Angiotensin II and AT$_{1a}$ Receptors in the Proximal Tubules of the Kidney: New Roles in Blood Pressure Control and Hypertension

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Abstract: Contrary to public perception, hypertension remains one of the most important public health problems in the United States, affecting 46% of adults with increased risk for heart attack, stroke, and kidney diseases. The mechanisms underlying poorly controlled hypertension remain incompletely understood. Recent development in the Cre/LoxP approach to study gain or loss of function of a particular gene has significantly helped advance our new insights into the role of proximal tubule angiotensin II (Ang II) and its AT$_1$ (AT$_{1a}$) receptors in basal blood pressure control and the development of Ang II-induced hypertension. This novel approach has provided us and others with an important tool to generate novel mouse models with proximal tubule-specific loss (deletion) or gain of the function (overexpression). The objective of this invited review article is to review and discuss recent findings using novel genetically modifying proximal tubule-specific mouse models. These new studies have consistently demonstrated that deletion of AT$_1$ (AT$_{1a}$) receptors or its direct downstream target Na$^+$/H$^+$ exchanger 3 (NHE3) selectively in the proximal tubules of the kidney lowers basal blood pressure, increases the pressure-natriuresis response, and induces natriuretic responses, whereas overexpression of an intracellular Ang II fusion protein or AT$_1$ (AT$_{1a}$) receptors selectively in the proximal tubules increases proximal tubule Na$^+$ reabsorption, impairs the pressure-natriuresis response, and elevates blood pressure. Furthermore, the development of Ang II-induced hypertension by systemic Ang II infusion or by proximal tubule-specific overexpression of an intracellular Ang II fusion protein was attenuated in mutant mice with proximal tubule-specific deletion of AT$_1$ (AT$_{1a}$) receptors or NHE3. Thus, these recent studies provide evidence for and new insights into the important roles of intratubular Ang II via AT$_1$ (AT$_{1a}$) receptors in basal blood pressure homeostasis and the development of Ang II-induced hypertension.

Keywords: angiotensin II; AT$_1$ receptor; hypertension; proximal tubule; sex differences

1. Introduction

Hypertension affects more than 46% of adults in the United States, significantly increasing their risk for cardiovascular diseases, stroke, and kidney failure [1–3]. Several classes of drugs are currently available to treat hypertension, including angiotensin-converting enzyme (ACE) inhibitors, angiotensin II (Ang II) receptor blockers (ARBs), calcium channel inhibitors, β-blockers, and loop diuretics. These antihypertensive drugs act to either inhibit the renin–angiotensin system (RAS), cause blood vessel vasodilatation, induce natriuresis and diuresis, or suppress sympathetic nerve activity [4–7]. Despite widespread treatments with these antihypertensive drugs, only approximately 50% of patients have attained adequate blood pressure control, while the rest continue to develop apparent...
treatment-resistant hypertension (aTRH) [6]. aTRH has been defined as uncontrolled blood pressure while taking three classes of antihypertensive drugs or taking four classes of antihypertensive drugs regardless of blood pressure control [4,5,7]. With nearly 20% of American adults affected by aTRH, the mechanisms of hypertension, whether controlled or poorly controlled, remain incompletely understood and warrants further studies [4].

The pressure-natriuresis response is one of the major mechanisms by which the kidneys control blood pressure and body salt and fluid homeostasis in response to the changes in renal arterial pressure [3,4,8]. A significant increase in arterial blood pressure is expected to trigger the pressure-natriuresis response that, in turn, alters interstitial hydrostatic pressure, proximal tubule Na\(^+\) transport, and renal medullary blood flow to induce diuresis and natriuresis responses. This is followed by blood pressure returning to control. Conversely, when blood pressure falls significantly, the pressure-natriuresis response is suppressed, antidiuretic and anti-natriuretic responses are augmented, and proximal tubule Na\(^+\) reabsorption is increased [3,4,8]. These responses work together to restore blood pressure back to control. However, how the pressure-natriuresis response is regulated and its potential mediators remain poorly understood. Recent studies suggest that the intratubular RAS, especially Ang II via activation of AT\(_1\) (AT\(_{1a}\)) receptors in the proximal tubules of the kidney, plays a key role in basal blood pressure control and the development of Ang II-induced hypertension by regulating proximal tubule Na\(^+\) reabsorption and the pressure-natriuresis response [9–11].

Recently, the XIII International Symposium on Vasoactive Peptides was held on 15–17 October 2021, which celebrated recent advances in studying the roles of vasoactive peptides, especially the renin–angiotensin system, in cardiovascular, neural, kidney, and blood pressure control, inflammation and the COVID-19 pandemic. Based on a lecture given at this symposium, the objective of this article is to briefly review and discuss recent studies using unique mouse models with proximal tubule-specific deletion (knockout) of Ang II type 1a receptors (AT\(_{1a}\)) or its major downstream target protein, the Na\(^+\)/H\(^+\) exchanger 3 (NHE3) in the kidney [12–16]. These studies have revealed important roles of AT\(_{1a}\) receptors, acting alone or via NHE3, in the proximal tubules in maintaining basal blood pressure homeostasis, the pressure-natriuresis response, and the development of Ang II-induced hypertension. This new knowledge improves our understanding of the renal mechanisms of blood pressure regulation as well as Ang II-induced hypertension and suggests that both AT\(_{1a}\) receptors and NHE3 may be potential therapeutic targets in treating hypertension in humans.

2. The Proximal Tubules Are Major Tubular Segments Expressing a Robust Renin–Angiotensin System in the Kidney

It has long been recognized that the proximal tubules express all major components of the RAS including key enzymes (i.e., renin and ACE) and sole substrate (i.e., angiotensinogen) that are required to generate Ang II and the receptors (i.e., AT\(_1\) and AT\(_2\)) that mediate the actions of Ang II [17–20]. The proximal tubules also express abundant enzyme (ACE2) and aminopeptidases (i.e., APA and APN) that metabolize Ang II or its downstream active fragments, Ang III or Ang (3–8) [21–24]. Ang II is the most potent effector of all angiotensin peptides and plays the most critical roles in the kidney to regulate blood flow, glomerular filtration, and tubular transport [13,25,26]. Most of the well-recognized effects of Ang II in the kidney are mediated by AT\(_1\) receptors [11,13,15,25–29], while AT\(_2\) receptors play a relatively moderate role in mediating the natriuretic response in the proximal tubules [16,30–33].

The molecular cloning of AT\(_1\) and AT\(_2\) receptors, and the development of Ang II receptor blockers (ARBs) for treatment of hypertension, diabetic nephropathy, and other kidney diseases represent one of the most significant breakthroughs in the Ang II receptor research field over last 3 decades.

The AT\(_1\) receptor is molecularly classified into two subtypes in rodents, AT\(_{1a}\) and AT\(_{1b}\), based on their molecular structures [34–37]. However, humans express only one AT\(_1\) receptor corresponding to AT\(_{1a}\) receptors in rodents. The AT\(_{1a}\) receptor was first
cloned from rat vascular smooth muscle cells with its cDNA encoding a 359 amino-acid protein, a molecular structure typical of seven transmembrane GPCR [36,37]. The AT_{1b} receptor was subsequently cloned from bovine adrenal cells with its cDNA sharing a 94% identical amino acid sequence of the AT_{1a} receptor [34,35]. Most AT_{1} receptors are of the AT_{1a} subtype accounting for >90% of the AT_{1} receptor family in the kidney and other tissues [25,26,28,38,39], whereas the AT_{1b} receptor accounts for approximately 5–10%, and its expression is mainly confined to the adrenal glands, the kidney, and brain [40–42]. Thus, it is not surprising that most of the well-recognized effects of Ang II are mediated by AT_{1a} receptors, whereas AT_{1b} receptors play a small role [13,25,26,28,38].

AT_{1} and AT_{2} receptors have been localized in the rat, mouse, and human kidney using quantitative in vitro or in vivo autoradiography (Figure 1) [43–48], in situ hybridization histochemistry [49–51], and immunohistochemistry, respectively [52–54]. The localization of AT_{1} (AT_{1a}) receptors in the kidney by radioreceptor binding and autoradiography is the gold-standard approach based on the direct GPCR and ligand pharmacology principles [43–48]. This technique has revealed a distinct anatomical distribution or localization of these receptors in the kidneys of rat, mouse, rabbit, monkey, or humans [43–47,55]. Specifically, autoradiographs show a very high density of AT_{1} receptors in the glomerulus, corresponding to mesangial cells, endothelial cells, and podocytes [43–46]. Although not as high as in the glomerulus, a moderately high level of AT_{1} receptors is localized in the intervening outer cortex corresponding to proximal convoluted tubules [44–47]. Interestingly, a high density of AT_{1} receptors is also localized in the longitudinal bands traversing the inner stripe of the outer medulla associated with the vasa recta bundles and type 1 renomedullary interstitial cells [47,56]. By contrast, the inner cortex, the outer stripe of the outer medulla, and the entire inner medulla especially toward the tip of the inner medulla express low to undetectable levels of AT_{1} receptors [43–48]. In comparison, the expression of AT_{2} receptors is very low throughout the kidney of adult rodents or humans except in the proximal tubules and blood vessels (Figure 1) [47,48]

Other techniques have also been used to localize AT_{1} and AT_{2} receptors in the kidney. In situ hybridization histochemistry offers a high sensitivity and specificity to localize AT_{1} (AT_{1a}) receptor mRNAs throughout the kidney including the blood vessels, glomerulus, proximal tubules, loop of Henle, distal tubules, and collecting ducts [49–51]. A recent study using the novel RNA scope technique reported similar findings throughout the kidney with AT_{1} receptor mRNAs observed in mesangial cell, juxtaglomerular cells, proximal tubule cells, interstitial cell, and late afferent and early efferent arterioles [57]. The localization of AT_{1} and AT_{2} receptor proteins in the kidney has also been studied, primarily using immunohistochemistry with AT_{1} and AT_{2} receptor antibodies [52–54]. The localization of AT_{1} (AT_{1a}) and AT_{2} receptor mRNAs or proteins in most, if not all, kidney cells or structures by these techniques may be different from the localization of AT_{1} receptor binding sites by radioreceptor or autoradiographic binding [43–48]. Although these techniques are very sensitive to detect AT_{1} and AT_{2} receptor mRNAs or proteins in the kidney, the specificity of these approaches, especially using commercially available AT_{1} or AT_{2} receptor antibodies for immunohistochemistry, remains controversial [58–60].
3. The Proximal Tubules Are Major Tubular Segments Expressing the Most Robust NHE3 Abundance in the Kidney, a Major Downstream Target of AT1 (AT1a) Receptor Activation

In contrast to the general perception that NHE3 is expressed in every tissue in the body, only two major organs or tissues express NHE3 most abundantly, i.e., the gastrointestinal tract (gut) and the kidney. In the gut, NHE3 is primarily expressed in the small intestines and much less in the stomach and large intestines or colon [61,62]. In the kidney, NHE3 is primarily expressed in proximal convoluted and straight tubules, and to a moderate extent, in the loop of Henle and is virtually not expressed in collecting ducts [61–66]. NHE3 is primarily localized on the apical membranes of the proximal tubules and loop of Henle under physiological conditions and mediates electroneutral Na⁺ entry into the cells from the lumen and H⁺ extrusion from the cells in the proximal tubules [67,68]. After entry into proximal tubule cells, intracellular Na⁺ ions are returned to the blood via the Na⁺/K⁺-ATPase, i.e., the Na⁺ and K⁺ pump on the basolateral membranes. Low blood pressure, loss of blood, sodium depletion, or salt wasting activate intratubular RAS expression.
and generation of Ang II that, in turn, activates AT₁ (AT₁a) receptors to stimulate NHE3 expression and increase Na⁺ reabsorption in the proximal tubules. These responses, along with other central neural, cardiovascular, and humoral factors help restore body salt and fluid balance and blood pressure homeostasis. By contrast, acute and chronic increases in blood pressure induces NHE3 endocytosis or redistribution from apical membranes, which augments the pressure-natriuresis response and induces diuresis and natriuresis that helps restore blood pressure to control [13–15].

NHE3 in the proximal tubules is the major downstream target of intratubular Ang II via AT₁ (AT₁a) receptors in the proximal tubules [15,69]. Physiologically, Ang II binds and activates AT₁ (AT₁a) and AT₂ receptors in the proximal tubules, with AT₁ (AT₁a) receptors playing a dominant role [13,15] and AT₂ receptors playing a smaller counterregulatory role [30,70]. Previous studies have shown that activation of AT₁ (AT₁a) receptors by Ang II mediates G protein-coupled, PKCα, IP₃, and Ca²⁺/calmodulin-dependent protein kinase II signaling to induce NHE3 expression and activity in cultured proximal tubule cells [55,71,72]. Other anti-natriuretic factors, such as glucocorticoids [73,74], glucagon, and insulin [75,76], also stimulate proximal tubule Na⁺ reabsorption, in part, by upregulating NHE3 expression in the kidney. Conversely, Ang II and its major metabolite Ang III reportedly activate AT₂ receptor-mediated NO/cGMP signaling to induce NHE3 endocytosis and increased urinary Na⁺ excretion [30,70]. The natriuretic peptide dopamine has also been shown to inhibit proximal tubule Na⁺ reabsorption and induces natriuresis by inhibiting NHE3 expression in the kidney [67,77–80]. Nevertheless, marked upregulation of intratubular Ang II/AT₁ (AT₁a) receptors/NHE3 signaling in the proximal tubules plays a more dominant role in the development of Ang II-induced hypertension.

4. AT₁ (AT₁a) Receptors in the Proximal Tubules of the Kidney Are Required for Maintaining Basal Blood Pressure Homeostasis

It has long been recognized that the proximal tubules play a key role in overall blood pressure regulation and the development of hypertension. This recognition is based on the fact that approximately 65–70% of Na⁺ and fluid is reabsorbed by the proximal tubules alone; thus, increases or decreases in proximal tubule reabsorption will exert a significant impact on blood pressure homeostasis [20]. Several previous studies have suggested that distal tubular segments may also play important roles in blood pressure regulation and the development of hypertension due to the fact of their ability to fully compensate for any increases or decreases in Na⁺ delivery from the proximal tubules [81–83]. However, recent studies from our and other labs suggest otherwise, because distal nephron segments fail to compensate for the loss of AT₁ (AT₁a) receptors or its major downstream target Na⁺ transporter NHE3 in the proximal tubules, leading to inhibition of proximal tubule Na⁺ reabsorption, natriuretic response, and lower basal blood pressure [13,15,27].

As mentioned previously, all major components of the RAS, including angiotensinogen, renin, ACE, and AT₁ and AT₂ receptors, have all been localized in the proximal tubules of the kidney [17–20]. Ang II concentrations in the proximal tubules are much higher than in the circulation under both the physiological and hypertensive states [84–87]. This may be due to several factors, including, but not limited to, (a) the expression of the substrate angiotensinogen and key enzymes renin and ACE for the generation of Ang II onsite; (b) the capacity of AT₁ (AT₁a) receptor- and the endocytic receptor megalin-mediated accumulation of circulating and tissue paracrine Ang II by the proximal tubules [88–90]; and (c) the feedforward regulatory mechanisms of intratubular RAS in the proximal tubules during the development of Ang II-induced hypertension [87,91]. Thus, it is expected that Ang II in the proximal tubules not only acts physiologically to stimulate proximal tubule reabsorption of sodium and fluid, maintain body sodium and fluid balance and basal blood pressure homeostasis, but also promotes sodium retention in hypertension via the actions of AT₁ (AT₁a) receptors [56,92,93].

Although previous studies have shown that Ang II has biphasic effects to regulate proximal tubule sodium transport based on in vivo micropuncture experiments [93,94] or
in vitro proximal tubule perfusion studies [78,95], whether these local effects alter systemic blood pressure has not been studied by these approaches. Whole body loss of function (deletion) or gain of function (overexpression) of the RAS may also be unable to determine the roles of intratubular Ang II and AT$_1$ (AT$_{1a}$) receptors in the proximal tubules of the kidney on basal blood pressure level and the development of Ang II-induced hypertension. At the whole kidney level, Crowley et al. were instrumental in demonstrating a key role of the kidney RAS in blood pressure control and in Ang II-induced hypertension using the cross-kidney transplantation approach between wild-type and whole-body Agtr1a$^{-/-}$ mice [10,96]. Their study elegantly showed that transplantation of Agtr1a$^{-/-}$ mouse kidneys into wild-type mice lowered basal blood pressure and attenuated Ang II-induced hypertension, whereas transplantation of wild-type mouse kidneys into Agtr1a$^{-/-}$ mice elevated blood pressure [10,96]. These studies directly support a key role for kidney AT$_1$ (AT$_{1a}$) receptors in blood pressure control and Ang II-induced hypertension, but the role of intratubular AT$_1$ (AT$_{1a}$) receptors in the proximal tubules was not determined in these studies.

Two other instrumental studies have determined the role of AT$_1$ (AT$_{1a}$) receptors in the proximal tubules of the kidney using the Cre/LoxP approach with two different “proximal tubule-specific” promoters [27,97]. Gurley et al. used the PEPCK-Cre/Agtr1a flox [27], whereas Li et al. used the KAP2-iCre/Agtr1a flox approach to generate proximal tubule-specific AT$_{1a}$-knockout mutant mice [97]. Both studies showed that intratubular AT$_{1a}$ receptors regulate blood pressure with or without compensatory expression of NHE3, sodium and phosphate cotransporter 2 (NaPi2), Na$^+$/K$^+$-ATPase, sodium chloride cotransporter (NCC), Na$^+$:K$^+$:Cl$^-$ cotransporter 2 (NKCC2), and epithelial sodium channel (ENaC) in proximal or distal nephron segments [27,97]. However, PEPCK has been known to express, to some extent, in tissues beyond the proximal tubules, including other segments of the nephron and epithelial cells in many other extra-renal tissues such as the liver, white and brown fat, jejunum, ileum, and sublingual gland [98,99]. Likewise, the expression of renal androgen-regulated protein, KAP2, is not only confined to the proximal tubules, but also expressed in the outer medulla of the kidney and other tissues that physiologically respond to androgen [100,101]. While these studies suggest an important role of AT$_1$ (AT$_{1a}$) receptors in the proximal tubules, the specificity of the PEPCK or KAP2 promoters to the proximal tubules remain to be further determined.

Against this background, our lab has recently taken a different approach to generate a new mutant mouse model (PT-Agtr1a$^{-/-}$) with proximal tubule-specific deletion of AT$_1$ receptors (AT$_{1a}$) in the kidney using the iL1-SGLT2-Cre/Agtr1a flox approach [16,28]. The scientific premise for using the iL1-SGLT2-Cre/Agtr1a flox approach was based on the findings that SGLT2, the sodium and glucose cotransporter, is more specific to the proximal tubules of the nephron and epithelial cells in many other extra-renal tissues such as the liver, white and brown fat, jejunum, ileum, and sublingual gland [98,99]. Likewise, the expression of renal androgen-regulated protein, KAP2, is not only confined to the proximal tubules, but also expressed in the outer medulla of the kidney and other tissues that physiologically respond to androgen [100,101]. While these studies suggest an important role of AT$_1$ (AT$_{1a}$) receptors in the proximal tubules, the specificity of the PEPCK or KAP2 promoters to the proximal tubules remain to be further determined.

With this new approach, our studies consistently showed that selective deletion of Agtr1a in the proximal tubules led to an appropriate 15 ± 3 mmHg decrease in basal systolic, diastolic, and mean arterial blood pressure in both male and female PT-Agtr1a$^{-/-}$ mice when compared to wild-type mice (Figure 2) [16,28]. By contrast, deletion of AT$_{1a}$ receptors in all tissues of the body led to an approximately 30 ± 5 mmHg lower systolic, diastolic, and mean arterial blood pressure in global Agtr1a$^{-/-}$ mice [11,16,28,55]. No significant sex differences in this basal blood pressure phenotype were observed in either global Agtr1a$^{-/-}$ or proximal tubule-specific PT-Agtr1a$^{-/-}$ mice [12,28]. The blood pressure-lowering effect in PT-Agtr1a$^{-/-}$ mice was associated with significant diuretic and natriuretic responses and increases in the basal glomerular filtration rate as well as the pressure-natriuresis response due to the inhibition of Na$^+$ and fluid reabsorption in the proximal tubules (Figure 2) [28,104,105]. Our data strongly support the hypothesis that intratubular Ang II via actions on AT$_{1a}$ receptors in the proximal tubules, indeed, play a key
role in maintaining basal blood pressure homeostasis, and loss of \( \text{AT}_{1a} \) receptor function in the proximal tubules would lower basal blood pressure.

![Figure 2](image.png)

**Figure 2.** Effects of global or proximal tubule-selective deletion of \( \text{AT}_{1a} \) receptors on basal systolic, diastolic, and mean arterial blood pressure (A–C), glomerular filtration rate (D), and natriuretic response (E) in whole-body \( \text{Agtr1a}^{-/-} \) or proximal tubule-specific \( \text{PT-Agtr1a}^{-/-} \) mice compared with wild-type mice. Note that \( \text{Agtr1a}^{-/-} \) mice showed that basal blood pressure was ~30 mmHg lower than wild-type mice, whereas \( \text{PT-Agtr1a}^{-/-} \) mice had basal blood pressure ~15 mmHg lower than wild-type mice, respectively. (F) shows no changes in 24 h urinary potassium excretion. * \( p < 0.05 \) or ** \( p < 0.01 \) vs. WT mice; *** \( p < 0.01 \) vs. global \( \text{Agtr1a}^{-/-} \) mice. Reproduced from Reference [28] with permission.

### 5. \( \text{AT}_{1} \) (\( \text{AT}_{1a} \)) Receptors in the Proximal Tubules of the Kidney Are Required for the Development of Ang II-Induced Hypertension

The development of Ang II-induced hypertension has been extensively studied in different animal models with \( \text{AT}_{1} \) (\( \text{AT}_{1a} \)) receptors playing the fundamental role [12,27,71,97,104]. \( \text{AT}_{1} \) (\( \text{AT}_{1a} \)) receptors are expressed widely, although to different levels, in the brain, adrenal glands, and cardiovascular and kidney tissues; thus, the mechanisms underlying Ang II-induced hypertension are expected to involve different tissues or pathways [16,39]. We and others have recently determined the direct contributions of intratubular Ang II via \( \text{AT}_{1} \) (\( \text{AT}_{1a} \)) receptors in the proximal tubules in the development of Ang II-induced hypertension by comparing global, kidney-, and proximal tubule-specific \( \text{Agtr1a}^{-/-} \) mice [10–12,16,27,28,97,104]. As discussed previously in the cross-kidney transplantation study, Crowley et al. reported that kidney \( \text{AT}_{1} \) (\( \text{AT}_{1a} \)) receptors played virtually equivalent roles in \( \text{AT}_{1} \) receptor actions in the kidney and in extrarenal tissues to determining the level of blood pressure and the development of Ang II-infused hypertension [10,96]. Gurley et al. showed that deletion of \( \text{AT}_{1a} \) receptors in mice significantly attenuated Ang II-induced hypertension in their mouse model generated using the PEPCK-Cre/\( \text{Agtr1a} \) flox approach [27]. By contrast, Li H. et al. demonstrated that Ang II action via the proximal tubule \( \text{AT}_{1a} \) receptors was not a significant component of the acute pressor action of increased circulating Ang II, i.e., in Ang II-induced hypertensive response and in their mouse model generated using
How increases in blood pressure by proximal tubule-specific overexpression of intracellular Ang II fusion protein were completely blocked, whereas Ang II-induced hypertension by systemic Ang II infusion was attenuated by only ~50% in PT-Agr1a<sup>−/−</sup> mice, remains
incompletely understood. However, both systemic and proximal tubule-specific AT₁ (AT₁a) receptor-dependent mechanisms are expected to mediate the development of Ang II-induced hypertension [10,13,26,27,29]. Deletion of AT₁ (AT₁a) receptors selectively in the proximal tubules of PT-Agtr1a⁻/⁻ mice only blocks AT₁ (AT₁a) receptor- and NHE3-mediated stimulation of proximal tubule Na⁺ reabsorption, but has no effects beyond the proximal tubules in the kidney and other extrarenal tissues [14,15,27,29,71]. Conversely, in the studies with proximal tubule-specific overexpression of intracellular Ang II fusion protein, intracellular Ang II does not induce systemic effects. This is because intracellular Ang II fusion protein only activates intracellular AT₁ (AT₁a) receptors confined to the proximal tubules to stimulate proximal tubule Na⁺ reabsorption, which elevates blood pressure [71,106,107]. Thus, deletion of AT₁ (AT₁a) receptors selectively in the proximal tubules of PT-Agtr1a⁻/⁻ mice is expected completely to block intracellular Ang II-induced blood pressure increases by blocking intracellular Ang II-stimulated proximal tubule Na⁺ reabsorption.

6. Sex Differences in Ang II-Induced Hypertension

Recently, sex differences in genetics, biology, physiology, and cardiovascular, kidney, and hypertensive diseases have attracted widespread attention and extensive research focus [108–113]. This is largely due to the recent mandates in the NIH Policies for Biomedical Research to consider and include gender- and/or sex-related factors as biological variables in all experimental designs [114–117]. Although great progress has been made in sex differences in many other research fields, sex differences in basal blood pressure control or in Ang II-induced hypertension have been inconsistent between different studies or animal models. Specifically, some sex differences have been found in the blood pressure or tubuloglomerular feedback (TGF) responses to Ang II [108–111], increases in NaCl cotransporter or ENaC expression in response to Ang II [118,119], the development of Ang II-induced hypertension [113], diabetic nephropathy [120], or abdominal aortic aneurysms [121]. However, whether sex differences are involved in intratubular Ang II and its AT₁a receptors in the proximal tubules in the development of Ang II-induced hypertension has not been studied previously.

Against this background, we have recently tested the hypothesis that there are significant sex differences in the roles of intratubular Ang II via AT₁a receptors in the proximal tubules during the development of Ang II-induced hypertension [12]. Specifically, we hypothesized that AT₁a receptors in the proximal tubules of female mice contribute less to Ang II-induced hypertension than those in male mice. To test this hypothesis, we directly compared the blood pressure, glomerular, and tubular responses to Ang II-induced hypertension in adult male and female wild-type and mutant mice with proximal tubule-specific knockout of AT₁a receptors (PT-Agtr1a⁻/⁻) [12]. When adult male and female wild-type and PT-Agtr1a⁻/⁻ mice were infused with or without an identical pressor dose of Ang II via osmotic pump for 2 weeks (1.5 mg/kg/day, i.p.), we found that basal systolic, diastolic, and mean arterial pressure were approximately 13 ± 3 mmHg lower, while basal 24 h urinary Na⁺ excretion was significantly higher in both male and female PT-Agtr1a⁻/⁻ mice than wild-type controls without significant sex differences in the same strain (Figure 4). Both male and female wild-type mice developed marked hypertension with similar magnitudes of the pressor responses to Ang II, also without significant sex differences. Furthermore, Ang II-induced hypertension was equally attenuated in male and female PT-Agtr1a⁻/⁻ mice with or without concurrent blockade of AT₁ receptors with losartan [28]. Finally, Ang II-induced glomerular and tubulointerstitial injury was attenuated in both male and female PT-Agtr1a⁻/⁻ mice. Taken together, our study shows that deletion of AT₁a receptors in the proximal tubules of the kidney attenuated Ang II-induced hypertension and kidney injury without revealing significant sex differences.
Ang II-induced hypertension was significantly attenuated in both male and female PT- (NPR proximal tubule function in mice [132]. However, reviewing and discussing the roles of pathway [131], whereas deletion of nephron PRR (ATP6AP2) has been shown to impair vasoactive factors may also be considered. One of these factors is G protein-coupled receptors for atrial natriuretic factor or peptide (ANP), especially NPR_A. ANP receptors (NPR_A) are expressed strongly in the proximal tubules in addition to the glomerulus, distal tubules, and collecting ducts [122–124], and ANP acts on NPR_A in the proximal tubules of the kidney to counteract the effects of Ang II by inhibiting proximal tubule Na+ reabsorption [125–127]. Thus, it is expected that deletion of AT1a receptors in the proximal tubules may augment the natriuretic response to NPR_A-mediated effects in the proximal tubules. However, no such study has been reported to determine whether ANP-induced natriuretic response is potentiated in PT-Agtr1a+/− mice. The other factor is dopamine receptors in the proximal tubules. Dopamine receptors are also expressed in the proximal tubules of the kidney, which mediate the natriuretic responses to dopamine administration and counteract the anti-natriuretic effects of AT1 (AT1a) receptors [128–130]. Prorenin receptors (PRR) are also expressed in the proximal tubules of the kidney in addition to the glomerulus, distal tubules, and collecting ducts [131,132]. The roles of PRR in the proximal tubules remain unknown. Soluble PRR reportedly promotes the fibrotic response in renal proximal tubule epithelial cells in vitro via the Akt/β-catenin/Snail signaling pathway [131], whereas deletion of nephron PRR (ATP6AP2) has been shown to impair proximal tubule function in mice [132]. However, reviewing and discussing the roles of NPR_A, dopamine receptors, or PRR in the proximal tubules of the kidney in details are beyond the scope of this article. Future studies are necessary to confirm whether NPR_A, PRR, or dopamine receptors are involved in mediating basal blood pressure and natriuretic phenotypes and in the development of Ang II-induced hypertension.
8. Conclusions and Perspectives

In summary, we have gained new insights into the important roles of intratubular Ang II and AT\(_1\) (AT\(_{1a}\)) receptors in the proximal tubules of the kidney in maintaining basal blood pressure homeostasis and the development of Ang II-induced hypertension. Significant progress has been made directly in a few key areas over the last three decades, but other studies may also independently contribute to the progresses. Specifically, early in vivo proximal tubule micropuncture experiments in rats [93,94] and in vitro isolated proximal tubule perfusion studies in rabbits or rats [78,95] established important milestones by demonstrating direct biphasic effects of Ang II on proximal tubule Na\(^{+}\) transport function. This was followed by subsequent loss or gain of AT\(_1\) (AT\(_{1a}\)) receptor function studies in mice using the kidney-cross transplantation between wild-type and Agtr1a\(^{-/-}\) mice, firmly confirming the critical role of kidney AT\(_1\) (AT\(_{1a}\)) receptors in blood pressure control and hypertension [10,96]. However, these studies have not be able to determine the direct role of intratubular Ang II and AT\(_1\) (AT\(_{1a}\)) receptors in the proximal tubules in blood pressure control and Ang II-induced hypertension. Recent development in the Cre/LoxP approach has significantly helped advance new insights into the role of proximal tubule Ang II and AT\(_1\) (AT\(_{1a}\)) receptors. This novel approach has provided us and others with an important tool to generate novel mouse models with proximal tubule-specific loss (deletion) or gain of the function (overexpression) [12–14,16,27,28,97]. These new studies have consistently demonstrated that deletion of AT\(_1\) (AT\(_{1a}\)) receptors [12,15,16] or its direct downstream target, NHE3, selectively in the proximal tubules of the kidney [13,14] lowers basal blood pressure, increases the pressure-natriuresis response, and induces natriuretic responses, whereas overexpression of an intracellular Ang II fusion protein or AT\(_1\) (AT\(_{1a}\)) receptors selectively in the proximal tubules increases proximal tubule Na\(^{+}\) reabsorption, impairs the pressure-natriuresis response, and elevates blood pressure [71,106,107]. Furthermore, since the development of Ang II-induced hypertension by systemic Ang II infusion or by proximal tubule-specific overexpression of an intracellular Ang II fusion protein was attenuated in mutant mice with proximal tubule-specific deletion of AT\(_{1a}\) [12,16,28] or NHE3 [13,69], we may conclude that intratubular Ang II via AT\(_{1a}\) and NHE3 in the proximal tubules play a key role in maintaining basal blood pressure and the development of Ang II-induced hypertension.

Author Contributions: Conceptualization: J.L.Z. and X.C.L.; Writing—draft preparations: A.P.d.O.L., S.M.N., X.C.L. and J.L.Z.; Participants in experiments: A.P.d.O.L., X.C.L., R.H. and S.M.N.; Review and editing: J.L.Z. and X.C.L.; Finalization: A.P.d.O.L., S.M.N., J.L.Z. and X.C.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported in part by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (2R01DK102429-03A1, 2R01DK067299-10A1, and 1R01DK102429-01) and the National Heart, Lung, and Blood Institute (1R56HL130988-01) to J.L.Z.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: All animal studies from the authors and cited in this article were approved by the IACUC of the University of Mississippi Medical Center and Tulane University School of Medicine.

Data Availability Statement: Not applicable.

Acknowledgments: All animal studies were performed with data collected in the laboratory of Jia Zhuo at the University of Mississippi Medical Center in Jackson, Mississippi, and Tulane University School of Medicine, New Orleans, Louisiana. We thank Isabelle Rubera and Michell Tauc from the Laboratoire de Physiomedecine Moleculaire, LP2M, UMR-CNRS 7370, Universite Cote d’Azur, Nice CEDEX 2, France (I.R., M.T.) for providing breeding pairs of iL1-sglt2-Cre mouse strain for us to generate proximal tubule-specific PT-Agtr1a\(^{-/-}\) mice in our studies. The contributions of our past and current technicians and postdoctoral fellows from their excellent technical assistance are greatly appreciated.

Conflicts of Interest: The authors declare no conflict of interest.
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