Glucose activates H\textsuperscript{+}-ATPase in kidney epithelial cells

Suguru Nakamura

Division of Nephrology, Hypertension, and Renal Transplant, Department of Medicine, University of Florida College of Medicine, Gainesville, Florida 32610

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Nakamura, Suguru. Glucose activates H\textsuperscript{+}-ATPase in kidney epithelial cells. Am J Physiol Cell Physiol 287: C97–C105, 2004; 10.1152/ajpcell.00469.2003.—The vacuolar H\textsuperscript{+}-ATPase (V-ATPase) acidifies compartments of the vacuolar system of eukaryotic cells. In renal epithelial cells, it resides on the plasma membrane and is essential for bicarbonate transport and acid-base homeostasis. The factors that regulate the H\textsuperscript{+}-ATPase remain largely unknown. The present study examines the effect of glucose on H\textsuperscript{+}-ATPase activity in the pig kidney epithelial cell line LLC-PK\textsubscript{1}. Cellular pH was measured by performing ratiometric fluorescence microscopy using the pH-sensitive indicator BCECF-AM. Intracellular acidification was contained 1 μM ethylisopropylamiloride and were K\textsuperscript{+} free to eliminate Na\textsuperscript{+}/H\textsuperscript{+} exchange and H\textsuperscript{+}/K\textsuperscript{+}-ATPase activity. After NH\textsubscript{3}/NH\textsubscript{4}\textsuperscript{+}-induced acidification, LLC-PK\textsubscript{1} cells had a significant pH\textsubscript{i} recovery rate that was inhibited entirely by 100 nM of the V-ATPase inhibitor concanamycin A. Acute removal of glucose from medium markedly reduced V-ATPase-dependent pH\textsubscript{i} recovery activity. Read- dition of glucose induced concentration-dependent reactivation of V-ATPase pH\textsubscript{i} recovery activity within 2 min. Glucose replacement produced no significant change in cell ATP or ADP content. H\textsuperscript{+}-ATPase activity was completely inhibited by the glycolytic inhibitor 2-deoxy-o-glucose (20 mM) but only partially inhibited by the mitochondrial electron transport inhibitor antimycin A (20 μM). The phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin (500 nM) abolished glucose activation of V-ATPase, and activity was restored after wortmannin removal. Glucose activates V-ATPase activity in kidney epithelial cells through the glycolytic pathway by a signaling pathway that requires PI3K activity. These findings represent an entirely new physiological effect of glucose, linking it to cellular proton secretion and vacuolar acidification.

Proximal tubule transporter NHE-3 and H\textsuperscript{+}/H\textsubscript{CO}_3\textsuperscript{–} exchange and H\textsuperscript{+}-ATPase transport is coupled with glycolysis in urinary epithelia. Steinmetz et al. (55) demonstrated close coupling of proton secretion with anaerobic lactate production in the turtle urinary bladder. Kurtz (29) found that H\textsuperscript{+}-ATPase activity in the rabbit S3 proximal tubule was inhibited by the sulphydryl reagent iodoacetate, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), suggesting dependence of the H\textsuperscript{+}-ATPase on glycolysis. In the genetic disorder hereditary fructose intolerance, caused by deficiency in the glycolytic enzyme aldolase B, fructose induces rapid, severe proximal renal tubular acidosis (35) thought to be caused by inhibition of residual aldolase activity from fructose metabolites (5).

Recently, studies have shown that H\textsuperscript{+}-ATPase binds directly to the glycolytic enzyme aldolase and colocalizes with the H\textsuperscript{+}-ATPase in two proton-transporting cell types: renal proximal tubule cells and osteoclasts (32). The functional importance of the interaction was demonstrated in Saccharomyces, in which deletion of the aldolase gene resulted in disassembly of V-ATPase (32), similarly to the disassembly observed after glucose removal (25), providing further evidence for direct coupling of V-ATPase with the glycolytic pathway. The present study shows that glucose activates V-ATPase activity in renal epithelial cells through a pathway requiring aerobic glycolysis, providing further evidence for coupling between V-ATPase activity and glycolysis.

METHODS

Cell culture. LLC-PK\textsubscript{1} cells were cultured in medium 199 supplemented with 10% (vol/vol) fetal bovine serum, 50 U/ml penicillin, and 50 μg/ml streptomycin. Cells were maintained in culture at 37°C in 5% CO\textsubscript{2} in air. The culture medium was replaced every 3 days, and cells were passaged only when confluent. LLC-PK\textsubscript{1} cell monolayers were grown on 40-mm-diameter coverslips and plated at a density of 6–8 × 10\textsuperscript{4} cells per dish (60 × 15 mm). Experiments were performed on confluent monolayers 3–6 days after cell passage.

Solutions. The standard HEPES-buffered solutions contained (in mM) 125 NaCl, 5 KCl, 1.2 MgSO\textsubscript{4}, 2 Na\textsubscript{2}HPO\textsubscript{4}, 32 HEPES, and 10.5 glucose, pH adjusted to 7.4 with NaOH. All experiments were performed in the nominal absence of CO\textsubscript{2}/HCO\textsubscript{3}\textsuperscript{–}. In the Na\textsuperscript{+}- and K\textsuperscript{+}-free solutions, N-methyl-D-glucammonium (NMDG\textsuperscript{+}) was substituted for Na\textsuperscript{+} and K\textsuperscript{+}, and the solutions were adjusted to pH 7.4 with Tris. In the 20 mM Na\textsubscript{2}H\textsubscript{3}O\textsubscript{4} solutions, either 20 mM NaCl or...
20 mM NMDG for Na+-free solutions was replaced by an equal concentration of NH₄Cl. In the calibration solution, which contained 10 μM of nigericin, NaCl was replaced by 105 mM of KCl and 20 mM of NMDG-Cl.

**In vitro microperfusion.** In vitro microperfusion was performed as described previously (36–40). The kidney from a male Sprague-Dawley rat was decapsulated, cross-sectioned, and placed immediately in a petri dish containing dissecting solution (i.e., glucose-free standard solution). Each section was cut into smaller wedges from the papillary tip to the cortex and transferred into a second petri dish containing dissecting solution maintained at 4°C under a dissecting microscope (Nikon SMZ-645). Proximal tubules were dissected under ×50 magnification. They were transferred to a lucite chamber containing bathing solution, which initially was maintained at room temperature. One end of the proximal tubule was pulled into an outer pipette. Once secure, the inner perfusion pipette was advanced, and the proximal tubule was opened with slight positive pressure. The opposite end of the proximal tubule was then pulled into a holding pipette. The proximal tubule was bathed in a low-volume laminar flow chamber. Solutions were continuously bubbled with 100% O₂ and delivered at the rate of 6 ml/min to the bathing chamber in water-jacketed lines at 37°C. Perfusion rates were maintained at 10–15 nl/min (microperfusion system; Vestavia Scientific, Birmingham, AL).

**Intracellular pH measurements.** LLC-PK₁ cells were grown to confluence on coverslips and incubated in the presence of 15 μM of BCECF-AM for 20 min at 37°C in culture medium as described above. After BCECF incubation, coverslips were placed in a closed perfusion chamber with a volume of 0.3 ml (Biopotech FS2; Bioptechs, Butler, PA) mounted on an inverted fluorescence microscope (Nikon TE-300). Solutions were preheated and delivered to the chamber at 37°C in lines that allowed any combination of four solutions at a flow rate of 5 ml/min. All experiments were started ~5 min after removal of BCECF-AM and were performed with the solutions at 37°C.

Intracellular pH (pHᵢ) in single LLC-PK₁ cells was measured by ratiometric fluorescence (50) using excitation at 440 and 490 nm and measurement of light emission at 520 nm (Intracellular Imaging, Cincinnati, OH). Intracellular acid loading was induced by the NH₄⁺/NH₃⁺ prepulse method (9, 41). Cells were exposed to 20 mM of NH₄Cl/NH₄⁺ for 5 min, and H⁺-ATPase activity was determined as the initial rate of pHᵢ recovery was measured in the absence of Na⁺, K⁺, and HCO₃⁻ after NH₄⁺/NH₃⁺ removal (41). The solutions also contained 1 μM ethylisopropylamiloride (EIPA) to eliminate Na⁺/H⁺ exchange (19).

pHᵢ was calculated on the basis of fluorescence ratios from an intracellular calibration curve constructed at the end of each experiment using the nigericin/high-K⁺ technique (60) and a linear calibration formula (440/490-nm ratio = a + b × pHᵢ) (60). Calibration analysis was performed separately for each cell. Rates of pHᵢ recovery (dpHᵢ/dt) were determined on the basis of the slopes of the linear regression lines of measurements taken during the first 3 min of recovery and expressed as change in pH units per second (30, 60).

**Determination of buffering capacity.** Intracellular buffer capacity (βᵢ) was determined with the formula βᵢ = [NH₄⁺]/pHᵢ, using the technique described by Boyarsky et al. (10) and calculated according to the method of Weintraub and Machen (62). In our system, βᵢ refers to the ability of intrinsic cellular components (excluding HCO₃⁻/CO₂) to buffer changes in pHᵢ and thus βᵢ values were estimated with the use of HEPES-buffered solutions.

βᵢ is defined as [base]/pHᵢ and is most precisely estimated in cells whose pHᵢ regulatory mechanisms are blocked. H⁺-HCO₃⁻ membrane transporters were blocked by a 0 mM Na⁺, 0 mM K⁺ solution plus 100 mM nigericin A. At steady-state pHᵢ, addition of 20 mM of NH₄Cl/NH₄⁺ (NH₄⁺ replacing NMDG⁺) caused a rapid increase in cell pH due to the influx of NH₄+ and subsequent generation of H⁺. Extracellular NH₄⁺ concentration ([NH₄⁺]) was then reduced in a stepwise manner to 0 mM (20, 10, 5, 2.5, and 0 mM) in the nominal absence of HCO₃⁻/CO₂. The rate of transmembrane H⁺ flux (J⁺ᵢ) was calculated by using the equation J⁺ᵢ = (dpHᵢ/dt) × βᵢ, where dpHᵢ/dt is the initial rate of pHᵢ recovery after an acid pulse and βᵢ is the cytosolic buffering capacity averaged for the respective pH interval.

**Materials.** Medium 199 was purchased from Mediatech (Herndon, VA). LLC-PK₁ cells were obtained from the American Type Culture Collection (Manassas, VA). Fetal bovine serum was purchased from GibCO-BRL (Gaithersburg, MD). BCECF-AM was obtained from Molecular Probes (Eugene, OR). Concanamycin A, nigericin, EIPA, 2-deoxy-D-glucose (2-DG), antimycin A, pyruvate, wortmannin, and other chemicals were purchased from Sigma (St. Louis, MO).

**Statistics.** Data are expressed as means ± SE where appropriate. Analysis of variance and the t-test were performed as appropriate to determine statistical significance. P < 0.05 was considered statistically significant.

**RESULTS**

**Na-independent concanamycin-sensitive and plasma membrane V-ATPase activity in LLC-PK₁ cells.** LLC-PK₁ cells possess several pH regulatory mechanisms that allow them to restore intracellular pH to baseline levels after acute intracellular acidification (19, 34, 53). To determine whether V-ATPase participates in pH recovery from intracellular acidification, pHᵢ changes were examined in confluent monolayers of LLC-PK₁ cells using the pH probe BCECF (50). Cells were maintained in Na⁺-containing solution, which was replaced by a Na⁺-free solution 5 min before initiation of pHᵢ measurements. After NH₃/NH₄⁺-induced acidification, in the presence of 10.5 mM glucose, Na⁺- and K⁺-independent pHᵢ recovery (dpHᵢ/dt) was observed at a rate of 13.8 ± 3.3 × 10⁻⁴ pH units/s (n = 23) (Fig. 1A). pHᵢ recovery was inhibited completely by the H⁺-ATPase inhibitor concanamycin A (100 nM) (Fig. 1B), indicating that Na-independent proton secretion in LLC-PK₁ cells is likely due to a plasma membrane V-ATPase. In the presence of concanamycin, Na⁺ addition caused pHᵢ to recover fully to baseline, likely by Na⁺/H⁺ antiport (19, 34), showing that concanamycin does not affect other pH recovery mechanisms.

Glucose activates H⁺-ATPase activity in LLC-PK₁ cells. H⁺-ATPase activity in LLC-PK₁ cells was inhibited markedly by removal of glucose from the medium (Fig. 2). To determine the time course of this effect, the pHᵢ recovery rate in the presence of 10 mM glucose (Fig. 2, period C) was determined and repeated measurements were performed of pHᵢ recovery rate 5 min after removal of glucose from the medium (Fig. 2, period E) and after replacement of glucose in the medium (period F). H⁺-ATPase-mediated pHᵢ recovery was undetectable 5 min after removal of glucose, and H⁺-ATPase activity returned rapidly after readdition of glucose. Recovery was detectable at 2.5 ± 0.6 min (n = 6) after readdition of glucose and half-maximal at 6.3 ± 0.7 min (n = 6). These data demonstrate that LLC-PK₁ cells contain glucose-activated plasma membrane H⁺-ATPase, the activity of which is dependent on the continual presence of glucose.

The glucose concentration dependence of H⁺-ATPase activation was determined by stimulating cells with varying (0–30 mM) concentrations of glucose after acute glucose removal (Fig. 3). The maximal effect of glucose was observed at 20 mM, with a Kₘ of 5.1 mM glucose. pH recovery rates were 3.4 ± 0.5 × 10⁻⁴ pH units/s for 0 mM glucose (n = 17 cells), which were significantly lower than those observed in the presence of glucose.
6.2 ± 0.9 × 10⁻⁴ pH units/s for 2.5 mM glucose (P < 0.02; n = 18), 9.2 ± 0.4 × 10⁻⁴ pH units/s for 5.5 mM glucose (P = 0.005; n = 21), 13.8 ± 0.7 × 10⁻⁴ pH units/s for 10.5 mM glucose (P < 0.00001; n = 23), 16.1 ± 1.6 × 10⁻⁴ pH units/s for 15 mM glucose (P = 0.082, not significant (NS); 10.5 vs. 15 mM; n = 19), and 17.2 ± 2.0 × 10⁻⁴ pH units/s for 20.5 mM glucose (P = NS, 10.5 vs. 20.5 mM; P = NS, 15 vs. 20.5 mM; n = 24), pH recovery rates at 25 and 30 mM glucose decreased from the maximum to 16.9 ± 3.5 × 10⁻⁴ pH units/s (P = NS; n = 15) and 16.3 ± 1.2 × 10⁻⁴ pH units/s (P = NS; n = 18), respectively. The concentration dependence of glucose-stimulated proton flux [calculated by using the equation \( J_H = \frac{d\text{pH}_i}{dt} \beta_i \), where \( \beta_i \) is buffering capacity as described in METHODS] was similar to that for pH recovery.

Amlal et al. (7) reported \( H^+\)-ATPase activity in LLC-PK₁ cells activated by hypotonicity. To determine whether glucose-induced \( H^+\)-ATPase activation was a result of changing the solution osmolarity in the present study, the effect of 10 mM mannitol was examined as a substitute for glucose, using the procedure shown in Fig. 2. As shown in Fig. 4, mannitol failed to activate \( H^+\)-ATPase activity, showing that glucose-induced \( H^+\)-ATPase activation is not due to an increase in solution osmolarity.
Glucose activates H⁺/H⁺-ATPase activity in isolated perfused rat PT S3 segments. Proximal tubules were perfused with Na⁺- and K⁺-free buffers, and the rate of pHi recovery was determined after 5 min of NH₄Cl incubation and removal, initially in 2.5 mM glucose, followed by 10.5 mM glucose. As shown in Fig. 5A, changing the glucose concentration from 2.5 to 10.5 mM produced a significant increase in pHi recovery rates, from 6.2 ± 3.4 × 10⁻⁴ to 16.9 ± 0.5 × 10⁻⁴ pH units/s (P < 0.05, n = 14; Fig. 5B). The results suggest that intact rat renal proximal tubule cells have glucose-activated plasma membrane H⁺-ATPase activity similar to that observed in LLC-PK₁ cells.

Glucose activation of H⁺-ATPase requires glycolysis. In principle, glucose could activate the H⁺-ATPase through metabolism, through signaling pathways, or both. To determine whether activation of H⁺-ATPase activity by glucose requires metabolism through glycolysis, the effect of 20 mM of 2-DG, a glycolytic inhibitor, was examined. As shown in Fig. 6, pretreatment with 2-DG eliminated glucose-activated H⁺-ATPase activity, suggesting that the response requires metabolism of glucose through glycolysis. Treatment with the mitochondrial electron transport inhibitor antimycin A (20 μM) partially inhibited activation of H⁺-ATPase by glucose (Fig. 7), suggesting that activation requires aerobic rather than anaerobic glycolysis. In support of this interpretation, it was found that 10 mM of pyruvate, the mitochondrial substrate for aerobic glycolysis, induced concanamycin-sensitive pH recovery, indicating activation of H⁺-ATPase activity (Fig. 8). In contrast, sodium acetate produced cytosolic alkalinization that was unaffected by concanamycin A (Fig. 9), probably by metabolism to bicarbonate in mitochondria. Alanine (10 mM) also was unable to activate H⁺-ATPase activity (Fig. 10).

Glucose activation of H⁺-ATPase requires phosphatidylinositol 3-kinase activity. In insulin-responsive tissues (11, 20, 59), lymphocytes (48), and other cells (11), signaling pathways...
involving phosphatidylinositol 3-kinase (PI3K) are involved in controlling glucose entry (59) and metabolism (20). To determine whether a PI3K-dependent signaling pathway contributes to the effect of glucose on H<sup>+</sup>/H<sub>11001</sub>-ATPase activity, the effect of 500 nM wortmannin, a PI3K inhibitor, was studied. As shown in Fig. 11, H<sup>+</sup>/H<sub>11001</sub>-ATPase activity induced by 10 mM of glucose was inhibited completely by wortmannin, and activity was restored after wortmannin removal.

**DISCUSSION**

These studies demonstrate that glucose is a potent and rapid activator of H<sup>+</sup>-ATPase-mediated proton secretion in both LLC-PK<sub>1</sub> cells and isolated renal proximal tubules. The glucose effect was concentration dependent, with a half-maximal effect at 5.1 mM of glucose and a maximal effect at 20 mM. Glucose-induced V-ATPase activation was rapidly reversible. V-ATPase activity was lost after 5–10 min of glucose removal and was restored rapidly by the readdition of glucose, with mean times of 2–3 min to initial recovery and 5.5–7 min to half-maximal recovery. The effect was not due to changes in extracellular fluid osmolarity, because mannitol produced no significant effect. Glucose-induced V-ATPase activation required metabolism of glucose through the glycolytic pathway, because it was inhibited entirely by 2-DG. Metabolism likely occurs through aerobic glycolysis because the mitochondrial complex III inhibitor antimycin partially inhibited V-ATPase activation. Pyruvate, an end product of the glycolytic pathway, activated V-ATPase activity in the absence of glucose, but alanine and acetate did not activate V-ATPase activity. These results extend those of the recent studies of the interaction of the V-ATPase with aldolase (32) and provide further evidence for coupling between V-ATPase activity and glycolysis.

The brewer’s yeast *Saccharomyces cerevisiae* has V-ATPase on the vacuolar membrane that is similar in structure and properties to mammalian V-ATPases (15, 17, 42, 56, 63). Yeast use glucose preferentially as a substrate for anaerobic glycolysis in ethanol production (23). Glucose removal induced rapid disassembly of yeast V-ATPase. Rapid reassembly occurred in a concentration-dependent manner with glucose retreatment (25, 45) and required metabolism of glucose beyond the formation of glucose 6-phosphate (45). A recent study (32) showed that V-ATPase binds directly to aldolase and that V-ATPase disassembly occurs in yeast strains that are deficient in aldolase and other glycolytic enzymes. Collectively, these experiments demonstrate that glycolysis is essential for assembly and function of the *Saccharomyces* V-ATPase.

Several studies have examined metabolic pathways supporting proton transport by V-ATPases in urinary epithelia (3, 29). In the turtle urinary bladder, a model epithelium resembling the mammalian kidney cortical collecting duct (2, 54) with an electrogenic plasma membrane V-ATPase (16, 65), glucose stimulated electrogenic V-ATPase-mediated proton transport (1, 3, 27, 55). Under standard conditions, transport was inhibi-

Fig. 6. Glycolysis is required for glucose-induced V-ATPase activation. Procedures used were identical to those in Fig. 2, except that glycolytic inhibitor 2-deoxy-D-glucose (2-DG; 20 mM) was added during second glucose treatment (period F), which completely inhibited V-ATPase activation.

Fig. 7. Effect of antimycin A on glucose-induced V-ATPase activation. Procedures used were identical to those in Fig. 2, except that mitochondrial electron transport inhibitor antimycin A (20 μM) was added during second glucose treatment (period F), which partially inhibited V-ATPase activation. Incomplete recovery of V-ATPase activity was observed after antimycin A removal (period G).
H+/H$_{1001}$-ATPase activity was identified in rabbit renal proximal tubules that was inhibited by the glycolytic inhibitor iodoacetate, suggesting coupling of activity with glycolysis (29). Proximal tubules have a low rate of lactate production (8), indicating that glucose metabolism in this segment occurs primarily by aerobic glycolysis. The present study shows that H+/H$_{1001}$-ATPase activity in rat proximal tubules is stimulated by increasing the extracellular glucose concentration, a response similar to the glucose-induced V-ATPase activation observed in LLC-PK$_1$ cells.

Nakamura et al. (39) previously examined the effect of glucose on the levels of ATP, ADP, and ATP-to-ADP ratio in serum- and/or glucose-starved LLC-PK$_1$ cells. Their study showed that incubation in glucose-free medium for 16 h reduced cell ATP content by 37% and increased ADP content slightly, changing the ATP-to-ADP ratio significantly from 2.53 to 1.22. However, stimulation with 10 mM glucose for periods ranging from 2.5 to 30 min produced no significant changes in ATP or ADP content. These results indicate that stimulation of V-ATPase activity by glucose, at least within short time intervals, likely does not occur by increasing ATP availability.

Recently, other studies have shown that ATP level remained practically unchanged when yeast cells were grown in either 2% or 0.025% glucose (44). Krauss et al. (28) demonstrated that under physiological conditions, hyperglycemia-induced mitochondrial superoxide production activates uncoupling protein 2, which decreases the ATP-to-ADP ratio.

Other previous studies have shown that glucose stimulates glycolysis and increases the activity of several glycolytic enzymes in LLC-PK$_1$ cells (18). The present study revealed that the glycolytic inhibitor 2-DG prevented glucose-induced V-ATPase activation in LLC-PK$_1$ cells. Taken together, these results suggest that glucose activates H+/H$_{1001}$-ATPase by stimulating glycolysis.

The present study shows that pyruvate also activated H+/H$_{1001}$-ATPase activity in the absence of glucose. It is possible that pyruvate could be converted to glucose through the gluconeogenic pathway and subsequently metabolized by glycolysis (51). In the presence of the H+/H$_{1001}$-ATPase inhibitor concanamycin A, it was observed that pyruvate-induced H+/H$_{1001}$-ATPase activity was inhibited (Fig. 8; pyruvate + CCA, period F) and that H+/H$_{1001}$-ATPase activity was restored by pyruvate after CCA removal (Fig. 8; pyruvate only, period G). As shown in Fig. 9, however, the H+/H$_{1001}$-ATPase inhibitor (i.e., CCA) had no effect on acetate-induced pH recovery (Fig. 9; NaAc + CCA, period F), likely due to a mechanism other than H+/H$_{1001}$-ATPase activity.

Fig. 8. Pyruvate activates V-ATPase in absence of glucose. Cells were treated with NH$_4$Cl in presence of 10 mM glucose to induce pH acidification (periods A and B) followed by removal of glucose and treatment with 10 mM pyruvate (period C), which produced significant pH recovery. After repeat pH acidification in presence of glucose (period D), cells were treated with glucose-free solution (period E), which produced no pH recovery, and then with 10 mM pyruvate containing 100 nM CCA (period F), which also produced no recovery. Partial pH recovery was observed after removal of CCA (period G).

Fig. 9. Sodium acetate (NaAc) alkalinizes intracellular pH but does not activate V-ATPase. Procedures used were identical to those in Fig. 9, except that 10 mM sodium acetate replaced pyruvate. Acetate produced alkalinization of pH (period C) that was not observed after acetate removal (period E) and was not affected by V-ATPase inhibitor CCA (period F).
involvement for the regulation of intracellular pH. The alkalization observed with acetate might be due to metabolic generation of alkali. Ishikawa et al. (22) showed that acetate induced cytosolic alkalization that was not affected by H⁺-ATPase inhibitors and probably was a result of mitochondrial metabolism of acetate to bicarbonate. Studies in the kidney (26, 51) have demonstrated the importance of such “futile” cycles of glycolysis and gluconeogenesis, which provide the capacity for rapid changes in glycolytic flux (24, 46). Net ATP consumption in futile cycles may be reduced by separation and compartmentalization of the glycolytic and gluconeogenic pathways (24).

Both pyruvate and acetate are metabolized by the citric acid cycle. The metabolism of acetate through acetyl coenzyme A-synthetase consumes one ATP and generates one less NADH and CO₂ (22) than does metabolism of pyruvate through pyruvate dehydrogenase. Acetate also inhibits some of the pathways for pyruvate metabolism (13). The observed differences between these two agents on V-ATPase activation strongly implicate mitochondrial metabolism in the activation pathway, consistent with the partial inhibition of activation observed with antimycin.

It is also significant that alanine was unable to activate V-ATPase. Although alanine can be converted to pyruvate by transamination (61, 64) and is a potential substrate for gluconeogenesis (33, 61), studies in both isolated renal proximal tubules (31, 47) and human volunteers (12, 57) have shown that alanine is a poor substrate for renal gluconeogenesis compared with lactate. These studies support the interpretation that substrate flux through the glycolytic pathway is required for V-ATPase activation. The results observed are not specific for V-ATPase, however, because Hering-Smith and Hamm (21) found that alanine was not an effective metabolic substrate for collecting duct Na⁺ transport in the absence of glucose.

The results of the present study indicate that PI3K activity is required for glucose activation of V-ATPase (Fig. 11). PI3K activity is required for several signaling pathways involved in glucose control, including glucose entry and glycogen metabolism (11, 20, 59). The signaling pathways downstream from PI3K and targets leading to V-ATPase activation remain to be determined in future studies.

In conclusion, glucose activates V-ATPase activity in renal epithelial cells through a pathway requiring aerobic glycolysis and PI3K activity, providing further evidence for coupling between V-ATPase activity and glycolysis.

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Current address of S. Nakamura: Dept. of Biological Sciences, Murray State Univ., 334 Blackburn Science Bldg., Murray, KY 42071-3346.

GRANTS

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Fig. 10. Alanine does not activate V-ATPase. Procedures used were similar to those in Fig. 4, except that 10 mM alanine replaced mannitol. No V-ATPase activity was observed with alanine in the absence of glucose (period F), but activity resumed after replacement of glucose (period G).

Fig. 11. Glucose-induced V-ATPase activation requires phosphatidylinositol 3-kinase (PI3K) activity. Procedures used were similar to those in Fig. 10, except that 10 mM glucose replaced acetate and PI3K inhibitor wortmannin (500 nM) replaced CCA. Glucose-induced V-ATPase activity was abolished by wortmannin (period F) and partially recovered after removal of wortmannin (period G).
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