Intratubular Renin-Angiotensin System in Hypertension

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The complexity of the intrarenal renin-angiotensin (Ang) system (RAS) continues to reveal itself as evidence accumulates demonstrating its robust independent regulation in the interstitial and intratubular compartments within the kidney.1–3 Early reports demonstrating the presence of Ang II receptors on the brush border of proximal tubules suggested physiological roles.4 However, because of the abundance of degrading enzymes on the brush border, the concentrations of angiotensin peptides were considered to be relatively low. Nevertheless, the abundance of luminal Ang II receptors throughout proximal and distal nephron segments sustained interest in the luminal actions of Ang II.5,6 Tubular perfusion studies indicating that luminal Ang II alters tubular sodium and volume reabsorption rate1,5,7,8 supported an important physiological role for luminal Ang II receptors.9

A paradigm shift occurred when it was discovered that the proximal intratubular concentrations of Ang I and II were much greater than their corresponding plasma concentrations.7,10,11 In addition, when proximal tubular fluid was incubated with excess renin, the resultant formation of Ang I indicated very high angiotensinogen (AGT) substrate availability in this segment.7,12 Furthermore, tubular fluid collected from downstream segments of perfused tubules also had Ang II concentrations similar to those in nonperfused tubules, thus supporting a local origin.11 These findings, along with the demonstration that proximal tubule cells express AGT mRNA and protein,13,14 established the foundation for the existence of a robust physiologically important tubular RAS.

Intratubular Ang II Receptors and Ang II Concentrations

The principal Ang II type receptor in adult kidneys is the Ang II type 1 (AT1) receptor,9 although Ang II type 2 receptors are upregulated in certain conditions15,16 and may also play a role in renin synthesis.17 Nevertheless, overall renal AT1 receptor abundance far exceeds Ang II type 2 receptor levels5,18 and AT1 receptors are widely distributed on luminal membranes throughout the nephron segments, including proximal tubule, thick ascending limb of Loop of Henle, macula densa, distal tubule, and collecting ducts (CDs).5,19 The regulation of intrarenal AT1 receptors is complex, because vascular AT1 receptors are downregulated, whereas tubular AT1 receptors are either sustained or upregulated by elevated Ang II levels.1,6,20,21

The presence of AT1 receptors on luminal membranes of various nephron segments generated interest in the Ang II concentrations available to activate the receptors.7,22–24 Proximal tubule fluid concentrations of Ang I and Ang II are in the range of 5 to 10 pmol/mL,2,7 which are similar to renal interstitial fluid concentrations.25 The tubular Ang II concentrations remain elevated in hypertension models, including Ang II–infused hypertension,26 Goldblatt hypertension,27 and TGR(mRen2) rats,28 suggesting their sustained actions on proximal reabsorption rate. The critical importance of kidney AT1 receptors in the regulation of normal blood pressure and development of hypertension has been demonstrated by studies showing that AT1a receptors in the kidneys are essential for normal blood pressure regulation and for mediating the hypertensive response to Ang II infusions.29,30 Furthermore, AT1a knockout mice fail to develop hypertension in response to unilateral renal arterial constriction.31,32

The tubular fluid Ang II concentrations in other nephron segments have not been measured because of difficulty in collecting sufficient fluid for analysis. Measurements made from urine samples collected under conditions where the major distal nephron transport systems were pharmacologically blocked suggest CD concentrations in the range of 0.5 pmol/mL for control mice with an ∼2-fold increase in Ang II–infused hypertensive mice.33 Increased urinary excretion rates of Ang II also occur in chronic Ang II–infused rats,34,35 and these were decreased during treatment with AT1 receptor blockers although the circulating Ang II concentrations were increased.35 These recent studies indicate that distal nephron Ang II is formed locally in the tubules at concentrations that are sufficiently high to influence distal nephron transport function, which has been shown to respond to Ang I and Ang II.7,22,23 Distal nephron Ang II was shown recently to enhance the sensitivity of the “connecting tubule glomerular feedback mechanism” that communicates signals between the connecting tubule (CNT) and the afferent arteriole.36 In contrast to the macula densa tubular glomerular feedback mechanism where Ang II augments its vasoconstriction capability,5,37 the

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effect of Ang II on the CNT feedback mechanism is afferent vasodilatation.36

AT₁ receptors are also responsible for internalizing Ang II, and the presence of substantial Ang II in endosomes in both control and Ang II infused hypertensive rats supports their internalization into a protected compartment that prevents degradation of some of the internalized Ang II.20 AT₁ receptor blockade prevents the internalization of the Ang II. Intracellular Ang II may activate various signaling pathways and also contribute to fibrogenic proliferative responses and microthrombosis.38–40 Internalized Ang II may also migrate to the nucleus to exert transcriptional effects.38,41 Ang II binding sites have been shown on nuclear membranes,41,42 and colocalization with nuclear markers suggests migration of the receptor complex to the nucleus.38,43

Augmentation of Intrarenal AGT in Hypertension
The seminal findings that AGT mRNA and protein are present in proximal tubule cells generated a great deal of interest regarding AGT intrarenal function.13,44–46 Chronic Ang II infusions augment intrarenal AGT mRNA and protein in proximal tubule cells in rats and mice.13,14,47,48 This effect is mediated via activation of AT₁ receptors, because it is prevented by treatment with ARBs.48,49 Ang II also stimulates AGT production in proximal tubule cell cultures.50 Thus, chronic infusions of Ang II in rats and mice lead to an augmentation of AGT expression contributing to greater generation and intrarenal production of Ang II (Figure 1). Importantly, this process seems to be self-limiting, because higher Ang II infusions do not stimulate AGT mRNA,48 and complex signaling mechanisms are activated to prevent uncontrolled positive feedback.51,52

Because the level of AGT is close to the Michaelis-Menten constant for renin, AGT levels can also control RAS activity; thus, upregulation of AGT levels may lead to elevated Ang peptide levels.53 Studies on rat and mouse models of hypertension have documented the effect of augmented AGT in the activation of the RAS.54–58 Genetic manipulations that lead to overexpression of the AGT gene cause hypertension.55,59 In human genetic studies, a linkage has been established between the AGT gene and hypertension.60–63 Enhanced intrarenal AGT mRNA and/or protein levels occur in experimental models of hypertension, including Ang II–dependent hypertensive rats13,14,47,49 and mice,58,56,64 Dahl salt-sensitive hypertensive rats,65 and spontaneously hypertensive rats,66 as well as in kidney diseases, including diabetic nephropathy,67–69 IgA nephropathy,70–72 and radiation nephropathy.73 Thus, increased intrarenal AGT contributes to the development and progression of hypertension and may be useful as a predictor of developing kidney disease.1,74 Although clearly related to activation of AT₁ receptors,49 the mechanism by which Ang II stimulates AGT mRNA and protein is complex and seems to require interactions with inflammatory factors, including interleukin 6, and increased oxidative stress.75–77

Urinary excretion rates of AGT provide an index of intratubular RAS status and are correlated with kidney Ang II levels in Ang II–dependent hypertensive rats.78,79 Furthermore, mice overexpressing AGT only in proximal tubules
have increased urinary Ang II excretion.77 Because of its potential importance in identifying Ang II–dependent hypertension in human subjects, direct quantitative methods to measure urinary AGT using a human/mouse/rat AGT ELISA were developed recently.80,81 Using this system, urinary excretion rates of AGT have been used as an index of intrarenal RAS status in patients with chronic kidney disease,74,82,83 diabetes mellitus,84,85 and hypertension.86–88 In a cross-sectional study, we reported that urinary AGT levels are significantly greater in hypertensive patients not treated with RAS blockers compared with normotensive subjects (Figure 2). Moreover, patients treated with RAS blockers showed reduced urinary AGT levels.87 In a population study, we showed that urinary AGT levels are correlated with high blood pressure in humans.88 Urinary AGT levels were significantly correlated with systolic and diastolic blood pressures, and high correlations between urinary AGT and blood pressure were shown in male subjects, especially in black male subjects.88 These recent translational studies strengthen the hypothesis that intratubular AGT exerts a crucial role in the development and progression of hypertension and kidney disease. The augmentation of proximal tubule AGT leads to spillover into the distal nephron segments providing substrate for additional generation of Ang I and subsequent formation of Ang II (Figure 3).

**Renin and (Pro)renin Receptor in the CDs During Ang II–Dependent Hypertension**

Renin is also produced by the principal cells of CNT and cortical and medullary CDs of mouse, rat, and human kidneys.89–91 Renin colocalizes with aquaporin 2.91 In response to chronic Ang II infusions, renin mRNA and protein levels increase in CNT and CDs.91 This effect contrasts with the effect of Ang II to suppress juxtaglomerular (JG) renin,92 but is also an AT1 receptor–mediated process.93 As shown in Figure 4, the stimulation of CD renin during Ang II–dependent hypertension occurs independent of blood pressure, because both nonclipped and clipped kidneys of Goldblatt hypertensive rats exhibit augmentation of renin synthesis and renin activity in the renal medulla, which is devoid of JG cells.94 Thus, CD renin is increased by Ang II in association with increased AGT spillover from the proximal tubules.95 In hypertensive models, the increased renin is primarily active renin,94 whereas in diabetic models, the increased CD renin is primarily (pro)renin.90 There is also an enhancement of angiotensin-converting enzyme (ACE) and inhibition of ACE2 gene expression associated with decreases in intrarenal Ang 1–7 levels,96,97 suggesting that suppression of ACE2 activity contributes to augmentation of intrarenal Ang II.

**Figure 2.** Urinary AGT (uAGT), expressed as ratio of uAGT/uCreatine, in normotensive and in hypertensive patients (HTN) treated with renin-Ang system blockers (RASB) and compared with those treated with other drugs. *P*<0.05 vs normotensive; †P<0.05 vs HTN-RASB. Data derived from Kobori et al.87

**Figure 3.** Cascade of intratubular RAS in Ang II–dependent hypertension. In Ang II–dependent hypertension, the kidney maintains intrarenal Ang II formation, enhanced proximal tubule AGT formation and spillover into distal nephron segments coupled with enhancement of CD renin and stimulation of tubular ACE (refer to text for relevant references). PT indicates proximal tubule; IC, intercalated cell; PC, principal cell; AA, afferent arteriole; EA, efferent arteriole.
The (pro)renin receptor, (P)RR, a 350-amino acid protein with a single transmembrane domain that binds renin or (pro)renin, increases the catalytic activity of renin and fully activates (pro)renin.99 (P)RR activation also elicits intracellular signals via extracellular signal–regulated kinase 1 and extracellular signal–regulated kinase 2 mitogen-activated protein kinase. (P)RR has been localized in glomerular mesangial cells, subendothelium of renal arteries, podocytes, macula densa cells, distal tubules, and CDs.98,99 (P)RR is predominantly expressed at the apex of the intercalated cells.100 An example of this localization is depicted in Figure 4. Recent findings have also shown that the full length form of (P)RR can be processed intracellularly by cleavage leading to a soluble form of (P)RR that can be secreted into the plasma and consequently bind renin.101 Although the function of (P)RR or soluble (P)RR in hypertensive conditions has not been established,102 data from various models suggest its contribution to hypertension, diabetes mellitus, and associated cardiovascular and renal diseases.90,103,104 These observations are of relevance in light of CD renin upregulation in Ang II–dependent hypertensive rats91,93,94 and renin and/or (pro)renin secretion by CD cells.89,90,94

Intrarenal ACE-Derived Ang II Formation in Hypertension

ACE is responsible for most conversion of Ang I to Ang II and is expressed on endothelial cells of the vasculature, on brush border of proximal tubule cells, glomeruli, and distal nephron segments, including inner medullary CDs.6,23,105–107 ACE knockout mice display very low arterial pressures coupled with an impaired capacity to generate Ang II, which is reflected as low levels of circulating and intrarenal Ang II, high levels of circulating Ang I,108 and failure to show increases in blood pressure in response to Ang I infusions.109

As shown in Figure 6, mice treated chronically with an ACE inhibitor show markedly attenuated responses in arterial pressure and lower intrarenal Ang II levels with low-dose infusions of Ang II that elicit a slow pressor response.64 Thus, endogenous ACE-derived Ang II formation contributes to the development of high local Ang II levels and hypertension induced by chronic Ang II infusions. To further determine the ability of kidney-specific ACE to augment intrarenal Ang II content and blood pressure, mice expressing ACE exclusively in the kidneys were infused chronically with Ang I.110 Kidney-specific ACE-derived Ang II formation increased Ang II content and led to the progressive development of hypertension, indicating that intrarenal ACE is a major contributor to the development of hypertension and increased...
intrarenal Ang II levels. Indeed, ACE expression is sustained or even augmented during Ang II–dependent hypertension. 106 and other models of kidney injury. 111

Perspectives

The results obtained to date indicate that increases in circulating or local Ang II concentrations elicit a positive augmentation of intrarenal AGT mRNA and protein leading to increased secretion of AGT into the tubular fluid. Together with the sustained or increased tubular ACE levels, the augmented AGT increases intratubular Ang II, which further augments sodium transport via stimulation of AT1 receptors. The augmented AGT production and secretion increase AGT delivered to the distal nephron segments, which can interact with renin and ACE produced by principal cells of CNT and CD cells to form more Ang II and stimulate distal transport activity. In a pathophysiologic environment, inappropriate stimulation of the intratubular RAS may be an important contributor to the development and maintenance of hypertension and associated renal injury. 112 Although this positive augmentation of intrarenal angiotensin by Ang II seems to be counterintuitive to normal feedback regulation, the process is primarily a local amplification mechanism to increase intratubular Ang II, thus effecting rapid homeostatic regulation of sodium reabsorption without equivalent increases in circulating Ang II. Furthermore, there are “brakes” in the system, as described earlier, to prevent uncontrolled positive feedback. 51

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None.

References


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