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H^+/Ca^{2+} Exchange in Rabbit Renal Cortical Endosomes

Shirley A. Hilden and Nicolaos E. Madias
Department of Medicine, Tufts University School of Medicine, and Division of Nephrology, New England Medical Center, Boston, Massachusetts 02111

Summary. We have examined the effect of second messengers on ATP-driven H^+ transport in an H^+ ATPase-bearing endosomal fraction isolated from rabbit renal cortex. cAMP (0.1 mM) had no effect on H^+ transport. Acridine orange fluorescence in the presence of 0.5 mM Ca^{2+} (+1 mM EGTA) was 19 ± 6% of control. Inhibition of ATP-driven H^+ transport by Ca^{2+} was concentration dependent; 0.25 and 0.5 mM Ca^{2+} (+1 mM EGTA) inhibited acridine orange fluorescence by ~50 and ~80%, respectively. Ca^{2+} also produced a concentration-dependent increase in the rate of pH-gradient dissipation. Ca^{2+} did not affect ATP hydrolysis. ATP-dependent Br^- uptake was virtually unchanged in the presence of 0.5 mM Ca^{2+} (+1 mM EGTA). These vesicles were also shown to transport Ca^{2+} in an ATP-dependent mode. Inositol 1, 4, 5-trisphosphate had no effect on ATP-dependent Ca^{2+} uptake. These results are consistent with the co-existence of an H^+ ATPase and an H^+/Ca^{2+} exchanger on these endosomes, the latter transport system using the H^+ gradient to energize Ca^{2+} uptake. Attempts to demonstrate an H^+/Ca^{2+} antiporter in the absence of ATP have been unsuccessful. Yet, when a pH gradient was established by preincubation with ATP and residual ATP was subsequently removed by hexokinase + glucose, stimulation of Ca^{2+} uptake could be demonstrated. A Ca^{2+}-dependent increase in H^+ permeability and an ATP-dependent Ca^{2+} uptake might have important implications for the regulation of vacuolar H^+ ATPase activity as well as the homeostasis of cytosolic Ca^{2+} concentration.

Key Words H^+/Ca^{2+} exchange · H^+ ATPase · vacuolar ATPase · H^+ transport · endocytosis

Introduction

Proton transport activity mediated by vacuolar H^+ ATPase, that is N-ethylmaleimide (NEM)- and N,N-dicyclohexylcarbodiimide(DCCD)-sensitive and vanadate- and oligomycin-insensitive H^+ ATPase, has been identified in numerous intracellular organelles such as endosomes, Golgi apparatus, lysosomes and plant tonoplasts (Schneider, 1987). The acidic interior created by the H^+ ATPase is considered of critical importance in carrying out an array of organellar functions including receptor-ligand dissociation, protein digestion, secretion and membrane and receptor recycling (Al-Awqati, 1986). Despite the structural and functional similarity of the vacuolar H^+ ATPase, there is considerable variability in the internal pH prevailing among the components of the vacuolar system (Schneider, 1987; Anderson & Orci, 1988). In fact, it has been considered likely that a functional link might exist between the operational diversity of the vacuoles and the variability in their internal pH.

Although the nature of the regulation of vacuolar pH remains largely unknown, several candidate mechanisms have been proposed. Anion channels associated with the H^+ ATPase represent a putative regulatory mechanism (Hilden, Johns & Madias, 1988). If the counterion (Cl^-) conductance were low, the H^+ ATPase would generate a membrane potential, whereas a pH gradient would be generated if the conductance were high. An example of this mechanism has been reported for the serotonin-storing secretory granules of thyroid parafollicular cells (Barasch et al., 1988). It was shown that secretagogues, such as thyrotropin, decrease the internal pH of the granules by opening a Cl^- channel present in parallel with an H^+ ATPase. Electrogenic transporters (such as Na^+, K^+ ATPase) have also been suggested as possible regulators of vacuolar acidification by modulating the membrane potential (Al-Awqati, 1986; Mellman, 1987; Stone & Xie, 1988; Cain, Sipe & Murphy, 1989; Fuchs, Schmid & Mellman, 1989). Thus, recent data have suggested that electrogenic Na^+ transport mediated by Na^+, K^+ ATPase reduces ATP-dependent proton transport in kinetically "early" endosomes isolated from Chinese hamster ovary cells (Fuchs et al., 1989). A third possible modulator of vacuolar acidification, Ca^{2+}, has been suggested. In a variety of organelles of plant cells and in rat parotid endoplasmic reticulum, evidence has been produced in support of an H^+/Ca^{2+} exchanger existing on the same vacuole as the H^+ ATPase and energized by the proton pump

(Hager & Hermsdorf, 1981; Rasi-Caldogno, de Michelis & Pugliarello, 1982; Zocchi & Hanson, 1983; Schumaker & Sze, 1985, 1986; Thévenod & Schulz, 1988). As a result of this secondary transport system, these vacuoles can accumulate Ca^{2+} and, therefore, could represent an intracellular Ca^{2+} storage site potentially responsive to another second messenger, inositol 1, 4, 5-trisphosphate (IP_3); IP_3 has been shown to release Ca^{2+} from a nonmitochondrial pool in rat kidney cortex (Thévenod et al., 1986). In fact, IP_3 results in Ca^{2+} release from oat root tonoplast, an organelle in which Ca^{2+} accumulation occurs as a result of co-existing H^+ ATPase and H^+/Ca^{2+} activities (Schumaker & Sze, 1987).

The present studies provide evidence suggesting that Ca^{2+} functions as a modulator of H^+ ATPase activity in an endosomal fraction isolated from rabbit renal cortex. Modulation appears to be mediated via activation of an H^+/Ca^{2+} exchanger that results in dissipation of the proton gradient.

Materials and Methods

H^+ ATPase Membrane Vesicle Preparation

Membrane vesicle (MV) preparation was carried out as described previously (Hilden et al., 1988). Briefly, New Zealand White rabbits were killed with Beuthanasia-D (Burns-Biotec Laboratories, Omaha, NE) and the kidneys were removed. Cortex was separated from medulla, minced and homogenized in a Teflon-glass homogenizer using 35 ml of homogenizing medium consisting of (in mM): 300 mannitol, 0.1 phenylmethylsulfonyl fluoride (PMSF), 1 EDTA, 25 tris (hydroxymethyl) aminomethane (Tris), pH 7.3. Initial homogenization used seven strokes with a loose-fitting pestle followed by 20 strokes with a tight-fitting pestle. The homogenate was diluted to 140 ml with homogenizing medium and centrifuged at 1,085 × *g* (3,000 rpm, Sorvall SS-34 rotor). The pellet (P_1) was discarded. The supernatant (S_1)was centrifuged at 34,800 × *g* (17,000 rpm) for 20 min. The pellet (P_2) was discarded. Mg gluconate (1 M) was added to the supernatant (S_2) so that the final concentration of Mg gluconate was 10 mM. This mixture was stirred on ice for 20 min. The resulting suspension was centrifuged at 34,800 × *g* for 20 min. The pellet (P_3) was the endosomal H^+ ATPase MV preparation and was resuspended in a small volume for transport or enzyme assays. The endosomal nature of this MV preparation has previously been documented (Hilden et al., 1988).

H^+ Transport

H^+ transport was measured with acridine orange (AO) as described previously (Hilden et al., 1988). Membrane vesicles were suspended in a medium containing (in mM): 100 mannitol, 100 K gluconate, 1 Mg gluconate, 5 N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid (HEPES), pH 7.0. The suspension was centrifuged and resuspended for transport. Membrane vesicles were left on ice until the assay. Vesicles (50 μl, 65–155 μg protein) were added to 3 ml of a medium containing (in mM): 100 mannitol, 100 KCl, 1 $MgCl_2$, 5 HEPES, pH 7.0, and 3 μM AO. The fluorescence of this mixture was measured in a Perkin-Elmer model L8-5 fluorescence spectrophotometer (excitation 490 nm, emission 530 nm). After stabilization of fluorescence, 20 μl of 50 mM Mg adenosine-triphosphate (ATP) was added, and the change in AO fluorescence was monitored as a function of time. AO is a fluorescent weak base that accumulates in acidic compartments. Intravesicular dye at high concentrations results in self-quenching and, therefore, a decrease in the fluorescent signal. Stimulation of H^+ pumping into vesicles by ATP leads to a decrease in the fluorescence intensity of AO. Effects of inhibitors or stimulators were studied by the addition of stock solutions before the addition of vesicles or after the addition of ATP. Initial rates of change in AO fluorescence are reported. Specific changes in this procedure are described in the figure legends.

$^{45}Ca^{2+}$ and $^{82}Br^-$ Uptake

Transport of these compounds was measured using the Millipore filtration technique (Hilden & Sacktor, 1979; Hilden et al., 1988). Specific details are included in the figure legends.

Materials

All chemicals were reagent grade and were purchased from Sigma (St. Louis, MO).

All experiments were done with at least three different membrane preparations. When presenting group results, all experiments performed were averaged, and results are reported as means ± SE.

Results

Effect of Second Messengers

The influence on H^+ transport of two different second messengers, cAMP and Ca^{2+}, was examined. The Table shows that 0.1 mM cAMP had no significant effect on H^+ transport by renal H^+ ATPase MV. Similarly, 0.1 mM dibutyryl cAMP had no effect (*data not shown*). In contrast, 0.5 mM Ca^{2+} (+1 mM EGTA) inhibited 80% of the initial ATP-stimulated change in acridine orange fluorescence by these vesicles. This was not a general permeability change of the vesicle membrane since the ATP-stimulated $^{82}Br^-$ uptake was not changed by addition of Ca^{2+}.

A more detailed picture of the Ca^{2+} inhibition is shown in Fig. 1 using AO to assay H^+ transport. Addition of ATP initiated a gradual decrease in AO fluorescence reaching a new steady state after several minutes. This decrease in AO fluorescence parallels the establishment of a pH gradient across the vesicle membrane. When Ca^{2+} was added to the assay mixture, the ability of ATP to stimulate the formation of an H^+ gradient was eliminated.

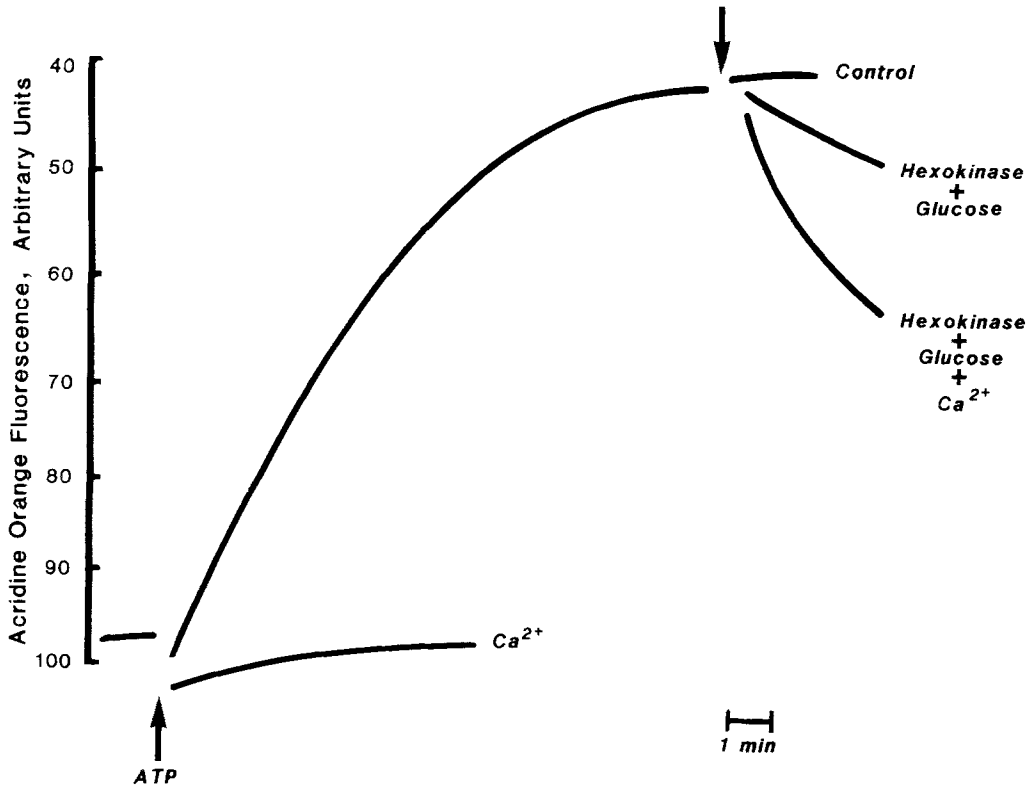

Fig. 1. Effect of Ca^{2+} on ATP-driven H^+ transport in H^+ ATPase membrane vesicles. H^+ ATPase membrane vesicles were equilibrated in a medium containing (in mM): 100 K gluconate, 100 mannitol, 1 Mg gluconate, 1 EGTA, 5 HEPES, pH 7.0. Membrane vesicles (50 μl) were added to 3 ml of a medium containing (in mM): 100 KCl, 100 mannitol, 1 Mg gluconate, 1 EGTA, 5 HEPES, pH 7.0, 0.003 acridine orange ± 1 $CaCl_2$. When fluorescence of acridine orange stabilized, stock MgATP was added so that the final concentration of MgATP was 0.3 mM. After several minutes (second arrow), hexokinase and glucose sufficient to hydrolyze all the original ATP was added with or without $CaCl_2$ (final concentration, 1 mM)

Table. Effect of Ca^{2+} and cAMP on acridine orange uptake and $^{82}Br^-$ uptake in H^+ ATPase membrane vesicles

	% of control	
	Acridine orange uptake	$^{82}Br^-$ uptake
0.5 mM Ca^{2+}	19 ± 6	94 ± 12
0.1 mM cAMP	86 ± 10	

Acridine orange fluorescence was measured as described in Fig. 1. Vesicles for $^{82}Br^-$ uptake were equilibrated in a medium containing (in mM): 100 K gluconate, 100 mannitol, 1 Mg gluconate, 1 EGTA, 5 HEPES, pH 7.0. Membrane vesicles (10 μl) were added to 100 μl medium + 0.5 mM $^{82}Br^-$ ± 0.5 mM Ca gluconate. Means ± SE are reported (n = 4).

Ca^{2+} Inhibition of H^+ Transport

This loss of ATP-driven H^+ transport in the presence of Ca^{2+} was concentration dependent as shown in Fig. 2. Inhibition of AO uptake was about 50% at 0.25 mM Ca^{2+} and about 80% at 0.5 mM Ca^{2+}.

Using the equation of Bulos and Sacktor (1979), the free Ca^{2+} concentrations in our system were calculated. At 0.75 mM Ca^{2+} + 1 mM EGTA, the calculated free-Ca^{2+} concentration was 0.6 μM. At 0.1 mM Ca^{2+} + 1 mM EGTA, the free-Ca^{2+} concentration was calculated as 0.02 μM. Thus, the responses observed in our experiments occurred over the range of free-Ca^{2+} concentration normally seen in the cytosol and the results reported herein might well have physiological significance.

Ca^{2+} Effect on ATP Hydrolysis and H^+ Permeability

The effect of Ca^{2+} on ATP-dependent H^+-gradient formation could be due to a direct effect of Ca^{2+} on the ATPase or to an effect of Ca^{2+} on the ability of the vesicle to maintain a pH gradient. The effect of 0.25–1.0 mM Ca^{2+} (+1 mM EGTA) on ATP hydrolysis was examined in gluconate or Cl^- medium. For

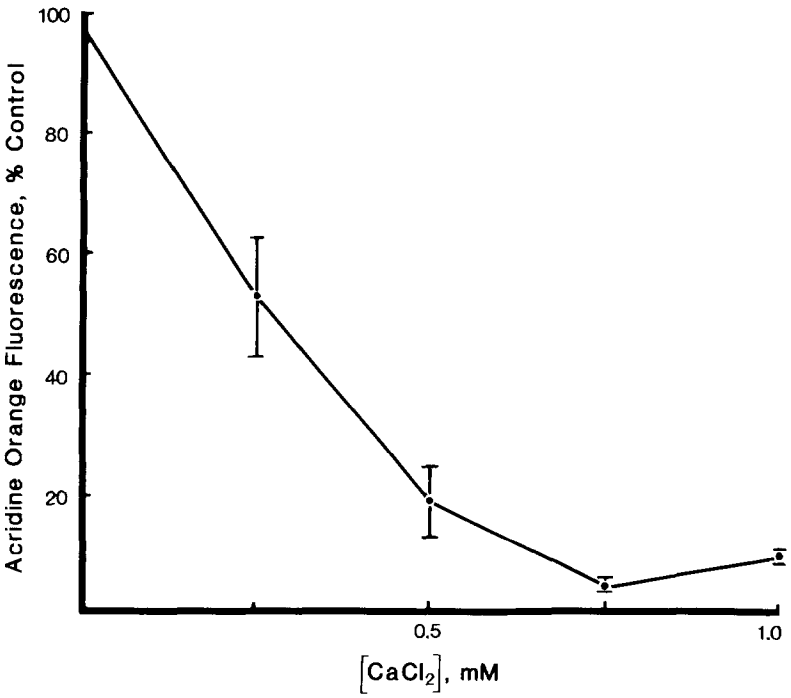

Fig. 2. Effect of Ca^{2+} concentration on ATP-driven H^+ transport in H^+ ATPase membrane vesicles. H^+ transport was measured as described in Fig. 1. Mean ± SE of three experiments at each Ca^{2+} concentration are reported

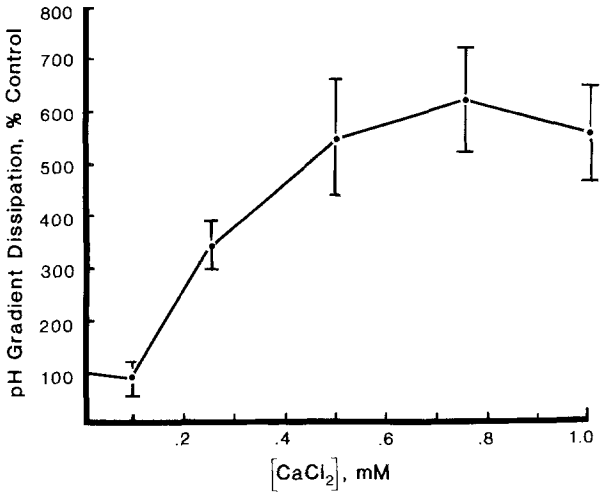

Fig. 3. Effect of Ca^{2+} concentration on pH-gradient dissipation in H^+ ATPase membrane vesicles. H^+ transport was measured as described in Fig. 1. After a pH gradient was established in the presence of ATP, hexokinase and glucose were added in an amount sufficient to remove all of the original ATP. The dissipation of the pH gradient in the absence of added Ca^{2+} was set at 100%. Variable amounts of Ca^{2+} were added and the pH-gradient dissipation measured. Mean ± SE of three experiments at each Ca^{2+} concentration are reported

example, ATP hydrolysis in the presence of 1 mM Ca^{2+} + 1 mM EGTA was 86 ± 5% of the control value measured in the absence of Ca^{2+} (n = 4). This was the maximum change seen in this type of experiment. This result indicates that there is no latent ATPase activity, which can be stimulated by Ca^{2+} to consume sufficient ATP such that the H^+ pump might be decreased because of lack of substrate. On the other hand, Ca^{2+} did appear to have an effect on the ability of these membranes to maintain a pH gradient as shown in Fig. 1. At the point when ATP had induced a steady-state H^+ gradient, hexokinase and glucose were added to remove the unhydrolyzed ATP. In the absence of other additions, the H^+ gradient began to decrease, slowly and steadily, indicating that ATP is necessary to maintain a stable H^+ gradient. When Ca^{2+} was added at the same time as hexokinase and glucose, the dissipation of the H^+ gradient was significantly enhanced. The stimulation of the rate of pH-gradient dissipation by Ca^{2+} was concentration dependent as shown in Fig. 3. pH-gradient dissipation had a dependence on Ca^{2+} similar to the Ca^{2+}-induced inhibition of H^+ transport shown in Fig. 2.

ATP-Dependent $^{45}Ca^{2+}$ Uptake

To further investigate the behavior of Ca^{2+} in this membrane, the uptake of $^{45}Ca^{2+}$ in the presence or absence of ATP was studied. Figure 4 shows that addition of ATP to the incubation mixture stimulated the uptake of $^{45}Ca^{2+}$ by these MV. Therefore, at a Ca^{2+} concentration (0.25 mM) which inhibited about 50% of the AO uptake (Fig. 2), vesicles were taking up Ca^{2+} in an ATP-dependent fashion. Figure 4 presents the results of a single experiment.

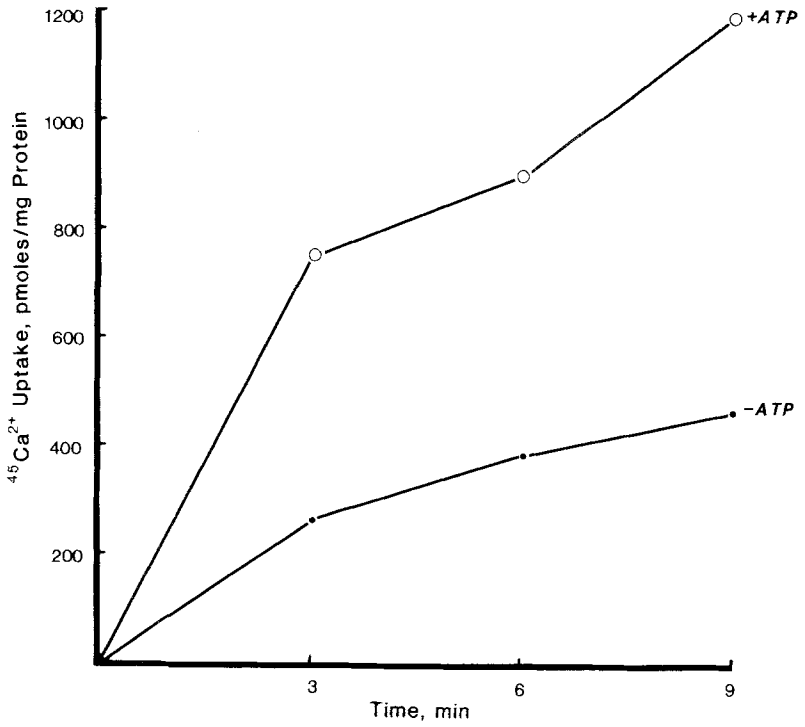

Fig. 4. ATP-dependent $^{45}Ca^{2+}$ uptake in H^+ ATPase membrane vesicles. Membrane vesicles were equilibrated in a medium containing (in mM): 100 KCl, 100 mannitol, 1 EGTA, 1 Mg gluconate, 5 HEPES, pH 7.0, ± MgATP (final concentration, 0.3 mM). Membrane vesicles (10 μl) were added to 100 μl equilibration medium + 0.25 mM $^{45}CaCl_2$. A typical experiment is reported

The average uptake of $^{45}Ca^{2+}$ by the MV in the absence of ATP at 3 min was 19 ± 2% of the uptake seen in the presence of ATP (n = 8). ATP-dependent $^{45}Ca^{2+}$ uptake was not inhibited by 0.05 or 0.5 mM vanadate suggesting that this uptake is not mediated by a vanadate-sensitive $E_1 - E_2$ ATPase, such as the sarcoplasmic reticulum Ca ATPase.

H^+/Ca^{2+} Exchange

A Ca^{2+}-sensitive H^+ transport and an ATP-dependent Ca^{2+} uptake have been reported in other H^+ ATPase MV (plant tonoplasts and rat parotid endoplasmic reticulum; Schumaker & Sze, 1985; Thévenod & Schulz, 1988). These reports suggested that two transport systems might produce these results: a primary transporter, an H^+ ATPase, which drives H^+ uptake and a secondary transport system; and an H^+/Ca^{2+} exchanger, which uses the H^+ gradient to energize Ca^{2+} uptake. Since an H^+/Ca^{2+} exchanger would depend on an H^+ gradient, the ATP-dependence of $^{45}Ca^{2+}$ uptake reported in Fig. 4 could reflect the requirement for the H^+ ATPase to produce a pH gradient by ATP hydrolysis.

If an H^+/Ca^{2+} exchanger exists in the renal cortical H^+ ATPase MV, it should be possible to demonstrate the exchanger in the absence of ATP. Initial attempts to demonstrate this exchanger by equilibrating the MV in acid pH media and measuring $^{45}Ca^{2+}$ uptake from neutral or basic transport media were unsuccessful. Experimental measurements suggest that the passive H^+ permeability of these MV is very low. Figure 5 reports a different approach. In this experiment, MV were equilibrated in the presence or absence of ATP. After 9 min (a time interval sufficient to produce a pH gradient, *see* Fig. 1), residual ATP was removed by adding hexokinase and glucose. 0.5 mM $^{45}CaCl_2$ was added and uptake of $^{45}Ca^{2+}$ was measured in vesicles which had or had not been pre-equilibrated with ATP to generate a pH gradient. If a pH gradient had been established by pre-equilibration with ATP, augmentation of the $^{45}Ca^{2+}$ uptake by the MV was observed. Figure 5 presents the results of a single experiment. Average uptake in vesicles equilibrated in the absence of ATP was 33 ± 9% of the uptake seen in vesicles pre-equilibrated with ATP ($^{45}Ca^{2+}$ uptake at 1 min following addition of hexokinase and glucose) (n = 3). Hexokinase and glucose, as used in these experiments, can remove 90% of the original ATP. Thus, the maximal residual ATP predicted after hexokinase and glucose treatment is on the order of 0.1 mM. We have shown that 0.1 mM ATP does not stimulate $^{45}Ca^{2+}$ uptake by the MV. Therefore, the $^{45}Ca^{2+}$ uptake seen after hexokinase and glucose addition is not due to a Ca ATPase activated by the remaining ATP. Rather, these results are consistent with an H^+ ATPase primary transport system co-existing on the same MV with a secondary transporter, an H^+/Ca^{2+} exchanger.

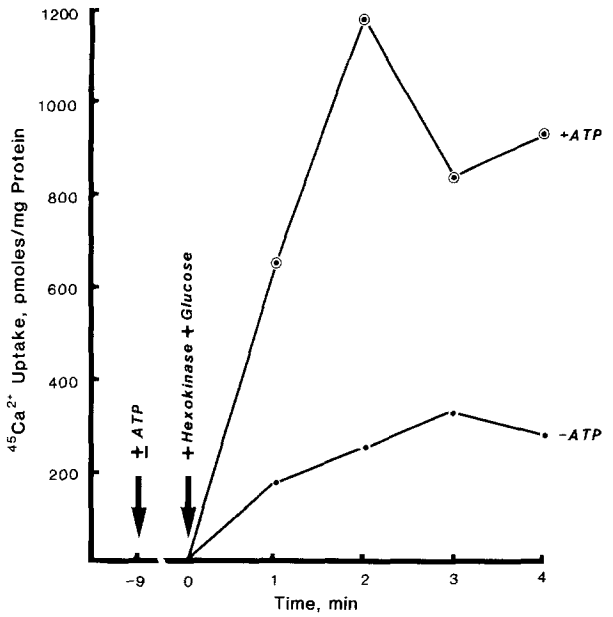

Fig. 5. Stimulation of $^{45}Ca^{2+}$ uptake by an ATP-established pH gradient after removal of residual ATP in H^+ ATPase membrane vesicles. ATP was added to medium containing membrane vesicles as described in Fig. 1. After 9 min, hexokinase + glucose were added as a degenerating system for ATP. Subsequently, 0.5 mM $^{45}CaCl_2$ (final concentration) was added to membrane vesicles which had or had not been subjected to initial incubation with ATP. A representative experiment is shown

Effect of IP_3 + GTP

Inositol 1, 4, 5-trisphosphate (IP_3) is believed to be a second messenger which can induce Ca^{2+} loss from intracellular Ca^{2+} stores such as endoplasmic reticulum. The effect of IP_3 on $^{45}Ca^{2+}$ uptake by these MV was studied. 1 μM IP_3 had no effect on $^{45}Ca^{2+}$ uptake in the presence of ATP (*data not shown*). If ATP was removed afer 9 min (by hexokinase + glucose), 1 μM IP_3 ± 50 μM guanosine 5-triphosphate (GTP) had no effect on $^{45}Ca^{2+}$ content.

Discussion

Effect of Ca^{2+}

In the present studies, the establishment of a pH gradient by vacuolar H^+ ATPase in rabbit renal cortical endosomal MV was inhibited by Ca^{2+} but not by cAMP. Such inhibition was concentration dependent and paralleled the effects of Ca^{2+} in augmenting dissipation of a proton gradient. The observed increase in proton-gradient dissipation, when combined with a lack of effect on ATP hydrolysis, suggests that Ca^{2+} inhibits H^+ transport in renal cortical endosomes by increasing the H^+ permeability of the membrane. The resulting increase in H^+ permeability was specific since Ca^{2+} or Cl^- (Br^-) uptake by the endosomal MV could still be measured (Fig. 4 and the Table). Taken together, these findings provide evidence in support of a role of Ca^{2+} in regulating H^+ ATPase activity in rabbit renal cortical endosomes.

Demonstration of H^+/Ca^{2+} Exchange

The Ca^{2+}-induced H^+ flux might be due to the presence of an H^+/Ca^{2+} exchanger in the endosomal membrane whose activity increases as the Ca^{2+} concentration rises. The observed ATP-dependent Ca^{2+} uptake (Fig. 4) is envisioned to result from an H^+ ATPase-induced proton gradient which then drives a secondary transporter, namely an H^+/Ca^{2+} antiporter; in this context, the increased Ca^{2+} uptake effected by ATP figures as an indirect consequence of ATP hydrolysis-dependent primary transport of H^+. The ability of an H^+ gradient to drive Ca^{2+} uptake in the absence of ATP (shown in Fig. 5) is in support of this explanation. This pH gradient-driven Ca^{2+} uptake could be demonstrated, however, only in the case that the proton gradient had originally been established by ATP addition. Attempts to establish a pH gradient by employing ionophores and/or incubation in low pH medium failed to demonstrate pH gradient-driven Ca^{2+} uptake. This situation has also been seen in endoplasmic reticulum (Thévenod & Schulz, 1988).

Co-Existence of H^+/Ca^{2+} Exchange with Vacuolar H^+ ATPase in Other Cells

An H^+/Ca^{2+} antiporter has been reported to co-exist with a vacuolar H^+ ATPase in numerous plant cells (Hager & Hermsdorf, 1981; Rasi-Caldogno et al., 1982; Zocchi & Hanson, 1983; Schumaker & Sze, 1985, 1986) as well as in rat parotid endoplasmic reticulum (Thévenod & Schulz, 1988). Differences from the present report do exist, however. In plant cells, H^+/Ca^{2+} antiport was demonstrated at much higher Ca^{2+} concentrations. For example, in the absence of a chelator such as EGTA, Schumaker and Sze (1985) demonstrated dissipation of an H^+ gradient by Ca^{2+} in oat root microsomes with a maximal effect appearing at 1 mM Ca^{2+}. In rat endoplasmic reticulum, an H^+ ATPase has been found to co-exist with an H^+/Ca^{2+} exchanger in the parotid gland but evidently not in the pancreas (Imamura & Schulz, 1985; Thévenod & Schulz, 1988). The functional implications of this diversity for the regulation of the H^+ ATPase have not been explored in detail. Of additional interest, it has been

suggested that these membranes also contain a vanadate-sensitive Ca^{2+} ATPase which apparently participates in Ca^{2+} uptake by endoplasmic reticulum (Imamura & Schulz, 1985; Thévenod & Schulz, 1988). In rat liver lysosomes, conflicting results for the effect of Ca^{2+} on proton pumping have been reported and, therefore, the presence of an H^+/Ca^{2+} exchanger is considered uncertain. In this regard, Dell' Antone (1988) has recently reported that substitution of Ca^{2+} for Mg^{2+} reduced the activity of the H^+ ATPase by 80% but that the proton pump functioned well in the presence of both Mg^{2+} and Ca^{2+} suggesting the absence of an H^+/Ca^{2+} antiporter. On the other hand, Moriyama, Takano and Ohkuma (1984) found that CaATP did not drive the H^+ pump but, rather, it supported DCCD-inhibitable ATP hydrolysis suggesting Ca^{2+}-induced uncoupling of ATP hydrolysis and H^+ pumping. Proton transport in the presence of both Mg^{2+} and Ca^{2+} was not tested by these investigators, however. These results suggest that the prevailing chemiosmotic gradients in H^+ ATPase-bearing membranous organelles might produce widely different results depending on the availability of other factors in the organelle, such as enzymes, transporters and receptors. In this regard, the presence or absence of an H^+/Ca^{2+} antiporter constitutes a specific example of a mechanism for regulation of vacuolar H^+ ATPase activity.

Effects of Ca^{2+} have been studied in other H^+ ATPase-bearing organelles besides those in which H^+/Ca^{2+} has been hypothesized. In chromaffin granules, CaATP was shown to be a substitute for MgATP in stimulating H^+ transport (Flatmark et al., 1985). However, similar to the present studies, when MgATP was the substrate for proton transport, 500 μM CaATP (+200 μM Ca EGTA) inhibited the proton pump by 40% suggesting the possibility of H^+/Ca^{2+} exchange. ATP hydrolysis was slightly inhibited in the same experimental condition. Xie and Stone (1988) have also studied the effects of CaATP or MgATP as substrates for H^+ ATPase. Using a purified H^+ ATPase from clathrin-coated vesicles, they demonstrated that MgATP supported both ATP hydrolysis as well as H^+ transport, whereas CaATP activated only ATP hydrolysis. These investigators have suggested that Ca^{2+} can support a partial reaction of the H^+ ATPase. However, when the purified pump was analyzed in the presence of both Mg^{2+} and Ca^{2+}, 0.1 mM Ca^{2+} had no effect on ATP hydrolysis when Mg^{2+} concentration was 1 mM (Fig. 2, Xie & Stone, 1988). Moreover, either 0.13 or 2.13 mM Mg^{2+} supported proton transport in the presence of 2.1 mM Ca^{2+} suggesting the absence of H^+/Ca^{2+} exchange in the purified enzyme. Xie and Stone (1988) contend that divalent cations can serve both a catalytic function (which Ca^{2+} can support) and a coupling function (which is not supported by Ca^{2+}). Sabolić, Haase and Burckhardt (1988) reported that the proton transport of rat liver endosomal H^+ ATPase was not stimulated by Ca^{2+}.

An H^+/Ca^{2+} antiporter has also been reported to reside in rat kidney basolateral membranes in which no H^+ ATPase has been identified. This exchange is believed to be mediated by a vanadate-sensitive ATP-driven Ca^{2+} pump (Tsukamoto, Tamura & Marumo, 1988). In our endosomal MV, vanadate has no effect on either H^+ transport or $^{45}Ca^{2+}$ uptake suggesting that our results do not reflect basolateral contaminants.

Most studies would, therefore, suggest that CaATP cannot support H^+ transport by the H^+ ATPase but that Ca^{2+} might have effects on acidification via an H^+/Ca^{2+} exchange or an uncoupling effect.

IP_3-Sensitive Ca^{2+} Storage Site

A nonmitochondrial Ca^{2+} storage site in rat renal cortical cells has been reported, which evidently responds to IP_3 by releasing Ca^{2+} (Thévenod et al., 1986). In our studies, the inability of IP_3 to change the Ca^{2+} content of rabbit renal cortical endosomes might indicate the absence of an IP_3 receptor on these membranes. The results suggest, therefore, that endosomes might not be part of the IP_3-sensitive intracellular Ca^{2+} storage system in these cells. The strength of this negative result is mitigated, however, by the observation that induction of Ca^{2+} efflux by IP_3 can be lost with time after organelle preparation (Thévenod & Schulz, 1988). Notwithstanding, our hypothesis is that an H^+/Ca^{2+} antiporter in these endosomes does not create an IP_3-sensitive Ca^{2+} storage site but, rather, is a means for H^+ ATPase-bearing vacuoles to respond to changes in cytosolic Ca^{2+}.

In conclusion, Ca^{2+} was found to decrease the pH gradient created by the vacuolar H^+ ATPase in a rabbit renal cortical endosomal fraction. The decrease of the pH gradient appears to be due to the presence of an H^+/Ca^{2+} exchanger in these membranes. Operation of the H^+/Ca^{2+} exchanger might have important implications for the regulation of vacuolar H^+ ATPase activity as well as the homeostasis of cytosolic Ca^{2+} concentration.

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