian cells [35–37], although this is controversial [38]. Another possible site for acidic calcium storage is in the contractile vacuole or associated membranes. The 'acidosomes' of the cellular slime mould *D. discoideum*, initially identified as a low-density vesicular fraction containing vacuolar $H^+$-ATPase activity [39] and which also have a $Ca^{2+}/H^+$-ATPase [33], are now thought to represent the contractile vacuole-associated reticular network, the spongiome [40,41]. A similar membrane system, probably involved in osmoregulation, could also be present in trypanosomatids [42]. $Ca^{2+}$ release from the acidic compartment in *D. discoideum* has been found to be sensitive to Ins(1,4,5)$P_3$ by some [43,44], but not by other authors [34], whereas in *T. brucei* Ins(1,4,5)$P_3$ does not release $Ca^{2+}$ from intracellular stores [15]. The involvement of $Na^+$ in $Ca^{2+}$ release from acidic compartments, as occurs in *T. brucei*, is an alternative that warrants further investigation.

We thank Dr. David A. Scott for helpful criticism, Dr. Li Zhong for help with the preparation of cells, and Dr. Rosana Catisti for help in some experiments. This work was supported in part by grants from the National Institutes of Health (AI-23258 and TW-00476) to R.D., and from the Fundação de Amparo a Ensino e Pesquisa da Universidade Estadual de Campinas to A.E.V.A.E.V. is a Biotechnology Career Fellow of the Rockefeller Foundation.

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Received 29 August 1995/25 October 1995; accepted 24 November 1995