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Novel aspects of the renin-angiotensin-aldosterone-system

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1. ABSTRACT

The renin-angiotensin-aldosterone system (RAAS) play a pivotal role in the progression of renal disease. The RAAS has become much more complex in recent years with the identification of novel peptides that exhibit biological activity. There are novel pathways of angiotensin II (ANG II) generation independent of angiotensin converting enzyme (ACE). ANG II bind to at least two different receptors and prorenin/renin also exerts pathophysiological effects through binding to specific receptor. ANG II itself has emerged as a multifunctional cytokine exhibiting many non-hemodynamic properties such as acting as a growth factor and profibrogenic and proinflammatory cytokine. These profibrogenic and proinflammatory effects are mediated by other factors such as transforming growth-factor beta (TGF-β) and chemoattractants that are induced in the kidney by ANG II. Increased aldosterone levels contribute to renal injury, independent of blood pressure or ANG II.. Numerous experimental and clinical studies have shown that ACE-inhibitors as well as AT1-receptor antagonists can prevent glomerulosclerosis and tubulointerstitial fibrosis.. This review will highlight some of these novel insights into the RAAS in regards to renal injury.

2. INTRODUCTION

The number of patients with end-stage renal failure is steadily increasing (1), an important challenge for nephrology. Diabetes mellitus type 2 and chronic glomerulonephritis represent the majority of diseases leading ultimately to dialysis-dependent end-stage renal disease. Furthermore, a decrease in renal transplantation and an increasing number of patients with chronic allograft failure requiring again renal replacement therapy contribute to the increasing number of dialysis patients. Although dialysis is possible for all patients in Western countries, people from other regions of the world that represent the fastest growing population of patients with end-stage renal diseases do not have such options. Particularly, the incidence of end-stage renal disease in older patients is increasing (1). Dialysis patients have an increased risk of cardiovascular diseases. In addition, patients with relatively minor impairment of renal functions also have an increased risk of cardiovascular diseases. Consequently, chronic kidney diseases have been labeled as a public health threat (1). A better understanding of the pathophysiology of chronic renal disease is necessary to develop innovative therapeutic strategies. Mechanisms involved in the progression of chronic renal disease include

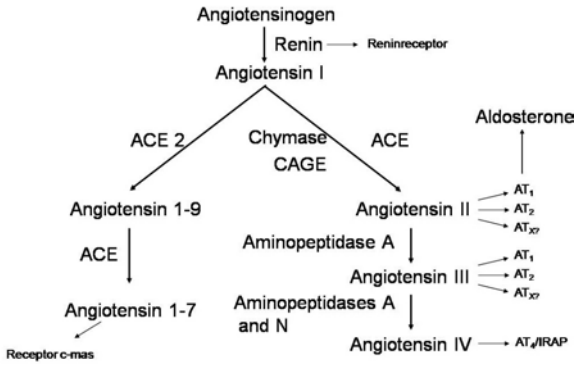

Figure 1. Overview of the RAAS. The system has become increasingly complex with alternative ways of ANG II formation besides ACE (chymase, CAGE), a second form of ACE (ACE2), and novel peptides such as angiotensin IV, angiotensin 1-9, and angiotensin 1-7. Clinically important could be the fact that ANG II can bind to AT2-receptors and angiotensin IV to AT4-receptors that are not antagonized by sartanes inducing proinflammatory and profibrotic effects (e.g. induction of chemokines, stimulation of PAI-1).

genetic factors, systemic and renal hemodynamic changes, metabolic acidosis, degree of proteinuria causing in turn injury of the tubulointerstitium, adaptive renal growth processes such as compensatory hypertrophy of surviving nephrons, infiltration of the renal parenchyma with inflammatory cells, local release of profibrogenic factors causing renal fibrosis, activation of intrarenal coagulation, processes of secondary hyperparathyroidism with high parathormone concentrations, and changes in serum lipid and glycosylated-protein levels (2). The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in many of these pathophysiological changes (3, 4). The RAAS has become considerably more complex in recent years with the discovery of new peptides, new pathways, and novel functions of RAAS components such as renin and aldosterone (5,6). The present contribution will highlight some of the more recent molecular insights into the complexity of the RAAS and its role in chronic renal disease.

3. THE DISCOVERY OF NEW RAAS COMPONENTS

The paradigm that served well for decades was that angiotensinogen is produced as a glycoprotein in the liver and is cleaved by renin released in renal juxtaglomerular cells to generate angiotensin I, which is in turn converted by the high angiotensin-converting enzyme (ACE) activity of the lungs into the active substance ANG II (7). ANG II, in turn, binds then to specific receptors in the adrenal cortex resulting in release of aldosterone (Aldo) as a main effector of this systemic hormone system. In this classical view, the cardinal function of the RAAS is maintaining blood pressure in situations of volume deficiency by ANG II-induced vasoconstriction and Aldo-mediated sodium retention in the collecting duct (7).

However, the RAAS has become quite complex in recent years and novel components of this network have been identified. Figures 1 provides an overview of our current understanding. It is generally appreciated that the key enzyme in generation of ANG II from angiotensin I is the angiotensin converting enzyme (ACE). However, it is not the only one. Other ANG II-generating enzymes include the serine protease chymase which is supposed to mediate more than 80% of ANG II formation in the heart and more than 60% in the vessels (6, 8). This is of particular interest because ACE inhibitors do not reduce chymase activity. Upregulation of chymase, mainly in the tubules, is observed in renal biopsies of patients with diabetic nephropathy (8). These findings indicate that under pathologic conditions, an upregulation of chymase occurs and increased local ANG II-generation can not be attenuated by ACE inhibitors. Mechanical stress of podocytes stimulates local ANG II synthesis by non-ACE pathways, presumably involving chymase (9).

A novel enzyme similar to ACE has been identified, called angiotensin-converting enzyme 2 (ACE2) (10). The ACE2 gene maps to the human X chromosome, and the amino-terminal domain of ACE2 shares approximately 40% sequence identity with ACE (11). Interestingly, ACE2 is a functional receptor for coronavirus (12). ACE2 is expressed predominantly in vascular endothelial cells, including those of the kidney (11). ACE2 has been also found in podocytes (13) and on tubular cells (14). In contrast to the "classic" ACE, which converts angiotensin I to the octapeptide ANG II, ACE2 cleaves one amino acid less off from angiotensin I so that in a first step angiotensin 1-9 is formed (15). Angiotensin 1-9 potentates ANG II mediated vasoconstriction on isolated rat aortic rings and has vasodepressor effects in conscious rats and augments bradykinin action on its B2 receptor by probably inducing conformational changes in the ACE/B2 receptor complex via interaction with ACE (16). 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 converting the peptide into inactive fragments is then mediated by ACE itself. Angiotensin 1-7 acts as a vasodepressor agent and is involved in apoptosis (17). Part of the vasodilator actions of angiotensin 1-7 are explained by Akt-dependent activation of endothelial nitric oxide synthase (18). Furthermore, it suppresses hypertrophy and has anti-fibrotic properties. Some of these mechanisms are mediated by angiotensin 1-7-induced inhibition of the mitogen activated kinase (MAP) pathways (19). The protein product of the c-mas gene is a receptor for angiotensin 1-7 (20). Several years ago when this relationship was not known, we found that overexpression of c-mas in tubular cells attenuated the ANG II-mediated increase in TGF-β expression (21). Thus, our earlier findings could be explained by increased actions of angiotensin 1-7 that counteract ANG II-mediated TGF-β expression.

The local expression of ACE2 correlates closely with the concentration of angiotensin 1-7 and leads to an, at least partial, antagonism of ANG II (22). Thus, ACE inhibition can lead to increased angiotensin 1-7 levels while

reducing ANG II in parallel (11). Indeed, infusion of ANG II into ACE2-deficient mice induced a significant higher blood pressure associated with higher ANG II levels (23). Interestingly, ACE2-deficient mice develop glomerulosclerosis and albuminuria at 12 months of age (24). These changes were prevented by an AT1-receptor antagonist (24). The findings suggest deletion of the ACE2 gene causes glomerulosclerosis by shifting the pathways away from angiotensin 1-7 formation towards unopposed ANG II production. In renal biopsies of patients with diabetic nephropathy an increase in ACE2 immunostaining was detected, whereas in early experimental diabetes ACE2 expression was suppressed (25-27). Although many important questions are not yet answered, the current findings suggest a unbalance of ACE and ACE2 expression during the course of diabetic nephropathy. A potential role of ACE2 has also been found in spontaneously hypertensive rats (SHR). The expression of ACE2 is significantly increased in SHR kidney at birth, but falls with the onset of hypertension compared with normotensive Wistar Kyoto rats (28).The developmental pattern of ACE2 expression in the SHR kidney is altered before the onset of hypertension (28). Since levels of the vasodilator angiotensin 1-7 levels are reduced in SHR (29), these findings indicate that reduced ACE2 expression in SHR may contribute to the hypertensive phenotype (30).

ANG II is metabolized by peptidases such as aminopeptidase A (APA) into angiotensin III and further into angiotensin IV (31). ANG IV binds to a specific receptor named AT4 (32). This receptor is widely expressed in the kidney including endothelial cells, and proximal and convoluted tubules (32,33). Recent evidence suggests that this receptor is identical with the enzyme insulin-regulated membrane aminopeptidase (IRAP; 34). AT4 receptor ligands such as ANG IV inhibit IRAP catalytic activity (34). ANG IV stimulates plasminogen activator inhibitor-1 (PAI-1) expression in proximal tubular and endothelial cells through AT4 receptors (35). Since PAI-1 reduces extracellular matrix turnover, ANG IV may induce renal fibrosis independently of activation of AT1 and AT2 receptors. ANG IV also activates the nuclear factor-□B pathways and stimulates transcription of proinflammatory genes through binding to the AT4 receptor (36). The close association of the AT4 receptor/IRAP with the inducible glucose transporter GLUT4 suggests that ANG IV (and presumably other AT4 receptor ligands) may modulate glucose uptake into cells (37). Other effects of ANG IV are release of NO and phosphorylation of focal adhesion kinase (34). ANG IV generating enzymes are upregulated in conditions with high ANG II and renal injury such as diabetic nephropathy and renal ablation, likely shifting more ANG II into the degradation pathway to ANG IV (38-40). However, effects depend on the expression of the AT4 receptor/IRAP. We found that overexpression of APA in mouse mesangial cells attenuated the proliferative effects of ANG II, suggesting that ANG IV does not modulate growth in these cells, in contrast to findings in endothelial and vascular smooth muscle cells (41).

Recently, two receptors for prorenin/renin have been identified (42). One, the manose-6-phosphate receptors is a clearance receptor and is involved in internalization of prorenin/renin without apparent signal transduction activation (42). The second receptor is a 350 amino acid protein that binds prorenin and renin with an equal affinity (42). Evolutionary analysis suggests that the receptor is a protein with two functionally distinct domains consisting of an evolutionary old part with the transmembrane domain and a newer, vertebrate-specific extracellular domain (43). Activation of this receptor by prorenin/renin leads to activation of MAP kinases (Erk 1, 2) and stimulation of profibrotic molecules such as PAI-I and TGF-β1(44). Overexpression of the prorenin/renin receptor in smooth muscle cells of transgenic rats induced high blood pressure (45). Thus, activation of the novel receptor by prorenin/renin induces direct pathophysiological changes independent of the generation of angiotensin fragments (46). There are currently renin inhibitors such as aliskiren on the market or in development that inhibit the enzymatic activity of renin and abolish angiotensin I generation (47). Although these substances are not direct prorenin/renin receptor antagonists, they can alter the structure of renin (48). It is therefore interesting to investigate further whether these substances may interfere with direct effects of renin through its putative receptor.

4. RECEPTORS FOR ANG II

The multiple effects of ANG II are mediated by different receptors. The two major ANG II receptors AT1 and AT2 are differentially expressed within in the kidney (49). Both are characterized by the configuration of a seven-transmembrane receptor, but share only around 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 kinases 1,2 (Erk 1,2), the phosphatidylinositol 3-kinase (PI3K)-dependent kinases Akt, and the mTOR/S6 kinase pathway (450-52). The traditionally described pathway of AT1 receptor signaling is activation of phospholipase C and the subsequent formation of diacylglycerol (DAG) and inositoltrisphophate, which lead to increase protein kinase C activity and an increase in levels of free intracellular calcium, respectively. Accumulating evidence indicates that important downstream effects of AT1 receptors are independent of classical G protein coupling (53). For example, AT1 receptor mediated endocytosis, tyrosine phosphorylation and activation of protein kinases occur independent of G proteins (53). ANG II-mediated phosphorylation of Smad1 is transduced through AT1 receptors involving Src (54). Lautrette *et al*. recently described a novel mechanisms by which ANG II transactivates the EGF receptor during renal injury (55). They found that ANG II-induced secretion of TGF-□ that binds to and activates the EGF receptor, explaining how ANG II through transactivation of the EGF receptor could exhibit tyrosine kinase activity (55). Similarly, ANG II mediates the extracellular cleavage of pro-heparin-binding EGF (HB-EGF) by activation of matrix metalloproteases with liberation of soluble HB-EGF that could activate the

EGF receptor (56, 57). AT1 receptor activation also stimulates release of reactive oxygen species (ROS) by a mechanism involving activation of the membrane-bound NAD(P)H-oxidase (58). ANG II activates NAD(P)H oxidase by different mechanisms. In vascular cells, caveolin 1 (a component of caveolae/lipid rafts that are cholesterol-enriched specialized membrane microdomains) is necessary for ANG II-mediated Rac1 and NAD(P)H oxidase activation and ROS generation (59). ANG II upregulates various NAD(P)H oxidase subunits including Nox1, p47phox, p67phox and p22phox through AT1 receptor activation (60-63). For example, ANG II infusion leads to blunted ROS formation and an attenuated blood pressure response in Nox1-deficient mice (64). Additionally, ANG II facilitates assembly of NAD(P)H oxidase subunits. ANG II has been found to induce serine phosphorylation of p47phox resulting in an increased binding of p47phox to p22phox (65). ANG II also stimulates Rac1 by disrupting the binding of Rac to the GDP dissociation inhibitor RhoGDI. Rac1 in turn binds to and activates Nox4, increasing ROS generation (66). In contrast to AT1 receptor activation, it appears that ANG II-mediated stimulation of AT2 receptors downregulates several NAD(P)H oxidase components (Nox1, p22phox, p67phox; 67). There is also evidence that ANG II-mediated transactivation of the EGF receptor depends on ROS. ANG II-mediated liberation of H_2O_2 can lead to EGF receptor transactivation, where H_2O_2 acts upstream of the receptor. Furthermore, ROS induces HB-EGF transcripts.

An AT1 receptor associated protein (ATRAP) has been isolated which interacts specifically with the C-terminal cytoplasmic domain of the receptor (68). In vitro studies showed that overexpression of ATRAP facilitates internalization of the AT1 receptor (68). In the kidney, ATRAP is widely expressed in tubules and in glomeruli (69). Dietary salt depletion significantly decreased the renal expression of ATRAP suggesting that the protein is a negative regulator of AT1 receptors by promoting endocytosis of receptors (69).

AT1 receptors can form dimers (70, 71). Heterodimers between AT1 receptors and bradykinin receptor (70) as well as AT1 receptor homodimers (71) have been found. Homodimers are formed by covalent binding at the glutamine residue 315 of the intracellular domain and are detected on monocytes of patients with essential hypertension (71). Apparently AT1 receptor hetero- and homodimers exhibit an increase in signal transduction activity after stimulation with ANG II. The number of AT1 and AT2 receptors is developmentally regulated and during maturation of the kidney, AT1 receptor expression becomes more abundant (49).

AT1 receptor expression is up-regulated by different stimuli like hypercholesteremia (72) or a change in osmolarity (73), but is suppressed by high ANG II concentrations or glitazones (74,75). N-acetylcysteine, an antioxidant reducing disulfide bonds, decreased ANG II binding to AT1 receptors (76). AT2 receptors are not suppressed by ANG II, but interestingly they are up-regulated in inflammatorily-modified and injured tissue (67). In particular, AT2 receptors are re-expressed in the kidney during renal injury and remodeling nephrons (67).

Almost all ANG II-induced physiologic and pathophysiological functions such as vasoconstriction, aldosterone release, stimulation of tubular transport, proinflammatory effects as well as profibrogenetic and growth stimulatory actions are mediated by AT1 receptors (75). The role of AT2 receptors seems less clear. Activation of AT2 receptors leads to a decrease in blood pressure through release of nitric oxide and activation of cGMP-dependent pathways, inhibits growth and induces differentiation, and is also involved in meditation of apoptosis (67, 77). AT2 receptor activation may, in addition to the well-known short negative feedback loop of the AT1 receptor to suppress renin, negatively influence renal renin production and can reduce blood pressure through this mechanism (78). However, recent evidence suggests that activation of NF-κB, an important proinflammatory transcription factor, is mediated by AT1 and AT2 receptors (79). Other studies have shown an anti-inflammatory effect of AT2 receptor activation via suppression of mitogen-kinase-dependent pathways (80). Agonistic antibodies against AT1 receptors have been identified in pregnant women with preeclampsia and in patients with secondary malign hypertension (81). These autoantibodies against AT1 receptors lead to a stimulation of the receptor (81). Some renal transplant patients with chronic allograft failure without classic HLA antibodies have such agonistic antibodies against the AT1 receptor and they were involved in vasculitis with destruction of the renal allograft (82).

Polymorphisms for different components of the RAAS like ACE, angiotensinogen or AT1 receptors have been described with controversial results (83), mainly explained by the different ethnic backgrounds of the study populations. In a large case-control study with more than 1000 patients, it was recently shown that ACE polymorphisms (haplotype defined by the D, rs4366_G and rs12449782_G alleles) were associated with diabetic nephropathy, but the association could not be confirmed in a family-based association study (84). Experimental overexpression of three copies of the ACE gene leads to aggravation of diabetic nephropathy in mice in comparison to reduced ACE gene expression (85). Thus, there is likely some genetic influence on the activity of the RAAS, but how this translates into the individual risk of predisposition or progression of renal disease remains unclear.

5. TISSUE SPECIFIC RAAS THAT CAN WORK INDEPENDENTLY

Over the last two decades local RAASs have been described operating independently from their systemic counterparts (86). For example, expression of local RAAS components have been found in the brain where they are involved in the regulation of thirst and salt appetite (87). A local RAAS including all its components has been shown in the proximal tubular cells of the kidney (88). Micropuncture studies have clearly shown that proximal tubular cells actively produce ANG II and also secrete angiotensinogen into the urine (88). Intraluminal

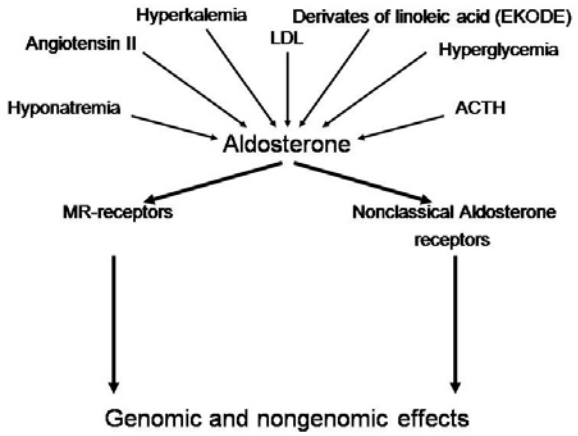

Figure 2. Aldosterone effects on the kidney. Various factors can stimulate synthesis and release of aldosterone. Aldosterone exhibits genomic and nongenomic effects. Classical mineralcorticoid receptors are involved in genomic effects whereas evidence exists that other receptors may be involved in the nongenomic effects of aldosterone not requiring gene transcription. Whether nongenomic effects of aldosterone are involved in the processes of renal fibrosis is currently unknown.

angiotensinogen can be converted in the distal tubules to ANG II and recent observations suggest that it leads to induction of sodium channels independently of Aldo (89). Hyperglycemia and proteinuria could stimulate local ANG II synthesis, mainly by oxygen species as signal transducers (90). Renal injury activates the local RAAS directly and indirectly. A reduction in calcitriol stimulates renin transcription accompanied by local increase of ANG II demonstrating an indirect activation (91).

Of clinical relevance is the observation that complete systemic inhibition of the ANG II formation by an ACE inhibitor is not accompanied by a significantly reduced intrarenal ANG II production (92). Intrarenal ANG II is found regionally compartmentalized (93). Intact ANG II is intracellulary found in endosomes derived from receptor mediated endocytosis. This might be an important mechanism, because observations in certain cells demonstrated that ANG II can be translocated into the nucleus where it directly regulates the gene transcription (94,95).

6. ALDOSTERONE

The classic understanding of Aldo as a hormone 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 (96). These Aldo effects have been explained as genomic effects due to increased transcription of different target genes after binding of Aldo to cytoplasmic receptors (Figure 2). Newer data provide evidence that non-genomic effects of Aldo, such as the activation of certain signal transduction pathways, occur in several organs including the kidney (97). These non-genomic effects of Aldo are defined by an insensitivity to inhibitors of transcription as well as a rapid time course that is incompatible with gene transcription and de novo protein synthesis (98). There is increasing evidence that these non-genomic Aldo effects are not transduced by the classical mineralcorticoid receptors and additional receptors for Aldo may exist (98). The activity of various ion transport systems (Na^+/K^+ ATPase, H^+-ATPase, Na^+/H^+ exchanger) are regulated by non-genomic actions of Aldo involving signaling pathways such as protein kinase C and MAP kinases (98). Whether non-genomic effects of Aldo and signaling through non-"classical" mineralcorticoid receptors contribute to renal injury is unknown (99). Aldo may be also generated in many other tissues besides the adrenal cortex, but this subject is a matter of debate because contradictory reports exists concerning the presence of aldosterone synthase and 11-β-hydroxylase in non-adrenal tissues (100). Besides the "classical" inducers of Aldo (ANG II, hyperkalemia, hyponatremia), Aldo synthesis is stimulated by LDL, derivates of linoleic acid (EKODE) and hyperglycemia (100). It has been postulated that renal tissue injury per se may facilitate the synthesis and release of Aldo (97).

In various animal models of renal diseases Aldo is involved in endothelial dysfunction, inflammation, proteinuria and fibrosis (100). Aldo increases the effect of ANG II, induces the generation of reactive oxygen species and leads to an acceleration of the ANG II-induced activation of MAP-kinases (101). In models of inflammatory renal injury, Aldo receptor antagonists prevented the expression of proinflammatory chemokines (100). On a cellular level Aldo stimulates proliferation of mesangial cells via the "classical" mineralocorticoid receptors (102) and podocytes are an additional target (103). Infusion of Aldo into uninephrectomized rats induces proteinuria accompanied by an increase in oxidative stress and enhanced expression of aldosterone effector kinase SgK1 (103). These effects were antagonized by eplerenone, a selective aldosterone receptor blocker (103). Aldo has been implicated in the enhanced expression of TGF-β in cell culture and various animal models (104). Microarray experiments with cells exposed to Aldo found that murine-double-minute type 2 (MDM2), a gene involved in anti-apoptosis and cell growth, is induced through mineralcorticoid receptors providing a new way how Aldo modulates tissue remodeling (105).

First clinical studies show that blockade of the Aldo receptors with spironolactone or eplerenone provides additional renal protection in humans even in the presence of ACE inhibition or AT1 receptor antagonism (106).

7. ACE-INHIBITORS AND AT1 RECEPTOR EFFECTS INDEPENDENT OF THE RAAS

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 ANG II formation in a model of ANG II-induced cardiac fibrosis (107).

Using endothelial cells, it has been demonstrated that the ACE inhibitors ramiprilat and perindoprilat increase CK2-mediated phosphorylation of serine^{1270} (108,109). Furthermore, ACE inhibitor treatment also 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 totally independent of the generation of ANG II (108).

Evidence is accumulating that some AT1-receptor antagonists such as telmisartan activate the peroxisome proliferator-activated receptor (PPAR)-γ, a well-known target for treatment of the metabolic syndrome and diabetes (110). Recent studies have indicated that in addition to antidiabetic properties, PPAR-γ activators may also improve renal disease, normalize hyperfiltration and reduce proteinuria (110,111). 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. Thus, it is possible that some of the protective effects of AT1 receptor blockers in slowing the progression of chronic renal disease are due to non-RAAS effects.

8. RENAL FIBROSIS AND THE RAAS

Micropuncture studies have shown that ANG II preferentially raises efferent glomerular resistance (112). The consequence is an increase in the glomerular capillary filtration pressure that results in a protected glomerular filtration rate despite an ANG II-induced decrease in renal plasma flow (112). Constriction of the efferent arterioles by ANG II increases peritubular capillary colloid osmotic pressure through a decrease in renal blood flow and an increase in filtration fraction (112). Since these changes increase interstitial fluid colloid osmotic pressure and tend to reduce interstitial fluid hydrostatic pressure, ANG II indirectly increases the driving force for fluid reabsorption across tubular cells (112).

Hostetter and colleagues found an increase in glomerular capillary pressure in many animals models of progressive nephron loss (113). Although these adaptive mechanisms may maintain indeed renal function in the early phase of chronic renal injury, glomerular hypertension and hyperfunction are ultimately detrimental to renal function and structure (114). An increase in capillary pressure may directly stimulate in glomerular cells through mechanical forces injury, proliferation, and production of extracellular matrix (115). The landmark study by Anderson and co-workers demonstrated that an ACE inhibitor limited glomerular injury in rats with experimentally induced reduction in renal mass (116). Thus, ANG II can induce glomerulosclerosis and tubulointerstitial fibrosis through hemodynamic mechanisms.

Numerous experimental studies in various animal models of acute and chronic renal diseases have demonstrated that ACE-inhibitors or AT1 receptor antagonists are nephroprotective The used animal models range from reduction of renal mass, Marfan syndrome, diabetic nephropathy, renal artery stenosis to calcineurin-inhibitor toxicity (117-122). ACE-inhibitors or AT1 receptor antagonists prevent in these models the development of glomerulosclerosis and tubulointerstitial fibrosis and may even induced regression of renal disease under certain circumstances. One of the first models in which the link between the RAAS, TGF-β1 and renal fibrosis was investiagted was unilateral ureteral ligation (121). In this model, TGF-β1 mRNA levels are increased in the obstructed kidney three days after surgery, but do not change significantly in the contralateral kidney (121). Treatment with an ACE-inhibitor or AT1 receptor antagonist significantly blunted this increase in TGF-β1 mRNA and the development of tubulointerstitial fibrosis (121-123).

Probably the most direct evidence that ANG II is involved in renal scarring stems from targeted overexpression of renin and angiotensinogen in rat glomeruli (124). Seven days after transfection, extracellular matrix was expanded in rats with glomerular renin and angiotensinogen overexpression without systemic hypertension (125). ANG II induces mRNA encoding the extracellular matrix proteins type I procollagen and fibronectin in cultured mesangial cells and also stimulates the transcription and synthesis of collagen type α1(IV) and α3(IV), but not type I, in cultured proximal tubular cells (126-128). The collagen α3(IV) expression is also stimulated by ANG II in podocytes (129). The stimulatory effects of ANG II on collagen expression depends on TGF-β1 expression (130-134). ANG II stimulates proliferation of cultured renal fibroblasts and increases mRNA expression of TGF-β, fibronectin and type I collagen (135).

For many years, prorenin was considered to be the inactive precursor of renin, without having a function of its own, although older studies suggests that prorenin is increased in the plasma of patients with diabetes mellitus and can be a marker of microvascular complications including nephropathy (136,137). A novel twist to the whole story comes with the recent observation that prorenin/renin alone, through a specific receptor, stimulates TGF-β1 in mesangial cells (44). In addition, direct activation of the prorenin/renin leads to stimulation mitogen-activated kinases (44). Certain transcription factors (promyelocytic zinc finger proteins) have been identified as direct interactions partners of the C-terminal domain of the prorenin/renin receptors (138). After receptor activation, this transcription factor is translocated into the nucleus (138). In a model of experimentally induced diabetes mellitus in AT1a receptor deficient mice, prorenin/renin receptor blockade prevented the development of diabetic nephropathy, clearly showing a contribution of prorenin/renin independently of ANG II (139). Treatment of hypertensive rats with one clipped kidney (Goldblatt model) with a vasopeptidase inhibitors increased plasma prorenin/renin and also upregulates the expression of the prorenin/renin receptors in the clipped kidney (140).This upregulation was associated with thickening of vessels and tubulointerstitial fibrosis suggesting that the prorenin/renin receptor play a profibrotic role in this model (140). In the traditional view,

prorenin is converted to renin before release by a proteolytic step in the juxtaglomerular cells involving various proteases. There is evidence that binding of prorenin to the prorenin/renin receptor leads to nonproteolytical conversion of prorenin into renin (42,43). Transgenetic overexpression of the prorenin/renin receptor resulted in elevated blood pressure, increased plasma Aldo levels and increased renal cyclooxygenase 2 expression (45). Thus, the prorenin /renin receptors contributes to renal fibrosis through direct (activation of TGF-β1) and indirect (conversion of prorenin into renin with subsequent ANG II generation) effects.

These findings raise the intriguing possibility that elevated renin, as a consequence of ACE inhibitor or AT1 receptor treatment, may directly contribute to renal fibrosis via TGF-β1 despite ANG II blockade. In animal models, blockade of the Aldo receptor with spironolactone reduced glomerulosclerosis and tubulointerstitial fibrosis (141). ANG II increases connective tissue growth factor (CTGF) in the kidney (133). CTGF is a novel fibrotic mediator and is stimulated by TGF-β. However, ANG II-induced CTGF expression also occurs independently of TGF-β(134).

A delicate balance between extracellular matrix synthesis and degradation under physiological conditions prevents fibrosis. ANG II induces via AT1 receptors PAI-1 and tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) (142,143). An induction of PAI-1 has been also described by ANG IV and Aldo. PAI-1 and TIMP-1 inhibit metalloproteinases and thereby matrix turnover resulting in accumulation of extracellular matrix. Ingenious experiments have demonstrated that more than a third of local fibroblasts in renal interstitial fibrosis originate from tubular epithelial through a process called epithelial to mesenchymal transition (EMT; 144). The molecular mechanism of EMT has been previously reviewed in detail (144). EMT may be important in later stages of renal disease progression leading to interstitial fibrosis and tubular atrophy because of vanishing epithelia cells. One important mediator of EMT is TGF-β1 and ANG II could contribute to EMT through induction of this profibrotic factor (145). EMT is antagonized by hepatocyte growth factor (HGF). Since ANG II suppresses HGF synthesis, ANG II may additionally foster EMT via a reduction of its antagonist (146).

9. CLINICAL EVIDENCE

A comprehensive analysis of all clinical studies showing that inhibition of the RAAS slows progression of chronic kidney disease is beyond the scope of this manuscript and the interested reader is referred to a previous review (147). However, a few key studies should be mentioned. In 1993 the Collaborative Study Group data reported of the first trial showing nephroprotection by ACE-inhibitors in patients with renal disease (148). The trial compared captopril with placebo in patients with type 1 diabetes. Captopril treatment was associated with a 50 percent reduction in the risk of the combined endpoints of death, dialysis, and transplantation (148). Importantly, inclusion of blood pressure into the study as a covariant did not alter the estimated reduction of risk of endpoints (148). The beneficial effect of the ACE-inhibitor was therefore not explained by the small differences in blood pressure control between the groups. In the „Ramipril Efficacy in Nephropathy Study“ (REIN) of the „Gruppo Italiano di Studi Epidemiologici in Nefrologia“ (GISEN) patients with chronic nondiabetic renal disease were classified according to baseline proteinuria, and assigned ramipril or placebo plus conventional antihypertensive therapy targeted to achieving diastolic blood pressure under 90 mmHg (149). In patients with proteinuria 1-3g/day progression to end-stage renal failure was significantly less common in the ramipril group, so was progression to overt proteinuria (149). Patients with highest proteinuria at baseline gained the most from the ramipril treatment. Importantly, blood pressure decreased in a similar way in the ramipril and placebo group after randomization (149).

Parving *et al*. administered the AT1 antagonist irbesartan to patients with type 2 diabetes who had early renal damage as manifested by microalbuminuria. The drug was associated with lower levels of proteinuria than the placebo (150). Diminution of proteinuria may indicate protection from ongoing kidney damage that would probably translate into the preservation of kidney function in the longer term (150). The two other studies by Brenner *et al*. and Lewis *et al*. used the AT1 antagonists irbesartan and losartan in patients with type 2 diabetes and established renal insufficiency (151,152). The AT1 antagonists led to lower levels of proteinuria, lower rates of decline in glomerular filtration rate and later onset of end-stage renal failure than the control medication. Similar protective effects of AT1 receptor blocker on renal function in humans have been found in patients with IgA nephropathy as well as in those with hypertensive nephropathy (153,154). There is accumulating evidence that the combination of ACE-inhibitors with AT1 receptor antagonists provide more complete inhibition of the RAAS leading to more renal protection, particular in patients with proteinuria (155,156). However, there are uncertainties concerning the adverse effects of such an approach (156).

The first clinical studies are appearing showing that blockade of the Aldo receptors provides additional renal protection even in the presence of ACE inhibition or AT1 receptor antagonism. For example, Bianchi and coworkers have shown in patients with nondiabetic renal disease that the addition of spironolactone significantly reduced proteinuria on top of an ACE-inhibitor or an AT1 receptor antagonist after 12 months (157). However, many of these studies have enrolled only a limited number of patients over a short time (a few months) and have looked at the surrogate parameter proteinuria (106, 157). The potential threat of hyperkalemia with such an approach and the confirmation of renoprotection with hard end-point parameters (e.g. doubling of serum creatinine) require further long-term clinical studies.

The oral renin inhibitor aliskiren has been shown to reduced effectively blood pressure in patients with essential hypertension (158). Studies investigating the effect of this novel drug on renal damage are under way.

10. CONCLUSION

Ten years ago, it was thought that all components of the RAAS have been identified. However, the system has become much more complex with the recent identification of novel peptides that exhibit biological activity and the identification of novel enzymatic pathways and new receptors. There is ample evidence that the RAAS plays a pivotal role in renal damage in many, if not all, kidney diseases. Therefore, inhibition of the RAAS is part of any strategy fighting the progression of chronic renal disease. The introduction of novel drugs (e.g. aliskiren) will help to more completely block the RAAS. However, the question which drugs should be primarily used and which combinations are superior is part of ongoing and upcoming clinical studies.

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