The proximal tubule in the pathophysiology of the diabetic kidney

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Submitted 13 December 2010; accepted in final form 10 January 2011

Vallon V. The proximal tubule in the pathophysiology of the diabetic kidney. Am J Physiol Regul Integr Comp Physiol 300: R1009–R1022, 2011. First published January 12, 2010; doi:10.1152/ajpregu.00809.2010.—Diabetic nephropathy is a leading cause of end-stage renal disease. A better understanding of the molecular mechanism involved in the early changes of the diabetic kidney may permit the development of new strategies to prevent diabetic nephropathy. This review focuses on the proximal tubule in the early diabetic kidney, particularly on its exposure and response to high glucose levels, albuminuria, and other factors in the diabetic glomerular filtrate, the hyperreabsorption of glucose, the unique molecular signature of the tubular growth phenotype, including aspects of senescence, and the resulting cellular and functional consequences. The latter includes the local release of proinflammatory chemokines and changes in proximal tubular salt and fluid reabsorption, which form the basis for the strong tubular control of glomerular filtration in the early diabetic kidney, including glomerular hyperfiltration and odd responses like the salt paradox. Importantly, these early proximal tubular changes can set the stage for oxidative stress, inflammation, hypoxia, and tubulointerstitial fibrosis, and thereby for the progression of diabetic renal disease.

Diabetic nephropathy; glucose transport; SGLT2; SGLT2 inhibitor; SGLT1; GLUT2; glomerular hyperfiltration; tubular injury; tubulointerstitial fibrosis; hypoxia; oxidative stress; salt paradox; hyperreabsorption; inflammation

DIABETES MELLITUS IS THE MAJOR cause of end-stage renal disease (143). About 20% of patients with either Type 1 or Type 2 diabetes (T1DM; T2DM) develop nephropathy after many years of diabetes, but we cannot predict which patient is affected or which genes and proteins are critically involved. It is urgent to better understand the events and molecular pathways that lead from the onset of diabetes to renal failure, and to identify earlier the patients at risk. Much has been learned about the role of the vasculature and the glomerulus, including mesangial cells and podocytes in the pathophysiology of the diabetic kidney (152, 183). This review focuses on proximal tubular changes that occur “early” in the diabetic kidney but have potential consequences for the long-term outcome.

Brownlee (24) proposed that the cell susceptibility to glucose-induced toxicity is determined by its expression of glucose uptake mechanisms and by the ability of these cells to down-regulate glucose uptake in the setting of hyperglycemia. Proximal tubular cells appear unable to decrease glucose transport rates adequately to prevent excessive changes in intracellular glucose when exposed to high glucose concentrations (94). Hyperglycemia not only exposes the tubular structures from the basolateral side but also enhances the amounts of glucose filtered by the glomeruli and thereby increases the tubular glucose load, exposure, and reabsorption. A better understanding of the role and regulation of renal glucose transport and handling in diabetes is important and may provide new therapeutic strategies like the current development of inhibitors of the Na+-glucose cotransporter SGLT2. Another notable phenotype of the early diabetic proximal tubule is that it grows. Tubular growth explains early functional changes in the diabetic kidney like the primary proximal tubular hyperreabsorption. Because of the physiology of tubuloglomerular communication, the latter forms the basis for the strong tubular control of glomerular filtration in the early diabetic kidney, including glomerular hyperfiltration. The molecular signature of proximal tubular growth in the diabetic kidney is unique and includes elements of cellular senescence, which may explain unusual responses like the “salt paradox” of the early diabetic kidney. Moreover, these molecular pathways are linked to fibrosis and inflammation and may contribute to and enhance the interaction of the diabetic milieu and albuminuria with the proximal tubular system. Thus, they may trigger renal oxidative stress and cortical interstitial inflammation, with the resulting hypoxia and tubulointerstitial fibrosis determining to a great extent the progression of renal disease. This review will discuss these issues in more detail. The interested reader is also referred to recent reviews on related topics (85, 110, 139, 147, 152). The pathophysiology and relevance of further downstream segments of the tubular and collecting duct system in
diabetes [e.g., glycogen deposits in thick ascending limb (TAL), insulin-induced salt retention in T2DM, or the renin angiotensin system in collecting duct] are beyond the scope of this review.

Hyperglycemia Enhances the Reabsorption of Glucose in the Proximal Tubule

Glucose entry into proximal tubular cells is insulin-independent, which makes proximal tubular cells particularly sensitive to hyperglycemia in diabetic conditions. The “bulk” of tubular glucose uptake across the apical membrane occurs in the “early” proximal tubule and is mediated by the low-affinity–high-capacity Na\(^+\)-glucose cotransporter SGLT2 (SLC5A2); in comparison, the high-affinity–low-capacity SGLT1 (SLC5A1) is thought to “clean up” most of the remaining luminal glucose in further distal parts of the proximal tubule (for reviews, see Refs. 126 and 174) (Fig. 1A). More recently, SGLT2 and SGLT1 protein expression has been directly localized in the brush border membrane of the early and later sections of the proximal tubule, respectively (14, 155). Moreover, Hummel et al. (60) expressed the human genes in HEK293 cells and confirmed that the Na\(^+\)-glucose coupling ratio equals a value of 1 for hSGLT2 and 2 for hSGLT1 (60) (Fig. 1B). Thus, Na\(^+\)-glucose uptake is electrogenic, with the ensuing depolarization being partly offset by luminal K\(^+\) exit (148, 149). Furthermore, hSGLT2 and hSGLT1 transport glucose with similar affinity (5 vs. 2 mM), whereas hSGLT1 has a greater concentrative power (60).

Micropuncture studies in knockout mice directly showed that SGLT2 is responsible for all glucose reabsorption in the early proximal tubule and, overall, is the major pathway of glucose reabsorption in the kidney, whereas mice heterozygous for SGLT2 showed no urinary glucose loss (155). The lack of SGLT2 suppressed the renal SGLT1 mRNA and protein expression by about 40%, which may be a mechanism of the late proximal segments to blunt the increase in glucose reuptake under conditions of increased luminal glucose delivery and uptake. Despite lacking SGLT2 and having suppressed SGLT1 expression, these mice have an increased absolute glucose reabsorption along the late proximal tubule and a mean fractional renal glucose reabsorption of ~40% (between 10 and 60%, varying inversely with the amount of glucose filtered). Preliminary studies in mice lacking SGLT1 showed normal renal SGLT2 protein expression and a significant, but minor, reduction in fractional renal glucose reabsorption from 99.8 to 96.9% (157). If SGLT1 is the major pathway for renal glucose uptake in mice lacking SGLT2, then its contribution to glucose uptake is significantly enhanced by inhibition of SGLT2 (Fig. 1A). Much of the evidence for the quantitative contribution of these proteins to renal glucose reabsorption in humans derives from the phenotype of subjects carrying gene mutations. Whereas mutations in SGLT1 are associated with intestinal glucose malabsorption with little or no glucosuria, individuals with gene mutations in SGLT2 have persistent renal glucosuria (126). Proximal tubular cells are not utilizing glucose for energy production. After being reabsorbed across the luminal membrane, glucose is transported across the basolateral membrane by low-affinity GLUT2 in the S1 segment, and by high-affinity GLUT1 in the S3 segment of the proximal tubule (172).

The level of protein expression in the cell membrane determines the capacity of glucose transport through SGLT2 and SGLT1. The renal cortex of streptozotocin (STZ)-diabetic rats was found to contain increased mRNA expression for SGLT1 and SGLT2 (164) and greater renal SGLT1 protein expression (165). Likewise, renal SGLT1 and SGLT2 mRNA levels in diabetic obese Zucker rats were higher than in age-matched lean rats (136). Primary cultures of human exfoliated proximal

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**Fig. 1.** Proximal tubular glucose transport in the normal and diabetic kidney. A: under euglycemic conditions ~97% of filtered glucose is reabsorbed via SGLT2 primarily in the early segments of the proximal tubule. A significant capacity of SGLT1 to reabsorb glucose in later segments of the proximal tubule is unmasked by SGLT2 inhibition (~40% of filtered glucose under normoglycemia; see numbers in parentheses); based on (155) and the assumption that apical tubular glucose uptake is significantly enhanced by inhibition of SGLT2 (Fig. 1A). Much of the evidence for the quantitative contribution of these proteins to renal glucose reabsorption in humans derives from the phenotype of subjects carrying gene mutations. Whereas mutations in SGLT1 are associated with intestinal glucose malabsorption with little or no glucosuria, individuals with gene mutations in SGLT2 have persistent renal glucosuria (126). Proximal tubular cells are not utilizing glucose for energy production. After being reabsorbed across the luminal membrane, glucose is transported across the basolateral membrane by low-affinity GLUT2 in the S1 segment, and by high-affinity GLUT1 in the S3 segment of the proximal tubule (172).

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tubular epithelial cells harvested from fresh urine of patients with T2DM showed an increased renal glucose uptake associated with increased mRNA and protein expression of SGLT2 and GLUT2 (117). In comparison, other studies showed unchanged expression, including SGLT1 protein (5, 34, 70) or reduced renal expression and activity of SGLT (7) in diabetic rodent models, and in vitro studies in renal proximal tubule cells indicated that high glucose-induced oxidative stress may explain the reduction in SGLT expression and Na⁺/glucose cotransport activity (53) (Fig. 1B). As the downregulation of SGLT1 in mice lacking SGLT2 (155), this could serve to limit renal glucose uptake and toxicity.

Upregulation of SGLT2 expression in diabetes has been linked to activation of ANG II AT1 receptors (102) and the transcription factor, hepatocyte nuclear factor HNF-1α (41). The serum and glucocorticoid-inducible kinase SGK1 is upregulated in proximal tubules in STZ-diabetic rats and in patients with diabetic nephropathy (75). Studies in mice indicated that SGK1 expression in the diabetic kidney contributes to stimulation of SGLT1 activity in proximal renal tubules (4). SGK1 could also facilitate proximal tubular glucose transport by stimulation of luminal K⁺ channels (KCNE1/KCNQ1), which maintain the electrical driving force (36, 148, 149) (Fig. 1B).

Upregulation of GLUT2 expression has been reported in renal proximal tubules in diabetic rats (29, 34, 70) and has been linked to transcriptional activity of both HNF-1α and HNF-3β (42). In contrast, GLUT1 appears to be downregulated in cortical tubules in diabetes (29, 34). Moreover, STZ-diabetes can target GLUT2 protein (but not GLUT1) to the brush border membrane of proximal tubules (87). The latter may be linked to protein kinase C PKCβ1 activation (49, 107, 108) and implicates facilitative glucose transport in the increased glucose reabsorption across the apical membrane in the diabetic kidney (Fig. 1B).

Multiple selective inhibitors of SGLT2 are currently in clinical trials to inhibit renal glucose reabsorption and increase renal excretion, thereby lowering blood glucose levels (95, 159). The long-term safety of this approach and whether these drugs lower the glucose-induced effects in cells expressing SGLT2 (which may slow or prevent the progressive nature of diabetic nephropathy) remains to be determined. Because of differences in the Na⁺:glucose coupling ratio, shifting glucose reabsorption from SGLT2 to SGLT1 is expected to attenuate the renal sodium loss in response to SGLT2 inhibition. All diabetic patients experience episodes of hyperglycemia and preventing the early proximal tubule from “seeing” these episodes of hyperglycemia through SGLT2 may attenuate the negative effects of glucose on renal structure and function (see below). However, blocking apical glucose entry via SGLT2 may simply increase basolateral or GLUT-mediated glucose entry during hyperglycemia. The consequences of shifting glucose reabsorption to later segments of the proximal tubule and enhancing the glucose load to the downstream tubular and collecting duct system deserves further investigation.

Unique Growth Phenotype of the Diabetic Proximal Tubule

The kidney, in general, and the proximal tubules, in particular, grow large from the onset of diabetes (30, 118, 131), and diabetic kidney growth has been linked to the development of nephropathy (16, 18, 77, 179). Proximal tubular growth involves an early period of hyperplasia, which precedes hypertrophy (59). Ornithine decarboxylase (ODC) is the rate-limiting enzyme in polyamine synthesis, which in the early diabetic kidney, is required for hyperplasia and most likely also for hypertrophy of the proximal tubule (33, 79, 105, 106, 141) (Fig. 2). Deng et al. (33) proposed that the increase in ODC expression in early diabetes mainly occurs in the distal nephron and that polyamines may pass from the distal to proximal tubule in a paracrine fashion to trigger proximal tubular growth. Further studies are needed to confirm these findings and explore what might trigger ODC expression in the distal tubule.

Early hyperplasia. DNA synthesis increases and peaks at day 2 in the proximal tubules of STZ-induced diabetes (119). Numerous growth factors have been implicated in this response, including IGF-I, HGF, PDGF, FGF, VEGF, EGF, and
diacyl glycerol (28, 171). Whereas all of these factors can induce ODC activity (128), downstream signaling events of IGF-I and VEGF also include activation of the phosphoinositide 3-kinase (PI3K)/Akt pathways (129). The rapid, yet transient, renal induction of IGF-I (38, 115) correlates with the upregulation of renal ODC expression and activity (33, 105, 141), induction of intracellular polyamines in the kidney cortex (33), and the early proliferative phase. Diabetes also activates PKC, which can produce a myriad of consequences, including a mitogen-induced early proliferation phase (23). In particular, diabetes can enhance proximal tubular activity of the PKCβ isoform (107), and PKCβ has been implicated in Akt activation in the renal cortex of diabetic rats (175). Activation of PI3K/Akt (133) and PKC pathways (58, 63) are both linked to ODC activation. In accordance with a role of PKCβ in kidney growth, the early diabetes-induced increase in kidney weight was blunted in mice lacking this PKC isoform (90).

Diabetic renal growth is associated with reduced phosphorylation of AMP-activated protein kinase (AMPK) (78). Phosphorylated AMPK inhibits the activity of mammalian target of rapamycin complex 1 (mTORC1) by phosphorylating and activating tuberous sclerosis complex (82). As a consequence, mTOR activity is enhanced in the diabetic kidney, and increasing AMPK phosphorylation reversed mTOR activation and inhibited renal growth without affecting hyperglycemia (78). Together, these studies propose a link between enhanced glomerular filtration and tubular expression of growth factors, activation of PKC, inhibition of AMPK, and activation of both mTORC1 and ODC in the early tubular proliferation in diabetes (Fig. 2).

Switch from hyperplasia to hypertrophy. The diabetic kidney switches early from hyperplastic to hypertrophic growth, e.g., at around day 4 in the model of STZ diabetes (33, 59), TGFβ1 is a critical mediator of this growth switch (52). In accordance, high glucose administered to primary tubule cells from TGFβ knockout mice induced an increased rate of proliferation relative to cells from wild-type littermates, but no hypertrophy (27). PKCβ can induce TGFβ in the diabetic kidney (61, 73, 74), and PKCβ1 is expressed in the proximal tubule (108) and activated in STZ diabetes (107). Further mechanisms implicated in high glucose-induced TGFβ expression and cellular hypertrophy in renal tubular cells of STZ-diabetic rats include ERK and p38 (45).

TGFβ can induce a G1 phase cell cycle arrest by induction of the cyclin-dependent kinase (CDK) inhibitor, p27Kip1 (p27) (69). Expression of p27 increases in response to hyperglycemia or diabetes, which, on the basis of studies in nontubular cells, can be attributed to induction by PKC (170) and TGFβ (69). Diabetes also increases the renal expression of the CDK p21 (6, 127). Consistent with a potential role of p21 in the switch from hyperplasia to hypertrophy in the diabetic kidney, loss of p21 increases tubular cell proliferation (6) (Fig. 2). Sustaining kidney hypertrophy and size in the long term of diabetes involves decreased proteolysis (40).

Tubular senescence in the early diabetic kidney. Senescence is a tumor suppressor mechanism that involves CDK inhibition to halt cells from replicating and passing on a potentially damaged genome (120). Transient induction of p21, p16⁰⁰ⁱ⁰⁴⁴ (p16), and/or p27 are involved in the prototypical senescent arrest or senescent-like growth arrest (8, 178). Recent studies by Satriano et al. (127) revealed that STZ-diabetic kidneys exhibited an early transient induction of growth-phase components followed by their suppression at day 10 after the onset of diabetes. This was concurrent with the induction of CDK inhibitors p16, p21, and p27 and markers of senescence, including expression of senescence associated beta-galactosidase activity in cortical tubules (127). Moreover, they showed that proximal tubule cells in culture transition to senescence in response to oxidative stress. Notably, kidneys of patients with T2DM and nephropathy display an accelerated senescent phenotype in tubule cells (163).

Senescent cells are fairly well differentiated but skewed in several aspects, including the release of inflammatory cytokines, production of growth factors and extracellular matrix (ECM), and resistance to apoptotic remodeling (9, 135). Whereas the senescent arrest of tubular cells may be triggered by gluco-toxic signals to prevent excessive proliferation, one may speculate that it also alters tubular function (e.g., the “salt paradox” of the diabetic kidney; see below) and contributes to the development of diabetic nephropathy (Fig. 2).

Primary Hyperreabsorption by the Diabetic Proximal Tubule

Hyperfiltering patients with T1DM (20, 54) or T2DM (89) have increased absolute and “fractional” proximal reabsorption, and enhanced fractional proximal reabsorption was confirmed in children with T1DM (98). Similarly, hyperfiltering rats with STZ-diabetes have increased absolute and “fractional” reabsorption in the nephron segments upstream of the macula densa (114, 146, 156). Because of the imperfect nature of glomerulo-tubular balance (GTB), i.e., load dependence of tubular reabsorption, an increase in glomerular filtration rate (GFR) should decrease “fractional” reabsorption and increase the distal delivery. If fractional reabsorption and GFR change in the same direction, there must be a “primary” change in tubular reabsorption. To more directly demonstrate “primary” hyperreabsorption, micropuncture was used to show that at the same level of single nephron GFR, proximal reabsorption was greater in rats with early STZ-diabetes compared with control (141, 150) (Fig. 3).
Tubular Growth and Sodium-Glucose Cotransport Contribute to Primary Proximal Tubular Hyperreabsorption in Diabetes

Dimethylarginine (DFO), an inhibitor of ODC, had been shown to attenuate kidney growth in early STZ-diabetic rats (106) and was used to test whether tubular growth per se contributes to the “primary” increase in proximal reabsorption in early diabetes mellitus. DFO not only attenuated kidney growth but eliminated the “primary” increase in proximal reabsorption in STZ-diabetic rats (141). Tubular growth in diabetes also involves PKCβ activation (see above). PKCβ1 is expressed in the brush border of proximal tubule (108), where it contributes to stimulation of sodium transport by ANG II (35). Moreover, the diabetes-induced activation of PKCβ1 in the diabetic kidney is inhibited by ACE inhibition (107). Further studies are needed to determine the role of ANG II and PKCβ1 for proximal tubular hyperreabsorption, which could contribute to the beneficial effects of inhibiting these systems in the diabetic kidney.

Modeling the effects of sodium-linked glucose transport on the active and passive components of proximal reabsorption predicts that modest hyperglycemia enhances sodium reabsorption in the proximal tubule (169). Bank and Ayne (15) performed microperfusion studies in STZ-diabetic rats and proposed that high glucose in the proximal tubular fluid stimulates sodium absorption through sodium-glucose cotransport. They found that increasing luminal glucose (from 100 to 500 mg/dl) induced significantly greater increases in sodium vs. glucose absorption on a molar basis, which may reflect, at least in part, the sodium:glucose coupling ratio of 2:1 for SGLT1 (in comparison, the ratio is 1:1 for SGLT2 (174)). Confirmation of increased SGLT1-mediated sodium transport was demonstrated with micropuncture in moderately hyperglycemic STZ-diabetic rats by applying the SGLT inhibitor, phlorizin, directly into the free-flowing early proximal tubule: in diabetic rats, phlorizin elicited a greater decline in absolute and fractional reabsorption up to the early distal tubule and abolished hyperreabsorption (156). Seyer-Hansen (130) had reported that in early STZ-diabetic rats, the glucose reabsorptive rate increased with kidney weight. Hence, the “primary” increase in proximal reabsorption in early diabetes is the combined result of tubular growth and increased sodium-glucose cotransport.

Primary Tubular Hyperreabsorption Contributes to Glomerular Hyperfiltration in Diabetes

The tubuloglomerular feedback (TGF) system senses changes in the concentration of Na+, Cl− and K+ at the luminal macula densa and induces reciprocal changes in single-nephron glomerular filtration rate (SNGFR) to stabilize electrolyte delivery to the distal tubule, where fine adjustments of reabsorption and excretion occur according to body needs. As outlined above, there is a “primary” increase in proximal reabsorption in early diabetes. Micropuncture in rats with superficial glomeruli allows collecting tubular fluid close to the macula densa. This approach revealed ambient early distal tubular concentrations of Na+, Cl− and K+ in nondiabetic rats of 21, 20, and 1.2 mM, respectively, and that in hyperfiltering STZ-diabetic rats, these concentrations were reduced by 20–28%, consistent with a “primary” increase in upstream reabsorption (156). The TGF system senses the decline in salt delivery and elicits an increase in GFR, which offsets a portion of the original error signal through negative TGF (Fig. 4A).

Tubular control of GFR has been proposed in dogs, where acute hyperglycemia increased GFR, but only if TGF were intact (173). Evidence for a primary hyperreabsorption upstream of the macula densa and a potential role in glomerular hyperfiltration was also obtained in diabetic patients, including studies that showed that fractional proximal reabsorption was elevated and positively correlated with GFR (20, 54, 162). Increasing the cortical interstitial concentrations of adenosine, which is a mediator of TGF (140), normalizes GFR in STZ-diabetic rats (154). Two studies have reported hyperfiltration in diabetic mice lacking an acute TGF response due to knockout of the adenosine A1 receptor (A1R−/−), and the authors concluded that this argues against the tubular control of GFR in diabetes (37, 125). The tubulo-centric principle invokes feedback from the tubule as the dominant controller of GFR in early diabetes but doesn’t require TGF to be the only controller. The theory still allows for additional primary defects in afferent arteriolar vasoconstriction and predicts such defects will be unmasked when feedback from the tubule is eliminated. In such a case, some degree of hyperfiltration would persist in the absence of A1R. Moreover, in one of the two studies, the nondiabetic A1R−/− mice but not the alloxan-diabetic A1R−/− mice were hypotensive compared with their WT controls during measurements of GFR (125). As a consequence, the higher GFR in normotensive alloxan-diabetic A1R−/− mice may have been the reflection of impaired renal autoregulation, which is a known trait of the TGF-less mouse (55). The other study used Akita-diabetic A1R−/− mice, which have blood glucose levels of 600 to 900 mg/dl, and are thus severely hyperglycemic (37). The resulting excessive glucose load to the proximal tubule may actually inhibit proximal reabsorption (169), in contrast to the primary increase in proximal tubular reabsorption with modest hyperglycemia (141, 156). As a consequence, TGF activation may serve to limit glomerular hyperfiltration during severe hyperglycemia. Furthermore, the severity of diabetes may determine the contribution of primary vascular vs. TGF-mediated influences of adenosine on GFR, and thus determine the net response to A1R blockade or knockout (153). In accordance with this discussion and the tubular control of GFR in diabetes, glomerular hyperfiltration was blunted when A1R−/− mice were exposed to STZ-induced moderate hyperglycemia (158).

As discussed above, sodium glucose cotransport and tubular growth contribute to enhanced proximal tubular hyperreabsorption in the diabetic kidney. Adding phlorizin to the early proximal tubule of diabetic rats not only increased early distal electrolyte concentration but induced a decisive reduction in SNGFR in diabetic rats (156). Likewise, application of DFM0 to reduce early diabetic tubular hypertrophy and hyperreabsorption also diminished glomerular hyperfiltration in direct proportion to the effect on kidney size (141). Since established tubular growth reverses slowly, glomerular hyperfiltration may endure in diabetic patients due to persistent tubular enlargement and hyperreabsorption, independent of the average blood glucose level. Moreover, patients may be heterogeneous in their response to hyperglycemia with regard to kidney growth, which may determine the resulting tubular hyperreabsorption and glomerular hyperfiltration.
The “primary” increase in tubular reabsorption in diabetes, in addition to reducing the TGF signal, can lower the hydrostatic pressure in Bowman space (\(P_{\text{Bow}}\)) (81, 154, 156). Enhanced reabsorption is expected to reduce \(P_{\text{Bow}}\) by lowering the flow rate through distal nephron segments where flow resistance is high (81). This reduction in \(P_{\text{Bow}}\) could make a contribution toglomerular hyperfiltration by increasing the effective glomerular filtration pressure (81, 156) (Fig. 4A).

Salt Paradox in the Diabetic Kidney—Link to Proximal Tubular Reabsorption and Growth

In normal subjects, GFR is either insensitive to dietary NaCl or changes in the same direction as NaCl intake (142, 150). In 1995, we reported that a low-salt diet (for 7–8 days after 6 wk of diabetes) increased renal blood flow, GFR, and kidney weight in male STZ-diabetic rats (160). Moreover, female rats with early (1 wk) or established (4–5 wk) STZ-diabetes respond with renal vasoconstriction in response to a high-NaCl diet (151). Because the negative impact of dietary NaCl on GFR in diabetes is counterintuitive with regard to salt balance, we refer to it as the “salt paradox.” The paradoxical effect of dietary salt was confirmed in STZ-diabetic mice (158) and Long Evans rats (76), and by micropuncture on the level of the single nephron in male (150) and female (unpublished observation) STZ-diabetic rats.

Importantly, Miller reported the same phenomenon in 1997 in young patients with uncomplicated T1DM: restriction of dietary sodium to 20 mmol/day lowered renal vascular resistance and increased effective renal plasma flow and GFR (91). Similarly, short-term moderate sodium restriction induced relative hyperfiltration in uncomplicated T1DM (84). Fewer data have been acquired on the early renal pathophysiology in T2DM. One study found that a high dietary NaCl intake for 5–7 days reduced renal plasma flow in hypertensive patients with T2DM (32). Another study found no significant effect on renal plasma flow or glomerular filtration rate by variation in NaCl intake for 9–14 days in patients with T2DM (26). Further studies are needed to elucidate whether the salt paradox is present in patients with T2DM in the early phase of the disease and in the absence of confounding complications.

The salt paradox in the diabetic kidney is explained by hypersensitivity of proximal reabsorption to dietary salt. Our experimental data indicate that the salt paradox is another manifestation of the strong tubular control of glomerular filtration in diabetes. Feeding a high-NaCl diet to diabetic rats led to a major “primary” decrease in proximal reabsorption, i.e., a change in reabsorption that is not attributable to GTB (Figs. 3 and 4C). By measuring concentrations of Na\(^+\), Cl\(^-\), and K\(^+\) in early distal tubular fluid in rats on a high- and low-NaCl diet, it was confirmed that this “primary” effect of dietary NaCl on tubular reabsorption is strongly linked to the TGF signal and the consequent reduction in GFR to dietary NaCl in diabetes. In comparison, nondiabetic rats on various NaCl diets manage salt balance with no significant primary effect on reabsorption upstream of the macula densa, and thus, a TGF-mediated inverse effect of dietary NaCl on GFR does not occur (150) (Figs. 3 and 4B), which, from a teleological standpoint, is appealing. Thus, the salt paradox arises in diabetes because the proximal tubule is strikingly sensitive to NaCl intake, making GFR a “slave” to tubular function via the physiology of tubuloglomerular communication. In accordance, the salt paradox is absent in STZ-diabetic mice lacking an intact TGF communication. In accordance, the salt paradox is absent in STZ-diabetic mice lacking an intact TGF response (158). Considering the need to maintain an effective circulating volume, the capacity to increase GFR by reducing distal salt delivery must be less than the capacity to reduce GFR through the systemic influences of salt depletion. Hence, if dietary salt restriction progresses to actual salt depletion, the salt paradox will become imperceptible. The clinical relevance of the salt paradox remains unclear, as well as its presence in type 2 diabetes (145).
Salt paradox in the diabetic kidney is linked to tubular growth. ANG II and renal nerves are the prominent effectors, which link proximal reabsorption to total body salt, but neither chronic renal denervation (17) nor chronic ANG II AT1 receptor blockade (160) prevented the rise in GFR in response to low-NaCl diet in STZ-diabetic rats. In early diabetes, hypertrophic proximal tubular cells are continuously pushed by mitogens and at the same time prevented from entering the cell cycle and have a senescent and possibly less differentiated phenotype (see above). A normal proximal tubule cell is programmed not to respond to every change in local hormones that contribute to salt balance, as this balance is normally taken care of in nephron segments downstream of the macula densa. The diabetic proximal tubule, however, may have lost this characteristic of a differentiated nephron segment, and as a consequence, responds strongly to dietary NaCl, forming the basis for the salt paradox. Indeed, and supporting a role of diabetic kidney growth, pharmacological inhibition of ODC and tubular growth prevented the salt paradox (92).

Proximal Tubular and Tubulointerstitial Injury in the Progression of Diabetic Kidney Disease

Glomerular mesangial expansion and podocyte loss are important early features of diabetic nephropathy (71, 152, 183). In comparison, the diabetic milieu and the prolonged interaction of albuminuria and other factors in the glomerular filtrate with the tubular system trigger renal oxidative stress and cortical interstitial inflammation, with the resulting hypoxia and tubulointerstitial fibrosis determining to a great extent the progression of renal disease (1, 19, 31, 47, 86, 88, 134). Moreover, the unique molecular mechanisms involved in the early growth phenotype of the diabetic kidney may begin to set the stage for long-term progression of diabetic kidney disease. A recent study showed that the regression of microalbuminuria in patients with T1DM is associated with lower levels of urinary tubular injury biomarkers, kidney injury molecule-1, and N-acetyl-β-D-glucosaminidase, consistent with the notion that tubular dysfunction is a critical component of the early course of diabetic nephropathy (144). Important factors contributing to tubulointerstitial injury include hyperglycemia, proteinuria, advanced glycation end products (AGEs), and chronic hypoxia, as recently reviewed (28, 71, 85, 134, 183) and briefly outlined in the following sections.

Interaction Between Proteinuria, Growth Factors, and the Proximal Tubular System Triggers Inflammation in the Diabetic Kidney

Proteinuria can result in tubular and interstitial damage by various components in the proteinuric urine, such as AGEs, transferrin, albumin, and albumin-bound fatty acids (57). Urinary albumin induces proinflammatory chemokines in proximal tubular epithelial cells, such as IL-8 (85, 138) and monocyte chemotactic protein 1 (MCP-1), the latter is mediated by NF-κB (168) (Fig. 5). These chemokines and macrophage infiltration have been implicated in the initiation of the pathological changes in STZ-diabetic rats and human diabetic nephropathy (57, 124).

Also, the interaction between growth factors, such as IGF-I, HGF, and TGFβ, and their respective receptors in the apical membranes in proximal and distal tubules and collecting ducts, enhances the levels of MCP-1, RANTES, and PDGF-β, which activate the proliferation of fibroblasts but also of macrophages (28, 57). In the diabetic kidney, the synergism of high glucose

![Fig. 5. Mechanisms of proximal tubular and tubulointerstitial injury in the diabetic kidney. Illustrated is the influence of hyperglycemia, luminal factors (derived from glomerular filtration and tubular release), tubular transport work, and peritubular blood flow on the interaction of proximal tubular cells with fibroblasts and inflammatory cells. TGFβ, chemokines, and the complex interactions between advanced glycation end products (AGEs), hypoxia and oxidative stress play key roles in the development of diabetic tubulointerstitial injury. ECM, extracellular matrix; RAGE, receptor for AGE; ROS, reactive oxygen species; SOD, superoxide dismutase. See text for further details. [Modified from (152).]
concentrations with cytokines, such as PDGF or the proinflammatory macrophage-derived cytokine, IL-1β, can stimulate TGFβ1 synthesis by proximal tubular cells (112, 113) (Fig. 5), indicating a close link between inflammation and the development of fibrosis in the diabetic kidney. Many proinflammatory mediators, including chemokines MCP-1 and RANTES, are under the tight control of the transcription factor NF-κB (51) and are responsive to reactive oxygen species (ROS) (100, 132) (Fig. 5). High glucose can induce macrophage inflammatory protein-3 alpha (MIP-3α) in human proximal tubular cells in a TGFβ1-dependent way (116). Moreover, MIP-3α was up-regulated in dilated tubules of diabetic rats, which were surrounded by T-lymphocytes. Notably, this up-regulation was attenuated in the presence of an ACE inhibitor (116).

Blockade of the renin-angiotensin-aldosterone system is currently the only clinically used anti-inflammatory strategy to treat diabetic nephropathy. Multiple nuclear hormone receptors have also been suggested to provide protection against metabolic, cardiovascular, and inflammatory diseases, including peroxisome proliferator-activated receptors, estrogen receptors, hepatic nuclear factor 4 alpha, and especially, the vitamin D receptor and the farnesoid X receptor have been implicated in the pathogenesis of diabetic nephropathy (167). There is first evidence for a potential direct role of these systems in the diabetic kidney, but the interpretation of renal outcomes in the setting of diabetes has been confounded by extrarenal effects surrounding the kidney (see above) to inflammation, as well as fibrotic changes and scarring (110, 180). In accordance, TGFβ1, activated by IGF-I, and IGF binding protein-3 (65). These interactions are modified by the tubular basement membrane components laminin and collagen type IV in the tubulointerstitium (80). Thus, there is complex cross-talk between proximal tubular cells, ECM proteins, and fibroblasts, and one may speculate that early changes and proximal tubular injury in diabetes affects these interactions and contributes to tubulointerstitial fibrosis (Fig. 5).

**Hyperglycemia, TGFβ, CTGF, and the Reciprocal Paracrine Activation Between Proximal Tubular Cells and Fibroblasts**

Ultrafiltered growth factors, such as IGF-I, HGF, and TGFβ, have been linked to proteinuria-associated interstitial fibrosis in the diabetic kidney (57) (Fig. 5). Moreover, high glucose concentrations can induce collagen gene transcription and secretion in vitro in murine cortical tubular cells (182). Exposure of primary cultures of human renal proximal tubular cells to high glucose induces cell growth and increases the amount of type IV collagen and fibronectin (66, 111) due to a net decrease in gelatinolytic activity (109).

Multiple growth factors have been implicated in the control of cell hypertrophy, proliferation, and survival, as well as renal matrix composition (28). The renal expression of TGFβ is elevated in human and experimental diabetic nephropathy (176) and links the early-growth phenotype of the diabetic kidney (see above) to inflammation, as well as fibrotic changes and scarring (110, 180). In accordance, TGFβ is relevant to the progression of renal disease, as suggested by studies in db/db mice, a model of T2DM, in which treatment with a monoclonal anti-TGFβ antibody prevented renal insufficiency (181).

Connective tissue growth factor (CTGF) is a proscerotic cytokine that is mainly induced by TGFβ, activated by IGF-I, and involved in the regulation of matrix accumulation (57, 166). CTGF mRNA levels were increased in the renal cortex of STZ-diabetic rats, and immunohistology localized the expression of CTGF protein, in particular, to dilated-appearing proximal tubules, where it colocalized with IGF-I (166), consistent with their proposed interaction. Glomerular ultrafiltrate from diabetic rats, which contained bioactive TGFβ and HGF, can induce CTGF expression in proximal tubular cells (166). Vice versa, CTGF may enhance the profibrotic effects of TGFβ (3, 50) (Fig. 5). Notably, urinary CTGF levels in diabetic patients correlate with the degree of microalbuminuria (46, 122), and using a specific CTGF antisense oligonucleotide, Guha et al. (50) demonstrated renoprotective effects in STZ-diabetes and db/db mice, including reduced renocortical expression of fibronectin, collagen (I and IV) and PAI-1, an inhibitor of matrix degradation, as well as lesser serum creatinine, proteinuria, albuminuria, and kidney growth.

In chronic kidney disease, the regions of active interstitial fibrosis predominantly exhibit a peritubular rather than perivascular distribution (10), indicating that injured proximal tubular cells may release fibrogenic signals to cortical fibroblasts. In fact, TGFβ1 can stimulate the release of preformed basic fibroblast growth factor from renal proximal tubular cells (67).

Importantly, there is evidence for reciprocal paracrine activation of proximal tubular cells and fibroblasts. Proximal tubular cells in the human kidney modulate the biological behavior of neighboring cortical fibroblasts through paracrine mechanisms, which include the production and release of the AB heterodimer of PDGF and TGFβ1 (64). Vice versa, studies in human renal fibroblasts indicated that they can modulate proximal tubule cell growth and transport via the secretion of IGF-I and IGF binding protein-3 (65). These interactions are modified by the tubular basement membrane components laminin and collagen type IV in the tubulointerstitium (80). Thus, there is complex cross-talk between proximal tubular cells, ECM proteins, and fibroblasts, and one may speculate that early changes and proximal tubular injury in diabetes affects these interactions and contributes to tubulointerstitial fibrosis (Fig. 5).

**Hypoxia, Oxidative Stress, and Tubulointerstitial Fibrosis in the Diabetic Kidney**

Abnormalities in oxygen metabolism have been implicated in the development and progression of diabetic nephropathy and include hypoxia, oxidative stress, nitrosative stress, and advanced glycation and/or carbonyl stress (44, 71, 93). A role of hypoxia in chronic renal disease was proposed by Fine et al. (39) and has been confirmed in human and animal models, including the diabetic kidney (96, 134). Renal hypoxia has been demonstrated in animal models of T1DM and T2DM (62, 103, 123), particularly in the outer medullary region, including the medullary TAL (121, 123). Hypoxia can be due to enhanced tubular oxygen consumption, as shown by ex vivo in cortical and medullary tubular cells of STZ-diabetic rats (103). Changes in vasoactive factors, such as ANG II and/or nitric oxide (NO), affecting postglomerular blood flow, as well as glomerular lesions and interstitial vascular rarefaction, further impair peritubular blood flow and oxygen delivery to the tubules. Impairment of renal function and concurrent anemia (13) and enhanced sodium transport load and oxygen consumption in remnant nephrons (97) can further enhance hypoxia (Fig. 5).

Defense against hypoxia involves the hypoxia-inducible factor (HIF), which induces various genes [e.g., erythropoietin, VEGF, hemoxygenase 1 (HO-1)] that can help to protect
Hypoxia has been implicated as a cause of oxidative stress in the diabetic kidney and in the pathophysiology of diabetic nephropathy (134). Most of the ROS are generated during mitochondrial oxidative phosphorylation, and smaller amounts are generated via the NADPH-oxidase system (48). In general, the cells in hypoxia depend on anaerobic glycolysis to generate ATP. However, the residual oxygen supply is used for oxidative ATP production via the Krebs cycle and electron transport chain, and the hypoxic conditions promote electron leakage from the mitochondrial electron transport chain, resulting in excessive ROS generation.

Vice versa, oxidative stress can enhance hypoxia. Diabetic rats upregulate the mitochondrial expression of uncoupling protein 2 in renal proximal tubular cells (43). This will induce mitochondrial uncoupling, which can attenuate diabetes-induced oxidative stress, but the resulting increase in O2 consumption may aggravate hypoxia (Fig. 5). Furthermore, overproduction of ROS, in part, due to activation of NADPH oxidase with translocation of p47phox to the membrane, limits NO generation in the diabetic kidney (12), which enhances hypoxia by affecting the use and supply of oxygen (104). Hypoxia is enhanced through NO quenching by AGEs (25, 161) or ROS (56), and NO captured by glucose (21). Superoxide enhances the Na-K-2Cl cotransporter activity in the TAL, which can further aggravate renal hypoxia (68). In accordance, treatment of STZ-diabetic rats with the antioxidant α-tocopherol prevented diabetes-induced disturbances in oxidative stress, oxygen tension, and oxygen consumption. Notably, diabetic hypertrophy and glomerular hyperfiltration were unaffected by α-tocopherol (103).

In vitro studies linked renal hypoxia to enhanced ECM: studies in human proximal tubular epithelial cells showed that hypoxia (1% O2, 24 h) increased total collagen production, which was related to decreased matrix metalloproteinase MMP-2 activity and increased tissue inhibitor of metalloproteinase-1 protein (101). Collagen IV mRNA levels decreased, while collagen I mRNA increased, suggesting induction of interstitial collagen. Although hypoxia stimulated TGFβ production, this did not appear to mediate the profibrogenic stimulus of hypoxia (101). Superoxide activated ERK-dependent fibrosis-stimulatory factor and ECM gene transcription have been implicated in STZ-diabetic rats (83). These findings link both hypoxia and oxidative stress to the tubulointerstitial accumulation of extracellular matrix and fibrosis in the diabetic kidney.

The role of HIF has been further assessed by the use of cobalt, which inhibits HIF degradation by the oxygen sensor prolyl hydroxylase (PHD). Application of cobalt for 20 wk to spontaneous hypertensive rats SHR/NDmc-r-cp rats, a hypertensive model of T2DM, reduced proteinuria and histological kidney damage without affecting hypertension and metabolic abnormalities (99). Cobalt increased expressions of HIF-regulated genes, including erythropoietin, VEGF, and HO-1, and reduced the renal expressions of TGFβ and AGE formation (99). The toxicity of cobalt prohibits its use in humans, but small molecular activators of HIF are currently being developed, including inhibitors of PHD1, which can induce hypoxia tolerance by reprogramming basal oxygen metabolism (11), without impairing the regulation of angiogenesis mediated by PHD2 (137).

**Perspectives and Significance**

The proximal tubule plays a vital role in the pathophysiology of the diabetic kidney. We are beginning to better understand the molecular basis of the complex interactions between the diabetic milieu and the proximal tubule and tubulointerstitium. Tubular glucose uptake is important for detrimental renal effects of diabetes, as well as glucose homeostasis, and inhibition of proximal tubular glucose reabsorption via SGLT2 is a promising approach to lower blood glucose levels. Ongoing studies are assessing the long-term safety of this approach and whether these drugs can reduce negative effects of glucose on cells expressing SGLT2 and attenuate the progressive nature of diabetic nephropathy. The outlined pathophysiological concept further identifies the unique early growth phenotype of the proximal tubule as a potential target for the prevention of early tubular hyperreabsorption and glomerular hyperfiltration, but, possibly more important, as an early link to tubulointerstitial inflammation, fibrosis, oxidative stress, hypoxia, and renal failure. In this regard, a better understanding of a proposed senescent phenotype of diabetic tubular cells is necessary. Tubular senescence may not only alter the tubular phenotype and contribute to the salt paradox, another manifestation of the strong tubular control of GFR in the diabetic kidney, but also trigger inflammation. Subjects may be heterogeneous in their proximal tubular capacity to sense and respond to hyperglycemia, resulting in variable degrees and qualities of kidney growth, which may contribute to the fact that some patients develop diabetic nephropathy, while others do not. A better understanding of the molecular mechanisms triggering diabetic kidney growth and of the key elements linking these early changes to inflammation, tubulointerstitial fibrosis, oxidative stress, and hypoxia is necessary, and such knowledge may help identify new diagnostic biomarkers and therapeutic approaches. Progress in this regard will also require the use of animal models that mimic the phenotypic and molecular signature of human diabetic kidney disease (22), and the follow up of these models into the later stages of diabetic nephropathy and renal failure.

**GRANTS**

The author’s work was supported by the National Institutes of Health (R01DK56248, R01HL094728, R01DK28606, R01GM66232, P30DK079337), the American Heart Association (GRNT3440038), the Department of Veterans Affairs, Bristol-Myers Squibb, and Astra-Zeneca.
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ACKNOWLEDGMENTS

The author’s research was supported by Bristol-Myers Squibb and Astra Zeneca.

DISCLOSURES

R1018 THE PROXIMAL TUBULE IN THE DIABETIC KIDNEY


R1020 THE PROXIMAL TUBULE IN THE DIABETIC KIDNEY

Review


165. Yoon G, Kim HJ, Yoon YS, Cho H, Lim IK, Lee JH. Iron chelation-induced senescence-like growth arrest in hepatocyte cell lines: associa-


