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SECONDARY HYPERTENSION: NERVOUS SYSTEM MECHANISMS (M WYSS, SECTION EDITOR)

Integrative Physiological Aspects of Brain RAS in Hypertension

Sharon D. B. de Morais[1] · Julia Shanks[1] · Irving H. Zucker[1]

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Abstract

Purpose of Review The renin-angiotensin system (RAS) plays an important role in modulating cardiovascular function and fluid homeostasis. While the systemic actions of the RAS are widely accepted, the role of the RAS in the brain, its regulation of cardiovascular function, and sympathetic outflow remain controversial. In this report, we discuss the current understanding of central RAS on blood pressure (BP) regulation, in light of recent literature and new experimental techniques.

Recent Findings Studies using neuronal or glial-specifc mouse models have allowed for greater understanding into the site-specific expression and role centrally expressed RAS proteins have on BP regulation. While all components of the RAS have been identified in cardiovascular regulatory regions of the brain, their actions may be site specific. In a number of animal models of hypertension, reduction in Ang II-mediated signaling, or upregulation of the central ACE2/Ang 1–7 pathway, has been shown to reduce BP, via a reduction in sympathetic signaling and increase parasympathetic tone, respectively. Emerging evidence also suggests that, in part, the female protective phenotype against hypertension may be due to inceased ACE2 activity within cardiovascular regulatory regions of the brain, potentially mediated by estrogen.

Summary Increasing evidence suggests the importance of a central renin-angiotensin pathway, although its localization and the mechanisms involved in its expression and regulation still need to be clarified and more precisely defined.

All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

Keywords Angiotensin · Renin · Sympathetic nervous system · Hypertension · Blood pressure · Brain

Introduction

Hypertension (HTN) is one of the leading causes of morbidity and mortality globally [1]. It accounts for 9.4 million deaths worldwide annually, with over half of all strokes and cases of ischemic heart disease attributable to high blood pressure (BP) [1, 2].

For the majority of patients, the development of HTN is dependent on a combination of genetic and environment factors. In many clinical and animal models, HTN presents with increased sympathetic outflow [3]. The increased sympathetic drive to the cardiovascular system and resultant increase in BP are termed "neurogenic hypertension." Disturbances in the central nervous system control of sympathetic outflow may induce neurogenic HTN. However, the pathophysiological mechanisms responsible for the increased sympathetic drive in HTN are unknown. The pathogenesis of HTN is complex and undoubtedly caused by multiple factors that regulate heart rate, cardiac output, and total peripheral resistance. Even after over 100 years of study, the mechanisms underlying the development of HTN are incompletely understood.

The renin-angiotensin system (RAS) plays an important role in cardiovascular homeostasis, body fluid regulation, and electrolyte balance and has recently been implicated as a metabolic regulator. The RAS has been shown to be key in the development of HTN [4–7] The classical RAS pathway, in brief, is composed of angiotensinogen (AGT), a precursor, synthetized in the liver, and renin, an aspartyl protease,

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✉ Irving H. Zucker
izucker@unmc.edu

[1] Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE 68198-5850, USA

released by the juxtaglomerular cells of the kidney (Fig. 1). Renin cleaves AGT generating the inactive decapeptide angiotensin I (Ang I). Ang I is activated by the angiotensin converting enzyme (ACE), located on endothelial cells of the lung and kidneys. ACE cleaves the carboxy-terminal His-Leu dipeptide of Ang I to generate the active octapeptide Ang II, the predominant effector protein of the RAS pathway. Binding of Ang II to the angiotensin II type 1 (AT1) receptor results in an increase in BP by inducing vasoconstriction, increases renal sodium reabsorption, and induces the release of aldosterone and arginine vasopressin (AVP) from the adrenal and pituitary glands, respectively, in addition to increasing central sympathetic outflow [8–10]. Two subtypes of AT1 receptors (AT1a and AT1b) have been identified in rodents [11, 12]; however, their distinct physiological actions have yet to be clarified. Conversely, Ang II binding to AT2 receptors induces vasodilation, apoptosis, cellular proliferation, and sympatho-inhibition, reduces sodium reabsorptium, and inhibits AVP release [8, 13–26].

Ang II is metabolized by aminopeptidase (AMN) A into Ang III (Ang 2–8), which is converted by AMN N into Ang IV (Ang 3–8). Alternatively, Ang II can be metabolized by ACE 2, generating Ang 1–7. Similar to Ang II, Ang III and its metabolites play an important role in AVP and aldosterone release, sympathetic hyperactivity, and BP regulation [19, 27–30]. However, Ang III has been shown to reduce sodium reabsorbance [31] and has a cardioprotective effect via AT2R [32]. Ang IV affects cognitive function, reduces neuronal apoptosis, and pro-inflammatory [33–37]. Ang 1–7 binds mainly to the Mas receptor whose actions counterbalance many of the deleterious effects of Ang II [38]. Therefore, ACE 2 and Ang 1–7 have been generally thought to be protective to the cardiovascular system.

In addition to endocrine and systemic actions of the RAS, which are now well described, all components of the RAS have now been identified in the brain. Although previously controversial, a number of recent articles have extensively reviewed the current literature on RAS control of central BP regulation providing positive evidence for its importance [23, 39–45]. A number of animal models have demonstrated the importance of the brain RAS on development and maintenance of HTN [46–52]. In addition, peripheral Ang II may gain entry into the central nervous system at sites with an intact blood-brain barrier [53]. Recent novel genetic and imaging techniques have allowed a greater understanding of the central RAS activity. This review will provide a broad overview of some of the most recent findings in this field.

Brain RAS/Systemic RAS Interactions

The difficulties in differentiating circulatory versus tissue RAS products limit the comprehension of both systems. However, genetic tools to generate transgenic animals

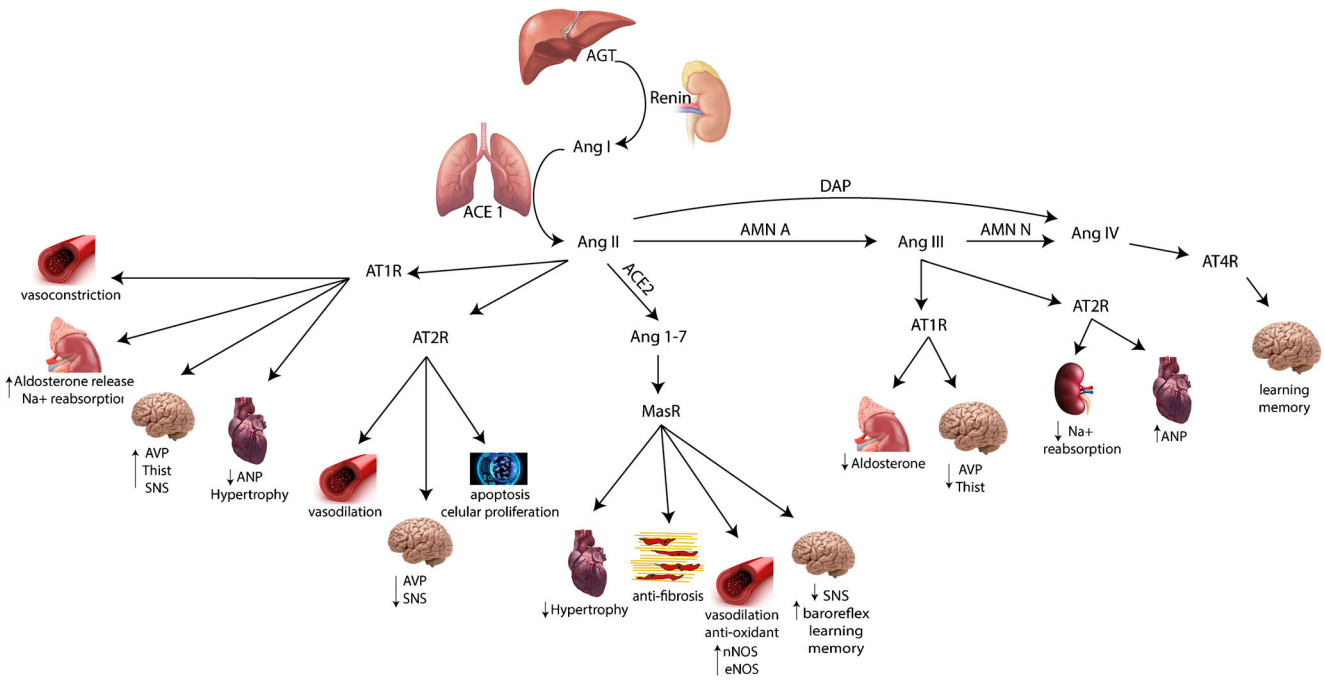

Fig. 1 The classical and systemic renin angiotensin system. ATG: angiotensinogen, Ang I: angiotensin I, Ang II: angiotensin II, Ang III: angiotensin III, Ang IV: Angiotensin IV, ACE: angiotensin converting enzyme, AMN A: aminopeptidase A, AMN N: aminopeptidase N, AT1R: AT1 receptor, AT2R: AT2 receptor, AT4R: AT4 receptor, DAP: dipepdyl aminopeptidase, Ren: Renin

targeting RAS tissue-specific products provide an important tool to dissect these systems.

In a recent review by Nakagawa and Sigmund [39], the authors point out that systemic BP regulation and renal-fluid regulation have generally been attributed to the circulating RAS [54]. Intrarenal Ang II is elevated in Ang II-induced HTN, independent of renal renin levels. The augmentation of intrarenal Ang II is due, in part, to uptake of circulating Ang II via an AT1R mechanism and to endogenous production of Ang II [55]. However, central Ang II activation induces sympathetic outflow (neurotransmission) [56–59]. AVP release has been attributed to tissue-specific RAS [23, 58, 60, 61]. The existence of both a local centrally acting and a peripheral systemically acting RAS makes understanding the intereactions and regulation between these two systems complex.

Neuronal and Glial RAS

Angiotensinogen

Astrocytes are the main site of AGT synthesis in the brain [62–66] and have been shown to contribute to elevated BP in rodents [50, 51] (Fig. 2). Moreover, AGT expression is responsive to Ang II in both SHR and Wistar astrocytes in vitro [67]. Its absence induces diabetes insipidus [68], and low glial AGT expression has been shown to play a role in the maintenance of diastolic function and exercise tolerance in rats [69].

There is also evidence for AGT expression in neurons [50, 65, 70–72]. Recently, Agassandian et al. [73] identified a population of neurons in the subfornical organ (SFO) that release AGT and possibly Ang I/II into the cerebral ventricle, when AGT and renin were overexpressed in two different transgenic mouse models.

To demonstrate the differential contribution of neuronal or glial cell AGT in sympathoexcitation, Sakai et al. [71] used a transgenic mouse model that expressed both renin and AGT genes under either neuronal- or glial-specific promoters. The authors showed that Ang II expression in the glial-specific renin/AGT overexpression mice was predominantly observed in the PVN and NTS, whereas in the neuron-specific mice, expression was predominatly observed in the RVLM. They showed that neuronal- and glial-specific Ang II generation exhibited different modulatory effects on arterial baroreflex function, a marker of sympathetic function, possibly due to regional differences in Ang II production.

Renin and Prorenin Receptor

Renin is the predominant rate-limiting step of the RAS cascade and was first identified in the brain in 1971 [74, 75]. However, in 1999, Lee-Kirsch et al. described a new isoform of renin, Renin-b, which is exclusively expressed in the brain. Renin-b evolves from an alternative promoter within exon 1 of the renin gene resulting in the loss of a signal peptide that allows the peptide to be constitutively active and remains intracellular [76]. The existence of intracellular renin is compelling to support the hypothesis that Ang II acts as a neurotransmitter [77].

Renin-b is expressed mainly in neurons [66, 78, 79] and has been identified in cardiovascular regulatory areas including the SFO, paraventricular nucleus (PVN), area postrema (AP), and rostral ventrolateral medulla (RVLM) [80, 81]. Centrally, its expression levels are significantly lower than in the periphery, making it difficult to study in the brain. However, the advancement of genetic tools in recent years has allowed for greater clarity in renin-b localization and function.

The importance of renin-b in the central RAS was indirectly demonstrated by Xu et al. (2011) [82]. These investigators knocked out secreted neuronal- and glial-renin, which had no effect on BP, HR, food, water and sodium intake, renal function, or metabolic rate. These data demonstrate that the secreted version of renin (Renin-a) is dispensable within the brain for cardiovascular, fluid, and metabolic homeostasis. Knockout of renin-b resulted in an increase in BP and sympathetic nerve activity and impaired baroreflex sensitivity as demonstrated by Shinohara et al. [6]. These authors proposed the hypothesis that in the brain, renin-b inhibits renin-a expression under normal conditions. However, in adverse conditions when Renin-b is reduced, renin-a is upregulated and initiates the RAS cascade to induce HTN. This idea is further supported by the observation that in response to deoxycorticosterone acetate (DOCA)–salt treatment to induce HTN, a switch in the expression of renin-b to renin-a has been shown [83].

In a recent controversial study, van Thiel et al. [41] failed to detect any level of renin mRNA (secreted or intracellular) from brain tissue of wild type, DOCA-salt treated, or Ang II infused mice. Moreover, the authors showed an increase in Ang II activity in renin knockout mice, suggesting that Ang II formation might occur by other proteases, such as cathepsin [84, 85]. In addition to the above cardiovascular effects, Shinohara et al. [6] recently demonstrated that Renin-b is also important in the regulation of energy homeostasis and thermogenesis. The authors showed an increase in resting metabolic rate and SNA to brown adipose tissue in renin-b null mice.

Renin-b can bind to a single transmembrane domain of the pro-renin receptor (PRR) [86] resulting in AGT-Ang II cleaving and RAS activation. The PRR is highly expressed in neurons, microgial, and astrocytes in the PVN, SFO, NTS, and area postrema [35, 86, 87]. PRR receptor overexpression is observed in hypertensive animal models [46, 87] and is thought to be regulated either directly by Ang II via an AT1 receptor-dependent pathway [86, 88] or indirectly via COX-2/PGE2 [89].

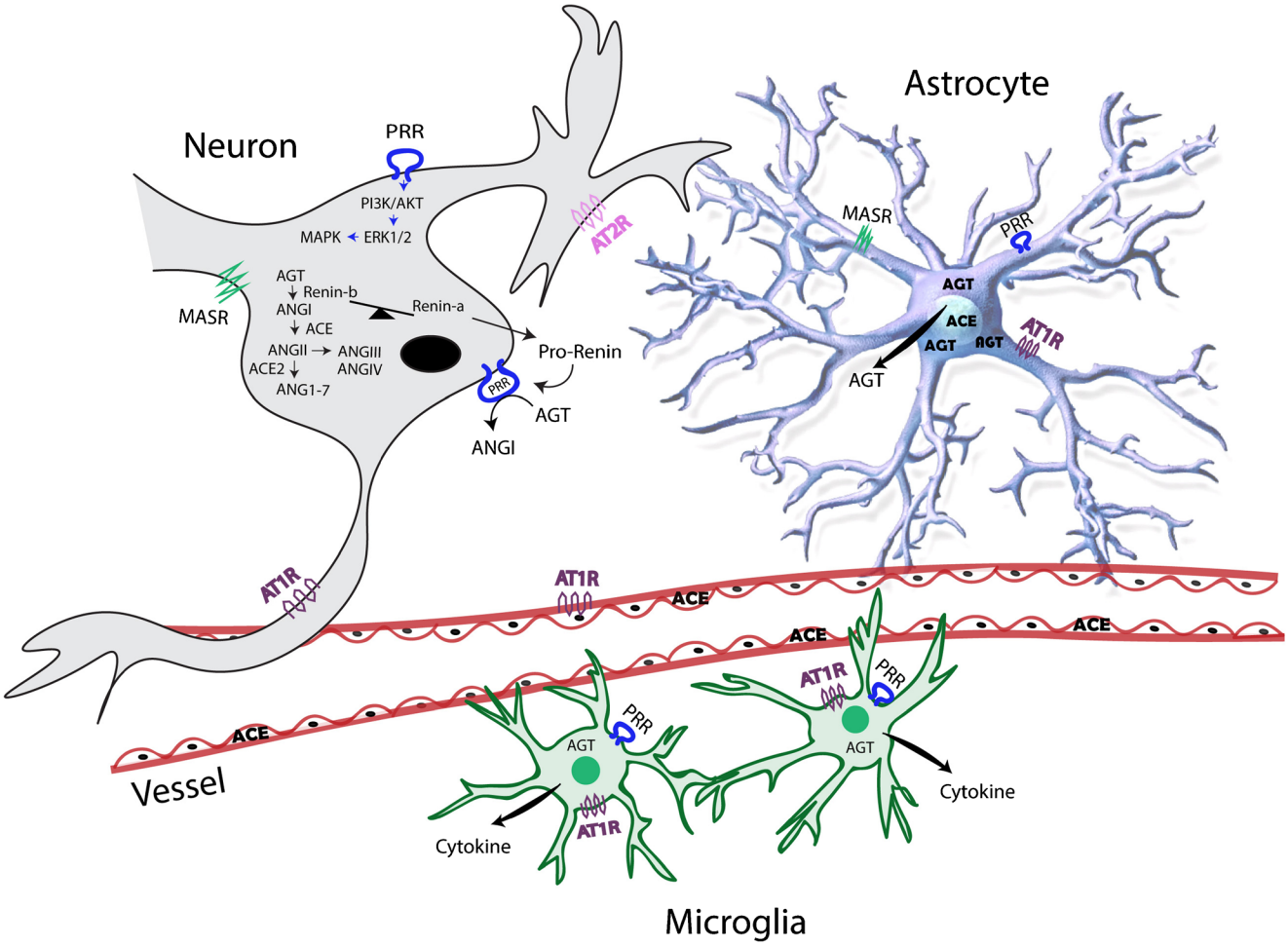

Fig. 2 Brain renin angiotensin system. An overview of the RAS components in central neurons, astrocytes, and microglial cells. ATG: angiotensinogen, Ang I: angiotensin I, Ang II: angiotensin II, Ang III: angiotensin III, Ang IV: Angiotensin IV, ACE: angiotensin converting enzyme, ACE2: angiotensin converting enzyme 2, AT1R: AT1 receptor, AT2R: AT2 receptor, MASR: Mas receptor, PRR: pro-renin receptor

The expression of the PRR in glial cells appears to be related to pro-inflammatory cytokine release and has been shown to contribute to neuroinflammation and the development of neurogenic HTN [90]. Moreover, neuron-specific PRR deletion reduced DOCA salt-induced HTN in knockout mice by reducing sympathetic tone and was associated with a reduction in Ang II levels [88]. These studies suggest that the PRR plays an important role in the central regulation of BP, although the presence of renin in the brain and the mechanisms involved in its expression still need to be clarified.

Angiotensin Receptors

Angiotensin receptors are expressed in multiple brain regions and have been largely reviewed [39, 40, 91]. Recent data showing optogenetic stimulation of AT1a expressing cells in the parvocellular neurosecretory neurons of the PVN promoted a rise in systolic BP, as well as activation of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes [23]. In addition, the presence of the AT2R in neurons at the prefrontal cortex, MnPO, portions of the amygdala, NTS and the AP, suggests a modulation not just of cardiovascular function but also of metabolism and stress responses [23].

Angiotensin-Converting Enzyme

Centrally, ACE is predominantly localized to within cerebral vasculature endothelium. However, lower expression levels have also been identified in neurons and astrocytes of cardiovascular regulatory regions including the choroid plexus, SFO, OVLT, hypothalamus, and basal ganglia [92–94].

Recently, Faulk et al. showed that ACE is increased in the median preoptic nucleus (MnPO) after 1 week of chronic intermittent hypoxia [95]. The MnPO projects to the PVN and modulates BP regulation. In this study, ACE was not localized to astrocytes.

Injection of human ACE into the brain of Sprgue-Dawley rats increased BP, sympathetic nerve activity, and

Ang II levels in cerebral spinal fluid [96]. It has been suggested that increases in central ACE activity or expression occur in hypertension, although the mechanisms behind this are unknown [97, 98].

ACE inhibition increases ACE mRNA expression both within the whole brain and isolated neuronal cultures, suggesting that neuronal ACE can contribute to the central ACE overexpression seen with ACE inhibition [99]. Although the exact role of cell type specific ACE expression within the brain and their role in BP regulation is still unknown, more recently, focus has shifted to examing the role of ACE2 in central BP regulation.

Angiotensin Converting Enzyme 2 and Ang 1–7 in BP Regulation

ACE2 transforms Ang II into Ang 1–7 by cleaving the carboxyterminal phenylalanine residue. Ang 1–7 signals predominantly through Mas receptors, but can also signal through the AT2R. In the peripheral circulation, Ang 1–7 is thought to predominantly regulate BP via nitric oxide (NO)-induced vasodilator effects [100, 101]. Centrally, the role of ACE2 and Ang 1–7 on BP regulation is less well understood. Central ACE2 has been shown to modulate NO synthesis, spontaneous baroreflex sensitivity (sBRS), and parasympathetic tone, suggesting a role for the development of neurogenic hypertension [14, 18, 19, 102, 103]. ACE2 expression and activity have been identified in multiple cardiovascular areas in the brain [47] and are thought to be the predominant enzyme involved in hydrolyzing central Ang II to Ang 1–7 in humans [104]. Central ACE2 downregulation has been observed in a number of animal hypertensive models [102, 105, 106]. On the other hand, neuronal overexpression of ACE2 has been shown to attenuate high BP in a multiple hypertensive models, including Ang II-induced, DOCA salt, and in the SHR [105, 107, 108].

Overexpression of ACE2 in the brain using a mouse model that expresses human ACE2 under a synapsin promoter, synhACE2 (SA), demonstrated that ACE2 overexpression attenuated the development of neurogenic hypertension, in a Ang II infusion model. This was partially mediated by preventing the decrease in sBRS and in parasympathetic tone usually mediated by Ang II in this model. Interstingly, no effect on sympathetic outflow was observed [103]. This protective effect of synhACE2 upregulation is proposed to be partially mediated through upregulation in expression and phosphorylation of nitric oxide synthase (NOS) in key BP regulation centers of the brain, such as the NTS and RVLM. This is consistent with early reports that showed Ang 1–7 increases NO release in the brain [109] and reinforces baroreflex sensitivity [110].

The role of ACE2/Ang 1–7 in central RAS and BP regulation has remained controversial. While predominantly thought of as producing depressor and bradycardic effects [111], Ang 1–7 appears to have a site-specific action within the brain. When injected into the PVN, Ang 1–7 enhances sympathetic outflow and cardiac sympathetic reflexes to a level comparable to that of Ang II infusion [112]. Previous methods of measuring ACE2 activity by determining Ang 1–7 formation from radio labeled Ang II [113, 114], or traditional fluorescent substrate techniques were nonspecific and difficult to quantify [115, 116]. New methods utilize a quenched fluorescent substrate of ACE2. ACE2 hydrolysis of a proline-lysine peptide bond removes the quenching effect, in the presence or absence of the ACE2 inhibitor DX600. This allows for an easy to use measurement of ACE2 activity across a variety of tissue and cell types [115], although DX600 may have issues with ACE2 sensitivity across different species [117].

This technique has recently been used by Xu et al. to determine if the downregulation of central ACE2 observed in HTN is due to increased ACE2 shedding into the cerebrospinal fluid (CSF) [106, 118, 119]. ACE2 is a transmembrane protein with the N-terminus located extracellularly and the C-terminus intracellularly [120]. The cleavage of the extracellular domain of ACE2 could result in the shed form of ACE2 (sACE2) being detectable in CSF. It is unknown if sACE2 has the same enzymatic activity as the membrane anchored form but it is proposed not to [119]. In this study, increased sACE2 was observed in the CSF of hypertensive individuals and positively correlated with systolic BP. This was normalized in individuals on antihypertensive treatments, which resulted in controlled HTN. The authors established that reduced ACE2 activity, measured using fluorescent quenching in the hypothalamus of the DOCA salt mouse, was correlated with an increase in ADAM17 (ADAM metallopeptidase domain 17) activity. ADAM17 is a disintegrin and metalloprotease originally described as a key sheddase in cytokine formation but is known to cleave a variety of membrane-bound proteins [121]. Its activity levels are dependent on the AT1R, not the elevation of BP [119]. Neither elevating BP with norepinephrine infusion nor in a central AT1 receptor knockout in the DOCA mouse model resulted in increased central ADAM17 activity compared to control [119]. Although the correlation between loss of central ACE2, increased sACE2, and increased ADAM17 within this study appears strong, there was no direct link showing that ADAM17 cleaved ACE2. Elevated sACE2 in human heart failure [122, 123], HTN [123], and severe acute respiratory syndrome [124] have been reported. It is possible that the loss of this compensatory arm of the central RAS axis may be contributing to sympatho-excitation, increased BP, and a negative prognostic outcome in these conditions. Preservation of ACE2 enzyme activity may be critical to inhibition of the Ang II-AT1R axis in the CNS.

Interestingly, in a corollary of the hypertensive state, the involvement of central ACE2 has been demonstrated in rabbits with pacing-induced heart failure in the RVLM and PVN [125] where its protein and message were reduced compared

to sham control animals. A follow-up study from the same investigators [126] showed that chronic intracerebroventricular infusion of Ang 1–7 resulted in a reduction in sympathetic nerve activity and enhanced arterial baroreflex function. Xiao et al. [127] clearly showed that heart failure mice that overexpress the human isoform of ACE 2 in the brain exhibit reduced sympathetic outflow as measured by NE excretion and direct recording of RSNA.

These data suggest that in cardiovascular states characterized by increased sympathetic outflow, ACE2 and Ang 1–7 play an important modulatory role. While the cellular mechanisms of the sympatho-inhbition are not completely clear, it is likely that a reduction in oxidative stress plays a role [128].

Sex Differences in Central RAS Regulation

Sex differences in BP regulation and in the progression of HTN have been shown in both clinical and animal models [129–131]. Female sex hormones, especially estrogen, have been shown to have a protective effect against the development of HTN and heart failure by direct modulation of the RAS, in the kidney, heart, vasculature, and CNS [130]. Recent studies have observed that brain expression of Ang 1–7, Mas receptors, and neuronal NOS is controlled by female sex hormones, and this may explain the reduced progression from HTN to other CV diseases in females [132].

Historically, studies on animal models of HTN have been carried out in males due to variances in hormonal cycles in females. This strategy has led to conflicting and inconsistent results in mechanisms related to neurogenic HTN in females. The National Institutes of Health and other major funding bodies have emphasized the need to carry out studies in both sexes in order to better define mechanistic differences between sexes [133]. Both classical estrogen receptor subtypes (ERα and ERβ) are expressed in key BP regulatory regions of the brain [134]. Estrogen replacement therapy is cardio protective in postmenopausal women and has previously been shown to decrease Ang II AT1R receptor binding and mRNA within the hypothalamus of female rats [135]. Several studies have examined the effects of sex differences on central Ang II-mediated neurogenic hypertension [130, 136, 137]. Moreover, a number of previously identified pre-conditioning stressors that sensitize rodents to subsequent Ang II-mediated hypertension [39] are thought now to be sex specific. When male rats are pre-treated with a sub-pressor dose of Ang II, subcutaneous or intracerebroventriular, prior to implantation of an osmotic minipump that will infuse the full dose of Ang II used to elicit Ang II-mediated hypertension, they developed a greater rise in BP than those rats that did not receive the sub-pressor Ang II pretreatment [138]. In this study, pretreatment with a sub-pressor dose Ang II resulted in increased expression of renin, Ang II, AT1R, AT2R, and ACE in the lamina terminalis of the forebrain. Conversely, females showed a slight reduction in BP during the pre-treatment with subpressor Ang II, and a significant but lower rise in BP during the high dose Ang II infusion phase which matched the BP rise seen in animals that had only received saline in the induction phase [136]. Interestingly, ovariectomized (OVX) females responded similar to the males, showing no change in BP during the induction phase, but a significant increase in BP during pressor phase. It was hypothesized that this difference in response between males and females was due to increased Ang 1–7-mediated signaling in the central nervous system (CNS) of females. Intracerebroventricular infusion of the Ang 1–7 antagonist A-779 in female rats during the induction phase prevented the attenuation in BP increase [136]. This suggests that part of the protective role of estrogen may be mediated by the central effects of Ang 1–7. Intracerebroventricular infusion of Ang 1–7 in male and OVX female rats reduced the induction of HTN but did not completely abolish it. If increased Ang 1–7 signaling mediates reduced HTN in females, it would be of interest to see if females have increased central ACE2 levels and a reduction in ACE2 shedding compared to male hypertensive animals.

Estrogen has previously been shown to prevent downregulation of expression of ACE and AT1R mRNA in the kidney, lung, aorta, heart, adrenal, and the PVN [139, 140]. The studies mentioned above imply estrogen modulate the Ang 1–7 pathway, and therefore would be implicated in preventing Ang II-mediated reduction in vagal tone [141]. Previous studies have suggested the antihypertensive effects of estrogen in the CNS to be due to attenuation of sympathetic outflow, although this appears to be region specific [142, 143]. Estrogen injection into the NTS or RVLM of male rats decreased BP and augmented baroreflex control of sympathetic nerve activity, whereas injection into the nucleus ambiguus, augmented baroreflex control of HR and increased vagal nerve activity, resulting in reduced HR with no change in BP [144]. Estrogen attenuation of sympathetic outflow is at least partially due to increase NO signaling. nNOS expression is higher in the SFO and PVN of female rats compared to male, and Ang II infusion further increased nNOS expression in intact females, with no change in expression in OVX females or males [145]. Estrogen has also been implicated in reducing sympathetic outflow by reducing central ROS production. Estrogen injected into the SFO inhibited Ang II induced hypertension, SFO nerve signaling, and intracellular ROS production [146]. Where increasing evidence suggests the importance of estrogen in modulating sex differences observed in neurogenic hypertension, its exact role and regional specificity still remain to be seen.

Summary

The RAS is highly complex, modulating cardiovascular, neuroendocrine, sodium homeostasis, and metabolism. Thought

once to predominantly act systemically through a number of endocrine, paracrine, and intracrine effects, the bulk of the evidence points to the role of a centrally acting RAS pathway which participates in the sympathetic hyperactivity observed in HTN and other cardiovascular disease states. The true role of the central RAS pathway and its regulation in both physiological and pathophysiological states is an active area of investigation. Recent evidence suggests a constitutively active brain-specific isoform of renin, renin-b that may regulate the central RAS axis. All components of the RAS have been located in central cell types including neurons, glia, and cerebral blood vessels. The stimuli (neuronal or humoral) that regulate each component are still unclear. Figure 2 shows the central components of the RAS and the potenitial cell locations of each component based on our current knowledge. Finally, some of the sex differences observed in neurogenic hypertension may be due to differential regulation of Ang 1–7 in females.

Compliance with Ethical Standards

Conflict of Interest Dr. Zucker reports grants from NIH, during the conduct of the study. Drs. De Morais and Shanks declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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