The renin–angiotensin system (RAS) is the best understood and arguably most important hormonal system in the control of blood pressure (BP) and the pathophysiology of hypertension. In the classical RAS, the enzyme renin cleaves its substrate angiotensinogen (Agt) forming the decapeptide angiotensin I that is in turn cleaved by angiotensin-converting enzyme (ACE) to produce the angiotensin II (Ang II), the biologically active peptide of the system. Although several other peptide products of Agt had been identified and studied, Ang II was generally accepted as the physiological and pathophysiological driving force of the classical RAS. Ang II activates its AT$_1$ receptor (AT$_1$R) to induce a large array of biological responses, including vasoconstriction, renal sodium (Na$^+$) reabsorption, cell proliferation, dedifferentiation, and growth and aldosterone secretion, increasing BP and contributing to the development of hypertension. However, we appreciate that the RAS is far more complex than the simple hormonal cascade represented by the classical system. In recent years, we have recognized several essential components and actions of the RAS, including those of local tissue RASs functioning independently of the circulating system, the (pro)renin receptor as a potential direct biological target for renin and prorenin, and 2 counter-regulatory pathways, the ACE-2–angiotensin (1–7)–Mas receptor cascade and the Ang type-2 receptor pathway. Indeed, our understanding of the RAS has been transformed into one of the most complex hormonal systems known with internal balance and interplay among its multiple enzymatic peptide and receptor constituents. This brief review will encompass selected recent publications identifying additional new components and actions of the RAS.

**New RAS Components**

**Angiotensin (1–12)**

Our traditional concept of the RAS cascade has been that the initial peptide cleaved from Agt is Ang I and that all other peptides are derived either directly or indirectly from Ang I catabolism. This concept has been recently challenged by the discovery of angiotensin (1–12) [Ang (1–12)], a peptide containing a 2-amino-acid (Leu$^{11}$-Tyr$^{12}$) C-terminal extension of Ang I. Initially isolated and identified from the rat small intestine, Ang (1–12) is present in high concentrations (comparable with those of Ang I and II) in other peripheral tissues, such as the aorta, heart, and kidneys, although its circulating levels are substantially lower than those of Ang I and II. Tissue levels of Ang (1–12) were unchanged after bilateral nephrectomy, renin inhibition, and Na$^+$ loading, which markedly reduced the circulating and tissue levels of Ang I and II and Na$^+$ restriction, which increased Ang I and II levels. The relatively higher tissue level of Ang (1–12) and its lack of change in response to systemic manipulation has suggested a possible physiological and pathophysiologic role in tissues, independently of the systemic circulation.

Initially, Ang (1–12) was found to induce a vasoconstrictor response in rat aorta that could be blocked either with an ACE inhibitor or AT$_1$R blocker, suggesting that Ang (1–12) might be a peptide precursor of Ang II. This concept has been validated, and interestingly renin has been demonstrated not to participate in Ang (1–12) formation or metabolism. Studies have indicated that in tissues, such as heart, instead of ACE, chymase seems to be the major Ang (1–12)–metabolizing enzyme. However, a recent study shows a different result for Ang (1–12) metabolism in the systemic circulation of normotensive and hypertensive rats, where ACE was shown to cleave the Leu$^{10}$-Leu$^{11}$ bond of Ang (1–12) to generate Ang I. Chymase, a chymotrypsin-like enzyme expressed in the secretory granules of mast cells, is generally accepted as the major catalyst for Ang I to II conversion in the heart and in the vasculature. The finding that different enzyme pathways for Ang II formation operate in cardiovascular tissues versus the systemic circulation supports the concept that tissue RASs may directly contribute to local tissue remodeling and disease. The enzyme(s) involved in the formation of Ang (1–12) from Agt are currently unknown; candidates include molecules of the kallikrein-kinin system, cathepsins, and chymase itself. The new pathways of Ang (1–12) formation and metabolism are depicted in Figure 1.

**Angiotensin A**

One of the major counter-regulatory arms of the RAS, generally opposing the actions of Ang II via AT$_1$Rs is the ACE-2–Ang (1–7)–Mas receptor pathway. Indeed, the activation...
of this pathway may be at least partially responsible for some of the beneficial effects of AT\(_1\)R blockade on vascular injury in hypertension.\(^{14}\) In addition to Ang (1–7), mass spectrometry has allowed an opportunity to identify other biologically active angiotensin peptides in plasma and tissues. Angiotensin A (Ang A) is such a peptide identified in human plasma in which Ang II has an N-terminal substitution of Ala\(^{1}\) for Asp\(^{1}\) [Ala\(^{1}\)-Ang II].\(^{15}\) Ang A is generated from Ang II by enzymatic decarboxylation of Asp\(^{1}\) in the presence of mononuclear leukocytes and was initially reported as having a similar affinity for AT\(_1\)R and AT\(_2\)R as Ang II.\(^{15}\) Subsequent studies in rats and genetically engineered mice confirmed that Ang A has similar pressor and renal vasoconstrictor effects as Ang II mediated by AT\(_1\)R.\(^{15}\) Although alamandine is a heptapeptide that differs from Ang (1–7) only by the presence of an N-terminal Ala\(^{1}\) [Ala\(^{1}\)-Ang (1–7)], alamandine can be synthesized in the rat heart from perfused Ang (1–7), but the enzyme responsible for endogenous alamandine formation under these circumstances remains unknown.\(^{17}\) Alamandine possesses many of the functional properties of Ang (1–7). In contrast to the vasoconstrictor actions demonstrated for Ang A, alamandine induces endothelium-dependent vasodilation in aortic rings that can be attenuated by NO synthase inhibitor N-nitro-L-arginine methyl ester.\(^{17}\) Similar to Ang (1–7), alamandine induces BP reduction when administered into the caudal ventrolateral medulla of anesthetized Fisher rats, as well as systemically in spontaneously hypertensive rats, and has cardiac antifibrotic properties in Sprague–Dawley rats.\(^{17}\) Although alamandine circulates in human plasma and is increased in patients with end-stage renal disease, its potential role in humans is unknown.\(^{17}\) The presumed endogenous biosynthetic pathway for alamandine is shown in Figure 1.

**Figure.** Schematic depiction of the renin–angiotensin system components and selected actions. The enzymes of the system are shown in red. Newly described enzymatic pathways are shown in yellow. Receptors are shown in the boxes. ACE indicates angiotensin-converting enzyme; Agt, angiotensinogen; Ang, angiotensin; APA, aminopeptidase A; AT\(_1\)R, angiotensin type-1 receptor; AT\(_2\)R, angiotensin type-2 receptor; MasR, Mas receptor; MrgD, Mas-related G-protein-coupled receptor; and PRR, (pro)renin receptor.

**Alamandine**

Another spectrographically identified Ang peptide is alamandine, a product of the catalytic hydrolysis of Ang A by human ACE-2.\(^{17}\) Alamandine is a heptapeptide that differs from Ang (1–7) only by the presence of an N-terminal Ala\(^{1}\) [Ala\(^{1}\)-Ang (1–7)]. Alamandine can be synthesized in the rat heart from perfused Ang (1–7), but the enzyme responsible for endogenous alamandine formation under these circumstances remains unknown.\(^{17}\) Alamandine possesses many of the functional properties of Ang (1–7). In contrast to the vasoconstrictor actions demonstrated for Ang A, alamandine induces endothelium-dependent vasodilation in aortic rings that can be attenuated by NO synthase inhibitor N-nitro-L-arginine methyl ester.\(^{17}\) Similar to Ang (1–7), alamandine induces BP reduction when administered into the caudal ventrolateral medulla of anesthetized Fisher rats, as well as systemically in spontaneously hypertensive rats, and has cardiac antifibrotic properties in Sprague–Dawley rats.\(^{17}\) Although alamandine

**Mas-Related G-protein–Coupled Receptor**

Unexpectedly, treatment with Mas receptor antagonist A-779 did not block alamandine-induced vasodilation, and alamandine-induced vasodilation was preserved in aortic rings from Mas receptor–deficient mice.\(^{17}\) However, alamandine-induced vasodilation was completely blocked by another Ang (1–7) antagonist D-Pro\(^{7}\)-Ang (1–7). This result suggested that, instead of binding to the Mas receptor, alamandine might exert its actions through another Mas-related receptor, MrgD.\(^{18}\) Indeed, alamandine was shown to bind to MrgD-transfected cells, and the binding was competitively inhibited by D-Pro\(^{7}\)-Ang (1–7) but not by A-779. In addition, alamandine released NO in MrgD- but not in Mas-transfected cells.\(^{17}\) Figure 1 displays the current position of MrgD within the RAS and its interactions with alamandine.

**Novel RAS Actions**

**Intrarenal Tissue RAS**

A functional intrarenal RAS was initially discovered in 1977\(^{19}\) and has been validated as important in the control of renal Na\(^{+}\) excretion and BP.\(^{20}\) Indeed, recent evidence has suggested a separate intratubular RAS in which Ang II formation is autoamplified by Ang II–induced upregulation of Agt, forming a positive feedback loop that is thought to play a role in renal tissue damage and hypertension.\(^{20,21}\) Several advances in our understanding of the mechanisms by which the intrarenal RAS functions contribute to the generation disease have recently been reported.

ACE is expressed in the kidneys predominantly at the apical surfaces of proximal tubule cells, but the role of ACE in local tissue Ang II production when the intrarenal RAS is activated had not been elucidated. Mice selectively lacking renal ACE exhibited markedly reduced natriuretic and hypertensive responses to intrarenal RAS activation in models with either
high- or low-circulating Ang II levels (Ang II infusion and reduced NO production, respectively). Selective removal of renal ACE decreased renal Ang II levels and renal Na+ and fluid reabsorption, shifting the pressure-natriuresis curve to the right (less sensitive). Ang II infusion in wild-type mice increased Na+ transporter expression and activity in the loop of Henle and more distal nephron sites, and these changes were largely eliminated in animals devoid of renal ACE. These observations strongly suggest that renal ACE is an essential component of the intrarenal RAS and that renal tubular Ang II generation is critical in the regulation of Na+ reabsorption and the pathophysiology of hypertension.

All of the components of the RAS, including the Ang substrate Agt mRNA, are present within the kidney, and intrarenal formation of Ang II has been repeatedly documented at the local tissue level independently of the systemic circulation. Because Agt mRNA had been demonstrated in the proximal tubules where Ang II formation occurs, it has been thought that intratubular Ang II is derived primarily from locally synthesized Agt. Surprisingly, however, recent studies in liver- and kidney-specific Agt knockout mice have demonstrated that, at least under basal conditions, the liver is the major source of renal Agt. Kidney-specific Agt knockout mice had renal levels of Ang and Ang II similar to those of wild-type mice, whereas liver-specific Agt knockout mice reduced circulating and renal Agt and Ang II to almost undetectable levels. These findings indicate that in normal nonhypertensive mice, most of the Agt present in proximal tubules are derived from the liver. A major unanswered question, however, is whether locally synthesized Agt significantly contributes to tubular Ang II in Ang II–dependent hypertension or in kidney disease states when the intrarenal RAS is activated.

The role and mechanisms of the intrarenal RAS in hypertension have been further explored using an elegant molecular approach using combined AT1 receptor and enhanced cyan fluorescent protein/Ang II selective transfer into the proximal tubules of AT1 receptor-null mice. This experimental manipulation resulted in a significant increase in BP likely because of an increase in proximal tubule Na+ and fluid reabsorption. Because enhanced cyan fluorescent protein/Ang II remains within the cell (is not secreted into the extracellular environment), these results suggest a role for an intracellular renal RAS within the proximal tubule in hypertension.

Subcellular Ang Systems

Complete self-contained functional intracellular RASs have been postulated for some time, with the primary focus being nuclear Ang peptides and receptors. In particular, a transgenic mouse model expressing intracellular Ang II, independently of secreted Agt or Ang peptides, demonstrated nuclear Ang II translocation accompanied by hypertension and renal thrombotic microangiopathy. Functionally, nuclear AT1Rs have been coupled to phosphoinositols-3 kinase and protein kinase C activation and ultimately to the production of reactive oxygen species. In contrast, both nuclear AT1Rs and Mas receptors have been linked to NO formation. In addition to a nuclear Ang system, a functional mitochondrial Ang system has been recently identified and characterized, wherein Ang II and predominantly AT1Rs are colocalized. The mitochondrial Ang system is functionally coupled to NO formation and the regulation of mitochondrial respiration. During the aging process, the expression of mitochondrial AT1Rs increases and that of AT1Rs decreases, suggesting the possibility that subcellular system might be related to chronic disorders of aging.

Adipose Tissue RAS

Another local tissue RAS thought to be important in the genesis of hypertension is the adipose tissue RAS. In adipocytes, Ang II biosynthesis seems to be driven by local cellular Agt production and subsequent enzymatic peptide cleavage by chymase and cathepsins. Both the systemic and the adipose RAS are activated in obesity, but whether the local adipose tissue RAS contributes to obesity-induced hypertension has remained unanswered. Recent studies using an innovative molecular approach indicate that the adipose RAS definitively contributes to the generation of obesity-induced hypertension. Selective conditional adipocyte Agt deficiency in mice, while not altering the degree of weight gain, prevented the increase in BP observed in control mice in response to a high dietary fat intake. Interestingly, high fat feeding induced a similar increase in circulating Agt in control and in knockout mice, indicating that changes in adipose tissue do not account for elevated circulating Agt in obesity. However, adipose-selective Agt disruption did prevent the increase in circulating Ang II observed in the control mice, indicating that adipocyte-derived Ang II is a primary contributor to the activation of the systemic RAS associated with obesity-induced hypertension. The findings of this study describe a new mechanism by which adipocytes affect the circulating RAS via modulation of Ang II biosynthesis. The precise enzymatic pathways responsible for increased circulating Ang II in obesity remain to be clarified in future research. Nevertheless, the primacy of adipocyte generation of Ang II suggests that RAS inhibition should be moved to the forefront of therapeutic considerations in obesity-induced hypertension.

AT1 Receptors

AT1Rs are generally recognized as functioning in a manner counter-regulatory to the actions of Ang II via AT1Rs. However, in contrast to AT1Rs, the role of AT1Rs in the control of Na+ excretion and BP has been less well defined. Recent significant advances in our understanding of the actions of AT1Rs have led to the identification of new therapeutic targets and the potential for AT1R agonist therapy in hypertension and several of its related disorders that are summarized here. AT1Rs are encoded by a gene on the X-chromosome. Several recent studies have demonstrated sex differences in the renal responses to AT1R activation, wherein greater renal vasodilation could be demonstrated in the female as compared with male rats. Indeed, the ratio of AT1R:AT1R expression in the renal vasculature is greater in females than in males. However, sex differences were not apparent at the level of renal tubule Na+ and fluid reabsorption. Thus, some but not all of the actions of AT1Rs are sex dependent.

AT1Rs have been shown to induce vasodilation in capacitance, as well as small resistance vessels, in a large number of studies. Recently, the vasodilator effect was demonstrated in a
The enzymatic pathways of endogenous alamandine biosynthesis, will clarify the regulation of alamandine production, and will uncover the control of expression and specific cell signaling mechanisms of MrgD receptors. Important questions will be how and why 3 separate AT,R counter-regulatory pathways [AT,R, Ang (1–7)/ACE-2/MasR, and alamandine/MrgD] are used and how they interrelate to each other if at all.

The local renal tissue RAS assumes increased physiological and pathophysiological import with recent demonstrations that Ang II generated locally by ACE and also intracellularly in the proximal tubule increases renal Na+ reabsorption and BP. However, these observations need to be placed in context with the new finding that hepatic (and not intrarenal) Agt seems to be the major source of intrarenal Ang II under basal conditions. The precise contribution of local intrarenal Ang II formation in Ang-dependent hypertension needs further clarification. Another local RAS, the adipocyte RAS, now seems as the major source of increased circulating Ang II in obesity. These findings suggest that RAS inhibition may become a primary therapeutic consideration in the treatment of obesity hypertension in humans. Exciting new evidence now points to the possibility that, in addition to local tissue RASs, functional-independent intracellular Ang systems exist both in nuclei and in mitochondria. Given the importance of these organelles in biological processes, it will be important to design both pharmacological and molecular probes to determine their potential roles in physiology, formation of disease and aging.

AT,R actions have steadily continued to be clarified, and we now know that these receptors inhibit Na+ reabsorption largely by interacting with Ang III rather than Ang II. Several studies are beginning to report AT,R actions using the nonpeptide receptor agonist C21, the results of which must be carefully interpreted according to dose and experimental conditions because of C21’s recently described off-target effects, particularly in the vasculature. AT,Rs have several newly described tissue protective actions in disease processes characterized by an activated RAS and even when RAS activity is low. The impressive neuroprotective actions of AT,R activation before and after stroke offer promise of preventive and therapeutic interventions that can be applied to human cerebrovascular disease in the future.

Far from definitive and complete, our knowledge of the RAS continues to unfold and become progressively more relevant to human physiology and disease. Undoubtedly, the next several years will yield fresh information on the RAS, providing vital opportunities for treatment and prevention of hypertension and related cardiovascular disorders.

Disclosures

None.

References


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Robert M. Carey

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