The renin–angiotensin system (RAS) is a coordinated hormonal cascade critical to the control of renal sodium (Na+) excretion and blood pressure (BP).1 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.1,2 The vast majority of actions of Ang II are transmitted via AT1Rs, including cellular dedifferentiation and proliferation; vasoconstriction, reduction of vascular compliance, cardiac contractility, increased renal tubule Na+ reabsorption; aldosterone, vasopressin, and endothelin secretion; salt appetite; and thirst and activation of the sympathetic nervous system.1,2 In contrast, AT2Rs generally oppose the actions of Ang II via AT1Rs under most circumstances.1–4

Another major regulatory system in cardiovascular and renal physiology is the peripheral dopaminergic system. Dopamine (DA) is mainly synthesized in renal proximal tubule cells (RPTCs) via the decarboxylation of L-dihydroxyphenylalanine that has been filtered at the glomerulus and transported into the RPTC across the apical brush border.5 Synthesized DA exits the cell mainly across the apical plasma membrane into the lumen, where it can bind to and activate specific DA D1-like receptors (D1-like R: D1 and D5).5,6 D1-like R activation induces vasodilation and inhibits renal Na+ reabsorption and actions, which also oppose those of Ang II via AT1Rs.

The purpose of this brief review is to summarize some of the key findings leading to our present concepts of AT1Rs and AT2Rs under most circumstances.1–4

Evidence for an Independent Functional Intrarenal RAS

Although renin was identified in the brain and the adrenal cortex in the late 1960s and early 1970s, the intrarenal RAS was the first independent functional tissue RAS to be described.7–9 The initial observations were from in vivo studies, which demonstrated that intrarenal inhibition of the RAS with angiotensin converting enzyme (ACE) inhibitors or Ang receptor blockers, at infusion rates that did not alter systemic BP during the experimental period, increased renal plasma flow, glomerular filtration rate, and Na+ and water excretion7 (Figure 1). These results were later confirmed by more rigorous approaches showing that small intrarenal doses of Ang receptor blocker, while not altering pressor responses to systemically administered Ang II, induced marked increases in renal hemodynamic and tubular function8,9 (Figure 2). Later, it was demonstrated that the mRNAs and proteins for all of the system components (renin, angiotensinogen [Agt], ACE, and AT1Rs) are localized in a site-specific manner within the kidney and that intrarenal formation of Ang II occurs independently of renal uptake of the peptide.1

Additional evidence for a separate renal tissue RAS came from studies showing that intrarenal Ang II levels were elevated in the nanomolar range in renal interstitial fluid compared with picomolar concentrations in plasma; that intrarenal Ang II concentrations were markedly increased compared with plasma levels in response to Na+ restriction; and that this response was blocked with intrarenal renin inhibition.10 Cellular studies demonstrated that Agt, Ang I, and Ang II could be colocalized with renin in proximal tubule and juxtaglomerular cells, that Ang peptides were released from these cells and that the release was regulated.11 Further functional studies demonstrated that combined intrarenal RAS blockade with low doses of ACE inhibitor, AT1R blocker, and renin inhibitor, although confined to the kidney, augmented major increases in renal function that were blocked with concurrent intrarenal Ang II administration.12 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.\(^\text{15,16}\)

**\(\text{AT}_2\text{R} \)** Expression and Cell Signaling Pathways

The \(\text{AT}_2\text{R}\) is a 7-transmembrane G-protein–coupled receptor, encoded on the X-chromosome, with only 34% amino acid sequence homology with the \(\text{AT}_1\text{R}\).\(^\text{2}\) \(\text{AT}_2\text{Rs}\) 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 \(\text{AT}_2\text{Rs}\) is substantially lower than that of \(\text{AT}_1\text{Rs}\) in the adult, \(\text{AT}_2\text{R}\) mRNA and protein can be easily detected in the adult kidney, adrenal cortex, heart, and vasculature and predominates over \(\text{AT}_1\text{Rs}\) in the uterus, ovary, adrenal medulla, and in discrete areas of the brain.\(^\text{17–20}\) Within the kidney, \(\text{AT}_2\text{Rs}\) are expressed predominantly in RPTCs and glomeruli.\(^\text{15,19}\)

The cell signaling mechanisms of \(\text{AT}_2\text{Rs}\) differ substantially from those of \(\text{AT}_1\text{Rs}\). \(\text{AT}_2\text{R}\) activation initiated by binding of Ang II to the receptor in the plasma membrane triggers G protein coupling of \(\text{Gi}\) and \(\text{Gi}\) 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 \(\text{AT}_2\text{Rs}\) opposes mitogen-activated protein kinase activation as a result of \(\text{AT}_1\text{R}\) activation. The opposing action of \(\text{AT}_1\text{Rs}\) and \(\text{AT}_2\text{Rs}\) on mitogen-activated protein kinases is
considered a fundamental signaling mechanism for receptor-receptor interactions.\textsuperscript{1-4} AT\textsubscript{2}Rs can also activate the phospholipase A\textsubscript{2} pathway leading to arachidonic acid release, and long-term AT\textsubscript{2}R activation can also increase the biosynthesis of ceremides, which can stimulate stress kinases and caspases to induce apoptosis.\textsuperscript{5}

**Vascular AT\textsubscript{1},R Actions and Mechanisms**

Overwhelming evidence currently exists that AT\textsubscript{2}Rs mediate vasodilation and oppose the AT\textsubscript{1},R-mediated vasoconstrictor actions of Ang II.\textsuperscript{21-35} AT\textsubscript{1},R-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. AT\textsubscript{1}R-induced vasodilation has also been demonstrated in large capacitance vessels such as the aorta and in the fetus.\textsuperscript{29-31} AT\textsubscript{1}R-stimulated vasodilation is mediated by a signaling cascade comprising bradykinin (BK), NO, and 3,5’-cyclic GMP (cGMP) (Figures 3 and 4).\textsuperscript{21,36-38} AT\textsubscript{1}R-mediated vasodilation is most readily demonstrated when AT\textsubscript{1}R antagonists are blocked with an AT\textsubscript{2}R antagonist.\textsuperscript{22,23,25,26} This is almost certainly because AT\textsubscript{1}R expression predominates over that of AT\textsubscript{2}Rs in the vasculature.\textsuperscript{42,43} AT\textsubscript{1}R-stimulated vasodilation is also augmented when the RAS is activated during Na\textsuperscript{+} restriction, Ang II infusion, or in renal vascular hypertension.\textsuperscript{21,22,44} Under all 3 circumstances, AT\textsubscript{1}Rs are upregulated, enhancing the vasodilator response to Ang II.\textsuperscript{18,21,44} Another condition which upregulates AT\textsubscript{1}R expression (by 300%) and unmasks its vasodilator action is increased pressure load from aortic banding.\textsuperscript{29,30} AT\textsubscript{1}R blockade with specific antagonist PD-123319 (PD) or BK receptor activation 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.\textsuperscript{29,30} Taken together, the results of these studies emphasize the likely importance of counter-regulatory AT\textsubscript{2}R upregulation and activation in circulatory disorders associated with chronic vasoconstriction via AT\textsubscript{1}R.

The vasodilator and depressor actions of AT\textsubscript{2}Rs are both acute and chronic and are not accompanied by desensitization, rendering these receptors a potential therapeutic target in hypertension.\textsuperscript{21,22} Indeed, the BP lowering effects of AT\textsubscript{2}R blockade may be mediated, at least in part, by AT\textsubscript{2}R activation as a result of increased renin biosynthesis and release, and increased Ang II that can act via unblocked AT\textsubscript{1}Rs.\textsuperscript{21,22} An example of this principle was demonstrated in diabetic, hypertensive humans in whom chronic AT\textsubscript{1}R inhibition upregulated vascular AT\textsubscript{2}R and facilitated a vasodilator response to Ang II in vitro.\textsuperscript{44} In addition, in spontaneously hypertensive rats during AT\textsubscript{1}R blockade, pharmacological activation of AT\textsubscript{2}Rs by Compound 21 (a nonpeptide AT\textsubscript{2}R agonist combined with an AT\textsubscript{1}R blocker in a nonpeptide AT\textsubscript{2}R agonist with >25,000-fold selectivity for AT\textsubscript{2}Rs over AT\textsubscript{1}Rs) resulted in decreased BP.\textsuperscript{55} These observations indicate the potential importance of a nonpeptide AT\textsubscript{2}R agonist combined with an AT\textsubscript{1}R blocker in the treatment of hypertension.

AT\textsubscript{1}R-mediated vasodilation and hypotension were confirmed in AT\textsubscript{1},R-null mice.\textsuperscript{45} Although baseline BP was similar between AT\textsubscript{1},R-null and wild-type mice, AT\textsubscript{1},R-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 AT\textsubscript{1}Rs in counter-regulating Ang II actions via AT\textsubscript{1}Rs. 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 AT\textsubscript{1},R-null mice.

**Intrarenal AT\textsubscript{2},R Actions and Mechanisms**

AT\textsubscript{1},R-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.\textsuperscript{45} These results suggested the possibility that intrarenal AT\textsubscript{2}Rs might increase renal Na\textsuperscript{+} excretion via BK, NO, and cGMP.\textsuperscript{46}
We subsequently explored and presented definitive evidence that intrarenal AT₂R activation mediates natriuresis.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, R blockade in rats induced a highly significant natriuresis that was abolished by intrarenal coadministration of AT, R-specific antagonist PD, indicating that the natriuretic effect of AT, R blockade is mediated by AT, R activation.⁴⁶

However, we were surprised to find that intrarenal Ang II infusion did not alter Na⁺ 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, R activation. Indeed, intrarenal infusion of des-aspartyl¹ Ang II (Ang III) into systemically AT, R-blocked rats induced a significant natriuretic response, which was abolished with intrarenal coinfusion of PD⁴⁶ (Figure 5). Intrarenal Ang III infusion in the absence of systemic AT, R blockade did not change Na⁺ excretion, similar to AT, R-mediated vascular responses.⁴⁶ In follow-up of this observation, we hypothesized that Ang II needs to be converted to Ang III to interact with AT, Rs 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, R blockade, intrarenal infusion of Ang III induced a natriuretic response that was markedly augmented by intrarenal coadministration of aminopeptidase N inhibitor 2-amino-methylsulfonyl-butane-thiol, methane-thiol (PC-18).⁴⁷ The PC-18-augmented natriuresis was abolished by intrarenal AT, R inhibition with PD, indicating an AT, R-mediated effect.⁴⁷ The necessity for conversion of Ang II to Ang III for AT, R-mediated natriuresis was confirmed by demonstrating that intrarenal administration of Ang II is only effective in inducing natriuresis when aminopeptidase N is blocked and that this response is abolished by intrarenal coadministration of aminopeptidase A inhibitor 3-amino-4-thio-butyl-sulfonic acid (EC-33).⁴⁸ Taken together, these studies demonstrate that Ang III is the preferred agonist for AT, R-mediated natriuresis. In systematic receptor binding studies, Ang III has been found to have about 30-fold selectivity over Ang II for AT₂Rs.⁴⁹ It was also shown that the renal expression of AT₁R mRNA was increased by intrarenal Ang II infusion, suggesting a role for AT₁R in mediating the natriuretic response.⁵⁰⁻⁵²

Recent in vivo studies have demonstrated that Ang III induces natriuresis via AT₂R activation in the renal proximal tubule by a NO/cGMP signaling mechanism.⁵³ In vitro studies also have recently shown that AT₁Rs reduce AT₂R function in the proximal tubule by the common NO/cGMP pathway and also reduce AT₂R mRNA via the ubiquitous transcription factor Sp1.⁵⁴ Ligand-activated AT₁Rs also heterodimerize with AT₂Rs, reducing their expression via protein–protein action in the plasma membrane.⁵³ Thus, AT₂Rs may oppose AT₁Rs by several pathways in the kidney.

The recent in vivo investigations cited above have confirmed that Ang III is the preferred endogenous ligand for the activation of renal AT₂Rs.⁵⁵ Unexpectedly, these studies were unable to elicit a natriuretic effect of Ang (1-7), which has counter-regulatory effects offsetting AT, R actions in other tissues.⁵⁶ No natriuretic response to Ang (1-7) was observed at equimolar doses as Ang III even in the presence of AT₂R blockade, ACE inhibition to reduce Ang (1-7) metabolism, or aminopeptidase A blockade to augment Ang (1-7) formation from Ang II via ACE-2.⁵⁷ However, these studies did demonstrate that intrarenal administration of aminopeptidase N inhibitor PC-18 induces natriuresis even in the absence of systemic AT₁R 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 Ang III/Ang II ratios during PC-18 administration, consistent with the role of Ang III in the augmented natriuretic effect.⁵⁸ These studies also demonstrated that systemic administration of the highly selective nonpeptide AT₂R agonist Compound 21 induces natriuresis that is abolished with intrarenal AT₁R antagonist PD in both male and female rats even in the absence of AT₁R 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 AT₁Rs in the thick ascending limb of Henle may contribute to the natriuretic response.⁵⁹⁻⁶¹ Ang II increases NO production in thick ascending limbs via activation of AT₁Rs, and NO inhibits the Na⁺ /K⁺2Cl⁻ cotransporter and reduces Na⁺ reabsorption in this nephron segment.⁵⁵ Whether this response requires Ang II conversion to Ang III awaits further study.

Figure 5. Angiotensin (Ang) III is the preferred endogenous AT₂R (Ang type 2 receptor) agonist mediating natriuresis. Urinary Na⁺ excretion (U, 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 AT₁R antagonist) (striped bars). Data are expressed as means±1 SE. *P<0.05, **P<0.01, ***P<0.001 from time control. Adapted from Padia et al.⁶²
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. Thus, the renal dopaminergic system is an important counter-regulatory system offsetting the antinatriuretic actions of AT₂Rs. 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 hypertension (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⁺ excretion. Similar to the AT₂R, D₁R mRNA is expressed only in low copy numbers in the kidney, and was difficult to demonstrate using standard molecular techniques. However, D₁R protein cellular distribution was further underscored by the demonstration that the natriuretic and diuretic effects of D₁-likeRs are dependent on the state of 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. 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. Elegant studies in mice with selective proximal tubule knockout of aromatic amino acid decarboxylase, the enzyme generating DA from L-dihydroxyphenylalanine, inducing intrarenal DA depletion have recently confirmed the importance of intrarenal DA in the control of Na⁺ excretion and BP. 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 AT₂Rs.

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. Similar to the AT₂R, D₁R mRNA is expressed only in low copy 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.

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. However, we were surprised to find that fenoldopam-induced natriuresis is also completely inhibited with intrarenal coinfusion of AT₂R antagonist PD. 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. 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. Because D₁-likeR signal via an adenylyl cyclase, cAMP, and protein kinase A pathway, we explored the role of these signaling processes...
in D₁-likeR–induced AT₃R recruitment to the apical plasma membrane and its necessity for the natriuretic response. In vivo experiments demonstrated that intrarenal administration of direct adenylyl cyclase activator forskolin together with 3-isobutyl-1-methylxanthine (IBMX) to inhibit its metabolism increased renal interstitial fluid levels of cAMP, stimulated AT₃R recruitment to the RPTC apical plasma membrane, and induced natriuresis that was abolished with AT₁R antagonist PD.⁷² Direct agonist stimulation of D₁-likeRs was not necessary for AT₃R-mediated natriuresis because forskolin/IBMX-induced AT₃R translocation and natriuresis persisted in the presence of D₁-likeR blockade with SCH-23390.⁷² Therefore, the mechanism by which AT₃Rs and D₁-likeRs interact during the mechanism by which AT₂Rs and D₁-likeRs interact during the natriuretic response.⁷²,⁷³ Recently, AT₃R-null animals were demonstrated to have increased longevity.⁷⁴ In current studies, the aging process is beginning to be linked to reduction in AT₃R and D₁ R expression and activation in different tissues and at the mitochondrial level.¹⁶,⁷⁵,⁷⁶ It is possible that D₁ and AT₃R 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. AT₃Rs and D₁Rs cooperatively oppose the vasoconstrictor and antinatriuretic functions mediated by Ang II at AT₁Rs. Reduced AT₃R 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 AT₃Rs and D₁Rs may provide therapeutic advantages or even preventive strategies in the presence or absence of AT₃R 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**


The Intrarenal Renin-Angiotensin and Dopaminergic Systems: Control of Renal Sodium Excretion and Blood Pressure
Robert M. Carey

Hypertension. 2013;61:673-680
doi: 10.1161/HYPERTENSIONAHA.111.00241
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/61/3/673

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/