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Intratubular Renin-Angiotensin System in Hypertension

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Abstract

It is well recognized that the renin-angiotensin system plays an important role in the regulation of arterial pressure and sodium homeostasis. Recent years, many studies have shown that local tissue angiotensin II levels are differentially regulated and cannot be explained on the basis of circulating concentrations. All of the components needed for angiotensin II generation are present within the various compartments in the kidney including the renal interstitium and the tubular network. The cascade of the renin-angiotensin system demonstrates three major possible sites for the pharmacological interruption of the renin-angiotensin system: the interaction of renin with its substrate, angiotensinogen, the angiotensin converting enzyme, and angiotensin II type 1 receptors. This brief article will focus on the role of the intratubular renin-angiotensin system in the pathophysiology of hypertension and the responses to the renin-angiotensin system blockade by renin inhibitors, angiotensin converting enzyme inhibitors and angiotensin II type 1 receptor blockers.

Keywords

Renin-angiotensin system; hypertension; kidney; angiotensin converting enzyme inhibitors; angiotensin II type 1 receptor blockers

INTRODUCTION

In recent years, the focus of interest on the role of the renin-angiotensin (Ang) system (RAS) in the regulation of arterial pressure and in the pathophysiology of hypertension has changed to a major emphasis on the role of the local/tissue RAS in specific tissues [1]. Various studies have demonstrated the importance of the tissue RAS in the brain [2], heart [3], adrenal glands [4], and vasculature [5], as well as in the kidney [6]. Presently, Ang converting enzyme (ACE) inhibitors (ACEI) or Ang II type 1 (AT1) receptor blockers (ARB) are recommended as first-line therapy for hypertensive patients with diabetic nephropathy [7]. The four large trials performed on type 2 diabetes showed that ARBs prevent the development of clinical proteinuria in microalbuminuric patients (IRbesartan in patients with type 2 diabetes and MicroAlbuminuria (IRMA) [8] and MicroAlbuminuria Reduction with VALsartan (MARVAL) studies [9]) and delay the progression of nephropathy towards end-stage renal failure in patients with overt nephropathy (Irbesartan Diabetic Nephropathy Trial (IDNT) [10] and Reduction of Endpoints in Non-insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) studies [11]). In the IDNT study, irbesartan showed the better renoprotective effect compared with amlodipine in patients at a late stage of type 2 diabetic nephropathy, independently from the blood pressure-lowering effects of the drugs [10,12]. Recently, the Diabetics Exposed to Telmisartan And enalaprIL (DETAIL) trial

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has directly compared ACEIs and ARBs in patients with type 2 diabetes, and shown the renoprotective effects of these drugs, respectively [13]. ACEIs and ARBs are effective on these patients because of their ability to block the local RAS [14]. Furthermore, Nakao *et al.* [15] reported that the combination of ACEIs and ARBs was significantly better than each individual drug in renal survival of non-diabetic patients with reduced renal function and moderate daily urine protein excretion. Locally generated Ang II may be involved in the pathogenic mechanisms of chronic renal diseases [16]. The beneficial effects of RAS blockers may be a consequence of the reduction of intraglomerular capillary pressure [17], and also, a result of their antiproteinuric effect [18], antiinflammatory [19], antiproliferative [20], and antifibrotic [21,22] properties. This brief article will focus on the role of the intratubular RAS in the pathophysiology of hypertension and the responses to RAS blockade.

INTRATUBULAR LOCALIZATION OF THE RENIN-ANGIOTENSIN SYSTEM COMPONENTS

Using *in situ* hybridization, Ingelfinger *et al.* [23] demonstrated that the angiotensinogen (AGT) gene was specifically present in the proximal tubules. Terada *et al.* [24] reported that AGT mRNA was expressed largely in the proximal convoluted tubules and proximal straight tubules, and that there were small amounts in glomeruli and vasa recta as revealed by reverse transcription and polymerase chain reaction. Richoux *et al.* [25] and Darby *et al.* [26,27] showed by immunohistochemistry that renal AGT protein is specifically located in proximal convoluted tubules. Kobori *et al.* [28] also showed that there was strong positive immunostaining for AGT protein in proximal convoluted tubules and proximal straight tubules, and weak positive staining in glomeruli and vasa recta; however, there was no staining in distal tubules or collecting ducts.

Yanagawa *et al.* [29] and Moe *et al.* [30,31] showed that renin mRNA and renin-like activity could be demonstrated in cultured proximal tubular cells. In addition, low but measurable renin concentrations in proximal tubule fluid have been reported in rats [32]. Interestingly, Prieto-Carrasquero *et al.* [33] recently reported that renin mRNA and protein are expressed in the principal cells of distal tubules of rats. Moreover, they demonstrated that renin in the distal tubular cells is upregulated by Ang II infusion and this upregulation depends on AT1 receptor activation [34]. They conclude that renin in distal tubular cells and renin in juxtaglomerular cells are separately regulated.

In terms of ACE, abundant expression of ACE mRNA [35] and protein [36] were shown in brush border of proximal tubules of human kidney. ACE has also been measured in proximal and distal tubular fluid but is more abundant in proximal tubule fluid [37].

There are two major types of Ang II receptors, AT1 receptor and type 2 (AT2) receptor, but there is much less AT2 receptors expression in adult kidneys [38,39]. AT1 receptor mRNA has been localized to proximal convoluted and straight tubules, thick ascending limb of the loop of Henle, cortical and medullary collecting duct cells, glomeruli, arterial vasculature, vasa recta, and juxtaglomerular cells [24]. In rodents, two subtypes of AT1 receptors ($AT1_A$ and $AT1_B$) have been demonstrated in the vasculature and glomerulus and in all nephron segments [39]. The $AT1_A$ receptor is the predominant subtype in nephron segments, whereas the $AT1_B$ receptor is more abundant than $AT1_A$ receptor in the glomerulus [40]. Studies using polyclonal and monoclonal antibodies to the AT1 receptors demonstrated that AT1 receptor protein has been localized on vascular smooth muscle cells throughout the vasculature, including the afferent and efferent arterioles and mesangial cells [41]. AT1 receptors are also present on proximal tubule brush border and basolateral membranes, thick ascending limb epithelia, distal tubules, collecting ducts, glomerular podocytes, and macula densa cells [38, 39,41].

INTRARENAL RENIN-ANGIOTENSIN SYSTEM IN HYPERTENSION

Many studies have demonstrated that Ang II vasoconstricts both pre-glomerular and post-glomerular arterioles. Ang II exerts powerful vascular effects that elicit decreases in renal blood flow and, to a lesser extent, in glomerular filtration rate, therefore, there is usually an increase in filtration fraction [42]. All of the components needed for Ang II generation are present within the various comportments in the kidney including the renal interstitium and the tubular network. Some of the interstitial Ang II is derived from locally formed AGT and may not be dependent on circulating Ang II or AGT. *In vivo* and *in vitro* studies have shown that Ang II stimulates intrarenal AGT mRNA localized in proximal tubular cells [28,43,44]. Studies in Ang II-infused rats have demonstrated that augmentation of intrarenal Ang II is due, in part, to uptake of circulating Ang II *via* an AT1 receptor mechanism and also to sustained or enhanced intrarenal production of Ang II [45]. Schunkert *et al.* [44] showed that plasma Ang II upregulates renal AGT gene expression and downregulates renal renin gene expression. Kobori *et al.* [46] demonstrated that there were significant increases in intrarenal AGT protein, as well as AGT mRNA level, in response to 2 weeks of Ang II infusion in rats. This augmentation mechanism may be responsible for sustained or enhanced generation of AGT, leading to continued intrarenal production of Ang II under conditions of elevated circulating concentrations. Kobori *et al.* [47] demonstrated that urinary AGT excretion rates directly relate to kidney Ang II content, but not plasma Ang II content and suggested that urinary excretion rate of AGT provides a specific index of intrarenal AGT production in Ang II-dependent hypertension [48]. This increase is not due to increased proteinuria or the development of hypertension since urinary protein excretion in volume-dependent hypertensive rats was significantly increased more than in Ang II-dependent hypertensive rats; however, urinary AGT excretion was significantly lower in volume-dependent hypertensive rats than in Ang II-dependent hypertensive rats. The increased amounts of intact AGT in urine in Ang II-dependent hypertension suggests augmented AGT levels throughout the nephron. To the extent that renin and ACE are available along the nephron, the AGT provides substrate for continued Ang I generation and Ang II conversion in segments beyond the proximal tubules [49–51]. The sustained increase in intrarenal Ang II in a setting of hypertension can lead to progressive renal injury, proliferation and fibrosis associated with activation of several major cytokines and growth factors [52–55].

The cascade of the RAS (Fig. 1) demonstrates three major possible sites of the pharmacological interruption of the RAS: the interaction of renin with its substrate, AGT, the ACE, and the AT1 receptors.

RENIN INHIBITORS

AGT is converted into Ang I by renin (Fig. 1). It has often been suggested that the renin step should be one of the most attractive targets for the RAS blockade for two important reasons: 1) the interaction of renin with its substrate, AGT, is a rate-limiting step [56], 2) renin has a species-specificity for its substrate [57].

Fisher and Hollenberg [58] examined renal plasma flow in healthy young men receiving a low sodium intake to activate the RAS. Renin expression in principal cells of collecting ducts is further increased in Ang II-dependent hypertension [33]. Dose-responses were evaluated for different types of blockade of the RAS: ACEIs (captopril, lisinopril, and ramipril) and renin inhibitors (enalkiren and zankiren). To their surprise, the renal vasodilator response to the renin inhibitor, enalkiren, exceeded their expectations. Enalkiren (A-64662, IC_{50} = 0.8 nM) induced a larger increase in renal plasma flow than captopril. Similar responses were observed with zankiren (A-72517, IC_{50} = 1 nM). El-Amrani *et al.* [59] also compared systematically the renal vascular response to a renin inhibitor, remikiren (R042-5892, IC_{50} = 0.8 nM), an ARB

(losartan), and an ACEI (lisinopril) in guinea pigs. The renin inhibitors display species specificity because renin structure varies with species. The guinea pig was selected because remikiren developed for primates, is also effective in the guinea pig. This study also showed that renin inhibitor induced a substantially larger increase in renal plasma flow, glomerular flow rate, diuresis, and natriuresis-as in the humans. The authors suggested that the potentiated response to renin inhibition could reflect greater lipophilicity and tissue penetration, leading to a local, intrarenal action at the site of Ang II production. Fisher *et al*. [60] suggested that renin inhibition is far more effective than ACE inhibition in blocking Ang II formation in the case of the kidney. Aliskiren (SPP100, IC_{50} = 0.6 nM) has the potential to become the first orally active renin inhibitor that provides a true alternative to ACEIs and ARBs in therapy for hypertension. Aliskiren acts as a transition state mimetic, inhibiting renin *via* hydrogen bonding of both the central hydroxy group and amino function to the catalytic Asp32 and Asp215 residues [61,62]. Aliskiren is one of the most potent renin inhibitors yet identified with high species specificity for primate renin [62]. Nussberger *et al.* [63] showed that aliskiren dose-dependently decreased plasma Ang II levels in humans following oral administration. Stanton *et al.* [64] showed that aliskiren inhibits the production of Ang I and II in healthy volunteers and reduces blood pressure. Gradman *et al.* [65] showed that the administration of aliskiren is as effective as the same amount of irbesartan in lowering blood pressure. Whether or not, renin inhibition by aliskiren results in protection from cardiovascular and renal diseases, similar to that seen for ACEIs and ARBs, needs to be researched.

ANGIOTENSIN CONVERTING ENZYME INHIBITORS

The principal action of ACE inhibition is a disruption of the conversion of Ang I to Ang II and consequently, inhibition of the Ang II effects, such as vasoconstriction, growth promotion and sodium reabsorption (Fig. 1). ACE primarily cleaves a C-terminal dipeptide from substrates and is also known as peptidyl dipeptidase A. Important physiological substrates of ACE are Ang I and bradykinin. Ang I is hydrolyzed by ACE to form the potent vasopressor octa-peptide Ang II. ACE hydrolyzes a wide range of polypeptide substrates, including substance P, luteinizing hormone-releasing hormone, acetyl-Ser-Asp-Lys-Pro, and neurotensin [66,67]. The specificity of ACE for Ang I is relatively low, and ACE inhibition triggers additional events as a result of protection of other peptides. ACE is also recognized as kininase II and ACEIs block the degradation of bradykinin which may also exert important effects [68].

Kinins act as endogenous vasodilators *via* stimulation of nitric oxide and release of vasodilatory prostaglandins. In the kidney, kinins contribute to the renal vasodilatory actions of ACEIs, mainly in the medullary circulation [69–73].

A recently described enzyme, termed ACE2, cleaves a single amino acid from Ang I to form Ang 1–9 and from Ang II to form Ang 1–7 [74,75]. Ang 1–7 exerts significant vasodilator and natriuretic actions that may partially counteract the effects of Ang II [76]. Despite sharing many biochemical properties with ACE, ACE2 is insensitive to classic ACEIs [74,77]. ACE2 expression is limited mainly to endothelial cells of the arteries, arterioles, and venules in the heart and kidney [74,75,77]. ACE2 is also expressed in renal tubular epithelium and vascular smooth muscle cells of the intrarenal arteries and coronary blood vessels [74,78]. Ang 1–7, an Ang fragment generated from Ang I by action of several endopeptidases, is another peptide degraded by ACE. Although, Ang 1–7 can interact with AT1 receptors, it has been suggested that the peptide is a ligand for novel receptors, which are different from AT1 receptors or AT2 receptors, and that the vasodilatory actions of Ang 1–7 are mediated by prostaglandins and nitric oxide leading to vasodilation, natriuresis and growth inhibition [79,80].

Multiple lines of evidence have suggested that an alternative pathway to the ACE exists for Ang II generation in the heart, large arteries, and the kidneys [81]. It has been well established

that in some patient treated with ACEIs, plasma levels of Ang II return to pre-treatment levels despite effective inhibition of plasma ACE [82,83]. This phenomenon has been attributed to actions of non-ACE enzymes that may convert Ang I to Ang II. For example, chymase is localized in heart, blood vessels, lungs and kidneys [84]. Takai *et al*. reported that rat vascular tissues contain ACE as the only Ang II-forming enzyme, while the vascular tissues of human, monkey, dog and hamster contain chymase in addition to ACE as Ang II forming enzymes [85]. Hollenberg *et al*. [14] also studied the response to Ang I in human and rabbits, and they observed marked species-specificity in vascular Ang II-forming pathways. They reported that although plasma Ang II formation is dependent on ACE, only 30–40% of the conversion of Ang I to Ang II in the human artery depends on this enzyme, whereas the rest depends on chymostatin. They also showed that Ang II formation in rodents appears to be almost entirely dependent on ACE, therefore, ACEIs will suppress Ang II completely in these animals, which contrasts with results in dogs, primates, and humans. Sadjadi *et al*. [86] showed that chymase activity is upregulated in the ischemic kidney of a two-kidney, one-clip renovascular hypertensive rat model. They also provided additional insight into the role of ACE-independent production of Ang II by the chymase pathway.

Hollenberg *et al.* also studied healthy, normotensive men under low salt intake [87]. They administrated Ang I at different doses. Then an ACEI, enalapril, was administered. During ACE inhibition, only the highest dose of Ang I raised plasma Ang II levels. Responses of plasma aldosterone concentration and blood pressure were in excellent accord with reduction in Ang II formation. However, the decrease in renal plasma flow was substantially less inhibited than expected, taking in account that ACE is also responsible for bradykinin degradation and thus vasodilator prostaglandins and nitric oxide accumulation. Data from this study showed that ACE inhibition led to non-uniform changes in the response to exogenous Ang I suggesting that intrarenal conversion of Ang I to Ang II also can occur by an ACE-independent pathway. The antihypertensive benefit of combining ACEI therapy with ARB therapy is most likely due to the blockade of ACE-independent pathway of Ang II generation.

ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKERS

Both ACEIs and ARBs target RAS, although their mechanisms of action differ considerably. While ACEIs currently occupy a prominent position in the therapeutic strategies used in hypertension, they are not necessarily the most logical or effective way to suppress the RAS. Blocking Ang II at the receptor levels is an attractive alternative, since ACEIs reduce but do not completely block the production of Ang II.

ARBs are specific non-peptide Ang II receptor antagonists. They work by blocking AT1 receptors at the tissue level. Several factors including the level of Ang II and the number of AT1 receptors available influence the actions of ARB. The number of AT1 receptors on the cell surface determines the magnitude of the blocker's effect. The powerful actions of intrarenal Ang II acting *via* stimulation of AT1 receptors on the vascular, glomerular, and tubular structures provide a synchronous cascade of effects contributing to the ability of the kidney to retain over 99% of the filtered sodium. The effects of Ang II not only on proximal nephron reabsorption but also on distal nephron transport function coupled with the associated actions of elevated aldosterone levels markedly increase the sodium-retaining capability of the kidney. The chronic high sodium diet has been reported to stimulate AT1 receptor mRNA expression in renal afferent arterioles [88]. Sodium depletion has been associated with increased number of AT1 receptors and an increased response to ARB. By binding AT1 receptors, an ARB decreases aldosterone, vasopressin, and catecholamine release ACE [89–94]. ARB also causes vascular vasodilation and inhibition of sodium and water reabsorption in the kidney. Collectively, these effects lead to a reduction in blood pressure [95].

Inhibition of AT1 receptors is associated with increases in renin secretion that leads to more Ang II formation. Accumulation of Ang II, as a result of ARB, can theoretically compete with ARB at the receptors and thus diminish therapeutic efficiency of the treatment. However this issue still remains controversial. Kobori *et al.* [96] have shown that intrarenal Ang II is independently regulated from plasma Ang II in Ang II-infused rat. They showed that an ARB, olmesartan, treatment decreased kidney Ang II levels while plasma Ang II concentration actually increased. In this model, kidney and urinary AGT levels were also inhibited by olmesartan and urinary AGT was closely linked to intrarenal Ang II in Ang II-infused rats.

The increase in Ang II after ARB allows stimulation of AT2 receptors. Activation of AT2 receptors is associated with increased tissue release of nitric oxide, guanylate cyclase, and tissue bradykinin [97]. In contrast to AT1 receptors, AT2 receptors have antigrowth properties and stimulate programmed cell death. Thus, the effects of AT2 receptors stimulation seem to counterbalance the effects of AT1 receptors.

Taking in account that ACEIs do not inhibit completely Ang II formation and that ARBs lead to Ang II accumulation with a possible underlying “escape mechanism,” should ACEIs and ARBs be used together? Ang II, generated by non-ACE mechanisms, could be inhibited at the receptor level by the ARB. While inhibition of the RAS *via* AT1 receptors could be strengthened, nitric oxide-dependent vasodilator pathways activated by ACEIs would remain intact. On the other hand, AT2 receptor-mediated actions could be activated by RAS shifted to AT2 receptors. Therefore, the addition of ACEIs to ARBs would be beneficial. The Randomized Evaluation of Strategies fOr Left Ventricular Dysfunction (RESOLVD) trial revealed that a combination of an ACEI and an ARB decreased blood pressure and improved the ejection fraction more than treatment with either drug alone in patients with congestive heart failure [98]. The Valsartan in Heart Failure Trial (Val-HeFT) demonstrated that a combination of an ACEI and an ARB reduced hospitalization for heart failure in patients with congestive heart failure by 30%, although no decrease in all-cause mortality was observed [99]. We are also waiting for the reports from ONgoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET) and Telmisartan Randomized AssessmeNt Study in aCEi iNtolerant patients with cardiovascular Disease (TRANSCEND). These trials are expected to provide new insights into the optimal treatment of hypertensive patients.

CONCLUSIONS

ARBs could be superior over ACEIs in terms of renal protection and as an antihypertensive agent. ARBs probably cause better inhibition of the effects of intrarenal RAS *via* AT1 receptors. However, ACEIs have a potential contribution through the stimulation of alternative vasodilation pathways. The potential beneficial effects of renin inhibitors appear to be not completely explored. Intrarenal RAS response to a combined therapy with ACEIs and ARBs may be beneficial in conditions with activated RAS.

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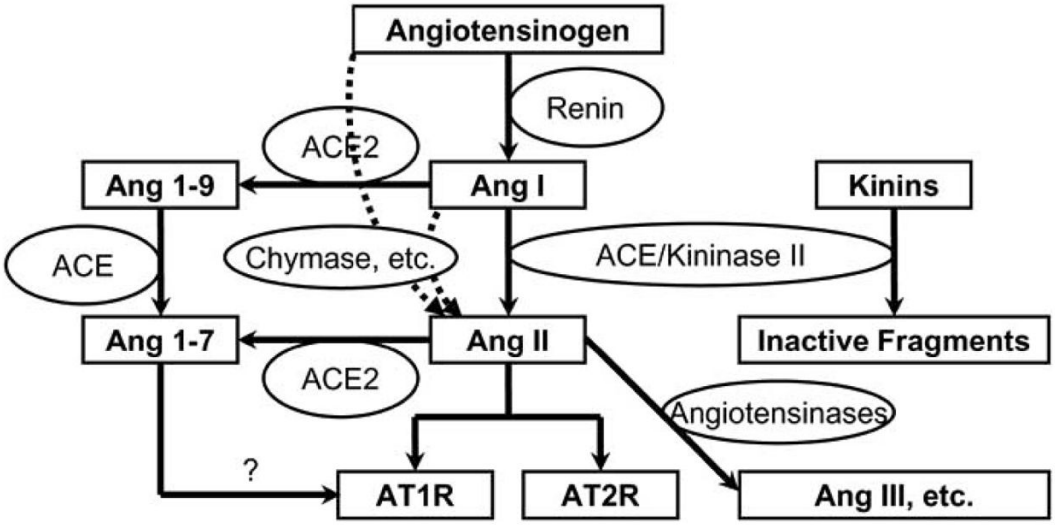

Fig. 1.
The cascade of the renin-angiotensin system and its interaction with the kinin system.