Published in final edited form as:

Contrib Nephrol. 2004 ; 143: 117–130.

Renal Renin-Angiotensin System

Atsuhiko Ichihara^a, Hiroyuki Kobori^c, Akira Nishiyama^b, and L. Gabriel Navar^c
H Suzuki and T Saruta

^a Keio University School of Medicine, Tokyo

^b Kagawa Medical University, Kagawa, Japan

^c Tulane University School of Medicine, New Orleans, La., USA

Abstract

The cardinal role of the intrarenal renin-angiotensin system (RAS) in the control of sodium excretion and the pathophysiology of hypertension continues to receive increased attention. In addition to its very powerful vasoconstrictor action, angiotensin (Ang) II exerts important actions on tubular transport function and several recent studies have emphasized the potential importance of actions of angiotensin peptides on receptors localized to the luminal membranes of both proximal and distal nephron segments. Furthermore, a strong case is being developed supporting the importance of local mechanisms regulating the activity of the RAS. This is due to the fact that all components of the RAS are strongly expressed in the kidneys.

Intrarenal Localization of Components of the RAS

Angiotensinogen

In situ hybridization studies have demonstrated that the angiotensinogen gene is specifically present in the proximal tubules [1]. Angiotensinogen mRNA is expressed largely in the proximal convoluted tubules and proximal straight tubules, and only small amounts are expressed in glomeruli and vasa recta as revealed by reverse transcription and polymerase chain reaction [2]. In addition, immunohistochemical studies have showed that renal angiotensinogen protein is specifically located in the proximal convoluted tubules by immunohistochemistry [3-5]. There is strong positive immunostaining for angiotensinogen protein in proximal convoluted tubules and proximal straight tubules, and weak positive staining in glomeruli and vasa recta; however, there is no perceptible staining in distal tubules or collecting ducts [6].

Renin

The juxtaglomerular apparatus (JGA) cells have abundant expression of renin mRNA [7] and protein [8,9], and renin is primarily generated in and secreted by the JGA to the circulating system [10]. The circulating renin acts on systemic angiotensinogen and also can enter organs and contribute to the activation of the local RAS [11]. Renin mRNA and renin-like activity have also been demonstrated in proximal and distal tubular cells [12-14]. In addition, low but measurable renin concentrations in proximal tubule fluid have been reported in rats [15]. Renin has been localized to collecting duct cells as well suggesting a role in the activation of angiotensin in the distal nephron. Thus, local renin may contribute to the activation of the local RAS as a praline/autocrine factor.

Angiotensin-Converting Enzyme (ACE)

In addition to its localization on endothelial cells of the renal microvasculature, there is abundant expression of ACE mRNA and protein in brush border of proximal tubules [16,17]. ACE has also been measured in proximal and distal tubular fluid but is more plentiful in proximal tubule fluid [18].

Angiotensin II Receptors

There are two major types of angiotensin II receptors type 1 (AT1) receptors and type 2 (AT2) receptors, but there is much less AT2 receptor expression in adult kidneys [19,20]. 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 [2]. In rodents, AT1 subtypes (AT1A and AT1B receptor subtypes) mRNAs have been demonstrated in the vasculature and glomerulus and in all nephron segments [20]. The AT1A receptor mRNA is the predominant subtype in nephron segments, whereas the AT1B receptor is more abundant than AT1A receptor in the glomerulus [21].

Studies using polyclonal and monoclonal antibodies to the AT1 receptor demonstrated that AT1 receptor protein is localized on vascular smooth muscle cells throughout the vasculature, including the afferent and efferent arterioles and mesangial cells [22], and on brush border and basolateral membranes of proximal tubules, thick ascending limb epithelia, distal tubules, collecting ducts, glomerular podocytes, and macula densa cells [19,20,22]. A recent study using confocal laser microscopy has shown the immunohistochemical localization of AT1 and AT2 receptors in isolated juxtaglomerular cells containing renin granules [9]. Both AT1 and AT2 receptors were detected not only on the cell surface but also in the cytoplasm, however, AT2 receptor signals indicated a lower expression level compared to AT1 receptor signals under normal conditions. These results suggest an important role of AT receptors in the functions of the JGA.

Effects of Angiotensin II on Juxtaglomerular Apparatus

In addition to its direct vasoconstrictor effects, the RAS exerts an important modulatory influence on the magnitude of the tubuloglomerular feedback (TGF) mechanism with high angiotensin levels causing increased TGF sensitivity. Enhanced TGF activity is associated with the development of systemic hypertension in several models of hypertension including two-kidney, one-clip Goldblatt hypertension [23], one-kidney, one-clip hypertension [24], hypertensive *ren-2* transgenic rats [25] and genetic hypertensive rats [26]. It has also been observed in the remnant kidneys of prehypertensive rats [27] and the kidneys of spontaneously hypertensive rats whose perfusion pressure is normalized by aortic coarctation [28]. In these animals, administration of ACE inhibitors or AT1 receptor blockers significantly reduce the sensitivity of the TGF mechanism [23-27], and peritubular infusions of Ang I and II enhanced the TGF activity [29]. Thus, Ang II contributes to the development of hypertension through its positive modulating effects on the TGF mechanism.

Recent studies have demonstrated the presence of neuronal nitric oxide synthase (nNOS) [30], cyclooxygenase-2 (COX-2) [31], and cytochrome P₄₅₀ [32] in macula densa and adjoining ascending loop of Henle cells. Nitric oxide (NO), and arachidonic acid metabolites have been shown to exert modulating roles on the TGF mechanism as shown in figure 1. NO derived from nNOS counteracts afferent arteriolar constriction during enhanced TGF activity [33]. In addition, eNOS-derived NO and nNOS-derived NO respectively inhibit the afferent and efferent arteriolar responses to exogenous Ang II [34]. In Ang II-induced hypertension, the ability of nNOS-derived NO to counteract the TGF-mediated afferent arteriolar constriction

is reduced [35]. Ang II stimulates superoxide production in vascular smooth muscle cells through phospholipase D-dependent pathways [36], and superoxide scavenges NO to form peroxynitrite (ONOO⁻), a short-lived and less potent vasorelaxant than NO [37]. Thus, superoxide may mediate the decreased ability of nNOS-derived NO to counteract the TGF-mediated afferent arteriolar constriction in Ang II-induced hypertension. This concept is supported by recent evidence that the superoxide dismutase mimetic, tempol, restores the reduced bioavailability of nNOS-derived NO in spontaneously hypertensive rats [38]. In addition, the expression of the nNOS gene and protein in the macula densa are upregulated in angiotensinogen-gene-knockout mice [39] and AT1 receptor-deficient mice [40]. Interestingly, the enhanced ability of nNOS-derived NO to counteract the TGF-mediated afferent arteriolar constriction observed in AT1 receptor-deficient mice [41], suggests that the modulation of TGF responses by Ang II is partially due to decreased activity of macula densa nNOS.

Ang II generates arachidonic acids from phospholipids by stimulating phospholipase A₂. The cyclooxygenase pathway is a major route of arachidonic acid metabolism in the kidney [42], and the arachidonic acid metabolites generated by the COX-2 adjacent to the macula densa counteracts the TGF-mediated afferent arteriolar constriction directly and indirectly through interacting with nNOS-derived NO [43,44]. In addition, superoxide interacts with arachidonic acid metabolites to form the vasoconstrictor, isoprostane. Because Ang II stimulates superoxide production in vascular smooth muscle cells, part of the effect of Ang II to modulate the TGF responses may be through the COX-2-dependent pathways.

The cytochrome P₄₅₀ pathway is also a route of arachidonic acid metabolism in the kidney [42], and 20-HETE generated by cytochrome P₄₅₀ has a constrictor effect on afferent arterioles [45] and thus may also contribute to the exaggerated TGF response in Ang II-dependent hypertension.

Ang II inhibits renin secretion in primary culture of juxtaglomerular cells [8,46]. A recent study demonstrated unique roles of AT1 and AT2 receptors in renin synthesis and secretion from juxtaglomerular cells [9]. Increased Ang II levels in the culture medium inhibited renin secretion from juxtaglomerular cells without affecting total intracellular renin content. In the presence of AT1 receptor blockers, however, increased medium Ang II levels reduced intracellular active renin content without affecting renin secretion or total intracellular renin content, and the reduced intracellular active renin content was resumed by add-on administration of AT2 receptor blockers. In addition, AT2 receptor decreased intracellular active renin content without affecting intracellular total renin content. These results suggest that AT1 receptors inhibit renin secretion from juxtaglomerular cells, while AT2 receptors inhibit conversion of inactive to active renin (prorenin processing) in juxtaglomerular cells.

Renal Hemodynamic Regulation by Intrarenal Angiotensin II

In Ang II-induced hypertension, acute administration of the AT1 receptor blocker, losartan, significantly increases cortical blood flow, total renal plasma flow, glomerular filtration rate, and urinary sodium excretion [47]. Thus, intrarenal Ang II plays an important role in regulating renal hemodynamics. Of interest, the renal responses to acute losartan were also observed in Ang II-infused rats treated chronically with losartan, although acute losartan decreased blood pressure only slightly, demonstrating adequate systemic vascular blockade, in these animal models [47]. Therefore, substantive intrarenal actions of Ang II can be maintained even when the systemic vascular AT1 receptors are effectively blocked.

When intrarenal Ang II influences renal hemodynamics, NO plays an important role in the modulation of renal responses to intrarenal Ang II. In Ang II-induced hypertension, intrarenal production of NO is similar to that in normotensive control rats [48] with an enhanced expression of eNOS and nNOS [49]. Both endogenous and exogenous NOs counteract afferent

and efferent arteriolar constrictor responses to Ang II [48]. Although afferent arteriolar responses to Ang II were enhanced in Ang II-induced hypertension [50], the buffering effects of endogenous NO on the Ang II-induced vasoconstriction was greater in afferent arterioles than in efferent arterioles [48] and thus contributes to maintaining the renal circulation under conditions of elevated systemic Ang II levels [51].

Renal Interstitial Function of Angiotensin II

The intrarenal content of Ang II is not distributed in a homogenous manner but is compartmentalized in both a regional and segmental manner [52]. It has been reported that Ang II is present at high concentrations in renal interstitial fluid (RIF) thus contributing to the disproportionately high total Ang II levels in the kidney [52]. Earlier measurements of renal lymph suggested that RIF Ang II concentrations were much higher than arterial or renal venous plasma concentrations [53]. More recent studies assessed RIF concentrations of Ang peptides using microdialysis probes implanted in the renal cortex [54-58]. Using this procedure, several studies demonstrated that RIF concentrations of Ang I and II are much higher than the corresponding plasma concentrations [54,55] (fig. 2a). We also found that acute ACE inhibition failed to lower the RIF Ang II concentrations significantly or decreased it only a small percentage, suggesting that much of the RIF Ang II may be derived from sites not readily accessible to ACE inhibitors [54,55] (fig. 2b,c). In addition, acute extracellular volume expansion lowered the plasma Ang I and II levels but failed to lower the RIF Ang I and II concentrations. Interestingly, interstitial infusion of Ang I significantly increased the RIF Ang II concentration, and this conversion was blocked by the addition of enalaprilat to the perfusate [54]. These results demonstrate that there is ACE activity in the interstitial compartment. However, the failure of ACE inhibitors to reduce endogenous RIF Ang II concentrations substantially suggests that much of the RIF Ang II is formed at sites not readily accessible to ACE inhibitors or is formed via non-ACE-dependent pathways such as cathepsin, chymase or tonin [52]. It was also demonstrated that the RIF Ang II levels in Ang II-infused hypertensive rats are augmented above control [56] (fig. 2d). These results suggest that at least part of the augmented Ang II content in the kidney from Ang II-infused hypertensive rats is distributed to the RIF. Zhuo et al. [59] showed that Ang II levels in renal cortical endosomes and intermicrovillar clefts are markedly increased in Ang II-infused hypertensive rats and that the increases in endosomal and intermicrovillar cleft Ang II levels were prevented by concurrent administration of candesartan. These results suggest that intracellular trafficking or accumulation of circulating and/or intrarenally formed Ang II into cortical tubular endosomes are enhanced during Ang II-dependent hypertension, and that this process is mediated by AT1 receptors. It is possible that accumulation of endosomal Ang II levels may result in translocation of part of the intracellular Ang II into the renal interstitial space during the development of Ang II-induced hypertension. In addition, Ang II-induced hypertension has also been associated with increased angiotensinogen (AGT) formation in the kidney [6]. Therefore, this pathway could also lead to de novo Ang II formation and secretion into the renal interstitial space. Treatment with AT1 receptor blockers prevents the cascade involving ligand-receptor activation and internalization [59] with subsequent stimulation of angiotensinogen synthesis and release [60], leading to reductions in RIF concentration of Ang II [56] (fig. 1d).

Several studies have indicated that RIF Ang II exerts biological effects, including the regulation of microvascular tone, tubular sodium reabsorption and the TGF mechanism [42]. In Ang II-dependent hypertension, the elevated Ang II concentrations acting on vascular and tubular basolateral receptors may contribute to enhanced sodium transport and impaired pressure natriuresis as well as the increased vascular resistance [42,61,62]. Micropuncture studies by Mitchell and Navar [63] demonstrated that peritubular capillary infusion of 10^{-7} mol/l Ang II resulted in increases in fractional proximal tubular fluid reabsorption and decreases in tubule

fluid flow, stop-flow pressure, and single nephron glomerular filtration rate. These results indicate that increases in the post-glomerular interstitial Ang II concentration can enhance proximal tubular reabsorption and increase preglomerular resistance. Studies using the juxtamedullary nephron preparation demonstrated that in Ang II-infused hypertension, afferent arteriolar responsiveness to Ang II administered from the interstitial side is significantly enhanced [50]. In addition, RIF Ang II levels may play an important role in the pathogenesis of tubulointerstitial changes when levels are inappropriately elevated [64]. It is likely that elevated RIF Ang II levels contribute to Ang II-dependent hypertension via multiple effects on the vasculature and the tubules leading to vasoconstriction, sodium retention and long-term proliferative and inflammatory responses.

Tubular Function of RAS

Angiotensin II

Ang II plays an important role in regulating proximal tubular reabsorptive function primarily via activation of AT1 receptors on both basolateral and luminal membranes [65]. This effect is mediated mainly by influencing the proximal sodium-hydrogen exchanger on the luminal membrane and the sodium-bicarbonate cotransporter on the basolateral membrane. Studies in isolated proximal tubular cells showed that Ang II stimulates Na^+/H^+ exchanger via AT1 receptors [66]. Although some AT2 receptors have been confirmed on proximal tubules [20], most functional studies suggest that the major effects of Ang II on proximal tubules are via AT1 receptors [67]. Recent studies have also indicated that Ang II contributes to the regulation of distal tubular sodium reabsorption rate [68]. This effect was blocked by either saralasin or losartan indicating that this effect also involves AT1 receptor activation [68]. These findings, together with the demonstration that AT receptors are present on the luminal membranes of distal nephron segments [22,69] indicate that luminal Ang II plays an important role in the regulation not only of proximal reabsorption rate but also of distal tubular reabsorptive function [52]. Peti-Peterdi et al. [70] demonstrated that Ang II directly stimulates epithelial sodium channel activity in the cortical collecting duct via AT1R. Furthermore, epithelial sodium channel gene expression in the cortical collecting duct is upregulated by chronic infusion of Ang II [71]. Thus, Ang II exerts important effects on tubular transport rate in distal as well as in proximal tubular segments via its action on both basolateral and luminal receptors [65].

Angiotensinogen

It has been clearly established that the liver is the primary source of circulating angiotensinogen and angiotensinogen is constitutively secreted into the circulation yielding concentrations much higher than the free Ang I and Ang II concentrations. Thus, in most species including primates and rodents, the rate of Ang I formation in the systemic circulation is determined primarily by the plasma renin activity. While angiotensinogen of liver origin may be an important source of substrate for intrarenal formation of the Ang peptides, it is now well recognized that angiotensinogen mRNA and protein are present in the kidney in proximal tubule cells. This finding, together with studies showing angiotensinogen in proximal tubule fluid and in the urine have led to the concept that much, if not all, of the angiotensinogen in the tubule and urine is of renal origin.

Lalouel and colleagues [72,73] have focused on intratubular RAS. Confluent monolayers of conditionally immortalized cells of murine proximal tubules were grown on semipermeable membranes separating apical and basolateral compartments. In monolayers with verified integrity, angiotensinogen was reproducibly detected in the apical but not in the basolateral compartment [72]. In two mouse strains, angiotensinogen protein has been detected in urine. Water deprivation induced significant activation of tubular expression of angiotensinogen

[73]. These data support the hypothesis that the intratubular angiotensinogen functionally exists and is regulated by pathophysiological conditions.

Sigmund and colleagues [74-76] developed a series of interesting models of inducible hypertension. Transgenic mice were generated in which a fragment of the kidney-specific androgen-regulated protein (KAP) promoter was fused to the human angiotensinogen (hAGT) gene. Renal expression of the transgene in female mice was undetectable under basal conditions but was strongly induced by administration of testosterone. In situ hybridization demonstrated that expression of hAGT mRNA in males and testosterone-treated females was restricted to proximal tubule epithelial cells in the renal cortex. Although there was no detectable hAGT protein in plasma, it was shown in the urine, consistent with a pathway of synthesis in proximal tubule cells and release into the tubular lumen [75]. Mouse angiotensinogen and hAGT have a similar structure; however, mouse renin is species-specific and cannot cleave hAGT [77]. Therefore the transferred hAGT was inactive in these mice, and these KAP-hAGT mice were normotensive. In double transgenic mice harboring both human renin gene and kidney-specific hAGT gene or systemic hAGT gene, plasma Ang II was elevated in the systemic model but not in the kidney-specific model. Nevertheless, blood pressure was markedly elevated in both transgenic mice. Acute administration of an AT1 receptor antagonist, losartan, into the circulation lowered blood pressure in the systemic model but not in the kidney-specific model [74]. These data support the hypothesis that the tissue-specific intratubular RAS can participate in the regulation of blood pressure independently of circulating RAS.

Kobori et al. [6] recently reported that Ang II-infused rats have increases in renal angiotensinogen mRNA and protein [78], and an enhancement of urinary excretion rate of angiotensinogen [79]. Chronic Ang II infusion to normal rats significantly increased urinary excretion rate of angiotensinogen in a time- and dose-dependent manner which was associated with elevation in kidney Ang II levels. Urinary excretion rate of angiotensinogen was closely correlated with systolic blood pressure and kidney Ang II content, but not with plasma Ang II concentration. Urinary protein excretion in volume-dependent hypertensive rats was significantly increased more than in Ang II-dependent hypertensive rats; however, urinary angiotensinogen excretion was significantly lower in volume-dependent hypertensive rats than in Ang II-dependent hypertensive rats [80]. Rat angiotensinogen was detected in plasma and urine before and after the injection of hAGT. However, hAGT was detected only in the plasma collected after the administration of hAGT but was not detected in the urine in Ang II-dependent hypertensive and or sham-operated normotensive rats. The failure to detect hAGT in the urine suggests limited glomerular permeability and/or tubular degradation [80]. These data support the hypothesis that urinary angiotensinogen provides a specific index of intrarenal angiotensinogen production in Ang II-dependent hypertension.

Renin

In addition to renin formed by the JGA cells, renin is synthesized by principal cells of connecting tubules and cortical collecting ducts [72,73]. Renin expression in connecting tubules was increased by sodium restriction [72]. Water deprivation induced significant activation in the tubular expression of renin [73]. These data support the hypothesis that intratubular renin functionally exists and is also regulated by pathophysiological conditions.

Prieto-Carrasquero et al. [81] recently reported that renin or renin-like immunoreactivity was enhanced in distal nephron segments of Ang II-dependent hypertensive rats while it was decreased in JGA. Concomitant with the enhancement of proximal tubular angiotensinogen, as described above, the increases in distal renin expression may help to explain the continued intrarenal formation of Ang II, which is observed in Ang II-dependent hypertension. This continued expression may contribute to the development and progression of high arterial

pressure in this model. Such changes demonstrate the differential regulation of renin expression in distal tubular cells from that in cells of the JGA.

References

1. Ingelfinger JR, Zuo WM, Fon EA, Ellison KE, Dzau VJ. In situ hybridization evidence for angiotensinogen messenger RNA in the rat proximal tubule. An hypothesis for the intrarenal renin-angiotensin system. *J Clin Invest* 1990;85:417–423. [PubMed: 2298917]
2. Terada Y, Tomita K, Nonoguchi H, Marumo F. PCR localization of angiotensin II receptor and angiotensinogen mRNAs in rat kidney. *Kidney Int* 1993;43:1251–1259. [PubMed: 8315939]
3. Richoux JP, Cordonnier JL, Bouhnik J, Clauser E, Corvol P, Menard J, Grignon G. Immunocytochemical localization of angiotensinogen in rat liver and kidney. *Cell Tissue Res* 1983;233:439–451. [PubMed: 6616576]
4. Darby IA, Congiu M, Fernley RT, Sernia C, Coghlan JP. Cellular and ultrastructural location of angiotensinogen in rat and sheep kidney. *Kidney Int* 1994;46:1557–1560. [PubMed: 7700005]
5. Darby IA, Sernia C. In situ hybridization and immunohistochemistry of renal angiotensinogen in neonatal and adult rat kidneys. *Cell Tissue Res* 1995;281:197–206. [PubMed: 7648615]
6. Kobori H, Harrison-Bernard LM, Navar LG. Expression of angiotensinogen mRNA and protein in angiotensin II-dependent hypertension. *J Am Soc Nephrol* 2001;12:431–439. [PubMed: 11181790]
7. Ichihara A, Kobori H, Miyashita Y, Hayashi M, Saruta T. Differential effects of thyroid hormone on renin secretion, content and mRNA in juxtaglomerular cells. *Am J Physiol* 1998;274:E224–E237. [PubMed: 9486151]
8. Ichihara A, Suzuki H, Murakami M, Naitoh M, Matsumoto A, Saruta T. Interactions between angiotensin II and norepinephrine on renin release by juxtaglomerular cells. *Eur J Endocrinol* 1995;133:569–577. [PubMed: 7581987]
9. Ichihara A, Hayashi M, Hirota N, Okada H, Koura Y, Tada Y, Kaneshiro Y, Tsuganezawa H, Saruta T. Angiotensin II type 2 receptor inhibits prorenin processing in juxtaglomerular cells. *Hypertens Res* 2003;26:915–921. [PubMed: 14714584]
10. Hackenthal E, Paul M, Ganten D, Taugner R. Morphology, physiology and molecular biology of renin secretion. *Physiol Rev* 1990;70:1067–1116. [PubMed: 2217555]
11. Re RN. Intracellular renin and the nature of intracrine enzymes. *Hypertension* 2003;42:117–122. [PubMed: 12860832]
12. Yanagawa N, Capparelli AW, Jo OD, Friedal A, Barrett JD, Eggena P. Production of angiotensinogen and renin-like activity by rabbit proximal tubular cells in culture. *Kidney Int* 1991;39:938–941. [PubMed: 2067210]
13. Henrich WL, McAllister EA, Eskue A, Miller T, Moe OW. Renin regulation in cultured proximal tubular cells. *Hypertension* 1996;27:1337–1340. [PubMed: 8641745]
14. Moe OW, Ujiiie K, Star RA, Miller RT, Widell J, Alpern RJ, Henrich WL. Renin expression in renal proximal tubule. *J Clin Invest* 1993;91:774–779. [PubMed: 7680667]
15. Leyssac PP. Changes in single nephron renin release are mediated by tubular fluid flow rate. *Kidney Int* 1986;30:332–339. [PubMed: 3537444]
16. Sibony M, Gasc JM, Soubrier F, Alhenc-Gelas F, Corvol P. Gene expression and tissue localization of the two isoforms of angiotensin I converting enzyme. *Hypertension* 1993;21:827–835. [PubMed: 8388857]
17. Schulz WW, Hagler HK, Buja LM, Erdos EG. Ultrastructural localization of angiotensin I-converting enzyme (EC 3.4.15.1) and neutral metalloendopeptidase (EC 3.4.24.11) in the proximal tubule of the human kidney. *Lab Invest* 1988;59:789–797. [PubMed: 2848979]
18. Casarini DE, Boim MA, Stella RC, Krieger-Azzolini MH, Krieger JE, Schor N. Angiotensin I-converting enzyme activity in tubular fluid along the rat nephron. *Am J Physiol* 1997;272:F405–F409. [PubMed: 9087685]
19. Wang ZQ, Millatt LJ, Heiderstadt NT, Siragy HM, Johns RA, Carey RM. Differential regulation of renal angiotensin subtype AT1A and AT2 receptor protein in rats with angiotensin-dependent hypertension. *Hypertension* 1999;33:96–101. [PubMed: 9931088]

20. Miyata N, Park F, Li XF, Cowley AW Jr. Distribution of angiotensin AT1 and AT2 receptor subtypes in the rat kidney. *Am J Physiol* 1999;277:F437–F446. [PubMed: 10484527]
21. Bouby N, Hus-Citharel A, Marchetti J, Bankir L, Corvol P, Llorens-Cortes C. Expression of type 1 angiotensin II receptor subtypes and angiotensin II-induced calcium mobilization along the rat nephron. *J Am Soc Nephrol* 1997;8:1658–1667. [PubMed: 9355068]
22. Harrison-Bernard LM, Navar LG, Ho MM, Vinson GP, El-Dahr SS. Immunohistochemical localization of Ang II AT1 receptor in adult rat kidney using a monoclonal antibody. *Am J Physiol* 1997;273:F170–F177. [PubMed: 9249605]
23. Huang WC, Bell PD, Harvey D, Mitchell KD, Navar LG. Angiotensin influences on tubuloglomerular feedback mechanism in hypertensive rats. *Kidney Int* 1988;34:631–637. [PubMed: 2848974]
24. Ploth DW, Mackenzie HS. Tubuloglomerular feedback in one-kidney, one-clip hypertensive rats. *Kidney Int* 1991;32:S115–S118.
25. Mitchell KD, Mullins JJ. Ang II dependence of tubuloglomerular feedback responsiveness in hypertensive ren-2 transgenic rats. *Am J Physiol* 1995;268:F821–F828. [PubMed: 7771510]
26. Brännström K, Morsing P, Arendshorst WJ. Exaggerated tubuloglomerular feedback activity in genetic hypertension is mediated by Ang II and AT1 receptors. *Am J Physiol* 1996;270:F749–F755. [PubMed: 8928835]
27. Wilke WL, Persson AEG. Captopril and tubuloglomerular feedback in remnant kidneys of prehypertensive rats. *J Am Soc Nephrol* 1992;3:73–79. [PubMed: 1391711]
28. Dilley JR, Arendshorst WJ. Enhanced tubuloglomerular feedback activity in rats developing spontaneously hypertension. *Am J Physiol* 1984;247:F672–F679. [PubMed: 6496694]
29. Mitchell KD, Navar LG. Enhanced tubuloglomerular feedback during peritubular infusions of angiotensin I and II. *Am J Physiol* 1988;255:F383–F390. [PubMed: 3414799]
30. Mundel P, Bachmann S, Bader M, Fischer A, Kummer W, Mayer B, Kriz W. Expression of nitric oxide synthase in kidney macula densa cells. *Kidney Int* 1992;42:1017–1019. [PubMed: 1280698]
31. Harris RC, McKanna JA, Akai Y, Jacobson HR, Dubois RN, Breyer MD. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *J Clin Invest* 1994;94:2504–2510. [PubMed: 7989609]
32. Ito O, Alonso-Galicia M, Hopp KA, Roman RJ. Localization of cytochrome P-450 4A isoforms along the rat nephron. *Am J Physiol* 1998;274:F395–F404. [PubMed: 9486235]
33. Ichihara A, Navar LG. Neuronal NOS contributes to biphasic autoregulatory response during enhanced TGF activity. *Am J Physiol* 1999;277:F113–F120. [PubMed: 10409304]
34. Ichihara A, Inscho EW, Imig JD, Navar LG. Neuronal nitric oxide synthase modulates rat renal microvascular function. *Am J Physiol* 1998;274:F516–F524. [PubMed: 9530268]
35. Ichihara A, Imig JD, Navar LG. Neuronal nitric oxide synthase-dependent afferent arteriolar function in angiotensin II-induced hypertension. *Hypertension* 1999;33:462–466. [PubMed: 9931148]
36. Touyz RM, Schiffrin EL. Ang II-stimulated superoxide production is mediated via phospholipase D in human vascular smooth muscle cells. *Hypertension* 1999;34:976–982. [PubMed: 10523394]
37. Liu S, Beckman JS, Ku DD. Peroxynitrite, a product of superoxide and nitric oxide, produces coronary vasorelaxation in dogs. *J Pharmacol Exp Ther* 1994;268:1114–1121. [PubMed: 8138924]
38. Ichihara A, Hayashi M, Hirota N, Saruta T. Superoxide inhibits neuronal nitric oxide synthase influences on afferent arterioles in spontaneously hypertensive rats. *Hypertension* 2001;37:630–634. [PubMed: 11230347]
39. Kihara M, Umemura S, Kadota T, Yabana M, Tamura K, Nyuui N, Ogawa N, Murakami K, Fukamizu A, Ishii M. The neuronal isoform of constitutive nitric oxide synthase is up-regulated in the macula densa of angiotensinogen gene-knockout mice. *Lab Invest* 1997;76:285–294. [PubMed: 9042165]
40. Kihara M, Umemura S, Sugaya T, Toya Y, Yabana M, Kobayashi S, Tamura K, Kadota T, Kishida R, Murakami K, Fukamizu A, Ishii M. Expression of neuronal type nitric oxide synthase and renin in the juxtaglomerular apparatus of angiotensin type-1a receptor gene-knockout mice. *Kidney Int* 1998;53:1585–1593. [PubMed: 9607188]
41. Ichihara A, Hayashi M, Koura Y, Tada Y, Sugaya T, Hirota N, Saruta T. Blunted tubuloglomerular feedback by absence of angiotensin type 1A receptor involves neuronal NOS. *Hypertension* 2002;40:934–939. [PubMed: 12468582]

42. Navar LG, Inscho EW, Majid DSA, Imig JD, Harrison-Bernard LM, Mitchell KD. Paracrine regulation of the renal microcirculation. *Physiol Rev* 1996;76:425–536. [PubMed: 8618962]
43. Ichihara A, Imig JD, Inscho EW, Navar LG. Cyclooxygenase-2 participates in tubular flow-dependent afferent arteriolar tone: Interaction with neuronal NOS. *Am J Physiol* 1998;275:F605–F612. [PubMed: 9755132]
44. Ichihara A, Imig JD, Navar LG. Cyclooxygenase-2 modulates afferent arteriolar responses to increases in pressure. *Hypertension* 1999;34:843–847. [PubMed: 10523371]
45. Imig JD, Zou AP, Stec DE, Harder DR, Falck JR, Roman RJ. Formation and actions of 20-hydroxyeicosatetraenoic acid in the renal microcirculation. *Am J Physiol* 1996;270:R217–R227. [PubMed: 8769805]
46. Ichihara A, Suzuki H, Matsukawa S, Kumagai K, Murakami M, Naitoh M, Ichikawa M, Matsumoto A, Saruta T. Different role of angiotensin II type 1 and type 2 receptors in renin release by interaction of angiotensin II and renal sympathetic nerve. *Clin Exp Nephrol* 1999;3:89–95.
47. Wang CT, Zou LX, Navar LG. Renal responses to AT1 blockade in angiotensin II-induced hypertensive rats. *J Am Soc Nephrol* 1997;8:535–542. [PubMed: 10495782]
48. Ichihara A, Imig JD, Inscho EW, Navar LG. Interactive nitric oxide-angiotensin II influences on renal microcirculation in angiotensin II-induced hypertension. *Hypertension* 1998;31:1255–1260. [PubMed: 9622138]
49. Navar LG, Ichihara A, Chin SY, Imig JD. Nitric oxide-angiotensin II interactions in angiotensin II-dependent hypertension. *Acta Physiol Scand* 2000;168:139–147. [PubMed: 10691792]
50. Ichihara A, Inscho EW, Imig JD, Michel RE, Navar LG. Role of renal nerves in afferent arteriolar reactivity in angiotensin-induced hypertension. *Hypertension* 1997;29:442–449. [PubMed: 9039140]
51. Chin SY, Wang CT, Majid DSA, Navar LG. Renoprotective effects of nitric oxide in angiotensin II-induced hypertension in the rat. *Am J Physiol* 1998;274:F876–F882. [PubMed: 9612324]
52. Navar LG, Harrison-Bernard LM, Nishiyama A, Kobori H. Regulation of intrarenal angiotensin II in hypertension. *Hypertension* 2002;39:316–322. [PubMed: 11882566]
53. Wilcox CS, Dzau VJ. Effect of captopril on the release of the components of the renin-angiotensin system into plasma and lymph. *J Am Soc Nephrol* 1992;2:1241–1250. [PubMed: 1317225]
54. Nishiyama A, Seth DM, Navar LG. Renal interstitial fluid angiotensin I and angiotensin II concentrations during local angiotensin-converting enzyme inhibition. *J Am Soc Nephrol* 2002;13:2207–2212. [PubMed: 12191964]
55. Nishiyama A, Seth DM, Navar LG. Renal interstitial fluid concentrations of angiotensins I and II in anesthetized rats. *Hypertension* 2002;39:129–134. [PubMed: 11799091]
56. Nishiyama A, Seth DM, Navar LG. AT1 receptor-mediated augmentation of renal interstitial fluid angiotensin II in angiotensin II-induced hypertension. *J Hypertens* 2003;21:1897–1903. [PubMed: 14508196]
57. Siragy HM, Howell NL, Ragsdale NV, Carey RM. Renal interstitial fluid angiotensin: Modulation by anesthesia, epinephrine, sodium depletion and renin inhibition. *Hypertension* 1995;25:1021–1024. [PubMed: 7737709]
58. Siragy HM, Carey RM. Protective role of the angiotensin AT2 receptor in a renal wrap hypertension model. *Hypertension* 1999;33:1237–1242. [PubMed: 10334818]
59. Zhuo JL, Imig JD, Hammond TG, Orenge S, Benes E, Navar LG. Ang II accumulation in rat renal endosomes during Ang II-induced hypertension: Role of AT(1) receptor. *Hypertension* 2002;39:116–121. [PubMed: 11799089]
60. Kobori H, Navar LG. Angiotensin II type 1 receptor-dependent augmentation of intrarenal angiotensinogen in angiotensin II-infused hypertensive rats (abstract). *Hypertension* 2003;42:414.
61. Wang CT, Chin SY, Navar LG. Impairment of pressure-natriuresis and renal autoregulation in Ang II-infused hypertensive rats. *Am J Physiol* 2000;279:F319–F325.
62. Wang CT, Navar LG, Mitchell KD. Proximal tubular fluid angiotensin II levels in angiotensin II-induced hypertensive rats. *J Hypertens* 2003;21:353–360. [PubMed: 12569266]
63. Mitchell KD, Navar LG. Superficial nephron responses to peritubular capillary infusions of angiotensins I and II. *Am J Physiol* 1987;252:F818–F824. [PubMed: 3034074]

64. Kim S, Iwao H. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacol Rev* 2000;52:11–34. [PubMed: 10699153]
65. Navar LG, Kobori H, Prieto-Carrasquero MC. Intrarenal angiotensin II and hypertension. *Curr Hypertens Rep* 2003;5:135–143. [PubMed: 12642013]
66. Saccomani G, Mitchell KD, Navar LG. Angiotensin II stimulation of Na⁺-H⁺ exchange in proximal tubule cells. *Am J Physiol* 1990;258:F1188–F1195. [PubMed: 2159715]
67. Navar LG, Mitchell KD, Harrison-Bernard LM, Kobori H, Nishiyama A. Intrarenal angiotensin II levels in normal and hypertensive states. *J Renin Angiotensin Aldosterone Syst* 2001;2:S176–S184.
68. Wang T, Giebisch G. Effects of angiotensin II on electrolyte transport in the early and late distal tubule in rat kidney. *Am J Physiol* 1996;271:F143–F149. [PubMed: 8760255]
69. Harrison-Bernard LM, Zhuo J, Kobori H, Ohishi M, Navar LG. Intrarenal AT1 receptor and ACE binding in Ang II-induced hypertensive rats. *Am J Physiol* 2002;281:F19–F25.
70. Peti-Peterdi J, Warnock DG, Bell PD. Angiotensin II directly stimulates ENaC activity in the cortical collecting duct via AT(1) receptors. *J Am Soc Nephrol* 2002;13:1131–1135. [PubMed: 11960999]
71. Beutler KT, Masilamani S, Turban S, Nielsen J, Brooks HL, Ageloff S, Fenton RA, Packer RK, Knepper MA. Long-term regulation of ENaC expression in kidney by angiotensin II. *Hypertension* 2003;41:1143–1150. [PubMed: 12682079]
72. Rohrwasser A, Morgan T, Dillon HF, Zhao L, Callaway CW, Hillas E, Zhang S, Cheng T, Inagami T, Ward K, Terreros DA, Lalouel JM. Elements of a paracrine tubular renin-angiotensin system along the entire nephron. *Hypertension* 1999;34:1265–1274. [PubMed: 10601129]
73. Lantelme P, Rohrwasser A, Gociman B, Hillas E, Cheng T, Petty G, Thomas J, Xiao S, Ishigami T, Herrmann T, Terreros DA, Ward K, Lalouel JM. Effects of dietary sodium and genetic background on angiotensinogen and renin in mouse. *Hypertension* 2002;39:1007–1014. [PubMed: 12019284]
74. Davisson RL, Ding Y, Stec DE, Catterall JF, Sigmund CD. Novel mechanism of hypertension revealed by cell-specific targeting of human angiotensinogen in transgenic mice. *Physiol Genomics* 1999;1:3–9. [PubMed: 11015555]
75. Ding Y, Davisson RL, Hardy DO, Zhu LJ, Merrill DC, Catterall JF, Sigmund CD. The kidney androgen-regulated protein promoter confers renal proximal tubule cell-specific and highly androgen-responsive expression on the human angiotensinogen gene in transgenic mice. *J Biol Chem* 1997;272:28142–28148. [PubMed: 9346970]
76. Ding Y, Sigmund CD. Androgen-dependent regulation of human angiotensinogen expression in KAP-hAGT transgenic mice. *Am J Physiol* 2001;280:F54–F60.
77. Fukamizu A, Sugimura K, Takimoto E, Sugiyama F, Seo MS, Takahashi S, Hatae T, Kajiwara N, Yagami K, Murakami K. Chimeric renin-angiotensin system demonstrates sustained increase in blood pressure of transgenic mice carrying both human renin and human angiotensinogen genes. *J Biol Chem* 1993;268:11617–11621. [PubMed: 8505294]
78. Kobori H, Harrison-Bernard LM, Navar LG. Enhancement of angiotensinogen expression in angiotensin II-dependent hypertension. *Hypertension* 2001;37:1329–1335. [PubMed: 11358949]
79. Kobori H, Harrison-Bernard LM, Navar LG. Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. *Kidney Int* 2002;61:579–585. [PubMed: 11849399]
80. Kobori H, Nishiyama A, Harrison-Bernard LM, Navar LG. Urinary angiotensinogen as an indicator of intrarenal angiotensin status in hypertension. *Hypertension* 2003;41:42–49. [PubMed: 12511528]
81. Prieto-Carrasquero MC, Harrison-Bernard LM, Seth DL, Navar LG. Distal tubule renin regulation in angiotensin II-induced hypertension. *FASEB J* 2002;16:A494.

Fig. 1.

Pivotal roles of angiotensin type 1 (AT1) receptors in the modulation of tubuloglomerular feedback responses. AA, arachidonic acid; ADMA, asymmetric, N^G-dimethyl-L-arginine; ATP, adenosine 5'-triphosphate; cGMP, guanosine 3',5'-cyclic monophosphate; COX-2, cyclooxygenase-2; CYP450, cytochrome P₄₅₀; eNOS, endothelial nitric oxide synthase; 20HETE, 20-hydroxyeicosatetraenoic acid; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; ONOO⁻, peroxynitrite; PGs, prostaglandins; PL, phospholipid; PLA₂, phospholipase A₂; PLC, phospholipase C; PLD, phospholipase D.

Fig. 2.

a Comparison of Ang I and II levels in plasma, total kidney and RIF. RIF concentrations of Ang I and II were calculated based on equilibrium rates determined in vitro [data derived from 55]. **b** Effects of intra-arterial infusion of enalaprilat (7.5 $\mu\text{mol/kg/min}$) on RIF concentrations of Ang II [data derived from 55]. **c** Effects of interstitial infusion of perindoprilat (10 mmol/l in perfusate) on RIF concentrations of Ang II [data derived from 54]. * $p < 0.05$ vs. **c**. **d** RIF concentrations of Ang II in normal rats (open bars), Ang II-infused hypertensive rats (hatched bars), and Ang II-infused rats treated with candesartan cilexetil (solid bars). * $p < 0.05$ vs. vehicle-infused rats. † $p < 0.05$ vs. Ang II-infused rats [data derived from 56].