Functional role of sodium glucose transporter in high glucose-mediated angiotensin type 1 receptor downregulation in human proximal tubule cells

Rekha Yesudas,1* Russell Snyder,1* Thomas Abbruscato,2 and Thomas Thekkumkara1
1Department of Biomedical Sciences, Texas Tech University Health Sciences Center, Amarillo, Texas; and 2Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Amarillo, Texas
Submitted 30 November 2011; accepted in final form 25 May 2012

Yesudas R, Snyder R, Abbruscato T, Thekkumkara T. Functional role of sodium glucose transporter in high glucose-mediated angiotensin type 1 receptor downregulation in human proximal tubule cells. Am J Physiol Renal Physiol 303: F766–F774, 2012. First published May 30, 2012; doi:10.1152/ajprenal.00651.2011.—Previously, we have demonstrated human angiotensin type 1 receptor (hAT1R) promoter architecture with regard to the effect of high glucose (25 mM)-mediated transcriptional repression in human proximal tubule epithelial cells (hPTEC; Thomas BE, Thekkumkara TJ. Mol Biol Cell 15: 4347–4355, 2004). In the present study, we investigated the role of glucose transporters in high glucose-mediated hAT1R repression in primary hPTEC. Cells were exposed to normal glucose (5.5 mM) and high glucose (25 mM), followed by determination of hyperglycemia-mediated changes in receptor expression and glucose transporter activity. Exposure of cells to high glucose resulted in downregulation of ANG II binding (4,034 ± 163.3 to 1,360 ± 154.3 dpm/mg protein) and hAT1R mRNA expression (reduced 60.6 ± 4.643%) at 48 h. Under similar conditions, we observed a significant increase in glucose uptake (influx) in cells exposed to hyperglycemia. Our data indicated that the magnitude of glucose influx is concentration and time dependent. In euglycemic cells, inhibiting sodium-glucose cotransporters (SGLTs) with phloretin decreased glucose influx by 28.57 ± 0.9123 and 54.33 ± 1.2022%, respectively. However, inhibiting SGLTs in cells under hyperglycemic conditions decreased glucose influx by 3.67 ± 2.906%, while GLUT-mediated glucose influx remained unaltered (57.67 ± 3.180%). Furthermore, pretreating cells with an SGLT inhibitor reversed high glucose-mediated downregulation of the hAT1R, suggesting an involvement of SGLT in high glucose-mediated hAT1R repression. Our results suggest that in hPTEC, hyperglycemia-induced hAT1R downregulation is largely mediated through SGLT-dependent glucose influx. As ANG II is an important modulator of hPTEC transcellular sodium reabsorption and function, glucose-mediated changes in hAT1R gene expression may participate in the pathogenesis of diabetic renal disease.

diabetes; gene expression; G protein-coupled receptors

DIABETES AND HYPERTENSION are two leading contributors to the development of diabetic nephropathy and end-stage renal disease. Several systemic and/or intrarenal networks of growth factors and cytokines may be modulated by diabetes. Activation of the renin-angiotensin system (RAS) is one of several factors believed to be involved in the development of diabetic nephropathy (37). The octapeptide ANG II is the primary effector molecule by which the RAS exerts its physiological actions through membrane receptors; the angiotensin type 1 receptor (AT1R) and type 2 receptor (AT2R) (1, 3). AT1 receptors are classic G protein-coupled receptors and play a critical role in the control of blood pressure and sodium homeostasis (1, 12). A potential link between ANG II and progressive diabetic pathology has been demonstrated in both clinical and experimental studies with angiotensin-converting enzyme (ACE) inhibitors and AT1R blockers (9, 26, 67). Thus it may be presumed that ANG II through binding and activation of the AT1R promotes the progression of diabetes-induced nephropathy in patients and experimental animal models. However, AT1R expression in the kidney is upregulated in the glomerular cells and downregulated in proximal epithelial cells in experimentally induced diabetes mellitus (13, 59) while insulin supplementation normalizes each tissue expression to nondiabetic controls, respectively. More specifically, in the proximal tubule the AT1R is downregulated from early onset to end-stage diabetic nephropathy. The functional significance of this decrease in the AT1R is not well understood, since studies have shown that inhibition of the AT1R prevents the progression of kidney disease. The proximal tubule is a major site of salt and water reabsorption, with up to 67% of the filtered load of sodium reabsorbed in this segment (18). In normal physiology, proximal tubule function is under the control of vasoconstrictor ANG II-stimulated reabsorption (17, 18). Therefore, alterations (increase/decrease) in AT1R expression have significant pathophysiological consequences.

Previously, we have identified a glucose-mediated transcriptional repressor element and demonstrated that high glucose alters the rate of transcription by interacting with glucose-inducible nuclear trans-acting factor(s) in human proximal tubule cells (54). However, the cellular mechanism by which glucose initializes these repressor effects on the hAT1 gene was not understood. There are two general classes of glucose transporters described in mammalian cells (44, 49), facilitative and sodium coupled. Currently, at least 6 sodium-dependent and 13 facilitative transporters are recognized (61). They exhibit different substrate specificities, transport affinities, developmental regulation, and tissue-specific expression (21, 58). Depending on the concentration gradient, facilitative glucose transporters (GLUTs) transport glucose in either direction across the cell membrane (39), while sodium-glucose cotransporters (SGLTs) transport substrates in a unidirectional manner (36). In particular, SGLT3 is reported as a glucose sensor in the human kidney (62). The kidney plays a major role in glucose homeostasis by reabsorbing filtered glucose. Glucose also influences many aspects of renal function including sodium-glucose cotransport, gluconeogenesis, activation of DNA and protein synthesis, and cellular hypertrophy (49). In the nephron, all filtered glucose is reabsorbed in the proximal tubule. Reabsorption of luminal glucose against a concentration gradient occurs via apical SGLTs (34, 64) and GLUTs (23,
Glucose then diffuses from the cells into the bloodstream via basolateral facilitative glucose transporters (14). SGLT1, 2 and GLUT1, 2 are the major glucose transporters active in proximal tubule epithelial cells (PTECs) (57, 62, 63). Studies have shown that 90% of filtered glucose is reabsorbed by the SGLT2 and GLUT2 in the proximal tubule (36, 61). Furthermore, diabetic nephropathy is reported to be associated with dysfunction of renal proximal tubular cells and alteration in glucose transporter functions under hyperglycemic condition (20).

The objective of this study was to determine the relationship of glucose transporters with respect to their initiation of a hyperglycemic induction of hAT1R downregulation. We selected hPTECs because 1) in diabetes mellitus hAT1R gene expression downregulates in the proximal tubule; 2) alterations in proximal tubule hAT1R expression results in marked changes in volume reabsorption, glomerular filtration rate, and renal vascular resistance; and 3) these cells are suitable model systems for investigating glucose-specific responses in the absence of insulin-like growth factors and other confounding variables. Our study demonstrates that hAT1R expression does reduce significantly upon exposure to high concentrations of glucose (25 mM). The participation of glucose transporters is not equally distributed to all transporter isoforms, but rather is due principally to increased uptake via SGLT, while the uptake due to GLUT remains roughly the same compared with cells exposed to normal (5.5 mM) concentrations of glucose. The results of this study are important to an understanding of the control of AT1R expression in the proximal tubule and the relationship this receptor may have in the progression of diabetic nephropathy.

MATERIALS AND METHODS

Materials. Cell culture reagents were purchased from Invitrogen (Carlsbad, CA). d-[3H]glucose and d-[1-14C]methyl α-glucopyranoside (αMG) were from PerkinElmer (Boston, MA) and 3H-ANG II was from Amersham (Piscataway, NJ). Losartan potassium was kindly provided by Merck Research Laboratories (Rahway, NJ). The AT1R (N-10) antibody was from Santa Cruz Biotechnology (Santa Cruz, CA), goat-anti rabbit IgG horseradish peroxidase conjugate was from Bio-Rad (Richmond, CA); nitrocellulose membrane was from Bio-Rad (Hercules, CA), Alexa Fluor goat anti-rabbit IgG, and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) were from Invitrogen. Phlorizin and phloretin, were from Sigma (St. Louis, MO). Real-time PCR reagents and equipment were from Applied Biosystems (Foster City, CA). Oligonucleotides were provided by Integrated DNA Technologies (Coralville, IA). Electrophoresis reagents were from Bio-Rad (Hercules, CA). Losartan potassium was kindly provided by Merck Research Laboratories (Rahway, NJ). Primary human PTECs (hPTECs) were obtained from Lonza (Walkersville, MD). Cells were maintained in renal epithelial cell basal medium (REBM) containing 0.5% FBS and REGM Single-Quots cocktail at 37°C in 5% CO2 under 100% humidity. Cultures were fed with fresh growth medium every 3 days. For the studies, cells were grown to 75–80% confluence, and the medium was replaced with DMEM with normal glucose (5.5 mM) containing 0.5% FBS and grown for another 24 h. For hyperglycemic conditions, cells were exposed to 25 mM glucose in DMEM containing 0.5% FBS for indicated times. Simultaneously, the control plates were replenished with fresh medium containing normal concentrations of glucose. d-Glucose was used in all experiments unless otherwise stated. In these studies, experiments were performed from passage 3 to passage 7, with no observed change in phenotype or proliferative activity (2).
Immunofluorescence microscopy. Proximal tubule cells were seeded in chamber slides and treated under indicated conditions. Cells were washed with ice-cold PBS and fixed with 4% paraformaldehyde in PBS, pH 7.4, for 22°C for 20 min, then permeabilized with 0.1% Triton X-100 in PBS for 5 min. Cells were blocked in 5% goat serum at 22°C for 1 h and incubated in hAT1R-specific primary antibody in 2.5% goat serum overnight at 4°C. After washing, cells were incubated with a fluorescence-tagged secondary antibody for 1 h at 22°C in the dark to prevent photodecay of the fluorophore. Nuclei were stained with 10 nM DAPI, and samples were mounted using ProLong Gold antifade mounting medium (Invitrogen, Eugene, OR). Image capture was performed at 22°C using an Olympus IX-81 microscope equipped with an Olympus SpectraMaster II monochromator light source and UltraPix camera (PerkinElmer) under a PlanApo 60x/1.40 oil-immersion objective. Images were analyzed using UltraView software (PerkinElmer). Other analyses were performed using an Olympus IX-81 microscope equipped with an Olympus U-CMAD3 camera under a PlanApo 60x/1.40 oil-immersion objective and analyzed using Slidebook software.

Cell viability assay. For the viability assay, high glucose- and phlorizin-treated cells were harvested by 2-min trypsinization followed immediately by anti-trypsin inactivation. Trypan blue solution (0.4%) was added to aliquots of cell suspension in a 1:1 ratio and allowed to stand for 5 min at room temperature. A hemocytometer was used to count live and dead cells, and the number of viable cells was calculated and expressed as a percentage.

Data analysis. Results presented in the study are triplicate values and representative of three or more independent experiments. Gel images and X-ray films were scanned on an Epson Expression 1600 Photoscanner using Adobe Photoshop CS3. Statistical significance was analyzed with GraphPad Prism software. Where appropriate, statistical differences were compared by use of ANOVA with post hoc Bonferroni tests or appropriate t-tests for parametric or nonparametric comparison. The values presented are means ± SE, and P < 0.05 was considered to be significant.

RESULTS

High glucose downregulates the hAT1R. Cell were exposed to normal-glucose and high-glucose medium for 48 h, and we measured the hAT1R-specific ANG II binding. The results show that cells exposed to the hyperglycemic condition downregulated hAT1R binding (Fig. 1A). After 48 h, there was a 53.28 ± 2.212% (P < 0.0001, n = 3) reduction in the hAT1R binding to normal-glucose and high-glucose medium for 48 h, and we measured the hAT1R-specific ANG II binding. The results showed that cells exposed to the hyperglycemic condition downregulated hAT1R binding (Fig. 1A). After 48 h, there was a 53.28 ± 2.212% (P < 0.0001, n = 3) reduction in the [3H]ANG II binding on cells grown under high glucose compared with the cells exposed to a normal concentration of glucose. To determine whether these changes in ANG II binding were hAT1R specific, we investigated binding with the AT1R antagonist losartan. The results showed that the hAT1R is the major subtype downregulated by high glucose treatment (hAT1R blockade yielded 55.00 ± 2.331% reduction in ANG II binding) (P < 0.0001, n = 3) in normal glucose losartan-treated cells and 51.66 ± 0.470% reduction (P = 0.0002, n = 3) in high glucose losartan-treated cells compared with normal glucose control (Fig. 1A). The high glucose-induced decrease in [3H]ANG II binding does not represent proximal tubule cell toxicity or accelerated cell death, as trypan blue cell viability assays showed that the viable cell count at 48-h high glucose exposure was similar to the control (P = 0.7725, n = 3) (Fig. 1B). To evaluate whether the observed changes are due to D-glucose itself, or rather a change in cellular osmolarity, the cells were exposed to 25 mM D-glucose, L-glucose, or mannitol. A binding study was then performed after 48-h incubation in these conditions (Fig. 1C). The results show that only D-glucose is capable of downregulating [3H]ANG II binding (binding reduced 55.34 ± 2.555%, P = 0.0005, n = 3), while L-glucose and mannitol both had no effect (P = 0.6718, n = 3; P = 0.6218, n = 3, for L-glucose and mannitol, respectively). Furthermore, an immunofluorescence study using a specific antibody directed against the hAT1R showed significantly less immunoreactivity at the cells’ surface when exposed to high...
glucose compared with normal glucose (Fig. 2A). We confirmed this observation by Western blot analysis, which displayed a 45.5 ± 8.242% decrease in hAT1R protein expression compared with control ($P = 0.0053$, $n = 3$) (Fig. 2, B and C). In a previous study (54), we have demonstrated that hAT1R promoter sensitivity to hyperglycemia results in transcriptional repression of hAT1R gene expression. To further validate the promoter studies and to correlate the high glucose-mediated downregulation of hAT1R binding and protein, we determined the hAT1R mRNA expression in normal glucose and high glucose conditions using dual RT-PCR. The results showed downregulation of hAT1R mRNA in hyperglycemic conditions compared with control (Fig. 3A). In total RNA from hPTECs under hyperglycemic conditions, AT1R mRNA expression decreased by 60.6 ± 4.643% ($P < 0.0001$, $n = 5$) (Fig. 3B). The above results demonstrate that cells exposed to high glucose showed downregulation of hAT1R protein and mRNA expression at 48 h, which is consistent with transcriptional repression.

Under hyperglycemia, glucose uptake is concentration and time dependent. To determine the magnitude of glucose influx in normal and hyperglycemic hPTECs, we performed dose-response and time course studies. Glucose uptake studies indicated that the glucose influx is concentration and time dependent (Fig. 4). The dose-response study with concentrations ranging from euglycemic (human) plasma glucose level of 5.5 mM to hyperglycemic concentration (25 mM) gave a linear plot (Fig. 4A). To determine the time course, cells were exposed to D-[$^3$H]glucose for up to 30 min. Upon comparison of normal and hyperglycemic cells, the uptake of glucose reached $V_{max}$ between 15 and 20 min in hyperglycemic cells.
compared with <5 min for euglycemic conditions. Under hyperglycemic conditions, glucose uptake was significantly increased (290.3 ± 17.32% relative to normal glucose control at 30 min; \( P < 0.0001, n = 3 \)) (Fig. 4B).

Increased glucose uptake in hyperglycemic hPTECs is mediated by SGLTs. To further investigate the role of glucose transporters in the increased glucose uptake in cells exposed to hyperglycemic conditions, we conducted glucose uptake studies while selectively inhibiting different glucose transporters. Phlorizin is reported as a potent competitive inhibitor for SGLT1, -2, and -3 with varying inhibitor constants (63). In previous studies, 0.5 mM phlorizin inhibited more than 95% of \( \alpha \text{MG} \) (a glucose analog) uptake in rabbit proximal tubule cells (48) and 88 ± 9% of \( \alpha \text{MG} \) uptake in hPTECs (56). Phlorizin has no known affinity or inhibitor effect on GLUT1–12 (5). Phloretin is a potent inhibitor of facilitative glucose transporters (32). Therefore, we used 0.5 mM phlorizin or 150 \( \mu \text{M} \) phloretin to inhibit D-glucose uptake, each concentration confirmed as effective in vitro to specifically inhibit respective transporters according to the literature. At a normal (5.5 mM) glucose concentration, cells incubated with phlorizin or phloretin showed decrease glucose uptake by 28.57 ± 0.9123 (\( P < 0.0001, n = 3 \)) and 54.33 ± 1.202% (\( P < 0.0001, n = 3 \)), respectively. In contrast, in high glucose (25 mM)-treated cells, phlorizin treatment decreased glucose influx by 53.67 ± 2.906% (\( P < 0.0001, n = 3 \)). However, GLUT-mediated glucose uptake remained the same in 25 mM glucose-treated cells as phloretin reduced glucose uptake by 57.67 ± 3.180% (\( P < 0.0001, n = 3 \)), or at a level not significantly different from euglycemic cells undergoing GLUT inhibition (\( P = 0.2066, n = 3 \)) (Fig. 5A). Additionally, we performed an uptake study using \( \alpha \text{MG} \) as a selective substrate for SGLT transport. The results show that under hyperglycemia, there is a significant increase in \( \alpha \text{MG} \) uptake (203.3 ± 9.132%, \( P < 0.0001, n = 3 \)), indicating that an increased capacity for glucose uptake is mediated by SGLT (Fig. 5B).

Hyperglycemia-induced hAT1R downregulation is mediated by SGLT. To determine the transporter-mediated effect of high glucose on native hAT1R expression, cells were exposed to phlorizin and phloretin for 48 h and measured for hAT1R-specific ANG II binding. Cell viability studies indicated that treatment with phlorizin or phloretin and 25 mM glucose did not significantly affect cell survival (data not shown). SGLT inhibition for 48 h displayed restoration to normal glucose hAT1R binding (mean difference between normal glucose...
control and 25 mM glucose combined with 0.5 mM phlorizin (8.661 ± 5.102%; \( P = 0.1089 \), \( n = 8 \)), while treatment with phloretin had no significant effect on ANG II binding (mean difference normal glucose control and 25 mM glucose combined with 0.15 mM phloretin, 51.41 ± 4.321%; \( P < 0.0001 \), \( n = 8 \)) (Fig. 6). To validate this observation, immunofluorescent microscopy was performed after 48-h phlorizin/phloretin treatment in hyperglycemic conditions. The results of this study demonstrated that hAT1R expression was restored to near control levels with treatment with 0.5 mM phlorizin but not with 0.15 mM phloretin (Fig. 7). We also observed that phlorizin-mediated restoration of hAT1R protein expression correlated with a restoration of hAT1R mRNA expression (mean difference between control and 25 mM glucose combined with 0.5 mM phlorizin, 7.777 ± 9.280%; \( P = 0.4161 \), \( n = 3 \)), while phloretin-treated cells still showed significant downregulation of hAT1R mRNA (mean difference between normal glucose control and 25 mM glucose combined with 0.15 mM phloretin, 51.39 ± 4.604%; \( P < 0.0001 \), \( n = 3 \)) (Fig. 8).

**DISCUSSION**

The RAS plays a major role in blood pressure regulation and electrolyte/extracellular fluid volume control. As a result, factors that influence this system have significant potential for pathological developments, particularly in the kidney (27). Diabetic nephropathy is a disease affecting approximately one-third of patients with type 1 diabetes as well as 20–40% of...
patients with type 2 diabetes (50, 66). Diabetic nephropathy leading to end-stage renal disease is also associated with a significantly increased risk for the development of cardiovascular disease (CVD) (8, 60). In the present study, we demonstrated that hyperglycemic conditions in hPTECs downregulated ANG II binding, which correlated with a reduction in both hAT1R protein and mRNA, and thus the study elucidates at least one mechanism of how glucose regulates endocrine signaling within the kidney. In previous studies, when mesangial cells were grown under hyperglycemic conditions, the AT1R-mediated increase in intracellular calcium and the contractile response were diminished (25). Similarly, in the renal microvasculature innervating the nephron, the effects of ANG II were significantly reduced in diabetic rats (24, 30). The current study’s findings were supportive of previous reports demonstrating in animal models that downregulation of ANG II binding was associated with AT1R mRNA and protein in diabetic rat proximal tubule cells (13, 40) as well as primary rabbit proximal tubule cells (45). By using the respective inhibitors of transcription and translation, actinomycin D and cycloheximide, Park and Han (45) suggested that there was a role for transcription and translation on glucose-mediated downregulation of ANG II binding in rabbit proximal tubule cells. Although we have not correlated transcriptional activity with the detected mRNA downregulation presented in this study, in a previous study we have shown the promoter of the hAT1R gene has a cis-acting glucose repressor element (GluRE) acting through trans-acting factors (54). Furthermore, there is significant evidence in support of hAT1R downregulation in diabetes. In humans, there is a significant reduction in ANG II-induced renal vascular resistance and mean arterial blood pressure in early type 1 diabetes mellitus (42).

The larger question posed by these studies and the present study is why the downregulation of a receptor, known to be upregulated during the pathogenesis of cardiovascular disease, should be inversely downregulated in the kidneys of hyperglycemic subjects, thereby exacerbating the nephropathy and eventually the progression of cardiovascular disease. One possible explanation may be simultaneous downregulation of the AT1R in the glomerulus, thereby removing the endogenous antagonistic effect that this receptor has with AT1R signaling (59). It was found that in streptozotocin-induced diabetic rats, AT1R and AT2R expression were significantly downregulated, with downregulation of the AT1R in the tubule epithelium and AT2R downregulation in the glomerulus, leading the authors to conclude that the reduced AT1R expression may be partially offset by the concomitant reduction in the AT2R and its counteracting effects, resulting in an AT1R-dominant response. Alternatively, the AT1R may have variable responsivity to ANG II among different cell types in the kidney. Patients have generally been found to be hyperresponsive to ANG II infusion (7), but the hemodynamic responsiveness to ANG II in the kidney has been reported as normal or even reduced (10, 16, 22), indicating that the receptor itself is either downregulated or becoming less sensitive to ligand stimulation. The origin of deregulated AT1R production is not resultant from a single stimulus during type 2 diabetes mellitus; hyperinsulinemia is known to act as a potent upregulator of hAT1R expression (4, 19, 29), but the overexpression of the receptor is believed to be consequent to reactive oxygen species resultant in insulin resistance, rather than the direct signaling by insulin itself (43).

In the study above, the authors found that fructose-fed rats were indeed hyperinsulinemic, hypertensive, and showed enhanced expression of the AT1R. However, upon exogenous infusion of insulin, AT1R expression and hypertension were entirely alleviated. It is possible that upon more efficient insulin signaling in an otherwise insulin-resistant system, reactive oxygen species production was minimized, leading to no further increase in AT1R production, but rather a return to homeostasis with the exception of circulating insulin titers.

However, insulin responsiveness is important to facilitative transport of glucose across different cell membranes, but as this study demonstrates, the increase in glucose uptake is mediated by sodium-coupled glucose transporters (i.e., non-GLUT) in hPTECs. In these cells, the distribution of SGLTs and GLUTs are site specific, with the GLUTs expressed basolaterally while the SGLTs are expressed in the brush border or luminal side, and the SGLTs mediating glucose influx from the renal filtrate and the GLUTs located at the basolateral border facilitating efflux to the surrounding tissues and vasculature (52). However, in diabetes it has been shown that GLUTs translocate to the brush border of the proximal tubule cells, diminishing their ability to efflux glucose to the basolateral tissues (41). In the present study, the hyperglycemic cells showed a 290.3% increase in glucose uptake compared with the euglycemic control. This result is consistent with a report in which it was demonstrated that SGLTs were upregulated in human exfoliated proximal tubular epithelial cells of patients with type 2 diabetes and markedly increased glucose uptake (46). However, in the current study SGLT-mediated glucose influx in hyperglycemia occurs within minutes of initial exposure to elevated glucose, suggesting an increased activity of the transporter without a significant change in the expression profile. The dose-dependent relationship between glucose concentration and observed uptake was linear within the time observed, and inhibiting the SGLTs reversed the downregulatory effect on the hAT1R. Based on the findings of the present study and previous observations in the literature, the proximal tubule cells in diabetes may take in more glucose, but the facilitative transporters requisited to remove the absorbed glucose to the basolateral tissues may be insufficient. With regard to insulin and expression of the hAT1R, our laboratory previously conducted gene promoter analysis and determined that insulin treatment of hPTECs resulted in a direct upregulation of hAT1R expression through a cis-acting element (64), which was consistent with other studies indicating that hyperinsulinemia upregulates hAT1R expression in hPTECs. However, the present study’s findings suggest that glucose uptake by sodium-coupled transport predominates in regulating hAT1R expression. Future studies in other tissue types should characterize insulin’s mixed effects in the regulation of the hAT1R with respect to its actions as a potentiator of glucose uptake and direct effect as a growth factor.

Human PTECs isolated from patients with type 2 diabetes showed a significant increase in glucose uptake, up to threefold, compared with those from healthy volunteers (46). One of the principal findings of the present study was that the increase in glucose uptake is primarily mediated by SGLTs, rather than the facilitative glucose transporters (GLUTs). Glucose transport in the diabetic kidney is upregulated in response to hyperglycemia and glycosuria (28). Sakhriani et al. (48) reported that 25% of d-glucose uptake by rabbit PTECs was...
mediated by the sodium-glucose transport process (48). Other researchers have found that phlorizin-induced inhibition of SGLT mediated a 28.8 ± 3.5% reduction in αMG uptake in hPTECs (31). SGLTs serve diverse purposes in human cells, acting as cotransporters, uniporters, glucosensors, water channels, and water transporters (63). ANG II is reported by Kawano et al. (33) to inhibit SGLT in renal proximal tubule epithelial cells; therefore, we may see how the respective receptor and transporter play a complex self-regulatory role, with each participant in the cascade having a positive feedback mechanism on the ligand or transport molecule involved and a negative feedback mechanism on the opposing strata. The transporter facilitates the high glucose transport to downregulate the receptor, whereas receptor stimulation results in downregulated glucotransporter expression. There are numerous factors that may affect the expression of SGLT in a given tissue, including proteinuria (35), hyperammonemia (47), and hyperglycemia itself (6, 46). While this is the first report of the role of SGLTs in the regulation of hAT1R expression, due to the inherent complexity of this system, the role of specific SGLTs and their respective long-term regulation of hAT1R expression require further investigation.

In conclusion, our study found that exposure of hPTECs to hyperglycemia downregulated the hAT1R. The observed effect of hyperglycemia on hAT1R gene expression was mediated through increased glucose transport via sodium-glucose transporters, which eventually has a downregulatory effect on hAT1R mRNA transcription and subsequently protein production. This study provides a unique mechanism demonstrating how the hAT1R may be regulated in hPTECs compared with other cell types in which SGLTs are absent, and facilitative glucose transport may result in a significantly different hAT1R expression profile.

GRANTS
This study was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK072140.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: R.Y. and R.S. performed experiments; R.Y., R.S., and T.T. analyzed data; R.S., T.A., and T.T. interpreted results of experiments; R.S. drafted manuscript; R.S., T.A., and T.T. edited and revised manuscript; and T.T. approved final version of manuscript.

REFERENCES
ROLE OF SGLT IN hAT1 EXPRESSION


