Angiotensin II and growth factors in the pathogenesis of diabetic nephropathy

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Angiotensin II and growth factors in the pathogenesis of diabetic nephropathy. The renin-angiotensin system (RAS) and growth factors mediate structural and functional changes during the course of diabetic nephropathy (DN). Studies in humans and experimental models with DN suggest their involvement in the development and progression of DN. Activation of renal tissue RAS and increased expression of growth factors have been demonstrated at early stages of the disease. Angiotensin II and growth factors alter renal hemodynamics and exert trophic changes in renal cells that eventually result in fibrosis through direct mechanisms or through the release of other mediators. Their effects are likely modulated by metabolic changes including high glucose and free fatty acids. While blockade of the RAS ameliorates DN in humans, such evidence for blockade of growth factors is still lacking. It is likely that susceptibility to the development of DN and therapeutic efficacy are modulated by genetic polymorphisms in components of the RAS and growth factors including their receptors and other target molecules. Approaches to understand the intricate relationship between these systems and the mechanism(s) by which they alter capillary permeability and result in structural changes are areas of fruitful investigation.

There is evidence that angiotensin II (Ang II) and growth factors play a role in mediating the hemodynamic and structural/metabolic manifestations of diabetic nephropathy (DN) [1]. In humans and experimental animals with DN, angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARBs) decrease glomerular injury, by decreasing systemic blood pressure and glomerular capillary pressure [1–3]. In glomerular, tubular and interstitial cells, Ang II induces protein synthesis, hypertrophy, proliferation and matrix expansion, suggesting non-hemodynamic mechanisms of Ang II-induced injury [2, 3]. As DN is characterized by low circulating renin, activation of local renal renin-angiotensin system (RAS) has been incriminated in renal injury [2, 3]. Additionally, the biological effects of Ang II may be mediated directly or through generation of other mediators, including growth factors [1–9]. Here, we summarize certain aspects of the role of the RAS and growth factors in DN. Detailed reviews can be found elsewhere [1–9].

THE RAS IN DN

Renin secretion by juxtaglomerular cells is the rate-limiting step for the generation of circulating Ang II. However, components of RAS also are expressed in many tissues where Ang II is generated locally [10–13]. Non-ACE pathways of Ang II generation exist and include chymostatin Ang II generating enzyme (CAGE), serine proteinase chymases, tissue plasminogen activator, cathepsin G and tonin [2, 7]. In the human kidney, virtually all Ang I generation is renin-dependent, but nearly 40% of Ang I may be converted to Ang II by pathways other than ACE. The contribution of non-ACE pathways to Ang I and Ang II generation may be substantially augmented in diabetes mellitus (DM) [2, 3, 12]. Hyperglycemia, elevated free fatty acids, and decreased (type 1 DM) or increased (type 2 DM) levels of insulin may regulate RAS in the kidney. However, direct evidence for activation of local RAS in DN is still lacking [2, 3]. ACE immunostaining is increased in glomeruli and may be decreased in proximal tubules in advanced DN. The effects of Ang II in DN are mediated via activation of two of its receptors, AT1 and AT2, the former being the predominant form. Renal expression of both receptors is down-regulated in DN [6]. However, the enhanced sensitivity to Ang II or to Ang II blockade in DN suggests that AT1-mediated signaling is regulated by other mediators such as nitric oxide (NO) [6]. Differential regulation of renal AT1 receptor expression by Ang II is cell-specific. Whereas in mesangial cells Ang II decreases mRNA and protein levels of AT1 receptors, opposite effects have been reported in proximal tubular epithelial cells [6, 14]. AT1 receptors in mesangial cells mediate cell contraction, increased production and decreased degradation of extracellular matrix (ECM), hy-
pertrophy, and increased production of growth factors. In podocytes, AT1 receptors may induce contraction of foot-processes and regulate glomerular filtration [6]. AT1 receptors promote apoptosis in mouse fibroblasts as well as production of the chemokine RANTES in rat glomerular endothelial cells [5, 6], suggesting a role of glomerular inflammatory cell infiltration in diabetes. In proximal tubular epithelial cells, activation of AT1 receptors promotes protein synthesis, contributing to hypertrophy and interstitial matrix expansion.

There is indirect evidence that Ang II alters the protein assembly of the podocyte slit diaphragm thereby regulating the glomerular capillary permselectivity. However, the role of slit diaphragm/podocyte proteins in DN has not been fully explored. In rats with spontaneous proteinuria, zonula occludens-1 (ZO-1), an important constituent of the podocyte foot process, is redistributed from its normal membrane location to the cytoplasm. Administration of ACEI prevents both glomerular redistribution of ZO-1 and proteinuria, suggesting a role for Ang II in regulation of glomerular permselectivity [15]. Moreover, diabetic SHR develop a deficiency of nephrin, which is restored by the use of the ARB irbesartan with attenuation of albuminuria [16]. In another model of nephrosis, progressive renal injury was associated with down-regulation of nephrin, which was totally prevented both by an ACEI and an ARB, suggesting that Ang II plays a role in modulating nephrin expression [17]. Furthermore, in an experimental model of DN, there is a decrease in the number of slit pores per unit length of glomerular basement membrane, indicative of podocyte foot-process broadening. Both an ACEI and an ARB attenuated these ultrastructural changes, suggesting that preservation of podocyte architecture could contribute to the renoprotective effects of blockade of the RAS in DN [18].

The mechanism by which the diabetic environment modulates the responses to Ang II remains largely unexplored. The hyperglycemic environment may potentiate the trophic effects of Ang II, likely via additive effects on protein kinase C (PKC) activation and expression of transforming growth factor β (TGF-β) [5, 6]. Ang II and high glucose have costimulatory actions on platelet-derived growth factor (PDGF) and TGF-β production, as well as on the accumulation of extracellular matrix components. Ang II and high glucose also inhibit intracellular proteinases in proximal tubules and nitric oxide synthase (NOS) expression [5]. In vascular smooth muscle cells, high glucose concentrations augment Ang II-induced JAK/STAT and ERK1/2 type mitogen-activated protein kinase activation [19]. In addition, there is a growing body of evidence suggesting that Ang II-induced generation of reactive oxygen species play a critical role in the hypertrophy and the matrix accumulation associated with DN. In mesangial cells, Ang II activates the hypertrophic serine-threonine kinase Akt/protein kinase B via a receptor-dependent pathway [20]. Early DN in rats is associated with augmented renal expression of p47^phox, a subunit of superoxide-generating NAD(P)H oxidase, as well as of the endothelial NOS (eNOS), and increased indices of systemic and renal oxidative/nitrosative stress. Importantly, these changes can be prevented with an ACEI or an ARB, indicating a role for Ang II-induced oxidative stress [21]. Inhibition of oxidative stress by compounds that block RAS may mask potential protective effects of antioxidants. The role of novel non-phagocytic NAD(P)H oxidases remains unexplored.

**GROWTH FACTORS**

Expression of several growth factors and cytokines is increased in DN and the biological activity of growth factors may be altered in diabetes. Renal TGF-β expression is increased in both type 1 and type 2 diabetes [8, 22]. Putative factors that regulate TGF-β expression include hyperglycemia, advanced glycated end-products (AGEs), mechanical stretch, Ang II, endothelin, lipids and products of oxidative stress [1]. High glucose concentration increases TGF-β1 expression and activity in mesangial and proximal tubular epithelial cells that is dependent on local RAS activation [4, 14]. TGF-β is a potent fibrogenic factor that modulates synthesis of both matrix proteins [5] and their receptors such as integrins and osteopontin. TGF-β also contributes to the apoptosis of podocytes. In db/db mice treated chronically with anti-TGF-β antibodies, renal function and structure are preserved but proteinuria persists [23]. Thus, at least in this model, it appears that TGF-β contributes to the structural changes but not to the increased glomerular permeability.

Connective tissue growth factor (CTGF) is another pro-sclerotic cytokine with increased renal and glomerular expression in DN. Synthesis of CTGF is stimulated by TGF-β, hyperglycemia, AGEs, mechanical stretch, and CTGF itself [1]. Vascular endothelial growth factor (VEGF) is a cytokine highly expressed in the kidney, primarily in podocytes but also in distal tubules and collecting ducts, with its major receptor, type 2 VEGF receptor, expressed on endothelial cells and cortical fibroblasts [24]. VEGF may contribute to the hemodynamic events in DN as anti-VEGF antibodies attenuate albuminuria and hyperfiltration [25]. Preliminary observations show that VEGF is augmented in renal cortex of mice in early stages of type 1 or type 2 DM, coinciding with hypertrophy (abstract; Senthil et al, *J Am Soc Nephrol* 12:566A–567A, 2001). Another growth factor, insulin-like growth factor 1 (IGF-1), may play an important role in the early development of renal hypertrophy in DN. Octreotide, an inhibitor of somatostatin, reduces kidney IGF-I expression and ameliorates renal hypertrophy and albuminuria [26]. Growth hormone may contribute directly or via IGF-1 to the renal hypertrophy in DN,
Much work is needed to elucidate molecular mechanisms by which these mediators regulate structure and function of renal cells in diabetes.

The observation that many patients with DN continue to progress to end-stage renal disease despite therapy including blockade of the RAS is a rationale to continue to explore the role of Ang II and growth factors in diabetic renal disease. Growth factor expression is not exclusively dependent on the RAS. Moreover, current drug dosage regimens use blood pressure control as the target, and there is poor documentation of blockade of tissue RAS. The regulation of renal tissue RAS components in diabetes remains largely unexplored. Mice deficient in tissue ACE or other components of the RAS may provide useful tools to explore the role of tissue RAS in end organ damage that complicates diabetes. Moreover, the role of a novel ACE homolog and biologically active metabolites of Ang I in diabetic end organ complications awaits investigation. Translational studies in humans to block deleterious effects of growth factors need to be explored, after thorough understanding of signaling molecules that mediate these effects. This will facilitate development of selective and safe inhibitors. There is genetic heterogeneity not only in the susceptibility to AGEs and oxidative stress DN, but also in responsiveness to drugs that block the RAS. Reproducible studies to explore genetic polymorphisms in components of the RAS, growth factors and their receptors are needed.

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FUTURE PERSPECTIVES

Disparate pathogenetic events that span the spectrum of hemodynamic, metabolic and genetic factors appear to be regulated by Ang II and growth factors (Fig. 1).