Published in final edited form as:

Clin Sci (Lond). 2018 July 16; 132(13): 1383–1401. doi:10.1042/CS20180121.

Intratubular and Intracellular Renin-Angiotensin System in The Kidney: A Unifying Perspective in Blood Pressure Control

Xiao C. Li^{1,*}, Dongmin Zhu^{1,2,*}, Xiaowen Zheng^{1,3}, Jiangfeng Zhang^{1,3}, Richard J. Roman¹, and Jia L. Zhuo¹

¹Laboratory of Receptor and Signal Transduction, Department of Pharmacology and Toxicology, Division of Nephrology, Internal Medicine, Cardiovascular-Renal Research Center, University of Mississippi Medical Center, Jackson, Mississippi, USA

²Guangxi University of Technology School of Medicine, Liuzhou, Guangxi

³Department of Emergency Medicine, 2nd Affiliate Hospital, Guangxi Medical University, Nanning, Guangxi, China

Abstract

The renin-angiotensin system (RAS) is widely recognized as one of the most important vasoactive hormonal systems in the physiological regulation of blood pressure and the development of hypertension. This recognition is derived from, and supported by, extensive molecular, cellular, genetic and pharmacological studies on the circulating (tissue-to-tissue), paracrine (cell-to-cell) and intracrine (intracellular, mitochondrial, nuclear) RAS during last several decades. Now, it is widely accepted that circulating and local RAS may act independently, or interactively, to regulate sympathetic activity, systemic and renal hemodynamics, body salt and fluid balance and blood pressure homeostasis. However, there remains continuous debate with respect to the specific sources of intratubular and intracellular RAS in the kidney and other tissues, the relative contributions of the circulating RAS to intratubular and intracellular RAS, and the roles of intratubular versus intracellular RAS to the normal control of blood pressure or the development of ANG II-dependent hypertension. Based on a lecture given at the recent XI International Symposium on Vasoactive Peptides held in Horizonte, Brazil, this article reviews recent studies using mouse models with global, kidney- or proximal tubule-specific overexpression (knockin) or deletion (knockout) of components of the RAS or its receptors. Although much knowledge has been gained from cell- and tissue-specific transgenic or knockout models, a unifying and integrative approach is now required to better understand how the circulating and local

Send Correspondence to: Jia L. Zhuo, M.D., Ph.D., Department of Pharmacology and Toxicology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505, USA, Phone: (601) 815-6765, Fax: (601) 984-1637, jzhuo@umc.edu.

*contribute equally as 1st author

Author Contribution

XCL, DZ and JLZ contribute to overall conception and design of the manuscript, acquisition of data, analysis and interpretation of data, drafting the article or revising it for important intellectual content, final approval of the version to be published, and agree to be accountable for all aspects of the work. XZ and JZ contribute to acquisition of data, analysis and interpretation of data, drafting and revising the article, and agree to be accountable for all aspects of the work. RJR contributes to revising the manuscript for important intellectual content, final approval of the version to be published, and agree to be accountable for all aspects of the work. All authors proofread and approved the manuscript.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

intratubular/intracellular RAS act independently, or with other vasoactive systems, to regulate blood pressure, cardiovascular and kidney function.

Introduction

Since the seminal discovery of the rate-limiting enzyme renin by Robert Tigerstedt and P. G. Bergman in 1898 [1] and the landmark study of Goldblatt et al. on the role of renin in the development of 2-kidney, 1-clip hypertension in 1934 [2], the renin-angiotensin system (RAS) has since been the most extensively studied endocrine (tissue-to-tissue), paracrine (cell-to-cell) and intracrine (intracellular) hormonal system. A critical role for the RAS in the regulation of arterial blood pressure, cardiovascular and kidney function, and the development of hypertension is now firmly established from studies using genetically modified animals [3–9] and human clinical studies using the pharmacological inhibitors of the system to target this system in hypertension and other cardiovascular and kidney diseases [10–16]. The classic endocrine and paracrine paradigms as a powerful vasoconstrictor, a stimulator of the release of aldosterone, and a renal sodium-retaining hormone have led to the one of the most successful drug discovery stories of the century, i.e., and the development of the inhibitors of angiotensin-converting enzyme (ACE) and renin, and the blockers of the type 1 angiotensin II (ANG II) (ARBs) and aldosterone receptors. Indeed, ACE and renin inhibitors, and ARBs and aldosterone receptor antagonists are the mainstays for the treatment of hypertension, stroke, heart failure, diabetic nephropathy, and other kidney diseases [10–16]. However, recent studies have also shown that the classical RAS paradigm has evolved significantly following discoveries of several new members, enzymes, or receptors of the RAS and their new roles, including prorenin receptors (PRR) [17,18], ACE2 [19,20], and ANG (1–7)/Mas receptors [21–24]. Thus, the key members of the classical RAS, including renin, ACE, ANG II and aldosterone, are no longer considered to be the only active effector molecules, but the classic renin/ACE/ANG II/AT₁ receptor axis still plays a predominant role in the regulation of arterial blood pressure, cardiovascular and kidney function, and the pathogenesis of hypertension [3–9]. The nonclassical pathways, such as the prorenin/prorenin receptor (PRR)/V-ATPase axis [18,25] and the intracrine (intracellular/mitochondria/nuclear) ANG II/AT₁ and AT₂ receptor axis [26–28] also appear to play an important role in the long-term transcriptional responses to the RAS stimulation. Conversely, the so-called protective arms of the RAS include the ACE2/ANG 1–7/Mas receptor axis, the aminopeptidase A (APA)/ANG III/AT₂ receptor axis, and the ANG IV/AT₄ receptor/IRAP axis serve counteracting roles of the renin/ACE/ANG II/AT₁ receptor axis [19–24]. Based on the lecture at the XI International Symposium on Vasoactive Peptides held in Belo Horizonte of Brazil in 2017, this article aims to review the new roles of intratubular and/or intracellular RAS uncovered using genetically modified animals with either overexpression or deficiency of one key enzyme, ANG peptide, or receptor of the RAS in the kidney, and discuss their physiological relevance and perspectives.

Intratubular RAS in the kidney: current consensus and debates

Most of the investigators agree that the RAS (RAS) plays an indispensable role in the cardiovascular and renal regulation, normal blood pressure homeostasis, and the

pathogenesis of hypertension [29–35]. There is also a general consensus that both circulating (endocrine) and local (paracrine) RAS act interactively to regulate vascular and sympathetic tones, renal pressure natriuresis response, and salt and water balance [29–35]. However, there are continuous debates with respect to: a) the origins of the intratubular and/or intracellular RAS [30,36–39]; b) the relative contributions of the circulating versus intrarenal RAS to the regulation of renal function [38–41]; c) the roles of intratubular RAS to the normal control of blood pressure and the development of ANG II-induced hypertension [29–31,42]; and d) the role of intracellular RAS [26–28,43–45]. Previously, it has been impossible to experimentally separate the roles of circulating versus local intratubular RAS due to the lack of global, kidney-, tubule or cell-specific genetically modified animal models. Furthermore, the findings that the renin derived from the kidney and angiotensinogen (AGT) derived from the liver are required for the activation of both the circulating and intrarenal/intratubular RAS further complicate the respective roles of the circulating, intrarenal, and intratubular RAS in the regulation of blood pressure, cardiovascular and kidney function.

The RAS in the kidney: roles of local intratubular expression and biosynthesis

Since 1980s, extensive molecular, biochemical and immunohistochemical studies have established that all components of the RAS are localized in the rodent and human kidney (Fig. 1) [29,30,46–48]. These include prorenin, renin, AGT, ANG I, ANG (1–7), ACE and ACE2, ANG II, ANG III or ANG IV proteins. More recent studies have demonstrated that prorenin, renin, and PRR are also expressed in the cortical and/or medullary collecting duct [18,49–52]. Furthermore, prorenin, renin, AGT, and ANG II proteins have been reported in the urine [51,53–55]. These findings have been taken as evidence for the local intratubular RAS in the kidney. The concept of a local intratubular RAS in the kidney is also support by well-recognized observations that the levels of ANG II peptides in the kidney [30,46,56–59], and the proximal tubular fluid compartment [56–58,60–62] are much higher than in the plasma. Navar's group has been instrumental to demonstrate high levels of AGT, ANG I, and ANG II protein levels in the proximal tubular fluid [29,55,63,64]. Other studies from his group and others have shown that AGT mRNA and proteins were expressed in the proximal tubule or proximal tubule cells [29,55,63,64]. Since ANG I and ANG II levels in the proximal tubular fluid are estimated at the nanomolar range, whereas the circulating ANG II level is at the femtomolar range, these studies suggest that ANG II may be produced intracellularly and then secreted into the proximal tubular fluid [30,37]. Whether ANG I and ANG II levels can be as high as nanomolar ranges in the kidney, proximal tubules, or proximal tubule and peritubular fluid compartments remains a subject of debate, given the difficulty in collecting adequate amounts of proximal tubular fluid samples and uncertainties about the specificity of antibodies especially in commercial radioimmunoassays. An alternative argument, however, may be that ANG I is generated in the proximal tubule fluid by renin and AGT that are filtered and taken up into the proximal tubule. ANG I generated in proximal tubule fluid or intracellularly may be converted to ANG II by the very high levels of ACE expressed on the apical membranes of the proximal tubule (see below).

Intratubular RAS in the kidney: roles of circulating, liver-derived angiotensinogen

In addition to local onsite expression or synthesis of the RAS discussed above, an alternative, prevailing view is that circulating AGT, ANG I and ANG II may be filtered and taken up in the proximal tubule of the kidney [38,39,41,58,65–69]. As the only known substrate for the rate-limiting enzyme renin, AGT is the largest peptide of the RAS, a ~62 kD protein similar to the molecular weight of albumin. Thus, like albumin, AGT was originally assumed to not be filtered by glomerulus, unless the glomerular filtration barrier is injured. Kobori et al. determined whether circulating AGT is a source of urinary AGT by infusing exogenous human AGT in control or ANG II-induced hypertensive rats [37,55]. No human AGT was detected in the urine of either sham control or ANG II-induced hypertensive rats in support of this view. Two possibilities may explain these findings; circulating human AGT may not have been filtered due to its poor glomerular permeability, and/or the filtered human AGT may have been metabolized in the renal tubules [37,55]. The data are consistent with their hypothesis that urinary AGT is primarily derived from AGT locally formed in the kidney tubules, rather than from the circulation in normal or ANG II-infused rats.

However, there is direct evidence that AGT may be filtered by the glomerulus and the filtered AGT is then taken up by proximal tubule cells [70]. Pohl et al. reported that immunoreactive or radiolabeled AGT found in the proximal tubule (S1) was due to its retrieval from the ultrafiltrate as well as the storage from endosomal and lysosomal compartments, whereas AGT mRNAs were detected mainly in the proximal straight tubule (S3) [70], just as it has emerged as the major uptake pathway for filtered albumin. The endocytic receptor megalin appears to play an important role in the proximal tubular uptake of AGT [70]. The study of Pohl et al. thus supports the concept that AGT mRNA is locally expressed mainly in the proximal straight tubule (S3). The direct evidence supporting the hypothesis that the liver is the primary source of circulating and intrarenal AGT came from the elegant study of Matsusaka et al. [38]. In this landmark study, these investigators generated mutant mice with hepatic-, kidney-specific, or double knockout of AGT to test their hypothesis [38]. Surprisingly, both AGT and ANG II protein levels in the kidney were similar in both wildtype and mutant mice with kidney-specific AGT knockout [38]. Conversely, AGT and ANG II protein levels in the kidney were almost abolished in mutant mice with liver-specific knockout of AGT [38]. These striking findings were highly unexpected, since kidney AGT and ANG II levels should be much lower in kidney-specific AGT knockout than wild-type mice, if the kidney is the primary source of intrarenal AGT expression. Alternatively, kidney AGT and ANG II levels should not have been reduced significantly in the liver-specific AGT knockout mice, if the kidney is the primary source of renal AGT. These authors further showed that AGT proteins were selectively located in megalin-expressing proximal tubule cells, consistent with the study of Pohl et al. [70]. Additional supporting data showed that genetic approach to induce podocyte injury, therefore damage the glomerular filtration barrier, increases urinary AGT protein excretion [38,71]. Taken together, the study of Matsusaka et al. provides the direct evidence that liver-

derived AGT is the primary source of renal AGT and ANG II proteins in the kidney and that glomerular injury may explain the apparent activation of the renal RAS in CKD models.

So, how can we best reconcile the studies of Kobori et al. [37,55] and Matsusaka et al. [38,71], and two different hypotheses on whether AGT mRNAs and proteins in the kidney are primarily due to local onsite expression and biosynthesis, or due to filtration of the circulating, liver-derived AGT? A careful analysis reveals that very different animal models and experimental conditions were used in those studies. While Kobori et al. focused on increased intratubular AGT mRNA expression and protein production during ANG II-induced hypertension [37,55], Matsusaka et al. focused on the glomerular filtration and proximal tubule uptake of liver-derived AGT using liver-specific AGT-KO mouse model with or without disruption of glomerular filtration barrier [38,71]. It is likely that under physiological conditions and with an intact endocytic receptor megalin-dependent uptake mechanism, the liver-derived AGT is the primary source of AGT proteins in the kidney, filtered by the glomerulus and taken up by the proximal tubule in the kidney, along with ANG I, ANG II, prorenin and renin (see later sections for details) (Fig. 2). However, in ANG II-dependent hypertension and kidney diseases association with activation of the local RAS, AGT mRNA expression and AGT protein biosynthesis may also be upregulated along the nephron in the kidney, independent of the circulating, liver-derived AGT [30]. A better approach for further studies to test this hypothesis is to measure intratubular AGT and ANG II levels using ANG II-infused, liver- or kidney-specific AGT knockout mice [38,71].

The specific roles of liver-derived or intratubular AGT in the regulation of basal blood pressure can be deduced from studies using mutant mouse models with liver-, kidney-, or tubule-specific overexpression or deletion of the AGT gene [38,72,73]. Ying et al. used the kidney androgen protein (KAP) gene promoter to study the effects of knock in of the mouse AGT gene in the proximal tubule on blood pressure [72]. KAP has been suggested to specifically express in the proximal tubule of the kidney, and its expression is increased by androgen stimulation [5,72,74]. Although AGT mRNA expression in the proximal tubule and urinary AGT proteins were significantly increased in mice with KAP-mediated AGT overexpression in the kidney, basal blood pressure remained normal under normal salt intake [72]. Since basal blood pressure remained unchanged in mice with KAP-mediated AGT overexpression in the kidney, Ramkumar et al. bred AGT-floxed mice with mice expressing the Pax8 rtTA and LC 1 transgenes to generate inducible nephron-wide AGT deletion in a follow-up study [40]. Blood pressure was found to be significantly lower in the nephron-specific AGT-KO mice than in wildtype mice, whether they were fed normal, low or high salt diet [40]. These conflicting results are inconsistent with the study of Matsusaka et al. [38]. In the latter study, the authors created three different strains of AGT-KO mice, the liver-specific, kidney-specific and liver-/kidney-double KO, and determined their basal blood pressure. Systolic blood pressure fell from ~120 mmHg in wildtype mice to ~75 mmHg in liver-specific AGT-KO mice or ~80 mmHg in liver-/kidney-double KO mice, respectively, but it was not significantly decreased in kidney-specific AGT-KO mice (~115 mmHg) (Fig. 3) [38]. While the marked hypotensive response is highly expected in liver-specific and liver and kidney double AGT-KO mice, the well-maintained blood pressure in kidney-specific AGT-KO mice was surprising. The reasons underlying the differences of the findings and conclusions reached from these studies remain unknown, but they may be due to the

differences of the approaches to generate AGT-floxed mice and express Cre in the tissue- or cell-specific manner [38,40,72]. However, the currently available evidence appears to support the concept that the liver is the primary source of systemic and intrarenal AGT and ANG II, and liver-derived AGT plays an important physiological role in basal blood pressure homeostasis [38]. This may also explain the close link between blood pressure and hepatorenal syndrome.

Intratubular RAS in the kidney: roles of intrarenal renin, prorenin and prorenin receptors

It is established beyond any doubt that prorenin and renin are synthesized and expressed in juxtaglomerular apparatus (JGA) and cells of afferent arterioles, and are secreted into the circulation in response to various stimuli [75–77]. It is this renin that initiates the biochemical cascade of the RAS activation in the kidney, circulation and other target tissues by cleaving AGT to form ANG I, which is then converted by ACE to the most potent peptide of the RAS, ANG II. Recently, it has been argued whether renin and prorenin in the kidney and urine may in fact come from the circulating sources, or be synthesized locally [41]. Roksner et al. used multiphoton microscopy to study whether fluorescently labeled renin and prorenin were filtered by the glomerulus in C57BL/6J mice before and after induction of doxorubicin-induced damage of the glomerular filtration barrier. These authors reported that nearly all fluorescently labeled renin and prorenin (37 kD) and prorenin (42 kD) were filtered by the glomerulus, and their filtration efficiency exceeded that of albumin (67 kD). The glomerular filtration of renin and prorenin was increased by disruption of the glomerular filtration barrier with doxorubicin. Nearly all of the filtered renin and prorenin were taken up along the nephron, primarily in the proximal tubule, and none appeared in the urine [41]. Furthermore, these authors found that overexpression of prorenin in the liver of CYP1a1-Ren2 transgenic rats increased circulating levels of prorenin by 200-fold, but prorenin was not detected in urine of these transgenic animals, suggesting it was completely reabsorbed along the nephron. However, urinary prorenin, renin, AGT, and albumin all were significantly increased in the urine of patients with Dent's disease or Lowes syndrome, which is associated with defective megalin-mediated uptake of low molecular weight of proteins in the proximal tubule [41]. These findings provide the evidence that circulating renin and prorenin are clearly filtered by the glomerulus and almost completely taken up in the proximal tubule of the kidney primarily through a megalin-dependent mechanism.

The roles of circulating and intratubular renin and/or prorenin in the regulation of blood pressure have been extensively investigated and confirmed in genetically modified animal models with either global or tissue-specific overexpression or deletion of renin. The early study of Mullins et al. first overexpressed the mouse renin gene, Ren-2, globally in a rats model, TGR(mRen-2)27, and reported that global overexpression of the renin gene induces fulminant hypertension [3]. TGR(mRen-2)27 rats showed significantly elevated circulating and kidney ANG II levels and upregulation of AT₁ receptor expression, rather than downregulation, in the kidney despite of severe hypertension, which were blocked by the AT₁ receptor antagonist losartan [3,78,79]. Thus, the TGR(mRen-2)27 rat represents a global renin overexpression, ANG II-dependent hypertensive rat model. To determine the

role of intratubular renin, Lavoie et al. overexpressed the human renin gene (hREN) with or without the human AGT gene, under the control of the kidney androgen-regulated promoter (KAP) [5,80]. Dual overexpression of the human renin and AGT genes in the proximal tubule significantly increased blood pressure, providing a proof of concept evidence for intratubular AGT and renin in basal blood pressure regulation [80]. Conversely, global deletion of the renin gene in mice led to significant phenotypes in the kidney and/or collecting ducts (49), and depletion of circulating PRA and caused marked hypotension [81]. These mice showed impaired renal morphology, and urinary concentration ability, as those observed in global AGT-KO or ACE-KO mice. In mice with Ren1c disruption (Ren1c^{-/-}), which is the only renin gene in C57BL/6J mice and humans [82], nearly 80% of the pups died of dehydration after birth. In those of surviving Ren1c^{-/-} mice, basal blood pressure was ~30 mmHg lower with markedly diluted urine, significant hydronephrosis, and fibrosis responses in the kidney [82]. Pentz et al. have ablated renin-expressing cells from the JGAs of the afferent arterioles during development by placing diphtheria toxin A chain (DTA) under control of the Ren1d mouse renin promoter [83]. They found that the kidneys were smaller with the development of hyperplastic or atrophic glomeruli and renal tubules. These studies suggest that systemic and JGA renin is not only important to maintain basal blood pressure, but is also required for the normal kidney and/or nephron development.

The roles of collecting duct (CD) renin and/or prorenin have been studied using collecting duct (CD)-specific renin transgenic, knockout, or prorenin receptor (PRR)-KO mice (49;50). Ramkumar et al. used the Cre/Lox recombination approach to generate mutant mice with CD-specific overexpression or deletion of renin to determine its role in the blood pressure regulation [49,50]. Under basal conditions, CD-renin expression increased 5-fold, whereas plasma renin was suppressed by more than 50%, but blood pressure was similar to wildtype mice [50]. Blood pressure increased significantly in response to high salt intake [50]. These authors also reported that there were no significant differences in basal blood pressure between wildtype and CD-specific renin-KO mice, but they did find attenuated blood pressure and epithelial Na⁺ channel (ENaC) responses to ANG II [49]. Whether intratubular and/or CD-PRR plays a role in maintaining blood pressure homeostasis is still controversial [52,84–86]. PRR is also termed Atp6ap2, ATPase H⁺-transporting lysosomal accessory protein 2 and a type 1 transmembrane protein and an accessory subunit of the vacuolar H⁺-ATPase (V-ATPase). In the kidney, PRR is primarily expressed in the intercalated cells and/or principle cells of CD in the medulla [87], which was upregulated during ANG II-induced hypertension [35,51,88]. Trepiccione et al. generated inducible, conditional deletion of Atp6ap2 throughout the nephron segments, but primarily in the intercalated cells of CD in the medulla [84]. These authors found that, apart from alterations in urine concentration and acid-base balance, nephron-specific Atp6ap2 depletion had no effects on intrarenal ANG II formation, sodium handling, and basal blood pressure regulation [84]. Using a similar approach, Ramkumar et al. developed an inducible renal tubule-wide PRR KO using the Pax8/LC1 transgene recombination approach. They found that there were no differences in basal blood pressure between control and PRR-KO mice [85]. Peng et al. generated CD-specific PRR-KO mice primarily in the principal cells using the PRR-floxed and AQP2-Cre approach, and also failed to find any effect on blood pressure [52]. By contrast, Prieto et al. generated CD-specific PRR-KO mice using the PRR-floxed and Hox-b7-Cre mice [86].

Interestingly, the latter CD-specific PRR-KO mice showed fewer nephrons or glomeruli, mild hypoplasia in the medulla, smaller kidney and lower kidney wt., etc., along with some abnormal morphological phenotypes pointing to impaired kidney development [86]. Significant functional abnormalities observed in these mice include decreased basal systolic blood pressure and glomerular filtration rate, and elevated diuretic and natriuretic responses without altering the expression and activities of the epithelial Na⁺ channel in the CD [86]. Overall, it remains difficult to reconcile the conclusions on the roles of CD-specific PRR in the regulation of blood pressure. The only consistent finding is that CD-specific PRR knockout attenuates ANG II-induced hypertension [52,85,86]. However, it is unlikely that CD-specific renin, prorenin, and PRR play a significant role in the normal regulation of blood pressure or kidney function.

Intratubular RAS in the kidney: roles of intratubular angiotensin-converting enzyme

Angiotensin-converting enzyme, ACE, a dipeptidyl carboxypeptidase I, kininase II or EC 3.4.15.1, plays a critical role in converting inactive ANG I to ANG II, as well as metabolizing bradykinin [89,90]. These dual roles of ACE have been well established in the regulation of cardiovascular and kidney function, and blood pressure homeostasis. Unlike AGT, renin, prorenin, ANG I and ANG II, which have been shown to be filtered by the glomerulus and taken up by the proximal tubule, it remains to be determined to what extent whether ACE in the kidney is derived from the systemic circulation or synthesized in the kidney. Previous studies using quantitative in vitro autoradiography have clearly demonstrated that ACE is expressed at the high levels and is found to localize in the apical membranes of the proximal tubule and endothelial cells of blood vessels in the kidney [91–93]. Since the radiolabeled ACE inhibitor Lisinopril selectively binds to, and inhibits the enzyme ACE, this technique has high specificity to localize intratubular ACE along the nephron segments in the rat and human kidney [91–93]. Most, if not all of the radiolabeled ACE inhibitor is found in the proximal tubule in the kidney. High resolution immunohistochemistry using the anti-human ACE antibody has been able to localize ACE in the microvilli and brush borders in the proximal tubule [94–96]. ACE immunostaining have also been visualized in the endoplasmic reticulum, mitochondria and nucleus [94]. In contrast, the glomerulus, distal tubules and the inner medullary collecting ducts express low levels of ACE [91,92,94–96]. These studies strongly suggest that intratubular ANG II may be generated at high levels in the proximal tubule, and much less is found in other nephron segments under physiological conditions.

The roles of intratubular ACE in the regulation of renal tubular transport may be best recognized through its primary action on intratubular ANG II formation in the proximal tubule of the kidney, which stimulates proximal tubular Na⁺ reabsorption and contributes to maintenance of salt and fluid balance [30,61,92,93,97–100]. Before the invention of genetically modified animal models, the role of ACE in the regulation of proximal tubular Na⁺ transport and blood pressure was demonstrated primarily using in vivo micropuncture and ACE inhibitors [61,92,93,97,98]. The effects of ACE inhibitors appear to be relatively consistent in previous studies. With genetically modified mouse models becoming available,

however, some confusing or unexpected roles of ACE on proximal tubular Na^+ transport and blood pressure have been reported [42,99,101–104]. For example, Hashimoto et al. used ACE2/2 mice, in which ACE expression was completely knocked out in the kidney [99], and found that the entire kidney and single nephron GFR were significantly reduced, and fractional proximal tubular reabsorption was increased significantly [99]. This study strongly implies that intratubular ACE presumably does not play a significant role in stimulating proximal fluid Na^+ transport [99]. In another study, Kessler et al. also reported that proximal tubular Na^+ reabsorption was not altered in mice with ACE-KO in the kidney [101]. Thus, the results of studies using the ACE-KO mouse models reached a completely different conclusion on the role of intratubular ACE in the regulation proximal tubular Na^+ reabsorption from those using ACE inhibitors or ANG II infusion in rats [61,92,93,97,98]. At present, it is difficult to reconcile these results. One plausible reason is that it is so technically difficult to perform in vivo micropuncture experiments in an anesthetized mouse, whose kidney, glomerular and proximal tubule size is only about 10% of those in an adult rat. It is nearly technically impossible to collect enough proximal tubule fluid from a single proximal tubule of a mouse for biochemical analysis of a single glomerular filtration rate and proximal tubule electrolyte transport. Proximal tubule fluid samples collected from many tubules and many mice would have to be pooled before any analyses, and mistakes may likely be introduced during the processes. The 2nd possibility may be due to other compensatory mechanisms being activated in the absence of intratubular ACE and/or ANG II to maintain proximal tubular function. The 3rd more plausible explanation would be that although intratubular or kidney ACE is knocked out, circulating ANG II is readily filtered by the glomerulus and taken up by the proximal tubule, and it maintains proximal tubular Na^+ transport in the ACE mutant mouse models [58,65,68,69,105–107]. The only way to exclude this possibility is to measure intratubular ANG II levels in these ACE-KO mice.

The roles of circulating or intratubular ACE in the kidney in the blood pressure control and the development of hypertension may be distinguished using global and kidney- or tubule-specific ACE-KO mouse models [7,42,104,108]. The hypotension phenotype is surprisingly consistent and reproducible in mutant mouse models with global deletion of AGT [6,109], renin [110], ACE [7,108], or total AT_1 receptors (AT_{1a} and AT_{1b}). These findings strongly support the scientific premise of the essential role of the renin/AGT/ACE/ANG II/ AT_1 receptor axis in the regulation of blood pressure. There was a consistent 20–30 mmHg fall in basal blood pressure in all of these global mutant mouse models [6,7,69,108–110]. However, since ACE is widely expressed in different tissues or cells, the global ACE-KO mouse model may not be suitable to determine the relative contribution of extrarenal versus kidney and/or intratubular ACE to blood pressure regulation. Bernstein's group has been instrumental to generate a number of ACE mutant mouse models to determine the role of kidney or intratubular ACE in the blood pressure regulation and the development of hypertension [7,42,103,104]. Esther et al. first generated ACE 2/2 mutant mice, in which circulating ACE levels are elevated but renal ACE are reduced, and found that ACE 2/2 mice had significantly lower blood pressure, urine concentrating defect, and increased proximal tubular reabsorption [7]. A different strain of ACE-KO mice, ACE 3/3 mice, overexpressed ACE in the liver, but completely lost endothelial ACE in all tissues [103]. Kidney ACE was reduced to just 14% that of wild-type mice, thus representing kidney-specific ACE-KO. Yet,

basal blood pressure, plasma ANG II, and kidney function were not different between ACE 3/3 and wildtype mice [103]. Two additional ACE-mutant mouse models were developed by the same group of investigators, one expressing ACE only in the kidney, ACE9/9 [104], and the other having ACE-KO in the entire kidney, ACE10/10 [42]. ACE 9/9 mice had lower plasma ANG II and hypotension [104], whereas ACE10/10 mice were able to maintain normal basal blood pressure (Fig. 4) [42]. These findings are consistent with those reported in kidney-specific AGT-KO mice, suggesting a key role of the circulating ACE and ANG II [38].

While it is well-recognized that both systemic and tissue ACE play an undisputed role in generating circulating and intrarenal ANG II, stimulating proximal tubule Na⁺ reabsorption, and maintaining blood pressure homeostasis [61,92,93,97,98], it remains difficult to reconcile the conflicting results between studies using genetically modified ACE mouse models and those using systemic or intrarenal blockade of ACE with inhibitors [61,92,93,97,98]. Questions remain with respect to why mice with endothelial ACE-KO may have normal [103] or lower basal blood pressure [104,111], whereas mice with ACE-KO in the proximal tubule or the entire kidney may be normotensive [42,111]. Similarly, what are the mechanisms underlying normal circulating or kidney ANG II levels even though ACE has been deleted systemically in the endothelium or locally in the kidney and/or the proximal tubule [42,103,104,112]? Thus, further studies are necessary to resolve these issues.

Intratubular RAS in the kidney: roles of intratubular and intracellular angiotensin II

Local generation versus uptake via endocytosis.

ANG II levels in the kidney are consistently higher than in the circulation in normal rats or mice [30,46,56–59], and are further increased in response to long-term systemic infusion of ANG II, even though plasma renin activity and renin in the kidney JGA are suppressed [58,63,65,113]. This phenomenon supports the concept that ANG II is generated locally within the kidney. Indeed, all major components of the RAS from AGT, renin, to ACE are expressed in the kidney to allow local generation of ANG II [29,30,46–48]. Moreover, AGT mRNA and protein expression are significantly augmented in the proximal tubule or the kidney, rather than downregulated, in ANG II-induced hypertension [30,63,64].

Augmentation of AGT expression provides further substrates for ANG II production in the kidney. In addition to the proximal tubule or the renal cortex, Prieto-Carrasquero's group showed increased renin and prorenin expression in the inner medullary CDs, therefore likely contributing to high intratubular ANG II levels in ANG II-dependent hypertension, such as 2K1C or ANG II-infused animal models [53,113–115]. Overall, a positive feed-forward loop has been proposed to explain higher intratubular ANG II levels in the kidney during ANG II-dependent hypertension [29,30,54,63,93,113].

There is also solid evidence to suggest that higher levels of ANG II in the kidney may also be due to uptake of circulating and/or extracellular ANG II via AT₁ (AT_{1a}) receptor-mediated mechanisms [58,66,68,69]. As discussed previously, ANG II levels in the plasma

and kidney are not different between wildtype mice and mice with the kidney- and/or proximal tubule-specific AGT- [38] or kidney ACE-KO [42,103,104,112]. This suggests that ANG II in the kidney is likely derived from the circulating ANG II via glomerular filtration and tubular uptake. Navar's, Danser's and our groups have previously determined whether the kidney and/or the proximal tubule take up circulating ANG II via AT₁ (AT_{1a}) receptor-mediated mechanisms [58,65,68,69,105,107,116]. Zou et al. used osmotic minipump to systemically infused ANG II for 2 weeks, and measured ANG II levels. They demonstrated AT₁ receptor-mediated accumulation of ANG II in the rat kidney [65,107]. Van Kats et al. systemically infused [¹²⁵I]-labeled ANG I or ANG II into anesthetized pigs, and showed that [¹²⁵I]-labeled ANG II, but not [¹²⁵I]-labeled ANG I, was accumulated in the kidney and adrenal glands, also via AT₁ receptor-mediated mechanisms [67,105]. In the kidney, high levels of internalized ANG II was primarily found in cortical endosomes [58,117], lysosomes [105], or the nuclei in the proximal tubule cells [105,106,118]. AT₁ receptor-mediated accumulation of ANG II in the kidney or proximal tubule cells may be blocked by the AT₁ receptor blocker losartan [106,119], siRNAs targeting AT₁ receptors [120], or in AT_{1a}-KO mice [68,69]. Thus, AT₁ (AT_{1a}) receptor-mediated uptake of ANG II has been confirmed and reproduced in cultured proximal tubule cells in vitro and in the proximal tubule of the kidney in vivo.

Novel role of intratubular ANG II in the blood pressure regulation.

Although it is well-recognized that the kidney plays a key role in the physiological regulation of blood pressure and the pathogenesis of hypertension, the precise contribution of intratubular ANG II is poorly understood [31,44,45,121–124]. In this regard, Coffman's group has been instrumental in studying the role of the kidney in the blood pressure regulation using the kidney cross-transplantation approach and genetically modified global or proximal tubule-specific AT_{1a}-KO mice [31,121,122]. Cross-transplantation of the kidneys between wild-type and global AT_{1a}-KO mice confirmed the importance of AT₁ receptors in the kidney in the development of ANG II-induced hypertension and cardiac hypertrophy [31]. Subsequent studies used the Cre/LoxP approach to generate proximal tubule-specific AT_{1a}-KO mice and showed that deletion of AT_{1a} receptors selectively in the proximal tubule decreased basal blood pressure by >10 mmHg, even though systemic and/or vascular AT_{1a} receptor expression and responses remained intact [123,124]. This probably reflects the fully life-time compensatory state of extra-proximal tubule AT_{1a} receptors or other vasoactive systems in response to AT₁ receptor deletion selectively in the proximal tubule. Instead, we used a short-term adenoviral transfer approach to rescue AT_{1a} receptors in global AT_{1a}-KO mice by overexpressing a full-length GFP-tagged AT_{1a} receptor gene (AT_{1a}R/GFP) selectively in the proximal tubule using a proximal tubule-specific sodium and glucose cotransporter 2 (sglt2) promoter [45]. This approach likely mimics a short-term systemic AT_{1a}-KO and proximal tubule-specific AT_{1a}-knock in mouse model, which expressed AT_{1a} receptors only in the proximal tubule of the kidney. In this model, blood pressure was elevated by 12 to 15 mmHg, 2 weeks after the rescue of AT_{1a} receptor expression in the proximal tubule [45]. These studies together provide evidence that the ACE/ANG II/AT_{1a} receptor axis in the proximal tubule of the kidney contributes about 10 to 15 mmHg to basal blood pressure in mice under physiological conditions (Fig. 5).

Novel role of intratubular intracellular ANG II in the blood pressure regulation.

Recently, the concept that there is a functional intracellular RAS in various tissues has gained traction [26–28,43,125]. It remains to be determined whether intracellular ANG II in the proximal tubule is formed within the cells or derived from AT₁ receptor-mediated internalization [30,39,126,127], and whether actions of intracellular ANG II is of detrimental or protective in the kidney and other tissues [28,118,128–130]. Indeed, we and others have shown that systemically administered [¹²⁵I]-labeled ANG I or [¹²⁵I]-ANG II accumulates inside the kidney cells [67,105,116,131,132], and that AGT, ACE, ANG II and ANG II receptors are found in rat renal cortical endosomes [58,62], mouse proximal tubule mitochondria [126,128,133], and rat or sheep renal cortical nuclei [118,134–136]. With the substrate, enzymes, and ANG II receptors all found inside cells, ANG II, formed intracellularly or internalized, is expected to play an important role in the kidney.

Indirect studies have previously shown that AT_{1a} receptor-mediated ANG II/AT₁ receptor internalization was associated with ANG II-stimulated uptake of ²²Na⁺ [137–139], inhibition of cAMP formation [119], NF-κB activation [140], and increased NHE3 expression in proximal tubule cells [120]. However, these studies were unable to differentiate the actions of internalized ANG II versus ANG II via the cell surface receptors. To overcome this technical problem, a number of studies on direct actions of intracellular ANG II have been reported in vascular smooth muscle cells [141,142], proximal tubule cells [143], freshly isolated renal cortical nuclei [118,129,135,136,144,145] or mitochondria [126,133]. In cultured VSMCs [141] or proximal tubule cells [143], we and Haller et al. reported that direct microinjection of ANG II into the cells induced cytosolic and/or nuclear calcium mobilization, whereas AT₁ receptor antagonists blocked this effect. In freshly isolated renal cortical nuclei, we showed that ANG II directly stimulated TGF-β, MCP-1, and NHE3 transcription responses [118], consistent with previous results in hepatic nuclei [144,145]. Similar nuclear transcriptional responses have been shown in cardiomyocytes or fibroblasts, in which intracellular ANG II regulated RNA synthesis and cell proliferation [129,146]. Furthermore, our lab, Cook et al., and Kumar and Baker demonstrated that overexpression of an intracellular ANG II fusion protein, stimulated cell proliferation in A10 VSMCs via p38MAPK signaling [147] or CHO cells [148], or increased NHE3 expression in proximal tubule cells [149]. Taken together, these studies suggest that intracellular ANG II contributes to cardiovascular and kidney diseases and hypertension. However, recent studies have suggested that intracellular ANG II may play a protective role in counteracting the detrimental effects of intracellular and/or nuclear ANG II [28,130,133,134]. Abadir et al. have localized ANG II and AT₂ receptors on mitochondrial inner membranes, and activation of AT₂ receptors by ANG II led to mitochondrial nitric oxide production and modulate mitochondrial respiration in rat and human cells [133]. In isolated sheep nuclei, Gwathmey et al. reported that ANG II activated AT₂ or ANG (1–7) receptors to increase NO production [134,135], whereas it acted on nuclear AT₁ receptors to increase super oxide production [150]. In the mitochondria, Wilson et al. demonstrated that ANG (1–7) was formed to modulate NO production via ANG (1–7)/Mas receptors [126]. Finally, Villar-Cheda et al. recently reported that activation of nuclear receptors by intracellular ANG II induced a number of protective responses in neurons, such as increases in AT₂ receptor and PGC-1α and IGF-1/SIRT1 expression [130]. Whether these so-called protective effects of

intracellular ANG II via activation of AT₂ receptors or ANG (1–7) via activation of the Mas receptors are sufficient to counteract the detrimental effects of intracellular, mitochondrial and nuclear ANG II via activation of AT₁ receptors remains to be investigated. It is likely that these two systems may interact to play important “Ying” and “Yang” effects in the regulation of central, cardiovascular and kidney function, and blood pressure, but the ANG II/AT₁ receptor axis likely plays a dominant role in the diseased states.

Although the above-mentioned proof of concept studies demonstrate a biological role of intracellular ANG II in cultured cells and other in vitro settings such as isolated endosomes, mitochondria, or nuclei, the role of intracellular ANG II in the blood pressure regulation remains to be further determined. One of the most critical issues is the technical difficulty to separate its role from that of extracellular counterpart that activates cell surface ANG II receptors and initiates downstream signaling events. Again, some important proof of concept experiments have been performed to prove a physiological role of intracellular RAS. Lavoie et al. generated two different strains of renin transgenic mice, with one expressing a secreted form of prorenin, and the other expressing a non-secreted form of human intracellular active renin, icREN selectively in the brain, driven by the astrocytic glial fibrillary acidic protein (GFAP) promoter, GFAP-icREN [151]. These authors further bred GFAP-icREN mice with transgenic mice expressing human AGT selectively in the brain using the same GFAP promoter to generate double transgenic mice expressing either the icREN or secreted renin. Interestingly, mean arterial pressure was significantly elevated by ~20 mmHg in GFAP-icREN mice. This response was blocked by ICV losartan administration [151]. Since there was no difference in blood pressure response between transgenic mice expressing human AGT and icREN and transgenic mice expressing the secreted form of active renin in the brain, this study provides solid evidence for a physiological role of intracellular RAS in the brain [151]. However the role of ANG II in astrocytes rather than neurons still remains to be determined. On the other hand, it has recently been debated whether there is actually a brain RAS, letting alone an intracellular system [152–154]. Danser’s group argued that renin in the brain may represent plasma renin that is taken up and trapped in the brain, whereas ANG II in the brain may represent ANG II taken up from blood rather than locally synthesized [152,153]. But Sigmund’ group has argued that there is compelling evidence for the de novo production of all components of the RAS in the brain [154]. Clearly, the unresolved issue in the brain just as in the kidney is how much ANG II accumulates versus is synthesized locally.

Cook et al. used a different transgenic approach to globally overexpress an intracellular ANG II fusion protein, ECFP/ANG II, in mice with the mouse metallothionein promoter targeting all tissues [43]. This fusion protein construct lacks a secretory signal, so it is not secreted outside the cells after its intracellular expression or synthesis [155], thus plasma ANG II was not altered in these mice [43]. However, basal blood pressure was significantly elevated by 16 mmHg, associated with renal injury with thrombotic microangiopathy or microthrombosis [43]. This model may be considered to be a global intracellular ANG II-knock in mouse model supporting the concept that intracellular ANG II has a physiological effect. We reasoned that ANG II taken up by the proximal tubule may escape the classical clathrin-coated pits/endosomes/lysosome degrading pathway, be transported to other organelles and act as an intracellular peptide. To test this hypothesis, we overexpressed the

non-secreted form of intracellular ANG II fusion protein, ECFP/ANG II [43,155], selectively in the proximal tubule of rats, C57BL/6J and global AT_{1a}-KO mice using the proximal tubule-specific sglt2 promoter [44,45,149]. The effect of ECFP/ANG II overexpression in the proximal tubule on basal blood pressure was determined for 4 weeks. In both rats and C57BL/6J mice, ECFP/ANG II overexpression increased blood pressure by about 15 to 20 mmHg for two weeks after introduction of the overexpression. These responses was completely blocked by losartan or in AT_{1a}-KO mice [44,45,149]. We further found that the ECFP/ANG II-induced hypertensive response was associated with increased phosphorylated ERK1/2 and NHE3 expression, augmentation of AGT expression, increased Na⁺ reabsorption in the proximal tubule, and a decrease in 24 h urinary sodium excretion [44,45,149]. Taken together, these proof of concept studies suggest that intracellular ANG II in the brain, cardiovascular tissues and the proximal tubule of the kidney likely play an important physiological role in the regulation of blood pressure independent of the circulating RAS and/or cell surface receptors.

Concluding remarks and perspective

In summary, the available evidence supports the concept of the circulating (endocrine), local tissue (paracrine), and intracellular RAS system in the kidney. All major components of the RAS, including AGT [5,38–40,55,71,72], renin and prorenin [17,18,25,35,41,49–54,157], ACE [7,8,42,90–96,98,99,104,111,112] and ACE2 [19,20,34,48,], ANG II and other ANG metabolites [37,46,47,55–61], AT₁, AT₂ and the ANG (1–7)/Mas receptors [79,92,93,123,124,131–136,156], have been identified, especially in the proximal tubule. Their respective sources and roles in the cardiovascular and kidney, and blood pressure regulation have been extensively investigated using molecular, cellular, genetic and pharmacological approaches during last three decades. The strongest evidence supporting the important roles of an intratubular RAS is that under physiological conditions, AGT, prorenin, renin, ACE, ANG II and AT₁ and AT₂ receptors are localized in the proximal tubule and/or the collecting duct, and the ANG II levels in the proximal tubule are much higher than can be explained by the circulating ANG II level. Moreover, the expression and the levels of intratubular and urinary AGT, prorenin, ACE, and ANG II proteins are significantly increased in the kidney in response to ANG II infusion in spite of suppression of the circulating RAS. By contrast, the strongest evidence supporting the important roles of the circulating RAS is that also under physiological conditions, the liver-derived AGT, and systemic prorenin and active renin, ANG I and ANG II are all filtered by the glomeruli and taken up by the proximal tubule. The maintenance of normal plasma ANG II levels and basal arterial blood pressure in the kidney- or proximal tubule-specific knockout of AGT or ACE further supports the roles of the circulating RAS. However, it may be too simplistic to try and distinguish between the actions of the circulating (endocrine) versus the local tissue (paracrine) or intracellular (intracrine) RAS, as their functions are intertwined and we “can’t see the forest for the trees”.

In reality, it still remains very difficult to separate the sources, contributions, and physiological roles of the intratubular/intracellular RAS from that of the circulating RAS due to the significant overlaps between two systems. A unifying or integrative hypothesis may be required to further improve our understanding the important roles of the circulating

and intratubular RAS in the cardiovascular, kidney and blood pressure regulation. It is possible that the intratubular RAS may not be active under physiological conditions, since circulating AGT, prorenin and renin, and ANG I may easily reach the proximal tubule through glomerular filtration, and ACE on the apical membranes rapidly converts ANG I to ANG II in the proximal tubule lumen. ANG II may also be filtered into the proximal tubule, where ANG II stimulates proximal tubular Na⁺ reabsorption and contributes to the maintenance of salt and water balance and normal blood pressure homeostasis. In ANG II-dependent hypertension, increased circulating ANG II may suppress the expression and production of hepatic AGT, prorenin and renin, and ACE, but it significantly increases the synthesis and secretion of AGT and ACE and the uptake of ANG II in the proximal tubule and the expression of prorenin, renin and PRR in the collecting duct. Augmentation of intratubular AGT, prorenin and renin, ACE and PRR expression, and increased uptake of extracellular ANG II into the mitochondria, endoplasmic reticulum, and nucleus in the kidney may represent important feed-forward mechanisms of ANG II-dependent hypertension.

Acknowledgements

This work was supported in part by grants from National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (2R01DK067299–10A), and NIDDK/National Institute of General Medical Sciences (2R01DK102429–03A), and National Heart, Lung, And Blood Institute (1R56HL130988–01) to Dr. Jia L. Zhuo. Dr. Dongmin Zhu is a visiting Associate Professor of Medicine from, and supported by a grant from Guangxi University of Technology School of Medicine, Liuzhou, Guangxi, China, whereas Drs. Xiaowen Zheng and Jianfeng Zhang are visiting Professors of Medicine from Guangxi Medical University, Nanning, Guangxi, China and was supported by a grant from National Natural Science Foundation of China (#81360290).

References

- (1). Tigerstedt R, Bergman PG (1898) Niere und Kreislauf. *Scand.Arch.Physiol* 8, 223–271.
- (2). Goldblatt H, Lynch J, Hanzal RF, Summerville WW (1934) Studies on experimental hypertension. 1. The production of persistent elevation of systolic blood pressure by means of renal ischemia. *J.Exp.Med* 59, 347–379. [PubMed: 19870251]
- (3). Mullins JJ, Peters J, Ganten D (1990) Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. *Nature*, 344(6266):541–4. [PubMed: 2181319]
- (4). Yang G, Merrill DC, Thompson MW, Robillard JE, Sigmund CD (1994) Functional expression of the human angiotensinogen gene in transgenic mice. *J. Biol. Chem* 269(51), 32497–502. [PubMed: 7798251]
- (5). Ding Y, Davisson RL, Hardy DO, Zhu LJ, Merrill DC., Catterall JF, et al. (1997) 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* 272(44), 28142–8. [PubMed: 9346970]
- (6). Kim HS, Kregg JH, Kluckman KD, Hagaman JR, Hodgin JB, Best CF, et al. (1995) Genetic control of blood pressure and the angiotensinogen locus. *Proc. Natl. Acad. Sci. U S A*, 92(7), 2735–9. [PubMed: 7708716]
- (7). Esther CR, Marino EM, Howard TE, Machaud A, Corvol P, Capecchi MR, et al. (1997) The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J. Clin. Invest* 99, 2375–85. [PubMed: 9153279]
- (8). Kregg JH, Kim HS, Moyer JS, Jennette JC, Peng L, Hiller SK, et al. (1997) Angiotensin-converting enzyme gene mutations, blood pressures, and cardiovascular homeostasis. *Hypertension*, 29(1 Pt 2), 150–7. [PubMed: 9039095]

- (9). Oliverio MI, Best CF, Kim HS, Arendshorst WJ, Smithies O, Coffman TM (1997) Angiotensin II responses in AT_{1A} receptor-deficient mice: a role for AT_{1B} receptors in blood pressure regulation. *Am. J. Physiol* 272(4 Pt 2), F515–F520. [PubMed: 9140053]
- (10). Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., et al. (2003) Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*, 42(6),1206–52. [PubMed: 14656957]
- (11). Carey RM, Whelton PK (2017) Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Synopsis of the 2017 American College of Cardiology/American Heart Association Hypertension Guideline. *Ann. Intern. Med* 2670318–3203.
- (12). McMurray JJ, Krum H, Abraham WT, Dickstein K, Kober LV, Desai AS, et al. (2016) Aliskiren, Enalapril, or Aliskiren and Enalapril in Heart Failure. *N. Engl. J. Med* 374(16),1521–32. [PubMed: 27043774]
- (13). Musini VM, Lawrence KA, Fortin PM, Bassett K, Wright JM (2017) Blood pressure lowering efficacy of renin inhibitors for primary hypertension. *Cochrane Database Syst. Rev* 4, CD007066. doi: 10.1002/14651858.CD007066. [PubMed: 28379619]
- (14). Jo SK, Rosner MH, Okusa MD (2007) Pharmacologic treatment of acute kidney injury: why drugs haven't worked and what is on the horizon. *Clin. J. Am. Soc. Nephrol.* 2(2), 356–65.
- (15). White WB, Weber MA, Sica D, Bakris GL, Perez A, Cao C, et al. (2011) Effects of the angiotensin receptor blocker azilsartan medoxomil versus olmesartan and valsartan on ambulatory and clinic blood pressure in patients with stages 1 and 2 hypertension. *Hypertension*, 57(3),413–20. [PubMed: 21282560]
- (16). Akioyamen L, Levine M, Sherifali D, O'Reilly D, Frankfurter C, Pullenayegum E, et al. (2016) Cardiovascular and cerebrovascular outcomes of long-term angiotensin receptor blockade: meta-analyses of trials in essential hypertension. *J. Am. Soc. Hypertens.* 10(1), 55–69. [PubMed: 26684588]
- (17). Nguyen G, Delarue F, Burckle C, Bouzahir L, Giller T, Sraer JD (2002) Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J.Clin.Invest* 109, 1417–1427. [PubMed: 12045255]
- (18). Yang T (2017) Unraveling the physiology of (Pro)renin receptor in the distal nephron. *Hypertension*, 69(4), 564–74. [PubMed: 28242715]
- (19). Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ. Res* 87(5), E1–E9. [PubMed: 10969042]
- (20). Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem* 275(43), 33238–43. [PubMed: 10924499]
- (21). Ferrario CM (2006) Angiotensin-converting enzyme 2 and angiotensin-(1–7): an evolving story in cardiovascular regulation. *Hypertension*, 47(3), 515–21. [PubMed: 16365192]
- (22). Santos RA, Simoes E, Silva AC, Maric C, Silva DM, Machado RP, et al. (2003) Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc. Natl. Acad. Sci. USA*, 100(14), 8258–63. [PubMed: 12829792]
- (23). Chappell MC (2007) Emerging evidence for a functional angiotensin-converting enzyme 2-angiotensin-(1–7)-MAS receptor axis: more than regulation of blood pressure? *Hypertension* 50(4), 596–9. [PubMed: 17785634]
- (24). Bader M, Alenina N, Andrade-Navarro MA, Santos RA (2014) MAS and its related G protein-coupled receptors, Mrgprs. *Pharmacol. Rev* 66(4), 1080–105. [PubMed: 25244929]
- (25). Danser AH, Deinum J (2005) Renin, prorenin and the putative (pro)renin receptor. *Hypertension*, 46, 1069–1076. [PubMed: 16186442]
- (26). Ellis B, Li XC, Miguel-Qin E, Gu V, Zhuo JL (2012) Evidence for a functional intracellular angiotensin system in the proximal tubule of the kidney. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 302(5), R494–R509. [PubMed: 22170616]
- (27). Gwathmey TM, Alzayadneh EM, Pendergrass KD, Chappell MC (2012) Novel roles of nuclear angiotensin receptors and signaling mechanisms. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 302(5), R518–R530. [PubMed: 22170620]

- (28). Re RN (2018) The role of intracellular angiotensin II. *Am. J. Physiol. Heart Circ. Physiol* 314(4), H766–H771. [PubMed: 29351452]
- (29). Kobori H, Nangaku M, Navar LG, Nishiyama A (2007) The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol. Rev* 59(3), 251–87. [PubMed: 17878513]
- (30). Navar LG, Kobori H, Prieto MC, Gonzalez-Villalobos RA (2011) Intratubular renin-angiotensin system in hypertension. *Hypertension* 57(3), 355–62. [PubMed: 21282552]
- (31). Crowley SD, Gurley SB, Oliverio MI, Pazmino AK, Griffiths R, Flannery PJ, et al. (2005) Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. *J. Clin. Invest* 115 (4), 1092–9 [PubMed: 15841186]
- (32). Coffman TM (2011) Under pressure: the search for the essential mechanisms of hypertension. *Nat. Med* 17(11), 1402–9. [PubMed: 22064430]
- (33). Sparks MA, Crowley SD, Gurley SB, Mirososou M, Coffman TM (2014) Classical Renin-Angiotensin system in kidney physiology. *Compr. Physiol* 4(3), 1201–28. [PubMed: 24944035]
- (34). Zhuo JL, Ferrao FM, Zheng Y, Li XC (2013) New frontiers in the intrarenal Renin-Angiotensin system: a critical review of classical and new paradigms. *Front. Endocrinol. (Lausanne)* 4, 166. doi: 10.3389/fendo.2013.00166. [PubMed: 24273531]
- (35). Yang T, Xu C. (2017) Physiology and pathophysiology of the intrarenal renin-angiotensin system: an Update. *J. Am. Soc. Nephrol.* (4), 1040–9. [PubMed: 28255001]
- (36). Kobori H, Ozawa Y, Satou R, Katsurada A, Miyata K, Ohashi N, et al. (2007) Kidney-specific enhancement of ANG II stimulates endogenous intrarenal angiotensinogen in gene-targeted mice. *Am. J. Physiol. Renal. Physiol.* 293(3), F938–F945. [PubMed: 17634399]
- (37). Navar LG, Satou R, Gonzalez-Villalobos RA The increasing complexity of the intratubular Renin-Angiotensin system. *J. Am. Soc. Nephrol* 23(7), 1130–2.
- (38). Matsusaka T, Niimura F, Shimizu A, Pastan I, Saito A, Kobori H, et al. (2012) Liver angiotensinogen is the primary source of renal angiotensin II. *J. Am. Soc. Nephrol* 23(7), 1181–9. [PubMed: 22518004]
- (39). Roman RJ, Fan F, Zhuo JL (2016) Intrarenal renin-angiotensin system: locally synthesized or taken up via endocytosis? *Hypertension*, 67(5), 831–3. [PubMed: 26928807]
- (40). Ramkumar N, Stuart D, Calquin M, Wang S, Niimura F, Matsusaka T, et al. (2016) Possible role for nephron-derived angiotensinogen in angiotensin-II dependent hypertension. *Physiol. Rep.* 4(1), e12675. [PubMed: 26755736]
- (41). Roksnoer LC, Heijnen BF, Nakano D, Peti-Peterdi J, Walsh SB, Garrelds IM, et al. (2016) On the origin of urinary renin: a translational approach. *Hypertension*, 67(5), 927–33. [PubMed: 26928805]
- (42). Gonzalez-Villalobos R, A, Janjoulia T, Fletcher NK, Giani JF, Nguyen MT, Riquier-Brison AD, et al. (2013) The absence of intrarenal ACE protects against hypertension. *J. Clin. Invest* 123(5), 2011–23. [PubMed: 23619363]
- (43). Redding KM, Chen BL, Singh A, Re RN, Navar LG, Seth DM, et al. (2010) Transgenic mice expressing an intracellular fluorescent fusion of angiotensin II demonstrate renal thrombotic microangiopathy and elevated blood pressure. *Am. J. Physiol. Heart. Circ. Physiol* 298, H1807–H1818. [PubMed: 20363893]
- (44). Li XC, Cook JL, Rubera I, Tauc M, Zhang F, Zhuo JL (2011) Intrarenal transfer of an intracellular cyan fluorescent fusion of angiotensin II selectively in proximal tubules increases blood pressure in rats and mice. *Am. J. Physiol. Renal. Physiol* 300, F1076–F1088. [PubMed: 21307128]
- (45). Li XC, Zhuo JL (2013) Proximal tubule-dominant transfer of AT_{1a} receptors induces blood pressure responses to intracellular angiotensin II in AT_{1a} receptor-deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 304, R588–R598. [PubMed: 23427083]
- (46). Carey RM, Siragy HM (2003) Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. *Endocr. Rev* 24(3), 261–71. [PubMed: 12788798]
- (47). Chappell MC (2012) Nonclassical renin-angiotensin system and renal function. *Compr. Physiol* 2(4), 2733–52. [PubMed: 23720263]

- (48). Zhuo JL, Li XC (2011) New insights and perspectives on intrarenal renin-angiotensin system: Focus on intracrine/intracellular angiotensin II. *Peptides*, 32(7), 1551–65. [PubMed: 21699940]
- (49). Ramkumar N, Stuart D, Rees S, Hoek AV, Sigmund CD, Kohan DE. (2014) Collecting duct-specific knockout of renin attenuates angiotensin II-induced hypertension. *Am. J. Physiol. Renal. Physiol* 307(8), F931–F938. [PubMed: 25122048]
- (50). Ramkumar N, Ying J, Stuart D, Kohan DE (2013) Overexpression of Renin in the collecting duct causes elevated blood pressure. *Am. J. Hypertens* 26(8), 965–72. [PubMed: 23702969]
- (51). Gonzalez AA, Lara LS, Luffman C, Seth DM, Prieto MC Soluble form of the (pro)renin receptor is augmented in the collecting duct and urine of chronic angiotensin II-dependent hypertensive rats. *Hypertension*, 57(4), 859–64. [PubMed: 21321306]
- (52). Peng K, Lu X, Wang F, Nau A, Chen R, Zhou SF, et al. (2017) Collecting duct (pro)renin receptor targets ENaC to mediate angiotensin II-induced hypertension. *Am. J. Physiol. Renal. Physiol* 312(2), F245–F253. [PubMed: 27122543]
- (53). Liu L, Gonzalez AA, McCormack M, Seth DM, Kobori H, Navar LG, et al. (2011) Increased renin excretion is associated with augmented urinary angiotensin II levels in chronic angiotensin II-infused hypertensive rats. *Am. J. Physiol. Renal. Physiol* 301(6), F1195–F1201. [PubMed: 21865264]
- (54). Prieto MC, Williams DE, Liu L, Kavanagh KL, Mullins JJ, Mitchell KD (2011) Enhancement of renin and prorenin receptor in collecting duct of Cyp11a1-Ren2 rats may contribute to development and progression of malignant hypertension. *Am. J. Physiol. Renal. Physiol* 300(2), F581–F588. [PubMed: 21068087]
- (55). Kobori H, Nishiyama A, Harrison-Bernard LM, Navar LG (2003) Urinary angiotensinogen as an indicator of intrarenal Angiotensin status in hypertension. *Hypertension*, 41(1), 42–9. [PubMed: 12511528]
- (56). Nishiyama A, Seth DM, Navar LG (2002) Renal interstitial fluid concentrations of angiotensins I and II in anesthetized rats. *Hypertension*, 39(1), 129–34. [PubMed: 11799091]
- (57). Siragy HM, Howell NL, Ragsdale NV, Carey RM (1995) Renal interstitial fluid angiotensin. Modulation by anesthesia, epinephrine, sodium depletion, and renin inhibition. *Hypertension*, 25(5), 1021–4. [PubMed: 7737709]
- (58). Zhuo JL, Imig JD, Hammond TG, Orengo S, Benes E, Navar LG (2002) Ang II accumulation in rat renal endosomes during Ang II-induced hypertension: role of AT₁ receptor. *Hypertension*, 39(1), 116–21. [PubMed: 11799089]
- (59). Shaltout HA, Westwood BM, Averill DB, Ferrario CM, Figueroa JP, Diz DI, et al. (2007) Angiotensin metabolism in renal proximal tubules, urine, and serum of sheep: evidence for ACE2-dependent processing of angiotensin II. *Am. J. Physiol. Renal. Physiol* 292(1), F82–F91. [PubMed: 16896185]
- (60). Braam B, Mitchell KD, Fox J, Navar LG (1993) Proximal tubular secretion of angiotensin II in rats. *Am. J. Physiol* 264(5 Pt 2), F891–F898. [PubMed: 8388654]
- (61). Navar LG, Lewis L, Hymel A, Braam B, Mitchell KD (1994) Tubular fluid concentrations and kidney contents of angiotensins I and II in anesthetized rats. *J. Am. Soc. Nephrol* 5(4), 1153–8. [PubMed: 7849257]
- (62). Imig JD, Navar GL, Zou LX, O'Reilly KC, Allen PL, Kaysen JH, et al. (1999) Renal endosomes contain angiotensin peptides, converting enzyme, and AT_{1A} receptors. *Am. J. Physiol* 277(2 Pt 2), F303–F311. [PubMed: 10444586]
- (63). Kobori H, Harrison-Bernard LM, Navar LG (2001) Enhancement of angiotensinogen expression in angiotensin II-dependent hypertension. *Hypertension*, 37(5), 1329–35. [PubMed: 11358949]
- (64). Kobori H, Harrison-Bernard LM, Navar LG Expression of angiotensinogen mRNA and protein in angiotensin II- dependent hypertension. *J. Am. Soc. Nephrol* 12(3), 431–9. [PubMed: 11181790]
- (65). Zou LX, Hymel A, Imig JD, Navar LG (1996) Renal accumulation of circulating angiotensin II in angiotensin II- infused rats. *Hypertension*, 27(3 Pt 2), 658–62. [PubMed: 8613220]
- (66). Zou LX, Imig JD, von Thun AM, Hymel A, Ono H, Navar LG (1996) Receptor-mediated intrarenal angiotensin II augmentation in angiotensin II-infused rats. *Hypertension*, 28(4), 669–77. [PubMed: 8843896]

- (67). van Kats JP, de Lannoy LM, Jan Danser AH, van Meegen JR, Verdouw PD, Schalekamp MA (1997) Angiotensin II type 1 (AT1) receptor-mediated accumulation of angiotensin II in tissues and its intracellular half-life in vivo. *Hypertension*, 30(1 Pt 1), 42–9. [PubMed: 9231819]
- (68). Li XC, Zhuo JL (2008) In vivo regulation of AT_{1a} receptor-mediated intracellular uptake of [¹²⁵I]-Val⁵-angiotensin II in the kidneys and adrenal glands of AT_{1a} receptor-deficient mice. *Am. J. Physiol. Renal. Physiol* 294, F293–F302. [PubMed: 18045833]
- (69). Li XC, Navar LG, Shao Y, Zhuo JL (2007) Genetic deletion of AT_{1a} receptors attenuates intracellular accumulation of angiotensin II in the kidney of AT_{1a} receptor-deficient mice. *Am. J. Physiol. Renal. Physiol* 293, F586–F593. [PubMed: 17537986]
- (70). Pohl M, Kaminski H, Castrop H, Bader M, Himmerkus N, Bleich M, et al. (2010) Intrarenal renin angiotensin system revisited: role of megalin-dependent endocytosis along the proximal nephron. *J. Biol. Chem* 285(53), 41935–46. [PubMed: 20966072]
- (71). Matsusaka T, Niimura F, Pastan I, Shintani A, Nishiyama A, Ichikawa I (2014) Podocyte injury enhances filtration of liver-derived angiotensinogen and renal angiotensin II generation. *Kidney Int.* 85(5), 1068–77. [PubMed: 24284520]
- (72). Ying J, Stuart D, Hillas E, Gociman BR, Ramkumar N, Lalouel JM, et al. (2012) Overexpression of mouse angiotensinogen in renal proximal tubule causes salt-sensitive hypertension in mice. *Am. J. Hypertens* 25(6), 684–9. [PubMed: 22378037]
- (73). Mancia G, De BG, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. (2007) 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J. Hypertens* 25(6), 1105–87. [PubMed: 17563527]
- (74). Ding Y, Sigmund CD (2001) Androgen-dependent regulation of human angiotensinogen expression in KAP-hAGT transgenic mice. *Am. J. Physiol. Renal. Physiol* 280(1), F54–F60. [PubMed: 11133514]
- (75). Taugner R, Hackenthal E, Nobiling R, Harlacher M, Reb G (1981) The distribution of renin in the different segments of the renal arterial tree: immunocytochemical investigation in the mouse kidney. *Histochemistry*, 73(1), 75–88. [PubMed: 7033188]
- (76). Celio MR, Inagami T (1981) Renin in the human kidney. Immunohistochemical localization. *Histochemistry*, 72(1), 1–10. [PubMed: 7026517]
- (77). Faraggiana T, Gresik E, Tanaka T, Inagami T, Lupo A (1982) Immunohistochemical localization of renin in the human kidney. *J. Histochem. Cytochem* 30(5), 459–65. [PubMed: 7042817]
- (78). Bader M, Zhao Y, Sander M, Lee MA, Bachmann J, Bohm M, et al. (1992) Role of tissue renin in the pathophysiology of hypertension in TGR(mREN2)27 rats. *Hypertension*, 19(6 Pt 2), 681–6. [PubMed: 1592468]
- (79). Zhuo JL, Ohishi M, Mendelsohn FA (1999) Roles of AT₁ and AT₂ receptors in the hypertensive Ren-2 gene transgenic rat kidney. *Hypertension*, 33(1 Pt 2), 347–53. [PubMed: 9931128]
- (80). Lavoie JL, Lake-Bruse KD, Sigmund CD (2004) Increased blood pressure in transgenic mice expressing both human renin and angiotensinogen in the renal proximal tubule. *Am. J. Physiol. Renal. Physiol* 286(5), F965–F971. [PubMed: 15075192]
- (81). Yanai K, Saito T, Kakinuma Y, Kon Y, Hirota K, Taniguchi-Yanai K, et al. (2000) Renin-dependent cardiovascular functions and renin-independent blood-brain barrier functions revealed by renin-deficient mice. *J. Biol. Chem* 275(1), 5–8. [PubMed: 10617578]
- (82). Takahashi N, Lopez ML, Cowhig JE, Jr., Taylor MA, Hatada T, Riggs E, et al. (2005) Ren1c homozygous null mice are hypotensive and polyuric, but heterozygotes are indistinguishable from wild-type. *J. Am. Soc. Nephrol* 16(1), 125–32. [PubMed: 15563565]
- (83). Pentz ES, Moyano MA, Thornhill BA, Sequeira Lopez M.L., Gomez RA. (2004) Ablation of renin-expressing juxtaglomerular cells results in a distinct kidney phenotype. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 286(3), R474–R483. [PubMed: 14563659]
- (84). Trepiccone F, Gerber SD, Grahammer F, Lopez-Cayuqueo KI, Baudrie V, Paunescu TG, et al. (2016) Renal Atp6ap2/(Pro)renin receptor is required for normal vacuolar H⁺-ATPase function but not for the renin-angiotensin system. *J. Am. Soc. Nephrol* 27(11), 3320–30. [PubMed: 27044666]

- (85). Ramkumar N, Stuart D, Mironova E, Bugay V, Wang S, Abraham N, et al. (2016) Renal tubular epithelial cell prorenin receptor regulates blood pressure and sodium transport. *Am. J. Physiol. Renal. Physiol* 311(1), F186–F194. [PubMed: 27053687]
- (86). Prieto MC, Reverte V, Mamenko M, Kuczeriszka M, Veiras LC, Rosales CB, et al. (2017) Collecting duct prorenin receptor knockout reduces renal function, increases sodium excretion, and mitigates renal responses in ANG II-induced hypertensive mice. *Am. J. Physiol. Renal. Physiol* 313(6), F1243–F1253. [PubMed: 28814438]
- (87). Advani A, Kelly DJ, Cox AJ, White KE, Advani SL, Thai K, et al. (2009) The (Pro)renin receptor: site-specific and functional linkage to the vacuolar H⁺-ATPase in the kidney. *Hypertension*, 54(2):261–9. [PubMed: 19546380]
- (88). Wang F, Lu X, Liu M, Feng Y, Zhou SF, Yang T (2015) Renal medullary (pro)renin receptor contributes to angiotensin II-induced hypertension in rats via activation of the local renin-angiotensin system. *BMC Med* 13, 278. doi: 10.1186/s12916-015-0514-1. [PubMed: 26554902]
- (89). Soubrier F, Alhenc-Gelas F, Hubert C, Allegrini J, John M, Tregear G, et al. (1988) Two putative active centers in human angiotensin I-converting enzyme revealed by molecular cloning. *Proc. Natl. Acad. Sci. USA*, 85(24), 9386–90. [PubMed: 2849100]
- (90). Bernstein KE, Martin BM, Bernstein EA, Linton J, Striker L, Striker G (1988) The isolation of angiotensin-converting enzyme cDNA. *J. Biol. Chem* 263(23), 11021–4. [PubMed: 2841312]
- (91). Chai SY, Allen AM, Adam WR, Mendelsohn FA (1986) Local actions of angiotensin II: quantitative in vitro autoradiographic localization of angiotensin II receptor binding and angiotensin converting enzyme in target tissues. *J. Cardiovasc. Pharmacol* 8(Suppl 10), S35–9.
- (92). Zhuo JL, Moeller I, Jenkins T, Chai SY, Allen AM, Ohishi M, et al. (1998) Mapping tissue angiotensin-converting enzyme and angiotensin AT₁, AT₂ and AT₄ receptors. *J Hyperten*, 16(12 Pt 2), 2027–37.
- (93). Harrison-Bernard LM, Zhuo JL, Kobori H, Ohishi M, Navar LG (2002) Intrarenal AT₁ receptor and ACE binding in ANG II-induced hypertensive rats. *Am. J. Physiol. Renal. Physiol* 282(1), F19–F25. [PubMed: 11739108]
- (94). Bruneval P, Hinglais N, Alhenc-Gelas F, Tricottet V, Corvol P, Menard J, et al. (1986) Angiotensin I converting enzyme in human intestine and kidney. Ultrastructural immunohistochemical localization. *Histochemistry*, 85(1), 73–80. [PubMed: 3015846]
- (95). Danilov SM, Faerman AI, Printseva OY, Martynov AV, Sakharov IY, Trakht IN (1987) Immunohistochemical study of angiotensin-converting enzyme in human tissues using monoclonal antibodies. *Histochemistry*, 87(5), 487–90. [PubMed: 2828286]
- (96). Schulz WW, Hagler HK, Buja LM, Erdos EG (1988) 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* 59(6), 789–97. [PubMed: 2848979]
- (97). Harris PJ, Navar LG, Ploth DW (1984) Evidence for angiotensin-stimulated proximal tubular fluid reabsorption in normotensive and hypertensive rats: effect of acute administration of captopril. *Clin Sci (Lond)*, 66(5), 541–4. [PubMed: 6368101]
- (98). Zhuo JL, Thomas D, Harris PJ, Skinner SL (1992) The role of endogenous angiotensin II in the regulation of renal haemodynamics and proximal fluid reabsorption in the rat. *J. Physiol.* 453, 1–13. [PubMed: 1464825]
- (99). Hashimoto S, Adams JW, Bernstein KE, Schnermann J (2005) Micropuncture determination of nephron function in mice without tissue angiotensin-converting enzyme. *Am. J. Physiol. Renal. Physiol* 288(3), F445–F452. [PubMed: 15494545]
- (100). Zhuo JL, Li XC (2013) Proximal nephron. *Compr. Physiol* 3(3), 1079–1123. [PubMed: 23897681]
- (101). Aronson PS (1983) Mechanisms of active H⁺ secretion in the proximal tubule. *Am. J. Physiol* 245(6), F647–F659. [PubMed: 6318566]
- (102). Bernstein KE, Ong FS, Blackwell WL, Shah KH, Giani JF, Gonzalez-Villalobos RA, et al. (2012) A modern understanding of the traditional and nontraditional biological functions of angiotensin-converting enzyme. *Pharmacol. Rev* 65(1), 1–46. [PubMed: 23257181]

- (103). Cole J, Quach DL, Sundaram K, Corvol P, Capecchi MR, Bernstein KE (2002) Mice lacking endothelial angiotensin-converting enzyme have a normal blood pressure. *Circ. Res* 90(1), 87–92. [PubMed: 11786523]
- (104). Gonzalez-Villalobos RA, Billet S, Kim C, Satou R, Fuchs S, Bernstein KE, et al. (2011) Intrarenal angiotensin-converting enzyme induces hypertension in response to angiotensin I infusion. *J. Am. Soc. Nephrol* 22(3), 449–59. [PubMed: 21115616]
- (105). van Kats JP, van Meegen JR, Verdouw PD, Duncker DJ, Schalekamp MA, Danser AH Subcellular localization of angiotensin II in kidney and adrenal. *J. Hypertens* 19(3 Pt 2), 583–9. [PubMed: 11327633]
- (106). Li XC, Zhuo JL Mechanisms of AT_{1a} receptor-mediated uptake of angiotensin II by proximal tubule cells: a novel role of the multiligand endocytic receptor megalin. *Am. J. Physiol. Renal. Physiol* 307(2), F222–F233. [PubMed: 24740791]
- (107). Zou LX, Imig JD, Hymel A, Navar LG Renal uptake of circulating angiotensin II in Val5-angiotensin II infused rats is mediated by AT₁ receptor. *Am. J. Hypertens* 11(5), 570–8. [PubMed: 9633793]
- (108). Kregg JH, John SW, Langenbach LL, Hodgins JB, Hagaman JR, Bachman ES, et al. (1995) Male-female differences in fertility and blood pressure in ACE- deficient mice. *Nature*, 375(6527), 146–8. [PubMed: 7753170]
- (109). Tanimoto K, Sugiyama F, Goto Y, Ishida J, Takimoto E, Yagami K, et al. (1994) Angiotensinogen-deficient mice with hypotension. *J. Biol. Chem* 269(50), 31334–7. [PubMed: 7989296]
- (110). Sharp MG, Fettes D, Brooker G, Clark AF, Peters J, Fleming S, et al. (1996) Targeted inactivation of the Ren-2 gene in mice. *Hypertension*, 28(6), 1126–31. [PubMed: 8952610]
- (111). Kessler SP, Hashimoto S, Senanayake PS, Gaughan C, Sen GC, Schnermann J (2005) Nephron function in transgenic mice with selective vascular or tubular expression of Angiotensin-converting enzyme. *J. Am. Soc. Nephrol* 16(12), 3535–42. [PubMed: 16221869]
- (112). Kessler SP, deS SP, Scheidemantel TS, Gomos JB, Rowe TM, Sen GC (2003) Maintenance of normal blood pressure and renal functions are independent effects of angiotensin-converting enzyme. *J. Biol. Chem* 278(23), 21105–12. [PubMed: 12777443]
- (113). Prieto-Carrasquero MC, Kobori H, Ozawa Y, Gutierrez A, Seth D, Navar LG AT₁ receptor-mediated enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. *Am. J. Physiol. Renal. Physiol* 289(3), F632–F637. [PubMed: 15870381]
- (114). Prieto MC, Botros FT, Kavanagh K, Navar LG (2013) Prorenin receptor in distal nephron segments of 2-kidney, 1-clip goldblatt hypertensive rats. *Ochsner J* 13(1), 26–32. [PubMed: 23532382]
- (115). Zhuo JL (2011) Augmented intratubular renin and prorenin expression in the medullary collecting ducts of the kidney as a novel mechanism of angiotensin II-induced hypertension. *Am. J. Physiol. Renal. Physiol* 301(6), F1193–F1194. [PubMed: 21993882]
- (116). van Kats JP, Schalekamp MA, Verdouw PD, Duncker DJ, Danser AH (2001) Intrarenal angiotensin II: interstitial and cellular levels and site of production. *Kidney Int* 60(6), 2311–7. [PubMed: 11737604]
- (117). Li XC, Hopfer U, Zhuo JL (2009) AT₁ receptor-mediated uptake of angiotensin II and NHE-3 expression in proximal tubule cells through the microtubule-dependent endocytic pathway. *Am. J. Physiol. Renal Physiol* 297(5), F1342–F1352. [PubMed: 19726542]
- (118). Li XC, Zhuo JL Intracellular ANG II directly induces in vitro transcription of TGF-beta1, MCP-1, and NHE-3 mRNAs in isolated rat renal cortical nuclei via activation of nuclear AT_{1a} receptors. *Am. J. Physiol. Cell Physiol* 294(4), C1034–C1045.
- (119). Li XC, Carretero OA, Navar LG, Zhuo JL (2006) AT₁ receptor-mediated accumulation of extracellular angiotensin II in proximal tubule cells: role of cytoskeleton microtubules and tyrosine phosphatases. *Am. J. Physiol. Renal Physiol* 291, F375–F383. [PubMed: 16478976]
- (120). Li XC, Zhuo JL (2007) Selective knockdown of AT₁ receptors by RNA interference inhibits Val⁵-Ang II endocytosis and NHE-3 expression in immortalized rabbit proximal tubule cells. *Am. J. Physiol. Cell Physiol* 293, C367–C378. 2007. [PubMed: 17428839]

- (121). Crowley SD, Gurley SB, Herrera MJ, Ruiz P, Griffiths R, Kumar AP, et al. (2006) Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney. *Proc. Natl. Acad. Sci. USA*, 103 (47),17985–90. [PubMed: 17090678]
- (122). Crowley SD, Zhang J, Herrera M, Griffiths RC, Ruiz P, Coffman TM (2011) The role of AT₁ receptor-mediated salt retention in angiotensin II-dependent hypertension. *Am. J. Physiol. Renal Physiol* 301(5):F1124–30. [PubMed: 21849491]
- (123). Gurley SB, Riquier-Brison AD, Schnermann J, Sparks MA, Allen AM, Haase VH, et al. AT_{1A} angiotensin receptors in the renal proximal tubule regulate blood pressure. *Cell Metab* 13(4), 469–75. [PubMed: 21459331]
- (124). Li H, Weatherford ET, Davis DR, Keen HL, Grobe JL, Daugherty A, et al. (2011) Renal proximal tubule angiotensin AT_{1A} receptors regulate blood pressure. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 301(4):R1067–77. [PubMed: 21753145]
- (125). Kumar R, Yong QC, Thomas CM, Baker KM Intracardiac intracellular angiotensin system in diabetes. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 302(5):R510–7. [PubMed: 22170614]
- (126). Wilson BA, Nautiyal M, Gwathmey TM, Rose JC, Chappell MC (2015) Evidence for a mitochondrial angiotensin-(1–7) system in the kidney. *Am. J. Physiol. Renal Physiol* 310(7):F637–F645. [PubMed: 26697984]
- (127). Zhuo JL, Kobori H, Li XC, Satou R, Katsurada A, Navar LG (2016) Augmentation of angiotensinogen expression in the proximal tubule by intracellular angiotensin II via AT_{1A}/MAPK/NF- κ B signaling pathways. *Am. J. Physiol. Renal Physiol* 310(10), F1103–F1112.
- (128). Wilson BA, Cruz-Diaz N, Su Y, Rose JC, Gwathmey TM, Chappell MC (2017) Angiotensinogen import in isolated proximal tubules: evidence for mitochondrial trafficking and uptake. *Am. J. Physiol. Renal Physiol* 312(5), F879–F886. [PubMed: 27903492]
- (129). Tadevosyan A, Xiao J, Surinkaew S, Naud P, Merlen C, Harada M, et al. (2017) Intracellular angiotensin-II interacts with nuclear angiotensin receptors in cardiac fibroblasts and regulates RNA synthesis, cell proliferation, and collagen secretion. *J. Am. Heart Assoc* 6(4),
- (130). Villar-Cheda B, Costa-Besada MA, Valenzuela R, Perez-Costas E, Melendez-Ferro M, Labandeira-Garcia JL (2017) The intracellular angiotensin system buffers deleterious effects of the extracellular paracrine system. *Cell Death Dis* 8(9), e3044. [PubMed: 28880266]
- (131). Zhuo JL, Alcorn D, McCausland J, Casley D, Mendelsohn FA (1994) In vivo occupancy of angiotensin II subtype 1 receptors in rat renal medullary interstitial cells. *Hypertension*, 23(6 Pt 2), 838–43. [PubMed: 8206614]
- (132). Zhuo JL, Alcorn D, Harris PJ, Mendelsohn FA (1993) Localization and properties of angiotensin II receptors in rat kidney. *Kidney Int.* 42, S40–S46.
- (133). Abadir PM, Foster DB, Crow M, Cooke CA, Rucker JJ, Jain A, et al. (2011) Identification and characterization of a functional mitochondrial angiotensin system. *Proc. Natl. Acad. Sci. USA*, 108(36):14849–54. [PubMed: 21852574]
- (134). Gwathmey T, Shaltout HA, Pendergrass KD, Pirro NT, Figueroa JP, Rose JC, et al. (2009) Nuclear angiotensin II - type 2 (AT₂) receptors are functionally linked to nitric oxide production. *Am. J. Physiol. Renal Physiol* 296, F1484–F1493. [PubMed: 19244399]
- (135). Gwathmey TM, Westwood BM, Pirro NT, Tang L, Rose JC, Diz DI, et al. (2010) Nuclear angiotensin-(1–7) receptor is functionally coupled to the formation of nitric oxide. *Am. J. Physiol. Renal Physiol* 299(5), F983–F990. [PubMed: 20810609]
- (136). Pendergrass KD, Averill DB, Ferrario CM, Diz DI, Chappell MC (2006) Differential expression of nuclear AT₁ receptors and angiotensin II within the kidney of the male congenic mRen2.Lewis rat. *Am. J. Physiol. Renal Physiol* 290(6), F1497–F1506. [PubMed: 16403834]
- (137). Schelling JR, Linas SL (1994) Angiotensin II-dependent proximal tubule sodium transport requires receptor-mediated endocytosis. *Am. J. Physiol* 266(3 Pt 1), C669–C675. [PubMed: 8166230]
- (138). Schelling JR, Hanson AS, Marzec R, Linas SL (1992) Cytoskeleton-dependent endocytosis is required for apical type 1 angiotensin II receptor-mediated phospholipase C activation in cultured rat proximal tubule cells. *J. Clin. Invest* 90(6), 2472–80. [PubMed: 1334976]

- (139). Thekkumkara TJ, Cookson R, Linas SL (1998) Angiotensin (AT₁A) receptor-mediated increases in transcellular sodium transport in proximal tubule cells. *Am. J. Physiol* 274, F897–F905. [PubMed: 9612327]
- (140). Zhuo JL, Carretero OA, Li XC Effects of AT₁ receptor-mediated endocytosis of extracellular Ang II on activation of nuclear factor- κ B in proximal tubule cells. *Ann. N.Y. Acad. Sci* 1091, 336–45.
- (141). Haller H, Lindschau C, Erdmann B, Quass P, Luft FC (1996) Effects of intracellular angiotensin II in vascular smooth muscle cells. *Circ. Res* 79(4), 765–72. [PubMed: 8831500]
- (142). Haller H, Lindschau C, Quass P, Luft FC (1999) Intracellular actions of angiotensin II in vascular smooth muscle cells. *J. Am. Soc. Nephrol Suppl* 11, S75–S83.
- (143). Zhuo JL, Li XC, Garvin JL, Navar LG, Carretero OA. Intracellular angiotensin II induces cytosolic Ca²⁺ mobilization by stimulating intracellular AT₁ receptors in proximal tubule cells. *Am. J. Physiol. Renal Physiol* 290, F1382–F1390.
- (144). Re R, Parab M (1984) Effect of angiotensin II on RNA synthesis by isolated nuclei. *Life Sci* 34, 647–51. [PubMed: 6199638]
- (145). Eggena P, Zhu JH, Clegg K, Barrett JD (1993) Nuclear angiotensin receptors induce transcription of renin and angiotensinogen mRNA. *Hypertension*, 22(4), 496–501. [PubMed: 8406654]
- (146). Tadevosyan A, Maguy A, Villeneuve LR, Babin J, Bonnefoy A, Allen BG, et al. (2010) Nuclear-delimited angiotensin receptor-mediated signaling regulates cardiomyocyte gene expression. *J. Biol. Chem* 285(29), 22338–49. [PubMed: 20463030]
- (147). Cook JL, Mills SJ, Naquin R, Alam J, Re RN (2006) Nuclear accumulation of the AT₁ receptor in a rat vascular smooth muscle cell line: effects upon signal transduction and cellular proliferation. *J. Mol. Cell Cardiol* 40(5), 696–707. [PubMed: 16516913]
- (148). Baker KM, Kumar R (2006) Intracellular angiotensin II induces cell proliferation independent of AT₁ receptor. *Am. J. Physiol. Cell Physiol* (5), C995–1001. [PubMed: 16774988]
- (149). Li XC, Hopfer U, Zhuo JL Novel signaling mechanisms of intracellular angiotensin II-induced NHE3 expression and activation in mouse proximal tubule cells. *Am. J. Physiol. Renal Physiol* 303(12), F1617–F1628.
- (150). Gwathmey TM, Pendergrass KD, Reid SD, Rose JC, Diz DI, Chappell MC (2010) Angiotensin-(1–7)-angiotensin-converting enzyme 2 attenuates reactive oxygen species formation to angiotensin II within the cell nucleus. *Hypertension*, 55(1), 166–71. [PubMed: 19948986]
- (151). Lavoie JL, Liu X, Bianco RA, Beltz TG, Johnson AK, Sigmund CD (2006) Evidence supporting a functional role for intracellular renin in the brain. *Hypertension*, 47(3), 461–6. [PubMed: 16446393]
- (152). van Thiel BS, Goes MA, Te RL, Severs D, Uijl E, Garrelds IM, et al. (2017) Brain renin-angiotensin system: does it exist? *Hypertension*, 69(6), 1136–44. [PubMed: 28396529]
- (153). Uijl E, Ren L, Danser AHJ (2018) Angiotensin generation in the brain: a reevaluation. *Clin. Sci. (Lond)*, 132(8), 839–50. [PubMed: 29712882]
- (154). Sigmund CD, Diz DI, Chappell MC No brain renin-angiotensin system: déjà vu all over again? *Hypertension*, 69(6), 1007–10.
- (155). Cook JL, Re R, Alam J, Hart M, Zhang Z (2004) Intracellular angiotensin II fusion protein alters AT₁ receptor fusion protein distribution and activates CREB. *J. Mol. Cell. Cardiol* 36(1), 75–90. [PubMed: 14734050]
- (156). Ferrao FM, Cardoso LHD, Drummond HA, Li XC, Zhuo JL, Gomes DS, et al. (2017) Luminal ANG II is internalized as a complex with AT₁R/AT₂R heterodimers to target endoplasmic reticulum in LLC-PK1 cells. *Am. J. Physiol. Renal Physiol* 313(2), F440–F449. [PubMed: 28468964]
- (157). Gonzalez AA, Lara LS, Prieto MC (2017) Role of collecting duct renin in the pathogenesis of hypertension. *Curr. Hypertens. Rep* 19(8):62. doi: 10.1007/s11906-017-0763-9 [PubMed: 28695400]

Figure 1:

The expression and localization of renin (A), angiotensin-converting enzyme (ACE) (B), angiotensin II type 1 (AT₁) (C), AT₂ (D), ANG (1–7) (E), and AT₄ receptors in human (A–D) or rat kidney (E & F). In the human kidney, active renin (silver grains, labeled by ¹²⁵I-H77, a renin inhibitor) is primarily localized in the juxtaglomerular apparatus (JGA) in the vascular pole of the glomerulus (A) [34][48], ACE primarily in the apical membranes of the proximal tubule (B, labeled by ¹²⁵I-351A, an ACE inhibitor) [91–93], AT₁ receptors in the glomerulus, proximal tubule, and the inner stripe of the out medulla (C, labeled by ¹²⁵I-

ANG II in the presence of PD123319, an AT_2 antagonist), and AT_2 receptors in intrarenal blood vessels and peritubular tissues (D, labeled by ^{125}I -ANG II in the presence of losartan, an AT_1 antagonist), respectively [79][131][132]. In the rat kidney, in addition to renin, ACE, AT_1 and AT_2 , the receptor binding sites for ANG (1–7), $AT_{(1-7)}$ labeled with ^{125}I -ANG (1–7) and ANG (3–8), AT_4 labeled with ^{125}I -ANG (3–8), are primarily localized in the cortex, corresponding to the proximal tubule [26][34].

Figure 2.

Under physiological conditions with normal blood pressure, glomerular filtration and permeability, and intact proximal tubular endocytic megalin- and AT_1 receptor-mediated uptake mechanisms, most of, if not all, major components of the RAS with a molecular wt. equaling or being smaller than albumin (~67 kDa), including AGT, prorenin, renin, ANG I and ANG II, are filtered by the glomerulus and taken up by the proximal tubule [38][39][41][70]. The reabsorption of these proteins and peptides is nearly complete. As a result very little is delivered to the distal nephron segments and are excreted into the urine. However, under pathophysiological conditions such as ANG II-induced hypertension, diabetes, Dent's disease and Lowe Syndrome, the glomerular structure and filtration barrier are damaged and/or there is a defected megalin-dependent endocytic uptake mechanism, most of the filtered or locally synthesized albumin, AGT, ANG I, ANG II, prorenin and renin are not taken up by the proximal tubule and are expected to appear in urine [38][39][41][70].

Figure 3.

Basal systolic blood pressure phenotype in conscious wildtype, liver AGT-KO, kidney AGT-KO, and dual liver and kidney AGT-KO mice. Please note that there is no difference in basal systolic blood pressure level between wildtype and kidney AGT-KO mice, whereas basal systolic blood pressure was significantly decreased in both liver AGT-KO and dual liver and kidney AGT-KO mice. Data were derived from references [38].

Figure 4. Basal systolic blood pressure phenotype in conscious wildtype, global ACE-KO [111], ACE2/2 with elevated plasma ACE [7], ACE3/3 with overexpression of ACE in the liver without kidney ACE [106], ACE9/9 with overexpression of ACE in the kidney [107], and ACE10/10 mice with kidney ACE knockout [42]. Please see the text for detailed discussion.

Figure 5.

Basal systolic blood pressure phenotype in conscious wildtype, global AT_{1a}-KO [9,31,70,113], proximal tubule-specific PT-AT_{1a}-KO [126,127], proximal tubule-specific overexpression of an intracellular ANG II fusion protein, PT-iANG II-KI [44,45,130], and global AT_{1a}-KO mice with proximal tubule-specific overexpression of AT_{1a} receptors, AT_{1a}-KO/PT-AT_{1a}-KI [45]. Please see the text for detailed discussion.