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Effects of High Glucose on Na,K-ATPase and Na/glucose Cotransporter Activity in Primary Rabbit Kidney Proximal Tubule Cells

Ho-Jae Han

Department of Veterinary Physiology, College of Veterinary Medicine, Chonnam National University, Kwangju, Korea

=ABSTRACT=

Renal proximal tubular hypertrophy and hyperfunction are known to be early manifestations of experimental and human diabetes. As the hypertrophy and hyperfunction have been suggested to be central components in the progression to renal failure, an understanding of their underlying causes is potentially important for the development of therapy. A primary rabbit kidney proximal tubule cell culture system was utilized to evaluate the possibility that the renal proximal tubular hypertrophy and hyperfunction observed *in vivo* in *diabetes mellitus*, can be attributed to effects of elevated glucose levels on membrane transport systems.

Primary cultures of rabbit proximal tubules, which achieved confluence at 10 days, exhibited brush-border characteristics typical of proximal tubular cells. Northern analysis indicated 2.2 ~ 2.3 and 2.0 kb Na/glucose cotransporter RNA species appeared in fresh and cultured proximal tubule cells after confluence, repectively. The cultured cells showed reduced Na/glucose cotransporter activity compared to fresh proximal tubules.

Primary cultured proximal tubule cells incubated in medium containing 20 mM glucose have reduced α-MG transport compared to cells grown in 5 mM glucose. In the proximal tubule cultures incubated in medium containing 5 mM or 20 mM glucose, phlorizin at 0.5 mM inhibited 0.5 mM α-MG uptake by 84.35% or 91.85%, respectively. The uptake of 0.5 mM α-MG was similarly inhibited by 0.1 mM ouabain (41.97% or 48.03% inhibition was observed, respectively). In addition, α-MG uptake was inhibited to a greater extent when Na^+ was omitted from the uptake buffer (81.86% or 86.73% inhibition was observed, respectively).

In cell homogenates derived from the primary cells grown in 5 mM glucose medium, the specific activity of the Na/K-ATPase (6.17±1.27 μmole Pi/mg protein/hr) was 1.56 fold lower than the values in cell homogenates treated with 360 mg/dl D-glucose, 20 mM (9.67±1.22 μmole Pi/mg protein/hr). Total Rb^+ uptake occured at a significantly higher rate (1.60 fold increase) in primary cultured rabbit kidney proximal tubule cell monolayers incubated in 20 mM glucose medium (10.48±2.45 nM/mg protein/min) as compared with parallel cultures in 5 mM glucose medium. Rb^+ uptake rate in 5 mM glucose medium was reduced by 28% when the cultures were incubated with 1 mM ouabain. The increase of the Rb^+ uptake by rabbit kidney proximal tubule cells in 20 mM glucose could be attributed primarily to an increase in the rate of ouabain-sensitive Rb^+ uptake (5 mM to 20 mM; 4.68±0.85 to 8.38±1.37 nM/mg protein/min). In conclusion, the activity of the renal proximal tubular Na,K-ATPase is elevated in high glucose concentration. In contrast, the activity of the Na/glucose cotransport system is inhibited.

Key Words: Kidney, Glucose, Na,K-ATPase, Na/glucose cotransporter

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INTRODUCTION

The renal tubular reabsorption of glucose is mediated by two major classes of transporters (Silverman, 1991; Elsas & Longo, 1992). Initially, luminal glucose is concentrated in tubules by apical Na/glucose cotransporters (SGLUTs). Afterwards, glucose reaches the blood space through basolateral facilitative glucose transporters (GLUTs) with low-Michaelis constant (Km) GLUT1 and high-Km GLUT2 (Dominguez, 1994). The α-MG uptake occurs by an energy-dependent transport system, which is inhibited by phlorizin (Fig. 1). The phlorizin-sensitive sugar transport system in LLC-PK_1 has been localized to the brush border. A similar system has been observed in the rabbit kidney proximal tubule cells (Taub, 1984). The accumulated sodium is actively transported out of the cell in exchange for potassium by the Na,K-ATPase on the basolateral membrane. Therefore, the Na,K-ATPase plays key roles in an organism's ability to regulate its plasma volume, electrolyte composition, and pH. The Na,K-ATPase contains two subunits, synthesized from separate genes: the α-subunit (α_1, α_2, and α_3, relative molecular mass 112 kDa) and the β-subunit (β_1 and β_2), which is glycosylated (35 kDa peptide). Several functional properties of the sodium pump, i.e., binding of ATP, formation of a phosphorylated intermediate, binding of ouabain, and ligand binding-dependent conformational changes, can be assigned to the α-subunit. No catalytic activity has yet been attributed to the β-subunit; however, αβ-heterodimer assembly appears requisite for transport of the Na,K-ATPase from the endoplasmic reticulum (Fambrough et al, 1994; Lingrel et al, 1994). Because the Na,K-ATPase mediates both K^+ influx and Na^+ efflux from the cell, the transport activity of this enzyme can be measured by evaluating ouabain-sensitive $^{42}K^+$ ($^{86}Rb^+$) influx or ouabain-sensitive efflux of preaccumulated $^{22}Na^+$ (Hoffman, 1986).

Renal failure is a major cause of death in diabetes. Approximately 30 to 45% of the patients with insulin-dependent *diabetes mellitus* (IDDM) develop end-stage renal disease and require either dialysis or renal transplantation for survival (Reddi & Camerini-Davalos, 1990). End-stage renal disease develops in about 5% of patients with non-insulin-dependent *diabetes mellitus* (NIDDM). The large majority of diabetic patients have this form of the disease. Thus, end-stage renal disease is an important clinical problem in patients with NIDDM. Moreover, hypertension and its macrovascular sequelae are significant problems in patients with NIDDM and may be linked to renal disease (Tung, 1988). Renal failure is considered to be the consequence not only of glomerulosclerosis, but also of lesions in the renal proximal tubule (Wardle, 1992; Yaqoob, 1994). The weight of the kidney increases within 60 hours after the appearance of hyperglycemia or glucosuria. The increase in kidney weight is due to cellular hypertrophy and hyperplasia but not to accumulation of water. Renal proximal tubular hypertrophy and hyperfunction have been established as being early manifestations of experimental and human diabetes (Seyer-Hansen, 1983). As the hypertrophy and hyperfunction have been suggested to be central components in the progression to renal failure, an understanding of their underlying causes is potentially important in the development of therapy. However, the mechanisms for renal hypertrophy and hyperplasia are not completely understood.

Alterations in the functional properties of renal proximal tubule epithelial cells in diabetes have been attributed to superphysiological concentrations of glucose. In renal proximal tubule cells, the activity of the apical Na/glucose cotransport sys-

tem has been reported to be reduced in the presence of elevated levels of glucose, whereas the activity of the Na/H antiport system and the Na,K-ATPase were elevated (Moran et al, 1983; Ku et al, 1986). Woods et al(1983) described a circulating, dialysable factor that maintains Na/Li exchange. In diabetic patients, metabolism of such a factor might be abnormal, thus causing changes in Na/Li countertransport.

This study was designed to examine the effects of exogenous glucose on the activity of the Na,K-ATPase and Na/glucose cotransport system in primary cultured rabbit kidney proximal tubule cells, using a primary rabbit kidney proximal tubule cell culture system. Primary rabbit kidney proximal tubule cell cultures are potentially powerful tools for studying the mechanism by which hormones and other effector molecules regulate renal growth and function *in vivo* (Taub, 1985). Primary rabbit kidney proximal tubule cell cultures can be used for testing of the physiological significance *in vitro* studies with established kidney cell lines as well as for growth and functional studies that are not possible with existing established cell lines. While the MDCK cell line has several functional properties typical of the distal tubules, the pig kidney cell line LLC-PK_1 exhibits a Na/glucose cotransport system typical of renal proximal tubule cells (Taub, 1984). However LLC-PK_1 lacks the hormone-sensitive adenylate cyclase and gluconeogenic capacity of proximal tubules cells (Amsler & Cook, 1982; Moran et al, 1983).

MATERIALS AND METHODS

Materials

Male New Zealand white rabbits (1.5 ~ 2.0 kg) were used for these experiments. Dulbecco's Modified Eagle's Medium (D-MEM) without glucose, F-12 nutrient mixture (Ham, 1800 mg/L, glucose) and soybean trypsin inhibitor were from Life Technologies (Grand Island, NY). Hormones, transferrin, and other chemicals were purchased from Sigma Chemical Corp. (St. Louis, MO). Class IV collagenase was from Worthington (Freehold, NJ). Guanidium isothiocyanate were obtained from Bethesda Research Laboratories (Gaithersburg, MD). Radionuclides, [^{14}C]Methyl-α-D-glucopyranoside(α-MG), 50 μCi, Rubidium-86 (^{86}Rb), α-^{32}P dCTP (3,000 Ci/mmol), and random priming labelling kits were purchased from Dupont/NEN. Restriction endonucleases were obtained from Promega, 1 kb DNA ladder from Life Technologies Inc., and Zeta Probe Blotting Membrane from Biorad. Liquiscint was obtained from National Diagnostics (Parsippany, NY). All others reagents were of the highest purity commercially available. Iron oxide was prepared by the method of Cook and Pickering (1958). Stock solutions of iron oxide in 0.9% NaCl were sterilized using an autoclave and diluted with phosphate buffered saline (PBS) prior to use.

Methods

Isolation of rabbit kidney proximal tubules and culture conditions

Primary rabbit kidney proximal tubule cell cultures were prepared by a modification of the method of Chung et al (1982). The basal medium, D-MEM/F12, pH 7.4, was a 50:50 mixture to Dulbecco's Modified Eagle's Medium and Ham's F12. The basal medium was further supplemented with 15 mM HEPES buffer(pH 7.4) and 20 mM sodium bicarbonate. Immediately prior to the use of the medium, the three growth supplements were added. Water utilized in medium preparation was purified by means of a MilliQ deionization system. To summarize, the kidneys of a male New Zealand white rabbit were perfused via the renal artery, first

with PBS, and subsequently with D-MEM/F12 containing 0.5% iron oxide (wt/vol), such that the kidney turned grey-black in color. Renal cortical slices were homogenized with 4 strokes of a sterile glass homogenizer, and the homogenate was poured first through a 253 μm and then a 83 μm mesh filter. Tubules and glomeruli on top of the 83 μm filter were transferred into sterile D-MEM/F12 medium containing a magnetic stir bar. Glomeruli (containing iron oxide) were removed with the stir bar. The remaining purified proximal tubules were briefly incubated in D-MEM/F12 containing 0.125 mg/ml collagenase (Class IV) and 0.025% soybean trypsin inhibitor. The tubules were then washed by centrifugation, resuspended in D-MEM/F12 containing the three supplements, and transferred into tissue culture dishes. Medium was changed one day after plating and every two days thereafter.

Primary rabbit kidney proximal tubule cells were maintained in a 37^{0}C, 5% CO_2-humidified environment in a serum-free basal medium supplemented with three growth supplements, 5 μg/ml insulin, 5 μg/ml transferrin and 5×10^{-8}M hydrocortisone (Chung et al, 1982).

Electron microscopy

Cells were grown on 35 mm tissue culture dishes and fixed with 2.5% (V/V) glutaraldehyde in 0.1 M sodium cacodylated (pH 7.2) for 1 hour. After washing in buffer, the cultures were then postfixed first in 1% osmium tetroxide in 0.1 M collidin buffer, enblock stained with uranyl acetate. The cultures were subsequently dehydrated in acetone and embedded in Epon mixture. Ultrathin sections were cut on an Ultracut E ultramicrotome equipped with a Dupont diamond knife and then double stained with uranyl acetate and lead citrate. The stained sections were then examined and photographed with a Hitachi-600 electron microscope.

Northern analysis of cellular RNA

Total RNA was isolated by the guanidium isothiocyanate/cesium chloride method (Han, 1993). RNA was isolated from either primary rabbit kidney proximal tubule cells grown to confluence or fresh proximal tubules prepared as described above. RNA (10 μg/sample) was fractionated by electrophoresis in formaldehyde gels containing 0.8% agarose and was transferred to Zeta Probe Blotting Membranes. Duplicate RNA samples on the gel were stained with ethidium bromide to verify the quality and the quantity of the RNA. A labelled probe for Na/glucose cotransporter cDNA was prepared from a 2.3 kb EcoRI fragment of PMJC 424 plasmid which containing rabbit Na/glucose cotransporter cDNA. This plasmid was obtained from Dr. Ernest Wright (Univ. of Cal. at LA). The restriction fragments were radiolabelled with alpha ^{32}P dCTP by the random primer method and were utilized for hybridization following the method of Church and Gilbert(1984). Standard stringent hybridization conditions were utilized.

α-methyl-D-glucoside uptake studies

Primary rabbit kidney proximal tubule cell cultures were grown to confluence in 35 mm dishes containing serum free D-MEM/F12 medium supplemented with insulin, transferrin, hydrocortisone. Subsequently, the cultures were incubated for 4 days in medium further supplemented with either 0 or 270 mg/dl D-glucose. Intact monolayers were then utilized for sugar uptake studies.

The nonmetabolized sugar, α-methyl-D-glucoside(α-MG), was utilized in the sugar uptake studies. Uptake determinations were conducted as described by Sakhrani et al(1984). In summary, before the uptake period, the monolayers were washed twice with uptake buffer containing (in

mM): NaCl, 136; KCl, 5.4; $CaCl_2$, 1.3; $MgSO_4$, 0.41; $MgCl_2$, 0.49; Na_2HPO_4, 0.44; KH_2PO_4, 0.44; HEPES, 5; glutamine, 2; and bovine serum albumin, 0.5 μg/ml. 1.5 milliliters of this buffer containing 0.5 mM α-MG and [^{14}C] α-MG(0.05 μ Ci/ml) were then added to the dishes. Other compounds as indicated were incubated in the uptake assay medium. The plates were incubated at 23^{0}C for the desired time period. At the end of the uptake period, the monolayers were rapidly washed three times with ice-cold isotonic mannitol. The cells were solubilized in 0.1 N NaOH and neutralized with HCl. To determine the [^{14}C] α-MG incorporated intracellularly, a half of each sample was removed and counted in a scintillation counter (Packard Co.), using liquid scintillation fluid. The remainder of each sample was used for protein determination by Bradford method (1976). The radioactive counts in each sample were then normalized with respect to protein and were corrected for zero-time uptake per mg protein (i.e., label not removed by washing procedure). All each uptake determinations were made in triplicate.

Na,K-ATPase assay and Rb^+ uptake

Primary rabbit kidney proximal tubule cells were grown to confluence in 35 mm dishes as described above. After achieving confluence the monolayers were further incubated for 4 days in medium further supplemented with either 0 mg/dl, or 270 mg/dl D-glucose.

Na,K-ATPase activity was assayed by a modification of the method of Jørgensen and Skou (1971). To summarize, samples (0.25 mg membrane protein) were preincubated in buffer containing 25 mM imidazole, 2 mM EDTA, and 0.6 mg/ml deoxycholate (1.0 ml total volume). After a 30 minute preincubation period, 100 μl aliquots were added to tubes containing 130 mM NaCl, 20 mM KCl, 3 mM ATP, 3 mM $MgCl_2$, and 30 mM histidine (pH 7.4). The samples were incubated for 10 minutes at 37^{0}C, and the reaction was stopped by the addition of 200 μl ice-cold 6% perchloric acid. The free inorganic phosphate released during the 10 minutes reaction period was determined and Na,K-ATPase activity (μmole Pi/mg protein/hr) was calculated.

For the studies of Rb^+ phosphate buffered saline uptake, confluent monolayers on 35 mm dishes were washed twice at 23^{0}C with 2 ml of uptake buffer (10 mM Tris-HCl, pH 7.4, and 140 mM NaCl). The cells were then submitted to a 5 minutes uptake period at 23^{0}C in uptake buffer containing 1 mM RbCl, 2 μCi $^{86}Rb^+$/ml, and 1 mM ouabain, where appropriate. At the end of the uptake period, the reaction mixture was removed by aspiration, and the monolayer was rapidly washed three times with ice-cold wash buffer (10 mM Tris-HCl, pH 7.4, and 100 mM $MgCl_2$). The cell cultures were solubilized in 0.1 N NaOH and neutralized with HCl (Taub et al, 1992). The next steps were conducted as described in α-MG uptake studies.

RESULTS

Characteristics of primary culture cells

The morphology of the cultures are shown in Fig. 2. After 10 days the monolayers demonstrated multicellular dome formation, indicative of vectorial sodium and water transport, with as many as three to four domes visible at low (×40) magnification (Fig. 2A). Dome formation was not observed in isolated cell colonies. Analysis by electron microscopy of several fields showed a homogenous cell population. Their ultrastructural features include numerous mitochondria and cell membrane-bound organelles with electron-dense cores. In addition, electron microscopy showed morphological polarity with conspicuous apical microvilli and well-defined tight junctions, char-

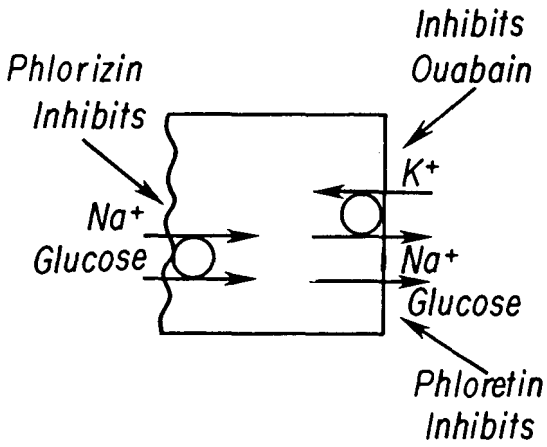

Fig. 1. Model of transepithelial glucose transport by renal proximal tubule cells. From Taub (1984) with permission. Glucose is transported with sodium across the apical membrane into the cell driven by the chemical gradient for sodium and cell-negative potential. Intracellularly-accumulated sodium is pumped out of cell by the Na,K-ATPase driven by hydrolysis of ATP to maintain the gradient for apical sodium entry. Potassium pumped into the cell by Na,K-ATPase leaks out across the basolateral membrane via a potassium conductance. A high intracellular potassium activity maintained by Na,K-ATPases associated with the basolateral potassium conductance supports cell-negative potential to drive apical sodium- coupled glucose transport.

acteristic of primary proximal tubule cells (Fig. 2B).

Identification of Na/glucose cotransporter

The expression of elaborated brush border (or apical membrane) is a distinctive property of renal proximal tubule cells *in vivo*, which has been observed to be diminished *in vitro*. Similarly, the activities of apical membrane proteins have been observed to be diminished after the cells have been placed in a cell culture environment. The influence of cell culture environment on the activity of Na/glucose cotransporter was examined. Activity of the Na/glucose cotranporter was investigated by comparing Northern hybridizations of intact proximal tubules and cultured proximal tubule cells. Fig. 3 shows that the intestinal Na/glucose carrier cDNA hybridized with mRNA of 2.2~2.3 kb and 2.0 kb in length in fresh and cultured proximal tubule cells, repectively. However, the activity of Na/glucose cotransporter was reduced in cultured proximal tubule cells (Fig. 3).

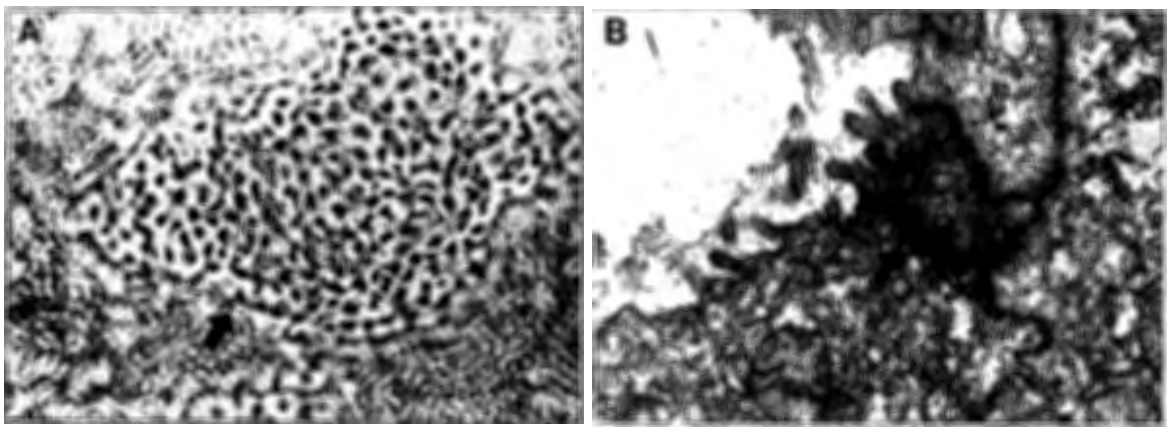

Fig. 2. Morphology of rabbit renal proximal tubule cells in primary culture. A: Dome formation by primary rabbit proximal tubule cell cultures. The cells in monolayer are out of focus when focusing on the cells in the dome (arrow). B: Electron micrograph of cultured rabbit kidney proximal tubule cells showing apical microvilli. Arrow indicate a tight junction at the apical surface.

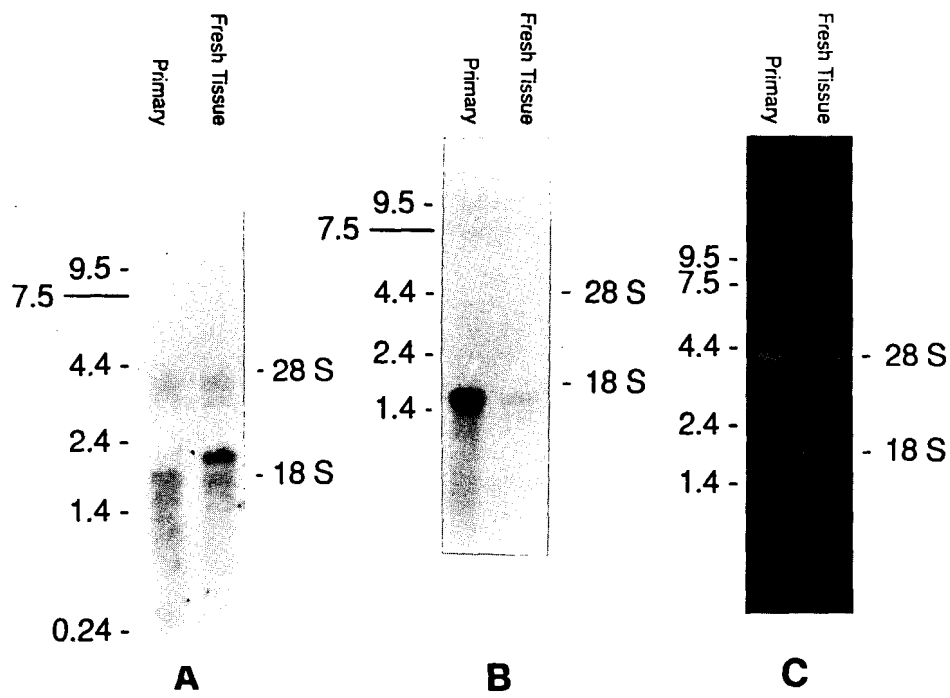

Fig. 3. Northern blot analysis of Na/glucose cotransporter mRNA from freshly isolated proximal tubules and primary cultured cells. The results are illustrated in A (Na/glucose cotransporter), B (β-actin as an internal control), and C (gel was stained with ethidium bromide)

Effect of glucose on α-methyl-D-glucoside uptake

An important function of the proximal tubule is the reabsorption of sugars from the lumen of the tubule back into the blood. This is mediated by a sodium-dependent glucose transport system, which is located on the apical surface of proximal tubule cells, as well as a sodium-independent transport system, located on the basolateral surface of the cells (Fig. 1). The Na-dependent glucose transport system is not observed on other segments of the nephron and consequently is a good proximal tubule marker. Phlorizin has been shown to block the Na-dependent glucose transport system localized on the apical surface of proximal cells.

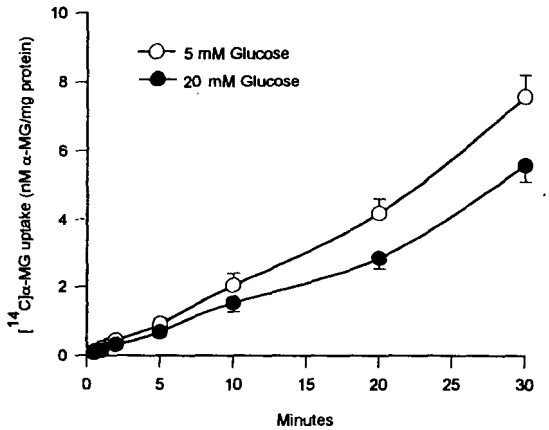

Fig. 4. Time course of uptake of 0.5 mM [^{14}C] α-MG into cultured proximal tubular cells treated with different glucose concentrations. Uptake was performed at 23^{0}C. Each point represent means ± S.E. of three independent determinations (n=9).

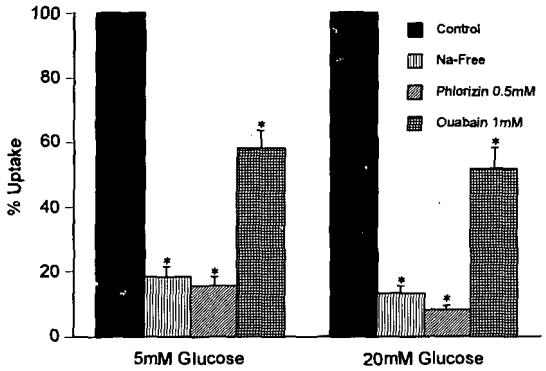

*Fig. 5. Effects of sodium, phlorizin and ouabain on uptake of 0.5 mM [^{14}C] α-MG in primary cultures of rabbit proximal tubule cells treated with different glucose concentrations. Uptake was performed at 23^{0}C for 10 minutes. Choline chloride replaced NaCl in Na-free buffer. Results are means ± S.E. of three independent determinations (n=9). *, p < 0.01 vs. control*

*Fig. 6. Effect of different glucose concentrations on Na,K-ATPase activity. Results are means ± S.E. of three independent determinations (n=9). *, p < 0.05 for 5 mM vs. 20 mM glucose.*

To determine effects of glucose on Na/glucose cotransporter activity, the transport of α-MG, a nonmetabolizable sugar, was studied. The time course of α-MG uptake by confluent primary cultured proximal tubule cells incubated in medium treated with 90 mg/dl D-glucose (5 mM) and 360 mg/dl D-glucose (20 mM) is shown in Fig. 4. The uptake in cells incubated in medium containing 5 mM glucose is linear over the first 20 minutes. In cells incubated in medium containing 20 mM glucose the uptake is linear for less than 10 minutes. α-MG transport was more greatly reduced in primary cultured proximal tubule cells incubated in medium containing 20 mM glucose than in those grown in medium 5 mM glucose.

Fig. 5 shows that in the proximal tubule cultures incubated in medium containing 5 mM or 20 mM glucose, phlorizin at 0.5 mM inhibited α-MG uptake by 84.35% or 91.85%, respectively. The uptake of α-MG was similarly inhibited by 0.1 mM ouabain (41.97% or 48.03% inhibition was observed, respectively). In addition, α-MG uptake was inhibited to a greater extent when Na^+ was deleted from the uptake buffer (81.86% or 86.73% inhibition was observed, respectively).

Effect of glucose on Na,K-ATPase activity and Rb^+ uptake

In cell homogenates derived from the primary cells grown in 5 mM glucose medium, the specific activity of the Na,K-ATPase (6.17±1.27 μmole Pi/mg protein/hr) was 1.56 fold lower than the values in cell homogenates treated with 20 mM (9.67±1.22 μmole Pi/mg protein/hr) (Fig. 6).

Rb^+ uptake into confluent monolayers of primary cultured rabbit proximal tubule cells treated with 90 mg/dl D-glucose (5 mM) or 360 mg/dl D-glucose (20 mM) was examined. Fig. 7 illustrates that in 5 mM glucose medium, Rb^+ uptake occured at 6.53±1.42 nM Rb^+/mg protein/min. The Rb^+ uptake rate in 5 mM glucose medium, was reduced by 28% when the cultures were incubated with 1 mM ouabain. The rate of Rb^+ uptake by primary cultured rabbit proximal tubule cells in 20 mM glucose medium, is also shown in Fig. 7. Total Rb^+ uptake occured at a significantly higher rate (1.60 fold increase) in primary cultured

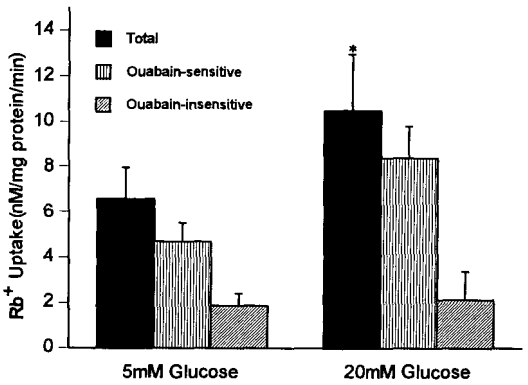

Fig. 7. *Effect of glucose and ouabain on Rb^+ uptake. Confluent rabbit kidney proximal tubule cells were incubated in media treated with different glucose concentrations for 4 days. Rb^+ uptake studies were conducted with uptake buffer containing $^{86}Rb^+$ (2 μCi/ml) for 5 minutes at 23°C. Both ouabain-treated and untreated monolayers were utilized in the Rb^+ uptake studies. All determinations were in three independent cultures and corrected for zero time uptake (n=9). The ouabain-sensitive component of Rb^+ uptake was calculated. Results are means ± S.E. of three independent determinations (n=9). *, $p < 0.05$ for 5 mM vs. 20 mM glucose.*

rabbit kidney proximal tubule cell monolayers maintained in 20 mM glucose medium (10.48± 2.45 nM Rb^+/mg protein/ min) as compared with parallel cultures in 5 mM glucose medium. The increase in the rate of the Rb^+ uptake by rabbit kidney proximal tubule cells in 20 mM glucose could be attributed primarily to an increase in the rate of ouabain sensitive Rb^+ uptake (5 mM to 20 mM; 4.68±0.85 to 8.38±1.37 nM Rb^+/mg protein/min).

DISCUSSION

The kidney simultaneously takes up and produces glucose. Renal carbohydrate metabolism is characterized by glycolysis, glucose oxidation, and the pentose phosphate pathway as well as gluconeogenesis and glycogenesis (Cryer, 1993; Holck & Rasch, 1993). Gluconeogenesis primarily occurs in the proximal tubular portion of the renal cortex where free fatty acids represent the major metabolic fuel that supplies the energy requirements of the cell. However, under certain conditions, including poorly controlled diabetes mellitus, prolonged fasting, and metabolic acidosis, renal gluconeogenesis can be enhanced markedly and a net output of glucose by the kidney is readily observed (Bank & Aynejian, 1990; Castellino & DeFronzo, 1990). Glucose-induced inhibition of cellular proliferation has been previously described for cultured renal proximal tubule cells and human endothelial cells (Ziyadeh, 1990). But several findings indicate that proximal tubule cells in culture undergo hypertrophy when exposed to elevated glucose concentrations. These differences may relate to the complex nature of the diabetic milieu *in vivo* as opposed to the controlled conditions of cell culture system.

The porcine kidney epithelial cell line LLC-PK_1 expresses a sodium-coupled glucose cotransporter (SGLUT1). Northern blot and PCR analysis of clonal cell populations indicated that SGLUT1 mRNA was not detectable in subconfluent cultures, but 2.2 and 3.9 kb SGLUT1 mRNA species appeared after cell confluence, accompanying expression of the transport activity (Yet et al, 1994). In this study, Northern analysis showed that 2.0 and 2.2 ~ 2.3 kb SGLUT mRNA species appeared in cultured proximal tubule cells and fresh proximal tubules, respectively. But the activity of SGLUT was reduced in cultured proximal tubule cells. Therefore, SGLUT gene expression of proximal tubule cells may respond to environmental conditions.

Na-dependent glucose influx has been the focus of the few studies of tubular glucose transport in diabetes. In this study, Na^+ dependent α-MG uptake was observed to be reduced in monolayers treated with 20 mM glucose, as compared with

monolayers maintained with 5 mM D-glucose. This observation would be consistent with the previous report of reduced Na/glucose cotransport activity in LLC-PK_1 cells treated with elevated D-glucose levels (Ohta et al, 1990). These results suggest that Na-dependent D-glucose reabsorption through the apical membrane in proximal tubular cells is regulated by the change in blood glucose level. The uptake of Na-dependent D-glucose by renal brush-border membrane vesicles (BBMV) isolated from streptozotocin-induced diabetic rats was decreased as compared with controls. Since a Vmax of 4.8 nmol/mg protein per 30 seconds in diabetic BBMV was significantly decreased as compared with that of controls (Vmax ≈ 7.0 nmol/mg protein per 30 seconds) without changing an apparent affinity for D-glucose, the decrease in the Na-dependent D-glucose uptake in diabetic rats is likely to be due to the reduction in the number of Na/glucose cotransport systems or a decrease in the magnitude of the Na^+ gradient across the plasma membrane (Yasuda et al, 1990). Also LLC-PK_1 cells cultured with 5mM glucose concentrations induced the increases of both islet activating protein(IAP)-catalysed ADP-ribosylation of Gi α and Na/glucose cotransport activity. Furthermore, this increase in the transport activity was blocked by the presence of IAP in a dose-dependent manner. These results suggest that there is a close relationship between the concentration of the heterotrimeric form of Gi protein (Gi $\alpha\beta\gamma$) and the activity of Na/glucose cotransport in LLC-PK_1 cells (Maruo, 1993). Glucose-dependent proximal tubular reabsorption of Na is increased in uncontrolled diabetes, reflecting higher glucose influx through SGLUT (Bank & Aynedjian, 1990). Probably, the upregulation of the renal tubular high Km GLUT2 gene compensates for the decrease in the tubule to blood glucose gradient. These changes could be involved in the pathogenesis of diabetic nephropathy.

The kidney enlarges in patients with IDDM, reflecting hypertrophy and hyperplasia (Reddi & Camerini-Davalos, 1990). The exact cellular events that lead to triggering or signalling the increase in renal tubular Na,K-ATPase in states of renal hypertrophy, such as diabetes and uninephrectomy, are not well understood. Prevention of renal enlargement is possible, in hyperglycemic rats, by effective insulin treatment (Rasch, 1979). It is not clear whether growth factors, glycemia, circulatory factors, or other factors account for renal enlargement. In the rabbit proximal convoluted tubules, maximal Na,K-ATPase activity is 33 to 38 pmol ATP/mm/min. Assuming that the molar Na^+/ATP ratio is 3 and that *in vivo* intracellular sodium concentration allows the pump to work around 30% of its Vmax, this value of ATPase activity can account for a transport capacity of 30 to 34 pEq Na^+/mm/min. Net sodium reabsorption along rabbit proximal convoluted tubule, as evaluated from the rate of isoosmotic water reabsorption, is 104 to 115 pEq/mm/min, from which only 30%, or 31 to 35 pEq/mm/min, is active. This is in good aggrement with the pumping capacity of Na,K-ATPase (Jacobson, 1979; McKeown et al, 1979).

Ouabain-sensitive Rb^+ uptake was elevated in primary cultures treated with 360 mg/dl D-glucose (20 mM) as compared with the rate observed in cultures maintained with 5 mM glucose. Such an observation could be explained by an increase in the affinity of the Na,K-ATPase for Rb^+ (ie by a decrease in the Km for Rb^+), and/or by an increase in the Vmax of the transport system. An increase in the Vmax for ouabain-sensitive Rb^+ uptake could be explained by an increase in the rate of transport of Rb^+ by each Na pump, and/or an increase in the number of active Na pumps. An increase in the rate of tranasport of Rb^+ by each Na pump may possibly be explained by an increase in intracellular Na^+ levels(a consequence of an

increase in the activity of the Na/H antiport system for example). An increase in intracellular Na^+ levels may also cause a reduction in the initial rate of sodium-dependent glucose uptake by the Na/glucose cotransport system.

The general increase in Na,K-ATPase might represent compensatory physiological changes in the kidney in response to the glucose osmotic diuresis and increased filtered sodium (Wald et al, 1993). Alternatively, from the standpoint of metabolism and transport, it is possible that in experimental diabetes, increased glucose and sodium in the renal luminal space may facilitate coupled sodium entry from the lumen, and that this increased sodium concentration in the tubular cells may accelerate water absorption which in turn could contribute to volume expansion, hypertension, and renal hypertrophy. To maintain sodium balance in the face of glomerular hyperfiltration and osmotic diuresis all tubular segments exhibited high Na,K-ATPase and therefore more energy had to be spent to fuel the Na pumps (Lynch & Balaban, 1987). This makes the kidney more susceptible to ischemic insults (Wald et al, 1990). Also, increase in Na,K-ATPase activity might, by a still unidentified mechanism, be an important contributor to the increased renal blood flow and glomerular filtration rate in diabetes. Whether the increased renal tubular Na,K-ATPase activity in streptozotocin-induced diabetic mellitus has a direct role in the pathogenesis of diabetic nephropathy remains to be determined.

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