Regulation of Intrarenal Angiotensin II in Hypertension

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Abstract—Intrarenal angiotensin II (Ang II) is regulated by several complex processes involving formation from both systemically delivered and intrarenally formed substrate, as well as receptor-mediated internalization. There is substantial compartmentalization of intrarenal Ang II, with levels in the renal interstitial fluid and in proximal tubule fluid being much greater than can be explained from the circulating levels. In Ang II–dependent hypertension, elevated intrarenal Ang II levels occur even when intrarenal renin expression and content are suppressed. 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 Ang II type 1 (AT1) receptor mechanism and also to sustained endogenous production of Ang II. Some of the internalized Ang II accumulates in the light and heavy endosomes and is therefore potentially available for intracellular actions. The enhanced intrarenal Ang II also exerts a positive feedback action to augment intrarenal levels of angiotensinogen (AGT) mRNA and protein, which contribute further to the increased intrarenal Ang II in hypertensive states. In addition, renal AT1 receptor protein and mRNA levels are maintained, allowing increased Ang II levels to elicit progressive effects. The increased intrarenal Ang II activity and AGT production are associated with increased urinary AGT excretion rates. The urinary AGT excretion rates show a clear relationship to kidney Ang II content, suggesting that urinary AGT may serve as an index of Ang II–dependent hypertension. Collectively, the data support a powerful role for intrarenal Ang II in the pathogenesis of hypertension. (Hypertension. 2002;39[part 2]:316-322.)

Key Words: renin-angiotensin system ■ angiotensinogen ■ receptors, angiotensin ■ hypertension, experimental

It is an honor and a privilege to be selected to present the Arthur C. Corcoran Memorial Lecture and I greatly appreciate this recognition that has been given to our research group. We all realize, however, that this year is very different for our country. The terrorist attacks have burned the date of September 11, 2001, indelibly in our minds. In the light of these tragic events, it has been difficult to move forward with the ordinary business of our lives. Compared with the sacrifices of the victims and their families, our activities have seemed irrelevant. Aside from moral and financial support, there is little that we personally can do. As scientists, however, we are comforted by the conviction that what we do collectively provides significant long-term benefits to the health and well-being of our citizens. Thus, going about our business and minimizing the disruptions is the most positive response that we can make. In doing so, however, we keep in mind that others less fortunate lost their lives or their loved ones while they were going about the ordinary business of their lives.

As a renal physiologist, receiving this Corcoran Award is particularly meaningful because Dr. Corcoran performed so many interesting studies related to renal function and the renin-angiotensin system (RAS). These and other accomplishments were reviewed by Dr. Frohlich in his Corcoran lecture paper. In perusing Corcoran’s work, one can quickly recognize that he was a pioneer in the emerging field of renal hormones. He studied the effects of renin on the kidney and participated in the discovery that renin, per se, was itself not a pressor agent, but that it had to work on a substance in the plasma that would activate a pressor factor, later identified and called angiotonin. He then performed physiological studies to demonstrate the effects of angiotonin on renal hemodynamics. His work spanned 3 decades and contributed greatly to our understanding of the pathophysiology of hypertension. My presentation is related, as it is focused on the regulation of intrarenal angiotensin II (Ang II) in hypertension. Interestingly, as we probe further into this system, we continue to get surprises that make us realize that we still do not have a complete understanding of the complex mechanisms regulating Ang II levels in the different compartments and regions in the kidney and how these are altered in hypertension.

We have seen a paradigm shift in recent years from a focus primarily on the role of the systemic RAS in the regulation of arterial pressure and in the pathophysiology of hypertension to an emphasis on local renin-angiotensin systems. Various studies have demonstrated the importance of the tissue RAS in the brain, heart, adrenal glands, and vasculature, as well as in the kidney. Indeed there is growing recognition that, in many forms of hypertension, there is a derangement in renal...
Monoclonal antibodies to the AT1 receptor have identified abundant AT1 receptors widely distributed throughout the vasculature and glomerulus and in all nephron segments, except for the thick ascending limb of the loop of Henle, cortical and medullary collecting duct cells, and glomeruli (G). Bar A, B, C = 50 μm; Bar D = 10 μm. From Harrison-Bernard et al.15

Figure 2. Renal AT1 receptor immunohistochemistry demonstrating positive staining in proximal tubules (PT), distal tubules (DT), connecting tubules (CD), and inner medullary collecting ducts (IMCD). A, Proximal tubular apical (single arrowhead) and basolateral (double arrowhead) staining. B, Higher-power magnification of inset A showing intense staining in the apical membrane of the CD cells and staining in the afferent arteriole (AA) vascular smooth muscle. Glomerulus (G). Bar A, B, C = 50 μm; Bar D = 10 μm. From Harrison-Bernard et al.15

The very high abundance of AT1 receptors in the kidney is complemented by tissue Ang II levels that are much higher than plasma levels. Compared with plasma levels, the Ang II tissue contents are much higher than can be explained on the basis of nonspecific equilibration between the plasma Ang II concentrations and the intrarenal extracellular fluid.33,26–28 In response to variations in dietary NaCl intake, there are closely associated changes in renal renin content, Ang I, and Ang II. However, the total kidney contents of Ang I and Ang II remain higher than the corresponding plasma concentrations, indicating that the intrarenal levels are not due to simple equilibration with circulating Ang II.3,26,28–30

As shown in Figure 3, Ang II kidney contents in several experimental models of angiotensin-dependent hypertension are 2 to 3 times higher than the plasma concentrations. This has been shown for 2-kidney 1-clip (2K1C) Goldblatt hypertension, Ang II–infused hypertension, and Ren2-TGR hypertension.29–31 In these models, the augmentation of intrarenal Ang II content is the consequence of several mechanisms. In addition to formation of intrarenal Ang II, the kidney accumulates Ang II from the circulation via an AT1-receptor–mediated process.31–33 Sustained elevations in circulating Ang II lead to progressive accumulation of intrarenal Ang II levels even though there is marked suppression of renin formation and release.34,35 Increased Ang II levels occur in renin-depleted kidneys of 2K1C Goldblatt hypertensive

Intrarenal Ang II Receptors

The complex and extensive actions of Ang II on renal function are due to the widespread distribution of Ang II receptors in various regions, nephron segments, and cell types within the kidney. As shown in Figure 1, there are 2 major types of Ang II receptors, type 1 (AT1) and type 2 (AT2), but the hypertensinogenic actions of Ang II are generally attributed to the AT1 receptor. Studies using polyclonal and monoclonal antibodies to the AT1 receptor have identified abundant AT1 receptors widely distributed throughout the kidney. AT1-receptor protein has been localized on vascular smooth muscle cells throughout the vasculature, including the afferent and efferent arterioles and mesangial cells.14,15 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.14–18 As illustrated in Figure 2, there is extensive luminal localization of AT1 receptors in both proximal and distal nephron segments and in both cortical and medullary regions. Interstitial cells in the medulla have abundant AT1 receptors. There is much less AT2-receptor immunostaining in adult kidneys, but it has been found in proximal tubules, collecting ducts, glomerular epithelial cells, and some of the vasculature.16–18 Similarly, AT1 mRNA has been localized to proximal convoluted and straight tubules,
Ang II–infused hypertensive rats,29,31,32 and Ren2-transgenic rats.37 Studies in Ang II–infused rats have demonstrated that the augmentation of intrarenal Ang II that occurs with chronic infusion of Ang II is dependent on an AT₁ receptor–mediated process because it can be prevented by concomitant treatment with AT₁-receptor blockers.31,33 In experiments in which Val₅-Ang II was infused into rats, it was possible to separate this isoform, using HPLC, from the endogenous Ang II isoform, which has isoleucine in the 5 position.32,33 After 2 weeks of infusion, up to two thirds of the intrarenal Ang II content was the exogenous Val₅-Ang II form. Furthermore, this accumulation was prevented by treatment with the AT₁-receptor blocker, losartan, in the drinking water.33 These data demonstrate that intrarenal accumulation of Ang II occurs via an AT₁-recepto–mediated mechanism, suggesting receptor-mediated endocytosis.

Compartmentalization of Intrarenal Ang II

The intrarenal content of Ang II is not distributed in a homogenous manner but is compartmentalized in both a regional and segmental manner.39 Ang II in the renal medulla is of interest because Ang II is thought to influence medullary hemodynamics to a greater extent than cortical hemodynamics.39,40 Receptor binding studies have demonstrated that the Ang II–receptor density is much greater in the medulla than in the cortex.41,42 Medullary Ang II levels are higher than the cortical levels in normal rats and increase further in Ang II–infused hypertensive rats.43,44 The combination of high Ang II levels in the medulla with the high density of Ang II receptors support a major role for Ang II in regulating hemodynamics and tubular function in the medulla.

Within the cortex, there is distribution of Ang II in the interstitial and tubular fluid as well as within the cells. The interstitial fluid contributes to the disproportionately high total Ang II levels. Earlier measurements of renal lymph suggested that interstitial fluid Ang II concentrations were much higher than arterial or renal venous plasma concentrations and supported the concept that Ang II is formed locally.45-46 More recent studies using microdialysis probes implanted in the renal cortex demonstrated that Ang II, as well as Ang I, concentrations in interstitial fluid are much higher than the plasma concentrations.47-49 Representative data are shown in Figure 4. Renal interstitial fluid Ang II concentrations were 3 to 5 pmoles/mL, whereas Ang I concentrations were lower, in the range of 1 pmole/mL, but still much higher than the corresponding plasma Ang I concentrations. Interestingly, angiotensin-converting enzyme (ACE) inhibition failed to lower the renal interstitial Ang II concentrations significantly, suggesting that much of the interstitial Ang II may be derived from sites not readily accessible to ACE inhibitors.49 In more recent studies, Nishiyama et al50 observed that renal interstitial Ang II levels are significantly elevated by about 2-fold in Ang II–infused rats.

Tubular Angiotensin II

In addition to the elevated Ang I and Ang II concentrations in renal interstitial fluid, proximal tubule fluid concentrations of Ang I and Ang II are much greater than the plasma concentrations.51-53 Braam et al52 showed that fluid samples collected from in vivo microperfused proximal tubule segments had Ang II concentrations similar to those measured in nonperfused tubules, demonstrating that the tubular fluid Ang II concentrations are not derived from the glomerular filtrate. It was also demonstrated that extracellular fluid volume expansion reduced plasma Ang II concentrations but did not reduce intratubular Ang II concentrations,52,54 Ang I and angiotensinogen (AGT), as well as Ang II, are present in proximal tubular fluid, thus indicating that intratubular Ang II could be formed from precursors secreted into the tubular lumen. Figure 5 shows average values for intratubular and interstitial Ang I and Ang II concentrations that have been found in anesthetized rats. Interestingly, the tubular fluid Ang I and Ang II concentrations are not reduced in several hypertensive models, including the nonclipped kidney of the Goldblatt-hypertensive rat, the Ren2-transgenic rat, and the Ang II–infused hypertensive rat, compared with control rats.56,37,55 Considering that, in all of these cases, the kidneys are markedly renin-depleted and exposed to elevated arterial pressures, the maintenance of high proximal tubular Ang I and Ang II concentrations reflects inappropriate intrarenal Ang II formation.

The Ang II concentrations in tubular fluid from other segments of the nephron remain unknown because of the technical difficulties of collecting sufficient volumes or the lack of access to these segments. However, several studies
support an important role for luminal Ang II in regulating reabsorptive function in distal nephron and collecting duct segments, as well as in proximal tubule segments. In the absence of direct data, urinary Ang II concentrations can be used as a rough approximation of distal tubule concentrations. Data from our laboratory indicate that urine Ang II concentrations in anesthetized rats are about 0.8 pmole/mL. Similar concentrations in the distal tubule or collecting duct fluid would be sufficient to exert an influence on transport function. Ang II concentrations as low as 10 fmole/mL have been reported to enhance distal tubular sodium transport. Therefore, the distal nephron represents a potentially important site for further control of sodium transport regulation by Ang II.

**Endosomal Accumulation of Ang II in Hypertension**

The demonstration that AT1 receptor blockade prevents the augmentation of intrarenal Ang II that occurs during chronic infusions of Ang II indicates a progressive AT1-receptor–mediated accumulation of Ang II in an intracellular compartment where it is protected from degradation. Until recently, however, there was no direct evidence that urine Ang II concentrations in anesthetized rats are about 0.8 pmole/mL. Similar concentrations in the distal tubule or collecting duct fluid would be sufficient to exert an influence on transport function. Ang II concentrations as low as 10 fmole/mL have been reported to enhance distal tubular sodium transport. Therefore, the distal nephron represents a potentially important site for further control of sodium transport regulation by Ang II.

**Figure 5.** Concentrations and sources of proximal tubule and interstitial AGT (Ao), Ang I, and Ang II. Ang II is also internalized into endosomes via the AT1 receptor. High interstitial Ang I and Ang II concentrations suggest local formation, but substrate may be from systemically delivered or locally produced AGT. AA indicates afferent arteriole; EA, efferent arteriole; JGA, juxtaglomerular cells.

**Figure 6.** Endosomal accumulation of Ang II in Ang II infused rats and prevention by concomitant treatment with the AT1 receptor blocker, candesartan (C). *P<0.05 versus control, †P<0.05 versus Ang II. From Zhuo et al.62

In addition, both AT1 receptors and ACE were found in these structures. ACE activity was important for the maintenance of Ang II contents in the endosomes and microvillar clefts because they were markedly reduced by acute ACE inhibition. These results demonstrate that Ang II is either formed or trafficked through intracellular endosomal compartments. However, a low salt diet, which stimulated the RAS, did not increase endosomal Ang II levels. In contrast, Zhuo et al demonstrated that chronic Ang II infusions lead to increased endosomal Ang II content. Renal cortical endosomes were harvested after 2 weeks of Ang II infusion in rats infused with Ang II and in rats concomitantly treated with candesartan, an AT1-receptor blocker. As shown in Figure 6, Ang II levels in both light and heavy endosomes were significantly increased after 2 weeks of Ang II infusion. Concurrent treatment with an AT1-receptor blocker prevented the increases in endosomal and total kidney Ang II levels. These data demonstrate that there is increased uptake and trafficking of Ang II into renal endosomes, mediated by AT1 receptors, in Ang II-dependent hypertension.

The role of the internalized Ang II remains unclear and several possibilities exist. As mentioned above, intracellular Ang II may exert cytosolic actions. Ang II could also be recycled and secreted to exert further actions by binding to Ang II receptors on the cell membrane. A particularly intriguing hypothesis is that Ang II may migrate to the nucleus to exert transcriptional effects. Recently, Chen et al transfected Chinese hamster ovary cells with an AT1 receptor fused with green fluorescent protein, which allowed visualization of trafficking of the internalized ligand-receptor complex. Ang II increased colocalization of green fluorescent protein with nuclear markers, suggesting migration of the receptor complex to the nucleus. Therefore, Ang II actions may include direct effects of intracellular Ang II to regulate nuclear signaling events.
Origins of Intrarenal Ang II

The finding that renal interstitial fluid Ang II concentrations are much higher than the plasma levels reflects substantial interstitial Ang II formation. This is easily explained because all of the components needed for Ang II generation are present within the renal interstitium.4,45,66 The importance of interstitial Ang II in regulating both hemodynamic and transport function is well established.4,67 Perfusion into peritubular capillaries of Ang I and Ang II exerts afferent and efferent arteriolar vasoconstriction, decreases in single nephron glomerular filtration rate, and increases in proximal fractional reabsorption rate.67 demonstrating diffusion of the peptides from the peritubular capillaries into the interstitium. However, the mechanisms regulating renal interstitial fluid Ang I and Ang II concentrations remain unclear. Although it has generally been thought that interstitial Ang II is of circulating origin, findings of substantial AGT mRNA and protein levels in renal proximal tubule cells have raised the possibility that some of the interstitial Ang II is derived from locally formed AGT and may not be dependent on circulating Ang II or AGT.68-70

Most of the intrarenal AGT mRNA and protein have been localized in proximal tubule cells, suggesting that AGT derived from proximal tubule cells provides the substrate for intratubular and interstitial Ang I and Ang II.23,71-75 Importantly, AGT mRNA levels and protein can be stimulated by Ang II. This paradoxical positive amplification mechanism, by which local production of substrate is enhanced by its own product, likely contributes to the progressive increases in intrarenal production of Ang II in hypertension,68,69,76 As shown in Figure 5, the AGT produced in proximal tubule cells may be directly secreted into the tubular lumen, in addition to producing its metabolites intracellularly and secreting them into the tubule lumen.74,75,77 Rohrwasser et al77 demonstrated luminal localization of AGT in proximal tubular cells in vivo and showed, in monolayer proximal tubule cell cultures, that most of the AGT was detected near the apical membrane. Proximal tubule AGT concentrations in anesthetized rats have been found to be in the range of 300 nmol/L, greatly exceeding the free Ang I and Ang II tubular fluid concentrations.44 Because of its size, it seems unlikely that much of the plasma AGT can filter across the glomerular membrane. The presence of AGT mRNA and protein in proximal tubule cells suggests that AGT is secreted directly into the tubule lumen.44,75,77-80 Ang I may be formed in the tubular lumen by renin or renin-like enzymes.71,81,82 Cultured proximal tubule cells have been found to produce renin and contain renin mRNA, thus suggesting that a low-level constitutive renin secretion may exist in proximal tubule cells. 71,81,83 In addition, low but measurable renin concentrations in proximal tubule fluid have been reported.64 Once Ang I is formed, conversion readily occurs by the ACE associated with the proximal tubule brush border.55-58 ACE has also been measured in proximal and distal tubular fluid but is more abundant in proximal tubular fluid.89 It seems clear that Ang II would have to be continuously produced or added to the proximal tubule fluid in view of the abundant angiotensinases found in the brush border.56,60 However, it remains uncertain how much of the peptides are formed intracellularly and how much are formed in the tubular fluid. It is also not clear if the AGT produced in proximal tubule cells is secreted both at luminal and basolateral sites or primarily into the tubular lumen.77,80

Augmentation of Intrarenal AGT Production

Although increased internalization of Ang II contributes to the increased intrarenal Ang II in the Ang II–infused model of hypertension, there is evidence that the overall Ang II levels are also due to Ang II–stimulated AGT production. Regardless of whether it is mediated by intracellular Ang II or activation of membrane receptors, in vivo and in vitro studies have shown that Ang II stimulates AGT mRNA.68,70,76 More recently, Kobori et al69 demonstrated that there were significant increases in intrarenal AGT protein, as well as AGT mRNA levels, in response to 2 weeks of Ang II infusion in rats fed a high-salt diet. This positive-feedback system may be responsible for sustained or enhanced generation of AGT, leading to continued intrarenal production of Ang II under conditions of elevated circulating concentrations. The intrarenally produced Ang II would be added to the Ang II that is internalized by the AT1 receptors, leading to the overall increased intrarenal Ang II contents.

Intact AGT in urine suggests its presence throughout the nephron, and, to the extent that renin and ACE are available along the nephron, there would have been Ang I generation and Ang II conversion in distal segments.77,80,91,92 Rohrwasser et al77 found that renin was present on the luminal side of connecting tubule cells in mice and human kidneys, suggesting that renin may also be secreted into the distal tubular fluid. Based on the presence of AGT in urine, it is possible that some of the proximally formed AGT that is secreted into the tubules flows into the distal nephron, allowing intraluminal Ang II formation to continue throughout the length of the nephron, with the residual AGT appearing in the urine.77,80,92 AGT has been detected at low nanomolar concentrations in urine from mice and human volunteers, and mice placed on low dietary salt intake showed increased urinary AGT levels.77 Using transgenic mice harboring the gene for human AGT fused to the kidney androgen-protein promoter, Ding et al80 demonstrated that human AGT was localized primarily to proximal tubule cells. They found abundant human AGT in mouse urine but only slight traces in the systemic circulation. More recently, Kobori et al93 evaluated the changes in urinary AGT excretion rates in Ang II–infused rats maintained on a high-salt diet to suppress basal AGT levels. In agreement with the previous study showing a stimulation of AGT mRNA levels and intrarenal AGT protein,69 this recent study demonstrated an approximately 4-fold increase in urinary AGT excretion rates in Ang II–infused rats.93 AGT was measured by both Western blot analysis and radioimmunoassay determination of generated Ang I after incubation with excess renin. Both techniques demonstrated enhanced urinary AGT levels, indicating that urinary AGT contained active AGT. Furthermore, there was a direct relationship between urinary AGT excretion rates and kidney Ang II contents. These data thus support the concept that, in angiotensin-dependent hypertension, there is increased AGT secretion by the proximal tubule cells, leading to a spillover of intact AGT into distal nephron segments. To the extent that there is available renin and ACE or other enzymes that can subserve similar functions, there would
be enhanced distal tubular formation of Ang II and increased Ang II–dependent stimulation of distal sodium reabsorption rate. Thus, it seems likely that urinary AGT concentrations or excretion rates reflect the distal nephron spillover of AGT and, accordingly, provide an index of the magnitude of the enhanced intrarenal AGT production in angiotensin-dependent hypertension.

Acknowledgments

We acknowledge the assistance provided by Debbie Olavarrieta in preparing the manuscript and figures. Research performed in the authors’ laboratories were supported by National Heart, Lung, and Blood Institute grant HL26371, by the American Heart Association (L.M.H.-B. and A.N.), the National Kidney Foundation, and Uehara Memorial Foundation (H.K.).

References

The document contains a list of scientific references related to hypotension and the renin-angiotensin system.

Some notable references include:

Regulation of Intrarenal Angiotensin II in Hypertension
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Hypertension. 2002;39:316-322
doi: 10.1161/hy0202.103821

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/39/2/316

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