The Renin-Angiotensin System and Diabetic Nephropathy

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Summary: The renin-angiotensin system (RAS) has key regulatory functions for blood pressure and fluid homeostasis. In addition, dysregulation of the system can have maladaptive effects to promote tissue injury in chronic diseases such as hypertension, heart failure, and kidney disease. These actions for the RAS to promote disease pathogenesis are especially apparent in diabetic nephropathy, the most common cause of end-stage renal disease in the United States. Evidence of a role for the RAS in diabetic nephropathy comes from studies in animal models and randomized clinical trials showing efficacy of angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers to slow the progression of renal disease. Widespread applications of these therapies to a range of renal diseases may have contributed to the recent reduction in the incidence rates for end-stage renal disease. We provide a general review of the RAS and its role in diabetic nephropathy.
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The renin-angiotensin system (RAS) is a multistep enzymatic cascade (Fig. 1) with potent effects to control blood pressure and fluid homeostasis. Angiotensinogen is the major substrate of this enzymatic cascade, serving as the source of all angiotensin peptides. Angiotensinogen in the circulation is synthesized primarily in the liver, but angiotensinogen also is expressed in a number of other tissues including the kidney. Renin specifically cleaves the 10 amino acids from the N-terminus of angiotensinogen to form angiotensin I. A substantial excess of angiotensinogen is present in serum and angiotensin-converting enzyme (ACE) is ubiquitous in the endothelium and plasma.[1] Accordingly, the amount of renin in the blood stream is a key rate-limiting step determining the level of angiotensin II and thus the activity of the system. The primary source of renin in the circulation is the kidney, where its expression and secretion are regulated tightly at the juxtaglomerular apparatus by 2 distinct mechanisms: a renal baroreceptor[2,3] and sodium chloride delivery to the macula densa.[4-6] Through these sensing mechanisms, levels of renin in plasma can be titrated incrementally in response to changes in blood pressure and salt balance.

The final step in the classical RAS pathway is conversion of angiotensin I to angiotensin II by ACE, which is the molecular target of ACE inhibitors. Somatic ACE is expressed as an ectoenzyme on the surface of endothelial cells throughout the body and is particularly abundant in the lung, intestine, choroid plexus, placenta, and on brush-border membranes in the kidney. A soluble form of ACE that circulates in plasma is formed by enzymatic cleavage of tissue-bound ACE at its transmembrane domain.[7] In addition to angiotensin I, other biologically active peptides are substrates for ACE. Perhaps the most important of these is bradykinin.[8] It has been shown that inhibition of the kininase actions of ACE contributes to the hemodynamic actions of ACE inhibitors.[9] Moreover, as discussed later, the actions of ACE to influence the

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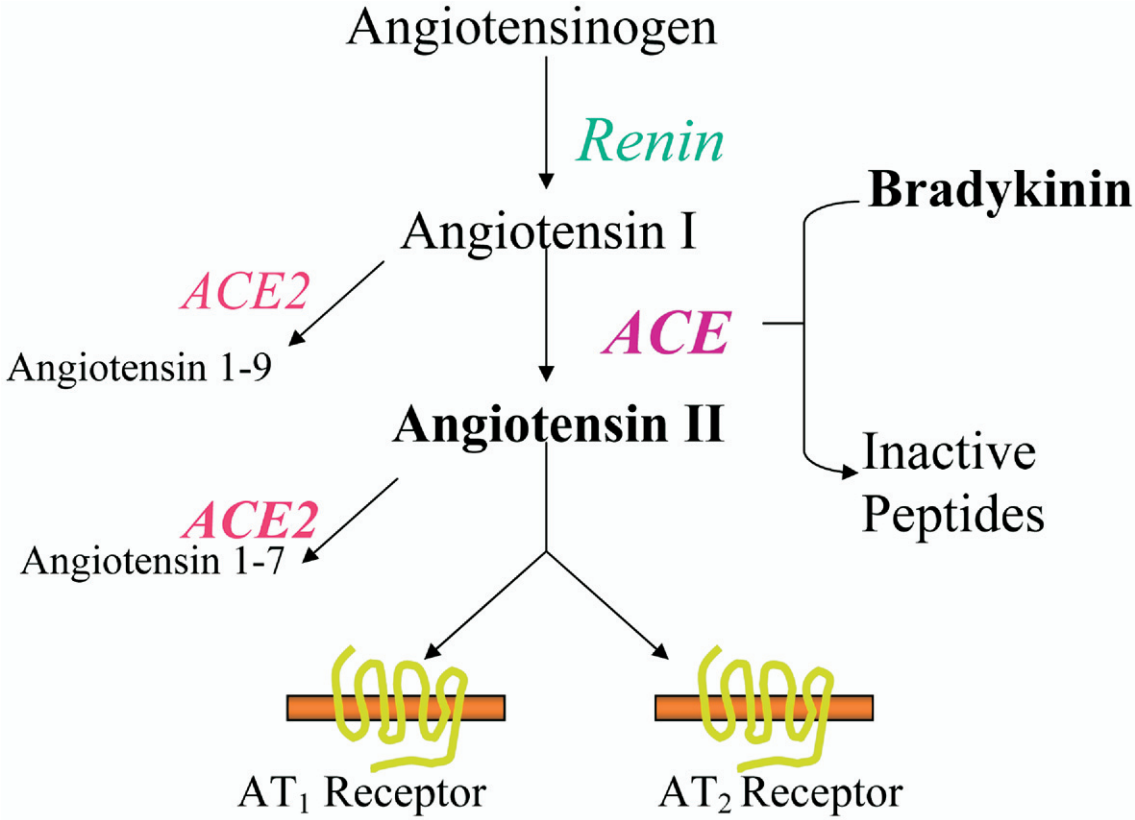

Figure 1. The RAS. Angiotensinogen is cleaved by renin to form angiotensin I, which then is cleaved by ACE to form angiotensin II. The effects of angiotensin II are mediated by specific cell surface receptors, AT_1 and AT_2, which exist in the kidney, adrenal glands, heart, and vascular smooth muscle. ACE also degrades bradykinin into inactive peptide fragments. ACE2 is a recently identified homolog of ACE that cleaves both angiotensin I and angiotensin II to form angiotensin 1-9 and angiotensin 1-7, respectively.

bradykinin system may affect susceptibility to kidney injury in diabetes.

Angiotensin II exerts its biological effects through specific cell-surface receptors, members of the 7-transmembrane family of receptors. The angiotensin receptors can be divided into 2 pharmacologic classes: type 1 (AT_1) and type 2 (AT_2), based on their differential affinities for various nonpeptide antagonists. Studies using these antagonists suggested that most of the classically recognized functions of the RAS are mediated by AT_1 receptors.[10] Gene targeting studies have confirmed these conclusions.[11] In contrast, the AT_2 receptor seems to function as a negative modulator of AT_1 receptor actions.[12-14] As discussed later, studies in animal models and clinical trials have suggested that the RAS promotes renal injury through the pathway of angiotensin II acting via AT_1 receptors.

RAS PROMOTES KIDNEY DISEASE IN ANIMAL MODELS OF DIABETES

A role for the RAS in diabetic nephropathy first was established clearly in a series of experiments from the Brenner laboratory during the 1980s in a rat model of diabetes induced by streptozotocin (STZ). Increased glomerular pressure was a consistent feature of this model, which was observed early in the course of diabetes and was associated with a relative increase in efferent arteriolar resistance compared with the afferent arteriole.[15] These changes in glomerular hemodynamics were associated with the development of proteinuria and pathologic features resembling human diabetic nephropathy including glomerulosclerosis.[16]

Because exaggerated efferent arteriolar constriction with increased glomerular pressure is

a characteristic hemodynamic footprint of angiotensin II in the glomerular microcirculation, it was suggested that this abnormal glomerular hemodynamic profile in diabetic rats might result from activation of the RAS.[16-18] To test this hypothesis, Zatz et al[17] performed experiments examining the effects of ACE inhibition on glomerular hemodynamics, proteinuria, and renal pathology in rats with STZ-induced diabetes. Enalapril treatment normalized glomerular pressures and reduced albumin excretion to levels similar to nondiabetic controls. Moreover, the development of glomerular sclerosis largely was prevented in the diabetic rats treated with enalapril. These studies suggest that diabetes stimulates the RAS, causing activation of AT_1 receptors by angiotensin II, producing an increase in glomerular hydrostatic pressure, proteinuria, and structural injury with sclerosis and fibrosis. The rat STZ model used in these studies falls short of reproducing many of the key features of human diabetic nephropathy including nodular glomerular sclerosis and end-stage renal disease. Nonetheless, findings in this model were sufficiently compelling to support clinical trials of RAS antagonists in human beings with diabetic nephropathy. As we discuss later, the results of the human trials closely paralleled the rat studies, showing dramatic beneficial effects of RAS inhibition to slow progression of renal damage in diabetic patients.

Brenner and associates concluded that glomerular hypertension in diabetic nephropathy had a critical causal role in the pathogenesis of kidney injury. Subsequently, it has been suggested that other nonhemodynamic actions of angiotensin II also might contribute to nephropathy pathogenesis. For example, activation of AT_1 receptors can trigger expression and release a range of proinflammatory and profibrotic mediators implicated in the progression of chronic kidney diseases. Most prominent among these is transforming growth factor-β (TGF-β). Activation of AT_1 receptors in a number of cell types leads to downstream activation of TGF- β expression. As discussed in the article by Zhu et al in this issue, TGF-β has multiple actions that have been shown to contribute to renal fibrosis and injury in diabetic nephropathy.[19-23] However, irrespective of the precise mechanism(s) by which RAS activation promotes kidney injury in diabetic nephropathy, the groundbreaking studies of Brenner et al provided a cogent rationale for using RAS inhibitors in patients with diabetic kidney disease.

ROLE FOR THE RAS IN DIABETIC NEPHROPATHY IN HUMAN BEINGS

Based on data from animal studies described previously, a series of randomized clinical trials were performed to evaluate the efficacy of ACE inhibitors and angiotensin-receptor blockers (ARBs) in patients with diabetic nephropathy. In aggregate, these studies provide compelling evidence for a key role of the RAS in the pathogenesis of diabetic nephropathy in human beings. In 1993, Lewis et al[24] compared the utility of captopril with standard antihypertensive therapy in type I diabetic patients with overt nephropathy characterized by reduced glomerular filtration rate and macroalbuminuria. Patients were treated to a blood pressure target of 140/90 mm Hg, although the achieved blood pressures were slightly, but significantly, lower in the captopril group. After an average of approximately 3 years of follow-up evaluation, treatment with captopril was associated with a dramatic and significant reduction in patients reaching the combined end point of death, initiation of dialysis, or transplantation.

Similar beneficial effects of ARBs subsequently were shown in patients with type II diabetes. Specifically, 2 large, prospective, randomized trials clearly showed that AT_1 receptor blockade significantly delays progression of renal disease in patients with type II diabetes and overt nephropathy. Published together, the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL)[25] trial and the Irbesartan Type 2 Diabetic Nephropathy Trial (IDNT)[26] both examined the efficacy of angiotensin-receptor blockade on the combined outcome of doubling of the baseline serum creatinine level, development of end-stage renal disease, and death. Both studies included a control group of patients treated with a conventional antihypertensive regimen not including ACE inhibitors or ARBs, and IDNT included a third arm in which the primary antihypertensive agent was the calcium-channel

blocker amlodipine. In both studies, antihypertensive therapy was adjusted to achieve a blood pressure goal of 140/90 mm Hg. The outcomes of the studies were virtually identical. That is, treatment with an ARB significantly reduced the occurrence of the primary end points. In IDNT, outcomes in the group treated with the ARB were also superior to the amlodipine-based regimen.

Taken together, data from these randomized prospective clinical trials provide strong evidence indicating that angiotensin II, acting through the AT_1 receptor, is a key pathway promoting progressive renal damage in patients with diabetic nephropathy. Other studies suggest a role for this pathway in incipient nephropathy. For example, Parving et al[27] showed that ARB treatment in patients with type II diabetes and microalbuminuria, but without other evidence of renal disease, prevented the development of overt nephropathy. As a consequence of the findings from this series of compelling clinical trials, the use of RAS antagonists has become the standard of care in diabetic patients with kidney disease and, largely by inference, in patients with chronic kidney disease of almost any cause. The widespread use of these agents has been suggested to be a major contributor to the recently observed slowing in the prevalence of end-stage renal disease in the United States.[28]

VARIANTS IN RAS GENES AND SUSCEPTIBILITY TO DIABETIC NEPHROPATHY

Clinical and epidemiologic studies have shown a clear genetic component for predisposition to developing diabetic nephropathy.[29-31] Although this has been an active area of investigation, the basis for genetic susceptibility to diabetic nephropathy remains poorly understood. One genetic polymorphism that has been associated with increased risk for nephropathy in patients with type I diabetes is the *D* allele of the *ACE* gene.[32] Because the *D* allele is associated with increased levels of ACE, it was hypothesized that the resulting high levels of ACE would enhance angiotensin II production, predisposing the kidney to nephropathy. To test this hypothesis, Huang et al[33] assessed the relationship between *Ace* gene copy number and renal complications in diabetes using genetically engineered mice carrying 1, 2, or 3 copies of the *Ace* gene. After induction of diabetes with STZ, urinary albumin excretion was significantly worse in the mice with 3 copies of the ACE gene compared with wild-type controls. Moreover, there was a significant correlation between plasma ACE levels and urinary albumin excretion ($r = .89$; $P < .01$). The higher levels of ACE in the 3-copy mice, which did not affect blood pressure at baseline, also caused blood pressure to increase in the presence of diabetes. These findings in the mouse model were consistent with the genetic association studies in human beings and suggested that increased levels of ACE played a causal role to increase the risk for diabetic nephropathy.

Other investigations raised questions, however, about whether the effects of increased ACE levels to promote kidney disease were related to enhanced production of angiotensin II, or other properties of ACE. For example, a series of computer simulations performed by Smithies et al suggested that increasing the levels of ACE would have very little impact on circulating angiotensin II levels.[34] Accordingly, these investigators hypothesized that the consequences of increased ACE levels to enhance the severity of diabetic nephropathy might relate to other properties of ACE, such as its actions as a kininase. To test this hypothesis, they studied the consequences of diabetes on kidney structure and function in animals lacking the bradykinin B2 receptor.[35] Generated by crossing with a genetic model of type I diabetes, the Akita mouse, diabetic B2 receptor–deficient mice had enlarged kidneys, significant albuminuria, and mesangial matrix expansion with glomerular sclerosis. The extent of these functional and pathologic abnormalities was significantly worse than diabetic animals with normal B2 bradykinin receptor expression. These studies identified a previously unappreciated role for the bradykinin system to protect against diabetic renal injury. Moreover, they indicated a critical role for the kininase actions of ACE to modulate renal pathology in diabetes and suggest that one of the beneficial consequences of ACE inhibition in diabetic nephrop-

athy is to enhance the activity of bradykinin at its B2 receptor.[35]

ACE2 AND DIABETIC NEPHROPATHY

By using genome-based strategies, homologues of ACE have been identified recently.[36,37] One of these, ACE2, shows more than 40% identity at the protein level with the catalytic domain of ACE.[36,37] Similar to ACE, ACE2 is expressed on the surface of certain endothelial cell populations. However, compared with the ubiquitous distribution of ACE, the expression pattern of ACE2 is more limited with the most abundant expression in the kidney, followed by the heart and testis.[36,37] Their substrate specificities also differ; the monocarboxypeptidase ACE2 hydrolyzes angiotensin II with high efficiency, but has much lower activity against angiotensin I.[36,38] Hydrolysis of angiotensin II by ACE2 generates another peptide with putative biological actions: angiotensin 1-7.[38] Accumulating evidence indicates that this peptide causes vasodilation, natriuresis, and may promote reduced blood pressures[39] via the Mas receptor.[40] It further has been suggested that ACE2 may be a major pathway for the synthesis of angiotensin 1-7.[41] Thus, the functions of ACE2 may be determined by its distinct actions to metabolize angiotensin II and to generate angiotensin 1-7.

Although the precise physiologic role of ACE2 is not clear, it originally was identified and cloned from a complementary DNA library prepared from ventricular tissue of a patient with heart failure.[36] Studies in ACE2-deficient mice have suggested a role for ACE2 in cardiac function[42,43] and in blood pressure regulation.[44] A third member of the ACE gene family, collectrin, was identified as a gene that is up-regulated in the subtotal nephrectomy model of chronic kidney disease.[45] Collectrin is highly homologous to the transmembrane portion of ACE2, but lacks the carboxypeptidase domain. Its physiologic functions have not yet been defined clearly, but it appears to have an essential role in renal amino acid transport.[46]

Because of its ability to modulate angiotensin peptide levels, there have been a series of studies addressing potential roles for ACE2 in the pathogenesis of diabetic nephropathy. For example, Tikellis et al[47] examined the expression of ACE2 in the kidney in diabetic nephropathy.[47] In the normal kidney, ACE2 protein and messenger RNA levels are highly expressed in renal tubules. However, these levels were decreased significantly in STZ diabetic rats. By contrast, ACE2 levels in the glomerulus were increased significantly in the diabetic kidney as assessed by in situ hybridization and immunohistochemistry.[47] These studies suggested that there may be complex modulation of ACE2 expression in diabetes. ACE2 expression also has been examined in a model of type II diabetes: the *db/db* mouse. In studies of young diabetic (*db/db*) mice, Ye et al[48] reported increased expression of ACE2 in the kidney that was accompanied by reduced expression of ACE. These investigators suggested that this pattern might act to protect the kidney during the early stages of diabetes. Batlle et al[49] also studied ACE2 expression in *db/db* and STZ-treated mice by using a novel fluorometric method to determine ACE and ACE2 activity concurrently in tissue samples. They also found increased ACE2 and decreased ACE activity in renal cortex from diabetic mice. Taken together, these studies suggest that expression of ACE2 may be modulated in diabetes. However, a role for ACE2 in the pathogenesis of diabetic nephropathy has not yet been established clearly.

RAS IN DIABETIC NEPHROPATHY: UNANSWERED QUESTIONS

Actions of the RAS to promote the pathogenesis of diabetic nephropathy were identified more than 2 decades ago. Nonetheless, a number of unanswered questions remain. One major question relates to the nature of RAS activation in diabetes. The most powerful evidence supporting the role of the RAS in diabetic nephropathy comes from studies in patients and animal models showing functional benefits of pharmacologic inhibitors of the RAS to ameliorate disease. However, in these same patients groups or animals models it is difficult to detect clear evidence of systemic activation of the RAS. For example, in patients with diabetes, plasma renin activity typically is normal or suppressed.[50-53] This has led to speculation that in diabetes, and perhaps other renal diseases,

there may be local activation of the RAS with enhanced levels of angiotensin II or exaggeration of its actions confined to key tissue compartments.[54,55] Such local activation may occur without detectable effects on the systemic RAS or plasma renin levels, but nonetheless is sufficient to cause pathology. Although this hypothesis first was articulated a number of years ago, direct scientific evidence for local activation of the RAS in kidney diseases is sorely lacking. Assuming that activation of the RAS occurs at some level during diabetes, another question relates as to which constituents of the diabetic milieu are responsible for activating the RAS. Because RAS-dependent kidney injury occurs in both type I and type II diabetes, insulin deficiency per se does not seem to provide a unifying mechanism here.

The efficacy of RAS antagonists to slow the relentless progression of kidney injury in patients with diabetic nephropathy is undeniable. However, the mechanisms of these beneficial actions also remain a matter of debate and a fertile area for investigation. Although direct measurements of glomerular hydrostatic pressure in human beings is not possible, studies using indirect assessments in clearance studies have shown an increased glomerular filtration rate in patients with early diabetes along with increased filtration fractions.[52] However, as in the studies in diabetic rats, a causal relationship between reduced glomerular pressures and renoprotection by RAS antagonists in patients with diabetic nephropathy has been difficult to prove directly. ACE inhibitors and ARBs also have potent actions to reduce proteinuria in patients with overt nephropathy. In clinical trials such as the RENAAL and the IDNT, high-grade proteinuria confers a poor prognosis for renal survival[25,26] and it has been suggested that proteinuria itself may have direct effects to promote tubulointerstitial injury and progressive loss of renal function. By this line of reasoning, the reduction of proteinuria associated with ACE inhibitors and ARBs may be a mechanism of renoprotection. Once again, direct evidence for this hypothesis is lacking. Furthermore, the precise mechanism by which RAS antagonism reduces proteinuria is not at all clear. It has been suggested that this may be related to reduced glomerular pressures per se,[16,56] preventing angiotensin (ang) II–dependent contraction of the mesangium,[57,58] or blockade of AT_1 receptor activation on glomerular epithelial cells.[59-62]

An alternative hypothesis is that the major benefit of RAS antagonists on the progression of nephropathy is primarily owing to improved control of systemic blood pressure. As mentioned previously, the groups treated with ACE inhibitor or ARB have lower blood pressures than the comparator groups in almost all of the diabetic nephropathy trials. Although these differences in blood pressure are quite small, typically less than 5 mm Hg, it long has been recognized that hypertension is an independent risk factor for the progression of kidney disease in diabetic nephropathy, and in epidemiologic studies, small differences in blood pressure can have a major impact on cardiovascular risk. Furthermore, studies using ambulatory blood pressure monitoring have suggested that RAS blockade can have profound effects to reduce blood pressures over 24 hours that may not be reflected in a single reading taken in the clinic.[63] Moreover, studies by Griffin and Bidani[64] in rat models of kidney disease also support the idea that the reduction of systemic blood pressure constitutes the major mechanism of renoprotection by RAS antagonists.

Questions about the role of the RAS in the pathogenesis of diabetic nephropathy have been lingering for years. Newer technologies for engineering of the mouse genome would seem to provide powerful tools for directly addressing some of these questions. As just one example, the ability to manipulate components of the RAS with rigorous temporal and spatial control within the kidney should provide insights into how and where the RAS is activated in diabetes. Yet there is one persistent obstacle: the lack of a good mouse model of diabetic nephropathy recapitulating the major features of diabetic nephropathy in human beings, namely robust proteinuria, nodular glomerulosclerosis, and progressive loss of renal function. Recent progress in this area, including work by the Animal Models of Diabetic Complications Consortium[65] (http://www.amdcc.org), has provided hope that a good murine model of

human diabetic nephropathy might be achievable. By combining new improved disease models with incisive genetic approaches, the mechanisms of kidney damage caused by RAS activation might be resolved at the molecular level. Such new insights would provide great promise for improving therapies in diabetic nephropathy.

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