J. Membrane Biol. 105, 197–205 (1988)

The Journal of
Membrane Biology

Na^+ and K^+ Fluxes Stimulated by Na^+-Coupled Glucose Transport: Evidence for a Ba^{2+}-Insensitive K^+ Efflux Pathway in Rabbit Proximal Tubules

M.J. Avison, S.R. Gullans, T. Ogino, and G. Giebisch
Departments of Molecular Biophysics & Biochemistry, and Physiology, Yale University School of Medicine, New Haven, Connecticut

Summary. Addition of glucose or the nonmetabolizable analogue α-methyl-D-glucoside to rabbit proximal tubules suspended in a glucose- and alanine-free buffer caused a sustained increase in intracellular Na^+ content (+43 ± 7 nmol · (mg protein)$^{-1}$) and a concomitant but larger decrease in K^+ content (−72 ± 11 nmol · (mg protein)$^{-1}$). A component of the net K^+ efflux was Ba^{2+} insensitive, and was inhibited by high (1 mM) but not low (10 μM) concentrations of the diuretics furosemide and bumetanide. The increase in intracellular Na^+ content is consistent with the view that the increased rates of Na^+ and water transport seen in the proximal tubule in the presence of glucose can be attributed (at least in part) to a stimulation of basolateral pump activity by an increased $[Na^+]_i$.

Key Words proximal tubule · Na^+ · K^+ · furosemide · bumetanide · glucose

Introduction

The reabsorption of glucose (and many other organic solutes) by the renal proximal tubule and small intestine is a two-step process consisting of Na^+-coupled uptake across the luminal membrane, followed by passive facilitated diffusion of glucose and active extrusion of Na^+ across the basolateral membranes (Schultz, Frizzell & Nellans, 1974; Ullrich, 1979; Schafer & Williams, 1985; Lang, Messner & Rehwald, 1986). Activation of this pathway results in a rapid and sustained increase in the rate of active Na^+ reabsorption (Burg et al., 1976; Gullans, Harris & Mandel, 1984). The simplest explanation for this stimulation of Na^+ reabsorption is that the enhanced rate of Na^+ entry causes an increase in $[Na^+]_i$,[1] which in turn stimulates the basolateral Na^+,K^+-ATPase. Unfortunately, experiments designed to assess the effects of Na^+-coupled transport of organic solutes on Na_i^+ have yielded ambiguous and even contradictory results. Thus previous studies have failed to detect a sustained increase in $[Na^+]_i$ (or intracellular Na^+ activity) following 3-O-methylglucose- (Lee & Armstrong, 1972) or galactose- (Hudson & Schultz, 1984) stimulated Na^+ reabsorption in amphibian small intestine; indeed, in one case (Lee & Armstrong, 1972) Na_i^+ was reported to fall. Conversely, Messner, Koller and Lang (1985) found that addition of 10 mM phenylalanine to the luminal perfusate of frog proximal tubules led to a substantial increase in Na_i^+, and White, Burnup and Ellingsen (1986) detected significant increases in Na_i^+ in *Amphiuma* small intestine following galactose or valine addition, but no significant increase following glucose addition. Finally, Morgunov and Boulpaep (1987) showed that luminal addition of 10 mM glucose caused an increase in Na_i^+ in perfused *Ambystoma* proximal tubule.

In the present study we have investigated the behavior of intracellular Na^+ following the addition of glucose or α-methyl-D-glucoside (αMG) to suspensions of rabbit cortical tubules, using ^{23}Na NMR to measure intracellular Na^+ content. We have also investigated the nature of the pathways of K^+ uptake and efflux prior to and following glucose addition by monitoring changes in $[K^+]_o$ with a K^+-sensitive electrode.

The results indicate that, following glucose addition to tubule suspensions (to 7 mM), intracellular Na^+ content increased by 43 ± 7 nmol · (mg protein)$^{-1}$ (+ ~30%) while intracellular K^+ content decreased by 72 ± 11 nmol · (mg protein)$^{-1}$ (− ~28%). The K^+ loss occurred at least in part via a Ba^{2+}-insensitive pathway. It was significantly blunted by 1 mM bumetanide or furosemide, although these drugs were ineffective at 10 μM.

[1] *Abbreviations:* $[Na^+]_i$—intracellular Na^+ concentration; $[K^+]_i$—intracellular K^+ concentration; $[Na^+]_o$—extracellular Na^+ concentration; $[K^+]_o$—extracellular K^+ concentration; Na_i^+—intracellular Na^+ activity; K_i^+—intracellular K^+ activity.

Table 1. Experimental solutions[a]

Solution	A	B	C	D
NaCl	115	115	80	115
$NaHCO_3$	25	25	25	25
NaH_2PO_4	2	2	2	2
$CaCl_2$	1	1	2	1
KCl	5	5	5	5
$MgSO_4$	1	1	1	—
$MgCl_2$	—	—	—	2
Glucose	5	—	—	—
Lactate	5	10	10	10
Alanine	1	—	—	—
HEPES	—	—	4	—
Dextran	0.6%	0.6%	0.6%	0.6%
Mannitol	25	—	—	—
$Na_7[Dy(P_3O_{10})_2]$	—	—	4	—
pH	7.4	7.4	7.4	7.4
Osmolarity	320	295	295	295

[a] All concentrations are in mM except dextran, which is weight %.

Materials and Methods

Renal cortical tubules were prepared by *in situ* collagenase infusion of both kidneys of female New Zealand white rabbits (2–3 kg) as described elsewhere (Balaban et al., 1980; Harris et al., 1982). This method gave tubules that were 90% proximal, had open lumens and were capable of active transcellular transport. The Ringer's used in the preparation of the tubules was solution A (Table 1). The tubules were stored on ice in this solution until used.

NMR Measurements

At the start of each experiment, tubules (2–3 g wet wt) were washed once then suspended in 30 ml of a glucose- and alanine-free buffer (B, Table 1) which had been gassed with 95%O_2/5%CO_2. This suspension was preincubated at 37°C for 30–35 min under a 95%O_2/5%CO_2 atmosphere. The tubules were then washed once and resuspended to 8 ml in a buffer (C, Table 1) containing the anionic shift reagent (SR) dysprosium tripolyphosphate (Gupta & Gupta, 1982).

^{23}Na NMR spectra were obtained using a Bruker WH360 wide bore spectrometer with a 20-mm probe tuned to 95.26 MHz. Each spectrum was the sum of 1976 transients of 512 data points each, collected in quadrature, zero-filled to 2K and digitally filtered (LB = −100, GB = 0.1) prior to Fourier transformation. With the 90° pulse and 70-msec repetition rate used, all components of the ^{23}Na spectrum were fully relaxed (Gullans et al., 1985).

At the start of an experiment, 8 ml of the tubule suspension (10–20 (mg protein) · ml^{-1}, suspended in solution C) were added to a 20 mm NMR tube containing a conical insert (for details *see* Avison et al., 1987). Antifoam B (Sigma, St. Louis, MO) was added to 0.1% to prevent frothing when the bubbler (PE-50 tubing) was inserted. This amount of antifoam had no adverse effects on tubule viability. A holder containing the K^+-selective and reference electrodes (*see below;* Avison et al., 1987), bubbler and addition lines was then inserted, and the assembled unit was lowered into the NMR probe. The conical insert, combined with gentle bubbling of 95%O_2/5%CO_2 from the base of the cone, gave good mixing and kept the tubules suspended and well oxygenated. The suspension was maintained at 37°C by passing warm air around the NMR tube.

^{23}Na NMR spectra and K^+-electrode output (if required) were collected as described previously (Avison et al., 1987) for a control period (glucose- and alanine-free), and then a bolus of glucose or αMG (56 μl of a 1-M solution) was added to the suspension to a final concentration of 7 mM without interruption of data acquisition. Data collection was continued until a new steady state was reached.

At the end of each experiment 1 ml of the suspension was rapidly centrifuged for 15 sec (12,000 × *g*) to separate the extracellular fluid for subsequent determination of the final values of $[Na^+]_o$ and $[K^+]_o$. A separate sample of the suspension was precipitated with 6% perchloric acid/1 mM EDTA and the pellet subsequently dissolved in 1 M NaOH/5% deoxycholate for determination of protein content.

K^+-Electrode Measurements

Net movements of K^+ across the tubular cell membranes were followed by measuring changes in $[K^+]_o$ using a K^+-selective and a reference electrode. In the NMR experiments we used modified electrodes supplied by Microelectrodes (Londonderry, NH) (K^+ electrode # MI 442, reference electrode # MI 402). The modifications have been described in a previous publication (Avison et al., 1987) and were necessary to prevent injection of radio-frequency noise which would otherwise degrade the ^{23}Na NMR measurement. The electrodes were connected to a high impedance electrometer (WP Instruments, New Haven, CT, Model # 725) and the output was digitized and recorded using an IBM CS9000 computer. The electrode slope and offset were determined before each experiment in buffers containing the SR. The slope was 49.3 ± 1.2 mV/decade $[K^+]$ from 0.1 to 10 mM $[K^+]$. The electrode's response time for a step in $[K^+]$ from 0.1 to 1 mM was 5–10 sec in a well-stirred solution when mixing times were negligible. In the NMR tube the response time, including mixing, was generally 10–20 sec.

In experiments where the NMR was not used, 3-ml aliquots of tubules suspended in solution D were preincubated for 30–35 min at 37°C under a 95%O_2/5%CO_2 atmosphere and then transferred to a chamber maintained at 37°C by a thermostatted water jacket. Antifoam B was added to 0.1% and the oxygen tension was maintained by gentle bubbling 95%O_2/5%CO_2 just below the surface of the suspension and stirring the suspension with a magnetic stirrer. K^+ fluxes were measured using a solid-state K^+-selective electrode (WPI Model #POT-1) in conjunction with the same type of reference electrode described above. The solid-state electrode had a slope of 58 mV/decade $[K^+]$, and its K^+/Na^+ selectivity was greater than 10^4. The overall response time, including mixing, for a step in $[K^+]$ from 0.1 to 1.0 mM was 2–5 sec. The same electrometer and computerized data acquisition system were used.

Other Measurements

Protein contents were measured by the Lowry assay (Lowry et al., 1951). $[Na^+]_o$ and $[K^+]_o$ were determined by flame photometry.

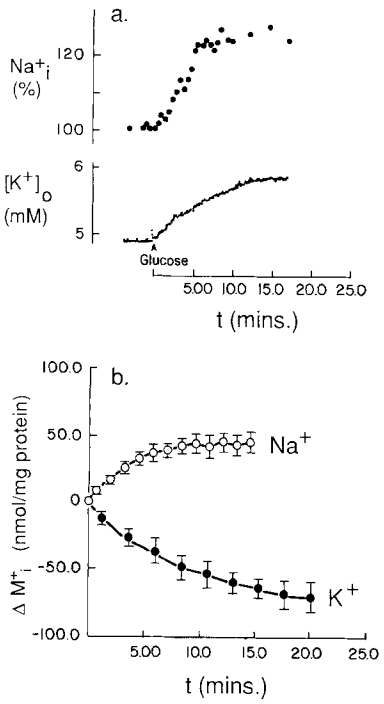

Fig. 1. Changes in tubule Na^+ and K^+ content following glucose addition. (*a*) A typical experiment showing the timecourse of the changes in Na^+_i and $[K^+]_o$ following addition of 7 mM glucose to a proximal tubule suspension. Na^+_i is plotted as % of the steady-state value prior to glucose addition. $[K^+]_o$ is in mM. (Note that increasing $[K^+]_o$ indicates a net efflux of K^+ from the tubules.) (*b*) Timecourse of the changes in intracellular ion content of proximal tubules following addition of 7 mM glucose at $t = 0$. Each point represents the mean of four experiments. Error bars are ± SE

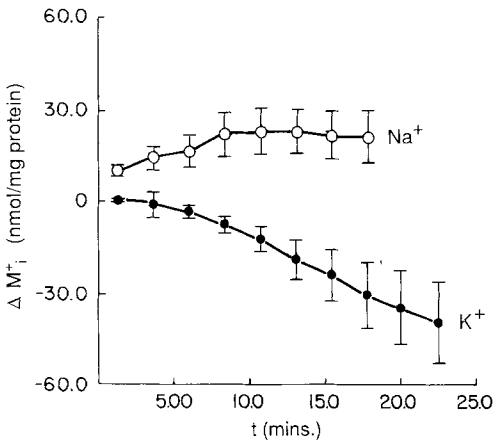

Fig. 2. Timecourse of the changes in intracellular ion content of proximal tubules following addition of 7 mM α-methyl-D-glucose at $t = 0$. Each point represents the mean of four experiments. Error bars are ± SE

Chemicals

All chemicals were reagent grade and were obtained from standard commercial sources. Sodium tripolyphosphate, and collagenase (Type IA) were obtained from Sigma (St. Louis, MO) and dysprosium chloride was obtained from Aldrich (Milwaukee, WI). Furosemide and bumetanide were dissolved at a concentration of 1 M in DMSO.

Data Analysis and Statistics

The Na^+ and K^+ fluxes and intracellular Na^+ contents were analyzed using methods described in Avison et al., (1987). Statistical comparisons were performed using the Student's *t* test, and the data are presented as means ± SE.

Results

Glucose-Stimulated Na^+ and K^+ Fluxes

The steady-state intracellular Na^+ content of rabbit cortical tubules suspended in glucose- and alanine-free buffer at 37°C was 145 ± 13 (nmol Na^+) · (mg protein)$^{-1}$ ($n = 12$). Addition of glucose to a final concentration of 7 mM caused intracellular Na^+ to increase by 43 ± 7 nmol · (mg protein)$^{-1}$ ($n = 7$) to a new steady-state value of 188 ± 79 (nmol Na^+) · (mg protein)$^{-1}$ ($n = 4$). The new steady state was reached in 8–10 min (Fig. 1).

The glucose addition stimulated a net loss of K^+ from the proximal tubules (Fig. 1). Initially the net K^+ efflux matched the net Na^+ uptake (i.e., $|\Delta Na^+| = |\Delta K^+|$); however, the tubules continued to lose K^+ for 5–10 min after the intracellular Na^+ content had stabilized, by which time intracellular K^+ had decreased by 72 ± 11 nmol · (mg protein)$^{-1}$ ($n = 4$).

α-Methyl-D-glucoside enters proximal tubule cells via the luminal Na^+-glucose cotransport system (Silverman, 1974), but is not metabolized (Reynolds & Segal, 1974). We therefore studied the effects of αMG addition to determine whether the Na^+ uptake and K^+ efflux observed following glucose addition were a consequence of Na^+-glucose cotransport, or of glucose metabolism.

Like glucose, αMG caused a net uptake of Na^+ and loss of K^+ by the tubules. However, the fluxes were smaller, and the K^+-efflux lagged the Na^+ uptake (Fig. 2). Thus the intracellular Na^+ content reached a new steady-state value in 8–10 min. The net Na^+ uptake was 23 ± 7 nmol · (mg protein)$^{-1}$ ($n = 4$). The αMG-induced K^+ loss initially lagged Na^+ uptake, but 15 min following αMG addition the net K^+ loss (24 ± 8 nmol · (mg protein)$^{-1}$ ($n = 4$)) matched the net Na^+ uptake (22 ± 8 nmol · (mg protein)$^{-1}$ ($n = 4$)). The K^+ loss continued, and even 22.5 min after αMG addition the intracellular K^+ content had not reached steady state. The mea-

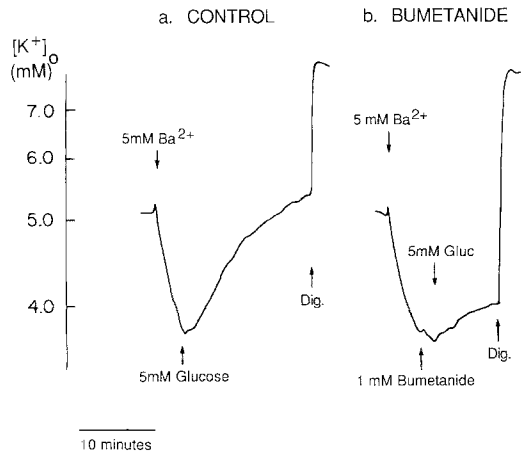

Fig. 3. Effects of Ba^{2+}, glucose, bumetanide (1 mM) and digitonin on tubule K^+ content. This is a typical experiment showing how inhibitor sensitivity of the glucose-stimulated K^+ loss was assessed. (*a*) In the control, tubules were equilibrated in glucose- and alanine-free medium at 37°C. Ba^{2+} was then added to block the principal K^+ efflux pathway, causing the tubules to accumulate K^+. Addition of glucose stimulated a K^+ efflux from the tubules. (*b*) The right panel shows that addition of 1 mM bumetanide prior to glucose addition inhibits the glucose-stimulated K^+ efflux seen in the control. Final addition of digitonin releases all the cytoplasmic K^+, allowing us to normalize the experiments

surement of intracellular Na^+ could not be continued beyond 22.5 min following the αMG addition due to loss of the shift, but at this time there was no sign of a significant decrease in intracellular Na^+ content.

Studies of Plasma Membrane K^+ Fluxes

To further characterize the nature of the glucose-stimulated K^+ efflux, we next examined the effects of various inhibitors of K^+ transport on basal and glucose-stimulated K^+ movements. Figure 3 illustrates our experimental strategy. Tubules were initially equilibrated in a SO_4^{2-}-free Ringer's containing 5 mM K^+ (Solution D, Table 1) at 37°C. (A SO_4^{2-}-free solution was necessary to avoid formation of insoluble $BaSO_4$ following Ba^{2+} addition.) 5 mM Ba^{2+} was then added to block the principal K^+ leak, leading to a large K^+ uptake by the cells. Once the intracellular K^+ content had reached a new steady-state value, one of two protocols was followed: (i) 5 mM glucose was added, to assess the Ba^{2+}-sensitivity of the K^+ loss seen in the NMR experiments following glucose addition (*see* Fig. 3*a*); (ii) DMSO (vehicle), furosemide or bumetanide was added prior to the glucose addition in order to assess the sensitivity of any Ba^{2+}-insensitive K^+ movements to these compounds (Fig. 3*b*). Finally, at the end of each experiment digitonin (0.7 mg · ml^{-1}) was added to selectivity dissolve the plasma membranes. From the change in $[K^+]_o$ following addition of digitonin, the cytoplasmic K^+ content prior to addition of glucose was determined. The results of these experiments are described below and summarized in Table 2 and Figure 4.

Table 2. Effects of Ba^{2+}, bumetanide, and furosemide on glucose-stimulated K^+ efflux[a]

	Initial K^+ efflux rate (nmol · (mg protein)$^{-1}$ · min^{-1})	Net K^+ loss (nmol · (mg protein)$^{-1}$
Control[b]	17.7 ± 3.2 (4)	71.8 ± 10.9 (4)
Ba^{2+}	25.7 ± 2.3 (3)	182.9 ± 22.8 (3)
Ba^{2+} + DMSO	26.1 ± 2.4 (7)	174.8 ± 18.0 (7)
Ba^{2+} + 10 μM F[c]	25.9 ± 1.7 (5)	158.4 ± 16.9 (5)
Ba^{2+} + 10 μM B[d]	27.2 ± 0.8 (6)	166.0 ± 7.6 (6)
Ba^{2+} + 1 mM F	6.6 ± 1.2 (6)	64.0 ± 8.4 (6)
Ba^{2+} + 1 mM B	12.5 ± 2.6 (5)	78.0 ± 12.4 (5)

[a] Effects of various inhibitors of K^+ transport systems on the initial rate of glucose-stimulated K^+ efflux and on the net K^+ loss. The experimental protocol is that illustrated in Fig. 3.
[b] In presence of shift reagent in NMR experiments.
[c] Furosemide.
[d] Bumetanide.

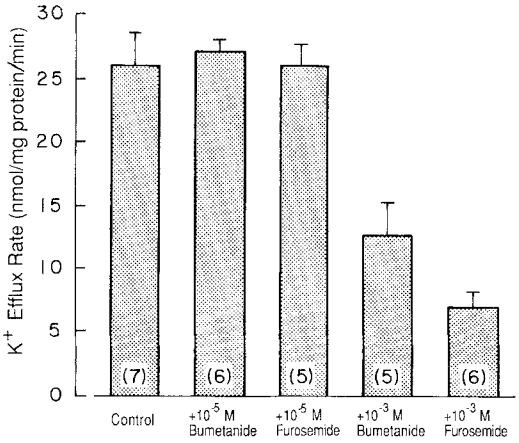

Fig. 4. Effects of bumetanide and furosemide on the rate of glucose-stimulated K^+ loss. The plot shows the effect of inhibitors on K^+ efflux rate following addition of 5 mM glucose to Ba^{2+}-blocked tubules. The experimental protocol is shown in Fig. 3. Data are from Table 2

Ba^{2+}

Since the principal K^+ leak in proximal tubule cells is Ba^{2+} sensitive (Soltoff & Mandel, 1986; this study), we first investigated the effect of Ba^{2+} on

the glucose-stimulated K^+ efflux. This resulted in an uptake of K^+ by the tubules (167.0 ± 5.0 nmol · (mg protein)$^{-1}$ (n = 29)). The initial rate of net K^+ uptake was 99.5 ± 3.8 nmol · min^{-1} · (mg protein)$^{-1}$ (n = 26). A new steady state was reached after 2–5 min, at which time 5 mM glucose was added. Despite the Ba^{2+} block, this glucose addition stimulated a large net K^+ loss whose initial rate corresponded to 25.7 ± 2.3 nmol · min^{-1} · (mg protein)$^{-1}$ (n = 3). Addition of DMSO, the vehicle for furosemide and bumetanide (*see below*), had no effect on the initial rate (26.1 ± 2.4 nmol · min · (mg protein)$^{-1}$ (n = 7)). Both these rates were greater than the initial rate measured in the NMR experiments in the absence of Ba^{2+} (17.7 ± 3.2 nmol · min^{-1} · (mg protein)$^{-1}$ (n = 4)). The net K^+ loss was also greater in the presence of Ba^{2+} (182.9 ± 22.8 nmol · (mg protein)$^{-1}$ (n = 3)) than in its absence (71.8 ± 10.9 (n = 4)). Again, DMSO had no effect on net K^+ loss from Ba^{2+}-blocked tubules (174.8 ± 18.0 nmol · (mg protein)$^{-1}$ (n = 7)).

Digitonin

From the change in $[K^+]_o$ following addition of digitonin, the cytoplasmic K^+ content prior to addition of glucose was determined to be 261.6 ± 8.1 nmol · (mg protein)$^{-1}$ (n = 29).

Furosemide and Bumetanide

The diuretic furosemide, and its analogue bumetanide, are known to inhibit a variety of Cl^--dependent cation transport processes (Lauf, 1985; Siebens, 1985). We therefore determined whether the Ba^{2+}-insensitive K^+ transport was sensitive to these diuretics.

At a concentration of 10 μM neither furosemide nor bumetanide had any effect on the net K^+ loss induced by glucose addition, nor on its initial rate. Furthermore, addition of these compounds at 1 mM to tubules at steady state, whether in the presence or absence of glucose, had small and less consistent effects on net K^+ movements. However, both the rate and degree of net K^+ loss observed following glucose addition were significantly reduced by the prior addition of 1 mM furosemide or bumetanide (Figs. 3*b* and 4, Table 2). In the presence of 1 mM furosemide, the initial rate of net K^+ loss was only 25% (6.6 ± 1.2 nmol · min^{-1} · (mg protein)$^{-1}$) of that observed in the presence of Ba^{2+} alone. The net K^+ loss was reduced to 37% of the Ba^{2+} value (64.0 ± 8.4 nmol · (mg protein)$^{-1}$ (n = 6) (Table 2, Fig. 4)). Likewise, in the presence of 1 mM bumetanide, the initial rate of K^+ efflux was 49% (12.5 ± 2.6 nmol · min^{-1} · (mg protein)$^{-1}$ (n = 5)) of the Ba^{2+}-blocked rate, and the net loss was 43% (78.0 ± 12.4 nmol · (mg protein)$^{-1}$ (n = 5)) of the Ba^{2+}-blocked K^+ efflux (Table 2, Fig. 4).

Discussion

It is known that addition of organic solutes such as glucose to the luminal perfusate of the proximal nephron leads to an increase in transepithelial Na^+ transport (Burg et al., 1976; Gullans et al., 1984), together with a rapid membrane depolarization (Cardinal, Lapointe & Laprade, 1984; Lang et al., 1986), followed in some cases, including rabbit proximal tubule, by a partial repolarization and an increase in Ba^{2+}-sensitive conductance of the basolateral membranes (Cardinal et al., 1984). It is less clear what effect these phenomena have on intracellular Na^+ and K^+.

In this study we have shown that the addition of glucose or a nonmetabolizable analogue, αMG, to suspensions of rabbit cortical tubules leads to a sustained rise in intracellular Na^+ content and a loss of intracellular K^+. A component of this net K^+ loss was Ba^{2+} insensitive. It was, however, inhibited significantly by 1 mM furosemide or bumetanide.

Intracellular Na^+

The increase in intracellular Na^+ content seen following addition of glucose is consistent with the operation of a Na^+-coupled glucose uptake system such as has been identified in renal brush border vesicles (Kinne, 1976; Kinne et al., 1975). Evidence that this Na^+ entry mechanism is active in cortical tubule suspensions comes from the observations of Gullans et al. that (i) addition of glucose to such a preparation causes an increase in ouabain-sensitive respiration, and (ii) phlorizin, a specific inhibitor of luminal Na^+-coupled glucose entry, blocks this stimulation (Gullans et al., 1984). The smaller increase in intracellular Na^+ seen following αMG addition is consistent with the smaller stimulation of ouabain-sensitive respiration observed by Gullans (1982), the higher K_M of αMG measured by Samarzija, Hinton and Fromter (1982) in the rat proximal tubule, and the smaller stimulation of Na^+ transport reported by Burg et al. (1976).

Since cellular volume changes were not measured in this study, the change in intracellular Na^+ content cannot be translated directly into changes in $[Na^+]_i$. However, for $[Na^+]_i$ to remain unchanged, the cellular volume must have increased by ~30% following glucose addition. Previous stud-

ies of luminal addition of organic solutes, albeit in different preparations of rabbit proximal tubules, have detected volume increases of 10% (Linshaw, 1980), and 14% (Cardinal et al., 1984), while we have calculated a value of ~20% from the data of Burg et al. (1976). It thus seems probable that $[Na^+]_i$ increased significantly following addition of glucose to the suspension, although confirmation must await a direct measurement of the cellular volume change under these conditions.

Our conclusion that the steady-state $[Na^+]_i$ is elevated in the presence of glucose is consistent with the idea that the increase in basolateral pump rate observed is a consequence (at least in part!) of an increase in $[Na^+]_i$. An earlier study by Khuri (1980) also showed that Na_i^+ increased in *Necturus* proximal tubules when glucose was present in the luminal fluid; however, the luminal perfusate contained tenfold more Na^+ when glucose was present, so the increased Na_i^+ may have been due as much to an increased driving force for Na^+ entry. The present study agrees with the results of Morgunov and Boulpaep (1987), who found that in perfused salamander proximal tubules Na_i^+ increased with increasing luminal glucose, saturating when the glucose concentration reached 5 mM, and Messner et al. (1985), who demonstrated a sustained rise in Na_i^+ following addition of 10 mM phenylalanine to the luminal perfusate in proximal tubules of frog kidney. Our findings contradict the study of Lee and Armstrong (1972), who could find no increase in Na_i^+ in bullfrog small intestine following replacement of 26 mM mannitol by the same concentration of 3-O-methyl-D-glucose in both serosal and mucosal buffers. They are also at odds with the study by Hudson and Schultz (1984), which found that replacement of 15 mM mannitol by the same concentration of galactose caused the short-circuit current across *Necturus* small intestine to increase three- to fourfold, despite a statistically insignificant rise in the steady-state Na_i^+. These authors did detect a transient rise in Na_i^+ following galactose addition, and speculated that the subsequent fall may be due to an increase in basolateral Na^+-pump activity and/or an increase in cell volume caused by accumulation of osmotically active galactose in the cytoplasm. It should be noted that the fourfold increase in Na^+ transport seen by Hudson and Schultz in *Necturus* small intestine is considerably larger than the maximal stimulation (~30%) seen in rabbit proximal tubule (Gullans et al., 1984). Furthermore, while the initial depolarization of the mucosal membrane seen following galactose addition to the mucosal buffer is followed by a repolarization (Hudson & Schultz, 1984), there is only a slow and incomplete repolarization of the luminal membrane of the rabbit proximal tubule following the luminal addition of glucose (Cardinal et al., 1984). Thus direct comparisons of the changes in intracellular Na^+ in these two tissues and their relation to the increased Na^+ transport rate following addition of organics may be inappropriate.

Intracellular K^+

Our data indicate that, following the addition of glucose or αMG, the increased rate of pump-mediated K^+ uptake is not only matched, but for a time is actually *exceeded* by the rate of K^+ efflux, leading to a net loss of K^+ from the tubules. Previous studies of proximal tubule cells have found only small changes in K_i^+ following the luminal application of organics (Messner et al., 1985; Lang et al., 1986), generally a slightly lower steady-state value in the presence of organics. Studies of other leaky epithelia found no significant difference in tissue K^+ in the presence or absence of various actively transported organic solutes (Schultz, Fuisz & Curran, 1966; Csaky & Esposito, 1969; Armstrong, Musselman & Reiteny, 1970), but did find reductions in intracellular K^+ concentration (Schultz et al., 1966; Csaky & Esposito, 1969; Armstrong, et al., 1970) and activity (Lee & Armstrong, 1972; Grasset, Gunter-Smith & Schultz, 1983; White et al., 1986). This reduction in K^+ concentration and activity has been attributed to the increase in cell volume caused by the accumulation of osmotically active organics. However, as Grasset et al. (1983) have pointed out, a change in cell volume cannot affect the steady-state K_i^+ unless it *directly* affects the rates of K^+ entry and/or exit.

Since a major leak pathway for K^+ in many epithelial cells including the proximal tubule is a Ba^{2+}-sensitive K^+ channel, we first investigated the possibility that the net K^+ loss observed following glucose addition was due to stimulation of a Ba^{2+}-sensitive leak. Our thoughts on this were guided by the work of Schultz and his collaborators on *Necturus* small intestine. This group showed that the addition of galactose caused a rapid depolarization of the mucosal membrane potential (ψ_{mc}), and a concommitant drop in r_m/r_s (the ratio of the mucosal to serosal membrane resistances). With time, however, ψ_{mc} repolarized and r_m/r_s rose, as a result of an increase in the Ba^{2+}-sensitive basolateral K^+ conductance (G_K^s) (Grasset et al., 1983; Lau, Hudson & Schultz, 1984). These responses to galactose were accompanied by a decline in the steady-state K_i^+ (Grasset et al.,1983). Lau et al. (1984) found that a similar increase in Ba^{2+}-sensitive G_K^s was induced by cell swelling, suggesting that the increase in G_K^s

seen following galactose addition is secondary to the intracellular accumulation of osmotically active galactose and water.

In the present study the initial rate of net K^+ loss observed following addition of 5 mM Ba^{2+} to a glucose- and alanine-free suspension of rabbit cortical tubules was 99.5 ± 3.8 nmol · min^{-1} · (mg protein)$^{-1}$ at 37°C. This rate matches the rate of pump-mediated K^+ uptake in the same preparation, as judged by the ouabain-sensitive respiration. (Assuming pump-mediated K^+ uptake/O_2 consumed is ~12 (Harris, Balaban & Mandel, 1980), and the ouabain-sensitive QO_2^0 = 8.5 (nmol O_2) · min^{-1} · (mg protein)$^{-1}$ (Gullans et al., 1984), the pump-mediated K^+ uptake rate is ~100 nmol · min^{-1} · (mg protein)$^{-1}$.) A recent report found that the initial rate of Ba^{2+}-induced K^+ uptake in rabbit cortical tubules was 108 ± 7 nmol · min^{-1} · (mg protein)$^{-1}$ in the presence of glucose and alanine (Soltoff & Mandel, 1986) and matched the initial ouabain-induced K^+ efflux (105 ± 5 nmol · min^{-1} · (mg protein)$^{-1}$), indicating that at steady state, in the presence or absence of glucose, the major path of K^+ egress in rabbit cortical tubules is the Ba^{2+}-sensitive K^+ leak.

The repolarization of the basolateral membrane seen in rabbit proximal tubule following addition of luminal glucose and alanine (Cardinal et al., 1984) suggests that some component of the net K^+ loss seen in the present study may involve the Ba^{2+}-sensitive pathway. However, since Biagi et al. (1981) have shown that Ba^{2+} completely blocks the K^+ conductance, our results indicate that a second Ba^{2+}-insensitive, furosemide and bumetanide-sensitive is also present, which is responsible for a significant fraction of the K^+ loss seen following glucose addition. In this regard, it is of interest to note that Brenner et al. (1969) have detected a direct effect of furosemide on proximal tubular Na^+ reabsorption in the rat proximal tubule. Thus under normal circumstances, both Ba^{2+}-sensitive and furosemide/bumetanide-sensitive pathways may have a role in the net K^+ loss observed following glucose addition.

Several observations suggest that the furosemide-sensitive pathway for K^+ efflux is normally small in the rabbit proximal tubule. First, addition of 10^{-4} M furosemide to the lumen of rabbit PCT's perfused with an ultrafiltrate of serum had no effect on fluid and Na^+ reabsorption, and addition of 10^{-3} M furosemide to the bath had no effect on electrical resistance or transepithelial potential difference (Burg et al., 1973). Second, our own data and those of Soltoff and Mandel (1986) indicate that the steady-state rate of ouabain-sensitive K^+ uptake by suspensions of rabbit cortical tubules is exactly matched by the steady-state Ba^{2+}-sensitive K^+ leak, both in the presence and absence of glucose. Finally, addition of 10^{-3} M furosemide to tubules at steady state had no significant effect, whether glucose was present or not.

Furosemide and bumetanide inhibit a variety of Cl^--coupled transport processes, including $Na^+/K^+/2Cl^-$ cotransport (Palfrey, Feit & Greengard, 1980), Cl^-/HCO_3^- exchange (Brazy & Gunn, 1976), Na^+/Cl^- cotransport (Larson & Spring, 1983), Ca^{2+}-dependent Cl^- channels (Evans et al., 1986) and K^+/Cl^- cotransport (Larson & Spring, 1984; Siebens, 1985). The sensitivity to furosemide and bumetanide strongly suggests, therefore, that the Ba^{2+}-insensitive K^+ loss is Cl^- dependent. Although we cannot distinguish between a directly and an indirectly coupled process, there are two observations which make a directly coupled process more likely. First, Sasaki et al. (1988) have recently directly observed Na^+-independent electroneutral KCl cotransport in the rabbit proximal straight tubule; second, the absence of a significant Cl^- conductance (Bello-Reuss, 1982) and the inability of Ba^{2+} to completely inhibit the K^+ loss make the concerted activation of K^+ and Cl^- channels unlikely.

K^+/Cl^- cotransport systems have been proposed or identified in a number of other cells. Guggino (1986) has identified such a cotransport system in distal tubules of the *Amphiuma* kidney. Corcia and Armstrong (1983) and Reuss (1983) demonstrated that in *Necturus* gallbladder Cl_i^- increased with increasing serosal K^+ activity and that this increase was not due to changes in basolateral membrane potential. Reuss further showed that the coupling of Cl_i^- to serosal K^+ did not require Na^+. Cell swelling has been shown to activate a bumetanide-sensitive loss of KCl from avian and toadfish red cells (for reviews *see* Lauf, 1985; Siebens, 1985). Finally, Lauf and his coworkers have reported a furosemide-sensitive, Cl^--dependent K^+ loss in osmotically enlarged low-K^+ sheep red cells (Lauf, 1985).

Conclusion

In this study we have examined the effects on intracellular Na^+ and K^+ content of activating the luminal Na^+/glucose cotransporter in the rabbit proximal tubule. Following the addition of saturating amounts of glucose or αMG, intracellular Na^+ content increased while intracellular K^+ content decreased. The increase in Na^+ probably reflected an increase in $[Na^+]_i$, since the increase in intracellular Na^+ content was greater than any increase in intracellular volume reported hitherto. A component of the decrease in K^+ content occurred via a Ba^{2+}-

insensitive pathway which was activated transiently following glucose or αMG addition. This K^+ loss was inhibited by 1 mM bumetanide or furosemide, suggesting that it is Cl^- dependent.

The work described was supported by grants from NIH (DK27121-09), and NSF (DMB 86-16310). This work was reported in part at the 18th Annual Meeting of the American Society of Nephrology, December 15–18, 1985.

References

Armstrong, W.McD., Musselman, D.L., Reiteny, H.C. 1970. Sodium, potassium, and water content of isolated bullfrog small intestinal epithelia. *Am. J. Physiol.* **219:**1023–1026

Avison, M.J., Gullans, S.R., Ogino, T., Giebisch, G., Shulman, R.G. 1987. Measurement of Na^+-K^+ coupling ratio of Na^+-K^+-ATPase in rabbit proximal tubules. *Am. J. Physiol.* **253:***C*126–*C*136

Balaban, R.S., Soltoff, S.P., Storey, J.M., Mandel, L.J. 1980. Improved renal cortical tubule suspension: Spectrophotometric study of O_2 delivery. *Am. J. Physiol.* **238:***F*50–*F*59

Bello-Reuss, E. 1982. Electrical properties of the basolateral membrane of the straight portion of the rabbit proximal renal tubule. *J. Physiol. (London)* **326:**49–63

Brazy, R.C., Gunn, R.B. 1976. Furosemide inhibition of chloride transport in human red blood cells. *J. Gen. Physiol.* **68:**583–599

Brenner, B.M., Keimowitz, R.I., Wright, F.S., Berliner, R.S. 1969. An inhibitory effect of furosemide on sodium reabsorption by the proximal tubule of the rat nephron. *J. Clin. Invest.* **48:**290–300

Burg, M., Patlak, C., Green, N., Villey, D. 1976. Organic solutes in fluid reabsorption by renal proximal convoluted tubules. *Am. J. Physiol.* **231:**627–637

Burg, M., Stoner, L., Cardinal, J., Green, N. 1973. Furosemide effect on isolated perfused tubules. *Am. J. Physiol.* **225:**119–124

Cardinal, J., Lapointe, J.-Y., Laprade, R. 1984. Luminal and peritubular ionic substitutions and intracellular potential of the rabbit proximal convoluted tubule. *Am. J. Physiol.* **247:***F*352–*F*364

Corcia, A., Armstrong, W.McD. 1983. KCl Cotransport: A mechanism for basolateral chloride exit in gallbladder. *J. Membrane Biol.* **76:**173–182

Csaky, T.Z., Esposito, G. 1969. Osmotic swelling of intestinal epithelial cells during active sugar transport. *Am. J. Physiol.* **217:**753–755

Evans, M.G., Marty, A., Tan, Y.P., Trautman, A. 1986. Blockage of Ca-activated Cl conductance by furosemide in rat lacrimal glands. *Pfluegers Arch.* **406:**65–68

Grasset, E., Gunter-Smith, P., Schultz, S.G. 1983. Effects of Na-coupled alanine transport on intracellular K activities and the K conductances of the basolateral membranes of *Necturus* small intestine. *J. Membrane Biol.* **78:**89–94

Guggino, W.B. 1986. Functional heterogeneity in the early distal tubule of the *Amphiuma* kidney: Evidence for two modes of Cl^- and K^+ transport across the basolateral cell membrane. *Am. J. Physiol.* **250:***F*340–*F*440

Gullans, S.R. 1982. Transport, respiration, and gluconeogenesis in the renal proximal tubule. Ph.D. Dissertation. Duke University, Durham, NC

Gullans, S.R., Avison, M.J., Ogino, T., Giebisch, G., Shulman, R.G. 1985. NMR measurements of intracellular sodium in the rabbit proximal tubule. *Am. J. Physiol.* **249:***F*160–*F*168

Gullans, S.R., Harris, S.I., Mandel, L.J. 1984. Glucose-dependent respiration in suspensions of rabbit cortical tubules. *J. Membrane Biol.* **78:**257–262

Gupta, R.K., Gupta, P. 1982. Direct observation of resolved resonances from intra- and extracellular sodium-23 ions in NMR studies of intact cells and tissues using dysprosium (III) tripolyphosphate as a paramagnetic shift reagent. *J. Magn. Reson.* **47:**344–350

Harris, S.I., Balaban, R.S., Mandel, L.J. 1980. Oxygen consumption and cellular ion transport: Evidence for adenosine triphosphate to O_2 ratio near 6 in intact cell. *Science* **208:**1148–1150

Harris, S.I., Patton, L., Barrett, L., Mandel, L.J. 1982. (Na^+,K^+)-ATPase kinetics within the intact renal cell. The role of oxidative metabolism. *J. Biol. Chem.* **257:**6996–7002

Hudson, R.L., Schultz, S.G. 1984. Sodium-coupled sugar transport: Effects on intracellular sodium activities and sodium pump activity. *Science* **224:**1237–1239

Khuri, R.N. 1980. Intracellular ion activity measurements in kidney tubules. *Curr. Topics Membr. Transp.* **13:**73–92

Kinne, R. 1976. Properties of the glucose transport system in the renal brush border membrane. *Curr. Topics Membr. Transp.* **8:**209–267

Kinne, R., Murer, H., Kinne-Saffran, E., Thees, M., Sachs, G. 1975. Sugar transport by renal plasma membrane vesicles. *J. Membrane Biol.* **21:**375–395

Lang, F., Messner, G., Rehwald, W. 1986. Electrophysiology of sodium-coupled transport in proximal renal tubules. *Am. J. Physiol.* **250:***F*953–*F*962

Larson, M., Spring, K. 1983. Bumetanide inhibition of NaCl transport by *Necturus* gallbladder. *J. Membrane Biol.* **74:**123–129

Larson, M., Spring, K. 1984. Volume regulation by *Necturus* gallbladder: Basolateral KCl exit. *J. Membrane Biol.* **81:**219–232

Lau, K.R., Hudson, R.L., Schultz, S.G. 1984. Cell swelling increases a barium-inhibitable potassium conductance in the basolateral membranes of *Necturus* small intestine. *Proc. Natl. Acad. Sci. USA* **81:**3591–3594

Lauf, P.K. 1985. K^+:Cl^- Cotransport: Sulfhydryls, divalent cations, and the mechanism of volume activation in a red cell. *J. Membrane Biol.* **88:**1–13

Lee, C.O., Armstrong, W.McD. 1972. Activities of sodium and potassium ions in epithelial cells of small intestine. *Science* **175:**1261–1264

Linshaw, M.A. 1980. Effect of metabolic inhibitors on renal tubule cell volume. *Am. J. Physiol.* **239:***F*571–*F*577

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275

Messner, G., Koller, A., Lang, F. 1985. The effect of phenylalanine on intracellular pH and sodium activity in proximal convoluted tubule cells of the frog kidney. *Pfluegers Arch.* **404:**145–149

Morgunov, N., Boulpaep, E.L. 1987. Electrochemical analysis of renal Na^+-glucose cotransport in salamander proximal tubules. *Am. J. Physiol.* **252:***F*154–*F*169

Palfrey, H., Feit, P.W., Greengard, P. 1980. cAMP-stimulated cation cotransport in avian erythrocytes: Inhibition by "loop" diuretics. *Am. J. Physiol.* **238:***C*139–*C*148

Reuss, L. 1983. Basolateral KCl cotransport in a NaCl-absorbing epithelium. *Nature (London)* **305:**723–726

Reynolds, R., Segal, S. 1974. Effects of dibutyryl cyclic AMP on α-methyl-D-glucoside accumulation in rabbit kidney. *Am. J. Physiol.* **226:**791–795

Samarzija, I., Hinton, B.T., Fromter, E. 1982. Electrophysiological analysis of rat renal system and amino acid transport: II. Dependence on various transport parameters and inhibitors. *Pfluegers Arch.* **393:**190–197

Sasaki, S., Ishibashi, K., Yoshiyama, N., Shiigai, T. 1988. KCl cotransport across the basolateral membrane of rabbit renal proximal straight tubules. *J. Clin. Invest.* **81:**194–199

Schafer, J.A., Williams, J.C., Jr. 1985. Transport of metabolic substrates by the proximal nephron. *Annu. Rev. Physiol.* **47:**103–125

Schultz, S.G., Frizzell, R.A., Nellans, H.N. 1974. Ion transport by mammalian small intestine. *Annu. Rev. Physiol.* **36:**51–91

Schultz, S.G., Fuisz, R.E., Curran, P.F. 1966. Amino acid and sugar transport in rabbit ileum. *J. Gen. Physiol.* **49:**849–866

Siebens, A. 1985. Cellular volume control. *In:* The Kidney: Physiology and Pathophysiology. D.W. Seldin and G. Giebisch, editors. pp. 91–115. Raven, New York

Silverman, M. 1974. The chemical and steric determinants governing sugar interactions with renal tubular membranes. *Biochim. Biophys. Acta* **332:**248–262

Soltoff, S.P., Mandel, L.J. 1986. Potassium transport in the rabbit proximal tubule: Effects of barium, ouabain, valinomycin, and other ionophores. *J. Membrane Biol.* **94:**153–161

Ullrich, K.J. 1979. Sugar, amino acid and Na^+ cotransport in the proximal tubule. *Annu. Rev. Physiol.* **41:**181–195

White, J.F., Burnup, K., Ellingsen, D. 1986. Effect of sugars and amino acids on amphibia intestinal Cl^- transport and intracellular Na^+, K^+, and Cl^- activity. *Am. J. Physiol.* **250:***G*109–*G*117

Received 11 April 1988