Divergent mechanism regulating fluid intake and metabolism by the brain renin-angiotensin system

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Submitted 12 October 2011; accepted in final form 1 November 2011

Sigmund CD. Divergent mechanism regulating fluid intake and metabolism by the brain renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol 302: R313–R320, 2012. First published November 2, 2011; doi:10.1152/ajpregu.00575.2011.—The purpose of this review is two-fold. First, I will highlight recent advances in our understanding of the mechanisms regulating angiotensin II (ANG II) synthesis in the brain, focusing on evidence that renin is expressed in the brain and is expressed in two forms: a secreted form, which may catalyze extracellular ANG I generation from glial or neuronal angiotensinogen (AGT), and an intracellular form, which may generate intracellular ANG in neurons that may act as a neurotransmitter. Second, I will discuss recent studies that advance the concept that the renin-angiotensin system (RAS) in the brain not only is a potent regulator of blood pressure and fluid intake but may also regulate metabolism. The efferent pathways regulating the blood pressure/dipsogenic effects and the metabolic effects of elevated central RAS activity appear different, with the former being dependent upon the hypothalamic-pituitary-adrenal axis, and the latter being dependent upon an interaction between the brain and the systemic (or adipose) RAS.

* Editorial review of this paper submitted by the Editor-in-Chief, Curt D. Sigmund, was handled by Deputy Editor, Willis K. Samson.

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I had the pleasure and honor at Experimental Biology 2011 to join a distinguished group of 17 scientists who were the past recipients of the Water and Electrolyte Homeostasis Section’s Ernest H. Starling Distinguished Lectureship. None of this could have been possible without the efforts of a talented and dedicated group of nearly 60 present and past undergraduate and graduate students, postdoctoral fellows, research assistants, and research scientists, who have been part of my laboratory for the 20 years since joining the faculty at the University of Iowa. I want to thank a continually growing cadre of collaborators and mentors both in Iowa and elsewhere who have enriched my science over the years; and the editorial team of AJP: Regulatory, Integrative and Comparative Physiology for making my term (ending in June 2013) as Editor an exciting one.

Tissue RAS

The concept that the RAS exists in many forms, a classical endocrine form, and a less well-understood tissue form has been around for over 30 years and continues to gain experimental support (reviewed in Ref. 40). The concept evolved from pharmacological and clinical data, indicating that the inhibitory activity of antihypertensive drugs, such as angiotensin converting enzyme (ACE) inhibitors, correlated better with inhibition of tissue ACE rather than plasma ACE, and that RAS inhibitors are effective antihypertensive agents even in patients with normal or low plasma renin activity, one index of the endocrine (also known as systemic or plasma) RAS (3, 14). Biochemical and molecular biological data, suggesting that components of the RAS were expressed in many tissues, reinforced the tissue RAS hypothesis. It is now clear, that all the components necessary for the production [renin, angiotensinogen (AGT), ACE, and ACE2] and action (AT1, AT2, mas receptors) of angiotensin peptides are expressed de novo and are present in many tissues including the kidney, blood vessels, heart, adipose tissue, adrenal gland, and the brain (40). This review will focus entirely on angiotensin II (ANG II) and the reader is directed elsewhere for a review of the ANG-(1–7) system, ACE2, and the mas receptor (76).

On a personal note, during my first interview for a faculty position at Iowa, the late Michael Brody suggested, that if you believe in the tissue RAS hypothesis, create transgenic mouse models (my model of choice) asking whether expression of ANG II only in tissues results in a change in arterial pressure. Much of my efforts over the past 20 years have been dedicated to that idea and the suggestion made by Dr. Brody during that interview. We have since shown that overexpression of RAS...
components, which generate ANG II only in the kidney, cause increased arterial pressure independent of changes in circulating ANG (8, 11–13, 38). These studies and conclusions have since been replicated and validated by others (24, 34, 56). More recently, Coffman’s laboratory showed that ablation of AT1 receptors, specifically in renal proximal tubule cells, causes decreased arterial pressure, a finding we also independently confirmed (28, 42).

Similar studies using constructs targeting expression of human renin (hREN) and human AGT (hAGT) to the brain, using either their endogenous promoters or glial- and neuron-specific promoters established that ANG II formed from both neuronal and glial sources of AGT can regulate cardiovascular function (9, 10, 48–50). We also published evidence that ANG II generated from glial and neuronal sources may have differential functions (58). Specifically, neuronal ANG reset the baroreflex to a higher pressure but did not alter reflex sensitivity, whereas glial ANG decreased sensitivity of the reflex. Since the ANG II generated in this model was likely extracellular, an alternative hypothesis is that the differences in baroreflex regulation observed in these mice was due to relative differences in regional expression of ANG targeted by the glial-specific (GFAP) or neuronal-specific (synapsin) promoters.

Despite a wealth of evidence from traditional transgenic mouse and rat models, and from advanced gene-targeting techniques supporting the importance of ANG II generation and action in the brain, the mechanisms governing the primary production of angiotensin peptides in the brain remains woefully incomplete (57, 61, 62, 65, among many other references). Largely, this is due to the inherent difficulties in the detection of renin in the brain.

Is Renin Expressed in the Brain?

One of the most controversial issues plaguing the brain RAS concept is whether renin is really expressed in the brain and is the enzyme responsible for the generation of ANG I from AGT. Perhaps the most compelling data supporting a renin-mediated mechanism for ANG peptide generation in the brain is genetic. It is now well established that there is a strict species-specific interaction between renin and AGT, so that transgenic mice and rats expressing hAGT are not expected to generate human ANG I or II (29). Based on this, we have hypothesized that if the sole mechanism for ANG peptide generation is renin dependent, then mice and rats expressing hAGT would not exhibit a phenotype; and indeed this is the case (22, 78). Of course, this assumes that the interaction between other processing enzymes and AGT is not species specific. Interestingly, the identification of another peptide derived from AGT [ANG-(1–12)] suggests there may be an alternative pathway for ANG production in tissues, including the brain (31).

From a biochemical standpoint, expression of the other components of the RAS are relatively abundant and easy to detect (i.e., AGT in glial cells), whereas direct detection of renin remains problematic. We recently determined that the level of renin mRNA in the brain is at least 10 Ct values (>1,000-fold) less than kidney (74). A renin-like activity was discovered in the dog and rat brain as far back as 1971 (18, 21); and the localization of renin was reported in both glial cells and neurons (15, 20, 30). We employed two approaches to address the problem of direct detection. The first was to use a reporter strategy in transgenic mice. As first reported by Ken Gross’s laboratory, mice expressing eGFP from the renin promoter exhibit the correct pattern of cell-specific expression in the kidney during development and in adults (33). We used the same model to examine the localization of eGFP as a surrogate reporter for renin expressing cells in the brain (37). This analysis revealed that the renin promoter was active in neurons of the cerebellum, hippocampus, dorsal motor nucleus of the vagus, inferior olivary nucleus, reticular formation, rostral ventrolateral medulla (RVLM), central nucleus of the amygdala, lateral parabrachial nucleus, mesencephalic trigeminal nucleus, bed nucleus of stria terminalis, and subfornical organ (SFO); and to a lesser degree in paraventricular nucleus (PVN), supraoptic nucleus (SON), arcuate nucleus, nucleus of the solitary tract (NTS), and other regions controlling cardiovascular function. In a follow-up study comparing renin expression (using the Ren-eGFP mice) with AGT expression [using AGT-LacZ mice, (77)] we found that cells expressing both genes were coexpressed in a number of regions of the brain, including the parabrachial nucleus and central nucleus of the amygdala, and were expressed in adjacent cells in the rostral ventrolateral medulla and the SFO (36). In another model where we crossed mice carrying a knock-in allele of cre recombinase (Ren-cre), graciously provided by R. Ariel Gomez with ROSA reporter mice, we observed β-gal staining (indicative of renin promoter activity) in the pons, medulla, cerebellum, and occasional cells of the hypothalamus (74).

The second approach to explore the mechanism of renin gene expression in the brain was to employ a novel transgenic model where we inserted a 160-kb region surrounding the hREN locus including the entirety of the renin gene, the upstream GOLT1A and KISS1 genes, and the downstream ETNK2 gene (63) (Fig. 1A). The rationale was simple: the larger the DNA fragment and the associated 5’ and 3’ flanking DNA, the better the chance that all regulatory elements needed to regulate the gene would be present. With respect to renin, this construct had ~75 kb of both 5’ and 3’ flanking sequences. We showed that expression of renin from this transgene, known affectionately as PAC160, was restricted to juxtагlomerular cells in the kidney and responded to the physiological cues that are known to regulate expression of the renin gene, such as high salt, ACE inhibition, and ANG II (63). At the tissue level, abundant expression was observed in the kidney and placenta, with much lower levels of expression elsewhere, including the brain. Anecdotally, we assayed for renin mRNA by RNase protection assay, and noted, what we thought then was an aberrantly shorter RNase protection assay product in the brain (64). We determined that this aberration was in fact a novel transcription start site that was only active in the brain, something we would have never detected if we had used quantitative PCR.

The relevance of this brain-specific alternative transcript became clear when it was also found by another laboratory and in multiple species (41). The transcript employs a novel promoter to initiate transcription at an alternative first exon termed exon-1b, which lacks the normal translation initiation codon found in exon-1a (Fig. 1B). The result is translation from an evolutionarily conserved ATG in exon-2, which results in a product lacking a signal sequence and the first third of the
prosegment, thus predicting the production of an active prorenin (41). Interestingly, brain-specific expression of this intracellular renin causes increased arterial pressure, at least when expressed in glial cells (39). Thus, in the brain, there are two potential forms of renin, a secreted form of renin derived from the classic precursor preprorenin, and an intracellular form derived from the novel transcript (Fig. 2). It is interesting to note that there is a developmental shift in expression whereby secreted renin predominates during fetal development and intracellular renin is the predominant form in adults (75).

We next sought to develop mouse models that could determine whether there is a physiological function for intracellular renin and to distinguish it from secreted renin (Fig. 3). This is facilitated by the unique structural organization of the renin gene, which provided an opportunity to alternatively “flox” (flank by loxP sites) the promoters and first exons encoding secreted and intracellular renin. We generated separate null and floxed alleles that could ablate or conditionally ablate secreted renin several years ago, whereas the floxed and null alleles that would allow us to do the same for intracellular renin were just recently completed and are in breeding. Complete deletion of secreted renin, even with preservation of intracellular renin, results in increased lethality, hypotension, and a decreased ability to concentrate urine (75). This is not particularly surprising as loss of secreted renin causes a complete loss of renin systemically (in kidney and plasma) and phenocopies a complete renin null (71). Brain-specific deletion of secreted renin, which is very low in adults, did not alter arterial pressure, although recent preliminary data suggests it may be required under some pathological conditions (74).

Fig. 1. Renin gene structure. A: schematic representation of PAC160 including the human renin (hREN) gene (blue), the kidney enhancer (KE; red), chorionic enhancer (CE; green), and neighboring genes (black arrows). Arrows denote the relative direction of transcription. [Adapted from Zhou et al. (81)]. B: schematic of the hREN gene showing the position of exon 1a (blue) and exon 1b (green) and their respective ATG initiating codons. C: evolutionary conservation of intracellular renin (icREN). The position of the signal peptide cleavage (S), referenced to the hREN sequence, and the icREN ATG initiation codon (I) are indicated by arrows. Details of the alignment was previously reported (39). [Adapted from Lavoie et al. (39)].

Fig. 2. Synthesis of icREN. The hypothesized biosynthetic pathway for icREN is shown. Classically, preprorenin is the primary translation product of the renin-a mRNA transcribed from the classical renin promoter and including exon 1a. Preprorenin is processed (yellow arrows) first by removal of the signal peptide and then by removal of the prosegment. Active renin is subsequently released into the systemic circulation. In the brain, a different renin mRNA, termed renin-b, is transcribed from an unknown promoter within intron 1 (in mice) or 6.2 kb upstream of the classical renin promoter (in humans) to result in a novel transcript lacking exon 1a and including exon 1b. There are no ATG sequences in exon 1b in rat, mouse, or human, and thus translation begins at the highly conserved ATG present in exon 2. This product encodes the entire active renin protein and two-thirds of the prosegment. It is unclear whether the prosegment is removed. Since the protein lacks a signal peptide, it is unlikely to be secreted. Additional studies are needed to determine whether this protein is stable, whether it remains intracellularly, and within which intracellular structures it resides. [Legend and figure reprinted from Grobe JL, et al. (27)].
Why would there be a need for intracellular renin? Overlooking any potential intracellular signaling mediated by the interaction of prorenin with the (pro)renin receptor intracellularly, one must presume that the main reason for a nonsecreted form of renin would be to generate angiotensin peptides intracellularly. Neurons, at least those in many cardiovascular control regions such as the SFO, PVN, NTS, and RVLM express angiotensinogen, the substrate of angiotensin, and the NTS (45). Nerve fibers positive for ang II immunoreactivity were reported in SFO, SON, PVN, median preoptic nucleus (MnPO), and posterior pituitary (19, 43, 44). ANG II has also been reported to be present in neuronal secretory vesicles, which perhaps are synaptic vesicles (53). If ANG II is to be considered a neurotransmitter it must 1) be synthesized in a presynaptic neuron, 2) released across the synapse, 3) have receptors in the synaptic cleft of the postsynaptic neuron, 4) cause activation of the postsynaptic neuron, and 5) be taken up or degraded (6). In an elegant review, Ferguson et al. (17) describe evidence suggesting that ANG II is a neurotransmitter. The question therefore is whether intracellular renin provides the missing link needed for the intraneuronal synthesis of ANG II. We eagerly await the mice carrying conditional and null alleles of the gene encoding intracellular renin to answer this fascinating question.

Physiological Significance of ANG Generation and Action in the Brain

One of our long-term goals is to understand where in the brain ANG peptides are generated, where they act, and how they influence ANG-dependent cardiovascular outputs. To accomplish this, we generated a series of transgenic mouse models overexpressing components of the human RAS in the brain (Fig. 4). We employed a multifaceted strategy to accomplish this. First, employing a construct expressing hAGT controlled by its endogenous promoter allowed us to retain the normal cellular specificity of AGT synthesis to ensure that ANG can only be derived from sites where the substrate is normally made. In brain, hAGT is widely expressed in glial cells, but also in neurons, particularly in those regions of the brain controlling cardiovascular function. This transgene comes in two “flavors”: A and A\textsuperscript{lox}. The A transgene is the wild-type hAGT construct, whereas the A\textsuperscript{lox} has been genetically manipulated so that it can be conditionally ablated in the presence of Cre-recombinase. We next employed a second transgene expressing hREN from the neuron-specific synapsin promoter (the sR mouse). This was based on the rationale that renin, although difficult to detect, is mainly expressed in neurons as we showed using reporter mice (36, 37). Cross breeding the mice produces the sRA and sRA\textsuperscript{lox} models, which both overexpress ANG selectively in the brain, with the latter allowing conditional ablation in response to cell-specific or region-specific administration of Cre-recombinase. The most recent iteration includes breeding sRA mice with AT1\textsuperscript{aR}\textsuperscript{lox} mice, providing a new model (sRA X AT1\textsuperscript{aR}\textsuperscript{lox}) to conditionally ablate ANG II AT1R in a cell-specific or region-specific manner in a model where ANG II is overexpressed. In the aggregate, these models provide powerful genetic tools to dissect the physiological relevance of ANG generation and ANG action.

We reported that sRA and sRA\textsuperscript{lox} mice are hypertensive and exhibit robust dipsogenic behavior, drinking as much as their body weight in a 24-h period (26, 57). The drinking response was AT1-dependent as it was blocked by intracerebroventricular losartan. Immunohistochemical staining revealed ANG-like immunoreactivity in the SFO, and ANG peptides were increased
in the anteroventral third ventricle (AV3V) and hypothalamus. Using an adenovirus encoding Cre-recombinase reported by us (68), and a method pioneered by the Davisson laboratory to site-specifically ablate any floxed gene in cardiovascular nuclei (65–67), we targeted ablation of ANG II overexpression by direct microinjection into the SFO (57). This resulted in a decrease in hAGT expression in the SFO, a loss of ANG-like immunoreactivity, and a significant decrease in water intake 2 days after injection that lasted through the remainder of the experiment (8 days). These results coupled with a similar study by Sinnayah et al. (65) clearly demonstrated that de novo synthesis of AGT and ANG II in the SFO is an important determinant of drinking behavior and arterial pressure regulation.

**A Novel Role for the Brain RAS in Metabolism**

The RAS has a well-recognized role in fluid and electrolyte homeostasis and blood pressure control, but its importance as a potent regulator of energy intake and expenditure is only now becoming appreciated. The level of RAS gene expression, and levels of plasma AGT and ACE, positively correlate with the severity of obesity in humans and animal models (2, 7, 16, 23, 46). Global knockout of, or pharmacological interference with renin, AGT, ACE, and AT1R, all result in lower body mass, altered body composition, and/or abnormal adipose development (4, 32, 35, 47, 69, 70, 82). Genetic knockout of AT2R and Mas receptors have opposing effects, as they do with cardiovascular end points (60, 79, 80). Contrary to these findings with genetic ablation or pharmacologic inhibition, peripheral or central infusion of ANG II results in reduced body mass either through a reduction in food intake or an elevation in metabolic rate (5, 54, 55).

Given these data, it was unclear what to expect from a model of brain RAS hyperactivity. Whatever the expectation, perhaps the most interesting observation was that the sRA and sRA\textsuperscript{lox} mice are exceptionally lean (26) (Fig. 5). They exhibit a 20% reduction in body mass and a marked reduction in subcutaneous and visceral adipose tissue. Their food intake is decreased by only a few percent, but when normalized to body mass is increased, suggesting that a change in energy intake cannot explain the decrease in body mass. Consequently, we focused our analysis on energy output, which revealed increases in both oxygen consumption at thermoneutrality and body tempera-

![Fig. 5. sRA mice are lean. An MRI scan revealed that sRA mice are exceptionally lean with reductions in both subcutaneous and visceral adiposity. [Reprinted from Grobe JL et al. (26)].](#)
ture. Sympathetic nerve activity (SNA) to both the interscapular brown (BAT) and perigenital white adipose tissue was markedly increased. The increase was so robust, there was no further increase in SNA to BAT in response to cold, whereas in control mice there was a stepwise increase in BAT SNA with graded exposure to cold. Increased sensitivity of oxygen consumption in sRA mice to propranolol evidenced the importance of the sympathetic nervous system.

Urinary aldosterone and corticosterone levels were exceptionally high in sRA mice, suggesting one or both of these pathways may play mechanistic roles in the cardinal phenotypes (hypertension, increased water and salt intake, and increased metabolic rate) observed in these mice. Interestingly, whereas adenectomy completely normalized the increased drinking and sodium input, it had no effect on metabolic rate, suggesting a dissociation of the efferent pathways controlling drinking and metabolism.

Studies performed in collaboration with Lisa Cassis (26) also revealed that plasma ANG II levels were reduced to 30% of normal; and renal renin mRNA was reduced by nearly 90%. This data was particularly important as it provided a possible answer to the quandary of why our brain-specific overexpression model exhibited the same lean phenotype as the RAS knockout models. Perhaps, decreased systemic (or adipose) RAS activity was a key. To test this, we infused sRA mice with a subpressor dose of ANG II for 8 wk and observed a decrease in oxygen consumption back to baseline levels. Similarly, many of the metabolic changes observed in renin null mice were reversed by ANG II infusion (70).

It is notable that like sRA mice, DOCA-salt treated mice exhibit increased brain RAS and decreased plasma RAS and also have elevated oxygen consumption compared with their untreated controls (25). The increase in metabolic rate is not caused by hypertension per se because it remained elevated after arterial pressure was decreased with hydralazine. However, the response is ANG II AT1R-dependent as it was blunted by intracerebroventricular losartan. Thus two factors appear essential to mediate the thermogenic responses observed in sRA (and perhaps DOCA-salt) mice (Fig. 6). First, increased central SNA to BAT and white adipose tissue caused by central AT1R activation increases heat production and oxygen consumption, a response inhibited by β-adrenergic blockade. Second, the thermogenic response is facilitated (and even requires) a decrease in circulating RAS activity (and perhaps adipose ANG), likely caused by hypertension-induced feedback inhibition of renal renin expression and release, because it can be efficiently blunted by restoration of peripheral ANG II. We are presently exploring the mechanisms by which this occurs. Thus, our data unifies the RAS gene ablation, pharmacological inhibition, and infusion studies to advance the concept that the brain RAS promotes increased energy expenditure when accompanied by decreased peripheral RAS activity and establishes a novel brain-systemic RAS connection.

ACKNOWLEDGEMENTS

The studies discussed in this review were performed by many students, postdoctoral fellows, and research scientists, with the assistance of a group of talented research assistants in my laboratory and the transgenic animal facility. There are too many to be named here. They, in particular the former trainees, more than anything else represent my scientific legacy. I hope to have the opportunity to continue training young scientists for years to come. I wish to point out the extraordinary efforts of Di Xu, Ph.D. who made the mice carrying null and floxed alleles of secreted renin. She struggled for years to advance the generation of the null and floxed alleles of intracellular renin, which, finally, are now available for our studies. I also want to acknowledge the extraordinary talents of Justin L. Grobe, Ph.D. who performed the studies linking the central RAS to metabolism and who is presently investigating the mechanisms by which this occurs.

GRANTS

The work presented in this review was funded by the National Institutes of Health and generously supported by the Roy J. Carver Trust.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

C.D.S. conception and design of research; C.D.S. prepared figures; C.D.S. drafted manuscript; C.D.S. edited and revised manuscript; C.D.S. approved final version of manuscript.

REFERENCES


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**Review**

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