J. Membrane Biol. 78, 257–262 (1984)

The Journal of
Membrane Biology
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Glucose-Dependent Respiration in Suspensions of Rabbit Cortical Tubules

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Summary. The effects of glucose on cellular respiration were examined in suspensions of rabbit cortical tubules. When glucose was removed from the bathing fluid, oxygen consumption (QO_2) decreased from 18.6 ± 0.8 to 15.7 ± 0.5 nmol O_2/mg protein·min ($P < 0.01$). The transported but nonmetabolized analogue of glucose, α-methyl-D-glucoside (αMG), was found to support QO_2 to the same extent as glucose. These observations were also evident in the presence of butyrate, a readily oxidized substrate of the renal cortex. Additional studies with nystatin and ouabain indicated that glucose-related changes in QO_2 were the result of changes in Na, K-ATPase associated respiration. The effect of glucose was localized to the luminal membrane since phlorizin (10^{-5} M), a specific inhibitor of luminal glucose-sodium cotransport, also significantly reduced QO_2 by $10 \pm 1\%$. Phlorizin inhibition of QO_2 was also evident in the presence of αMG but was abolished when glucose was removed from the bathing medium. Finally, measurement of NADH fluorescence showed that addition of glucose (5 mM) to a tubule suspension causes an oxidation of NAD. These data are all consistent with glucose acting to increase respiration by stimulating sodium entry at the luminal membrane (via glucose-sodium cotransport) followed by increased sodium pump activity and its associated increase in mitochondrial respiration.

Key Words glucose transport · transport and metabolism · oxygen consumption · phlorizin

Introduction

The transport of isosmotic fluid by the renal proximal tubule can be enhanced by the presence of organic solutes such as glucose in the luminal fluid (Burg, Patlak, Green & Villey, 1976; Ullrich, 1979). Transport and electrophysiological evidence favors the notion that glucose promotes sodium transport by increasing luminal sodium entry via a sodium-solute cotransport mechanism (Ullrich, 1979; Frömter, 1982). However, studies of *in vivo* glucose oxidation have suggested that a selective linkage to sodium transport exists, involving glucose as a metabolic fuel for proximal fluid transport (*see* Cohen & Kamm, 1976). The present investigation directly addresses the primary role of glucose in the stimulation of sodium transport and cellular respiration in a suspension of rabbit cortical tubules.

Brush border membrane vesicle studies have shown previously that the uphill entry of D-glucose into the renal proximal tubule is sodium-dependent, electrogenic, stereospecific, saturable, and inhibitable by phlorizin (Frasch et al., 1970; Chesney, Sacktor & Kleinzeller, 1974; Silverman, 1974*a*, *b*, 1976; Hilden & Sacktor, 1979; Turner & Silverman, 1977, 1978; Brazy & Dennis, 1978; Turner & Moran, 1982). Accordingly, transport studies of isolated perfused rabbit proximal convoluted tubules have shown that removal of glucose or addition of phlorizin (10^{-5} M) to the perfusate can reduce net fluid absorption by about 20% (Burg et al., 1976; Kokko, 1973; Brazy & Dennis, 1978). Further, phlorizin (10^{-5} M) was shown to be ineffective when added in the absence of glucose or when added to the peritubular bathing medium (Brazy & Dennis, 1978). Given these observations, one would predict that altering glucose availability to the renal proximal tubule should cause commensurate changes in cellular respiration as a result of changes in Na, K-ATPase turnover.

In the present investigation, suspensions of rabbit cortical tubules were used to monitor respiratory changes associated with alterations in glucose availability. This preparation is ideally suited to this study because it contains almost exclusively proximal tubules and the lumens of the tubules are open so that the luminal brush border membrane is freely accessible to the extracellular fluid (Balaban, Soltoff, Storey & Mandel, 1980; Arthus, Bergeron & Scriver, 1982; Vinay, Gougoux & Lemieux, 1982). The results indicate that glucose-related respiration can account for 10 to 16% of

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the oxygen consumption of the proximal tubule. Furthermore, alterations in respiration appear to be related to an increase in glucose-dependent sodium transport and not to the ability of glucose to behave as a metabolic substrate.

Materials and Methods

Suspensions of Cortical Tubules

Cortical tubule suspensions were prepared according to Balaban et al. (1980). Briefly, female New Zealand White rabbits were anesthesized with ether and cannulated via the aorta to permit the kidneys to be flushed with 80 mg/dl collagenase (Sigma Type I, Sigma Chemical Co., St. Louis, MO). The kidneys were then excised and the cortex was dissected in ice-cold Ringer's solution. At 4 °C the tubules were dispersed, washed several times, and centrifuged on a cushion of 25% Ficoll (400,000 mol wt, Pharmacia Fine Chemicals AB, Uppsala, Sweden) to remove nonvital cells and cellular debris. The Ringer's solution used throughout these procedures contained (mM) sodium chloride (105), sodium bicarbonate (25), potassium chloride (5), magnesium sulfate (1), monosodium phosphate (2), calcium chloride (1.5), sodium lactate (10), D-glucose (5), L-alanine (1), and 0.6% (wt/vol) dialyzed dextran (T-40, Pharmacia, Piscataway, NJ). Three final washes were performed in the solution to be used for the experiment. These solutions were identical to that above except for glucose, which was either absent, present (5 mM), or replaced by 5 mM α-methyl-D-glucopyranoside (αMG), as noted in the figures and tables. The final pellet was resuspended to a concentration of 3–5 mg tubular protein/ml.

Oxygen Consumption Measurements

Cortical tubule suspensions were preincubated for 30–35 min at 37 °C in a shaker bath under an atmosphere of 95% O_2/5% CO_2 to maintain the pH at 7.4. Oxygen consumption rates (QO_2) were measured polarographically in a closed 1.6-ml chamber (Gilson Medical Electronics, Middleton, WI) using a Clark electrode (Yellow Springs Instrument Co., Yellow Springs, OH). The oxygen tension was recorded as a function of time and the slope indicated the QO_2. Oxygen consumption rates are expressed according to protein content which was measured with the biuret method (Gornall, Bardawill & David, 1949).

Fluorometric Measurements

Fluorometric measurements of NADH were performed on the tubule suspensions as described by Balaban et al. (1980). This method allows simultaneous measurements of NADH fluorescence and QO_2 in a single, well-mixed suspension of cortical tubules. Briefly, incident light from a mercury arc lamp (366 nm) was focused on the suspension. Using two photomultiplier tubes, NADH was measured as the difference between the 450 nm and the 366 nm signals such that a decrease in 450 nm fluorescence indicated a net oxidation of NADH.

Chemicals

All chemicals were analytical grade and obtained from standard commercial sources. α-methyl-D-glucopyranoside was obtained from Pfanestiehl Laboratories (Waukegan, IL). Phlorizin was obtained from Pfaltz and Bauer (Stamford, CT). Nystatin was obtained from Calbiochem. Behring (La Jolla, CA) and was dissolved in dimethylsulfoxide.

Statistics

Data are expressed as the mean ± SE and statistical comparisons were made using the Student's t test.

Results

Oxygen consumption rates were measured in the presence or absence of glucose. As shown in Table 1, removal of glucose from the bathing medium reduced the rate of respiration from 18.6 ± 0.8 to 15.7 ± 0.5 nmol O_2/mg protein·min ($P<0.01$). (The glucose-free medium actually contained 50–100 μM glucose as a result of gluconeogenesis during the pre-incubation.) When butyrate (1 mM), a readily metabolized substrate (Weidemann & Krebs, 1969; Harris, Balaban, Barrett & Mandel, 1981) was added to the bathing medium, removal of glucose still reduced QO_2 from 25.4 ± 0.9 to 19.8 ± 0.8 nmol O_2/mg protein·min ($P<0.002$). This observation suggests that the inhibition of QO_2 by removal of exogenous glucose is not the result of the tubules experiencing a substrate limitation.

To explore further the role of glucose in the inhibition of QO_2, tubules were suspended in a medium in which glucose was replaced by αMG, a transported (Silverman, 1976) but nonmetabolized (Reynolds & Segal, 1974) analogue of glucose. As shown in Table 2, in the presence of glucose QO_2 was 19.7 ± 1.0 nmol O_2/mg protein·min and was unchanged in the presence of αMG at 20.5 ± 0.8 nmol O_2/mg protein·min. Similarly, when exogenous butyrate was added to the medium, QO_2 in the presence of glucose (27.8 ± 0.5 nmol O_2/mg protein·min) was not significantly different from that in the presence of αMG (28.1 ± 0.9 nmol O_2/mg protein·min). These data further indicate that glucose-related changes in QO_2 are related to the transport of glucose.

Measurements of oxygen consumption were also performed following the additions of nystatin and ouabain to the tubule suspensions. Nystatin, a cation-selective ionophore, has been shown to stimulate Na, K-ATPase-associated respiration to maximal levels in cortical tubule suspensions. This stimulation requires that the sodium pump and its coupling mechanism to mitochondrial oxidative phosphorylation be fully intact. In addition, the level to which nystatin will stimulate respiration depends on substrate availability. As shown in Table 3, nystatin-stimulated QO_2 (nmol O_2/mg protein·min) was 25.6 ± 1.6 in the presence of glucose, 25.5 ± 1.1 in the absence of glucose, and 25.9 ± 0.7 in the presence of αMG, indicating that respiration associated with increased turnover of the Na, K-ATPase was unaffected by perturbations in glucose

Table 1. Oxygen consumption rates of tubule suspensions in the presence and absence of glucose

Experimental condition	Oxygen consumption rate ($nmol\ O_2$/mg protein·min)	
	Glucose	Glucose-free
Control	18.6 ±0.8 (10)	15.7[a] ±0.5 (12)
+Butyrate	25.4 ±0.9 (6)	19.8[b] ±0.8 (5)

Tubule suspensions were prepared in the presence or absence of glucose (5 mM) as noted. +Butyrate indicates that the medium was supplemented with butyrate (1 mM). All values are mean±SE with the number of observations in parentheses.

[a] $p<0.01$
[b] $p<0.002$

Table 2. Oxygen consumption rates of tubule suspensions in the presence of glucose or α-methyl-D-glucoside

Experimental condition	Oxygen consumption rate ($nmol\ O_2$/mg protein·min)	
	Glucose	α-methyl-D-glucoside
Control	19.7 ±1.0 (13)	20.5 ±0.8 (14)
+Butyrate	27.8 ±0.5 (9)	28.1 ±0.9 (11)

Tubules were prepared in the presence of either glucose (5 mM) or α-methyl-D-glucoside (5 mM), as noted. +Butyrate indicates that the control medium was supplemented with butyrate (1 mM). All values are mean±SE with the number of observations in parentheses.

Table 3. Nystatin-stimulated and ouabain-limited QO_2 of tubule suspensions

Experimental condition	Oxygen consumption rate ($nmol\ O_2$/mg protein·min)		
	Glucose	Glucose-free	α-methyl-D-glucoside
+Nystatin	25.6 ±1.6 (4)	25.5 ±1.1 (4)	24.9 ±0.7 (4)
+Ouabain	6.8 ±0.2 (4)	7.2 ±0.3 (4)	6.9 ±0.2 (4)

Tubules were prepared in the presence or absence of glucose (5 mM) or in the presence of α-methyl-D-glucoside (5 mM), as noted. Oxygen consumption rates were measured in the presence of nystatin (>40 μg/mg protein) and in the presence of ouabain (5×10^{-5} M). All values are the mean±SE with the number of observations in parentheses.

Table 4. Effects of 10^{-5} M phlorizin on QO_2 in cortical tubule suspensions

Experimental condition	Change in QO_2 (E/C)		
	Glucose	Glucose-free	α-methyl-D-glucoside
Control	0.90[a] ±0.01 (12)	0.98 ±0.01 (6)	0.92[a] ±0.01 (6)
+Butyrate	0.92[a] ±0.01 (10)	1.00 ±0.02 (5)	0.92[b] ±0.02 (5)

Changes in oxygen consumption were measured following the addition of 10^{-5} M phlorizin. E/C represents the ratio of the experimental (phlorizin) QO_2 (E) to the control (initial) QO_2 (C). The various conditions are identical to those in Table 1. All values represent means±SE with the number of observation in parentheses

[a] $P<0.001$.
[b] $P<0.02$.

availability. Oxygen consumption rates in the presence of ouabain were similarly unaffected by glucose substitutions, indicating that these perturbations affected the Na, K-ATPase-activated component of respiration.

Phlorizin (10^{-5} M), a known inhibitor of Na-glucose cotransport (Silverman, 1976), was found to reduce QO_2 to 0.90 ± 0.01 of the control rate but had no effect in the absence of glucose (0.98 ± 0.01) (Table 4). When αMG was substituted for glucose, phlorizin reduced QO_2 to 0.92 ± 0.01 of the control rate, a level comparable to that observed in the presence of glucose. Similar degrees of inhibition of QO_2 by phlorizin were observed in the presence of butyrate (Table 4). Furthermore, nystatin-stimulated respiration and ouabain-inhibited respiration were unaffected by phlorizin, indicating that phlorizin inhibited respiration by altering glucose-related Na, K-ATPase activity and not by causing nonspecific effects on metabolism.

In another series of experiments, the addition of glucose to glucose-free tubule suspensions stimulated respiration in a dose-dependent manner (Fig. 1). A single reciprocal (Eadie-Hofstee) plot of the data (Inset Fig. 1) indicates that QO_2 could be maximally stimulated by 11.1% and the K_m for glucose was 2.3 mM ($r=0.76$).

As a final test of the mechanism by which glucose stimulates respiration, pyridine nucleotide flu-

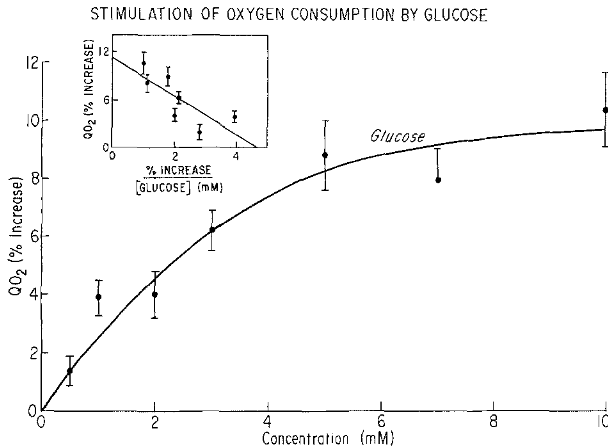

Fig. 1. Dose-dependent stimulation of oxygen consumption by glucose. Tubule suspensions were pre-incubated for 30 minutes in lactate (10 mM) and alanine (1 mM). *Inset:* A single-reciprocal (Eadie-Hofstee) plot of glucose stimulation of oxygen consumption. In both figures each point represents the mean ± SE of 5 suspensions

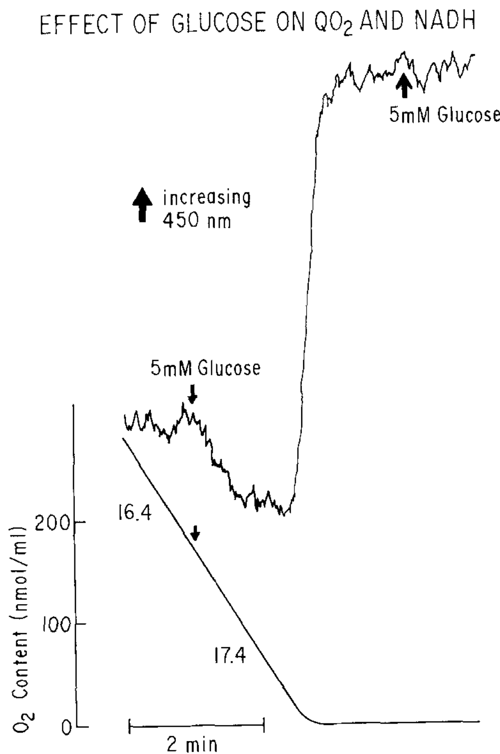

Fig. 2. Response of oxygen consumption and NADH fluorescence to additions of 5 mM glucose. The tubule suspension was pre-incubated for 30 min in the presence of lactate (10 mM) and alanine (1 mM). Glucose additions were made at the arrows. The numbers near the oxygen consumption line are the oxygen consumption rates at those points in nmol O_2/mg protein · min. An oxidation of NAD is represented by a downward deflection of the 450 nm signal. The tubule suspension reached anoxia as indicated by the flat oxygen consumption rate. At anoxia, the NADH fluorescence increased, indicating that the NAD in the cell had become totally reduced as is characteristic of anoxia. The second addition of 5 mM glucose was made during anoxia to demonstrate that the previously observed oxidation of NADH by glucose was not an optical artifact

orescence was measured in cortical tubule suspensions (*see* Mandel, 1982). One would predict that if glucose stimulation of QO_2 was the result of increased substrate delivery to the respiratory chain there would be a net reduction of NAD. On the other hand, if glucose-stimulated respiration was caused by an increased luminal sodium entry followed by increased Na, K-ATPase activity, there would be a net oxidation of NAD. A representative experiment is shown in Fig. 2 where the addition of glucose (5 mM) to a glucose-free suspension of tubules caused a marked decrease in 450 nm fluorescence or a net oxidation of NAD. This addition of glucose also caused a concomitant increase in QO_2 from 16.4 to 17.4 nmol O_2/mg protein · min. Anoxia caused a characteristic reduction in NAD. The addition of 5 mM glucose during anoxia caused no change in NADH fluorescence, demonstrating that the previously observed oxidation was not an optical artifact. A comparable oxidation of NAD was also caused by the addition of 5 mM αMG to the tubule suspensions (data not shown).

Discussion

The present investigation has demonstrated that glucose can stimulate respiration in suspensions of rabbit cortical tubules. Based on several criteria, it appears that glucose increases respiration by increasing luminal sodium entry which, in turn, activates the basolateral sodium pump. Since the sodium pump is tightly coupled to mitochondrial oxidative phosphorylation (Harris et al., 1981; Mandel & Balaban, 1981), this series of events increases respiration. Such an explanation is based in part on numerous observations in other comparable preparations.

In isolated perfused rabbit proximal convoluted tubules glucose removal from the perfusing and bathing solutions has been shown to decrease isosmotic fluid absorption by 25% or 0.27 nl/mm · min (Burg et al., 1976). Comparable observations have been made by others in the rabbit and rat proximal convoluted tubule (Brazy & Dennis, 1978; Ullrich, 1979). In cortical tubule suspensions, removal of glucose from the bathing medium caused QO_2 to decrease by 16% or 2.9 nmol O_2/mg protein · min (Table 1). Assuming that only 70% of the cellular respiration is associated with Na, K-ATPase activity (Balaban et al., 1980), this decrease in QO_2 would represent a 23% decrease in respiration related to sodium transport. Therefore, the magnitude of the observed changes in fluid transport and QO_2 following removal of glucose are comparable.

The glucose analogue, α-methyl-D-glucoside, has been shown to interact preferentially with the sodium-dependent luminal entry pathways of the rabbit and rat kidney (Kolinska, 1970; Segal, Rosenhagen & Rea, 1973; Silverman, 1976) and the chicken intestine (Kimmich & Randles, 1981). Furthermore, αMG is not metabolized by the rabbit renal cortex (Reynolds & Segal, 1974). Addition of αMG to the glucose-free perfusate of an isolated perfused rabbit proximal convoluted tubule was shown to increase net fluid absorption by 0.37 nl/mm·min (Burg et al., 1976), not significantly different from the glucose-induced stimulation. Similarly, substitution of αMG for glucose in suspensions of cortical tubules (Table 1) did not significantly affect cellular respiration, suggesting that glucose-related respiration is associated with its cotransport with sodium and not its ability to be metabolized.

Studies with phlorizin provided strong evidence that glucose-stimulated respiration depends on the luminal entry of glucose. Phlorizin is an inhibitor of glucose transport in the renal proximal tubule (Brazy & Dennis, 1978; Vick, Diedrich & Baumann, 1973). Phlorizin acts at the luminal membrane to inhibit glucose transport in the renal proximal tubule (Vick et al., 1973; Silverman, 1976; Brazy & Dennis, 1978). Studies of brush border membrane vesicles have shown that phlorizin binds to a high affinity (K_a=0.2–8 μM), sodium-dependent site and is not itself transported (Frasch et al., 1970; Chesney et al., 1974; Silverman, 1974*b*, 1976). In isolated perfused proximal convoluted tubules, phlorizin (10^{-5} M) has been shown to reduce fluid transport by 18% or 0.14 nl/mm·min (Brazy & Dennis, 1978). This concentration of phlorizin had virtually no effect when added to the bathing fluid or when added to the perfusate in the absence of glucose. In comparison, addition of phlorizin (10^{-5} M) to suspensions of rabbit cortical tubules reduced QO_2 by 10% (Table 3). This phenomenon was evident when glucose was replaced by αMG, but removal of glucose blocked the inhibition by phlorizin. The magnitude of this inhibition is comparable to that seen following removal of glucose from the suspension (Table 1) or to that seen for phlorizin inhibition of fluid transport in rabbit proximal tubules (Brazy & Dennis, 1978). Since phlorizin inhibits sodium-dependent glucose uptake at the luminal membrane, glucose-induced changes in respiration appear to be related to the cotransport of sodium.

Evidence that glucose-related QO_2 was related to a stimulation of Na, K-ATPase activity was garnered from studies with nystatin and ouabain. Under all three experimental conditions, 5 mM glucose, glucose-free, and 5 mM αMG, nystatin-stimulated and ouabain-inhibited respiration were unaffected (Table 3). These observations indicate that glucose increased respiration by augmenting the turnover of the sodium pump and not by increasing the supply of cellular energy. Furthermore, glucose, and αMG-induced respiration were fully inhibitable by ouabain, indicating that they acted to deliver more sodium to the sodium pump.

Previous studies have shown that sodium entry at the luminal membrane is the rate-limiting step in transepithelial transport in the proximal tubule (Spring & Giebisch, 1977; Harris et al., 1981). Furthermore, glucose can accelerate the uptake of sodium into brush border membrane vesicles (Hilden & Sacktor, 1979; Thierry, Poujeol & Ripoche, 1981). Therefore, evaluation of the kinetics of glucose stimulation of QO_2 should provide a description of the sodium-glucose cotransport site at the luminal membrane. Accordingly, stimulation of QO_2 by glucose was a saturable function which displayed a V_{max} of 11.1% and a K_m of 2.3 mM glucose (Fig. 1). This K_m for glucose is in the range of that observed in isolated perfused tubules (Barfuss & Schafer, 1981) and brush border membrane vesicles (Hilden & Sacktor, 1979). However, recent studies of the heterogeneity of glucose transport along the proximal tubule (McKeown, Brazy & Dennis, 1979; Barfuss & Schafer, 1981) have shown that the cortex contains two types of transport sites. Kinetic studies of brush border membrane vesicles have indicated that preparations derived from the whole cortex display curvilinear kinetic plots (Turner & Silverman, 1977, 1978). Recent observations indicate that the renal cortex contains two types of glucose transport sites, a low affinity, high capacity site in the outer cortex (early proximal tubule) and a high affinity, low capacity site in the outer medulla (late proximal tubule) (Turner & Moran, 1982). The current investigation was performed with a mixture of proximal tubule segments derived from the whole cortex and could therefore display curvilinear kinetics on an Eadie-Hofstee plot (Inset Fig. 1). Unfortunately, within our experimental error it is not possible to reliably determine whether the kinetics reflect two sodium-dependent glucose transport sites. We can only state that the data appear to be curvilinear as one would predict, and this could explain the relatively low correlation coefficient (r=0.76).

Mandel (1982) recently described how measurements of pyridine nucleotide fluorescence can be used to distinguish the primary mode by which a solute can stimulate oxidative metabolism. In this regard, the addition of 5 mM glucose to the cortical tubule suspension caused an oxidation of the

NADH (Fig. 2). This observation confirms that glucose-induced respiration is the result of an increased energy demand by the cell rather than an increased delivery of energy to a substrate-deprived system. Since this energy demand is inhibitable by ouabain (Table 3), we conclude that glucose promotes cellular respiration by increasing luminal sodium entry which then accelerates the turnover of the ATP-dependent Na, K-ATPase at the basolateral membrane.

In conclusion, glucose addition to glucose-free suspensions of cortical tubules maximally increased oxygen consumption rates by about 10%. Evaluation of this response by a variety of criteria indicated that it was the result of an increase in glucose-mediated sodium transport rather than glucose acting as a metabolic substrate.

We thank Lauren Patton and Melinda McCord for excellent technical assistance and Dr. P.C. Brazy for valuable discussions. This investigation was supported by U.S. Public Health Service grant AM-26816 and a grant from the North Carolina Heart Association.

References

Arthus, M.-F., Bergeron, M., Scriver, C.R. 1982. Topology of membrane exposure in the renal cortex slice: Studies of glutathione and maltose changes. *Biochim. Biophys. Acta* **692**:371–376

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

Barfuss, D.W., Schaffer, J.A. 1981. Differences in active and passive glucose transport along the proximal tubule. *Am. J. Physiol.* **290**:*F*322–*F*332

Brazy, P.C., Dennis, V.W. 1978. Characteristics of glucose-phlorizin interactions in isolated proximal tubules. *Am. J. Physiol.* **234**:*F*279–*F*286

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

Chesney, R., Sacktor, B., Kleinzeller, A. 1974. The binding of phlorizin to the isolated luminal membrane of the renal proximal tubule. *Biochim. Biophys. Acta* **332**:263–277

Cohen, J.J., Kamm, D.E. 1976. Renal metabolism: Relation to renal function. *In:* The Kidney, B.M. Brenner and F.C. Rector, editors. pp. 126–214. Saunders, Philadelphia

Frasch, W., Frohnert, P.P., Boxe, F., Baumann, K., Kinne, R. 1970. Competitive inhibition of phlorizin binding by D-glucose and the influence of sodium: A study on isolated brush border membrane of rat kidney. *Pfluegers Arch.* **320**:265–284

Frömter, E. 1982. Electrophysiological analysis of rat renal sugar and amino acid transport: I. Basic phenomena. *Pfluegers Arch.* **393**:179–189

Gornall, A.G., Bardawill, C.J., David, M.M. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**:751–766

Harris, S.I., Balaban, R.S., Barrett, L., Mandel, L.J. 1981. Mitochondrial respiratory capacity and Na^+- and K^+-dependent adenosine triphosphatase-mediated ion transport in the intact renal cell. *J. Biol. Chem.* **256**:10319–10328

Hilden, S.A., Sacktor, B. 1979. D-glucose-dependent sodium transport in renal brush border membrane vesicles. *J. Biol. Chem.* **254**:7090–7096

Kimmich, G.A., Randles, J. 1981. α-methylglucoside satisfies only Na^+-dependent transport system of intestinal epithelium. *Am. J. Physiol.* **241**:*C*227–*C*232

Kokko, J.P. 1973. Proximal tubule potential difference: Dependence on glucose, HCO_3 and amino acids. *J. Clin. Invest.* **52**:1362–1367

Kolinska, J. 1970. Kinetics of sugar transport in rabbit kidney cortex *in vitro:* movement of D-galactose, 2-deoxy-D-galactose and α-methyl-D-glucoside. *Biochim. Biophys. Acta* **219**:200–219

Mandel, L.J. 1982. Use of noninvasive fluorometry and spectrophotometry to study epithelial metabolism and transport. *Fed. Proc.* **41**:36–41

Mandel, L.J., Balaban, R.S. 1981. Stoichiometry and coupling of active transport to oxidative metabolism in epithelial tissues. *Am. J. Physiol.* **240**:*F*357–*F*371

McKeown, J.W., Brazy, P.C., Dennis, V.W. 1979. Intrarenal heterogeneity for fluid, phosphate, and glucose absorption in the rabbit. *Am. J. Physiol.* **237**:*F*312–*F*318

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

Segal, S., Rosenhagen, M., Rea, C. 1973. Developmental and other characteristics of α-methyl-D-glucoside transport by rat kidney cortex slices. *Biochim. Biophys. Acta* **291**:519–530

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

Silverman, M. 1974*b*. The *in vivo* localization of high affinity phlorizin receptors to the brush border surface of the proximal tubule in dog kidney. *Biochim. Biophys. Acta* **339**:92–102

Silverman, M. 1976. Glucose transport in the kidney. *Biochim. Biophys. Acta* **457**:303–351

Spring, K. R., Giebisch, G. 1977. Kinetics of Na^+ transport in *Necturus* proximal tubule. *J. Gen. Physiol.* **70**:307–328

Thierry, J., Poujeol, P., Ripoche, P. 1981. Interactions between Na^+-dependent uptake of D-glucose, phosphate, and L-alanine in rat renal brush border membrane vesicles. *Biochim. Biophys. Acta* **647**:203–210

Turner, R.J., Moran, A. 1982. Heterogeneity of sodium-dependent D-glucose transport sites along the proximal tubule: Evidence from vesicle studies. *Am. J. Physiol.* **242**:*F*406–*F*414

Turner, R.J., Silverman, M. 1977. Sugar uptake into normal human renal brush border vesicles. *Proc. Natl. Acad. Sci. USA* **75**:2825–2829

Turner, R.J., Silverman, M. 1978. Sugar uptake into brush border vesicles from dog kidney: II. Kinetics. *Biochim. Biophys. Acta* **511**:470–486

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

Vick, H., Diedrich, D.F., Baumann, K. 1973. Re-evaluation of renal tubular glucose transport inhibition by phlorizin analogs. *Am. J. Physiol.* **224**:552–557

Vinay, P., Gougoux, A., Lemieux, G. 1982. Isolation of a pure suspension of rat proximal tubules. *Am. J. Physiol.* **241**:*F*403–*F*411

Weidemann, M.J., Krebs, H.A. 1969. The fuel of respiration of rat kidney cortex. *Biochem. J.* **112**:149–166

Received 1 September 1983; revised 4 November 1983