Angiotensin III/AT$_2$ Receptor/NHE3 Signaling Pathway in the Proximal Tubules of the Kidney: A Novel Natriuretic and Antihypertensive Mechanism in Hypertension

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The renin-angiotensin system (RAS) is well recognized as one of the most important circulating, tissue, and intracellular vasoactive hormonal systems in maintaining normal cardiovascular, blood pressure, and kidney function; and it is also important in the pathogenesis of cardiovascular, hypertensive, and renal diseases.[1–3] Extensive research during the past several decades has firmly established that the RAS superfamily consists of at least several important axes, which play diverse roles in the blood pressure, cardiovascular and renal regulation, and diseased processes. The most important axis of the RAS is the classic angiotensinogen/renin/angiotensin-converting enzyme (ACE)/angiotensin II/AT$_1$ receptor axis.[1–4] This classic axis represents the powerful vasopressor system, which is not only required for maintaining normal cardiovascular, blood pressure, and renal homeostasis, but also plays a key role in the development of hypertension and various cardiovascular and kidney diseases, when the system is overactivated. The inhibitors of renin and ACE and the blockers of angiotensin II AT$_1$ and aldosterone receptors are primarily designed to target this axis to treat hypertension and cardiovascular and kidney diseases. Although not known as a vasoconstrictor or vasopressor, the prorenin/renin/prorenin receptor (Atp6ap2)/mitogen-activated protein kinase extracellular signal-regulated kinase 1/2/V-ATPase axis

exerts certain harmful effects similar to the classic angiotensinogen/renin/ACE/angiotensin II/AT$_1$ receptor axis.[5,6] By contrast, there are at least 2 other important axes of the RAS, which include the angiotensin II/aminopeptidase A/angiotensin III/AT$_2$/NO/cGMP axis and the angiotensin I/angiotensin II/ACE2/angiotensin (1-7)/*Mas* receptor axis.[7–9] The latter 2 RAS axes serve as one of the most important vasodepressor axes and the cardiorenal protective arms of the RAS, which act directly or indirectly to oppose the harmful effects of the renin/ACE/angiotensin II/AT$_1$ receptor axis and the prorenin/renin/prorenin receptor axis. The focus of this editorial is on one important role of the angiotensin II/aminopeptidase A/angiotensin III/AT$_2$/NO/cGMP axis in the proximal tubules of the kidney in the regulation of blood pressure, pressure-natriuresis response, and the development of hypertension.

In the kidney, all major components of the RAS, including renin, angiotensinogen, ACE, angiotensin II, AT$_1$, and AT$_2$ receptors, are expressed or localized in the proximal tubules of the kidney.[1–3] In addition, circulating renin, angiotensinogen, angiotensin I, angiotensin II, and angiotensin III are also readily filtered by the glomerulus and taken up by the proximal tubules.[10,11] Thus, angiotensin II can be generated on-site in the proximal tubules or taken up by the proximal tubules via AT$_1$ receptor-mediated endocytosis, with local angiotensin II levels ranging from high femtomole to lower nanomole concentrations.[1,3,10,11] In the proximal tubules, angiotensin II binds to cell surface G-protein–coupled AT$_1$ (AT$_{1a}$) receptors on apical and basolateral membranes, as well as intracellular (mitochondria and nuclear) AT$_1$ (AT$_{1a}$) receptors, and activates key downstream G$_{q/11}$/phospholipase C/IP$_3$ (inositol 1,4,5-trisphosphate)/[Ca^{2+}]$_i$/protein kinase C signaling pathways. This, in turn, induces the expression and activity of the Na$^+$ transporters or cotransporters on the apical membranes, such as NHE3 and the Na$^+$/glucose cotransporter 2, and on the basolateral membranes, such as the Na$^+$/K$^+$-ATPase and the sodium and bicarbonate cotransporter, NBCe1-A, in the proximal tubules. NHE3 is directly and indirectly responsible for reabsorbing ≈50% of the glomerular

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filtered Na$^+$ load, contributing to the maintenance of normal blood pressure homeostasis or the salt-retaining response in hypertension.[12] However, angiotensin II is metabolized in the proximal tubules by aminopeptidase A to generate the heptapeptide angiotensin III by removing an amino acid at the N-terminal, whereas angiotensin III is further metabolized to form angiotensin (3−8) (angiotensin IV) by aminopeptidase N.[9] As a weaker agonist of the parent peptide angiotensin II, angiotensin III is much less potent in its vasopressor activity (≈30−40% of angiotensin II), but is as potent as angiotensin II in stimulating the biosynthesis and release of aldosterone from adrenal glands or vasopressin from the brain. Angiotensin II and angiotensin III bind and activate both AT$_1$ and AT$_2$ receptors in the proximal tubules. Although the actions and signaling mechanisms underlying angiotensin II/AT$_1$ receptor-mediated blood pressure and cardiovascular and kidney responses have been extensively investigated and well recognized, those of AT$_2$ receptor-mediated effects remain incompletely understood.

In the current issue of the *Journal of the American Heart Association* (*JAHA*), Kemp et al[13] mechanistically tested a novel hypothesis that there is a defective response in angiotensin III–induced, AT$_2$ receptor-mediated natriuretic response and defective downstream signal mechanisms in young prehypertensive spontaneously hypertensive rats (SHRs), which may contribute to the development of hypertension in these animals. This study was built strongly on the scientific premise that angiotensin III, not angiotensin II, is the predominant endogenous agonist or ligand for AT$_2$ receptor-induced natriuretic response in normotensive Wistar-Kyoto (WKY) rats, but this angiotensin III/AT$_2$ receptor signaling is defective not only in adult SHRs, but also in prehypertensive, 4-week-old male and female SHRs. There are several strengths in the study of Kemp et al[13] that should be commended. First, the hypothesis tested is novel in that angiotensin III is the predominant ligand for AT$_2$ receptors, and activation of AT$_2$ receptors by angiotensin III may be considered to be a therapeutic target to promote the pressure-natriuresis response in hypertensive animals or humans. Second, the experimental approaches to test their hypothesis were rigorous in that male and female 4-week-old prehypertensive SHRs and normotensive WKY rats were used as the animal models; and an AT$_1$ receptor blocker, candesartan, was used to first block AT$_1$ systemically to exclude the actions of predominant AT$_1$ receptors before angiotensin III was infused directly into the interstitial space of the left kidney.[13] The contralateral right kidney was used as a control. The latter approach is especially important because, although both AT$_1$ and AT$_2$ receptors are expressed in the proximal tubules of the kidney, AT$_1$ receptors predominate AT$_2$ receptors in a ratio of ≈8:1 in the proximal tubules of the kidney.[14] Thus, this may be the key reason why it is

so difficult to demonstrate AT$_2$ receptor-mediated cardiovascular, blood pressure, and renal responses in the presence of predominant AT$_1$ (AT$_{1a}$) receptors. Moreover, infusion of angiotensin III or other pharmacological agents directly into the renal cortical interstitial space is also a good approach to exclude the systemic effect of angiotensin III on blood pressure that may obscure the natriuretic effect of AT$_2$ receptor activation by local angiotensin III. Third, the authors were able to mechanistically determine kidney angiotensin II and angiotensin III levels using the high-performance liquid chromatography–based enzyme immunoassays, isolation of proximal tubule cell apical membranes for Western blots of NHE3 and AT$_2$ receptor proteins, and high-resolution confocal immunofluorescent imaging of NHE3, AT$_2$, and αNKA (Na$^+$/K$^+$-ATPase) localization in the renal cortex of young SHRs and WKY rats, further strengthening the scientific impacts of their findings.[13] Overall, the authors nicely demonstrated that angiotensin III induced a significant natriuresis response in 4-week-old normotensive WKY rats, but not in 4-week-old prehypertensive SHRs, even before the hypertension is developed. Because angiotensin III levels were basically similar in WKY rats and SHRs, this would exclude the possibility of increased angiotensin III degradation as a possible cause for a defective natriuresis response in young SHRs. In young WKY rats, the authors further demonstrated that angiotensin III induced the translocation of AT$_2$ receptors to apical plasma membranes and internalization of NHE3 from apical membranes and Na$^+$/K$^+$-ATPase from basolateral membranes of renal proximal tubule cells, an underlying mechanism of angiotensin III/AT$_2$ receptor-mediated natriuretic response in these animals. By contrast, angiotensin III failed to induce the translocation of NHE3 or Na$^+$/K$^+$-ATPase in the proximal tubules of young SHRs, therefore supporting the presence of defective angiotensin III/AT$_2$ receptor signaling. These results support the authors' hypothesis that, even in prehypertensive SHRs, the angiotensin III/AT$_2$ receptor signaling is already impaired, which may at least, in part, explain that blood pressure begins to increase in SHRs after the age of 5 weeks.[13] Overall, this is a well-designed, well-performed, and mechanistic study by the authors, and the results provide new insights into the potential roles of the angiotensin III/AT$_2$ receptor signaling in the physiological regulation of proximal tubule Na$^+$ reabsorption and blood pressure homeostasis by promoting the natriuretic response.

However, the study of Kemp et al also has a potential limitation in the experimental approach, as rightly acknowledged by the authors, in that their experiments were performed during the AT$_1$ receptor blockade by candesartan.[13] Thus, the natriuretic effect of the angiotensin III/AT$_2$ receptor activation can be elicited only in the absence of angiotensin II/AT$_1$ receptor signaling in the proximal tubules of the kidney. It is not known whether the natriuresis was entirely because of the

angiotensin III/AT$_2$ receptor activation or AT$_1$ receptor block-ade. This limitation may be inadequate to reveal the true physiological significance of angiotensin III/AT$_2$ receptor-mediated natriuresis in normotensive WKY rats and prehyper-tensive SHRs. To overcome this limitation, it may perhaps be necessary to consider performing similar experiments using global as well as proximal tubule-specific AT$_2$ receptor knockout mice infused with angiotensin III. However, few, if any, studies have attempted to take advantage of these tissue-specific, genetically modified animal models to test their hypothesis.

Nevertheless, the results of Kemp et al are significant and translationally relevant for the prevention or treatment of hypertension in human studies.[13] Hypertension currently affects ≈46% of adults in the United States, and only 50% of hypertensive patients have their blood pressure under adequate control.[15] It is necessary to further identify additional mechanisms of hypertension and develop new pharmacological strategies to manage hypertension. The angiotensin III/AT$_2$/NHE3 signaling pathway, as identified by Kemp et al,[13] and the angiotensin II/AT$_1$ receptor/NHE3 signaling pathway may act as 2 important counteracting YIN and YANG hormonal systems to regulate proximal tubule Na$^+$ reabsorption and maintain basal blood pressure homeostasis. Angiotensin II acts as a YANG factor to activate AT$_1$ (AT$_{1a}$) receptors and increase the expression and activity of NHE3 and Na$^+$/K$^+$-ATPase in the proximal tubules, which promotes proximal tubule Na$^+$ reabsorption, impairs the pressure-natriuresis response, and contributes to salt retention in hypertension.[16] By contrast, angiotensin III may act as a YIN factor to activate AT$_2$ receptors to inhibit the expression and activity of NHE3 and Na$^+$/K$^+$-ATPase to promote the natriuresis response and lower blood pressure.[9,13,17] It is, therefore, attractive to propose that pharmacological approaches may be used to either increases angiotensin III generation by targeting aminopeptidase A or aminopeptidase N or directly activate the AT$_2$ receptor signaling pathway to oppose the salt-retaining effect of the angiotensin II/AT$_1$ receptor signaling in the proximal tubules. Indeed, this concept is supported by previous studies showing that angiotensin III is the preferred agonist for AT$_2$ receptors in human coronary arteries, adrenal glands, and the kidney.[17–19] We and others have previously shown that, although most of AT$_2$ receptor expression rapidly and markedly decreases in many target tissues before reaching adulthood, the expression of AT$_2$ receptors persists in the adrenal medulla, the proximal tubules of the kidney, and the vasculature of the human kidney.[14] Furthermore, a nonpeptide AT$_2$ receptor agonist, compound 21, has been shown to significantly promote the natriuretic response in SHRs or obese Zucker rats. Taken together, these proof-of-concept studies support the scientific premise that the angiotensin III/AT$_2$ receptor/NHE3 signaling pathway may be considered to be a novel

proximal tubule-specific, natriuresis-promoting regulating mechanism to maintain normal body salt and fluid balance and blood pressure, as well as in hypertension.[20]

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Disclosures

None.

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