

The Increasing Complexity of the Intratubular Renin-Angiotensin System

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The presence of receptors for angiotensin II (AII) on the luminal membranes of various nephron segments has been well established for many decades.^{1,2} Originally their function remained unclear because tubular fluid AII concentrations were thought to be quite low due to the presence of various degrading enzymes on the brush border of proximal tubular cells. However, a series of reports in the 1990s demonstrated that the proximal tubular concentrations of AI and AII are in the nanomolar range and much higher than can be explained by tubular fluid reabsorption or equilibration with the circulating levels.^{3,4} These findings led to a paradigm shift in our concepts regarding the role of luminal AII receptors in various nephron segments, and it is now well accepted that intraluminal AII and other angiotensin peptides exert various actions on transport systems in essentially all nephron segments predominantly through activation of AT1 receptors.^{5–10}

In further studies, proximal tubular fluid samples were incubated with excess renin to determine substrate availability. The resultant AI concentrations demonstrated that the proximal tubular fluid angiotensinogen (AGT) concentrations are also very high¹¹ and greater than the circulating concentrations, indicating that it is unlikely the tubular AGT concentrations are derived from filtered AGT, in particular considering the limited permeability of the AGT because of its large size.¹¹ Using *in situ* perfusion of proximal tubules with artificial tubular fluid with the delivery of filtrate blocked, Braam *et al.*⁴ collected the tubular fluid from downstream segments and found these also had elevated AII concentrations in the nanomolar range, supporting tubular secretion of AII or its precursors. Studies on isolated perfused tubules from S2 segments indicated that AII is produced intracellularly and secreted preformed into the tubular lumen, supporting the presence of intact AGT in isolated proximal tubule segments.¹² These findings, along with

clear evidence for the presence of AGT mRNA and protein in proximal tubular cells,^{13–15} provided the foundation for the concept that the intratubular AII concentrations are derived primarily from locally synthesized substrate. The local production of AGT in the proximal tubule was supported by Terada *et al.*,¹⁴ who demonstrated the presence of a large signal for AGT mRNA in microdissected proximal convoluted and straight tubules. *In vitro* studies have also consistently demonstrated mRNA encoding AGT in proximal tubular cell lines extracted from proximal tubule segments.^{15,16} However, Pohl *et al.*¹⁷ recently reported predominant localization of mRNA encoding AGT to S3 segments.

The findings of kidney tubular mRNA encoding AGT notwithstanding, the kidney's ability to produce AGT is dwarfed by that of the liver, the organ primarily responsible for the maintenance of circulating AGT concentrations. In addition, it is well recognized that filtered AGT can be taken up by proximal tubule scavenger receptors such as megalin and cubilin,^{17,18} or by a putative-specific AGT receptor on proximal tubular epithelial cells.¹⁹ Thus, various studies demonstrated that AGT is internalized by these transporters, which is consistent with observations that megalin binds and internalizes AII.¹⁸

The important question of the quantitative contribution of either source to total renal AGT content remained unresolved because of the lack of definitive tools needed to provide the answer. The paper by Matsusaka *et al.*²⁰ in this issue of *JASN* provides critical data obtained in mice that had either liver AGT or the kidney AGT mRNA expression deleted genetically. Their results show that kidney-specific AGT-null mice have kidney AGT protein and AII levels similar to those in control mice, whereas the liver-specific AGT-null mice have markedly lower or undetectable levels of AGT in the proximal tubules and kidney AII. Further studies confirmed that AGT uptake by proximal tubular cells is mediated by megalin and that disruption of the filtration barrier markedly increases tubular and urinary AGT protein. The results thus indicate that, in normal nonhypertensive mice, most of the AGT protein present in proximal tubules is of liver origin. This finding is not unexpected, as the kidney levels of AGT protein in normal rats and mice are rather low, especially in comparison to the enhanced levels that develop in response to chronic AII infusions.^{21–23} In addition, AGT synthesis and secretion is a constitutive process, so cells expressing mRNA encoding AGT probably do not store the protein, but secrete it into the tubular fluid.

To the extent that the proximal tubule AII concentrations in mice are as high as have been found in rat proximal tubular fluid,²⁴ the results presented by Matsusaka *et al.* pose the interesting dilemma regarding the cellular pathways by which the increased intratubular AII levels are derived from the AGT taken up from the circulation by the proximal tubular cells. AII may be formed intracellularly from AGT reabsorbed from either the renal interstitium or tubular fluid, or the internalized AGT may be resecreted into the tubular lumen where

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peptide generation takes place. Thus, a major experimental and conceptual challenge that emerges from these findings is to understand the cellular and molecular trafficking that occurs to accomplish these formidable processes.

Because megalin is a scavenger receptor, its prevailing function has been generally considered to be the uptake of peptides and proteins that cross the glomerular filtration barrier and targeting them to degradation by the lysosomal pathway.²⁵ However, new advances indicate that megalin may also act as a signaling molecule.^{26,27} Thus, the internalization of AGT may result in intracellular formation of AI and AII and/or signaling. These intracellularly generated angiotensins may be secreted into proximal tubular fluid. Although some of the internalized AGT may be resecreted into the proximal tubular fluid, an alternative pathway is that a part of the AGT-megalín complex is released by the megalín ectodomain shedding, leading to increases in AGT and AII concentrations in proximal tubular fluid.

A critical issue not addressed by Matsusaka *et al.* is what the quantitative and functional contribution of either source of AGT is during hypertensive states or renal diseases.²⁸ Studies in chronic AII-infused animals showed increased AGT protein accumulation in all segments of the proximal tubule.^{21,23,29} These results led to the hypothesis of a robust and independent tubular renin-angiotensin system where augmentation of proximal tubular AGT leads to increased intratubular secretion of AGT and greater intratubular formation of AI and AII to act on luminal AT₁ receptors and stimulate tubular transport.²⁴ Although Matsusaka *et al.* confirm that AGT in the urine is derived, in large part, from the mRNA encoding AGT present in latter segments of the proximal tubules, these levels are extremely low and can be markedly upregulated to yield many fold greater increases in urinary AGT concentrations as found in AII-infused animals.^{21,24,30} Urinary AII levels are markedly augmented when there is a stimulus for AGT overexpression in renal proximal tubules, demonstrating that AGT produced in proximal tubules contributes to intrarenal AII elevation and increased urinary AGT excretion.³¹ Thus, the study by Matsusaka *et al.* does not address contributions of intrarenal mRNA encoding AGT and locally synthesized AGT to tubular AII in AII-dependent hypertension or in various kidney diseases.²⁸

The augmentation of proximal tubule AGT protein during hypertensive states cannot be attributed to megalín uptake, because it occurs despite substantial megalín downregulation by activated Extracellular signal regulated kinase1/2 and, in many instances, in advance of overt proteinuria.³² Furthermore, deoxycorticosterone-salt rats have proteinuria but do not have increased urinary AGT.³³ Thus, the data support an important role of proximal tubule AGT, generated locally in hypertension. In this setting, it is possible that mRNA encoding AGT extends beyond the S3 segment to the convoluted segments as has been shown *in vitro*. Furthermore, these *in vitro* experiments show that AII and other factors including proinflammatory cytokines such as IL-6 and IFN- γ , glucocorticoids,

reactive oxygen species, and glucose synergistically or independently stimulate the expression of mRNA encoding AGT mainly through NF- κ B and JAK-STAT pathways in cultured proximal tubular cells.^{34,35}

In summary, the report by Matsusaka *et al.* makes an important contribution to our understanding of the ever increasing complexity of the intrarenal renin-angiotensin system by demonstrating that, in nonstimulated states, liver AGT and not kidney AGT, is mainly responsible for kidney AII and proximal tubule AGT. However, the study does not address the influence of augmented mRNA encoding AGT in the kidneys that occurs in AII-dependent hypertension, diabetes, and certain other renal diseases.^{24,28}

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DISCLOSURES

None.

REFERENCES

- Douglas JG, Hopfer U: Novel aspect of angiotensin receptors and signal transduction in the kidney. *Annu Rev Physiol* 56: 649–669, 1994
- Navar LG, Harrison-Bernard LM, Imig JD, Mitchell KD: Renal actions of angiotensin II and AT₁ receptor blockers. In: *Angiotensin II Receptor Antagonists*, edited by Epstein M, Brunner HR, Philadelphia, Hanley & Belfus, Inc., 2000, p 189
- Seikaly MG, Arant BS Jr, Seney FD Jr: Endogenous angiotensin concentrations in specific intrarenal fluid compartments of the rat. *J Clin Invest* 86: 1352–1357, 1990
- Braam B, Mitchell KD, Fox J, Navar LG: Proximal tubular secretion of angiotensin II in rats. *Am J Physiol* 264: F891–F898, 1993
- Navar LG, Harrison-Bernard LM, Wang C-T, Cervenka L, Mitchell KD: Concentrations and actions of intraluminal angiotensin II. *J Am Soc Nephrol* 10[Suppl 11]: S189–S195, 1999
- Baum M, Quigley R, Quan A: Effect of luminal angiotensin II on rabbit proximal convoluted tubule bicarbonate absorption. *Am J Physiol* 273: F595–F600, 1997
- Zhuo JL, Li XC, Garvin JL, Navar LG, Carretero OA: Intracellular ANG: Intracellular ANG II induces cytosolic Ca²⁺ mobilization by stimulating intracellular AT₁ receptors in proximal tubule cells. *Am J Physiol Renal Physiol* 290: F1382–F1390, 2006
- 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* 13: 1131–1135, 2002
- Kovács G, Peti-Peterdi J, Rosivall L, Bell PD: Angiotensin II directly stimulates macula densa Na-2Cl-K cotransport via apical AT(1) receptors. *Am J Physiol Renal Physiol* 282: F301–F306, 2002

10. Komlosi P, Fuson AL, Fintha A, Peti-Peterdi J, Rosivall L, Warnock DG, Bell PD: Angiotensin I conversion to angiotensin II stimulates cortical collecting duct sodium transport. *Hypertension* 42: 195–199, 2003
11. Navar LG, Imig JD, Zou L, Wang C-T: Intrarenal production of angiotensin II. *Semin Nephrol* 17: 412–422, 1997
12. Zhou Y, Boron WF: Role of endogenously secreted angiotensin II in the CO₂-induced stimulation of HCO₃ reabsorption by renal proximal tubules. *Am J Physiol Renal Physiol* 294: F245–F252, 2008
13. Darby IA, Congiu M, Fernley RT, Sernia C, Coghlan JP: Cellular and ultrastructural location of angiotensinogen in rat and sheep kidney. *Kidney Int* 46: 1557–1560, 1994
14. Terada Y, Tomita K, Nonoguchi H, Marumo F: PCR localization of angiotensin II receptor and angiotensinogen mRNAs in rat kidney. *Kidney Int* 43: 1251–1259, 1993
15. 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* 85: 417–423, 1990
16. Satou R, Gonzalez-Villalobos RA, Miyata K, Ohashi N, Urushihara M, Acres OW, Navar LG, Kobori H: IL-6 augments angiotensinogen in primary cultured renal proximal tubular cells. *Mol Cell Endocrinol* 311: 24–31, 2009
17. Pohl M, Kaminski H, Castrop H, Bader M, Himmerkus N, Bleich M, Bachmann S, Theilig F: Intrarenal renin angiotensin system revisited: Role of megalin-dependent endocytosis along the proximal nephron. *J Biol Chem* 285: 41935–41946, 2010
18. Gonzalez-Villalobos R, Klassen RB, Allen PL, Navar LG, Hammond TG: Megalin binds and internalizes angiotensin II. *Am J Physiol Renal Physiol* 288: F420–F427, 2005
19. Pan N, Luo J, Kaiser SJ, Frome WL, Dart RA, Tewksbury DA: Specific receptor for angiotensinogen on human renal cells. *Clin Chim Acta* 373: 32–36, 2006
20. Matsusaka T, Niimura F, Shimizu A, Pastan I, Saito A, Kobori H, Nishiyama A, Ichikawa I: Liver angiotensinogen is the primary source of renal angiotensin II. *J Am Soc Nephrol* 23: 1181–1189, 2012
21. Kobori H, Prieto-Carrasquero MC, Ozawa Y, Navar LG: AT1 receptor mediated augmentation of intrarenal angiotensinogen in angiotensin II-dependent hypertension. *Hypertension* 43: 1126–1132, 2004
22. Kobori H, Harrison-Bernard LM, Navar LG: Expression of angiotensinogen mRNA and protein in angiotensin II-dependent hypertension. *J Am Soc Nephrol* 12: 431–439, 2001
23. Gonzalez-Villalobos RA, Seth DM, Satou R, Horton H, Ohashi N, Miyata K, Katsurada A, Tran DV, Kobori H, Navar LG: Intrarenal angiotensin II and angiotensinogen augmentation in chronic angiotensin II-infused mice. *Am J Physiol Renal Physiol* 295: F772–F779, 2008
24. Navar LG, Kobori H, Prieto MC, Gonzalez-Villalobos RA: Intratubular renin-angiotensin system in hypertension. *Hypertension* 57: 355–362, 2011
25. Christensen EI, Verroust PJ, Nielsen R: Receptor-mediated endocytosis in renal proximal tubule. *Pflügers Arch* 458: 1039–1048, 2009
26. Biemesderfer D: Regulated intramembrane proteolysis of megalin: Linking urinary protein and gene regulation in proximal tubule? *Kidney Int* 69: 1717–1721, 2006
27. Christ A, Terryn S, Schmidt V, Christensen EI, Huska MR, Andrade-Navarro MA, Hübner N, Devuyst O, Hammes A, Willnow TE: The soluble intracellular domain of megalin does not affect renal proximal tubular function in vivo. *Kidney Int* 78: 473–477, 2010
28. Kobori H, Nangaku M, Navar LG, Nishiyama A: The intrarenal renin-angiotensin system: From physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev* 59: 251–287, 2007
29. Gonzalez-Villalobos RA, Satou R, Ohashi N, Semprun-Prieto LC, Katsurada A, Kim C, Upchurch GM, Prieto MC, Kobori H, Navar LG: Intrarenal mouse renin-angiotensin system during ANG II-induced hypertension and ACE inhibition. *Am J Physiol Renal Physiol* 298: F150–F157, 2010
30. Lara LS, McCormack M, Semprun-Prieto LC, Shenouda S, Majid DS, Kobori H, Navar LG, Prieto MC: AT1 receptor-mediated augmentation of angiotensinogen, oxidative stress, and inflammation in ANG II-salt hypertension. *Am J Physiol Renal Physiol* 302: F85–F94, 2012
31. Ying J, Stuart D, Hillas E, Gociman BR, Ramkumar N, Lalouel JM, Kohan DE: Overexpression of mouse angiotensinogen in renal proximal tubule causes salt-sensitive hypertension in mice. *Am J Hypertens* 25: 684–689, 2012
32. Tojo A, Onozato ML, Kurihara H, Sakai T, Goto A, Fujita T: Angiotensin II blockade restores albumin reabsorption in the proximal tubules of diabetic rats. *Hypertens Res* 26: 413–419, 2003
33. Kobori H, Nishiyama A, Harrison-Bernard LM, Navar LG: Urinary angiotensinogen as an indicator of intrarenal angiotensin status in hypertension. *Hypertension* 41: 42–49, 2003
34. Satou R, Miyata K, Gonzalez-Villalobos RA, Ingelfinger JR, Navar LG, Kobori H: Interferon- γ biphasically regulates angiotensinogen expression via a JAK-STAT pathway and suppressor of cytokine signaling 1 (SOCS1) in renal proximal tubular cells. *FASEB J* 26: 1821–1830, 2012
35. Navar LG, Prieto MC, Satou R, Kobori H: Intrarenal angiotensin II and its contribution to the genesis of chronic hypertension. *Curr Opin Pharmacol* 11: 180–186, 2011

See related article, "Liver Angiotensinogen Is the Primary Source of Renal Angiotensin II," on pages 1181–1189.

Renal Nerves and CKD: Is Renal Denervation the Answer?

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A role for renal nerves in the regulation of renal function has been recognized since the time of Claude Bernard.¹ It has also been clear that renal nerves have an intimate relationship with many forms of experimental hypertension,² and surgical splancnicectomy, with consequent renal denervation, was a recommended form of therapy for hypertension in humans before the introduction of effective pharmacologic agents,³ despite the occurrence of serious side effects including orthostatic hypotension, erectile dysfunction, and gastrointestinal symptoms. Interest in renal denervation as an approach to the management of resistant hypertension in humans has recently been renewed with the introduction of an endovascular device, which uses the energy of a radiofrequency signal to ablate adjacent nerves in the vascular wall. When applied in a systematic manner in the lumens of the renal arteries, this approach results in sustained reduction in BP in patients with resistant hypertension.⁴

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