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New insights into angiotensin receptor actions: from blood pressure to aging

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Abstract

Purpose of the review—The renin-angiotensin system (RAS) is critical for cardiovascular control, impacting normal physiology and disease pathogenesis. Although several biologically active peptides are generated by this system, its major actions are mediated by angiotensin II acting through its type 1 (AT1) and type 2 (AT2) receptors. Along with their effects to influence blood pressure and hemodynamics, recent studies have provided evidence that angiotensin receptors influence a range of processes independent from hemodynamic effects.

Recent findings—This review is focused on new molecular mechanisms underlying actions of AT1 receptors to influence vasoconstriction, inflammation, immune responses, and longevity. Moreover, we also highlight new advances in understanding functions of the AT2 receptor in end-organ damage, emphasizing the AT2 receptor as a potential therapeutic target in cardiovascular diseases.

Summary—Here we review recent advances in understanding the role of angiotensin receptors in normal physiology and disease states, focusing on their properties that may contribute to blood pressure regulation, end-organ damage, autoimmune disease and longevity.

Keywords

Angiotensin receptors; hypertension; aging; vascular function; immunity

Introduction

The renin-angiotensin system (RAS), a master physiological regulator, has been the subject of intensive study for over one-hundred years. The RAS is a hormonal cascade whereby the protein substrate angiotensinogen is successively metabolized by renin and angiotensin converting enzyme (ACE) to form angiotensin (Ang) II, its major biologically active peptide. Ang II acts in many tissues including kidney, heart, blood vessels, brain, and lymphatic organs, through binding and activation of receptors, which belong to the large family of G-coupled (GPCR), 7 trans-membrane (7TM) spanning receptors. The angiotensin receptors can be separated pharmacologically into two distinct classes: type 1 (AT1) and type 2 (AT2), and these receptors have been cloned and sequenced from many species [1, 2]. The consensus from studies using pharmacological AT1 and AT2 receptor antagonists [3] and

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genetic studies in mice is that the dominant cardiovascular actions of the RAS are mediated by the AT1 receptor [4, 5]. While humans have only a single AT1 receptor gene (*AGTR1*) encoding a single AT1 receptor isoform, rodents have two AT1 receptor subtypes, AT1A and AT1B, encoded by distinct genes [6]. While the two murine AT1 receptor subtypes are highly homologous and share similar affinities to Ang II, the AT1A receptor predominates in most tissues [3], representing the closest murine homologue to the single human AT1 receptor. On the other hand, expression of the AT2 receptor is highest during fetal development [7, 8], decreasing in most tissues to very low levels in adults. In general, the functions of the AT2 receptor tend to oppose actions of the AT1 receptor.

Here we will review some recent advances highlighting novel functions of the RAS to influence normal physiology and disease states, focusing on responses mediated by the two classes of angiotensin receptors, AT1 and AT2.

AT1 receptor signaling in blood pressure homeostasis

The classical actions of the RAS, and in particular its cardiovascular effects, are elicited by activation of AT1 receptors. The efficacy of specific AT1 receptor blockers (ARBs) in treating hypertension, slowing progression of chronic kidney disease, and reducing cardiovascular risk [9] reflects the important role of this receptor in a variety of disorders [10, 11]. Similarly, targeted deletion of the major murine AT1 receptor (AT1A) receptor causes a marked reduction of blood pressure and salt sensitivity in mice, confirming its importance in cardiovascular control [4, 5, 12, 13]. Accordingly, defining the precise functions of this receptor is likely to provide key insights into normal physiological functions of the RAS as well as critical pathophysiological pathways.

One well-recognized function mediated by AT1 receptors is to trigger intense vasoconstriction [14]. These actions are mediated by direct effects of AT1 receptors in vascular smooth muscle cells (VSMCs) [15, 16] along with indirect effects of AT1 receptor activation of pathways in the CNS linked to peripheral vasoconstriction [17]. Recently, Guilluy and associates [18**] identified a novel signaling pathway linking AT1 receptor activation to vascular smooth muscle cell contraction. Specifically, these authors found that activation of JAK2 results in phosphorylation of a specific guanine nucleotide exchange factor, *Arhgef1*, triggering RhoA signaling and activation of Rho kinase [19, 20], which inhibits myosin light chain phosphatase, thereby promoting VSMC contraction.

When *Arhgef1* was specifically deleted from VSMCs, acute vasoconstrictor responses to Ang II were abrogated, whereas responses to other vasoconstrictors such as phenylephrine and endothelin were preserved [18**]. Likewise, the hypertensive response to chronic infusion of Ang II was significantly attenuated. These responses were also inhibited by administration of a specific inhibitor of JAK2. The virtually complete protection from Ang II-dependent hypertension observed in this study was somewhat surprising and seems to contradict previous studies from our laboratory using a kidney cross-transplantation model, which indicated that AT1A receptors in the kidney and their effects to regulate renal sodium excretion play a predominant role in the development of Ang II-dependent hypertension [12].

One possible unifying explanation would be that AT1 receptor actions in the renal vasculature have critical actions to influence kidney function in hypertension. On the other hand, our preliminary studies show that cell-specific elimination of AT1 receptors in the renal proximal tubule epithelium provides substantial protection from Ang II-dependent hypertension (Gurley et al, unpublished data). Nonetheless, the identification of a pathway requiring JAK2 and *Arhgef1* that mediates AT1 receptor dependent vasoconstriction and demonstration of its physiological significance is a major advance. Understanding the

relative contributions of vascular versus renal epithelial actions of AT1 receptors to chronic blood pressure homeostasis will be an interesting topic for future studies.

AT1 receptor activation and autoimmune diseases

Beside its well-defined hemodynamic actions, evidence has emerged indicating that some of the consequences of AT1 receptor activation contributing to target organ damage involve non-hemodynamic pathways. One of these may be modulation of the immune system [21-23]. Direct actions of AT1 receptors to affect lymphocyte functions have been long recognized [24, 25]. Moreover, recent studies, using animal models of inflammatory autoimmune diseases have provided solid evidence that direct cellular actions of AT1 receptor may have profound influence on the course of autoimmune and inflammatory diseases [26**, 27*, 28**, 29**]. For example, in myelin-oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-EAE), a mouse model of multiple sclerosis, components of the RAS, including renin, ACE and AT1 receptors are upregulated both in activated lymphocytes and inflamed tissues. Furthermore, treatment with an ACE inhibitor or ARB delays the onset or attenuates manifestations of MOG-EAE [28**, 29**], through actions that are clearly independent of blood pressure. AT1 receptor inhibition reduced the absolute number of antigen presenting cells (APC) expressing CD11c and CD11b, along with a number of APC-related chemokines such as CCL2, CCL3 and CXCL 10 and consequently impaired APC migration [29**]. Further, ARB administration suppressed autoreactive TH1 and TH 17 cells by inhibiting the canonical NF κ B1 transcription factor complex while increasing CD4+FoxP3+ T regulatory cells [28**].

In addition to impacting autoimmune demyelinating disease, effects of angiotensin II on T regulatory cells may also be relevant to cardiovascular diseases. In this regard, adoptive transfer of T regulatory cells ameliorated cardiac fibrosis and improved cardiac function in Ang II-dependent hypertension without affecting blood pressure [30*]. Moreover, AT1 receptor activation increases TGF- β expression and signaling in the CNS by activating the TGF- β -activating protease thrombospondin-1 (TSP-1), leading to an increased inflammatory response and an inflammatory T-cell phenotype [27*]. Similar to its effects on chemokine-dependent migration of APCs [29**], AT1 receptor activation also promotes rapid mobilization and migration of undifferentiated splenic monocytes towards injured tissue in response to myocardial infarction [31*].

A recent study identified powerful non-hemodynamic actions of AT1 receptors in another autoimmune disease model, murine systemic erythematosus lupus (SLE) [26**]. In contrast to MOG-EAE, AT1 receptors in lymphocytes are not responsible for affecting disease pathogenesis, instead, it is a population of AT1 receptors on glomerular podocytes in the kidney that promote renal inflammation and injury in lupus. This study utilized the MLR-Fas lpr/lpr mouse model for SLE that develops an aggressive, diffuse proliferative glomerulonephritis resembling lupus nephritis in humans. To define the role of AT1A receptors in lupus, MLR-Fas lpr/lpr mice were generated lacking the AT1A receptor. Surprisingly, AT1A receptor-deficient MLR-Fas lpr/lpr had increased early mortality with accelerated proteinuria, glomerular inflammation and pathology. However, increased disease severity was not a consequence of AT1A receptor deficiency in immune cells, since transplantation of AT1A-deficient bone marrow did not affect survival. When lupus mice lacking AT1A receptors were given losartan, which blocks both AT1A and AT1B receptors, markers of kidney disease, including proteinuria, glomerular pathology, and cytokine mRNA expression, were reduced. In the mouse kidney, AT1B receptors are expressed primarily by podocytes. This indicates that activation of residual populations of glomerular AT1B receptors, most likely in podocytes, was the mechanism responsible for the more severe renal disease in the AT1A receptor deficient MLR-Fas lpr/lpr mice. This study illustrated the potent capacity for cellular actions of glomerular AT1 receptors in isolation to

promote proteinuria, to stimulate pro-inflammatory cytokine expression and to induce structural injury *in vivo* in a manner that is independent of systemic hypertension or other hemodynamic perturbations. By inference, these findings suggest that attenuation of AT1 actions in podocytes may be one mechanism behind the beneficial effects of RAS blockade in glomerular diseases.

AT1 receptors influence aging

Large clinical trials have demonstrated beneficial effects of AT1 receptor blockade in cardiovascular disease that are achieved by lowering blood pressure, slowing the progression of atherosclerosis, and attenuating end-organ damage [32, 33] resulting in decreased morbidity and mortality. Such studies have confirmed the utility of AT1 receptor blockade in patients with cardiovascular and kidney diseases. A recent study suggests that beneficial, lifeprolonging effects of AT1 receptor blockade might also extend into the general population, through actions to slow the aging process [34**]. Specifically, Benigni and associates showed that elimination of the AT1A receptor in mice prolonged life span by ~28% compared to genetically matched wild-type controls. Reflecting the versatile actions of the AT1 receptor, enhanced longevity was associated with improved cardiovascular morphology, reduced ROS production, attenuated mitochondrial loss, and enhanced expression of survival genes. Specifically, levels of nicotinamide phosphoribosyltransferase (Nampt) and sirtuin-3 (Sirt3) were enhanced in mice lacking AT1A receptors and these changes have been associated with improved mitochondrial survival and reduced oxidative stress [35, 36]. Conversely, administration of Ang II decreased Nampt and Sirt3 levels *in vitro*. In summary, this study suggested that actions of the AT1 receptor contribute to the aging process by promoting reactive oxygen production and mitochondrial damage, and that inhibition of these actions by ARBs may promote longevity.

New insights in AT2 receptor mediated actions

Defining the precise functions of the AT2 receptors has been challenging due to its low levels of expression, the very robust actions of the AT1 receptor, and, until recently, a relatively limited selection of small molecule agonists and antagonists. Initial studies using mice with target deletion of the AT2 receptor gene [37] suggested that the AT2 receptor did not have a major role in normal physiological regulation. Subsequent studies, however, indicated that expression of the AT2 receptor increases under pathological conditions such as myocardial infarction, stroke and pancreatic fibrosis [38*, 39, 40]. Furthermore, activation of the AT2 receptor triggers nitric oxide (NO) release [41*] and inhibits NF- κ B [42*] and, the JAK/STAT signaling pathway [43], actions that would potentially counteract effects of the AT1 receptor and promote cardiovascular protection [39]. In addition, AT2 receptor activation directly antagonizes AT1 receptor mediated actions by forming heterodimers with the AT1 receptor [44]. In support of this premise are studies in AT2 receptor-deficient mice challenged with cardiovascular disease models, showing acceleration of pathology. Along with increases in cerebral infarct size [40] and an acceleration in the progression of atherosclerosis [45], recent studies demonstrated increased pancreatic fibrosis in an experimental model of pancreatitis [38*] and aggravated renal injury in subtotal nephrectomized AT2 receptor-knockout mice [46*]. In this latter study, AT2 receptor-deficient mice had exaggerated mortality with increases in albuminuria, renal fibrosis, glomerular injury, lymphocyte infiltration, and chemokine expression compared to controls after renal ablation, whereas blood pressure and RAS metabolites were similar between the groups. As discussed above, the lack of potent and selective AT2 receptor agonists and antagonists limited the scope of research into AT2 receptor functions. However, in 2004, Wan and associates [47] developed a highly selective, non-peptide AT2 receptor agonist, called Compound 21 (C21) allowing more direct studies of specific effects of AT2 receptor stimulation *in vivo*. For example, AT2 receptor activation by chronic C21

administration improves cardiac function after myocardial infarction, with reduced infarct size, ameliorated remodeling and reduced inflammatory responses [39]. Similarly, inflammatory responses following exposure of fibroblasts to Ang II *in vitro* were markedly attenuated with simultaneous administration of the AT₂ receptor agonist. One mechanism of these antiinflammatory actions may be through the inhibition of TNF- α induced IL-6 production and inhibition of NF- κ B activity [42*].

AT₂ receptor mediated effects on vascular function are more complex. For example, in spontaneously hypertensive rats (SHR), specific AT₂ receptor blockade had no effect on vascular reactivity in coronary arteries but was associated with reduced vasoconstriction of mesenteric arteries in response to Ang II [48]. On the other hand, in coronary arteries of humans [49] and normotensive rats [48], AT₂ receptor blockade amplified Ang II-dependent vasoconstriction. In SHR and control rats, AT₂ receptor stimulation with the small molecule agonist C21 caused a vasodilation *in vitro* [50]. In this study, acute administration of C21 also decreased blood pressure in SHRs and this effect was potentiated by concomitant AT₁ receptor blockade [50]. The AT₂ receptor-agonist had no effect on blood pressure in conscious normotensive rats when given acutely or in rats after myocardial infarction when administered chronically [39].

Conclusion

A series of recent studies have provided new insights into broad functions of the RAS acting through its AT₁ and AT₂ receptors. These studies highlight and emphasize the diverse role of the RAS in physiology and disease pathogenesis, and these roles can be separated based on distinct functions of the angiotensin receptors. For the AT₁ receptor, a key role for the Rho kinase-signaling cascade to mediate vasoconstriction has been identified and suggests potential new targets for the treatment of hypertension and vascular disease. A robust effect of AT₁ receptors to modify inflammation and immune responses in autoimmune diseases has been clearly defined, which may also have therapeutic significance. Based on a number of pathways linked to AT₁ receptor activation, a role for this receptor to promote aging has been recently described, suggesting potential benefits of RAS antagonists to promote longevity. With regard to the AT₂ receptor, the consensus of emerging data is that this receptor exerts anti-inflammatory, anti-apoptotic and blood pressure lowering actions. In cardiovascular diseases, these actions would be expected to have beneficial consequences. Recent work indicating relatively potent actions of small molecule AT₂ agonists in pre-clinical models is impressive and suggests a potential therapeutic role in the treatment of hypertension and target end-organ damage that might enhance the actions of currently available RAS inhibitors.

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