

Renin-Angiotensin-Aldosterone System and Progression of Renal Disease

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Inhibition of the renin-angiotensin-aldosterone system (RAAS) is one of the most powerful maneuvers to slow progression of renal disease. Angiotensin II (AngII) has emerged in the past decade as a multifunctional cytokine that exhibits many nonhemodynamic properties, such as acting as a growth factor and profibrogenic cytokine, and even having proinflammatory properties. Many of these deleterious functions are mediated by other factors, such as TGF- β and chemoattractants that are induced in the kidney by AngII. Moreover, understanding of the RAAS has become much more complex in recent years with the identification of novel peptides (e.g., AngIV) that could bind to specific receptors, elucidating deleterious effects, and non-angiotensin-converting enzyme (ACE)-mediated generation of AngII. The ability of renal cells to produce AngII in a concentration that is much higher than what is found in the systemic circulation and the observation that aldosterone may be engaged directly in profibrogenic processes independent of hypertension have added to the complexity of the RAAS. Even renin has now been identified to have a "life on its own" and mediates profibrotic effects *via* binding to specific receptors. Finally, drugs that are used to block the RAAS, such as ACE inhibitors or certain AngII type 1 receptor antagonists, may have properties on cells independent of AngII (ACE inhibitor-mediated outside-inside signaling and peroxisome proliferator-activated receptor- γ stimulatory effects of certain sartanes). Although blockade of the RAAS with ACE inhibitors, AngII type 1 receptor antagonists, or the combination of both should be part of every strategy to slow progression of renal disease, a better understanding of the novel aspects of the RAAS should contribute to the development of innovative strategies not only to completely halt progression but also to induce regression of human renal disease.

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One of the major challenges in today's nephrology is the increasing number of patients with ESRD. Moreover, it has been appreciated more recently that patients with already relatively minor impairment of renal functions have an increased risk for cardiovascular diseases, long before ESRD is reached. Therefore, a better understanding of the pathophysiology of chronic renal disease is mandatory to develop strategies to prevent the progression of renal disease or even to restore renal function by inducing regression (1). Regardless of the primary entity, progression of renal disease is characterized by pathomorphologic changes that comprise early renal inflammation, followed by subsequent tubulointerstitial fibrosis, tubular atrophy, and glomerulosclerosis (1). The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in many of the pathophysiological changes that lead to progression of renal disease. This article highlights some of the more recent molecular insights into the complexity of the RAAS and its role in chronic renal disease. Because of space restriction, the many clinical studies that deal with the RAAS are not covered, and the reader is referred to excellent reviews (2,3).

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How the RAAS Was Seen in the Past

Traditionally, the RAAS was considered as an endocrine system with angiotensinogen, produced in the liver, that is cleaved by renin released from renal juxtaglomerular cells (4). By this way, angiotensin I (AngI) is generated, which, in turn, is further cleaved by angiotensin-converting enzyme (ACE) activity of the lungs into the active form of AngII. AngII then binds to specific receptors in adrenal cortex, resulting in release of aldosterone. In this classical view, the cardinal function of the RAAS is maintaining of BP by AngII-induced vasoconstriction and aldosterone-mediated sodium retention in the collecting duct (4). However, the RAAS has become complex in recent years, and novel components of this network have been identified. Figure 1 provides an overview of our current understanding.

Generation of AngII and Other Peptides

The most widely known enzyme that is capable of AngII formation is ACE, but it is not the only one. Other AngII-generating enzymes include the serine protease chymase, which is supposed to mediate >80% of AngII formation in the heart and >60% in the vessels (5). ACE inhibitors do not reduce chymase activity. Upregulation of chymase, mainly in the tubules, is observed in renal biopsies of patients with diabetic nephropathy (5). These findings indicate that under pathologic

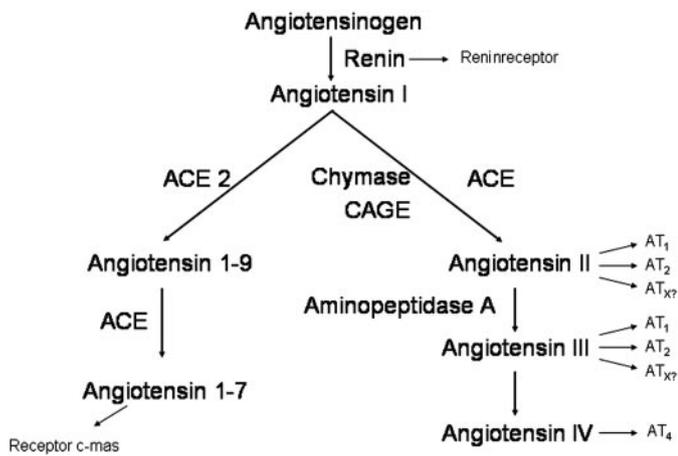


Figure 1. Overview of the renin-angiotensin-aldosterone system (RAAS). The system has become increasingly complex with alternative ways of angiotensin II (AngII) formation besides angiotensin-converting enzyme (ACE; [Chymase, chymostatin-sensitive AngII-generating enzyme [CAGE]], a second form of ACE (ACE2), and novel peptides such as AngIV, angiotensin 1-9, and angiotensin 1-7. Clinically important could be that AngII can bind to AngII type 2 (AT₂) receptors and AngIV to AT₄ receptors that are not antagonized by sartanes, inducing proinflammatory and profibrotic effects (*e.g.*, induction of chemokines, stimulation of plasminogen activator inhibitor-1).

conditions, an upregulation of chymase occurs and increased local AngII generation cannot be attenuated by ACE inhibitors. Mechanical stress of podocytes stimulates local AngII synthesis by non-ACE pathways that presumably involve chymase (6), yet the exact role of chymase-mediated AngII formation for renal disease in humans is unclear, and ACE inhibitors clearly slow progression of disease.

A novel enzyme similar to ACE, called angiotensin-converting enzyme 2 (ACE2), has been identified (7). ACE2 is expressed predominantly in vascular endothelial cells, including those of the kidney (8). In contrast to the “classic” ACE, which converts AngI to the octapeptide AngII, ACE2 cleaves one amino acid less from AngI so that in a first step, angiotensin 1-9 is formed (7). Angiotensin 1-9 is thought to potentiate AngII-mediated vasoconstriction on isolated rat aortic rings and to have vasodepressor effects in conscious rats. It also was found that angiotensin 1-9 augments bradykinin action on its B2 receptor probably by inducing conformational changes (9). In a second step, angiotensin 1-9 can be converted to angiotensin 1-7 by the “classic” ACE. A major pathway of angiotensin 1-7 degradation’s converting the peptide into inactive fragments is mediated by ACE itself. Angiotensin 1-7 is known to act as a vasodepressor agent and is involved in apoptosis and growth arrest. The protein product of the c-mas gene is a receptor for angiotensin 1-7. Further experimental studies show anti-inflammatory and antifibrotic effects of angiotensin 1-7. The local expression of ACE2 correlates closely with the concentration of angiotensin 1-7 and leads to an, at least partial, antagonism of AngII. Thus, ACE inhibition can lead to increased angiotensin 1-7 levels while reducing AngII in parallel (9). However, one

should be aware that the majority of data for angiotensin 1-7 stems from the cardiovascular system, and the relevance for renal disease is unclear.

AngII is metabolized by peptidases, such as aminopeptidase A, into AngIII and further into AngIV (10). AngIII interacts with AngII type 1 (AT₁) and AT₂ receptors, albeit with a lower affinity, and exhibits principally similar effects as AngII. AngIV binds to a specific receptor called AT₄, which is widely expressed in the kidney, including endothelial cells and proximal as well as convoluted tubules. The AT₄ receptor, by protein purification and peptide sequencing, has been identified to be insulin-regulated aminopeptidase (11).

AngII Receptors

The multiple effects of AngII are mediated by different receptors. The two major AngII receptors, AT₁ and AT₂, are differentially expressed within in the kidney (12). Both are characterized by the configuration of a seven-transmembrane receptor but share only approximately 30% homology on the protein level. AT₁ receptors are coupled to heterotrimeric G proteins and mediate different, mainly second-messenger signal transduction pathways, such as activation of phospholipases, inhibition of adenylate cyclase, stimulation of tyrosine phosphorylation, extracellular signal-regulated kinases 1 and 2, the phosphatidylinositol 3-kinase-dependent kinase Akt, and the mammalian target of rapamycin/S6 kinase pathway (12). AT₁ receptor activation also stimulates release of reactive oxygen species by a mechanism that involves activation of the membrane-bound NAD(P)H oxidase.

Lautrette *et al.* (13) recently described a novel mechanisms by which AngII transactivates the EGF receptor during renal injury in a model of chronic AngII infusion over 2 mo. They found that AngII induced secretion of TGF- α that binds to and activates the EGF receptor, explaining how AngII through transactivation of the EGF receptor could exhibit tyrosine kinase activity (13).

AT₂ receptors are involved in an increase in intracellular protein phosphatase activity (14). The number of AT₁ and AT₂ receptors is developmentally regulated, and during maturation of the kidney, AT₁ receptor expression becomes more abundant.

AT₁ receptor expression is upregulated by various stimuli, such as hypercholesterolemia or a change in osmolarity, but is suppressed by high AngII concentrations or glitazones (12). N-acetylcysteine, an antioxidant that reduces disulfide bonds, decreased AngII binding to AT₁ receptors (15). AT₂ receptors are not suppressed by AngII, but, interesting, they are upregulated in injured tissue and during inflammation (14). In particular, AT₂ receptors are re-expressed in the kidney during renal injury and remodeling nephrons. The ability of AT₁ receptors to form homodimers as well as heterodimers with other receptors such as the bradykinin receptor results in a significant acceleration of signal transduction activity after AngII stimulation (16).

Almost all AngII-induced physiologic and pathophysiologic functions, such as vasoconstriction, aldosterone release, stimulation of tubular transport, proinflammatory effects, and profi-

brogenic and growth stimulatory actions, are mediated by AT_1 receptors (12). The role of AT_2 receptors seems less clear. Activation of AT_2 receptors leads to a decrease in BP through release of nitric oxide, inhibits growth and induces differentiation, and also is involved in mediation of apoptosis (14). Recent evidence suggests that activation of NF- κ B, an important proinflammatory transcription factor, is mediated by AT_1 and AT_2 receptors. The question of which pathophysiologic effects are mediated through the AT_2 receptor is of clinical relevance because not all important pathophysiologic functions of AngII may be antagonized by AT_1 receptor blockers.

Agonistic antibodies against AT_1 receptors have been identified in pregnant women with preeclampsia and in patients with secondary malignant hypertension (17). These autoantibodies against AT_1 receptors lead to a stimulation of the receptor (18). Some renal transplant patients who have chronic allograft failure without classic HLA antibodies have such agonistic antibodies against the AT_1 receptor, and they were involved in vasculitis with destruction of the renal allograft (18).

Polymorphisms for different components of the RAAS, such as ACE, angiotensinogen, or AT_1 receptors, have been described with controversial results (19), mainly explained by the different ethnic backgrounds of the study populations. Huang *et al.* (20) induced diabetes in mice that had one, two, or three copies of the ACE gene. Twelve weeks later, the three-copy diabetic mice had increased BP and overt proteinuria. Proteinuria was correlated to plasma ACE level in the three-copy diabetic mice. Thus, a modest genetic increase in ACE levels leads to aggravation of diabetic nephropathy in mice in comparison with reduced ACE gene expression (20). These data indicate that there likely is some genetic influence on the activity of the RAAS, but how this translates into the individual risk for predisposition or progression of renal disease remains unclear.

Local RAAS

During the past two decades, local RAAS have been described to operate independent from their systemic counterpart (21). A local RAAS including all its components could have been shown in the proximal tubular cells of the kidney (Figure 2). Proximal tubular cells actively produce AngII and also secrete angiotensinogen into the urine (21). Intraluminal angiotensinogen may be converted in the distal tubules to AngII, and recent observations suggest that it leads to induction of sodium channels independent of aldosterone (22). Hyperglycemia and proteinuria could stimulate local AngII synthesis, mainly by oxygen species as signal transducers (12). Renal injury activates the local RAAS directly and indirectly. For example, a reduction in calcitriol stimulates renin transcription accompanied by local increase of AngII, demonstrating an indirect activation (12).

Of clinical relevance is the observation that complete systemic inhibition of the AngII formation by an ACE inhibitor is not accompanied by a significantly reduced intrarenal AngII production (23). Intrarenal AngII is found regionally compartmentalized (24). Intact AngII is found in endosomes that are

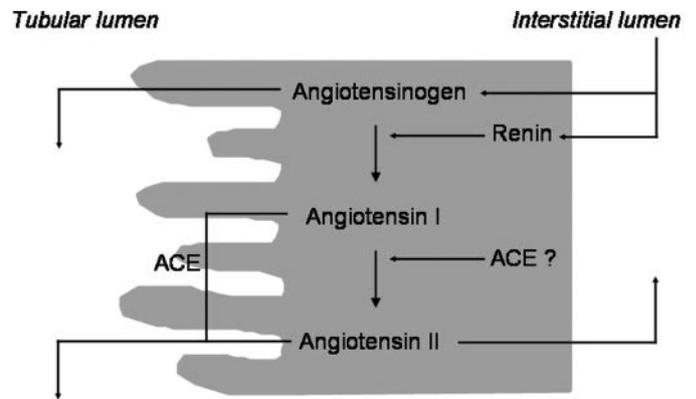


Figure 2. Proximal tubular cells as an example of a local RAAS. Tubular cells could generate AngII in nanomolar concentrations and secrete into the tubular fluid as well as the interstitial space. Furthermore, tubular cells secrete intact angiotensinogen into the tubular fluid. Cells also could take renin and angiotensinogen up from the circulation, indicating a close interaction with the systemic RAAS. Although proximal tubular cells have ACE in their brush border membranes, it is controversial whether intracellular ACE contributes to AngII formation.

derived from receptor-mediated endocytosis. This might be an important mechanism, because observations in certain cells demonstrated that AngII can be translocated into the nucleus, where it directly regulates the gene transcription (12). AngII has many diverse effects on renal cells, some of which are depicted in Figure 3.

Proteinuria

Besides hypertension, proteinuria is one of the most important risk factors for the progression of renal diseases. As outlined in detail in the accompanying article by Remuzzi *et al.*, increased tubular absorption of filtered proteins induces tubulointerstitial inflammation, ultimately resulting in tubular atrophy, interstitial fibrosis, and loss of renal function. The RAAS plays an important role in many of the pathophysiologic pro-

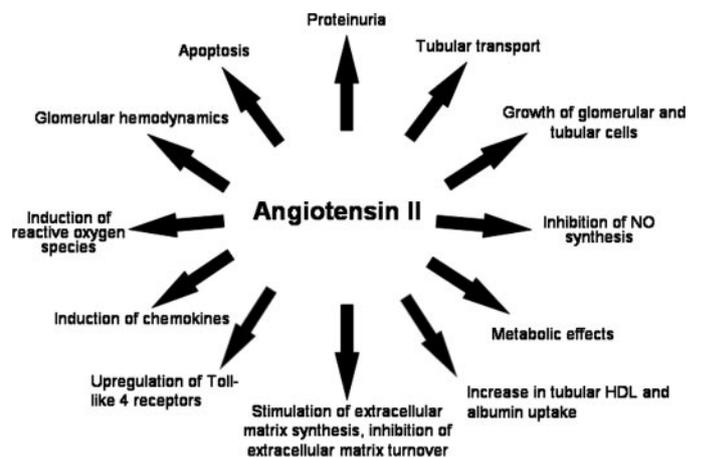


Figure 3. AngII is a cytokine with many effects on the kidney, clearly beyond the classical function as a hemodynamic mediator.

cesses that are associated with proteinuria. First, AngII is a mediator of proteinuria. It preferentially raises efferent glomerular arteriole resistance. AngII induces TGF- β 1 in the various renal cells (25). Sharma *et al.* (26) recently showed that TGF- β 1 impairs the autoregulation by afferent arterioles. Because afferent arterioles respond to an increase in arterial pressure with vasoconstriction, impaired autoregulation in the presence of TGF- β 1 leads to an elevation in transcapillary pressure, particularly during systemic hypertension. Thus, AngII directly (efferent vasoconstriction) and indirectly (TGF- β 1-mediated impaired afferent arteriole autoregulation) enhances capillary filtration pressure.

Moreover, AngII exhibits direct effects on the integrity of the ultrafiltration barrier. It has been shown that AngII decreases the synthesis of negatively charged proteoglycans and additionally suppresses nephrin transcription (12,27). Because intact nephrin–nephrin signaling is important for the survival of podocytes, AngII-mediated suppression of nephrin results in podocyte apoptosis. Vascular endothelial growth factor (VEGF) also could be important in increasing the permeability of the ultrafiltration barrier. Neutralization of VEGF with an antibody reduces proteinuria by one half in a model of diabetic nephropathy (28). AngII stimulates VEGF expression through AT₁ and AT₂ receptor. The increase of VEGF expression through AT₂ receptors presumably is mediated by an increase in hypoxia-inducible factor 1 α because AT₂ receptor activation led to a downregulation of prolyl hydroxylase 3, an enzyme that is important for initiating the degradation of hypoxia-inducible factor 1 α (29). AngII-induced synthesis of the α 3 chain of collagen type IV, the principal ingredient of the glomerular basement membrane, is mediated by VEGF and TGF- β 1 (30). Consequently, AngII through hemodynamic and nonhemodynamic mechanisms increases proteinuria.

In the proximal tubule, albumin and other ultrafiltered proteins are reabsorbed by endocytosis involving megalin and cubulin (31). AngII stimulates albumin endocytosis in proximal tubule cells *via* AT₂ receptor-mediated protein kinase B activation. However, an increase in tubular albumin reabsorption activates the tubular RAAS, leading to a vicious circle (32). Albumin uptake induces a deluge of proinflammatory and profibrogenic cytokines such as RANTES, monocyte chemoattractant protein-1, IL-8, endothelin, and TGF- β 1 (33). This stimulates the migration of immune-competent cells into the interstitium.

Inflammation

AngII activates through AT₁ and AT₂ the proinflammatory transcription factor NF- κ B (34). In addition, AngIII and AngIV can stimulate NF- κ B (33). It therefore is obvious that sartanes could block only some proinflammatory effects of the RAAS. The Rho kinase pathway is involved in AngII-mediated NF- κ B activation. Furthermore, AngII stimulates the transcription factor Ets, and this factor is a critical regulator of vascular inflammation with T cell and macrophages/monocytes recruitment to the vessel wall (35). We recently demonstrated another mechanism for how AngII could contribute to renal inflammation. AngII upregulates on mesangial cells Toll-like 4 receptors that

bind LPS (36). This AngII-mediated Toll-like 4 receptor upregulation resulted in enhanced NF- κ B activation (36). The recruitment of inflammatory cells into the glomerulus as well as into the tubulointerstitium plays a pivotal role in progression of chronic renal disease. AngII stimulates upregulation of adhesion molecules such as vascular cellular adhesion molecule-1, intracellular adhesion molecule-1, and integrins, allowing circulating immune cells to adhere on capillaries. NF- κ B-mediated transcription of chemokines, including monocyte chemoattractant protein-1, RANTES, and other chemokines, then is responsible for renal tissue infiltration with leukocytes. In addition, AngII may directly stimulate proliferation of lymphocytes (33). It is interesting that lymphocytes are an active source of AngII, further amplifying proinflammatory effects (37). It is obvious that the AngII-mediated proinflammatory effects amplify the pathophysiologic changes that are induced by proteinuria.

Growth Effects and Apoptosis

AngII stimulates proliferation of mesangial cells, glomerular endothelial cells, and fibroblasts. In contrast, the peptide induces hypertrophy of proximal tubular cells by a p27^{Kip1}-mediated cell-cycle arrest (38,39). Proliferation of glomerular cells and fibroblasts could enhance structural renal damage and fibrosis. AngII-induced tubular hypertrophy, although initially an adaptive response to loss of functional nephrons, is over the long term maladaptive and likely fosters development of tubular atrophy and interstitial fibrosis. Moreover, AngII induces apoptosis under certain conditions *in vivo* and *in vitro* (39). Experimental evidence suggests that AT₁ and AT₂ receptors are involved in this effect. The decision of whether cells undergo growth stimulatory effects (proliferation, hypertrophy) or rather apoptosis may depend on the presence of additional growth factors and cytokines and the activation state of the cell. Furthermore, the AngII concentration may play a role, but why the peptide mediates growth stimulatory effects under certain experimental conditions and induces apoptosis in other settings is not completely understood.

Fibrosis

Probably the most direct evidence that AngII is involved in renal scarring stems from targeted overexpression of renin and angiotensinogen in rat glomeruli (25). Seven days after transfection, extracellular matrix (ECM) was expanded in rats with glomerular renin and angiotensinogen overexpression without systemic hypertension. AngII induces mRNA encoding the ECM proteins type I procollagen and fibronectin in cultured mesangial cells and also stimulates the transcription and synthesis of collagen type α 1(IV) and α 3(IV) but not type I in cultured proximal tubular cells (25). The stimulatory effects of AngII on collagen expression depends on TGF- β 1 expression. AngII stimulates proliferation of cultured renal fibroblasts and increases mRNA expression of TGF- β , fibronectin, and type I collagen. A novel twist to the whole story is the recent observation that renin alone, through a specific receptor, stimulates TGF- β 1 in mesangial cells (40). These findings raise the intriguing possibility that elevated renin, as a consequence of ACE

inhibitor or AT₁ receptor treatment, may contribute directly to renal fibrosis *via* TGF- β 1 despite AngII blockade. AngII increases connective tissue growth factor (CTGF) in the kidney (41). CTGF is a novel fibrotic mediator and is stimulated by TGF- β . However, AngII-induced CTGF expression also occurs independent of TGF- β (41).

A delicate balance between ECM synthesis and degradation under physiologic conditions prevents fibrosis. AngII induces *via* AT₁ receptors plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor of metalloproteinases-1 (TIMP-1). PAI-1 and TIMP-1 inhibit metalloproteinases and thereby matrix turnover, resulting in accumulation of ECM. Through stimulating the expression of PAI-1 in the proximal tubules *via* the AT₄ receptor, AngIV can play a role in the development of renal fibrosis independent from the activation of AT₁ and AT₂ receptors (42). Because of upregulation of AngIV, which generates enzymes under conditions with high local AngII concentrations and in diabetic nephropathy (10), accelerated degradation of AngII into AngIV could activate AT₄ receptors, inducing PAI-1.

Ingenious experiments have demonstrated that more than one third of local fibroblasts in renal interstitial fibrosis originate from tubular epithelial cells through a process called epithelial-to-mesenchymal transition (EMT). The molecular mechanism of EMT was reviewed previously in detail (43). EMT may be important in later stages of renal disease progression, leading to interstitial fibrosis and tubular atrophy because of vanishing epithelial cells. One important mediator of EMT is TGF- β 1, and AngII could contribute to EMT through induction of this profibrotic factor (43). EMT is antagonized by hepatocyte growth factor. Because AngII suppresses hepatocyte growth factor synthesis, AngII additionally may foster EMT *via* a reduction of its antagonist (44).

ACE Inhibitor and AT₁ Receptor Effects Independent of the RAAS

Recent evidence suggests that ACE inhibitors as well as AT₁ receptor blockers can influence cellular functions independent of inhibition of the RAAS. For example, ACE inhibitors block the hydrolysis of N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP). Some protective effects of ACE inhibitor (*e.g.*, inhibition of fibrosis, reduction of inflammatory cell infiltration) are the result of the inhibition of Ac-SDKP hydrolysis rather than inhibition of AngII formation in a model of AngII-induced cardiac fibrosis (45). Whether similar mechanisms are operative in the kidney remains unclear.

Using endothelial cells, it has been demonstrated that the ACE inhibitors ramiprilat and perindoprilat increase CK2-mediated phosphorylation of serine¹²⁷⁰ (46). Furthermore, ACE inhibitor treatment increased the activity of N-terminal kinase in endothelial cells. These provocative findings indicate that ACE inhibitors may mediate cellular function by “outside-in” signaling directly through ACE, an effect that is totally independent of the generation of AngII (46).

Evidence is accumulating that some AT₁ receptor antagonists, such as telmisartan, activate the peroxisome proliferator-activated receptor- γ (PPAR- γ), a widely known target for treatment of the metabolic syndrome and diabetes (47). Recent

studies have indicated that in addition to antidiabetic properties, PPAR- γ activators may improve renal disease, normalize hyperfiltration, and reduce proteinuria (47). The PPAR- γ -activating properties of certain sartanes do not require the presence of AT₁ receptors and are caused by the molecular structure of the specific sartanes (47). Therefore, it is possible that some of the protective effects of AT₁ receptor blockers in slowing the progression of chronic renal disease are due to actions that are independent of the RAAS action.

What about Aldosterone?

The classic understanding of aldosterone as a hormone that is produced in the adrenal cortex, which is involved in the reabsorption of sodium and the secretion of potassium and protons in the collecting duct, needs to be extended. These aldosterone effects have been explained as genomic effects that are caused by increased transcription of different target genes after binding of aldosterone to cytoplasmic receptors. Newer data provide evidence that nongenomic effects of aldosterone, such as the activation of certain signal transduction pathways, occur in several organs, including the kidney (48). Aldosterone also is generated in many other tissues besides the adrenal cortex. In various animal models of renal diseases, aldosterone is involved in endothelial dysfunction, inflammation, proteinuria, and fibrosis (48). Aldosterone increases the effect of AngII, induces the generation of reactive oxygen species, and leads to an acceleration of the AngII-induced activation of mitogen-activated protein kinases (49). These findings indicate that blockade of mineralocorticoid receptors presumably is beneficial even in situations with high AngII, because common signal transduction pathways between the two systems are interrupted. The first clinical studies seem to be showing that blockade of the aldosterone receptors provides additional renal protection even in the presence of ACE inhibition or AT₁ receptor antagonism. However, the potential threat of hyperkalemia with such an approach requires further clinical studies to test the safety of this treatment.

Conclusion

The RAAS has come a long way since we described more than 15 yr ago structural and profibrotic effects of AngII on renal cells (38). This fascinating system has become increasingly complex, and the search for new members still continues. AngII has emerged from a vasoconstrictor to the major multifactorial peptide involved in the progression of renal disease. A comprehensive understanding of the RAAS with novel pharmacologic approaches to interfere with its members (*e.g.*, the renin inhibitor askiren) hopefully will provide tools to halt progression completely and induce regression of renal disease.

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