Activation of a local renin angiotensin system in podocytes by glucose

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Durvasula RV, Shankland SJ. Activation of a local renin angiotensin system in podocytes by glucose. Am J Physiol Renal Physiol 294: F830–F839, 2008. First published January 23, 2008; doi:10.1152/ajprenal.00266.2007.—ANG II is a critical mediator of diabetic nephropathy. Pharmacologic inhibition of ANG II slows disease progression beyond what could be predicted by the blood pressure lowering effects alone, suggesting the importance of nonhemodynamic pathways of ANG II in mediating disease. Podocyte injury and loss arecardinal features of diabetic nephropathy. Mounting evidence suggests that the podocyte is a direct target of ANG II-mediated signaling in diabetic renal disease. We have tested the hypothesis that high glucose leads to the activation of a local angiotensin system in podocytes and delineated the underlying pathways involved. Cultured podocytes were exposed to standard glucose (5 mM), high glucose (40 mM), or mannitol as an osmotic control. ANG II levels in cell lysates were measured in the presence or absence of inhibitors of angiotensin-converting enzyme (captopril), chymase (chymostatin), and renin (aliskiren) activity. The effects of glucose on renin and angiotensin subtype 1 receptor expression and protein levels were determined. Exposure to high glucose resulted in a 2.1-fold increase ANG II levels mediated through increased renin activity, as exposure to high glucose increased renin levels and preincubation with Aliskiren abrogated glucose-induced ANG II production. Relevance to the in vivo setting was demonstrated by showing glomerular upregulation of the prorenin receptor in a podocyte distribution early in the course of experimental diabetic nephropathy. Furthermore, high glucose increased angiotensin subtype 1 receptor levels by immunofluorescence and Western blot. Taken together, the resultant activation of a local renin angiotensin system by high glucose may promote progressive podocyte injury and loss in diabetic nephropathy.

METHODS

Cell culture. Experiments were performed utilizing early passage growth-restricted, conditionally immortalized mouse podocytes as we have previously reported (7). Cells were exposed to standard glucose (5 mM) and escalating doses of high glucose (25 and 40 mM) or mannitol-containing media (5 glucose + 20 mM mannitol and 5 glucose + 35 mM mannitol) as an osmotic control.

Primary antibodies. Immunofluorescence studies were performed for renin using a purified goat polyclonal anti-renin primary antibody generated against an internal region of renin of murine origin (Santa Cruz Biotechnologies). Immunofluorescence studies for AT,R were performed utilizing a purified rabbit polyclonal anti-AT,R antibody (Santa Cruz Biotechnologies). In vivo staining for the prorenin receptor was performed using antisera derived from rabbit (Asie Suisse Laboratories, Harrisburg, OR), and colocalization to the podocyte was determined using a monoclonal mouse anti-podocalyxin antibody (Fitzgerald Industries International, Concord, MA). Western blot analysis for AT,R was performed using a mouse monoclonal anti-AT,R primary antibody (Chemicon, Temecula, CA). A rabbit polyclonal anti-DAF/CD55 antibody (Santa Cruz Biotechnologies) was used as a loading control.

Measuring mRNA levels. Renin mRNA expression in cultured podocytes was verified by semiquantitative RT-PCR (8), utilizing the following primer sequences: renin: 5’TTCCTCGTCTTTTGACCACACT3’ and 3’CCACAGCCCTCTTCATCAT5’; and β-actin: 5’GGTGCGCCTCTACACCAACCA3’ and 3’CTCTTTGATGTCACGCACA5’. Podocyte expression of the prorenin receptor was validated in both primary cultured rat podocytes (14) (5’CACA-TTGCTCACGTCCTGCCGTCAGT3’ and 3’GTCACCAGGATGTGTGCAAT-3’) as well as conditionally immortalized mouse podocytes (5’TGTGGATGACTTGGGAACGC3’ and 3’CACAAGGATGTGCGAAATG5’).

Real-time PCR was performed utilizing commercially available primers and TaqMan probes (Applied Biosystems) for angiotensinogen (Assay ID#Mm00599662_m1), renin (Assay ID#Mm02342889_g1), and...
β-actin (product #4352933E). Amplification was performed using the Stratagene Mx3005P real-time PCR system utilizing the universal thermal cycling parameters optimized by Applied Biosystems. Analysis of relative gene expression was performed using the ΔΔCt method (27) with 5 mM glucose serving as the reference group.

Western blot analysis. To determine AT1-R levels in plasma membrane fractions, subcellular fractionation was performed as described previously (35). Culture plates were washed and harvested by manual scraping in HES buffer (255 mM sucrose, 20 mM HEPES pH 7.4, and 1 mM EDTA) before homogenization via sonication. Cell debris was removed from the homogenate by centrifugation at 1,300 rpm for 15 min. The supernatant was then centrifuged for 30 min at 19,000 g to yield a pellet containing the plasma membrane fraction, which was then resuspended in HES buffer supplemented with protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN).

Protein concentration was determined by BCA protein assay kit (Pierce, Rockford, IL) according to the manufacturer’s directions, and Western blot analysis for AT1-R was performed as we have previously reported (7). The chromagen 5-bromo-4-chloro-3-inodyl phosphate/nitro blue tetrazolium (Sigma, St. Louis, MO) was used for detection of the resultant bands. Blots were reprobed with antibody for β-actin as a loading control or, alternatively, CD55 to confirm purity of the plasma membrane fraction as recently reported (48). Densitometric quantitation was performed using Image J software (National Institutes of Health), and results were corrected to the appropriate loading control.

Immunofluorescence studies. Growth-restricted podocytes were seeded onto collagen type IV-coated glass coverslips. Cells were fixed with 2% paraformaldehyde/4% sucrose solution, permeabilized with 0.3% Triton solution, and stained for AT1-R or renin as described previously (8). Specificity of staining was confirmed by preincubating the primary antibody in a fivefold excess of the peptide used for generating the primary antibody or alternatively omission of the primary antibody. Cells that had four or more discrete cytoplasmic granules stain for renin were considered to be “renin positive.”

Measuring ANG II levels. ANG I and II levels were measured by competitive and highly specific ELISA with minimal (<1%) cross-reactivity for other angiotensin-related peptides or renin substrate (Peninsula Laboratories, San Carlos, CA) as we have previously reported (7). Studies were repeated in the presence of selective inhibitors (or vehicle where applicable) of ACE activity (captopril), chymase activity (chymostatin, Sigma), and renin activity (aliskiren, kindly provided by Novartis Pharmaceuticals, Basel, Switzerland). For all inhibitor studies, cells were preincubated for 60 min and doses were chosen based on what has been reported in the literature and manufacturers’ recommendations.

Tissue levels of prorenin receptor in an experimental model of diabetic nephropathy. Relevance of the cell culture results to the in vivo setting was determined by studying archival tissue from the streptazotocin model of diabetic nephropathy. Specifically, hyperglycemia was induced in six male Sprague-Dawley rats weighing 200 g by an intraperitoneal injection of streptozotocin (70 mg/kg body wt) while controls were injected with 10 mM citrate, pH 5.5 (vehicle for streptozotocin). Animals were killed on day 4 and 28 and biopsies fixed in formalin. After an antigen retrieval step in boiling citric acid (pH = 6.0) for 10 min, immunostaining was performed for the prorenin receptor based on recent reports (31) in the literature. A minimum of 15 consecutive glomeruli were analyzed per section from each animal, and prorenin receptor staining was measured using Image J software and expressed as the percentage of glomerular area with positive staining.

Statistical analysis. Unless otherwise noted, all experiments were repeated on at least three separate occasions. Western blots were run using protein harvested on each occasion, and densitometric analysis was performed in triplicate on each blot. To pool results from multiple ELISA assays, ANG II concentrations obtained in each individual experiment were adjusted to ANG II measurement under basal (5 mM glucose) conditions and expressed in arbitrary units. Specifically, the measured concentration of each individual replicate from each test group (including the basal reference group) was converted into a relative concentration based on the average of the reference (5 mM) group, thereby allowing data from separate studies to be pooled and presented graphically with error bars representing the standard deviation. Statistical analysis on data obtained was performed using paired t-test or ANOVA with a Bonferroni-Dunn correction (Statview 5.0, Abacus Concepts, Berkeley, CA). A P value <0.05 was considered statistically significant.

**RESULTS**

High glucose increases ANG II production in conditionally immortalized mouse podocytes. Podocytes are known to possess the metabolic machinery necessary for autologous synthesis of ANG II (21), and we have previously demonstrated that mechanical strain leads to increased ANG II generation by podocytes in vitro (7). Accordingly, to determine the effects of glucose on ANG II production by podocytes, growth-restricted conditionally immortalized mouse podocytes were cultured in media containing standard glucose (5 mM), escalating doses of high glucose (25 and 40 mM), or mannitol as an osmotic control. Protein was harvested from whole cell lysates at 24 h, and ANG II levels were measured by competitive ELISA. As shown in Fig. 1, exposure to high glucose increased ANG II levels in a dose-dependent manner. Specifically, incubation in 25 and 40 mM glucose media resulted in a 1.4-fold (P = 0.003) and 2.1-fold (P < 0.001) increase in ANG II concentration relative to the 5-mM glucose reference group. In contrast, exposure to equivalent concentrations of mannitol did not have any significant effect on ANG II levels, thereby excluding a nonspecific osmotic effect. Because exposure to 40 mM glucose resulted in greater generation of ANG II, this concentration was used for all subsequent in vitro studies.

Glucose-induced increase ANG II by podocytes is independent of ACE activity. ACE is known to be a critical determinant of systemic ANG II generation. ACE inhibitors represent an effective way to antagonize the angiotensin axis by blocking the conversion of ANG I to ANG II in circulation. However, in

![Fig. 1. High glucose (glc) increases ANG II production by cultured podocytes in a dose-dependent fashion. Growth-restricted conditionally immortalized mouse podocytes were cultured under conditions of standard (5 mM) or escalating doses of high glucose (glc: 25 and 40 mM). ANG II levels were measured in whole cell lysate by competitive ELISA. Exposure to 25 mM glucose resulted in a 1.4-fold increase in ANG II levels at 24 h (*P = 0.003) while incubating cells in 40 mM glucose resulted in a 2.1-fold increase (#P < 0.001). In contrast, exposure of podocytes to mannitol (mann) as osmotic controls did not significantly increase ANG II production.](http://ajprenal.physiology.org/)

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local tissue angiotensin systems, a role for non-ACE pathways (including chymases) is being increasingly recognized (25, 37). To determine the relative importance of the ACE pathway on glucose-induced ANG II generation, cultured podocytes were preincubated in standard media containing either captopril (pharmacologic inhibitor of ACE) or chymostatin (chymase inhibitor) before exposure to high glucose. As shown in Fig. 2, preincubation with captopril was unable to antagonize glucose-induced ANG II generation. In contrast, chymostatin reduced glucose-induced ANG II production in a dose-dependent fashion, suggesting that high glucose increases the local production of ANG II in podocytes through non-ACE pathways.

High glucose increases AT1R levels in conditionally immortalized mouse podocytes. The AT1R is responsible for mediating many of the deleterious nonhemodynamic effects of ANG II on tissue injury. Pharmacologic blockade of AT1R slows disease progression in patients with diabetic nephropathy (2, 24). To determine the effects of high glucose on AT1R levels in podocytes, cells were cultured in media containing standard glucose, high glucose, or mannitol and AT1R protein levels were determined by immunofluorescence staining. As shown in Fig. 3, exposure to high glucose resulted in an increase in the intensity of AT1R staining at 24 h (B) compared with standard glucose (A) or mannitol (C). Similar results were obtained after 48 h of exposure to high glucose conditions (data not shown). Specifically, AT1R staining was concentrated in a plasma membrane distribution. To verify this finding, subcellular localization was performed, and protein was isolated from the plasma membrane domain of podocytes exposed to standard vs. high glucose for 24 h. Western blot analysis of the plasma membrane fraction (bottom), confirmed increased levels of AT1R after exposure to high glucose. To ensure equal loading of protein, decay accelerating factor (CD-55), a complement regulatory protein restricted to the plasma membrane domain, was used as a loading control. When densitometric analysis was performed and results were adjusted for decay accelerating factor, exposure of podocytes to high glucose resulted in a 2.5-fold increase in AT1R levels compared with standard glucose conditions (P < 0.001). In contrast, protein levels and subcellular distribution of AT2R were unchanged after exposure to high glucose (results not shown). Taken together, our results indicate that high glucose leads to activation of a local angiotensin system in podocytes by increasing ANG II generation as well as augmenting AT1R density in the plasma membrane.

High glucose increases renin levels in conditionally immortalized podocytes. Having demonstrated that high glucose increases ANG II generation in cultured podocytes, we wished to delineate the underlying mechanisms. Specifically, we asked whether glucose could increase renin levels, thereby augmenting the conversion of angiotensinogen into ANG I. To confirm renin gene expression by conditionally immortalized mouse podocytes, total RNA was isolated and RT-PCR was performed. Growth-restricted conditionally immortalized mouse podocytes were cultured under conditions of standard glucose (5 mM), or mannitol and fixed in formalin/sucrose. Immunofluorescence staining revealed faint staining for AT1R along the plasma membrane under standard glucose conditions (A). After exposure to high glucose for 24 h, an increase in AT1R staining intensity along the plasma membrane was noted (arrows, B). In contrast, exposure of podocytes to 40 mM mannitol as an osmotic control did not have an appreciable effect on AT1R immunostaining (C). Subcellular fractionation was performed, and AT1R levels were measured in the plasma membrane fraction by Western blot analysis (bottom). When adjusted for the housekeeping protein decay accelerating factor, densitometric analysis confirmed a 2.5-fold increase in AT1R levels at 24 h in response to high glucose compared with standard glucose conditions (*P < 0.01). DAF, decay accelerating factor.

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Fig. 2. Glucose increases podocyte production of ANG II via non-ACE pathways. Growth-restricted conditionally immortalized mouse podocytes were preincubated for 60 min in the presence of the ACE inhibitor captopril vs. the nonselective chymase inhibitor chymostatin before incubating cells in 40 mM glucose. ANG II levels were measured in whole cell lysate by competitive ELISA. While preincubination with captopril had no effect on glucose-induced production of ANG II (P > 0.001 vs. 5 mM glucose), preincubation with chymostatin returned ANG II to basal levels in a dose-dependent manner (#P < 0.01 vs. 40 mM glucose).

Fig. 3. High glucose increases angiotensin subtype 1 receptor (AT1R) protein levels of cultured podocytes in a plasma membrane distribution. Growth-restricted conditionally immortalized mouse podocytes were cultured under conditions of standard glucose (5 mM), high glucose (40 mM), or mannitol and fixed in formalin/sucrose. Immunofluorescence staining revealed faint staining for AT1R along the plasma membrane under standard glucose conditions (A). After exposure to high glucose for 24 h, an increase in AT1R staining intensity along the plasma membrane was noted (arrows, B). In contrast, exposure of podocytes to 40 mM mannitol as an osmotic control did not have an appreciable effect on AT1R immunostaining (C). Subcellular fractionation was performed, and AT1R levels were measured in the plasma membrane fraction by Western blot analysis (bottom). When adjusted for the housekeeping protein decay accelerating factor, densitometric analysis confirmed a 2.5-fold increase in AT1R levels at 24 h in response to high glucose compared with standard glucose conditions (*P < 0.01). DAF, decay accelerating factor.
formed. DNA isolated from mouse kidney cortex served as a positive control while brain, liver, and connective tissue served as negative controls. As shown in Fig. 4, renin mRNA was detected in podocytes with tissue-specific distribution. Using real-time quantitative PCR, we next determined the effects of glucose on renin mRNA expression. As shown in Fig. 5A, culturing podocytes in the presence of high glucose for 8 and 24 h resulted in a 4.8-fold (*P < 0.002) and 2.8-fold (#P < 0.05) increase in renin mRNA expression compared with standard glucose. While exposure to mannitol resulted in a more modest (though significant) increase in renin mRNA expression at 8 h, this was not sustained at 24 h, suggesting that the glucose-induced increase in renin mRNA expression was not due to a nonspecific osmotic effect alone. In contrast to the effect of glucose on renin gene expression, exposure to high glucose had no effect on angiotensinogen mRNA expression at 8 or 24 h (Fig. 5B).

To determine the effects of glucose on renin protein levels, immunofluorescence studies were performed. As depicted in Fig. 6A, a fraction of growth-restricted conditionally immortalized podocytes normally stain positive for renin-containing cytoplasmic granules (top, left). The intensity of staining was largely abrogated by preincubating the primary antibody with an excess of blocking peptide (top, right), thus confirming specificity of the staining reaction. By counterstaining nuclei of all cells with DAPI, we then determined the effects of glucose on the percentage of renin-containing cells (Fig. 6B). As shown graphically in Fig. 6B, exposure to high glucose increased the number of cells staining positive for renin-containing cytoplasmic granules by 34 and 91% at 24 and 48 h, respectively, compared with standard glucose (#P < 0.05), whereas exposure to mannitol as an osmotic control had no effect (data not shown).

High glucose increases renin activity in conditionally immortalized podocytes. To determine whether the glucose-induced increase in renin mRNA and protein levels was associated with an overall increase in renin activity, preincubation studies were performed with aliskiren, a pharmacologic inhibitor of human renin that is also active against murine renin, and ANG II concentration was measured after exposure to high glucose. Aliskiren was used at a concentration of 20 nM based on published reports of its IC50 (44). As shown in Fig. 7, preincubation with aliskiren abrogated glucose-induced ANG II production in a dose-dependent fashion. In contrast, preincubation with aliskiren had only a negligible effect on ANG II production under basal (5 mM) glucose conditions. To further support that glucose-induced increase in ANG II generation is mediated via an increase in renin activity, competitive ELISA was performed for ANG I. As demonstrated in Fig. 8, exposure to high glucose resulted in a 1.46-fold increase (P < 0.006), compared with standard glucose conditions, whereas exposure to mannitol had no significant effect. Taken together, our results indicate that high glucose increases local ANG II production by directly increasing renin levels and activity.

Glomerular prorenin receptor levels are increased in an experimental model of diabetic nephropathy. Having demonstrated increased renin activity by cultured podocytes after incubation in high glucose-containing media, we wished to
demonstrate relevance of our cell culture studies to the in vivo setting. A specific receptor for renin has recently been characterized, which not only amplifies the efficiency of angiotensinogen conversion to ANG I at the cell membrane surface but also initiates a series of MAPK signaling events that promote the development of renal fibrosis independent of the actions of ANG II (14, 31). Binding both renin and prorenin with equal affinity, expression of the prorenin receptor has been established in a number of organs including the kidney (32). While its expression has been confirmed in mesangial cells, to date there have been no studies of the prorenin receptor in the podocyte. Accordingly, we queried whether prorenin receptor levels may be increased in podocytes in an experimental model of diabetic nephropathy. To first determine whether podocytes express the prorenin receptor, total RNA was isolated from conditionally immortalized mouse podocytes while cDNA derived from mouse kidney cortex served as a positive control. As shown in Fig. 9A, RT-PCR confirmed mRNA expression of the prorenin receptor in cultured mouse podocytes, as well as an established primary rat podocyte cell line. To determine whether the receptor protein may be detected within podocytes in vivo, immunofluorescence staining was performed in formalin-fixed tissue sections harvested from rat kidney using commercially available antiserum specific for the prorenin receptor. As shown in Fig. 9B, glomerular staining for the receptor was noted in both a mesangial and podocyte distribution, the specificity of which was confirmed with the omission of the primary antibody. Furthermore, double staining with synaptopdin as a specific podocyte marker confirmed colocalization of the prorenin receptor with podocytes in vivo. Immunohistochemistry for the prorenin receptor was then performed in archival tissue harvested from streptazotocin-injected (diabetic) rats while citrate-injected animals served as controls. Staining was performed 2- and 4-wk after disease induction as these early time points represent a stage by which animals were hyperglycemic but before the development of overt nephropathy. As shown in Fig. 10, basal levels of prorenin receptor staining are present in the glomerulus of a
ANG II is emerging as a critical mediator of podocyte injury in diabetic nephropathy. Studies in both type I and type II diabetic patients have firmly demonstrated that with progression of renal disease, a reduction in podocyte number ensues, which correlates strongly with the development of proteinuria and glomerulosclerosis (33, 39). Elevated glucose levels have been shown to be directly toxic to podocytes (5, 6, 10, 11, 16, 20, 29, 40) with excess cellular uptake likely triggering a cascade of maladaptive intracellular signaling events as recently reviewed (19). Studies by Coward et al. (4) have identified the presence of insulin-responsive GLUT-1 and GLUT-4 glucose transporters in the foot processes of podocytes in human glomerular sections, thus providing clues as to the mechanisms underlying glucose toxicity of the podocyte within the diabetic milieu.

ANG II is emerging as a critical mediator of podocyte injury in diabetic renal disease. Blocking ANG II has become a cornerstone in the management of diabetic nephropathy and affords renal protection beyond blood pressure lowering effects alone. However, plasma activity of renin, which mediates the rate-limiting step in the pathway of ANG II generation, is typically low in patients with diabetic nephropathy. Attempts to resolve this apparent paradox of the low renin state of diabetic nephropathy have resulted in the growing awareness of local tissue angiotensin systems, which play an important role in mediating disease through a series of nonhemodynamic effects including apoptosis, hypertrophy, matrix accumulation, and fibrosis. Accordingly, delineating mechanisms of the regulation of local angiotensin system in diabetic nephropathy is of utmost importance.

We report that exposing growth-restricted conditionally immortalized mouse podocytes to media containing high glucose increased ANG II production in a dose-dependent fashion compared with standard glucose or mannitol. While preincubation studies with the ACE inhibitor captopril failed to abrogate glucose-induced increase in ANG II, a nonselective chymostatin restored ANG II levels to baseline, thus enforcing the importance of non-ACE pathways in the local generation of ANG II in podocytes. We asked whether glucose might stimulate the angiotensin axis more proximally by increasing renin levels. Renin is classically expressed by the juxtaglomerular apparatus where it is stored in secretory granules. Renal hypoperfusion stimulates renin secretion, which then catalyzes the conversion of angiotensinogen to ANG I and ultimately the main effector peptide ANG II (23). Beyond the juxtaglomerular apparatus, renin is also expressed by a variety of other tissues and may be regulated by a number of different stimuli including glucose (42). In this study, we made the novel observation that exposure to high glucose upregulates renin mRNA expression in podocytes and increases the relative proportion of renin-positive cells. Such recruitment of renin expression by cells in a variegated manner in response to physiologic stimuli has been reported in the literature (30). Furthermore, we found that high glucose increased ANG I production, yet failed to increase gene expression of the angiotensinogen substrate. To definitively demonstrate that glucose increases overall renin activity in podocytes, preincubation studies were performed using the selective renin antagonist aliskiren. While aliskiren had only minimal activity in...
suppressing ANG II generation under basal (5 mM glucose) conditions, it strongly attenuated glucose-induced increase in ANG II. Taken together, our results suggest that a major pathway by which glucose increases ANG II generation in podocytes is by directly increasing overall renin activity, likely due to increased enzyme levels.

In the present study, we also demonstrated that exposure of podocytes to high glucose increased plasma membrane levels of AT$_1$R, the principal ligand receptor for ANG II, which is known to mediate many of the deleterious effects of ANG II in progressive renal disease. While the recent study reported by Yoo and et al. (47) likewise demonstrated increased production of ANG II and upregulation of the AT$_1$R in podocytes after exposure to high glucose, our results are discordant as to the underlying mechanism. Whereas we are reporting an increase in renin activity in response to high glucose with no changes in angiotensinogen expression, the study by Yoo et al. identifies increased levels of the angiotensinogen substrate in a transcriptionally dependent fashion without changes in renin activity. It should be noted that significant differences exist with regards to experimental design, which may suggest that the discrepant findings. Most notably, their studies were performed using 30 mM glucose containing media under serum-free conditions, whereas our experiments were performed under higher ambient glucose concentrations (40 mM) and in media containing 10% FBS. Furthermore, differences exist with regards to the methods by which renin was measured. While Yoo et al. used ANG I generation in the presence of an excess of angiotensinogen substrate as a marker of renin activity, we measured changes in mRNA and protein levels of renin, while also determining renin activity by measuring ANG I generation in the presence of a specific pharmacologic inhibitor of renin. Utilizing a longer preincubation period with captopril, Yoo et al. were also able to show a partial effect of ACE inhibition on glucose-induced ANG II generation. We recognize that if uptake of captopril in our in vitro studies was incomplete due perhaps to our shorter preincubation, then maximal suppression of ACE activity may not have been achieved. Although Yoo et al. demonstrated an effect of chymostatin on inhibiting ANG II generation as we also report, this is in contrast to recent studies by Liebau et al. (26). However, it should be emphasized that the latter studies were conducted using a human podocyte line in the absence of glucose stimulation.

While conversion of angiotensinogen to ANG I in circulation requires cleavage by mature renin, accumulating evidence suggests that renin or its proenzyme precursor (prorenin) may additionally interact with a tissue-specific receptor to fuel progression of diabetic kidney disease (41). Nguyen et al. (31) have characterized a specific receptor capable of binding prorenin to induce a conformational change and expose the enzymatic cleft responsible for cleavage of the angiotensinogen substrate. Binding to its receptor results in a fivefold increase in the catalytic efficiency of membrane-bound prorenin, thereby providing an additional mechanism for increased local ANG II
generation (31). Additionally, ligand interaction of the receptor with prorenin activates MAPK signaling pathways to trigger a series of profibrotic responses independent of the angiotensin axis (14, 15). Studies (15) from an experimental model of diabetic nephropathy have demonstrated increased prorenin activity within the glomeruli, which parallels our in vitro observations of increased renin activity in podocytes exposed to high glucose. Furthermore, infusion in diabetic rats of a decoy peptide that interferes with the interaction of prorenin with its ligand receptor ameliorated proteinuria and prevented the development of glomerulosclerosis. The importance of prorenin receptor activation in progression of glomerular disease independent of the angiotensin system is underscored by the prorenin receptor overexpressing transgenic rat that develops spontaneous proteinuria and glomerulosclerosis, which remained refractory to treatment with an ACE inhibitor but did respond to infusion of a prorenin receptor antagonist (18). Accordingly, our study for the first time of podocyte expression of the prorenin receptor and increased glomerular expression in early diabetic kidney disease is of particular relevance.

How does ANG II fuel podocyte injury in diabetic nephropathy? It is likely that multiple pathways are involved. We have previously demonstrated that mechanical strain, the result of elevated intraglomerular capillary pressure, increases local ANG II production, thereby promoting podocyte apoptosis in an AT1R-dependent fashion. Definitive proof of the deleterious effects of AT1R-mediated signaling of the podocyte comes from the transgenic AT1R-overexpressing rat, which spontaneously develops podocyte injury, proteinuria, and glomerulosclerosis (12). It is likely that ANG II also has direct effects on permeability of the glomerular filtration barrier (34). While diabetic nephropathy is characterized by reduced levels of nephrin, pharmacologic inhibition of ANG II restores nephrin levels to baseline (1, 22) and reverses foot process effacement (28). In addition, angiotensin receptor antagonists attenuated VEGF expression in diabetic rats and prevented the development of proteinuria (17). Furthermore, ANG II may lead to an overall loss of anionic charge of the glomerular basement membrane in diabetic nephropathy by modulating heparin sulfate proteoglycan synthesis of the podocyte (3). ANG II also increases p27 levels in diabetic glomeruli and cultured podocytes to induce cellular hypertrophy (45). Recent studies (20) have implicated a synergistic role for glucose and ANG II in mediating podocyte hypertrophy by induction of reactive oxygen species (20). Ultimately the resultant injury and loss of podocytes fuels the future development of glomerulosclerosis.

In conclusion, we have shown that high glucose directly increases ANG II generation through enhanced renin activity.
and upregulates AT1R levels in cultured podocytes. Furthermore, our results suggest that glomerular expression of the prorenin receptor is increased in vivo which may further lead to activation of a local angiotensin system to fuel podocyte injury and loss in diabetic nephropathy. Our overall proposed schema is shown in Fig. 11. Our findings could have significant implications for treatment strategies of diabetic renal disease. Beyond traditional ACE inhibitors or angiotensin receptor blockers, to maximally suppress the local angiotensin system in podocytes, targeting renin activity may be an effective strategy.

GRANTS

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