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New Insights and Perspectives on Intrarenal Renin-Angiotensin System: Focus on Intracrine/Intracellular Angiotensin II*

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Abstract

Although renin, the rate-limiting enzyme of the renin-angiotensin system (RAS), was first discovered by Robert Tigerstedt and Bergman more than a century ago, the research on the RAS still remains stronger than ever. The RAS, once considered to be an endocrine system, is now widely recognized as dual (circulating and local/tissue) or multiple hormonal systems (endocrine, paracrine and intracrine). In addition to the classical renin/angiotensin I-converting enzyme (ACE)/angiotensin II (Ang II)/Ang II receptor (AT₁/AT₂) axis, the prorenin/(Pro)renin receptor (PRR)/MAP kinase axis, the ACE2/Ang (1-7)/Mas receptor axis, and the Ang IV/AT₄/insulin-regulated aminopeptidase (IRAP) axis have recently been discovered. Furthermore, the roles of the evolving RAS have been extended far beyond blood pressure control, aldosterone synthesis, and body fluid and electrolyte homeostasis. Indeed, novel actions and underlying signaling mechanisms for each member of the RAS in physiology and diseases are continuously uncovered. However, many challenges still remain in the RAS research field despite of more than one century's research effort. It is expected that the research on the expanded RAS will continue to play a prominent role in cardiovascular, renal and hypertension research. The purpose of this article is to review the progress recently being made in the RAS research, with special emphasis on the local RAS in the kidney and the newly discovered prorenin/PRR/MAP kinase axis, the ACE2/Ang (1-7)/Mas receptor axis, the Ang IV/AT₄/IRAP axis, and intracrine/intracellular Ang II. The improved knowledge of the expanded RAS will help us better understand how the classical renin/ACE/Ang II/AT₁ receptor axis, extracellular and/or intracellular origin, interacts with other novel RAS axes to regulate blood pressure and cardiovascular and kidney function in both physiological and diseased states.

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Keywords

Angiotensin converting enzyme 2 (ACE2); angiotensin II (Ang II); AT₁ receptor signaling; insulin-regulated aminopeptidase (IRAP); Mas receptor; (Pro)renin receptor (PRR)

1. Introduction

Since renin, the rate-limiting enzyme of the renin-angiotensin system (RAS), was first discovered by Robert Tigerstedt and Bergman more than a century ago [210], the RAS is now well recognized as a dual vasoactive system, acting as both a circulating endocrine system and a local tissue paracrine system [25,77,111]. A local RAS has been demonstrated in nearly every target tissues including the kidney, adrenal glands, heart, blood vessels, pancreas, liver, brain, and even adipose tissues. The localization and functional properties of a local RAS in various target tissues have been reviewed elsewhere [12,29,40,61,65,117,236]. The objective of this review therefore focuses solely on the new insights and perspectives on the endocrine, paracrine and intracrine RAS in the kidney. In the kidney, all major components of the RAS, including the precursor angiotensinogen, the rate-limiting enzyme renin and angiotensin I-converting enzyme (ACE), and the receptors for angiotensin II (Ang II), AT₁ and AT₂, Ang (1-7) and Ang (3-8) have been demonstrated (Fig. 1) [29,35,107,153,163,238]. Although there are continuous debates on whether renin and angiotensinogen are synthesized or taken up in tissues other than the juxtaglomerular apparatus (JGA) or hepatocytes, respectively, there is a consensus that the presence of renin, angiotensinogen and ACE in tissues is necessary for local formation of Ang II independent of circulating Ang II. In spite of recent discovery of several new components of the RAS, such as prorenin or (Pro)renin receptor, PRR [168,169], the second ACE (ACE2) [51,68], biologically active angiotensin fragments such as Ang (1-7) and Ang (3-8) (Ang IV) [35,82], and the novel receptors for Ang (1-7), Mas [187], and Ang IV, AT₄ [3], Ang II is still and will remain to be the principle effector of the RAS in the kidney and other tissues. Likewise, although five major classes of Ang receptors, AT_{1a}, AT_{1b} and AT₂ for Ang II, the Mas receptor for Ang (1-7), and AT₄ for Ang IV have been cloned, the AT₁ (AT_{1a}) receptor remains to be the principal receptor that mediates the majority of the known actions of Ang II in the kidney [30,39,76,172,199,235,239]. Nevertheless, new progress has indeed been made in several areas during last several years. First, what we have known as the classical RAS for many decades has now expanded to include prorenin/PRR [168,169] and ACE2 [51,68] (Fig. 2). Second, there are renewed interests in the physiological and pathophysiological roles of several Ang II metabolites including Ang III, Ang (1-7) and Ang IV [33,82,173]. Third, the so-called intracrine/intracellular RAS or Ang II, which was previously considered not to be physiologically important, has been recently shown to have blood pressure-increasing effects in rodents [135,180]. The critical review or timely update of these new developments in the RAS research may provide new insights and perspectives into both classic and novel roles of the RAS in the physiological regulation of blood pressure, cardiovascular and kidney functions, and in the development of hypertension, cardiovascular and kidney diseases.

2. Overview of the prorenin/PRR/MAP kinases ERK 1/2 axis in renal physiology and diseases

2.1. Localization and roles of prorenin in the kidney

The molecular biology, structure, biochemistry and the roles of prorenin and (Pro)renin receptors (PRR) in physiology and diseases have been critically reviewed elsewhere [26,58,166,198]. In the current article, only the progress on the kidney- and blood pressure-

related prorenin and PRR research is briefly reviewed. The kidney was initially thought to be the only tissue to produce renin in JGA cells. Indeed, renin was found to decrease markedly after the kidneys were removed in humans and animals [27,28]. However, it is now recognized that prorenin is also produced in extrarenal tissues including adrenal glands, reproductive tissues such as ovary and testis, and the retina of eyes [26,59,226]. A physiological or pathophysiological role of prorenin is indicated by the observations that the levels of prorenin may be 100-time higher in ovarian follicular fluid and amniotic fluid in physiology, and in vitreous fluid of diabetic retinopathy [59,226]. In the kidney, renin and prorenin are primarily located in JGA cells, afferent arteries and interlobular arterioles, where prorenin may be activated by sodium depletion [60,202,205,237]. Despite of its presence in the kidney (or other tissues), prorenin was not previously considered to have a biological role due to its insignificant intrinsic enzymatic activity [26]. For example, when recombinant human prorenin was infused into rhesus monkeys or rats transgenic for human angiotensinogen, it failed to increase blood pressure [132,159]. Likewise, blood pressure was either unchanged or decreased in rats transgenic for rat or human prorenin with ~ 60 to 400-fold increases in plasma prorenin levels [154,220]. These studies strongly suggest that prorenin may not exert a physiological role in blood pressure, cardiovascular and renal regulation.

2.2. Localization and roles of (Pro)renin receptors (PRR) in the kidney

The discovery of PRR rapidly changes the dynamics of prorenin research in the kidney and other tissues [26,58,166,198]. The term of the (Pro)renin receptor may be confusing, because both renin and prorenin recognize and bind this same receptor, and activate the same intracellular signaling pathway, the MAP kinases ERK1/2. It is not quite sure whether it is renin or prorenin that physiologically activates this PRR. The PRR is a 350-amino acid protein with a single transmembrane domain and was first pharmacologically characterized in glomerular mesangial cells [168] and subsequently cloned by Nguyen et al. in 2002 [169]. PRR was later found structurally identical to CAPER protein (endoplasmic reticulum-localized type 1 transmembrane adaptor precursor) and vacuolar H⁺-ATPase (V-ATPase) [1,57,198]. The interactions between PRR and V-ATPase are particularly relevant in the renal physiology and diseases. V-ATPase is present on the membranes of intracellular endosomes, lysosomes and endoplasmic reticulum, where V-ATPase activity plays an important role in maintaining an acidification environment of endosomal and lysosomal compartments, and therefore in intracellular pH homeostasis and G protein-coupled endocytosis and recycling. In contrast to its initial localization and characterization in human mesangial cells [168], mRNAs and immunoreactive proteins of PRR were recently localized primarily in the distal nephron segments especially in collecting ducts [1,198]. However, PRR is in fact expressed widely in different tissues.

2.3. Signaling mechanisms underlying the activation of the prorenin/PRR/MAP kinases ERK 1/2 axis

Activation of PRR by prorenin or renin has been shown to induce diverse biological and pathophysiological responses ranging from activation of MAP kinases ERK1/2, p38 MAP kinase, TGF- β 1, PAI-1, COX-2, PI3 kinase and fibronectin, to proteinuria and nephropathy [165,166,198]. Most if not all of studies on prorenin and PPR suggest that renin and prorenin bind PRR and activates MAP kinases ERK1/2 independent of Ang II production and/or activation of AT₁ (AT_{1a}) receptors [166,167]. It has been further suggested that this pathway may play an important role in cardiovascular and kidney diseases in which the classical renin and ACE inhibitors or AT₁ receptor blockers (ARBs) are ineffective [164,165]. However, this conclusion remains to be further established due to several reasons [26,57,80,198]. First, there is evidence that prorenin binding to PRR induces non-proteolytic activation of prorenin to elicit full renin enzymatic activity [13,161,169].

Activation of prorenin on the cell surface almost certainly leads to Ang II formation on the cell membrane, which in turn activates downstream MAP kinases ERK1/2 signaling via an AT₁ receptor-dependent mechanism. The renin- and/or Ang II-dependent activation of MAP kinases ERK1/2 appears to have been well established. Ang II also appears to stimulate V-ATPase activity in rat proximal tubule cells via mechanisms involving tyrosine kinase, p38 MAPK, and PI3K [31]. Since PRR is structurally identical to CAPER protein (endoplasmic reticulum-localized type 1 transmembrane adaptor precursor) and vacuolar H⁺-ATPase (V-ATPase), intracellular PRR may also mediate the intracellular actions of prorenin or renin [1,57,198]. Second, in most published studies nanomolar concentrations of prorenin have been used to induce PRR activation in vitro, whereas only femtomolar to picomolar concentrations of prorenin are reported in the circulation and tissues [26,57]. Thus physiological roles of PRR remain uncertain. Third, renin and ACE inhibitors and ARBs were not simultaneously used to block the entire RAS cascade before in vitro or in vivo studies involving prorenin or PRR overexpression were performed, and Ang II production was rarely determined in those studies. And finally, a specific PRR inhibitor or antagonist remains to be developed, which will play an important role in defining the physiological and pathophysiological roles of prorenin and PRR. The results of the so-called Handle Region Peptide (HRP) to block PRR at best have been mixed and often remain controversial. Both positive and negative effects of this decoy peptide in blocking PRR have been reported [104,80]. Even if HRP is indeed effective in blocking prorenin and PRR interactions, its clinical relevance remains unknown due to its peptide properties. The renin-specific inhibitors have been developed to treat hypertension and cardiovascular and kidney diseases. Whether the renin inhibitors are therapeutically superior to classical ACE inhibitors or ARBs remains an issue of continuous debates. If prorenin and PRR indeed play important physiological and pathophysiological roles in blood pressure regulation and pathologies of cardiovascular, renal and diabetic diseases, the development of orally active PRR-specific inhibitors to block prorenin-induced activation of PRR will be highly necessary.

3. Overview of the ACE2/Ang (1-7)/Mas receptor axis in renal physiology and diseases

3.1. Roles of ACE2 in Ang (1-7) formation in the kidney and other tissues

Recently, there has been an explosion of renewed interest in studying the biological, physiological and pathophysiological role of the angiotensin metabolite, Ang (1-7), following the discoveries of ACE2 and the Mas receptor specific for Ang (1-7) [35,82,106,186,195]. Ang (1-7) is a heptapeptide of the RAS, which may be generated in two different ways. First, a number of enzymes in tissues including neprilysin [EC 3.4.24.11], prolyl oligopeptidase [EC 3.4.24.26], and thimet oligopeptidase [EC 3.4.24.15] may cleave three end amino acids, Phe-His-Leu, from the decapeptide Ang I [Ang (1-10)] to form Ang (1-7) [35,82]. In the kidney, neprilysin appears to be the major enzyme to process Ang I to Ang (1-7) [35,82]. Second, it is now recognized that Ang (1-7) can also be generated by an alternative enzyme named ACE2 [69,212]. ACE2 was first cloned by Donoghue et al. from 5' sequencing of a human heart failure ventricle cDNA library in 2000 [69]. It shares 42% of the genomic structure of ACE and is expressed not as widely as ACE in that ACE2 is localized mainly in the heart, kidney and testis in humans [69,130,201,223]. Both ACE and ACE2 are membrane-bound metallopeptidases and require both Zn²⁺ and Cl⁻ for optimal activation [35,82]. However, the biochemical activities of ACE2 are different from those of ACE. For example, the main functions of ACE are to convert the inactive Ang I to form the active octapeptide Ang II [Ang (1-8)] and to degrade bradykinin. Additionally, ACE also appears to be a major route for Ang (1-7) metabolism in the plasma and proximal tubules of the kidney [35,82]. By contrast, ACE2 acts as a monocarboxypeptidase to cleave only a single residue or amino acid from the carboxyl terminus of Ang I to form Ang (1-9)

[69], or degrade Ang II into Ang (1-7) [35,82]. ACE2 is not inhibited by classical ACE inhibitors, suggesting that ACE2 represents a novel and independent member of the RAS [35,82]. Interestingly, Ang (1-9) may further be cleaved by other endopeptidases to generate Ang (1-7) or other fragments, of which Ang (1-7) by far generates most of exciting investigations among all angiotensin metabolites.

3.2. Localization of Ang (1-7) receptors or the Mas receptors in the kidney

The specific binding sites or receptors for the heptapeptide Ang (1-7) had been elusive until Santos et al. identified the G protein-coupled receptor encoded by the Mas protooncogene as the Ang (1-7) receptor [187]. The association between the Mas protooncogene and Ang II receptors or Ang II-induced signalling is not entire new, since it has been suggested that the Mas gene may encode an Ang II receptor [109] and modulate AT₁ receptor-mediated intracellular Ca²⁺ signalling [5]. However, Santos et al. showed that genetic deletion of the Mas receptor abolished Ang (1-7) binding in the mouse kidney and prevented in vitro and in vivo vascular and renal responses to Ang (1-7) stimulation [187]. There are questions remained as to whether the Mas receptor is specific only to Ang (1-7) or involves multiple receptor binding sites. For example, specific ¹²⁵I-Ang (1-7) binding in kidney sections as demonstrated by Santos et al. was not much different between wild-type and Mas-KO mice [187]. Furthermore, even at pharmacological concentrations (10 μM) unlabeled Ang (1-7) or Ang (1-7) antagonist A-779 could still only displace approximately 40% of ¹²⁵I-Ang (1-7) binding [187]. This raises the possibility that the Mas receptor is only partially specific to Ang (1-7) and additional receptor(s) may be involved. Nevertheless, there is evidence that the Mas receptor appears to mediate the known biological and physiological effects of Ang (1-7) in different tissues [35,82,187].

In the kidney, the Mas receptor is widely expressed in intrarenal blood vessels such as afferent arterioles and tubular epithelium especially in proximal tubules, where the known biological and physiological effects of Ang (1-7) have been extensively investigated [35,82] (for a more extensive review). Using ¹²⁵I-Ang (1-7) as the ligand for its receptors, we found that specific ¹²⁵I-Ang (1-7) receptor binding is localized primarily in the inner cortex and the outer stripe of the outer medulla in the rat kidney, which may be completely displaced by unlabeled Ang (1-7) (Fig. 1). This anatomical localization suggests that Ang (1-7) receptors are likely expressed predominantly in proximal tubules.

3.3. Roles of Ang (1-7) receptors or the Mas receptors in the kidney

In general, the activation of ACE2/Ang (1-7)/the Mas receptor axis largely opposes the known actions of the renin//ACE/Ang II/AT₁ receptor axis through inhibition of MAP kinase- or cyclooxygenase-2 (COX-2)-dependent pathways, or stimulation of nitric oxide (NO)/cGMP-dependent pathway [35,82,106,186] (Fig. 3). While the renin/ACE/Ang II/AT₁ receptor axis acts to raise blood pressure [53,108,137], cause renal vasoconstriction, decrease renal blood flow (RBF) and glomerular filtration rate (GFR) [32,98,117], stimulation of proximal tubule sodium transport [43,162,240] and increase urine concentration [138,170] etc., the ACE2/Ang (1-7)/the Mas receptor instead acts to reduce blood pressure, dilate intrarenal blood vessels, increase RBF and GFR, inhibit proximal tubule transport, and induce diuresis [35,82] (for a comprehensive review). Similarly, the renin/ACE/Ang II/AT₁ receptor axis is widely implicated in the pathogenesis and progression of acute and chronic kidney diseases, including hypertension and diabetic nephropathy [52,77,142,158]. By contrast, overexpression of ACE2 to increase Ang (1-7) production or stimulation of the Mas receptor by Ang (1-7) in target tissues may be used to counter the pathological role of the renin/ACE/Ang II/AT₁ receptor axis. Indeed, impairment of the ACE2/Ang (1-7)/the Mas receptor axis may be associated with increases in blood pressure [23,88] or acute renal ischemic and reperfusion injury [55].

It should be emphasized, however, that the activation of the ACE2/Ang (1-7)/the Mas receptor axis may not be involved in established hypertension in SHRSP or TGR (mRen2)27 models [114], but may be associated with stimulation of growth-stimulatory pathways in human mesangial cells [248], increased blood pressure and adverse cardiac remodelling in rats with subtotal nephrectomy [219], accelerated STZ-induced diabetic nephropathy [197], or Ang II-induced epithelial-to-mesenchymal transformation [24]. Ang-(1-7) also reportedly stimulated water transport in rat inner medullary collecting duct via interactions with vasopressin V2 receptors [150]. These effects of Ang (1-7) are quite similar to those induced by the activation of the renin/ACE/Ang II/AT₁ receptor axis. It is tempting to speculate that these diverse effects of Ang (1-7) may be mediated by, or interacted with, different receptors that recognize the heptapeptide and may be expressed differentially in different cell types in the kidney. Further studies are required to establish the physiological and therapeutical roles of this novel ACE2/Ang (1-7)/the Mas receptor axis in hypertension, cardiovascular and renal diseases.

4. Overview of the Ang IV/AT₄/IRAP axis in renal physiology and diseases

4.1. Roles of aminopeptidases A and N in Ang IV formation in the kidney and other tissues

Ang (3-8), also termed Ang IV, is another biologically active Ang II fragment that has been a subject of extensive studies during recent years. Ang IV is primarily derived from Ang II or Ang III through the enzymatic actions of aminopeptidases A and N to cleave one or two N terminal amino acids [8,9]. The Ang IV/AT₄ receptors and their biological and pharmacological properties and tissue distribution have been comprehensively reviewed [61]. In the current article, we will focus on the Ang IV/AT₄ receptor axis in the kidney, because Aminopeptidases A and N are particularly abundant in this organ, especially in apical membranes of proximal nephron where these enzymes degrade Ang II to form Ang IV [174]. Ang IV can also be generated in the glomeruli of the kidney [9].

4.2. Hemodynamic and tubular effects of Ang IV in the kidney

In the kidney, Ang IV has been shown to induce diverse biological and physiological effects, which are often inconsistent or controversial. For example, Ang IV was initially shown to induce renal cortical vasodilatation probably through nitric oxide independent of AT₁/AT₂ receptors [44,203]. However, subsequent studies showed that Ang IV remains a potent renal vasoconstrictor in the kidney via activation of AT₁ receptor [83,133,218,229,230]. One of the most significant studies is that genetic over-expression of Ang IV in the brain of a transgenic mouse model leads to hypertension that is reversed by an AT₁ receptor antagonist [148]. Some of Ang IV-induced intracellular signaling pathways also appear to resemble those elicited by Ang II via AT₁ receptor activation. For instance, Ang IV was reported to increase intracellular calcium in mesangial cells [34,133], human proximal tubules cells [94], opossum kidney cells [74], or Mardin-Darby bovine kidney epithelial cells [95]. Conversely, Ang IV either increased [54] or had no effect on cAMP production or intracellular calcium in kidney cells [37].

We have recently studied whether Ang IV exerts systemic and renal cortical effects on blood pressure (MAP), renal microvascular smooth muscle cells (VSMCs), or glomerular mesangial cells (MC) and, if so, whether AT₁ receptor signaling is involved [133]. We demonstrated that in anesthetized rats, systemic infusion of Ang II, Ang III, or Ang IV caused dose-dependent increases in MAP and decreases in renal cortical blood flow. Ang II also induced dose-dependent reductions in renal medullary blood flow, whereas Ang IV did not. Ang IV-induced MAP response and renal cortical vasoconstriction were completely abolished by the AT₁ receptor blocker losartan [133]. When Ang IV (1 nmol/kg/min) was infused directly in the renal artery, renal cortical blood flow was reduced by >30%, and the

response was also blocked by losartan. Furthermore, unlabeled Ang IV displaced ^{125}I -labeled $[\text{Sar}^1, \text{Ile}^8]\text{Ang II}$ binding, whereas unlabeled Ang II (10 μM) also inhibited ^{125}I -labeled $\text{Nle}^1\text{-Ang IV (AT}_4\text{)}$ binding in a concentration-dependent manner (Fig. 1). In freshly isolated renal VSMCs, Ang IV (100 nM) increased intracellular Ca^{2+} concentration, and the effect was blocked by losartan and U-73122, a selective inhibitor of phospholipase C/inositol trisphosphate/ Ca^{2+} signaling (1 μM) [133]. In cultured rat MCs, Ang IV (10 nM) induced MAP kinases ERK 1/2 phosphorylation also via AT_1 receptor- and phospholipase C-activated signaling. Our results suggest that, at nanomolar concentrations, Ang IV can increase MAP and induce renal cortical effects by interacting with AT_1 receptor-activated signalling pathways [133].

4.3. Insulin-regulated aminopeptidase as the receptor for Ang IV (AT_4)

The identification of the AT_4 receptor to be insulin-regulated aminopeptidase (IRAP) may open new opportunities to further study the roles of Ang IV via this unique receptor [3]. IRAP is a type II integral membrane spanning protein associated with the M1 family of aminopeptidases and GLUT4 vesicles in insulin-responsive cells [3,33]. Albiston et al. have recently mapped the distribution of both GLUT4 and IRAP in the kidney [4]. IRAP and GLUT4 immunostaining was localized, but not overlapped, in proximal and distal tubules and thick ascending limbs in the cortex. GLUT4 staining appears to be associated with intracellular vesicles, whereas IRAP staining was predominantly associated with the apical membrane [4]. IRAP immunoreactivity was also localized in the principal cells of the inner medulla collecting ducts (IMCD), with limited overlap with the vasopressin responsive water channel aquaporin-2 (AQP-2) [4]. The role of IRAP in the regulation of renal hemodynamics or renal tubular transport has not been investigated. The localization of IRAP and specific ^{125}I -Ang IV binding sites in the renal cortex suggests that the Ang IV/ AT_4 /IRAP axis may play an important role in the regulation of glucose uptake or transport in proximal tubules of the kidney [4,96,133]. Mice deficient in IRAP (IRAP $^{-/-}$) have been generated [116] and may be a useful model for further studies of the physiological and pathophysiological roles of the Ang IV/ AT_4 /IRAP axis in the kidney.

5. New insights into the classical renin/ACE/Ang II/ AT_1 receptor axis in renal physiology and diseases: focus on intracrine/intracellular Ang II in proximal tubules

5.1. Overview of the knowledge gap on the roles of intracrine/intracellular Ang II

Angiotensin II is undoubtedly the major effector of the renin/ACE/Ang II/ AT_1 receptor axis in long-term cardiovascular, renal and blood pressure regulation (Fig. 1 & Fig. 2). There is also no doubt that extracellular (circulating and paracrine) Ang II plays the classical roles of Ang II through activation of cell surface GPCRs (Fig. 3) [25,29,61,111,152,211,213]. Thus, no wonder many have assumed that: 1) Ang II only needs to activate cell surface receptors to induce all of its responses, 2) all renin and ACE inhibitors and ARBs would block all responses to extracellular Ang II to produce the same beneficial effects, and 3) an intracrine/intracellular Ang II system may not be required or involved in the regulation of cardiovascular and renal physiology or diseases.

However, recent research suggests that these views may be revised for a number of reasons [49,65,119,121,179,246]. First, extracellular Ang II is continuously internalized with the receptors immediately after it activates cell surface receptors. There is evidence that activated agonist/receptor complex internalized into endosomes may continue to transmit Ras/MAPK signaling from endosomes [18,50,149,160,176]. Second, Ang II exerts powerful and long-lasting genomic or gene transcriptional effects, which may be independent from

the well-recognized systemic effects mediated by cell surface receptors [19,110,121,193]. In vitro and in vivo, Ang II induces the expression/transcription of growth factors and proliferative cytokines including nuclear factor- κ B (NF- κ B) [21,141,142,185], monocyte chemoattractant protein-1 (MCP-1) [141,204,242], TNF- α [204], and TGF- β 1 [113,225,227]. Genomic effects induced by Ang II may be in part mediated by interactions with intracellular and nuclear mineralocorticoid receptors (MR) [19,110,131,156]. Although systemic hemodynamic responses to Ang II often occur in seconds or minutes, the genomic responses to Ang II including cellular growth, mitogenic and proliferative effects occur ranging from hours, weeks to months [124,156,224]. Thus the genomic effects of Ang II are probably mediated by intracellular Ang II system. Third, not all ARBs, ACE or renin inhibitors are created equal to block both extracellular and intracellular Ang II systems. Due to their structures, lipophilic properties, and affinity with AT₁ receptors, ARBs may differ in their ability to enter the cells to block intracellular AT₁ receptors [123]. Different effects of ARBs on uric acid metabolism, cell proliferation, oxidative stress, nitric oxide production and PPAR- γ activity have been reported [120,123]. For example, losartan has been shown to internalize with AT_{1a} and AT_{1b} receptors, albeit at a slower rate than Ang II [17,45], and to block intracellular and nuclear effects of Ang II [48,49,90,141,247]. Furthermore, telmisartan (less so for losartan or Irbesartan) is a partial activator of liver-specific peroxisome proliferator-activated receptor γ (PPAR- γ) in addition to blocking AT₁ receptors [78,192]. Finally, although beneficial effects of ACE and renin inhibitors and ARBs are well documented, some patients continue to progress to hypertension and develop cardiovascular and renal complications despite being treated with one or two of these inhibitors [122,151]. This raises the possibility that the actions of intracellular Ang II may not be adequately blocked or other mechanisms may be involved [11]. Thus it is highly important to study the roles and underlying mechanisms of actions of intracellular Ang II and develop next generation of multifunctional ARBs that not only block cell surface but also cytoplasmic and nuclear AT₁ receptors, and/or non-AT₁ receptor-dependent mechanisms.

5.2. AT₁ (AT_{1a}) receptor-mediated uptake of Ang II by proximal tubules as a source of intracellular Ang II

GPCR-mediated endocytosis of extracellular Ang II plays an important role in the regulation of cellular responses to circulating and paracrine Ang II stimulation in target cells. The current dogma holds that the primary purpose of AT₁ receptor-mediated endocytosis of Ang II is to desensitize cellular responses to continuous stimulation of cell surface AT₁ receptor by extracellular Ang II by moving the Ang II/AT₁ complex into the cells [81,85,103,233]. After endocytosis, Ang II is expected to be delivered first to endosomes and then to lysosomes for degradation, whereas the receptor recycles back to the cell surface [81,100,103]. Thus it has been long held that internalized Ang II plays little, if any, role in the regulation of cellular responses to Ang II. However, recent evidence suggests that the endosomal system may serve as a legitimate platform for the uptake of agonists via receptor-mediated endocytosis and continuous signaling from activated agonist/receptor complex in many cells [160]. Ras and mitogen-activated protein kinase (MAPK) signalling for several GPCRs including AT_{1a}, vasopressin V₂, and β_2 adrenergic receptors (β_2 AR) have been reported in endosomal membranes [18,50,160], the endoplasmic reticulum and the Golgi independent of plasma membrane-initiated signalling [2,41,160]. We and others have recently shown that circulating and paracrine Ang II was taken up by the kidney via AT₁ (AT_{1a}) receptor-mediated endocytosis, and that high levels of internalized Ang II and AT_{1a} receptors were found in the endosomal compartments [105,196,217,221,245,246,249]. We have further demonstrated for the first time that deletion of AT_{1a} receptors markedly blocked intracellular uptake of [¹²⁵I]Val⁵-Ang II [140] or unlabeled Val⁵-Ang II in the kidney of AT_{1a}-KO mice [137]. However, these studies focused only on the entire kidney and the nephron segmental localization of Ang II uptake has not been determined.

Whether circulating and paracrine Ang II is taken up by proximal tubules from the luminal or the interstitial fluid compartments via apical (AP) or basolateral (BL) membrane AT₁ (AT_{1a}) receptor-mediated endocytosis has not been well studied. High density and high affinity Ang II receptors are localized in both AP and BL membranes of proximal tubule cells [22,70,76]. Ang II is known to stimulate both AP and BL membrane Ang II receptors to induce well-documented biphasic responses in proximal tubule sodium transport [43,97,101,222,240]. However, there is little information on AT_{1a} or AT_{1b} receptor-mediated uptake of extracellular Ang II from AP or BL membrane compartments. Becker et al. expressed a rabbit AT₁ receptor in LLC-PKC14 cells, a porcine proximal tubule cell line, and showed that AT₁ receptor-mediated endocytosis of [¹²⁵I]-Ang II was strikingly different in AP and BL membranes [14]. [¹²⁵I]-Ang II was internalized more rapidly and dominantly in AP membranes than in BL membranes [14]. By contrast, Thekkumkara et al. found that AT_{1a} receptors were internalized 4-times faster in BL membranes than in AP membranes of opossum kidney (OK) cells [207]. We also demonstrated that extracellular Ang II was taken up via AT₁ receptor-mediated endocytosis by rabbit proximal tubule cells [134,139,244,247], but the roles of AP vs. BL membrane AT_{1a} receptors in mediating the uptake of extracellular Ang II by proximal tubules in the kidney have not been determined. Multi-photon intravital microscopic functional imaging may be an ideal tool to study AT_{1a} receptor-mediated Ang II uptake by proximal tubules of the kidney in vivo.

In addition to AT₁ (AT_{1a}) receptor-mediated mechanisms, other factors may also regulate the uptake of extracellular Ang II by proximal tubules. Rodent kidneys express AT_{1a} and AT_{1b} receptors and both receptors undergo ligand-induced endocytosis in non-renal cells transiently expressing these mutant receptors [45,147,188]. Yet it remains unknown whether in the absence of dominant AT_{1a} receptors, AT_{1b} receptors play any role in mediating Ang II uptake in proximal tubules. We recently reported that losartan had a small but significant effect on intrarenal uptake of [¹²⁵I]Val⁵-Ang II in AT_{1a}-KO mice, suggesting a small role for AT_{1b} receptors [140]. Furthermore, AP membranes of proximal tubules express abundant endocytic receptor megalin, which plays a crucial role in mediating low molecular weight (LMW) protein uptake [42,118,231]. Deletion of megalin in mice led to the development of LMW proteinuria [118,129]. Interestingly, megalin also binds and internalizes Ang II in immortalized yolk sac cells (BN-16 cells) [86]. Whether megalin plays any role in mediating the uptake of Ang II by proximal tubules is virtually unknown.

5.3. Canonical vs. noncanonical endocytic pathways in mediating Ang II uptake by proximal tubules

Although we demonstrated recently that AT₁ receptors play a major role in mediating the uptake of extracellular Ang II by proximal tubule cells in vitro and circulating Ang II in mouse kidneys in vivo [134,137,139,140,244,247], the underlying cellular mechanisms and endocytic pathways involved have not been determined. In VSMCs, cardiomyocytes and COS-7 cells, GPCRs including β_2 AR, AT_{1a}, EGFR, and insulin receptors are internalized via the canonical clathrin-coated pits-dependent pathway [6,81,177,209,232]. Clathrin-coated pits play an important role in invaginating and pinching off the plasma membranes to form coated vesicles and this process is powered by GTPase/dynamin with the vesicles being targeted to endosomes [178]. GPCR kinases (GRKs), small GTP-binding proteins, such as Rab5, and β -arrestins are reportedly involved in clathrin-dependent AT_{1a} endocytosis [6,56,81,175,194,234]. However, dominant-negatives, siRNAs or knockout targeting dynamin, GRKs or β -arrestins have little effects on AT_{1a} receptor endocytosis in some studies, suggesting that alternative (non-canonical) pathways may also be involved in AT_{1a} receptor endocytosis [103,177]. AT_{1a} receptors have been shown to internalize into lipid rafts/CAV-1 or non clathrin-coated vesicles in non-renal cells [215,250].

It is not known whether the canonical clathrin-coated pits/dynamin/ β -arrestins or other non-canonical mechanisms mediate AT_{1a} receptor endocytosis or uptake of extracellular Ang II by proximal tubule cells. In LLC-PK14 cells expressing the rabbit AT₁ receptor, Becker et al. showed that Ang II-induced AT₁ receptor endocytosis in AP and BL membranes was significantly inhibited by phenylarsine oxide (PAO), an inhibitor of tyrosine phosphatases [7,87], but not by pertussis toxin [14,15]. Shelling and Linas showed that colchicine, an inhibitor of cytoskeleton microtubules [79], inhibited the effects of Ang II on rat proximal tubule cells by blocking Ang II receptor-mediated endocytosis [189,190]. Recently, we demonstrated that depletion of clathrin-coated pits with 400 mM sucrose or siRNA knockdown of clathrin light or high chain subunits had no effects on AT₁ receptor-mediated uptake of Val⁵-Ang II, but colchicine or siRNA knockdown of microtubule-associated proteins MAP-1A or MAP-1B markedly inhibited AT₁ receptor-mediated uptake of Val⁵- or FITC-labeled Ang II by proximal tubule cells [134,136,244,246]. These studies suggest that AT₁ receptor-mediated uptake of Ang II by proximal tubule cells may involve the noncanonical microtubule-dependent endocytic pathway, rather than the canonical clathrin-dependent mechanisms [134,244,246]. However, the cellular mechanisms underlying the microtubule-dependent uptake of Ang II by proximal tubule cells remain to be elucidated.

Two important motifs in the 3rd cytoplasmic loop of the AT_{1a} receptor have been identified to be critical for AT_{1a} receptor internalization [102,208]. Triple alanine mutation or deletion of the motif Ser³³⁵-Thr³³⁶-Leu³³⁷ in the cytoplasmic tail of the receptor impaired ~95% of AT₁ receptor internalization [102]. Furthermore, a distinct region of the cytoplasmic tail may also be involved in AT_{1a} receptor endocytosis [208]. Mutation or truncation of the motif Leu³¹⁶ to Tyr³¹⁹ led to 2.5-fold decreases in rate and >70% reduction in maximal internalization [208,209]. Although these motifs are well conserved across various species, whether they are involved in AT_{1a} receptor-mediated Ang II uptake by proximal tubule cells have not been investigated.

How Ang II and AT₁ receptors are internalized into the endosomal compartments and transported to other organelles or the nucleus in proximal tubule cells also remains to be determined [38,46,141,157]. Radiolabeled Ang II has been found in peri-nuclear regions of rat VSMCs and cardiac myocytes [182] or the Golgi of adrenal cells after systemic administration [16]. Cook et al. demonstrated that Ang II induced AT_{1a} receptor translocation to the nuclei of hepatocytes and VSMCs [46,47]. In HEK 293 cells stably expressing the AT_{1a} receptor, internalized AT_{1a} receptors were observed in peri-nuclear regions and the nuclei after stimulated by Ang II [38,157]. Recently, we also observed high levels of internalized FITC-labeled Ang II in perinuclear regions and the nucleus, which was completely inhibited by colchicine and siRNA knockdown of MAP-1A [134,136,139,141]. This suggests that the microtubule-dependent pathway may play an important role in nuclear trafficking of internalized Ang II and AT₁ receptors in proximal tubule cells. Furthermore, a nuclear localization sequence (NLS, KKFKKY, aa307-312) within the AT_{1a} receptor was recently identified to be important for nuclear trafficking and activation of AT_{1a} receptors by Ang II [157].

5.4. In vitro effects of intracellular vs. extracellular Ang II in proximal tubule cells and signalling mechanisms

In cultured proximal tubule cells or isolated proximal tubule perfusion, exogenous Ang II stimulates the expression of NHE-3 [139,141], AP insertion of NHE-3 [72,139], Na⁺/H⁺ exchanger activity [20,84,112,143,181], or NHE-3-induced ²²Na⁺ uptake [72,93,101,214]. The signaling mechanisms by which exogenous Ang II increases the expression and activity of NHE-3 in proximal tubule cells are well documented [73,115,191,222]. Ang II activation of cell surface GPCRs increases IP3 turnover and [Ca²⁺]_i, generation of DG, and subsequent activation of PKC, leading to either inhibition or stimulation of proximal tubule sodium

transport by extracellular Ang II [36,101,145,146,191]. The known downstream signaling pathways for extracellular Ang II that are either activated or inhibited by the PLC/[Ca²⁺]_i/PKC signaling include calcium-dependent calcineurin activation [128], cAMP-dependent PKA [101,144,206], [Ca²⁺]_i-independent PLA₂ [14,15,75], phosphatidylinositol-3 kinase (PI 3-kinase) [72], c-Src/MAP kinases ERK 1/2 [75] and activation of NF-κB [21,142,184,244]. Thus extracellular Ang II is not the focus of the current review.

Neither the roles of intracellular Ang II nor the signaling mechanisms involved in the regulation of the NHE-3 expression, AP membrane insertion, and the Na⁺/H⁺ exchanger activity by intracellular Ang II has been well studied in proximal tubule cells. It is widely held that in order to induce intracellular actions, extracellular Ang II must bind to cell surface receptors and activate intracellular signaling mechanisms [62,71,213,241]. However, there is accumulating evidence that internalized Ang II may act as an intracellular peptide to regulate proximal tubule function. For instance, blockade of AT₁ receptor endocytosis inhibits PKC activation, formation of IP₃, and AP membrane Na⁺ flux [189,190]. Moreover, AP membrane AT₁ receptor endocytosis is associated with activation of phospholipase A₂ (PLA₂) [14,15]. In OK cells expressing AT_{1a} receptors, AT₁ receptor endocytosis is associated with inhibition of BL membrane adenylyl cyclase and increases in AP membrane Na₊ uptake [206,207]. Recently, we showed that AT₁-mediated uptake of extracellular Val⁵-Ang II was also associated with inhibition of basal and forskolin-stimulated cAMP accumulation [134], Ang II-stimulated NHE-3 expression and AP membrane insertion [139], and Ang II-induced activation of NF-κB in proximal tubule cells [142,244]. Although these studies suggest that AT₁ receptor-mediated uptake of extracellular Ang II may play an important role in the regulation of proximal tubule functions, they by no means confirmed whether these effects were due to activation of intracellular receptors.

The challenge in proving a role for intracrine/intracellular Ang II is primarily due to the lack of approaches that can distinguish between the responses induced by intracellular Ang II acting on cytoplasmic or nuclear receptors and those evoked by extracellular Ang II via activation of cell surface receptors. To overcome this difficulty, Haller et al. microinjected Ang II directly into rat VSMCs to induce intracellular and nuclear calcium responses to Ang II [91,92]. De Mello et al. dialyzed Ang II or renin directly into hamster cardiomyocytes and demonstrated that Ang II enhanced the L-type calcium currents [63,64]. Single cell microinjection or microdialysis of Ang II directly into the cells may provide an innovative approach for the proof of concept studies. We have confirmed that intracellular microinjection of Ang II directly into single rabbit proximal tubule cells also induced intracellular [Ca²⁺]_i responses [243,246,247]. We found that co-microinjection of the AT₁ blocker losartan abolished the [Ca²⁺]_i response induced by microinjected Ang II but not completely by extracellular Ang II [247]. We further showed that Ang II directly stimulated nuclear AT_{1a} receptors to increase in vitro transcription of mRNAs for TGF-β1, MCP-1 and NHE-3 in freshly isolated rat renal cortical nuclei [141]. Likewise, Ang II was found to increase nitric oxide or super oxide production in isolated renal cortical nuclei via AT₁ or AT₂ receptors [89,90]. These studies strongly suggest that intracellular Ang II may activate cytoplasmic and nuclear AT₁ receptor to produce important intracellular or genomic effects in proximal tubule cells.

The other novel strategy to determine the effects of intracellular Ang II may be to express intracellular Ang II proteins in proximal tubule cells with or without expression of the wild-type or mutant Ang II receptors. Cook et al. recently transfected rat VSMCs or hepatocytes with a cyan fluorescent intracellular Ang II protein (ECFP/Ang II) and/or a rat yellow fluorescent AT_{1a} receptor (AT1R/EYFP), and demonstrated that intracellular Ang II stimulated VSMC proliferation via activation of cAMP response element-binding protein (CREB), p38 MAP kinase and MAP kinases ERK 1/2 phosphorylation [46,48]. The

construct encodes the Ang II protein without possessing secretory signal sequences and thus its expression will not lead to extracellular secretion [46,48]. Baker and Kumar have also expressed an intracellular Ang II (pcDNA/TO-iAng II) in CHO cells and interestingly they found that the intracellular Ang II induced cell proliferation independent of AT₁ receptors [11,119]. How intracellular Ang II induces CHO cell proliferation without activation of AT₁ receptors is not fully understood. Future studies should be designed to elucidate the signaling mechanisms by which intracellular Ang II induces short-term biological and long-term genomic effects in cardiovascular and renal systems.

5.5. Physiological effects of intracellular vs. extracellular Ang II on proximal tubule transport and blood pressure

Whether intracellular and/or internalized Ang II may physiologically regulate proximal tubular sodium transport and therefore blood pressure has also not been well studied so far. The progress has been stymied in this field due to the lack of suitable animal models that express an intracellular Ang II protein and the technical challenges in distinguishing between the effects induced by intracellular vs. extracellular Ang II. Reudelhuber's group first generated transgenic mice expressing an Ang II-producing fusion protein in cardiomyocytes to distinguish the effects between circulating and paracrine Ang II [216,228]. The expression of the Ang II-releasing fusion protein in cardiomyocytes was controlled by the α myosin heavy chain promoter. Cardiac Ang II levels are 10-fold higher than wild-type mice without elevating circulating Ang II [216,228]. Because this cardiac-specific Ang II construct contains a signal peptide sequence from human prorenin and a furin cleavage site, Ang II fusion protein will be cleaved by furin, released into the secretory pathway, and secreted into the cardiac interstitium [216,228]. Thus, Ang II secreted from cardiomyocytes activates cell surface rather than intracellular receptors to induce paracrine effects. Baker et al. used an adenoviral vector encoding an intracellular Ang II peptide to study the hypertrophic effect of cardiac-specific Ang II [10]. Intra-cardiac injection of this vector in mice surprisingly resulted in cardiac hypertrophy 48 h later, without affecting blood pressure and circulating Ang II levels [10]. How this intracellular Ang II rapidly induces cardiac hypertrophy and the effect is not blocked by the AT₁ receptor blocker losartan is not known, but it may suggest an AT₁ receptor-independent effect [10,11].

No attempt had been made to express an intracellular Ang II protein in any cell type of the kidney to study the physiological roles of intracellular Ang II until recently. It is not clear whether the in vitro effects of internalized or intracellular Ang II can be reproduced in proximal tubules of the kidney to regulate proximal tubule sodium and fluid reabsorption, promote salt retention and alter blood pressure in animals. Expression of an intracellular Ang II protein selectively in proximal tubules of the kidney under the control of a proximal tubule cell-specific promoter would be an ideal approach to test this hypothesis. Sigmund's group first used the kidney androgen-regulated protein gene (KAP) to drive tissue-specific expression of human angiotensinogen and renin in the kidney [66,67,125]. The KAP gene is widely expressed in the kidney, with its expression reportedly confined in proximal tubules and being regulated by androgen and estrogen [66,155,200]. Alternatively, Coffman's group generated transgenic mice in which AT_{1a} receptors was expressed under the control of the gamma-glutamyl transpeptidase (γ GT) promoter, which is specific for proximal tubule cells [126]. These approaches may be useful to investigate sexual dimorphic regulation of angiotensinogen expression or the roles of AT_{1a} receptors in proximal tubules. However, they may not differentiate the roles of intracellular vs. extracellular Ang II in an animal model [66,125,126], because both intracellular and extracellular pathways may be involved. Potential ectopic expression of these transgenes in tissues other than proximal tubules of the kidney under the control of these promoters may also pose problems for the selective expression of target genes in proximal tubules. Most recently, transgenic mice expressing an

intracellular fluorescent fusion of Ang II, ECFP/AII, have been generated using the mouse metallothionein promoter [180]. Global overexpression of this transgene in the brain, heart, kidney, liver, lung and testes led to increases in blood pressure and induced microangiopathy in the kidney [180]. This is the first transgenic mouse model demonstrating blood pressure in animals.

In collaboration with Dr. Julia Cook of Ochsner Clinic Foundation and Dr. Isabelle Rubera of University of Nice-Sophia, France, we have developed an adenoviral construct (Ad-sgt2-ECFP/AII), which encodes the cyan fluorescent fusion of Ang II protein, ECFP/AII [46,48]. We used the sodium and glucose co-transporter 2 promoter, sglt2, to drive the expression of ECFP/AII selectively in proximal tubules of the rat and mouse kidneys [183]. The sglt2 is expressed almost exclusively in early segment(s) of proximal tubules in the kidney [99]. We demonstrated that blood pressure was significantly increased by proximal tubule-specific transfer of ECFP/AII in a time-related manner [135]. The net increases in systolic blood pressure 1, 2 or 4 weeks after ECFP/Ang II transfer averaged between 15 mmHg to 30 mmHg. These levels of increased blood pressure are similar to those reported in transgenic mice globally expressing ECFP/Ang II [180] or in AT_{1a}-KO mice with the knockin of AT_{1a} receptors selectively in proximal tubules [127], but much lower than those induced by chronic infusion of exogenous Ang II in rats or mice [137,171,245]. Since we did not observe ectopic expression in extrarenal tissues and the expressed ECFP/AII was not released into the tubular lumen or interstitial compartment, this effect is proximal tubule- and intracellular specific. Furthermore, because the blood pressure-elevating effect of proximal tubule-selective transfer of ECFP/AII was blocked by losartan and prevented in AT_{1a}-KO mice, our study suggests that AT₁ (AT_{1a}) receptors likely mediate this blood pressure-increasing effect [135].

6. Future Perspectives of the evolving endocrine, paracrine and intracrine RAS in cardiovascular, renal and hypertension research

In summary, the great progress has been made in the RAS research field since renin was first discovered by Tigerstedt and Bergman more than a century ago. The RAS, once considered as an endocrine system, is now recognized as dual (circulating and local/tissue) or multiple systems (endocrine, paracrine and intracrine). In addition to the classical renin/ACE/Ang II/Ang II receptor axis, we have now added several new axes to the RAS family, namely the prorenin/PRR/MAPK axis, the ACE2/Ang (1-7)/Mas receptor axis, and the Ang IV/AT₄/IRAP axis. With the addition of these new members, the roles of the evolving RAS have been extended far beyond the regulation of blood pressure, aldosterone synthesis, and body fluid and electrolyte homeostasis. Indeed, novel actions for each member of the RAS are continuously discovered in physiology and diseases. However, many challenges still remain in the RAS research despite of more than one century's research. It is expected that the research on several areas of the expanded RAS will continue to play a prominent role in cardiovascular, renal and hypertension research. For example, it still remains poorly understood how the prorenin/PRR/MAPK axis, which appears to play a very limited role in physiology, is activated in many diseases such as diabetes. It is also not quite certain whether pharmacological blockade of PRR can provide additional benefits beyond the well-established renin and ACE inhibitors and ARBs in preventing and treating cardiovascular, renal and diabetic complications. Although the ACE/Ang (1-7)/Mas receptor axis appears to play a counter-regulatory role to buffer the effects of the renin/ACE/Ang II/AT₁ receptor axis, the development and clinical relevance of the orally active agonists or compounds that aim to metabolize Ang II to increase Ang (1-7) production or to activate the Mas receptor still await clinical trials. In term of the Ang IV/AT₄/IRAP axis, its cardiovascular and renal effects appear to be similar to the activation of the renin/ACE/Ang II/AT₁ receptor axis, albeit at much higher concentrations. The focus on the Ang IV/AT₄/IRAP axis should

therefore be directed to its role and signalling mechanisms by which it regulate glucose uptake in health and diabetes via interactions with GLUT4. Finally, there is a renewed interest in the biology, biochemistry and physiological roles of the intracrine/intracellular RAS. It remains poorly understood how extracellular Ang II is internalized and after internalization, escapes the lysosome degradation pathways and trafficks to other intracellular organelles and the nucleus, whether and how internalized Ang II can transmitting signaling from endosomes independent of cell surface GPCR-mediated signaling, and whether and how internalized/intracellular Ang II interacts with the nuclear receptors to regulate growth and transcriptional responses. The additional challenges ahead are to develop novel strategies or compounds to block intracellular and nuclear actions of intracrine/intracellular Ang II to treat hypertension, cardiovascular and kidney diseases.

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Research Highlights

- The renin-angiotensin system is now evolving as an endocrine, paracrine and intracrine system.
- The renin/ACE/Ang II/AT₁ receptor axis is the most recognized cascade of the entire system.
- The prorenin/(Pro)renin receptor/MAP kinases ERK 1/2 axis is activated in diseased states.
- The ACE2/Ang (1-7) Mas receptor axis opposes the effects induced by Ang II via AT₁ receptors.
- Intracrine/intracellular Ang II plays important roles in cardiovascular and renal regulation.

Figure 1.

In vitro autoradiographic mapping of: (A) active renin binding in juxtaglomerular apparatus in the dog kidney pretreated with sodium depletion using the radiolabeled renin inhibitor, ^{125}I -H77 [202,237], (B) angiotensin I-converting enzyme binding in proximal tubules of the rat kidney using ^{125}I -351A [98,238], (C) AT₁ receptor in the presence of the AT₂ receptor blocker PD123319 or (D) AT₂ receptor binding in the presence of the AT receptor blocker losartan using ^{125}I -[Sar¹,Ile⁸]-Ang II [236,239,241], (E) Ang (1-7) receptor binding in the rat kidney using ^{125}I -Ang (1-7) as the radioligand, and (F) Ang IV receptor binding in the rat kidney using ^{125}I -Ang (3-8) [133,238]. The levels of binding are indicated by color calibration bars with red representing the highest, whereas blue showing the lowest levels of enzyme or receptor binding. G: glomerulus. IM: inner medulla. IS: inner stripe of the outer medulla. JGA: juxtaglomerular apparatus. P: proximal tubule.

Overview of The Evolving Renin-Angiotensin System

Figure 2.

Overview of the expanded and evolving renin-angiotensin system. (1): the classical angiotensinogen/renin/ACE/Ang II/AT₁ receptor axis. (2): the prorenin/(Pro)renin receptor/MAP kinases ERK 1/2 axis. (3) the ACE2/Ang (1-7)/Mas receptor axis. (4) the Ang IV/AT₄/IRAP axis. APA: aminopeptidase A. APN: aminopeptidase N. IRAP: insulin-regulated aminopeptidase.

Angiotensin receptor classification & signaling mechanisms

Receptor:	AT₁	AT₂	Ang (1-7)	AT₄
Subtype:	AT _{1a} & AT _{1b}	No	No	No
Structure:	7-TM 359 aa	7-TM 364 aa	7-TM Mas R	M1 family aminopeptidase (IRAP)
Signaling:	Gq/11 PLC-β IP ₃ , Ca ²⁺ PKC MAPK JAK/STAT	G proteins PTP BK NO/cGMP	MAPK COX-2 BK NO/cGMP	GLUT4/ glucose uptake Enhanced learning & Memory
Localization:	Kidney, liver, heart, vessel, adrenal and brain.			

Figure 3.

Angiotensin receptor classification into four different classes and their signalling mechanisms. AT₁: type 1 receptor specific for Ang II including type 1a and type 1b. AT₂: type 2 receptor specific for Ang II. Mas receptor: specific for Ang (1-7). AT₄: type 4 specific for Ang IV. 7-TM: seven transmembrane domains. BK: bradykinin. cGMP: cyclic guanosine monophosphate. COX-2: cyclooxygenase-2. Gq/11: a G protein subunit. IP₃: inositol trisphosphate. JAK/STAT: janus kinase/signal transducer and activator of transcription. MAPK: mitogen-activated protein (MAP) kinases. NO: nitric oxide. PKC: protein kinase C. PLC-β: phospholipase C-β. PTP: protein tyrosine phosphatase.

Figure 4.

AT₁ receptor-dependent effects of Ang IV in the rat kidney. Top: mean arterial blood pressure (MAP) response to Ang IV in anesthetized rats. Middle: renal cortical blood flow response (CBF) to Ang IV, as determined by laser-doppler flowmeter in anesthetized rats. Bottom: intracellular calcium response to Ang IV in freshly isolated rat preglomerular vascular smooth muscle cells (VSMCs). All three responses to Ang IV were blocked by losartan, suggesting AT₁ receptor-mediated effects for Ang IV in the kidney. Data are derived from [133]

Figure 5.

Intracellular microinjection of Ang II directly into single rabbit proximal tubule cells induces time-dependent increases in intracellular calcium levels (0 to 120 seconds), which is blocked by co-microinjection of losartan (Los), an AT_1 receptor blocker, but not by PD123319 (PD), an AT_2 receptor blocker. ** $p < 0.01$ vs. Control. ++ $p < 0.01$ vs. Ang II. Data are derived from [246,247].

Figure 6.

Intrarenal adenoviral transfer of an intracellular cyan fluorescent fusion of Ang II protein, ECFP/Ang II, selectively in proximal tubules of the rat or mouse kidney increases systolic blood pressure in rat and wild-type mice. A: a proximal tubule-specific promoter for the sodium and glucose co-transporter 2 was used to drive ECFP/Ang II expression selectively in proximal tubules (PT) [135]. B: ECFP/Ang II expression in a representative, freshly isolated proximal tubule. C: proximal tubule-specific transfer of ECFP/Ang II in wild-type (WT) mouse kidneys increased blood pressure, which was blocked in mice deficient of AT_{1a} receptors (AT_{1a}-KO). ** $p < 0.01$ vs. Control in WT mice. ++ $p < 0.01$ vs. WT mice at baselines and after ECFP/Ang II transfer. The data are derived from [135].