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Effects of High Glucose Levels on the Protein Kinase C Signal Transduction Pathway in Primary Cultured Renal Proximal Tubule Cells

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=ABSTRACT=

Diabetes mellitus is associated with a wide range of pathophysiologic changes in the kidney. This study was designed to examine the mechanisms by which glucose modulates the expression of polarized membrane transport functions in primary cultured rabbit renal proximal tubule cells. Results are as follows:

The rate of 30 minute Rb^+ uptake was significantly higher(137.76±5.40%) in primary renal tubular cell cultures treated with 20 mM glucose than that of 5 mM glucose. Not the level of mRNA for the α subunit of Na, K-ATPase but that of β subunit was elevated in primary cultures treated with high glucose. The initial rate of methyl-α-D-glucopyranoside(α-MG) uptake was significantly lower(71.91±3.02%) in monolayers treated with 20 mM glucose than that of 5 mM glucose. There was a tendency of an increase in phlorizin binding site in cells treated with 5 mM glucose. However, 3-O-methyl-D-glucose(3-O-MG) uptake was not affected by glucose concentration in culture media. TPA inhibited Rb^+ uptake by 63.61± 1.94 and 45.80±1.36% and α-MG uptake by 48.54±3.69 and 41.87±6.70% in the cells treated with 5 and 20 mM glucose, respectively. Also TPA inhibited mRNA expression of Na/glucose cotransporter in cells grown in 5mM glucose medium. cAMP significantly stimulated α-MG uptake by 114.65±5.70% in cells treated with 5mM glucose, while it did not affect α-MG uptake in cell treated with 20 mM glucose. However, cAMP inhibited Rb^+ uptake by 76.69± 4.16 and 66.87±2.41% in cells treated with 5 and 20 mM glucose, respectively.

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. High glucose may in part affect the activity of the Na,K-ATPase and the Na/glucose cotransport system by controlling the protein kinase C and/or A signal transduction pathway in primary cultured renal proximal tubule cells.

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

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INTRODUCTION

Renal failure is a major cause of death in diabetes. Diabetes mellitus is associated with a wide range of pathophysiologic changes in the kidney, including an increase in glomerular filtration rate and renal blood flow, basement membrane thickening, and generalized hypertrophy(Hostetter, 1991). In the renal proximal tubule not only metabolic changes are occurring(the rate of ammoniogenesis and gluconeogenesis is increased), but also alterations in reabsorptive functions are observed.

Whereas activity of Na/H antiport system is increased, but that of Na/glucose cotransport system is decreased in animals with type I diabetes(Meury et al., 1994, Harris, et al., 1986). A decrease in Na/glucose cotransport activity has been reported in brush border membrane vesicles of diabetic rats, indicating that an intrinsic change has occurred in transporters in the plasma membrane. A direct involvement of glucose in the inhibition of the Na/glucose cotransport system has been indicated in studies on the LLC-PK_1 cell line. Moran et al(1983) showed that prolonged incubations of LLC-PK_1 cells with high glucose resulted in a reduction in Na/glucose cotransport activity, as well as the number of glucose transporters. In streptozotocin-induced diabetic rats, renal hypertrophy and increased renal Na,K-ATPase activity develop with a similar time course. As the Na,K-ATPase is a major consumer of metabolic energy and it plays central role in many renal transport processes, an increase in Na,K-ATPase activity has been proposed as an essential component of the renal hypertrophy and hyperfunction observed in diabetes mellitus(Lingrel et al., 1994, Fambrough et al., 1994, Ku et al., 1986, Ku & Meezan, 1984). The mechanisms by which the Na,K-ATPase activity is increased and the Na/glucose cotransport activity is decreased in diabetes, are poorly understood, although hyperglycemia has been proposed as an underlying cause. It has been reported that high concentration of glucose induces hypertrophy in the SV40 transformed mouse cell line designated as MCT(mouse cortical tubule)(Ziyadeh et al., 1990).

A number of experimental studies have indicated that the activities of the renal proximal tubular Na,K-ATPase and Na/glucose cotransport are both regulated by the protein kinase C pathway(Doucet, 1992). The Na/glucose cotransport and Na/phosphate cotransport activities in primary rabbit kidney proximal tubule cells are inhibited by phorbol esters (Freidlander & Amiel, 1989). Phorbol esters such as phorbol 12-myristate 13-acetate(TPA) specifically bind to protein kinase C, and are potent activators of the enzyme(Nishizuka, 1986, 1984). Diacylglycerol(DAG) analogues, oleoylacetylglycerol (OAG), and 1,2-dioctanoyl-sn-glycerol(DiC8), also inhibit the Na/glucose cotransport in microperfused proximal convoluted tubules(Baum & Hays, 1988), suggesting that protein kinase C is involved in the TPA inhibition of Na/glucose cotransport. The involvement of other signal transduction pathways, such as the cyclic AMP pathway has also been proposed (Breton et al., 1994).

Primary cultures of rabbit kidney proximal tubule cells were used in this study. These cells retain many differentiated functions distinctive of the renal proximal tubule cells(Han et al., 1996). This study was carried out to examine the mechanisms by which glucose modulates the expression of polarized membrane transport functions.

MATERIALS AND METHODS

Materials

Male New Zealand white rabbits (1.5~2.0 kg) were used. 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). 12-O-tetradecanoylphorbol 13-acetate (TPA), 8-bromoadenosine 3':5'-cyclic monophosphate (8-BrcAMP), 3-isobutyl-1-methyl-xanthine(IBMX) and other chemicals were purchased from Sigma

Chemical Corp. (St. Louis, MO). Class IV collagenase was from Worthington (Freehold, NJ). Guanidium isothiocyanate was obtained from Bethesda Research Laboratories (Gaithersburg, MD). Radionuclides, [^{14}C]methyl-α-D-glucopyranoside (α-MG), ^{14}C-3-O-methyl-D-glucose, [^{3}H]phlorizin, 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 was from Life Technologies Inc., and Zeta Probe Blotting Membrane was 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 solution of iron oxide in 0.9% NaCl was 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(if not indicated). The basal medium was further supplemented with 15 mM HEPES buffer(pH 7.4) and 20 mM sodium bicarbonate. Immediately prior to use of the medium, three growth supplements(5 μg/ml insulin, 5 μg/ml transferrin and 5×10^{-8} M hydrocortisone) were added. Water utilized in medium preparation was purified by means of a MilliQ deionization system. The kidneys of a rabbit were perfused via the renal artery, first with PBS, and then with D-MEM/F12 containing 0.5% iron oxide (wt/vol), such that the kidney turned grey-black in color. Renal cortical slices were prepared and homogenized with 4 strokes of a sterile glass homogenizer. 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 stiring bar. Glomeruli (containing iron oxide) were removed with the stiring bar. The remaining 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 cell cultures were maintained in a 37°C, 5% CO_2-humidified environment in a serum- free basal medium supplemented with three growth supplements (Chung et al, 1982).

α-methyl-D-glucoside uptake and phlorizin binding study

Primary renal proximal tubule cells 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 used for glucose uptake studies. A nonmetabolized glucose analogue, α-methyl-D-glucoside(α-MG), was used as a substrate. The uptake was determined as described by Sakhrani et al(1984). In brief, before the uptake period, monolayers were washed twice with an 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(0.5 μCi/ml) were then added to the dishes. Other compounds as indicated were incubated in the uptake assay medium. The monolayers were incubated at 37°C for the desired time period. At the end of the uptake period, they were rapidly washed three times with an ice-cold isotonic mannitol solution.

The cells were solubilized in 0.1 N NaOH and neutralized with HCl. To determine the [^{14}C] α-MG retained in cells, a half of the sample was mixed with liquid scintillation cocktail and counted in a scintillation counter (Packard Co.). The remainder of the sample was used for protein determination by Bradford method (1976). The radioactive counts of samples were then normalized with respect to protein and were corrected for zero-time uptake per mg protein (i.e., the radioactivity not removed by washing procedure). The Na-independent glucose uptake was measured by [^{14}C]3-O-methyl-D-glucose uptake. All uptake determinations were made in triplicate.

The binding of ^{3}H-phlorizin to intact monolayers was determined as described by Amsler and CooK (1982). The ^{3}H-phlorizin binding was measured over a 30 minute period in a buffer containing 140mM NaCl. Phlorizin was added at concentrations ranging from 1×10^{-5} to 1×10^{-3} M.

Rb^{+} uptake study

Primary renal proximal tubule cells were grown to confluence in 35 mm dishes as described above. After reaching a confluence the monolayers were further incubated for 4 days in the medium further supplemented with either 0 mg/dl, or 270 mg/dl D-glucose. For the study of Rb^{+} uptake, monolayers were washed twice at 23°C with 2 ml of uptake buffer (10 mM Tris-HCl, pH 7.4, and 140 mM NaCl). The cells were then incubated for 30 minutes at 37°C in uptake buffer containing 1 mM RbCl, 1 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, neutralized with HCl(Taub et al., 1992), and then analyzed for radioactivity.

Northern analysis of cellular RNA

Primary cultures for Northern analysis of Na/glucose cotransporter were grown to confluence in 100 mm dishes containing 5 mM glucose medium as described above. Subsequently, the cultures were incubated for 4 days in the same medium further supplemented with TPA(100 ng/ml). The medium was changed daily. Primary cultures for Northern analysis of Na,K-ATPase were grown to confluence in 100 mm dishes containing glucose-free medium. Subsequently, the cultures were incubated for 4 days in the same medium further supplemented with either 300 mg/dl D-glucose, 100 mg/dl D-glucose, or no glucose. The medium was changed daily. On day 4 the cultures were used for RNA preparation. Total RNA was isolated by the guanidium isothiocyanate/cesium chloride method(Han, 1993). RNA 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. The blots were hybridized overnight at 65°C as described by Church and Gilbert(1984).

A labelled probe for Na/glucose cotransporter cDNA was prepared from a 2.3 kb EcoR 1 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). ^{32}P labelled Na,K-ATPase probes were prepared from restriction fragments of appropriate cDNA's, using the random primer method. The probes utilized included a) an EcoR 1 fragment derived from plasmid pKS α 1.5, which contained a 1.5 kb dog kidney α subunit cDNA, b) a 958 base pair EcoR 1 fragment derived from plasmid pUC 18.B6, which contained a 729 base pair coding region of dog kidney β subunit cDNA(obtained from Dr. Alicia McDonough, USC). Inserts were removed from above plasmids by restriction endonuclease digestion, and purified by means of agarose gel electrophoresis. Following ^{32}P-labelling by the random priming method, probes with a specific activity greater than 10^{7} cpm/μg DNA were used for hybridization study.

RESULTS

Effect of high glucose on the activity of the Na,K-ATPase

The effect of exogenous glucose on the activity of Na,K-ATPase in primary cultured rabbit renal proximal tubule cells was determined. The activity of the Na,K-ATPase was determined by Rb^+ uptake. Rb^+ uptake in primary cultures treated with 20 mM glucose was significantly increased to 137.76± 5.40% of the value in cultures maintained with 5mM glucose at 30 minutes(Fig. 1, $P<0.05$).

Effects of high glucose on the activity of Na/glucose cotransport system and phlorizin binding

The activity of Na/glucose cotransport system may also be influenced by exogenous glucose. In order to evaluate this possibility, glucose uptake study was conducted. A nonmetabolized glucose, α-methyl-D-glucoside(α-MG), was utilized as a substrate. The rate of 1mM ^{14}C-α-MG uptake was determined over a 30 minute period in the presence of 140 mM NaCl. α-MG uptake in monolayers treated with 20 mM glucose was significantly decreased to 71.91±3.02% of the value in monolayers maintained with 5 mM glucose(Fig. 2, $P<0.05$). A decrease in α-MG uptake may be associated with a reduction in the number of Na/glucose cotransport systems. This possibility was evaluated by measuring ^{3}H-phlorizin binding. The results, however, indicated that there was no significant increase in binding site in cells treated with 5mM glucose(Fig. 3). 3-O-MG uptake was not affected by glucose concentration in culture media(Fig. 4).

Effects of TPA on Rb^+ uptake and α-MG uptake

Primary cultures of rabbit kidney proximal tubule cells were grown to confluence in normal glucose (5 mM) D-MEM/F12 medium supplemented with insulin, transferrin and hydrocortisone. Confluent monolayers were treated with 270 mg/dl D-glucose,

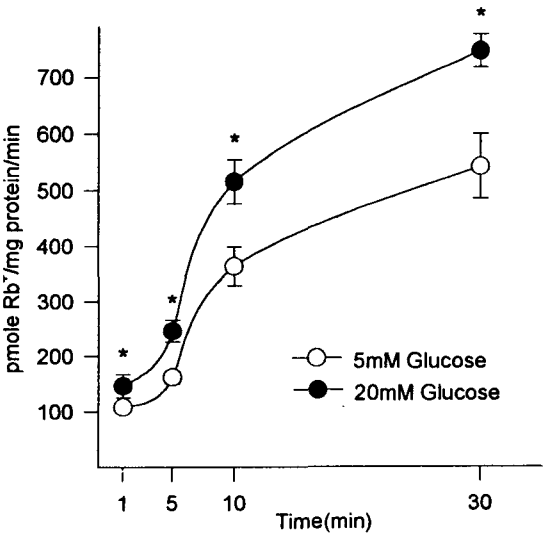

*Fig. 1. Time courses of $^{86}Rb^+$ uptake into cultured proximal tubular cells treated with different glucose concentrations. Each point is the mean ±S.E. of 9 separate experiments performed on 3 different cultures. *, $P<0.05$ vs. 5 mM glucose.*

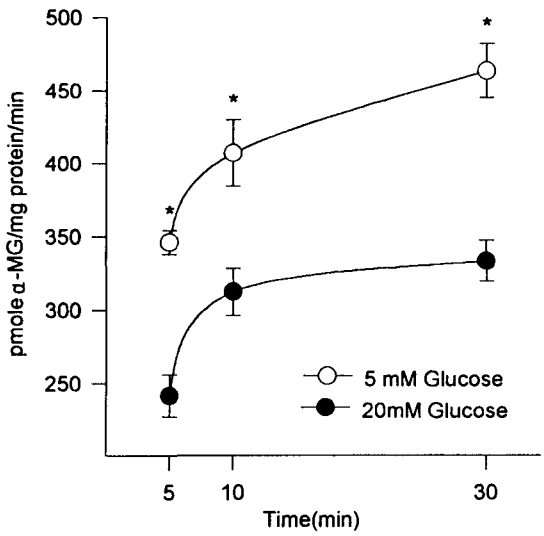

*Fig. 2. Time courses of ^{14}C-α-MG uptake into cultured proximal tubular cells treated with different glucose concentrations. Each point is the mean ±S.E. of 9 separate experiments performed on 3 different cultures. *, $P<0.05$ vs. 20 mM glucose.*

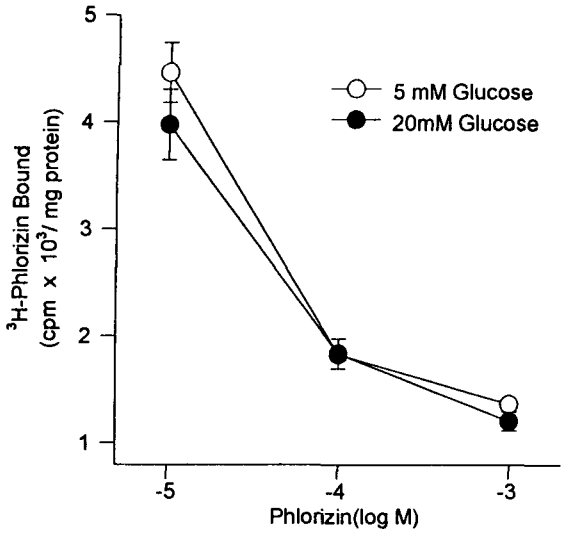

Fig. 3. Effect of glucose on ^{3}H-phlorizin binding in cultured proximal tubular cells treated with different glucose concentrations. Phlorizin was added to the incubation medium at concentrations ranging from 1×10^{-3} to 1×10^{-5}M throughout uptake period. Uptake was measured at 37°C for 30 minutes. Each point is the mean ±S.E. of 9 separate experiments performed on 3 different cultures.

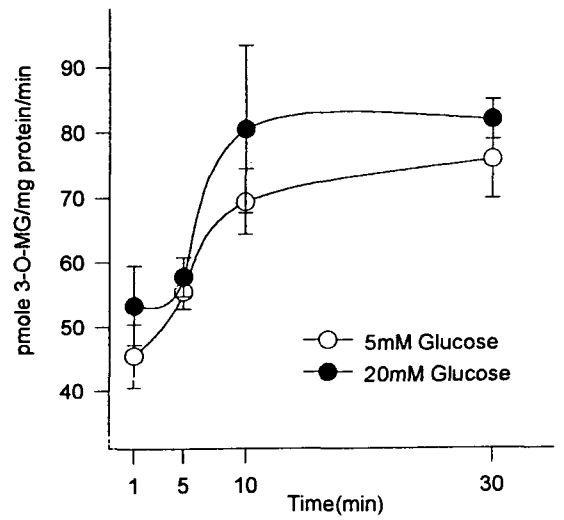

Fig. 4. Time courses of ^{14}C-3-O-MG uptake into cultured proximal tubular cells treated with different glucose concentrations. Each point is the mean±S.E. of 9 separate experiments performed on 3 different cultures.

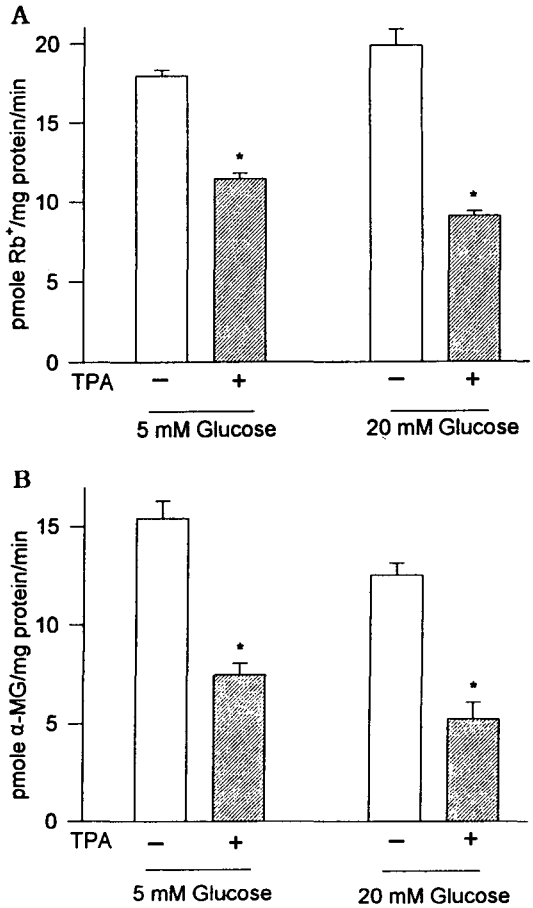

Fig. 5. Effects of TPA on $^{86}Rb^+$ uptake(A) and ^{14}C-α-MG uptake(B).
*Primary cultures of rabbit kidney proximal tubule cells were grown to confluence in 5mM glucose D-MEM/F12 medium. Subsequently, the cultures were incubated for 4 days in medium further supplemented with either 0 or 270 mg/100ml D-glucose. Primary cultures were then incubated either in the presence or absence of 100ng/ml TPA over 4-day period. Uptake was measured at 37°C for 30 minutes. Values are the mean±S.E. of 9 separate experiments performed on 3 different cultures. *, P<0.05 vs. control(5 mM or 20 mM glucose).*

or with TPA, or with D-glucose in combination with TPA over a 4-day time period. Monolayers in normal glucose medium were used as control(Fig. 5). Results indicated that TPA inhibited Rb^+ uptake (63.61±1.94% of that of 5 mM glucose, 45.80±

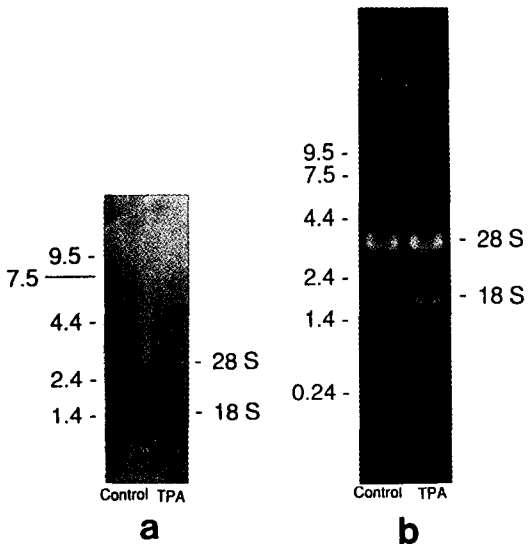

Fig. 6. Northern blot analysis of Na/glucose cotransporter mRNA from primary rabbit kidney cells. Total RNA(10 μg/lane) was subjected to electrophoresis in 0.8% agarose gels. One set of samples from the gel was transferred to a Zeta probe blotting membrane. The blot was hybridized with a Na/glucose cotransporter cDNA probe. The blot was then wahsed and exposed to X-ray film for 4 days(a). The other set of RNA samples was stained with ethidium bromide as illustrated in b.

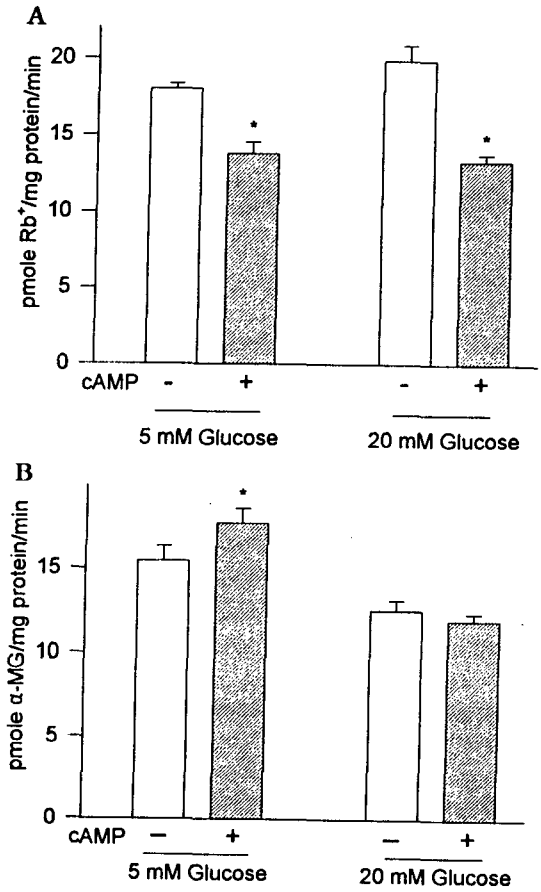

Fig. 7. Effects of 8-Br cAMP on $^{86}Rb^{+}$ uptake(A) and ^{14}C-α-MG uptake(B).
*Primary cultures of rabbit kidney proximal tubule cells were grown to confluence in 5 mM glucose D-MEM/F12 medium. Subsequently, the cultures were incubated for 4 days in medium further supplemented with either 0 or 270 mg/100ml D-glucose. Primary cultures were then incubated either in the presence or absence of 200 μg/ml 8-Br cAMP over 4-day period. Uptake was measured at 37°C for 30 minutes. Values are the mean ±S.E. of 9 separate experiments performed on 3 different cultures. *, P<0.05 vs. control(5 mM or 20 mM glucose).*

1.36% of that of 25 mM glucose) and α-MG uptake (48.54±3.69% of that of 5 mM glucose, 41.87± 6.70% of that of 20 mM glucose) in the cells treated with 5 mM or 20 mM glucose(P<0.05). Northern analysis was performed to evaluate the possibility that the effect of TPA on Na/glucose cotransport was the mRNA level. The level Na/ glucose cotransporter mRNA was decreased in primary cultures treated with TPA(Fig. 6).

Effects of cAMP on Rb^{+} and α-MG uptakes

In the next series of experiments, we evaluated that 8-bromocyclic AMP affects the Na,K-ATPase and the Na/glucose cotransport system. Confluent monolayers were treated with 270 mg/dl D-glucose, with 8-bromocyclic AMP, or with D-glucose in combination with 8-bromocyclic AMP over a 4-day period. Monolayers in normal glucose medium were used as control(Fig. 7). cAMP significantly stimulated α-MG uptake on cells treated with 5 mM glucose (114.65±5.70% of that of 5 mM glucose)(P<0.05),

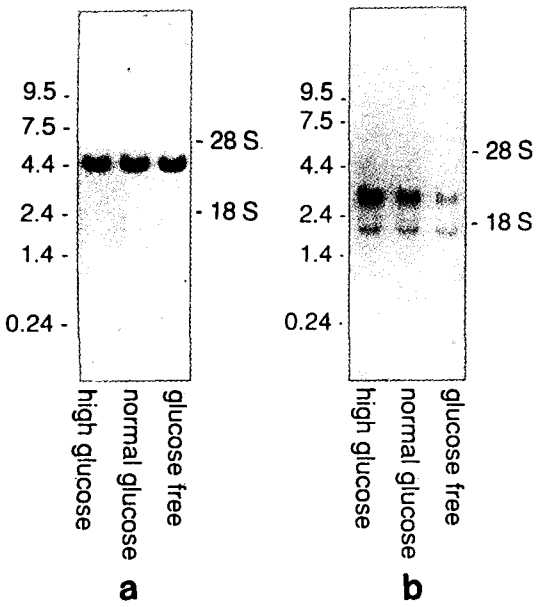

Fig. 8. Northern blot analysis of Na,K-ATPase mRNA from primary rabbit kidney cells. RNA samples(10μg/lane) were run on 0.8% agarose gels, and transferred to Zeta probe blotting membranes. The blots were first hybridized with a ^{32}P-cDNA probe for the α subunit of the dog Na,K-ATPase. Subsequently, the blots were stripped of the α subunit cDNA probe, and were hybridized with a ^{32}P-cDNA probe for the β subunit of the dog Na,K-ATPase. The results are illustrated in a(α-Na,K-ATPase) and b(β-Na,K-ATPase).

while did not affect in cells treated with 20 mM glucose. However, cAMP inhibited Rb^+ uptake(76.69 ±4.16% of that of 5 mM glucose, 66.87±2.41% of that of 20 mM glucose) in cells treated with 5 mM or 20 mM glucose.

Effects of glucose on Na,K-ATPase mRNA

Northern analysis was performed to evaluate the possibility that the effect of glucose on Na,K-ATPase was an the mRNA level. Primary cultures were grown to confluence in glucose-free medium supplemented with either 300 mg/dl D-glucose, 100 mg/dl D-glucose, or no glucose. Although the level of mRNA for α subunit was not elevated, that for β subunit was elevated in primary cultures treated with high glucose(Fig. 8).

DISCUSSION

The expression of Na/glucose cotransport system in the renal tubule cell cultures is regulated by the concentration of glucose in the growth medium. In the present study, the rate of α-MG uptake was observed to be lower in monolayers treated with high glucose(20 mM) than that of monolayers maintained with normal glucose(5 mM). This result is consistent with observations in LLC-PK_1 cells (Moran et al., 1983). Yasuda et al(1990) found that a decrease of renal cortical brush-border membrane vesicle Na/glucose cotransporter activity in streptozotocin-treated rats correlated with a reduction in cotransporter site determined by phlorizin binding. Because cotransporter activity recovered when plasma glucose was reduced either by insulin replacement or by 3 days of starvation, these results suggested that the reduction in cotransporter expression resulted from a hyperglycemia. Our results demonstrated that the number of phlorizin binding sites in cells grown in 5 mM glucose is slightly higher than that in cells grown in 20 mM glucose. Therefore, decreased glucose transport in 20 mM glucose conditions can be partly attributed to a decrease in the number of transporting sites available. In fibroblasts, a low concentration of glucose or glucose depletion was found to stimulate Na^+ independent glucose transport by enhancing the induction of glucose transporters(Stanton & Seifter, 1988). In the present study, however, we did not find any effect of glucose concentration in the culture media on the 3-O-MG uptake.

The present study shows that Na,K-ATPase mRNA and Rb^+ uptake increased in a high glucose condition. These results suggest that increase in the mRNA level may result in an increased rate of biosynthesis of the Na,K-ATPase, and its subsequent insertion into the plasma membrane. As a consequence the Rb^+ uptake(total Na,K-ATPase activity) would be increase. The present study along with our

previous studies support a close association between the development of diabetic renal hypertrophy and increased renal tubular Na,K-ATPase activity(Han, 1995).

To examine the mechanisms by which glucose modulates transport functions of proximal tubule cells, we have attempted to examine the involvement of TPA and cAMP. The present study demonstrates that TPA and high glucose inhibit the α-MG uptake in primary rabbit kidney proximal tubule cells. These results support the hypothesis that high glucose affects the activity of α-MG transport system by activating the protein kinase C pathway. Protein kinase C activity has been shown to increase in the luminal brush border of proximal tubules from diabetic rats(Hise & Mehta, 1988). High glucose has been shown to activate protein kinase C in mesangial cell cultures, further suggesting that intracellular free Ca^{2+} may be increased during hyperglycemia. On the other hand, there is the possibility that protein kinase C may directly modulate the function of Na/glucose cotransporter. There are three potential kinase C sites on SGLT1: Ser-303, Ser-418, and Ser-562, all of which are predicted to be intracellular. A rapid loss of SGLT1 mRNA occurs following treatment of confluent LLC-PK_1 cultures with TPA(Hediger & Rhoads, 1994). Results of the present study show that TPA inhibited Na,K-ATPase activity in primary cultured proximal tubule cells. Mechanisms by which activation of protein kinases lead to Na,K-ATPase inhibition are not clear. Bertorello et al(1991) reported that PKC can phosphorylate the catalytic subunit of purified shark rectal gland Na,K-ATPase in vitro. Another possibility is that the kinases interact, directly or through intermediary steps, with other mediators that participate in the signaling cascade in kidney cells(e.g., PLA2)(Nishizuka, 1992, Hirata, 1981).

The present study shows that 8-Br cAMP caused an increase in the activity of Na/glucose cotransport system. This observation is consistent with previous results obtained in the LLC-PK_1 cell line(Miller & Heath, 1989). Previously, IBMX(cAMP phosphodiesterase inhibitor) was found to stimulate the maturation of Na/glucose cotransport system in LLC-PK_1 cells. SGLT1 mRNA was also increased after treatment of cultures with IBMX, an inducer of Na/glucose cotransport activity(Yet et al., 1994). In our experiments, the Na/glucose cotransporter mRNA was not increased after treatment of cultures with 8-Br cAMP(data not shown). In this case, glucose may exert the effect at the posttranscriptional level. In contrast, phorbol esters delay the maturation of the Na/glucose cotransport system in LLC-PK_1(Amsler & Cook, 1982). However conflicting data have been reported regarding the modulation of the Na,K-ATPase by signaling pathways in proximal tubule. In rat kidneys, Giesen et al(1984) demonstrated that an increase in endogenous cAMP production led to an activation of Na,K-ATPase in renal cortical homogenates and an inhibition of Na,K-ATPase in medullary homogenates. In agreement with the study of Giesen et al., Bertorello and Aperia(1988) demonstrated that DBcAMP and forskolin significantly increase Na,K-ATPase activity in rat proximal convoluted tubules. In contrast, our result demonstrated an inhibition of Na,K-ATPase of primary cultured rabbit renal proximal tubule cells by cAMP. Such a discrepancy in cAMP effect on Na,K-ATPase may be attributed to a species difference. The mode of action of cAMP in inhibiting the Na,K-ATPase could be the result of a change in the substrate concentration, disorder of exocytotic insertion of pump molecules, inhibition of a latent pool, or modulation of the kinetics of the pumps already present in the membrane. Therefore, the mechanisms by which high glucose lead to change of renal transport functions are not clearly explained from these results. Further studies are need to elucidate effects of high glucose on intracellular signal transduction pathways.

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. High glucose may partly affect the activity of Na,K-ATPase and

Na/glucose cotransport system by controlling the protein kinase C and/or A signal transduction pathway in primary cultured renal proximal tubule cells.

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