Regulatory Mechanisms of Na^+/glucose Transporters in Renal Proximal Tubule Cells

Ho Jae Han, DVM, Ph.D.

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

Glucose is a key fuel and an important metabolic substrate in mammals. A number of mechanisms contribute to the changes in cellular functions that occur in response to exposure of endogenous factors. These include increased cytokines and growth factors as well as the glucose specific effects due to increased intracellular level of glucose. The kidneys play a major role in the regulation of plasma glucose levels, and ever increasing attention is now being given to renal glucose transporters as the implications including diabetes mellitus. The primary rabbit renal proximal tubule cells (PTCs) culture system utilized in my study has well recognized to retain *in vitro* the differentiated phenotype typical of the renal proximal tubule, including a polarized morphology and distinctive proximal tubule transport. Thus, I examined the effects of several factors on Na^+/glucose cotransporters in PTCs. ANG II, EGF, epinephrine, TCDD, high glucose, bee venom, partially inhibited [^{14}C]-α-methyl-D-glucopyranoside (α-MG) uptake, whereas BSA stimulates α-MG uptake in PTCs. On the other hand, caffeic acid, ginsenosides, and estrogens protected oxidative stress-induced inhibition of α-MG uptake.
Key Words : Kidney, Na^+/glucose cotransporter, Glucose

Glucose has been observed to have dramatic effects on the functional properties of epithelial cells and kidneys play a major role in the regulation of plasma glucose levels[1, 2]. In addition, glucose is actively reabsorbed by specific transporters across the brush-border membranes of proximal tubular cells[3]. The number of known glucose transporters in the kidney and small intestine has expanded considerably by many researchers. Alteration of glucose transporter induced changes of regulatory mechanism in renal function[4, 5]. However, the functional and molecular changes of transport systems by several factors are still unknown. Thus, this review focuses on regulatory mechanisms of glucose transporters in renal proximal tubule cells.

Corresponding author : Ho Jae Han, Department of Veterinary Physiology, College of Veterinary Medicine, Chonnam National University
Tel : 82-62-530-2831 Fax : 82-62-530-2809
E-mail : hjhan@chonnam.ac.kr

Glucose uptake into the cells belong to one of the most extensively studied fields of cell physiology

The major function of membrane proteins is their role as transporters. Many molecules enter or leave the cell using transport proteins. We will classify transport proteins into two categories: channels and carriers. Channel proteins create water-filled passageways that link the intracellular and extracellular compartments. Carrier proteins bind to the substrates that they carry but never form a direct connection between the intracellular and extracellular fluid. Movement of substances across cell membranes with the aid of a carrier protein is known as mediated transport. If mediated transport is passive, molecules move down their concentration gradient, and net transport stops when concentrations are equal inside the cell and out, the process is known as facilitated diffusion. If protein-mediated transport requires ener-

gy from ATP or another outside source and moves a substance against its concentration gradient, the process is known as active transport. Both passive and active forms of mediated transport demonstrate three properties: specificity, competition, and saturation[6, 7] Glucose is a key fuel in mammals and an important metabolic substrate. It is obtained directly from the diet, principally following the hydrolysis of ingested disaccharides and polysaccharides, and by synthesis from other substrates in organs such as the liver. Glucose derived from the diet is transferred from the lumen of the small intestine, and both dietary glucose and glucose synthesized with in the body have to be transported from the circulation into target cells. Why does body have both a facilitated diffusion glucose transporters (GLUTs) and a Na^+/glucose cotransporter (SGLTs) for glucose uptake? All cells use GLUTs to bring glucose into cells from the extracellular fluid. In addition, GLUTs can move molecules in either direction across a membrane, depending on the concentration gradient. In contrast, the SGLTs can move substrate only into the cell because it must follow the Na^+ gradient. Consequently, the SGLTs are found only on transporting epithelial cells that use it to bring glucose into the body from the external environment.

Classification of glucose transporters

Sugar absorption/reabsorption in the mammalian body is mediated by specific transporters. The number of known glucose transporters has expanded considerably over the past 2 years. At least three, and up to six, Na^+/glucose cotransporters (SGLT1-SGLT6; gene name SLC5A, 11 genes) have been identified[8-10]. The first of this type of glucose transport protein to be cloned was the high-affinity transporter from rabbit intestine, SGLT1 (11). SGLT1 has a limited tissue expression and is found essentially on the apical membranes of small-intestinal absorptive cells (enterocytes) and renal proximal straight tubules (S3 cells). Consequently, a single missense mutation in the SGLT1 gene causes clinical Glc/Gal malabsorption[12]. A second Na^+/glucose transporter, SGLT2, is of low affinity and is predominantly expressed on the apical membrane of renal convoluted proximal tubules (S1 and S2 cells)[13, 14]. Renal reabsorption of Glc is considered to occur mainly through SGLT2, because a homozygous nonsense mutation and compound heterozygous mutations in the SGLT2 gene were recently found in patients with renal glucosuria[15, 16]. In addition, a specific inhibitor of this transporter induces glucosuria in rats[17, 18]. A pig renal amino acid co-transporter (SAAT1) has been reclassified as a low-affinity glucose co-transporter and finally renamed pSGLT2[19]. SGLT3, found as a humam ortholong of porcine SGLT2 and formerly called SAAT1[19, 20], recently underwent a reevaluation of its function[21]. These results of the reevaluation suggested that SGLT3 is not glucose transporter, but rather a glucose sensor in the plasma membrane of cholinergic neurons, skeletal muscle, and other tissues. Additional members, SGLT4-6, have been assigned but await complete functional and structural characterization. Similarly, thirteen members of the family of facilitative sugar transporters (GLUT1-GLUT12 and HMIT; gene name SLC2A, 13 genes) are now recognized. These various transporters exhibit different substrate specificities, kinetic properties, and tissue expression profiles (Table 1)[22]. The class I facilitative transporters contain GLUT1-4, and these have been comprehensively characterized in terms of structure, function, and tissue distribution. The class II facilitative transporters are headed by the fructose transporter GLUT5, and include GLUT7, GLUT9 (neither of which has been functionally characterized) and GLUT11. The class III facilitative transporters comprise five members : GLUT6, GLUT8, GLUT10, GLUT12, and HMIT.

The number of distinct gene products, together with the presence of several different transporters in certain tissues and cells (for example, GLUT1,

Table 1. The Glucose Transporter (GLUT) Family of Facilitative Sugar and Polyol Transporters (Gene name SLC2A)

Isoform	Previous name	Class	Main tissue localization	Insulin sensitive?	Functional characteristics (transport)	Present in skeletal muscle?*	Present in white adipose tissue?*
GLUT1	—	□	Erythrocytes, brain, ubiquitous	No	Glucose	Yes	Yes
GLUT2	—	□	Liver, pancreas, intestine, Kidney	No	Glucose (low affinity); fructose	No	No
GLUT3	—	□	Brain	No	Glucose (high affinity)	No	Yes (m)
GLUT4	—	□	Heart, muscle, WAT, BAT, brain	Yes	Glucose (high affinity)	Yes	Yes
GLUT5		□	Intestine, testes, Kidney	No	Fructose; glucose (very low affinity)	Yes	Yes
GLUT6	GLUT9	□	Brain, spleen, leucocytes	No	Glucose	No	n.d
GLUT7		□	n.d	n.d	n.d	n.d	n.d
GLUT8	GLUT X1	□	Testes, brain and other tissues	No (yes in blastocytes)	Glucose	Yes (m)	Yes (m)
GLUT9	GLUT X	□	Liver, kidney	n.d	n.d	No	n.d
GLUT10		□	Liver, pancreas	No	glucose	Yes (m)	No
GLUT11·	GLUT 10	□	Heart, muscle	No	Glucose (low affinity); fructose (long form)	Yes (m)	No
GLUT12	GLUT 8	□	Heart, prostate muscle, small intestine, WAT	Yes	n.d	Yes	Yes
HMIT		□	Brain	n.d	H^+-myo inositol	No (m)	Yes (m)

WAT, white adipose tissue; BAT, brown adipose tissue; m, mRNA only; n.d.. not determined; HMIT, H^+-coupled myo-inositol transpoter

*The presence of each transporter in skeletal muscle and WAT is shown since these are the major sites of insulin-stimulated glucose uptake.

*GLUT11 occurs in two splice variants; a short form (low-affinity glucose transport) and a long form (which may be a fructose transporter). (Wood and Trayhurn, 2003)

GLUT4, GLUT5, GLUT8, GLUT12 and HMIT in white adipose tissue), indicates that glucose delivery into cells is a process of considerable complexity[22]. The expression of several sugar transporter isoforms in individual tissues, and indeed cells, is a reflection of the different characteristics of each of the various transporters, and provides a high degree of specificity in the control of glucose uptake under different physiological conditions (i.e., a wide range of glucose concentrations). Therefore, the mechanisms by which external and internal signals regulate glucose uptake into the cells belong to one of the most extensively studied fields of cell physiology.

Regulatory mechanisms of SGLTs in renal proximal tubule cells

The kidneys play a major role in the regulation of plasma glucose levels, and ever increasing attention is now being given to renal glucose transporters as drug targets in the treatment of patients with diabetes mellitus[2]. Each day, ~180 g of D-glucose

are filtered from plasma by the kidneys, and this is all normally reabsorbed back into the blood in the proximal tubules[8]. The model for glucose transport across the tubule is similar to that first proposed for the small intestine; i.e., glucose is first accumulated within the epithelium by SGLTs in the brush-border membrane and then is transported out of the cell across the basolateral membrane by GLUTs. Genetic studies suggest that two different genes regulate SGLTs, and there is evidence from animal studies to suggest that the major bulk of sugar is reabsorbed in the convoluted proximal tubule by a low-affinity, high-capacity transporter and that the remainder is absorbed in the straight proximal tubule by a high-affinity, low-capacity transporter (Table 2, 3)[8].

Experimental model

A convenient means to evaluate the effects of several factors including hormones and growth factors on glucose transporters of renal proximal tubule cells is by means of *in vitro* studies with differentiated cell cultures[23]. The primary rabbit renal proximal tubule cells (PTCs) culture system which was utilized in my study has well recognized to retain in vitro the differentiated phenotype typical of the renal proximal tubule, including a polarized morphology and distinctive proximal tubule transport[24-26]. In my reports, I also demonstrated that a Na^+-dependent and phlorizin-sensitive glucose transporter is present in the PTCs[27-30]. Therefore, PTCs in hormonally defined, serum-free culture conditions would be a powerful tool for studying the alteration of glucose transporters activity in renal proximal tubule cells. Now, I will introduce my several data about regulatory mechanisms of SGLTs in renal proximal tubule cells.

Table 2. Na^+/glucose Cotransporter Genes

	SGLT1	SGLT2	SGLT3
Locus name	SLC5A1	SLC5A2	SLC5A4
Locus ID	6523	6524	6527
Alternatives	D22S675		DJ90G24.4/ SAAT1
Unigene	Hs 1964	Hs 9003	Hs 130101
Accession nos. (refseq)	NP00334	NP003032	AAB61732
OMIM	182380	182381	
Chromosome (refseq)	22p13.1	16p11.2	22p121-123
Size	72 kb		37 kb
Exons (no.)	15		15
Protein (residues)	664	672	659

ID, identification; SGLT, Na^+-glucose cotransporter (Wright; 2001).
Web address; http://www.ncbi.nlm.nih.gov80/LocusLink

Effects of several regulatory factors on glucose transporters

1. ANG II

Angiotensin II (ANG II) in the proximal tubule cells is associated with the pathogenesis of the diabetic nephropathy, since high glucose downregulated ANG II binding due to the increase of ANG II synthesis. SGLTs are also related to the development of diabetic nephropathy. ANG II and SGLTs have been reported to be associated with the onset of diverse renal diseases. However, the effect of ANG II on

Table 3. Transport Properties of SGLTs

	SGLT1	SGLT2	SGLT3*
$K_{0.5}$ (D-glucose; mM)	0.4	2	6
$K_{0.5}$ (Na^+; mM)†	3	100	1.5
Coupling (Na^+/glucose)‡	2	(1)	2
Turnover no., s^{-1}§	60		60
Phlorizin Ki, μM	0.22	1	9
Sugar selectivity	D-glc ~ D-gal	D-glc ~ D-gal	D-glc ~ D-gal
Na^+ uniport	+		+
Water cotransport	+		+

Abbreviations : $K_{0.5}$, affinity value; glc, glucose; gal, galactose
*Properties of pig SGLT3. Those for SGLT1 and SGLT2 are for human.
†Determined at -150 mV, but the voltage for SGLT2 was not specified.
‡Determined using Na^+ and sugar fluxes under voltage clamp, except for SGLT2.
§Taken from the maximum rate of Na^+/glucose cotransport at saturating voltages (-150 mV) and the number of transporters estimated from SGLT charge movement (Q_{max}) (Wright; 2001).

SGLTs activity was not elucidated. Thus, the effect of ANG II on ^{14}C-α-methyl-D-glucopyranoside (α-MG) uptake and its related signal pathways were examined in the primary cultured rabbit renal PTCs. ANG II inhibited α-MG uptake in time- and concentration- dependent manner and decreased protein level of SGLTs. ANG II-induced inhibition of α-MG uptake was blocked by losartan, AT_1 receptor blocker, but not by PD 123319, AT_2 receptor blocker. The role of tyrosine kinase phosphorylation and arachidonic acid were involved in ANG II-induced inhibition of α-MG uptake. The effects of ANG II on AA release and α-MG uptake also were abolished by staurosporine and bisindolylmaleimide I (PKC inhibitors) or PD 98059 (p44/42 MAPK inhibitor), but not SB 203580 (p38 MAPK inhibitor), respectively. Indeed, ANG II increased p44/42 MAPK activity. ANG II-induced activation of p44/42 MAPKs was blocked by staurosporine (Fig. 1)[24].

2. EGF

The kidney is one of the main sites of synthesis of EGF. EGF receptors have been found in the proximal tubule at high levels, suggesting that EGF may play a role in modulating renal function. To date there is little information concerning the effect of EGF on the SGLTs in renal proximal tubule cells. On the other hand, EGF has been observed to increase the activity of the Na^+/H^+ antiport system and the Na^+/HCO_3^- cotransport system in primary rat kidney proximal tubule cell cultures, and to inhibit sodium, potassium, and chloride transport in the ra-

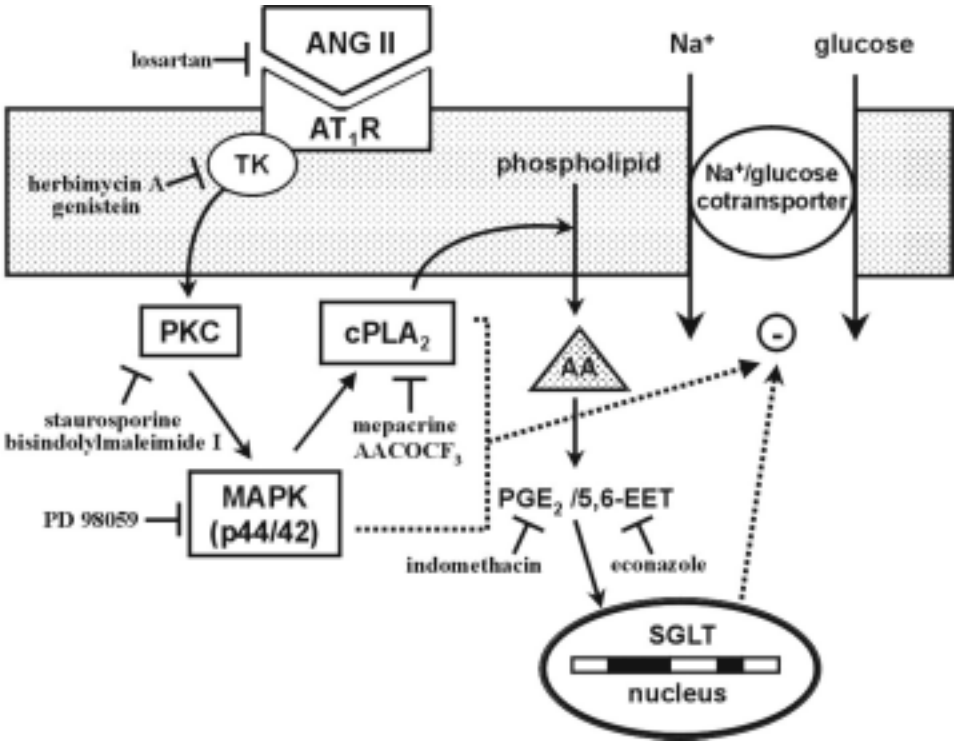

Fig. 1. *The hypothesized model of inhibition of Na^+/glucose cotransporters (α-MG uptake) by ANG II in renal proximal tubule cells. ANG II binding to AT1 receptor stimulates TK or PKC which induces p42/44 MAPK activation. Subsequently, p44/42 MAPK activation induces the release of [^{3}H]-AA which may be metabolized to cyclooxygenase and cytochrome P-450 metabolites such as PGE2 and 5,6-EET. These molecules may induce the decrease of SGLTs expression, which is involved in inhibition of the α-MG uptake. AT1R; angioteins II receptor type 1, TK; tyrosine kinase, PKC; protein kinase C, MAPK (p44/ 42); p44/42 mitogen activated protein kinase, $cPLA_2$; cytosolic phospholipase A_2, AA; arachidonic acid, PGE_2; prostaglandin E_2, 5,6-EET; 5, 6 epoxy-eicosatrienoic acids. Solid line; proposed pathway, dash line; suspected pathway (Han et al., 2004a).*

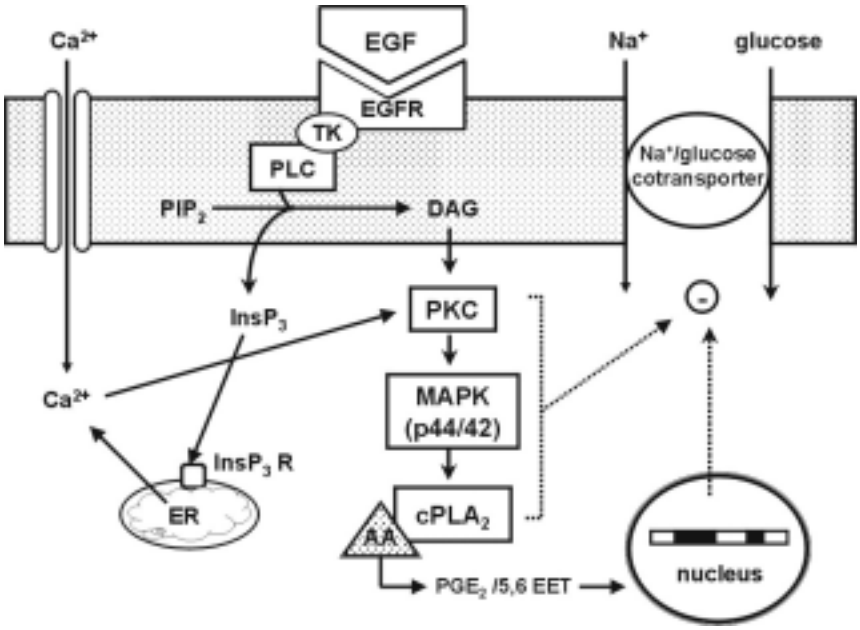

Fig. 2. *The hypothesized model of inhibition of Na^+/glucose cotransporters (α-MG uptake) by EGF in renal proximal tubule cells. EGF binding to EGF receptor is coupled to the activation of TK, PLC and PKC which induces p44/42 MAPK activation. Subsequently, p44/42 MAPK activates $cPLA_2$ which stimulates AA release, which may be metabolized to cyclooxygenase and cytochrome P-450 metabolites (PGE_2 or 5,6-EET et al). These molecules may induce some new protein synthesis including SGLT1, which involved in inhibition of Na^+/glucose cotransporters. In addition, there is a possibility that protein kinases may directly regulate the activity of Na^+/glucose cotransporters. EGFR; EGF receptor, TK; tyrosine kinase, PLC; phospholipase C, PIP_2; phosphatidylinositol 4,5-bisphosphate, $InsP_3$; inositol trisphosphate, DAG; diacylglycerol, PKC; protein kinase C, MAPK (p44/42); p44/42 mitogen activated protein kinase, $cPLA_2$; cytosolic phospholipase A2, AA; arachidonic acid, COX; cyclooxygenase, cP450; cytochrome P-450 epoxygenase, PGE_2; prostaglandin E_2, 5,6-EET; 5, 6 epoxy-eicosatrienoic acids. Solid line denotes the proposed pathway and the dotted line is the suspected pathway (Han et al., 2004b).*

bbit cortical collecting duct. Thus, the effect of EGF on α-MG uptake and its related signaling pathways were examined in primary cultured rabbit renal PTCs. EGF (50 ng/ml) was found to inhibit α-MG uptake, a distinctive proximal tubule marker. The EGF effect was blocked by EGF receptor antagonist or tyrosine kinase inhibitors. In addition, the EGF-induced inhibition of α-MG uptake was blocked by phospholipase C inhibitors as well as PKC inhibitors. EGF was also observed to increase inositol phosphate formation. Furthermore, both the EGF-induced inhibition of α-MG uptake and increase of arachidonic acid (AA) release were blocked by $AACOCF_3$ (cytosolic phospholipase A_2 inhibitor), indomethacin (cyclooxygenase inhibitor), and econazole (cytochrome P-450 epoxygenase inhibitor). I examined the involvement of mitogen activated protein kinases (MAPKs) in mediating the effect of EGF on α-MG uptake. Indeed, EGF increased phosphorylation of p44/p42 MAPK and the EGF-induced inhibition of α-MG uptake as well as the stimulatory effect of EGF on AA release was blocked by PD 98059, suggesting a causal relationship. However, inhibitors of PKC also prevented the EGF-induced increase of AA release (Fig. 2)[25].

Both EGF and oxidative stress contribute to the initiation and progression of renal proximal tubular dysfunction under pathophysiologic conditions. Thus,

study was performed 1) to examine both the individual, and the combined effects of H_2O_2 and EGF on α-MG uptake in PTCs, and 2) to elucidate the involvement of p44/42 MAPK and phospholipase A_2 in mediating these actions. Both H_2O_2 and EGF inhibited α-MG uptake individually, while the combination of H_2O_2 and EGF further potentiated the inhibitory effect on α-MG uptake, which was elicited by each agent. H_2O_2 not only caused a rapid increase in the phosphorylation of p44/42 MAPK, but also promoted the translocation of $cPLA_2$ from the cytosolic to particulate fraction, and stimulated cellular AA release. EGF similarly activates phosphorylation of p44/42 MAPK and stimulates AA release. When PTCs were exposed to 100 μM H_2O_2 and 50 ng/ml EGF simultaneously, a further increase in the phosphorylation of p44/42 MAPK, of AA release, and of prostaglandin E_2 (PGE_2) production was elicited as compared with the effects of each individual agonist alone. Moreover, the additive phosphorylation of p44/42 MAPK, AA release, and PGE_2 production by H_2O_2 and EGF was almost completely inhibited by the p44/42 MAPK inhibitor, PD 98059[29].

3. Epinephrine

The extensive sympathetic innervation of the kidney is important in the physiological regulation of all aspects of renal functions. Increased renal sympathetic nerve activity results in renal vasoconstriction, with decreased glomerular filtration rate and renal blood flow, and increased renal vascular resistance; increased renal tubular reabsorption of sodium and water throughout the nephron; and increased renal release of renin and norepinephrine. In addition, there are direct adrenergic effects on proximal tubule functions. Epinephrine has known to be a very important factor in the regulation of renal sodium excretion. However, the effect of epinephrine on SGLTs was not fully elucidated. Thus, I examined effect of epinephrine on α-MG uptake and its related signal pathways in the primary cultured rabbit renal PTCs. Epinephrine inhibited α-MG uptake in a time- and dose-dependent manner and also decreased SGLT1 and SGLT2 protein level. Both phentolamine and propranolol completely prevented epinephrine-induced inhibition of α-MG uptake. The epinephrine-induced inhibition of α-MG uptake was blocked by SQ 22536 or myristoylated PKA inhibitor amide 14-22 and epinephrine increased the intracellular cAMP content. In western blotting analysis, epinephrine increases phosphorylation of p44/42 and p38 MAPKs and PD 98059 or SB 203580 blocked the effect of epinephrine. In addition, epinephrine increased AA release and PGE_2 production and effects of epinephrine on α-MG uptake and AA release were blocked by staurosporine and bisindolylmaleimide I or mepacrine and $AACOCF_3$. Indeed, epinephrine translocated PKC or $cPLA_2$ from cytosol to membrane fraction[31].

4. TCDD and estradiol-17β

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a highly toxic environmental toxicant that alters cell proliferation and function. The mechanism of action of TCDD is very similar to that proposed for steroid hormones. TCDD alters renal glomerular function as well as anion transport in proximal tubules. Of particular interest to this report is the effect of TCDD on the SGLTs, which is a typical marker of renal proximal tubule cell function. Estrogens are noted for their ability to stimulate cell proliferation in various tissues. However, little is known about any interaction between TCDD and estradiol-17β that affects renal proximal tubule cell proliferation and SGLTs activity. Thus, the effects of TCDD and E_2 on [^{3}H]-thymidine incorporation and on α-MG uptake were investigated in the primary rabbit kidney PTCs. TCDD inhibited [^{3}H]-thymidine incorporation and c-fos transcripts in real-time RT-PCR, whereas E_2 stimulated them. Aryl hydrocarbon receptor agonists, β-naphthoflavone and polychlorinated biphenyls synergistically increased the TCDD-induced inhibition of [^{3}H]-thymidine incorporation. However, the AhR antagonist, β-naphtho-

flavone as well as E_2 blocked TCDD-induced inhibition of [^{3}H]-thymidine incorporation. TCDD specifically inhibited α-MG uptake and its effect was due to V_{max} value but not K_m value. Indeed, TCDD decreased SGLT1, 2 protein level compared with control. In addition, TCDD-induced inhibition of α-MG uptake was blocked by α-NF or E_2[28].

5. High glucose

An elevated glucose concentration lead to an increase in reactive oxygen species production as well as to the attenuation of free radical scavenging molecules. Free radicals play an important role in the pathogenesis of diabetic nephropathy by their severe cytotoxic effects, such as lipid peroxides (LPO) formation and protein denaturation in cell membrane followed by the alteration of the membrane fluidity, enzyme properties, and ion transports. A direct involvement of glucose in the inhibition of the SGLTs has been established in studies on the LLC-PK1 cells. In addition, there is a report that oxidant altered SGLTs' activity in LLC-PK1 cells. However, the mechanisms by which the SGLTs' activity is altered in diabetic states, are poorly understood although hyperglycemia has been proposed as an underlying cause. Although recent data have shown that nuclear factor-kappa B (NF-κB) is activated in tubules and glomeruli in various experimental models of renal injury, biological significance of NF-κB activation in diabetic renal injury is not clear. Thus, study performed to investigate involvement of NF-κB in high glucose-induced SGLTs' dysfunction in primary cultured renal PTCs. Treatment with 25 mM glucose for 48 hr increased NF-κB DNA binding activity by five times compared to 5 mM glucose. The specificity of the binding reaction was confirmed by competition studies. The level of NF-κB p65 protein by treatment with 25 mM glucose for 48 hr was increased by about 2-fold compared to 5 mM glucose in the nuclear extracts. Also, western blot analysis of IκB-α protein showed that levels of IκB-α protein were clearly decreased after incubation with 25 mM glucose compare to 5 mM glucose. To examine the relationship of NF-κB and oxidative stress or PKC in the high glucose-induced inhibition of α-MG uptake, PKC inhibitors and antioxidants effectively blocked high glucose-induced activation of NF-κB although these themselves had no significant effects[32].

Oxidative stress plays an important role in the pathogenesis of renal diseases such as diabetic nephropathy. The metabolism of excessive intracellular glucose may involve a number of processes. One consequence of excessive intracellular glucose levels is an increased rate of oxidative phosphorylation under hyperglycemic conditions, while another consequence is an increase in the metabolism of glucose to sorbitol by aldose reductase. In addition, hyperglycemia may result in the activation of NADPH oxidase, the production of superoxide anion, and hydrogen peroxide (H_2O_2). Thus, I investigate the mechanisms responsible for the H_2O_2 production which occurs as the consequence of hyperglycemia, and the effect of H_2O_2 on the activity of SGLT in primary cultures of PTCs. When primary PTCs were cultured in the presence of high glucose, one consequence was that the Na^+/glucose cotransport system was inhibited, as indicated by uptake studies utilizing α-MG, a non-metabolizable analogue of D-glucose. Pretreatment of the cultures with either 1) aminoguanidine or pyridoxamine [inhibitors of the accumulation of Advanced Glycation End products (AGEs)], 2) rotenone (an inhibitor of the mitochondrial electron transport chain), or 3) apocynin or diphenylene iodonium (DPI) (inhibitors of NADPH oxidase) blocked the observed changes which occurred as a consequence of the incubation of the PTCs with high glucose. Included amongst these changes were the observed increase in H_2O_2 levels, as well as an increase in lipid peroxide (LPO) production, and a decrease both in the activity of catalase and in the level of glutathione (GSH), endogenous antioxidants.

The high glucose-induced decrease in the level of the Na^+/glucose cotransporter was similarly prevented by either aminoguanidine, rotenone, or apocynin. Thus, the inhibitory effect of high glucose on both the level of the Na^+/glucose cotransport system and the activity of the Na^+/glucose cotransport system can be explained, at least in part, as being due to the effects of the H_2O_2, the consequent formation of AGEs, the increase in mitochondrial metabolism, and in NADPH oxidase activity in the PTCs. Other related changes observed in the PTCs which could be reversed by treatment with either aminoguanidine, pyridoxamine, rotenone, apocynin, or DPI included an increase in TGF-β1 secretion, and the activation of the NF-κB signal transduction pathway[30].

GLUT5 was overexpressed in the kidney (proximal tubules and mesangial cells) and sciatic nerve (Schwann cells and axons) of streptozotocin-induced diabetic rats, and intestine (jejunum) in diabetic humans. In contrast, GLUT5 expression fell substantially (by ~75%) in adipocytes of streptozotocin-diabetic rats and was accompanied by a reduction in fructose uptake by approximately 50%. These reports support that glucose could be an important regulator of GLUT5 activity. However, the precise signal that regulates fructose transport in renal proximal tubule cells under high glucose conditions is not yet known although fructose has been recommended as a substitute for glucose in the diets of diabetic people. Thus, I investigated that effect of high glucose on fructose uptake and its signaling pathways in primary cultured rabbit renal PTCs. Glucose inhibited the fructose uptake in a time- and dose-dependent manner. Indeed, 25 mM glucose for 48 hr decreased GLUT5 protein level. Twenty-five mM glucose-induced inhibition of fructose uptake was blocked by pertussis toxin, SQ 22536 (an adenylate cyclase inhibitor), and myristoylated amide 14-22 (protein kinase A inhibitor). Indeed, 25 mM glucose increased the intracellular cAMP content. Furthermore, 25 mM glucose-induced inhibition of fructose uptake was prevented by neomycin or U 73122, and staurosporine or bisindolylmaleimide I. In fact, 25 mM glucose increased the total PKC activity and translocation of PKC from the cytosolic to membrane fraction. In addition, PD 98059 but not SB 203580 and mepacrine or $AACOCF_3$ blocked 25 mM glucose-induced inhibition of fructose uptake. Results of western blotting using the p44/42 MAPK and GLUT5 antibodies were consistent with the results of uptake experiments[26].

6. Bee venom

Human envenomation caused by bee stings has been reported to cause acute renal failure and the pathogenetic mechanisms of these renal functional changes are still unclear. Bee venom is also a complex mixture of enzymes and proteins. Thus, the study was conducted to examine the effects of bee venom (Apis mellifera) on apical transporters' activity and its related signal pathways in primary cultured renal proximal tubule cells. Bee venom (≥1 μg/ml) increased lipid peroxide formation over 30 min. The increase in AA release and LPO formation and the inhibition of α-MG uptake induced by bee venom (1 μg/ml) and melittin (a major component of bee venom; 0.5 μg/ml) were blocked by N-acetyl-L-cysteine, vitamin C and vitamin E, anti-oxidants. Bee venom- and melittin-induced increases in LPO formation and inhibition of α-MG uptake were significantly prevented by mepacrine and $AACOCF_3$, phospholipase A_2 inhibitors. In addition, nordihydroquaiareic acid (a lipoxygenase inhibitor) and econazole but not indomethacin prevented bee venom- and melittin-induced increases in LPO formation and inhibitiron of α-MG uptake[33].

7. Albumin

Albumin is not simply an inert piece of cargo reabsorbed and degraded by the proximal tubular epithelium. A growing body of evidence implicates albumin has an important regulatory function in renal proximal tubule cells. However, it remains to be de-

termined if albumin can have an effect on SGLTs in the renal proximal tubule cells as well as the intracellular signal molecules involved. In my study, the effect of bovine serum albumin (BSA) on α-MG uptake and its related signal molecules were examined in the primary cultured rabbit renal PTCs. BSA significantly increased uptake of α-MG, a distinctive proximal tubule marker, as well as expression level of SGLT1, 2 proteins. Neomycin or U 73122, BAPTA/AM or TMB-8 (intracellular Ca^{2+} mobilization inhibitors) completely abolished BSA-induced increase of α-MG uptake. BSA significantly increased IPs accumulation, but did not affect Ca^{2+} uptake. Effect of BSA on α-MG uptake was blocked by PD 98059, but did not SB 203580. BSA increased phosphorylation of p44/42 MAPK in a time-dependent manner. Antioxidants significantly blocked BSA-induced increase of H_2O_2 formation and α-MG uptake. BSA activated NF-αB translocation into nucleus. PDTC, SN50, and TLCK (NF-κB inhibitors) also completely blocked BSA-induced increase of α-MG uptake, NF-κB p65 and phospho IκB-α activation [27].

8. Protection of SGLTs against oxidative stress

Oxidative stress has been implicated as a primary cause of renal failure in certain renal diseases. Indeed, renal proximal tubule is a very sensitive site to oxidative stress and retains functionally fully characterized transporters. It has been reported that ginsenosides have a beneficial effect on diverse diseases including oxidative stress. However, the protective effect of ginsenosides on oxidative stress has not been elucidated in PTCs. Thus, I examined the effect of ginsenosides on oxidative stress-induced alteration of apical transporters and its related mechanism in PTCs. In my study, H_2O_2 ($>10^{-5}$ M) inhibited α-MG uptake in a dose-dependent manner ($p<0.05$). It also inhibited Pi and Na^+ uptake. At a concentration of 20 μg/mL, total ginsenosides significantly reduced H_2O_2-induced inhibition of apical transporters. In contrast, protopanaxadiol (PD) and protopanaxatriol (PT) saponins exhibited a less preventive effect than total ginsenosides ($p<0.05$). Furthermore, I examined its action mechanism. H_2O_2 increased LPO formation, AA release, and Ca^{2+} uptake. These effects on H_2O_2 were significantly prevented by total ginsenosides and PD or PT sanponins. However, total ginsenosides appear to be more protective than PD and PT saponins ($p<0.05$)[34].

The protective effect of caffeic acid (CA) against oxidative stress-induced inhibition of proximal tubule apical transporter was also investigated. In this study, 10^{-4} M H_2O_2 did not affect cell viability regardless of incubation time. However, it decreased apical transporters' activity such as Na^+/glucose cotransporter, Na^+/Pi cotransporter, and Na^+/H^+ antiporter in the proximal tubule cells. CA ($>10^{-6}$ M) prevented H_2O_2-induced inhibition of apical transporters. Thus, I investigated its action mechanism. CA also prevented H_2O_2-induced lipid peroxides formation, AA release, and Ca^{2+} uptake[35].

The effect of oxidants and protective effect of sex steroid hormones on Na^+/glucose cotransporter of renal proximal tubular cells is not yet elucidated. Thus, I examined the effect of sex steroid hormones against tert-butyl hydroperoxide (t-BHP)-induced alteration of Na^+/glucose cotransporter activity in PTCs. t-BHP inhibited α-MG uptake in dose-dependent manner. t-BHP-induced inhibition of α-MG uptake was due not to K_m but to the decrease of V_{max}. t-BHP-induced inhibition of α-MG uptake was significantly blocked by estradiol-17β, but not by progesterone and testosterone. This protective effect was not blocked by estrogen receptor antagonist of transcription and translation inhibitor. In addition, 0.5 mM t-BHP increased AA release and Ca^{2+} uptake. These effects of t-BHP were also significantly blocked by estradiol-17β, but not by progesterone and testosterone. Protective efficacy of estradiol-17β on t-BHP-induced inhibition of α-MG uptake is exhibited between antioxidants and iron chelators[36].

In addition the study was undertaken in order to

examine the effect of various oestrogens on t-BHP-induced cell injury and changes in apical transporters in primary cultured rabbit renal proximal tubule cells. Compared with control, t-BHP (0.5 mmol/L; 1 h) decreased cell viability (62%) and glutathione (GSH) content (60%) and increased LPO formation (309%), AA release (193%) and Ca^{2+} influx (168%). The protective potency of various oestrogens for these parameters is dependent on the precise oestrogenic structure, with 2-hydroxy-oestradiol-17β (2-OH-E_2) and 4-OH-E_2, both catecholic oestrogens, or diethylstilbesterol (DES) being more potent than oestradiol (E_2), oestriol or oestradiol-17α, all phenolic oestrogens ($p<0.05$). These cytoprotective effects of oestrogens occur at concentrations above 10 micromol/L and are not dependent on classical oestrogen receptors and gene transcription and translation. In addition, various oestrogens have different preventative effects against t-BHP-induced inhibition of α-MG uptake, inorganic phosphate (Pi) and Na^+ uptake, consistent with the results of cell injury. In contrast, the potency against t-BHP-induced changes in cell viability, LPO, GSH content and transporter function of the antioxidants taurine and vitamin C is similar to that of phenolic oestrogens, whereas that of the iron chelators deferoxamine and phenanthroline is similar to that of catecholic oestrogens[37].

Conclusion

ANG II and EGF partially inhibited α-MG uptake via PLC/PKC, p44/42 MAPKs and PLA_2 signaling pathways in the PTCs. H_2O_2-induced inhibition of α-MG uptake in the renal proximal tubule is mediated through a modulation of the EGF signaling pathway, promoting further phosphorylation of p44/42 MAPK, activation of PLA_2. Epinephrine partially inhibits the α-MG uptake through PKA, PKC, p44/42, p38 MAPK, and $cPLA_2$ pathways. On the other hand, high glucose inhibits the fructose uptake through cAMP, PLC/PKC, p44/42 MAPK, and $cPLA_2$ pathways. In addition, inhibition of α-MG uptake by high glucose is the consequence of an increase in the level of LPO, H_2O_2, and TGF-β1 secretion, and the activation of the NF-κB signal transduction pathway. TCDD and bee venom partially inhibited α-MG uptake. However, BSA stimulates α-MG uptake and its action is partially correlated with PLC, MAPK, or NF-κB signal molecules. On the other hand, caffeic acid, ginsenosides, and sex hormones protected oxidative stress-induced inhibition of α-MG uptake. Therefore, these findings may provide new insights into the pathophysiological mechanisms as well as functional regulation of Na^+/glucose cotransporters in renal proximal tubule cells

Acknowledgements

This research was supported by Grant SC 14032 from the Stem Cell Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, Republic of Korea and Korea Research Foundation (KRF 2003-005-E00012).

References

1) Jung JC, Lee SM, Kadakia N, Taub M : Growth and function of primary rabbit kidney proximal tubule cells in glucose-free serum-free medium. *J Cell Physiol* **150:**243-250, 1991
2) Oku A, Ueta K, Arakawa K, Kano-Ishihara T, Matsumoto M, Adachi T, Yasuda K, Tsuda K, Saito A : Antihyperglycemic effect of T-1095 via inhibition of renal Na^+-glucose cotransporters in streptozotocin-induced diabetic rats. *Biol Pharm Bull* **23:**1434-1437, 2000
3) Cheung PT, Hammerman MR : Na^+-independent D-glucose transport in rabbit renal basolateral membranes. *Am J Physiol* **254:**F711-F718, 1988
4) Heilig CW, Brosius FC 3rd, Henry DN : Glucose transporters of the glomerulus and the implications for diabetic nephropathy. *Kidney Int Suppl* **60:** S91-S99, 1997
5) Wiesener MS, Munchenhagen PM, Berger I, Morgan NV, Roigas J, Schwiertz A, Jurgensen JS, Gruber G, Maxwell PH, Loning SA, Frei U, Maher

ER, Grone HJ, Eckardt KU : Constitutive activation of hypoxia-inducible genes related to overexpression of hypoxia-inducible factor-1alpha in clear cell renal carcinomas. *Cancer Res* **61:**5215-5222, 2001
6) Thorens B : Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. *Am J Physiol* **4:**G541-G553, 1996
7) Wright EM, Hirsch JR, Loo DD, Zampighi GA : Regulation of Na^+/glucose cotransporters. *J Exp Biol* **200:**287-293, 1997
8) Wright EM : Renal Na^+-glucose cotransporters. *Am J Physiol Renal Physiol* **280:**F10-F18, 2001
9) Uldry M, Thorens B : The SLC2 family of facilitated hexose and polyol transporters. *Pflugers Arch* **447:**480-489, 2004
10) Wright EM, Turk E : The sodium/glucose cotransport family SLC5. *Pflugers Arch* **447:**510-518, 2004
11) Hediger MA, Coady MJ, Ikeda TS, Wright EM : Expression cloning and cDNA sequencing of the Na^+/glucose co-transporter. *Nature* **330:**379-381, 1987
12) Turk E, Zabel B, Mundlos S, Dyer J, Wright EM : Glucose/galactose malabsorption caused by a defect in the Na+/glucose cotransporter. *Nature* **350:** 354-356, 1991
13) Wells RG, Pajor AM, Kanai Y, Turk E, Wright EM, Hediger MA : Cloning of a human kidney cDNA with similarity to the sodium-glucose cotransporter. *Am J Physiol* **263:**F459-F465, 1992
14) Kanai Y, Lee WS, You G, Brown D, Hediger MA : The human kidney low affinity Na^+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. *J Clin Invest* **93:**397-404, 1994
15) Van den Heuvel LP, Assink K, Willemse, M, Monnens L : Autosomal recessive renal glucosuria attributable to a mutation in the sodium glucose cotransporter (SGLT2). *Hum Genet* **111:**544-547, 2002
16) Calado J, Soto K, Clemente C, Correia P, Rueff J : Novel compound heterozygous mutations in SLC5A2 are responsible for autosomal recessive renal glucosuria. *Hum Genet* **114:**314-316, 2004
17) Isaji M, Fujikura H, Nishimura T, Fushimi N, Tatani K, Katsuno K, Hiratochi M, Nakabayashi T, Miyata H : Pioneering development of a novel selective inhibitor of low affinity sodium/glucose cotransporter (SGLT2): a new approach to improving diabetic mellitus. *FASEB J* **15:**A214, 2001
18) Katsuno K, Fujimori Y, Takemura Y, Fujikura H, Iyobe A, Teranishi H, Miyata H, Isaji M : KGT-1251, a novel selective SGLT2 inhibitor, has antidiabetic effect in diabetic rodent models. *FASEB J* **15:**A214, 2001
19) Mackenzie B, Panayotova-Heiermann M, Loo DD, Lever JE, Wright EM : SAAT1 is a low affinity Na^+/glucose cotransporter and not an amino acid transporter. A reinterpretation. *J Biol Chem* **269:** 22488-22491, 1994
20) Kong CT, Yet SF, Lever JE : Cloning and expression of a mammalian Na^+/amino acid cotransporter with sequence similarity to Na^+/glucose cotransporters. *J Biol Chem* **268:**1509-1512, 1993
21) Diez-Sampedro A, Hirayama BA, Osswald C, Gorboulev V, Baumgarten K, Volk C, Wright EM, Koepsell H : A glucose sensor hiding in a family of transporters. Proc Natl Acad Sci USA **100:**11753-11758, 2003
22) Wood IS, Trayhurn P : Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br J Nutr* **89:**3-9, 2003
23) Taub M : The use of defined media in cell and tissue culture. *Toxico in Vitro* **4:**213-225, 1990
24) Han HJ, Park SH, Lee YJ : Signaling cascade of ANG II-induced inhibition of α-MG uptake in renal proximal tubule cells. Am J Physiol Renal Physiol **286:**F634-F642, 2004a
25) Han HJ, Park YJ, Lee YJ, Taub M : Epidermal growth factor inhibits ^{14}C-α-methyl-D-glucopyranoside uptake in renal proximal tubule cells: involvement of PLC/PKC, p44/42 MAPK, and $cPLA_2$. *J Cell Physiol* **199:**206-216, 2004b
26) Park SH, Lee YJ, Lim MJ, Kim EJ, Lee JH, Han HJ: High glucose inhibits fructose uptake in renal proximal tubule cells: involvement of cAMP, PLC/PKC, p44/42 MAPK, and $cPLA_2$. *J Cell Physiol* **200:**407-416, 2004c
27) Han HJ, Oh JY, Lee YJ : Effect of albumin on ^{14}C-α-methyl-D-glucopyranoside uptake in primary cultured renal proximal tubule cells: Involvement of PLC, MAPK, and NF-κB. *J Cell Physiol* **202:** 246-254, 2005a
28) Han HJ, Lim MJ, Lee YJ, Kim EJ, Jeon YJ, Lee JH : Effects of TCDD and estradiol-17β on the proliferation and Na^+/glucose cotransporter in renal proximal tubule cells. *Toxicol in Vitro* **19:**21-30, 2005b
29) Han HJ, Lee YJ, Park JY, Kim EJ, Lee JH, Taub ML : Effect of EGF on H_2O_2-induced inhibition of α-MG uptake in renal proximal tubule cells: Involvement of MAPK and AA release. *J Cell Physiol* 2005c [Epub ahead of print].
30) Han HJ, Lee YJ, Park SH, Lee JH, Taub M : High glucose-induced oxidative stress inhibits Na^+/glucose cotransporter activity in renal proximal tubule cells. *Am J Physiol Renal Physiol* 2005d [Epub ahead of print].
31) Kim EJ, Lee YJ, Lee JH, Han HJ : Effect of epinephrine on α-methyl-D-glucopyranoside uptake in renal proximal tubule cells. *Cell Physiol Biochem*

14:395-406, 2004

32) Han HJ, Jeon YJ, Lee YJ : Involvement of NF-κB in high glucose-induced alteration of α-methyl-D-glucopyranoside (α-MG) uptake in renal proximal tubule cells. *Cell Physiol Biochem* **13:**375-384, 2003

33) Han HJ, Park SH, Lee JH, Yoon BC, Park KM, Mar WC, Lee HJ, Kang SK : Involvement of oxidative stress in bee venom-induced inhibition of Na^+/glucose cotransporter in renal proximal tubule cells. *Clin Exp Pharmacol Physiol* **29:**564-568, 2002

34) Han HJ, Yoon BC, Park SH, Park JY, Oh YJ, Lee YJ, Park KM : Ginsenosides protect apical transporters of cultured proximal tubule cells from dysfunctions induced by H_2O_2. *Kidney Blood Press Res* **25:**308-314, 2002

35) Han HJ, Park SH, Park KM, Yoon BC, Kim TS, Lee JH : Effect of caffeic acid on apical transporters' dysfunction of renal proximal tubule cells under oxidative stress in vitro. *Planta Med* **68:**483-486, 2002

36) Han HJ, Park SH, Park HJ, Lee JH, Lee BC, Hwang WS: Effects of sex hormones on Na^+/glucose cotransporter of renal proximal tubular cells following oxidant injury. *Kidney Blood Press Res* **24:**159-165, 2001

37) Han HJ, Park SH, Park HJ, Park KM, Kang JW, Lee JH, Lee BC, Hwang WS : Effect of various oestrogens on cell injury and alteration of apical transporters induced by tert-butyl hydroperoxide in renal proximal tubule cells. *Clin Exp Pharmacol Physiol* **29:**60-67, 2002