The renin–angiotensin system (RAS) is a coordinated hormonal cascade critical to the control of renal sodium (Na⁺) excretion and blood pressure (BP). Angiotensin II (Ang II), the principal RAS effector peptide, binds to 2 distinct receptors, the Ang type-1 receptor (AT₁R) and the Ang type-2 receptor (AT₂R) with high affinity. The vast majority of actions of Ang II are transmitted via AT₁Rs, including increased renal tubule Na⁺ reabsorption; aldosterone, vasoconstriction, reduction of vascular compliance, cardiac contractility, cellular dedifferentiation and proliferation; vasoconstriction under most circumstances. 1–4

Activation of the sympathetic nervous system. 1,2 In contrast, AT₂Rs generally oppose the actions of Ang II via AT₁Rs. The key findings leading to our present concepts of AT₂Rs and oppose those of Ang II within the kidney or the systemic circulation. 1,2 Expression of Agt either in the kidney or the systemic circulation. 13 Expression of Agt within the kidney induced chronic hypertension. 13 Within the kidney, there is now substantial evidence for a separate intratubular RAS in which Ang II formation is autoamplified by Ang II-induced upregulation of Ang II. 13 Within the kidney, augmented major increases in renal function that were blocked with concurrent intrarenal Ang II administration. 13 Altogether, these studies provided strong support for the existence of an independent functional intrarenal RAS.

Definitive molecular evidence for an independent intrarenal RAS and its importance in the control of BP was obtained using a transgenic mouse model overexpressing Agt either in the kidney or the systemic circulation. 13 Expression of Agt selectively within the kidney induced chronic hypertension independently of the endocrine RAS. 13 Within the kidney, there is now substantial evidence for a separate intratubular RAS in which Ang II formation is autoamplified by Ang II-induced upregulation of Agt, creating a positive feedback loop that may play a role in renal tissue damage and hypertension. 14 Current studies are also providing evidence for intracellular...
RASs that are independently functioning in a specific subcellular compartment. Such subcellular RASs have recently been described both within nuclei and mitochondria.\textsuperscript{15,16}

**AT\textsubscript{2}R Expression and Cell Signaling Pathways**

The AT\textsubscript{2}R is a 7-transmembrane G-protein–coupled receptor, encoded on the X-chromosome, with only 34% amino acid sequence homology with the AT\textsubscript{1}R.\textsuperscript{2} AT2Rs are expressed ubiquitously at very high levels in the fetus but decline precipitously in the neonatal period in most, but not all tissues. Although the expression of AT2Rs is substantially lower than that of AT1Rs in the adult, AT2R mRNA and protein can be easily detected in the adult kidney, adrenal cortex, heart, and vasculature and predominates over AT1Rs in the uterus, ovary, adrenal medulla, and in discrete areas of the brain.\textsuperscript{17–20} Within the kidney, AT1Rs are expressed predominantly in RPTCs and glomeruli.\textsuperscript{13,19}

The cell signaling mechanisms of AT2Rs differ substantially from those of AT1Rs. AT2R activation initiated by binding of Ang II to the receptor in the plasma membrane triggers G protein coupling of Gi\textsubscript{2} and Gi\textsubscript{3} via the third intracellular loop of the receptor. G protein coupling initiates the activation of phosphotyrosine phosphatases, which dephosphorylate and inactivate mitogen-activated protein kinases, including extracellular-regulated kinase-1 and -2. Phosphotyrosine phosphatase activation can also occur through a non–G protein–coupled mechanism. Mitogen-activated protein kinase inhibition via AT2Rs opposes mitogen-activated protein kinase activation as a result of AT1R activation. The opposing action of AT1Rs and AT2Rs on mitogen-activated protein kinases is
considered a fundamental signaling mechanism for receptor-receptor interactions.1–4 AT2R can also activate the phospholipase A2 pathway leading to arachidonic acid release, and long-term AT2R activation can also increase the biosynthesis of ceremides, which can stimulate stress kinases and caspases to induce apoptosis.5

Vascular AT1R Actions and Mechanisms
Overwhelming evidence currently exists that AT1Rs mediate vasodilation and oppose the AT1R-mediated vasoconstrictor actions of Ang II.21–35 AT1R-mediated vasodilation has been demonstrated in small resistance arteries of the mesenteric, uterine, adrenal, coronary, and peripheral circulations in many animal models and in humans. AT1R-induced vasodilation has also been demonstrated in large capacitance vessels such as the aorta and in the fetus.29–31 AT1R-stimulated vasodilation is mediated by a signaling cascade comprising bradykinin (BK), NO, and 3,5'-cyclic GMP (cGMP) (Figures 3 and 4).21,36–38 AT1Rs increase NO and cGMP production either by increasing BK production with activation of BK B2 receptors or by direct activation of NO production independently of BK.39–41

AT1R-mediated vasodilation is most readily demonstrated when AT1Rs are blocked with an AT1R antagonist,22,23,25,26 This is almost certainly because AT1R expression predominates over that of AT2Rs in the vasculature.42,43 AT1R-stimulated vasodilation is also augmented when the RAS is activated during Na+ restriction, Ang II infusion, or in renal vascular hypertension.21,22,44 Under all 3 circumstances, AT2Rs are upregulated, enhancing the vasodilator response to Ang II.18,21,44 Another condition which upregulates AT1R expression (by 300%) and unmasks its vasodilator action is increased pressure load from aortic banding.29,30 AT2R blockade with specific antagonist PD-123319 (PD) or BK B2 receptor inhibition with icatibant restores the diminished Ang II contractile responses and abolishes the 9-fold increase in aortic cGMP stimulated by Ang II under these circumstances.29,30 Taken together, the results of these studies emphasize the likely importance of counter-regulatory AT2R upregulation and activation in circulatory disorders associated with chronic vasoconstriction via AT1Rs.

The vasodilator and depressor actions of AT2Rs are both acute and chronic and are not accompanied by desensitization, rendering these receptors a potential therapeutic target in hypertension.23,25 Indeed, the BP lowering effects of AT2R blockade may be mediated, at least in part, by AT2R activation as a result of increased renin biosynthesis and release, and increased Ang II that can act via unblocked AT2Rs.21,22 An example of this principle was demonstrated in diabetic, hypertensive humans in whom chronic AT1R inhibition upregulated vascular AT2Rs and facilitated a vasodilator response to Ang II in vitro.44 In addition, in spontaneously hypertensive rats during AT1R blockade, pharmacological activation of AT2Rs by Compound 21 (a nonpeptide AT1R agonist with >25,000-fold selectivity for AT2Rs over AT1Rs) resulted in decreased BP.25 These observations indicate the potential importance of a nonpeptide AT2R agonist combined with an AT1R blocker in the treatment of hypertension.

AT1R-mediated vasodilation and hypotension were confirmed in AT1R-null mice.45 Although baseline BP was similar between AT1R-null and wild-type mice, AT1R-null mice demonstrated marked and sustained hypersensitivity to the pressor actions of infused Ang II over the course of 7 days, emphasizing the importance of AT1Rs in counter-regulating Ang II actions via AT1Rs. Ang II pressor hypersensitivity was accompanied by a highly significant reduction in baseline and Ang II-stimulated renal interstitial levels of BK, NO, and cGMP in AT1R-null mice.

Intrarenal AT1R Actions and Mechanisms
AT1R-null mice also had marked antinatriuresis (and inhibition of pressure-natriuresis) during the chronic Ang II infusion that was not present in wild-type mice.45 These results suggested the possibility that intrarenal AT1Rs might increase renal Na+ excretion via BK, NO, and cGMP.45

Figure 3. Angiotensin (Ang) II releases renal bradykinin (BK) by AT1R (Ang type 2 receptor) activation. Renal interstitial fluid (RIF) cGMP levels in response to intravenous infusion of Ang II; Losartan, an AT1R antagonist; PD, PD-123319, an AT1R antagonist; and combinations in Sprague-Dawley rats. Control vehicle infusions, white bars; experimental agent infusions, black bars. Data are expressed as means±1 SE. *P<0.0001 from control; ++P<0.05, +++P<0.0001 from Ang II alone. Data derived from Siragy et al23 and Siragy and Carey.26

Figure 4. Angiotensin (Ang) II releases renal cyclic GMP (cGMP) by AT1R activation. Renal interstitial fluid (RIF) cGMP levels in response to intravenous infusion of Ang II; Losartan, an AT1R antagonist; PD, PD-123319, an AT1R antagonist; and combinations in Sprague-Dawley rats. Control vehicle infusion data, white bars; experimental agent infusion, black bars. Data represent mean±1 SE. *P<0.001 from vehicle or time control; ++P<0.001 from Ang II alone. Adapted from Siragy and Carey25 and Siragy and Carey26 with permission.
We subsequently explored and presented definitive evidence that intrarenal AT\textsubscript{2}R activation mediates natriuresis.\textsuperscript{46-48} These studies were enabled by the technique of renal interstitial microinfusion of pharmacological agents, which affords direct evaluation of the intrarenal mechanisms governing renal function without systemic hormonal or hemodynamic influences. Selective intrarenal AT\textsubscript{2}R blockade in rats induced a highly significant natriuresis that was abolished by intrarenal coadministration of AT\textsubscript{2}R-specific antagonist PD, indicating that the natriuretic effect of AT\textsubscript{2}R blockade is mediated by AT\textsubscript{2}R activation.\textsuperscript{46}

However, we were surprised to find that intrarenal Ang II infusion did not alter Na\textsuperscript{+} excretion even at high infusion rates. This finding provoked a question as to whether a downstream metabolite of Ang II might be required for renal AT\textsubscript{2}R activation. Indeed, intrarenal infusion of des-aspartyl\textsuperscript{1} Ang II (Ang III) into systemically AT\textsubscript{2}R-blocked rats induced a significant natriuretic response, which was abolished with intrarenal coadministration of AT\textsubscript{2}R-selective antagonist PD,\textsuperscript{46} similar to AT\textsubscript{1}R-mediated vascular responses.\textsuperscript{46} In follow-up of this observation, we hypothesized that Ang II needs to be converted to Ang III to interact with AT\textsubscript{2}R within the kidney. Ang II is converted to the heptapeptide Ang III by aminopeptidase A, and Ang III is converted to the hexapeptide Ang IV by aminopeptidase N. In the presence of systemic AT\textsubscript{2}R blockade, intrarenal infusion of Ang III induced a natriuretic response that was markedly augmented by intrarenal coadministration of aminopeptidase N inhibitor 3-amino-4-thio-butyl-sulfonic acid (EC-33).\textsuperscript{48} Taken together, these studies demonstrate that intrarenal administration of aminopeptidase N inhibitor PC-18 induces natriuresis even in the absence of systemic AT\textsubscript{2}R blockade. Furthermore, renal interstitial Ang peptide levels during Ang III administration with and without PC-18 demonstrated a marked augmentation of renal interstitial and tissue Ang III concentrations and AT\textsubscript{2}R/Ang II ratios during PC-18 administration, consistent with the role of Ang III in the augmented natriuretic effect.\textsuperscript{52} These studies also demonstrated that systemic administration of the highly selective nonpeptide AT\textsubscript{2}R agonist Compound 21 induces natriuresis that is abolished with intrarenal AT\textsubscript{2}R antagonist PD in both male and female rats even in the absence of AT\textsubscript{2}R blockade, suggesting the potential for this compound as a natriuretic/diuretic agent in the treatment of disorders associated with extracellular fluid volume expansion and hypertension.

Recent studies also have suggested that AT\textsubscript{2}R in the thick ascending limb of Henle may contribute to the natriuretic response.\textsuperscript{54,55} Ang II increases NO production in thick ascending limbs via activation of AT\textsubscript{2}R, and NO inhibits the Na\textsuperscript{+}/K\textsuperscript{+}/2Cl\textsuperscript{−} cotransporter and reduces Na\textsuperscript{+} reabsorption in this nephron segment.\textsuperscript{55} Whether this response requires Ang II conversion to Ang III awaits further study.

![Figure 5. Angiotensin (Ang) III is the preferred endogenous AT\textsubscript{2}R (Ang type 2 receptor) agonist mediating natriuresis. Urinary Na\textsuperscript{+} excretion (U\textsubscript{Na}V) in anesthetized Sprague-Dawley rats in response to direct renal interstitial infusion of vehicle (white bars), Ang II (black bars), Ang III (gray bars), or Ang III +PD (PC-123319, an AT\textsubscript{2}R antagonist) (striped bars). Data are expressed as mean±1 SE. *P<0.05, **P<0.01, ***P<0.001 from time control. Adapted from Padia et al.\textsuperscript{46}](image-url)
**Intrarenal Dopaminergic System**

The renal dopaminergic system is a major hormonal system controlling renal Na+ excretion and BP. D1-likeR activation inhibits renal Na+ reabsorption through an adenylyl cyclase-cAMP mechanism. In both humans and experimental animals, highly selective D1-likeR agonist fenoldopam elicits a substantial natriuretic response that is based almost exclusively on inhibition of renal proximal tubule Na+ reabsorption.5,56–60 Thus, the renal dopaminergic system is an important counter-regulatory system offsetting the antinatriuretic actions of AT1Rs. Indeed, fenoldopam was demonstrated to be close to ideal as an antihypertensive agent in that it normalized BP without reflex tachycardia and induced natriuresis in patients with primary hypertension61 (Figure 6). In spite of its low bioavailability, these and other favorable observations led to Food and Drug Administration approval for emergency treatment of hypertension in intensive care settings.

The physiological importance of the renal dopaminergic system in the control of Na+ excretion was demonstrated initially during the 1980s. Studies using intrarenal arterial administration of highly selective D1-likeR antagonist SCH-23390 revealed that, similar to the intrarenal RAS, DA synthesized within the kidney acts in a local cell-to-cell (paracrine) manner exclusively at the renal proximal tubule to control Na+ excretion62,63 (Figure 7). These studies demonstrated that 60% of basal Na+ excretion in the Na+-replete state is controlled by intrarenal dopaminergic mechanisms acting at the proximal tubule. These observations were later confirmed using renal interstitial infusion of D1R antisense oligodeoxynucleotides to inhibit receptor expression directly.64 The importance of the renal dopaminergic system in the control of Na+ excretion was further underscored by the demonstration that the natriuretic and diuretic effects of D1-likeRs are sized within the kidney acts in a local cell-to-cell (paracrine) manner exclusively at the renal proximal tubule to control Na+ excretion62,63 (Figure 7). These studies demonstrated that 60% of basal Na+ excretion in the Na+-replete state is controlled by intrarenal dopaminergic mechanisms acting at the proximal tubule. These observations were later confirmed using renal interstitial infusion of D1R antisense oligodeoxynucleotides to inhibit receptor expression directly.64 The importance of the renal dopaminergic system in the control of Na+ excretion was further underscored by the demonstration that the natriuretic and diuretic effects of D1-likeRs are dependent on the Na+ balance. In Na+-deplete states, D1-likeR-mediated natriuresis does not occur, whereas in normal or high Na+ states D1-likeRs induce a robust natriuretic response.65 Additional evidence for the physiological importance of renal dopaminergic control of Na+ excretion included the observation that renal DA production is increased during Na+ surfeit but reduced during Na+ depletion.65 Elegant studies in mice with selective proximal tubule knockout of aromatic amino acid decarboxylase, the enzyme generating DA from L-dihydroyphenylalanine, inducing intrarenal DA depletion have recently confirmed the importance of intrarenal DA in the control of Na+ excretion and BP.66 Taken altogether, these studies strongly support the physiological importance of renal DA and D1-likeRs as counter-regulatory systems limiting, at least in part, the Na+-retaining actions of intrarenal Ang II via AT1Rs.

In the mid-1990s, with antibodies directed toward the extracellular domain of D1Rs, receptor protein was localized in the renal proximal tubule and in several other cells and tissues.67–69 Similar to the AT1R, D1R mRNA is expressed only in low copy numbers and was difficult to demonstrate using standard molecular techniques. However, D1R protein cellular distribution was later confirmed using more sensitive in situ amplification of D1R mRNA.70

**D1R/AT2R Interactions**

Renal interstitial administration of fenoldopam in Na+-loaded rats elicits a robust natriuretic response that is abolished with intrarenal coadministration of D1-likeR antagonist SCH-23390.71 However, we were surprised to find that fenoldopam-induced natriuresis is also completely inhibited with intrarenal coinfusion of AT2R antagonist PD71 (Figure 8). To explore the possible mechanism of AT2R involvement in D1-likeR-induced natriuresis, studies were performed to determine the intracellular trafficking of AT2Rs in RPTCs.71,72 In vivo administration of fenoldopam was associated with translocation of AT2Rs from intracellular sites to the apical plasma membranes of RPTCs. Fenoldopam-induced AT2R translocation to the apical plasma membrane and natriuresis were abolished in the presence of microtubulin inhibitor nocodazole but were unaffected by actin microfilament inhibitor cytochalasin D, suggesting that microtubules are required for the translocation process.72 Because D1-likeRs signal via an adenylyl cyclase, cAMP, and protein kinase A pathway, we explored the role of these signaling processes.
in D1-like receptor-mediated AT2R translocation and natriuresis persisted in the presence of D1-like receptor antagonist SCH-23390.72 Therefore, the mechanism by which AT1Rs and D1-like Rs interact during high Na+ conditions to induce natriuresis is D1-like R-CAMP signaling, which provides the necessary stimulus for AT1R translocation and natriuresis. We also demonstrated that similar to agonist-stimulated D1 receptor recruitment to the apical plasma membrane, AT1Rs are translocated via microtubules to the apical plasma membrane, where they are required to induce the natriuretic response.73,74

Recently, AT1R-null animals were demonstrated to have increased longevity.75 In current studies, the aging process is beginning to be linked to reduction in AT1R and D1R expression and activation in different tissues and at the mitochondrial level.16,75,76 It is possible that D1R and AT1R pharmacological activation may provide a new target for the reversal of certain aspects of the aging process and for the extension of lifespan in the future.

Conclusions and Perspectives

In conclusion, the intrarenal RAS and dopaminergic system play a major critical role in cardiovascular and renal function, the subject of this brief review. AT1Rs and D1Rs cooperatively oppose the vasoconstrictor and antinatriuretic functions mediated by Ang II at AT1Rs. Reduced AT1R expression and activity may contribute to the initiation and acceleration of disease processes including hypertension, edema-forming states, and inflammation/fibrosis, leading to cardiovascular and renal damage. Conversely, pharmacological activation of AT1Rs and D1Rs may provide therapeutic advantages or even preventive strategies in the presence or absence of AT1R blockade. Increased understanding of Ang and DA receptor functions and interactions currently provides hope for improved treatment and prevention of hypertension and other Na+/fluid retaining states and for the extension of healthier lives in the future.

Disclosures

None.

References


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