

Brain renin angiotensin in disease

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Abstract A brain renin angiotensin system (RAS) and its role in cardiovascular control and fluid homeostasis was at first controversial. This was because a circulating kidney-derived renin angiotensin system was so similar and well established. But, the pursuit of brain RAS has proven to be correct. In the course of accepting brain RAS, high standards of proof attracted state of the art techniques in all the new developments of biology. Consequently, brain RAS is a robust concept that has enlightened neuroscience as well as cardiovascular physiology and is a model neuropeptide system. Molecular biology confirmed the components of brain RAS and their location in the brain. Transgenic mice and rats bearing renin and extra copies of angiotensinogen genes revealed the importance of brain RAS. Cre-lox delivery in vectors has enabled pinpoint gene deletion of brain RAS in discrete brain nuclei. The new concept of brain RAS includes ACE-2, Ang1–7, and prorenin and Mas receptors. Angiotensin II (ANG II) generated in the brain by brain renin has many neural effects. It activates behavioral effects by selective activation of ANG II receptor subtypes in different locations. It regulates sympathetic activity and baroreflexes and contributes to neurogenic hypertension. New findings implicate brain RAS in a much wider range of neural effects. We

review brain RAS involvement in Alzheimer's disease, stroke memory, and learning alcoholism stress depression. There is growing evidence to consider developing treatment strategies for a variety of neurological disease states based on brain RAS.

Keywords Brain renin angiotensin · Hypertension · Stroke · Stress · Alzheimer's · Alcoholism · Depression · Stress · Hippocampus

Introduction—history

Brain renin and its effects have been established by a wide range of studies and techniques over the past 37 years. It is now not only accepted as a model neuropeptide system but it also has clinical implications for a variety of brain related disorders and their treatments.

Renin has a long and fascinating history. What began with its discovery in 1898 by Tiegerstedt and Bergman, who named it a kidney hormone [1], was forgotten for 40 years. However, by 1971, renin was recognized as an essential enzyme of the kidney renin angiotensin (AGT) aldosterone system. Its biochemistry, components, their location, and its function had been established. So when Detlev Ganten [2] proposed that there was renin in the brain independent of kidney renin, it required high standards of proof. Each time a new, more precise tool has become available, the role of brain renin angiotensin system (RAS) has been revisited. Early data was based on radioimmunoassay and enzyme assays [2, 3]. About that time, angiotensin II (ANG II) had become very interesting, physiologically and behaviorally [4]. When ANG II is injected into the brain, it elicits a pressor response and, if water is available, a drinking response. The behavioral act

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of drinking obviously requires neural circuits in the brain. It was reasoned that the effects of ANG II in the brain were isolated from circulating RAS by the blood-brain barrier (BBB). However, circumventricular organs (CVOs) lack a BBB and the circulating RAS and the endogenous brain RAS appear to have a complex interface in the CVOs [5, 6].

Brain studies

To prove a brain RAS existed, neurophysiological techniques were required. Felix and Phillips [7] showed that ANG II activated neurons in the brain and in the subformal organ (SFO), and a peptide antagonist of ANG II, saralasin, inhibited ANG II-induced activity in those neurons. This suggested that ANG II is a nonclassical neurotransmitter. Taking the concept a step further, it was reasoned that if ANG II, formed by brain renin, was involved in hypertension, then inhibiting brain RAS would lower blood pressure. Simply injecting saralasin alone into the brain of stroke prone hypertensive rats (spSHR) temporarily reduced high blood pressure [8]. This occurred even in the absence of kidney renin when the spSHR rats were bilaterally nephrectomized [8]. The finding was later confirmed by molecular inhibition of ANG II synthesis in the brain [9]. It meant that brain RAS plays a role in neurogenic hypertension.

Immunocytochemistry with renin antibodies showed staining in neurons [10, 11]. Renin-like activity was evident in the hypothalamus, pituitary, and pineal glands. Antibodies to ANG II revealed a clear distribution of ANG II-like staining in the hypothalamus and CVOs and in higher concentration in SHR [12]. What was needed were actual measurements of the renin and ANG II proteins. This was achieved with high-performance liquid chromatography [13, 14] and direct protein assay [15]. Brain levels of ANG II were higher than circulating levels of ANG II, suggesting independence of the two systems.

Having shown that ANG II was in the brain, the next level of proof required demonstrating that it had been synthesized in the brain. Molecular biology was used to measure and locate mRNA for AGT and renin in the brain [16, 17]. Stornetta et al. [18] identified glial cells as the site of synthesis of AGT using *in situ* hybridization mRNA.

Receptors

Mendelsohn et al. [19], using autoradiography with pseudocolor imaging, were able to map the entire distribution ANG II receptor binding sites in the brain. This proved that

there were sites within the brain that had evolved to be activated by ANG II made in the brain. Minute injections of ANG II into key brain nuclei showed that ANG II could differentially produce pressor effects, elicit drinking, release vasopressin, inhibit the baroreflex, and increase sympathetic nervous system activity [20, 21]. The confirmation that there was a separate brain RAS, independent of the circulating RAS, gave rise to the more generalized concept of local or tissue angiotensins in most other tissues including heart, blood vessels, and, ironically, the kidney [21]. Whitebread et al. [22] and Chiu et al. [23] reported that there were two subtypes of the ANG II receptor, AT₁ and AT₂. From this has sprung not only insight into genes involved in brain function but also the highly successful angiotensin receptor blockade class of antihypertensive drugs. The development of the transgenic mice [24] was relevant to brain RAS with transgenic mice overexpressing AGT by a metallothionein promoter [25]. Transgenic mice, overexpressing AGT in brain and liver, led to high blood pressure [26, 27]. But, what about renin? Renin exists in only one form in humans, Ren-1, but in two forms in mice, Ren-1 and Ren-2. After inserting a mouse *Ren-2* gene into a transgenic rat [TGR(mREN-2)27], Mullins et al. [28] reported fulminant hypertension in transgenic rats, even though circulating ANG II levels were low. The homogeneous offspring developed malignant hypertension and had high mortality. The heterozygous offspring had end organ damage in the heart and kidney with extreme hypertension. Hypertension disappeared when the brains of these mice was anesthetized. Curt Sigmund's group [29] developed transgenic mice that overexpressed human renin and human AGT. With either a glial fibrillary acidic protein promoter or a synapsin-1 promoter, to stimulate gene expression in neurons or glial cells, hypertension and increased drinking resulted.

Gene deletion and inhibition

Transgenic animals must undergo embryonic development. If the inserted gene or the deleted gene of the transgenic animal is not embryonically lethal, it may be compensated for by other genes duplicating its role. This may account for the surprisingly benign effects of AT₂ knockout transgenic mouse [30, 31]. To overcome this possibility, we developed gene suppression methods in adult animals using *in vivo* RNA-based inhibition. Antisense inhibition of AGT mRNA or AT1R mRNA oligonucleotides delivered to the brain reduces hypertension in SHR [32]. Antisense inhibition of AT1R specifically diminishes AT1 receptors in the paraventricular nucleus and Organum vasculosum of the lamina terminalis [33]. Functionally, AT1 antisense inhibited the effects of ANG II in the brain including the pressor

response, vasopressin release, and drinking [34]. In the double transgenic mice developed by Sigmund and colleagues [35], to overexpress both human renin and human AGT so that they constantly have high ANG II levels, antisense to AT1R mRNA dramatically reduced the blood pressure.[Phillips et al., unpublished].

In an elegant study, Davisson et al. [36], using a combination of AT_{1A}- and AT_{1B}-deficient mice, showed that AT_{1A} receptors are mainly involved in central blood pressure (BP) control, while AT_{1B} receptors mediate the drinking response.

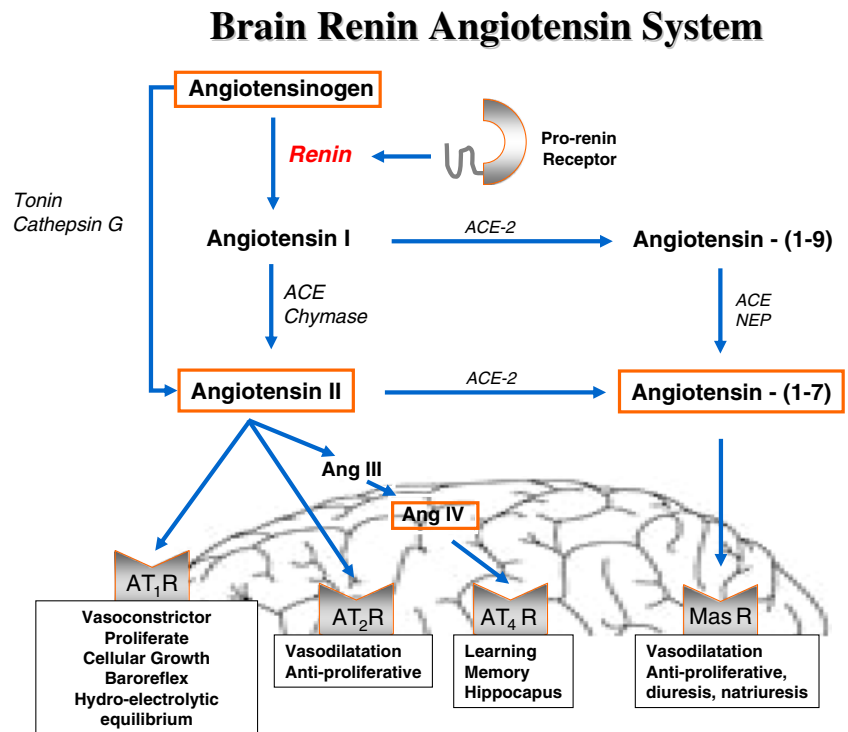
The question of which genes and exactly where in the brain cardiovascular effects are regulated may now be answered with greater precision. Davisson and colleagues, who previously demonstrated uptake of vectors into specific location of the brain, reported an approach to gene deletion in mice with the Cre-lox system [37]. Cre, a bacteriophage P1-derived DNA recombinase, serves to recombine precisely defined DNA sequences that have been “floxed” by loxP sequences prior to embryonic development. Because Cre recombines the loxP sequences, the floxed segment is deleted and rendered nonfunctional. Sinnayah et al. [37] microinjected Cre with a promoter into selective targets, such as the SFO and supraoptic nucleus, delivered by adenovirus vectors or feline immunodeficiency virus. With this technique, the Sigmund group have demonstrated the presence of brain RAS and its physiological and blood pressure effects [38, 39].

The new RAS

The classical brain RAS components must now include ANG III, ANG IV, ANG (1–7), ACE2, prorenin, and Mas receptors (Fig. 1). ANG III is a metabolite of ANG II found in brain and cerebrospinal fluid. It appears to act on the AT1 receptor. ANG IV is an endogenous agonist that has been identified in the brain in areas distinct from those where AT1 and AT2 are located and probably acts on an AT4 receptor. An action of brain RAS is implied in memory and learning by the finding that stimulation of AT4 receptors potentiates the release of acetylcholine from hippocampal brain slices [40]. ACE 2 generates ANG (1–9) from Ang I [41, 42]; ACE 2 is a human ACE homolog that differs greatly from ACE in substrate specificity and its activity is not modified by ACE inhibitors [41]. ACE 2 is widely expressed in a variety of tissues including brain [41–44].

Mice lacking the *ACE 2* gene showed elevation in plasma ANG II level [45] and enhanced pressor response to ANG I. ANG (1–7) is formed from ANG II via the action of several tissue-specific endopeptidases or from ANG I via ACE 2. ANG (1–7) can be metabolized by ACE to ANG (1–5); it is a peptide with no known biological activity. ANG (1–7) produces its biological effects such as vasodilatation, diuresis, natriuresis, and antitrophic via the Mas receptor [46]. Mas receptor stimulation also induces to production of nitric oxide (NO) and prostacyclin. This new

Fig. 1 Brain Renin Angiotensin System: the schematic figure summarizes the formation of components of the brain RAS; angiotensin II (1–8) [ANG II] ad its metabolites ANG III, ANG IV and ANG (1–7). Although the main enzymatic pathways are mediated by renin and angiotensin converting enzyme ACE or ACE2, there are other potential pathways, but they have not been shown to be physiologically active. The major neural effects of angiotensins are mediated by AT1, AT2, AT4, prorenin, and Mas receptors



view of the RAS suggests that overexpression of ACE-2 and ANG (1–7) receptors are potential targets for therapies. Another new concept has been proposed by J. Paton that baroreflexes can be controlled by systemic ANG II through activation of AT1 receptors on endothelial cells in capillaries close to the nucleus tractus solitarius (NTS). The AT1 stimulation releases NO, which passes across blood vessels into NTS neurons and triggers the reflexive decrease in heart rate [47, 48]. This points to a complex interface between the brain RAS and the systemic RAS in controlling vital cardiovascular functions.

Prorenin and renin

Prorenin receptors were first cloned by Nguyen et al. [48]. They bind prorenin with higher affinity than renin [49]. Tissue prorenin [50, 51] is active and renin per se has cellular effects independent of ANG II generation [48, 52]. Prorenin receptors may help to explain the presence of tissues RAS, where the generation of ANG II is independent of systemic RAS.

There are two forms of renin expressed in the brain of rodents and humans: secreted prorenin and nonsecreted renin. This suggests that renin is constitutively active [53]. Using transgenic mice that express nonsecreted active renin or secreted prorenin in the brain, BP and drinking volume were increase similarly in both models. BP was normalized by an intracerebral ventricular injection of losartan in both models, whereas the same dose given systemically had no effect. These data support the concept of an intracellular form of renin in the brain, which may promote functional changes in fluid homeostasis and BP regulation [53].

ACE 2, ANG (1–7), and mas receptors

ACE 2 is a carboxypeptidase that counterregulates the vasoconstrictors effects of ANG II to degrade it to the vasodilator peptide ANG (1–7) [54]. The first functional evidence of the ANG (1–7) in the brain showed that the peptide stimulates the release of vasopressin, equivalent to that obtained with ANG II [55]. In the central nervous system, ANG (1–7) acts as neuromodulator, especially in areas related to tonic and reflex control of arterial pressure. Its effects are blocked by a Mas receptor antagonist A-779 [56]. Becker et al. [57] showed Mas receptor in cardiovascular and hydroelectrolytic control areas of the rat brain. ANG-(1–7) appears to act through the Mas receptor.

The distribution of ACE 2 is widespread in the mouse brain, predominantly in neurons. ACE 2 mRNA is found in rat medulla oblongata, and ACE 2 overlaps with compo-

nents of the RAS in the same areas [43, 58]. ACE-2, ANG (1–7), and Mas receptors are all localized in brain areas related to the control of cardiovascular function.

Brain RAS effects in neurological disease

The physiological effects of central ANG II are mainly mediated by AT1R, including vasoconstriction effects, vasopressin release, retention of salt and water, cell growth, and stimulation of aldosterone from the adrenal gland [21]. However, via AT2, the ANG II mediates a wide variety of actions, including vasodilatation, inhibition of cellular growth, cell differentiation, and apoptosis. In addition to the brain RAS role in neurogenic hypertension discussed above, evidence is accumulating that brain RAS is involved in Alzheimer's disease, stroke, memory and learning, alcoholism, depression and, emotional stress.

Alzheimer's disease

Alzheimer's disease (AD) is an age-related, neurodegenerative disease with a prevalence of 5–10% in individuals over 65 years [59]. The dementia presents as primary indicator with memory loss following progressive cognitive impairment and personality changes [69]. Two hypotheses have been proposed to explain the pathogenesis of AD [60]. The amyloid plaque (AP) hypothesis postulates that AP is generated by proteolytic activity of the β - and γ -secretase processing the amyloid precursor protein (APP). However, although AP was observed in transgenic mice with mutations of *APP* and presenilin-1 (*PSI*) genes, the mice did not exhibit neurodegeneration or memory loss observed in AD patients. Supporting the presenilin hypothesis, it postulates the neurodegenerative and behavioral effects independent of AP. An increase in the ACE activity and alteration in others components of brain RAS has been demonstrated in AD [61–64]. The beneficial effects of ACE inhibitors and AT1 blockers have been shown in animals and humans suggesting reducing brain RAS activity is important. There are many effects of decreased ANG II activity including reduced blood pressure, less acetylcholine release, and increase of substance P—a substrate of ACE which is reported to increase neprilysin activity, a recognized amyloid β degrading enzyme [59].

Stroke

Treatment with ACE inhibitors and AT1 antagonists has been shown to prevent stroke in spSHR and in salt-loaded Dahl salt-sensitive rats [65]. Some clinical trails (SYST-EUR, Systolic Hypertension in Europe; PROGRESS,

Perindopril Protection Against Recurrent Stroke) and randomized studies using antihypertensive treatments have been performed using different combinations of ACE inhibitors: AT1 receptor antagonists, β -blockers, and diuretics treatments [61–64]. AT1 receptor antagonists normalized the expression of cerebrovascular nitric oxide synthase, cerebrovascular compliance, and protected cerebrovascular flow [66]. Similarly, cerebral blood flow was increased by ANG (1–7) and reduced by a selective ANG (1–7) antagonist A-779 [67]. These data suggest that ANG (1–7) could have protective effects in ischemia. In general, the results of treatment show a reduction of BP, stroke, vascular dementia, and cognitive decline [64]. Zhou et al. [68] showed evidence of an active local RAS in brain microvessels influenced by circulating prorenin and ANG II with a predominant localization to the microvessel endothelium. Kagiya et al. [69] induced stroke with middle cerebral artery occlusion in rats and found that local ANGII levels and AT2 receptor concentrations increased. They concluded that brain RAS and AT2 receptors increase apoptosis in cerebral ischemia.

Memory and learning

Several studies have indicated brain RAS in memory function. AT1 antagonist treatment reduced anxiety and improve learning, spatial working memory, and motor performance in the aged rat [70–73]. DeNobel et al. [70] reported that intracerebroventricular infusion of renin disrupts passive avoidance learning. Coadministration of ACE inhibitor (captopril) or AT1 antagonist attenuates this deficit induced by renin. AT2 receptor antagonist was not effective. ACE inhibitors have been shown to enhance memory learned by fear or habituation [71]. Recently, Bonini et al. [72] showed that the intrahippocampal infusion of ANG II in rats induces amnesia. The effect was completely reversible and did not alter locomotion activity, exploratory behavior, or anxiety state. This effect of ANG II was blocked by the AT2 antagonist but not by losartan.

Long-term potentiation (LTP) is a correlation of learning and memory processes. ANG IV and ANG (1–7) increase the excitability and LTP of neurons of the hippocampus [73]. Furthermore, Mas receptor knockout mice do not show alteration of hippocampal LTP [73]. A picture emerges of inhibitory influence by ANG II acting at the AT1 receptor, and a facilitatory role by ANG IV acting at the AT4 to increase excitability, LTP, associative and spatial learning, and memory. Although, the ANG II participation in memory consolidation is controversial, results suggest that ANG IV and ANG 1–7 via Mas receptor in the brain are involved in cognitive behavior.

Alcohol intake

ANG II induces drinking, but does it induce alcohol drinking? Losartan, the AT1 receptor blocker, has been reported to block toxic effects of low doses of ethanol [74]. Maul et al. [75] showed that in AT1 knockout mice, ANG II affected alcohol consumption, but AT2 receptor and bradykinin receptors are not involved [75]. Furthermore, ethanol-induced inhibition of hippocampal LTP is mediated by AT1 receptors [76]. Recently, Sommer et al. [77], using rats treated with spirapril—a blood-brain-penetrating inhibitor of ACE—or transgenic rats [TGR(ASrAOGEN)680] with reduced central AGT expression, showed that increased ethanol consumption was associated with an upregulation of AGT transcript levels and a downregulation of local AT1 mRNA. Taken together, these data suggest that reducing brain RAS could be a strategy for treating alcoholism.

Depressive illness

The etiology of depression involves several neurotransmitter systems. The first evidence that brain RAS may be important in depression was observed in hypertensive patients undergoing captopril treatment. Depressed patients in the group noted reduced depression [78]. In this context, RAS polymorphisms have been investigated for their association with depression, including the AGT T allele, ACE D allele, and AT1R C allele, which are associated with markedly higher plasma AGT and ACE activity and with greater responsiveness to ANG II at lower concentrations. The ACE D allele was more prevalent in depressed patients of Japanese population [79]. AGT M allele was associated with increased susceptibility for bipolar affective disorder in Brazilian patients [80]. Recently, Saab et al. [81] observed that AT1 C allele was prevalent in a depressed Lebanese population. RAS polymorphisms associated with depression are, in some cases, associated with suicidal behavior [82]. Among males, the risk for suicide in I/I homozygotes was about 80% higher than in subjects with other genotypes. ACE I/D polymorphism may also increase the vulnerability to suicide [82]. However, not all studies have found associations of these polymorphisms with depression, so the jury is still out.

Stress

Brain RAS distribution of AT1 receptors follows the hypothalamic–pituitary–adrenal axis. During stress, both the peripheral and the central ANG II systems are stimulated, with increases in AT1 receptors expression and ANG II levels. Different types of stress have been

associated with peripheral sympathetic nerve stimulation, increased plasma renin activity, and higher circulating ANG II [83]. AT1R are increased in brain by isolation stress induced by short periods of isolation and to 24-h isolation stress. AT1 receptor blocker prevented this response and the ulcerations of the gastric mucosa produced by isolation stress [66]. Peng and Phillips [84, 85] demonstrated that in cold-induced hypertension, AGT and ANG II in the brain predominate and in different brain areas, AT1R are upregulated and AT2R downregulated. AT1 antisense treatment reduced the cold-stress-induced high blood pressure [84]. Experimental studies also showed that pretreatment with losartan provide protection from stress induced by immobility or forced swimming [86]. ANG II is an important stress hormone, and blockade of AT1 receptors might be considered for stress-induced disorders. To inhibit central AT1 receptors may be impractical, but the hypothesis of Paton suggests inhibition of AT1R in brain capillaries could be an alternative if NO is involved [47, 87].

Conclusion

Detlev Ganten's bold hypothesis in 1971 has proven correct. The brain RAS is a model for neuropeptide processing and action in the brain. Brain RAS has neuronal effects far beyond cardiovascular and fluid homeostasis and may be developed for therapies for neurological dysfunctions

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