

The Role of the Brain Renin-Angiotensin System in Neurodegenerative Disorders

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Abstract: The primary function of the renin-angiotensin system (RAS) is to maintain fluid homeostasis and regulate blood pressure. Several components of the RAS, namely angiotensinogen, angiotensin converting enzyme, angiotensin II and their receptors, are found in the CNS suggesting the possibility of a localized RAS in the brain. Cognitively disabling neurodegenerative disorders such as Alzheimer's disease or vascular dementia show vascular lesions, and the brain RAS has been suggested to contribute to the disease process. The aim of this brief review is to summarize the current state of research in this field with emphasis on RAS-related alterations during the course of neurodegenerative disorders.

Keywords: Renin-angiotensin system, Angiotensin II, III, IV, Angiotensin converting enzyme, AT1 and AT2 receptors, Alzheimer's disease dementia.

I. THE RENIN-ANGIOTENSIN SYSTEM (RAS):

More than 100 years ago the first component of the renin-angiotensin system (RAS), renin, isolated from kidney extracts, was found to induce a vasopressor response when injected to rabbits [1]. Today, the RAS is identified as a complex enzymatic pathway generating several active peptides which control fluid homeostasis, blood pressure, hormone secretion, behavioral and cognitive responses [2-5]. Renin, a proteolytic enzyme released from the juxtaglomerular apparatus of the kidney when arterial blood pressure decreases, acts on the inactive precursor angiotensinogen to form the decapeptide angiotensin (Ang) I (Table 1). Ang I, in turn, is hydrolyzed by the action of angiotensin converting enzyme (ACE), a zinc metalloproteinase also known as dipeptidyl carboxypeptidase I (DCP I), at the carboxy terminal dipeptide His-Leu to form the active octapeptide Ang II [6]. ACE, which is present in the endothelial cells of the blood vessels, has also an additional effect to degrade bradykinin, an active vasodilator.

Ang II, a potent vasoconstrictor peptide, was isolated by two independent research groups in 1940 [7, 8]. In 1961 it was demonstrated that Ang II has not only peripheral actions, but also induces a centrally mediated hypertensive response [9]. Central injections of Ang II were found to strongly stimulate drinking behavior [10]. Today, it is well established that Ang II exerts a number of central actions modulating autonomic nervous system activity [11], the hypothalamic-pituitary-adrenal axis and vasopressin secretion [12], stimulation of thirst [13] and baroreflex control [14], and has, besides its antidepressant and anxiolytic effects, some impact on learning and memory [4].

In further steps of Ang metabolism Ang II is converted to the heptapeptide Ang III by the enzymatic action of

aminopeptidase A, a zinc metalloproteinase, that cleaves the N-terminal aspartyl residue (Table 1) [15, 16]. Ang III, on the other hand, is cleaved by aminopeptidase B to form the 3-8 hexapeptide fragment of Ang II, called Ang IV [17]. Finally, Ang IV is degraded by aminopeptidase N and dipeptidylaminopeptidase into NH₂-terminal deleted peptides (Table 1). Besides affecting blood flow regulation, Ang IV has been implicated primarily in modulation of exploratory behavior and processes attributed to learning and memory [17].

II. THE RAS IN THE CENTRAL NERVOUS SYSTEM

Active components of the RAS, such as Ang II, do not cross the blood-brain barrier [18], and peripheral RAS can only directly influence those cerebral regions, such as circumventricular areas, that lack the blood-brain barrier [19]. Therefore, blood-borne Ang II has been postulated to interact with specific receptors in the neurons of circumventricular organs which may project to many other brain regions behind the blood-brain barrier [20]. The subfornical organ or organum vasculosum laminae terminalis belongs to the circumventricular organs effected by circulatory Ang II causing salt appetite, thirst and vasopressin secretion [21], and the action of peripheral Ang II on the area postrema increases blood pressure [22].

Beyond the actions of peripheral RAS components in certain regions of the central nervous system (CNS), an independent RAS exists in the brain. Early observations in the 1970 in dog and rodent brains suggested the possibility of a centrally localized RAS [23-26]. Renin was isolated in the dog brain [23, 24], and Ang II binding sites were determined in rat brain [26, 27]. Immunohistochemical experiments revealed the distribution of angiotensinogen, Ang I, Ang II and renin in several brain regions of rats [28, 29]. Ang II immunoreactivity was localized both to neurons and vessels in the brainstem, cerebellum and hypothalamus, whereas angiotensinogen and Ang I were found in cellular localization in hypothalamic nuclei [28, 29]. In a next step

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Table 1. The Renin-Angiotensin System (RAS)

| RAS components | Transforming enzymes | Amino acid sequences |
|----------------------|--|--|
| Angiotensinogen ↓ | <i>Renin</i> | Asp-Arg-Val-Tyr-Ile-His- Pro-Phe-His-Leu-Leu-Val Tyr-Ser |
| Angiotensin I ↓ | <i>Angiotensin converting enzyme (ACE)</i> | Asp-Arg-Val-Tyr-Ile-His- Pro-Phe-His-Leu |
| Angiotensin II ↓ | <i>Aminopeptidase A</i> | Asp-Arg-Val-Tyr-Ile-His- Pro-Phe |
| Angiotensin III ↓ | <i>Aminopeptidase B</i> | Arg-Val-Tyr-Ile-His-Pro-Phe |
| Angiotensin IV | | Val-Tyr-Ile-His-Pro-Phe |

the synthesis of several RAS components, namely renin and angiotensinogen, within the brain were confirmed by the identification of their messenger RNA sequences [30, 31]. In addition, in human brain Ang II immunoreactivity has been shown in the basal ganglia, cortex, hypothalamus, thalamus, brainstem and cerebellum [32-34], and Ang binding sites are present in the forebrain, substantia nigra, basal ganglia, cerebellum, cortex, thalamus and hippocampus [35-37]. Thus, Ang II in the brain has been suggested to act as a neurotransmitter regulating thirst, drinking, antidiuretic hormone secretion, facilitating vasopressor effects and hormone secretion such as adrenocorticotrophic and luteinizing hormones [38, 39]. Ang II is also involved in the regulation of neurotransmitters such as noradrenaline and 5-hydroxytryptamine (5-HT) [38], and inhibits acetylcholine release [40, 41]. Today, it is well established that the brain has its own intrinsic RAS with all its components present in the CNS.

III. ANGIOTENSIN RECEPTORS:

Angiotensin peptides exert their functions through specific receptors, namely AT1, AT2 and AT4 (Table 2) [2, 4, 5, 17, 37, 42, 43]. The AT1, a seven- transmembrane receptor of 40-42 kDa coupled by guanyl nucleotid binding

proteins to phospholipase C, calcium and cAMP second messenger systems [43] appears to mediate classic angiotensin functions such as the control of fluid homeostasis, blood pressure, cyclicity of reproductive hormones and sexual behavior [37]. The human AT1 gene is located on chromosome 3q [43]. In rodents there are two subtypes of AT1, AT1_A and AT1_B, coded by different genes, but they have identical pharmacological effects [43]. AT2 is also a seven-transmembrane receptor of 42 kDa sharing a 32-34% homology with the AT1 [4]. The human AT2 gene is localized on chromosome X with the cytogenic location q23-q24 [43]. The AT2 has been suggested to be involved in brain development, apoptosis, vascular growth, blood flow control and NMDA receptor modulation [4, 37]. An AT3 angiotensin binding site has been identified, which causes a marked cGMP stimulation, but, so far, no physiological function could be attributed to it [44]. Finally, Ang IV has been found to bind to a specific binding site different from AT1 and AT2 which has been designated AT4 [45]. Ang IV binds only with very low affinity to AT1 and AT2, whereas, vice versa, Ang II displays a very low affinity to AT4 [46]. AT4 has been postulated to mediate the effects of Ang IV not only on the regulation of blood flow, but also on the modulation of central motor and sensory activities, and, in addition, on learning and memory [17].

Table 2. Angiotensin Receptors

| | AT1 | AT2 | AT4 |
|--------------------|------------------------------------|------------------------------------|------------------|
| Main ligand | Ang II | Ang II | Ang IV |
| Structure | 7 transmembrane | 7 transmembrane | Trimer |
| Molecular size | 40-42 kDa | 42 kDa | 165; ~60; ~80kDa |
| Amino acids | 359 | 363 | Unknown |
| Receptor subtypes | AT1 _A ,AT1 _B | AT2 _A ,AT2 _B | Likely |
| G-protein coupling | Yes | Likely | Unlikely |

Modified from references 17 and 42

In the rodent brain high concentrations of Ang II binding sites are found in the septum, and, in decreasing order, in the thalamus, midbrain, area postrema, medulla, hypothalamus, striatum, cerebellum, hippocampus and cortex [26]. In recent differential experiments the highest density of AT1 has been found on neurons of the lamina terminalis, hypothalamic paraventricular nucleus and nucleus of the solitary tract, but AT1 is also present in the circumventricular organs, hindbrain, substantia nigra, lateral parabrachial nucleus, periaqueductal gray, intermedio-lateral column and dorsal horn of the spinal cord, amygdala, bed nucleus of the stria terminalis, cortex and hippocampus [47]. AT1 is localized to presynaptic nerve terminals, and is also present on glial cells in the brain. Dehydration, hypertension and stress can up- or down-regulate AT1 expression [48, 49]. AT2, on the other hand, is located particularly in the thalamus and cerebellum [47, 50]. A role during cerebral development has been attributed to AT2, since AT2 is primarily expressed in young animals [51]. In adults, AT2 counteracts some AT1-mediated effects [52].

AT4 distribution in the brain differs from that for AT1 and AT2 [17]. AT4 expression has been found in the cortex, hippocampus, amygdala, thalamus, hypothalamus, caudate nucleus, basal nucleus of Meynert, nucleus accumbens, lateral olfactory tract, ventral tegmental area, substantia nigra, superior colliculus, periaqueductal gray, cerebellum, inferior olivary nucleus, lateral vestibular nucleus, locus coeruleus, spinal cord, and the motor trigeminal and fascial nuclei [17]. AT4 binding has been localized both to neurons and astrocytes [53]. The distribution of AT4 in brain areas highly implicated in memory function emphasizes the putative role of AT4 in cognitive processes [17].

IV. ANGIOTENSIN EFFECTS ON COGNITION AND BEHAVIOR:

Initial findings suggested that central Ang II injections in rodents before or after a conditioned avoidance test facilitated learning and retention [54]. Ang II was suggested to directly act on central RAS receptors to enhance associative memory and learning possibly with differential effects on acquisition, storage and recall. Since the selective AT1 antagonist losartan was found to abolish the Ang II-induced improvement in object recognition, the cognition-improving effects of Ang II were suggested to be transmitted by AT1 [55]. However, subsequent contradictory findings showed that losartan was also able to facilitate spatial and short-term working memory, and to reverse scopolamine-induced cognitive deficits [56].

Investigations using ACE inhibitors, on the other hand, supported the hypothesis that Ang II suppression may have cognitive enhancing effects [4]. These drugs were found to enhance learning in rats [57]. Daily administration of captopril, an ACE inhibitor, improved retention and learning deficits in aged mice [57]. Ang II inhibits acetylcholine release [41]. Therefore, enhanced acetylcholine release may be responsible for the cognitive improvement after the administration of ACE inhibitors decreasing Ang II levels [40, 41]. Indeed, findings that Ang II injections suppress long-term potentiation in the hippocampus [58] and amygdala, and that this effect is AT1-mediated [59], suggest that direct

Ang II effects on AT1 may cause cognitive impairment. Thus, the long-term treatment of hypertension with captopril [60], but not with enalapril, another ACE inhibitor [61], improves quality of life in elderly humans, providing additional clinical evidence for the positive effects of Ang II suppression. However, there is also evidence for a bimodal action of Ang II on learning, showing an inhibitory action at low doses, and a facilitatory effect at higher doses [62].

Ang IV and AT4 agonists have been postulated to be positively implicated in memory acquisition and retrieval [2, 17]. Central administration of Ang IV stimulates exploratory locomotor behavior, improves recall in passive avoidance situations and facilitates memory retention in rodents [63, 64], whereas AT4 agonists are able to reverse scopolamine- or bilateral perforant pathway lesion-induced memory deficits [64]. Recently, AT4 has been isolated and has shown to be the insulin-regulated aminopeptidase (IRAP) [65]. IRAP is a metallopeptidase and has been shown to cleave a number of peptides in the brain. The distinct facilitating effects of Ang IV on memory have been related to its binding to IRAP and inhibition of IRAP's enzymatic activity [65].

Brain RAS may also be involved in the course of affective disorders [4]. Clinical experiments suggest that antidepressant drugs reduce Ang II function via a post-receptor action, and ACE inhibitors exhibit antidepressive effects [4]. A modulating effect of Ang II on anxiety has been also reported [4]. Central Ang II administration initially causes a decrease in exploratory behaviour in rats reflecting increased anxiety followed by increased exploratory behavior [4, 66, 67]. Interestingly, peripheral administration of Ang II ACE inhibitors causes first anxiety-like behavior, and later anxiolytic-like effects. Dopaminergic pathways may play a role in the anxiety-modulating effects of Ang II [66], but an additional involvement of GABAergic pathways has been also suggested, since Ang II was found to potentiate the actions of GABA [68].

V. ANGIOTENSIN CONVERTING ENZYME (ACE) GENE POLYMORPHISM IN ALZHEIMER'S DISEASE (AD):

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in elderly people. Whereas mutations in amyloid precursor protein and presenilin genes have been associated with early onset familial AD cases, so far, only the polymorphic variation of the apolipoprotein E has been established as a genetic risk factor for late onset and sporadic forms of AD [69]. In the search for other markers for AD, ACE gene polymorphism has attracted attention [3, 4, 70-76]. The ACE protein is encoded by a 21-kilobase, 26-exon gene located on chromosome 17 at q23 and reveals an insertion (I)/deletion (D) polymorphism in intron 16. A polymorphism in which the deletion rather than the insertion of a 287-base-pair sequence in intron 16 of the human ACE gene was confirmed to be associated with increased serum ACE activity [77]. Circulating ACE levels in humans with the homozygote DD polymorphism have been found to be twice as high as the ACE II genotype [78], and higher ACE activity in the plasma is closely associated with a higher

prevalance of hypertension, ischemic heart disease, and lacunar strokes in the brain leading to cognitive disturbances [79]. ACE directly effects aggregation, deposition and fibril formation of AD amyloid β -peptide (A β) [80], a neurotoxic peptide forming extracellular and vascular deposits in AD brain which aggravates the disease process. ACE was found to significantly inhibit A β aggregation and retard A β fibril formation [80]. ACE was also able to protect cells from A β -induced neurotoxicity. Therefore, ACE has been suggested to affect susceptibility to AD. Indirect evidence for this is the increased ACE in cortical areas and caudate nucleus of AD patients [34, 81, 82]. An additional factor attributing ACE a role in neurodegenerative disorders may be the fact that ACE is closely linked to vascular diseases [83], and vascular pathologies in the brain contribute to the progress of cognitive decline.

The data investigating the association between ACE gene polymorphism and AD is controversial in several aspects [3, 4, 70-76, 84-86]. Some studies showed a strong association between ACE ID and II genotypes and AD [70, 87, 88], which was also supported in a recent meta-analysis summarizing the previous studies [84]. In a cohort of 350 AD patients ACE I allele frequency has been found to be increased 28% [87], and the same increase was also confirmed both in AD cases and Parkinson patients with coexisting AD pathology in another study [88]. However, several other clinical studies have failed to confirm the association between the ACE I allele and AD [71, 72, 74, 76]. A recent study in autopsy-confirmed AD cases found no increase in the ACE I allele in AD cases and the presence of ACE I allele was not associated with increased amounts of A β deposits in the brain [72]. Ethnic differences, on the other hand, have been suggested to account for the discrepancies of study results, since studies in Asian populations seem to point to a relation to the ethnic background [76], and confirm an association between ACE I allele increase and AD [70].

An increased ACE D allele prevalence and a reduced ACE I allele prevalence were found in patients with age-associated memory impairment [89]. In addition, in a 4-year study investigating the cognitive decline in 1168 aged subjects with different ACE I/D genotypes, there was a strong association between a homozygote ACE DD allele and the lowest scores in cognitive performance tests when compared to subjects with ID and II alleles [90]. At the end of the study survey the subjects with ACE DD allele showed also significantly greater cognitive decline than the subjects in other groups. Several studies in AD patients have detected the ACE D allele more frequently in the diseased population and thus suggest that bearing at least one ACE D allele may be a risk factor for AD [73, 75, 76]. However, there are also studies showing no association between AD and ACE D allele [71, 74]. Interestingly, there is also data showing that the ACE D allele is more prevalent in centenarians suggesting it may confer longevity [91] i.e. even attributing a neuroprotective effect to the ACE D allele [92]. Besides ethnic differences the allele combination may also account for these contradictory results, since homozygote allele combinations DD and II have been associated with AD, but not the heterozygote allele combination ID [84]. Or, as recently suggested in a study showing that AD patients with

the ACE DD genotype revealed more white matter lesions in the frontal and temporal cortex when compared with ID and II genotypes, ACE D allele-related pathology may represent a significant co-morbidity, also shared by other disorders, rather than forming an integral part of the AD process itself [93].

In summary, there is some indications for a possible impact of ACE gene polymorphism on AD-related pathology, but many questions remain to be elucidated. It seems to be too early to determine ACE gene polymorphisms as a reliable marker for AD.

VI. ALTERATIONS IN BRAIN RAS IN AD AND OTHER NEURODEGENERATIVE DISORDERS:

Several studies have investigated the AD-related RAS alterations in the brain [33, 34, 81, 82, 94]. ACE activity was found to be elevated in the cortex [34, 81, 82], medial hippocampus, parahippocampal gyrus and caudate nucleus of AD patients [81]. In addition, AT1 [34] and AT2 [94] have also been found to be increased in AD cortex suggesting an augmented brain RAS activity during the disease process. Since the suppressing effect of Ang II on acetylcholine release is well established [40, 41], and acetylcholine is one of the major neurotransmitter systems heavily implicated in AD pathology, the enhanced RAS activity may represent an additional factor contributing to cognitive impairment in AD. Interestingly, neurons containing Ang II found in the striatum and hippocampus of AD patients were found to participate in AD-related plaque formations, and Ang II immunoreactive neurons in those patients were mostly distorted showing neurodegenerative changes [33]. Ang II acting as a neuropeptide in those brain regions may be directly involved in AD pathology.

Not only neuronal RAS seems to be altered in AD, but also ACE and Ang II found in perivascular localization are distinctly increased in AD, possibly pointing to an underlying microvascular pathology where RAS is involved [33, 34]. Whereby, RAS components found in association with vessels in those patients can also be peripheral origin diffusing through destroyed blood-brain barrier in the vessel walls. Vascular deposits of A β are often the cause of microvascular pathology in AD. Nevertheless, a vascular pathology has been associated with AD, and vascular risk factors may not only lead to vascular dementia, but may also increase the risk of developing AD [95]. RAS alterations seem to represent a pathological link common to different types of dementia.

Alterations in RAS receptors have been found also in other neurodegenerative disorders such as Parkinson's disease and Huntington's disease [94]. In Parkinson's disease patients, AT1 was significantly decreased in the caudate nucleus, putamen and substantia nigra, whereas, in Huntington's disease patients, AT1 was found to be slightly decreased in putamen, relative to matched controls [94]. AT2 levels in the caudate nucleus, on the other hand, was decreased in Parkinson's patients and increased in Huntington's disease patients. Since the receptor alterations were massive, the authors have concluded that RAS receptors in the basal ganglia are principally localized on neurons which undergo degeneration during the disease

process and that these neurons are probably dopaminergic [94]. The brain RAS seems to decisively contribute to the pathology of the dopaminergic nigrostriatal pathway in these patients.

Components of the brain RAS have been found to exert distinct neuroprotective effects and, therefore, have been taken into consideration as a potential therapeutic option in neurodegenerative disorders [80, 96-98]. Ang II and IV have been shown to have anti-apoptotic effects in neurons [96], and, as mentioned above, ACE significantly protects from A β -related cellular toxicity inhibiting the fibril formation and aggregation of A β in a dose dependent manner [80]. In addition, AT₂ appears to be involved in brain development and neuronal regeneration, and AT₂ transmitted effects regulate ionic fluxes, cell differentiation and axonal regeneration in neurons [97, 98]. These direct neuroprotective, plaque-retarding and regenerational effects may provide a novel therapeutic approach to treating or preventing neurodegeneration.

CONCLUSION

Taken together, there is growing evidence that the central RAS is involved in the disease process of neurodegenerative disorders at many levels. The last word as to whether ACE gene polymorphism is a risk factor for AD is not pronounced, but the brain RAS seems to play a distinct role in cognitive symptoms, vascular pathology and neurodegeneration seen not only in AD, but also in other neurodegenerative disorders. At least the clinical experiences with ACE inhibitors in the prevention of hypertension and cerebrovascular diseases raise expectations for considering the RAS as a novel therapeutic target for cognitive dysfunction. Therefore, preventive therapeutic strategies involving RAS-regulating substances may attract further attention.

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