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COMMENTARY

THE BRAIN RENIN–ANGIOTENSIN SYSTEM: A MODEL FOR THE SYNTHESIS OF PEPTIDES IN THE BRAIN

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Peptides in the brain are presently a subject of active research and rapid progress for several reasons: 1) Refined biochemical techniques have made the extraction and characterization of even small amounts of peptides from brain tissue possible. 2) Specific and highly sensitive radioimmunoassays have allowed meaningful measurements of peptides from small functional brain nuclei. 3) Immunohistochemical methods have permitted us to study the distribution and localization of peptides in the nervous system. 4) New and important biological functions of peptides within the nervous system have been discovered. Research has been stimulated especially on opioid peptides[1], pituitary peptides and their fragments[2], releasing factors[3, 4], substance P[5], neurotensin, prolactin, angiotensin and other peptides (for review see:[6–12]). One of the surprising results of these research efforts has been that peptides with previously assumed specific localization and function, have now been discovered in a variety of organs and tissues implying new and hitherto unsuspected functions.

The renin–angiotensin system (RAS) was originally linked to the kidney, to mechanisms of renal hypertension and to sodium and volume regulation[13]. It has subsequently been demonstrated that renin can be found at even higher concentrations in uterus, salivary glands, in various other tissues and in tumors[13–18].

The brain was the first extrarenal tissue in which all components of the complex RAS enzyme system have been systematically investigated. Furthermore, the brain RAS may be considered a model enzyme system for the biosynthesis of brain peptides. This paper will therefore confine itself to a discussion of recent findings and selected aspects of the brain RAS. For a complete discussion the reader is referred to earlier reviews[17, 19–22]. Preference is given to recent review articles which refer to earlier original papers.

ANGIOTENSIN EFFECTS ON THE BRAIN

The RAS of the kidney has been widely studied[13, 23, 24]. Decrease of renal perfusion pressure due to e.g. renal artery stenosis stimulates the release of the enzyme renin (E.C. 3.4.99.19) from the kidney into the blood. The protein substrate angiotensinogen is synthesized mainly in the liver and is also secreted into the blood. Renin then cleaves the decapeptide angiotensin I (ANG I) from angiotensinogen. ANG I is then converted to angiotensin II (ANG II) by the ANG I-converting enzyme. Stimulation of ANG II receptors in the arteries and in the adrenal gland produce vasoconstriction and secretion of mineralocorticoid hormones, which lead to salt and water retention and increase of arterial blood pressure (Fig. 1)[13, 23, 24].

In cross perfusion experiments it was first shown that angiotensin could also elevate blood pressure by stimulation of ANG II-sensitive sites in the brain[25]. The *area postrema* situated on the ground of the fourth brain ventricle in close vicinity to the *calamus sciptorius* and *nucleus tractus solitarius* is probably involved in the mediation of these effects[26–28]. Injection of ANG II directly into the brain ventricles has also been found to produce an increase in arterial blood pressure. Evidence has been given that this effect is probably mediated by different angiotensin-sensitive sites, as ANG II applied directly on the *area postrema* from the fourth brain ventricle had no effect on blood pressure[20, 28–30]. The type of cardiovascular response elicited by intravenous (i.v.) angiotensin or by injection of ANG II into the brain ventricles (i.v.t.) has been found to be different. First, stimulation of the *area postrema* via the vertebral artery has led to an increase of heart rate, while i.v.t. ANG II has increased blood pressure without affecting heart rate. Second, i.v. administration of ANG II has elicited a fast, short-lasting blood pressure response while the i.v.t. effects on blood pressure have had a lag time and have lasted longer. Third, the blood pressure increases to a given dose of ANG II have been higher by the i.v. than by the i.v.t. route, the maximal blood pressure increases rarely exceeding 30–40 mm Hg even at doses of 1000 pmol/kg ANG II i.v.t. Fourth, anaesthesia has been found to reduce or abolish the i.v.t. ANG II effect on blood pressure; this has not been the case if ANG II was given i.v. Fifth, tachyphylaxis to ANG II has occurred rapidly after i.v. but not after i.v.t. administration. Finally, ANG II i.v.t. has had no acute effect on kidney function, whereas i.v. ANG II has caused a decrease in plasma flow, glomerular filtration rate and Na^+-excretion[17, 20, 28–30, 34].

These differences between central and

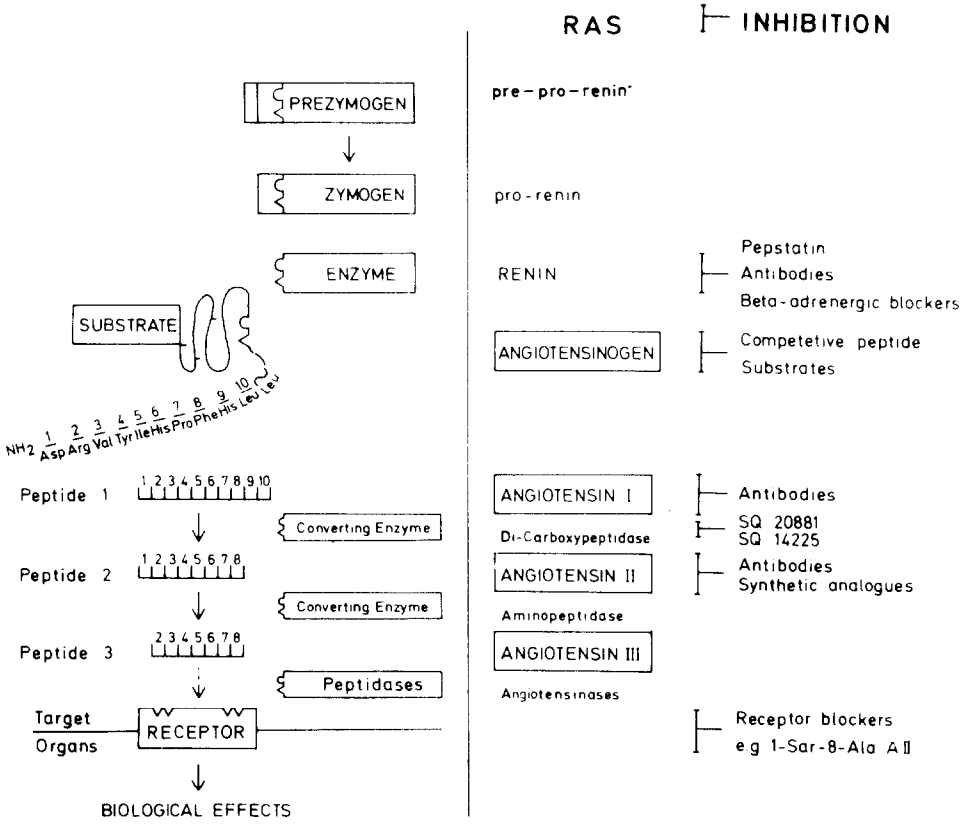

Fig. 1. Schematic outline of the renin–angiotensin system (RAS). The left part represents the components of the enzyme system in general terms. The occurrence of prezymogen and zymogen forms of renin are not proven. In the middle part, the specific nomenclature of the RAS is given. The right part indicates possibilities of pharmacological interferences with the RAS at different steps of the enzymatic cascade.

peripheral effects of ANG II on blood pressure imply that they are mediated by stimulation of different receptors and that the blood–brain barrier prevents circulating plasma ANG II from reaching the angiotensin-sensitive sites in brain.

Apart from the effects on arterial blood pressure, drinking behaviour can be induced by ANG II in water-satiated animals of various species. This response is more easily and more reliably obtained by administration of ANG II into the brain ventricles than by i.v. injection or infusion, but both routes are effective (for review see: [20, 31–33]). ANG II i.v.t. has consistently produced a release of antidiuretic hormone (ADH) [20, 34–36]; the release of ADH following i.v. administration of ANG II, on the other hand, is still a matter of controversy [20, 37]. Further effects of ANG II in the brain such as the release of adrenocorticotropic hormone (ACTH) and effect on memory are discussed elsewhere in more detail [17, 19, 20, 38].

ANGIOTENSIN-SENSITIVE SITES IN THE BRAIN

The brain areas most sensitive for the blood pressure effects of ANG II are the *area postrema*, the *nucleus submedialis*, the *hypothalamus* and tissue surrounding the anteroventral part of the third ventricle (AV3V). The *area postrema* is stimulated by circulating plasma ANG II. The blood–brain barrier is incomplete in this region and the histology indicates chemoreceptor properties of the cells. The fact that the *area postrema* is not stimulated by i.v.t. ANG II indicates that a CSF–brain barrier prevents its stimulation from the CSF side [26–30, 34, 39].

ANG II administered into the brain ventricles of cats appears to act on the *nucleus submedialis* which lies adjacent to the cerebral aqueduct at a plane between 6 and 4 mm anterior to Horsley–Clarke zero. In rats the ANG II pressor response was not abolished if contact with the *nucleus submedialis* was prevented [20, 25, 34].

Severs *et al.* [34] proposed that the i.v.t. pressor response could be mediated by action of ANG II on hypothalamic nuclei, release of ADH from the posterior pituitary and by increase of sympathetic tone. This hypothesis was supported by experiments showing that the pressor response to i.v.t. ANG II was significantly reduced in rats with hereditary hypothalamic diabetes insipidus; these rats are not capable of producing ADH [17, 19, 40]. That i.v.t. ANG II can indeed release ADH has been shown by several authors [19, 20, 34–36].

Another area sensitive to i.v.t. ANG II is the tissue surrounding the anteroventral third ventricle (AV3V) [32, 41]. In the rat with AV3V electrolytic periventricular tissue ablation both drinking and pressor responses to i.v.t. injections of ANG II were abolished [42]. The pressor responses to i.v.

ANG II were reduced in conscious but not in anaesthetized rats with AV3V lesions. This suggests contribution of an anaesthesia-sensitive central mechanism to the pressor response of plasma ANG II[41, 42].

The i.v.t. ANG II dipsogenic responses were reduced in AV3V lesioned animals but were the same in rats with diabetes insipidus which exhibited a blunted blood pressure response[17, 42, 43]. These findings suggest a dissociation of brain sites mediating the centrally induced blood pressure response and the drinking response. The *organum vasculosum lamina terminalis* in the anterior third ventricle, the subfornical organ situated at the *foramen intraventriculare* Monroi and the median preoptic area are the most sensitive areas that mediate drinking behaviour[31–33].

ANGIOTENSIN RECEPTORS

Biological effects of peptides are generally mediated by interaction with membrane-bound macromolecules i.e. receptors. ANG II receptors in the classical target organs of the RAS, the adrenal cortex and arteries have been well studied[44]. ANG II receptors in the brain have been described only recently[9, 45–48]. They satisfy the criteria of specificity, high affinity, saturability and reversibility of binding. Adrenal gland and brain have shown the highest levels of ANG II binding of all organs studied; however, angiotensin receptor-binding sites in the brain have approximately one order of magnitude greater affinity for ANG II than those in the adrenal cortex. The dissociation constant for ANG II at rat brain receptors is between 0.2 to 0.9 nM. The specificity of the receptors as tested with various synthetic ANG II analogues and related peptides was similar in the brain and in the adrenal gland. ANG II antagonists and ANG II peptide fragments competed with the binding sites and their potency *in vitro* correlated with their potency *in vivo*. Subcellular fractionation studies indicated that over 90 per cent of the ANG II binding was associated with particulate matter and was concentrated in the microsomal fraction[9, 45, 47].

Angiotensin binding in rat brain has been found to be most prevalent in the *area postrema*, *midbrain*, *thalamus*, *hypothalamus* and *septum*. These regions had previously been shown to mediate biological effects of ANG II. In calf brain, ANG II receptors have also been localized in the cerebellar cortex and deep nuclei of the cerebellum[9, 45, 47]. So far, no biological correlate is obvious for this latter localization. Using microelectrodes to record intracellular activity, concentration-dependent spiking activity was elicited by ANG II from organ-cultured supraoptic nucleus neurons. Specific ANG II antagonists blocked this effect[48]. Phillips *et al.*[46] applied ANG II to individual cells of the cat subfornical organ by microiontophoresis. The increased spiking frequency following ANG II could be blocked by the ANG II antagonist [Sar^1, Val^5, Ala^8]-ANG II-octapeptide (Saralasin). Saralasin also tended to slow down the spontaneous activity of neurons. This has been interpreted to mean that endogenous brain ANG II maintains the spontaneous firing rate of neurons[20, 46]. Presynaptic localization of ANG II receptors has been claimed but more work is necessary in order to obtain a more precise definition of the nature of these brain ANG II receptors[49].

THE BLOOD–BRAIN BARRIER (BBB)

To answer the question of whether angiotensin receptors in brain are stimulated by blood-borne ANG II or by ANG II generated within the brain itself, the permeability of the BBB and of the blood–CSF barrier must be examined. Previous studies have attempted to determine the distribution of ANG II in the brain after i.v. injection of radioactively labelled ANG II[50–52]. However, these experiments cannot adequately determine whether plasma ANG II penetrates into the brain for several reasons. First, when ^{125}I-labelled angiotensin was used, the tracer does not correspond to the endogenous peptide as indicated by its reduced biological activity. Second, angiotensin is extremely rapidly degraded into fragments by angiotensinases in plasma and tissue (the half-life of ANG II in plasma is 30 sec). The radioactivity in plasma and tissue is, therefore, almost certainly not due to the intact ANG II molecule even if extremely short time sequences are studied. Third, the amino acid tyrosine in position 4 of the ANG II molecule usually carries the radioactive tracer. Tyrosine, however, is actively taken up into the brain by selective carrier mechanisms of the BBB[53]. Fourth, if the specific activity of the radioactive ANG II is not sufficiently high, large amounts need to be injected. These large doses of ANG II produce increases in blood pressure which, in turn, increase the permeability of the BBB[20].

We have confirmed previous findings that radioactivity can be found in brain and CSF following i.v. injections of ^{125}I and ^{3}H-labelled ANG II and ANG I; however, this radioactivity has been found to be entirely related to inactive degradation products of angiotensin[17, 20, 52]. Biologically more relevant results may be obtained by the simultaneous measurements of ANG I and ANG II in CSF and plasma. We have measured angiotensin in CSF and in plasma of normotensive rats and rats with different kinds of experimental hypertension. ANG II was also infused i.v. in subpressor and graded pressor doses into rats and dogs. No parallelism between plasma and CSF angiotensin levels was found and we have, therefore, concluded that ANG II does not penetrate freely from blood into the brain[19, 54].

These results seem to be in contradiction with reports indicating that i.v.t. saralasin could block the dipsogenic effect of raised peripheral angiotensin levels[55, 56] and that i.v. saralasin could antagonize effects of i.v.t. ANG II[57]. However, in these latter experiments extremely high doses of ANG II and saralasin have been used. These doses lead to abrupt increases in arterial blood pressure with consequent disruption of the integrity of the BBB. Phillips *et al.*[58] have shown that horseradish peroxidase could be detected in periventricular tissue and other brain sites 5 min following the simultaneous i.v. injection of 2 μg

ANG II and the marker enzyme, while at the lower dose of 10 ng ANG II with less than 35 mm Hg blood pressure increase, there was no crossing of the horseradish peroxidase from blood to the brain. Impermeability of the blood–CSF barrier was also found for other components of the RAS, i.e. renin and angiotensinogen[17, 19].

ANGIOTENSINOGEN

The presence of angiotensinogen, the high molecular weight precursor of angiotensin, has been first demonstrated in brain tissue of dogs[59] and was confirmed in brain tissue of rabbits and sheep[60, 61] and in CSF of rats, dogs, sheep and man[19, 22, 60]. Biochemical characterization of brain and plasma angiotensinogen has indicated that they are not identical. Isoelectric focussing has revealed differences in the isoelectric point profiles of angiotensinogen in brain, CSF and plasma. These appear to be mainly due to differences in the content of sialic acid[60]. The origin of brain and CSF angiotensinogen is unresolved. Though plasma contamination can probably be excluded[59, 61], it is still possible that there is slow penetration of the protein from plasma into the brain or first into CSF and then into the brain. Specific angiotensinogen levels in CSF are about 50 times higher than in plasma[19, 22, 60] and active uptake mechanisms would have to be postulated for the transport of angiotensinogen from plasma into CSF. Furthermore, experimentally induced changes in plasma angiotensinogen do not parallel changes in CSF angiotensinogen[19, 62]. Finally, the biochemical differences between plasma and brain angiotensinogen[60] are arguments in favour of its local synthesis in the brain. It appears that angiotensinogen levels in brain may be rate-limiting for angiotensin formation, since injection of exogenous angiotensinogen into brain tissue has elicited drinking[63]. This is not the case if angiotensinogen is injected into the CSF, since CSF contains little or no renin. Components of the RAS in CSF probably represent an overflow from brain tissue ("sink function") and normally play no regulatory role. CSF measurements may still be a valuable diagnostic tool for assessment of the activity of a stimulated brain RAS.

BRAIN RENIN

Renin-like enzymes that form ANG I from natural angiotensinogen have been measured in brain tissue of rats, desert rats (*meriones shawi shawi Duvernoy*), dogs, sheep and man[17, 19, 22, 64–69]. Various nomenclatures have been used[17, 18] and uncertainty concerning the exact nature of the enzyme has resulted from the fact that brain renin has not been obtained in pure form. Purification of renin from tissue is difficult because, like kidney renin, brain renin is an acid protease and closely associated with other proteases. These can degrade the enzyme during the purification procedure. All renin preparations available to date, including human kidney renin distributed as international standard by the World Health Organization, have been found to be contaminated with "cathepsin D-like" acid protease activity[70]. The use of appropriate enzyme inhibitors during all purification steps and the introduction of affinity chromatography have facilitated the purification of renins. Pepstatin has proven particularly useful as a ligand for affinity chromatography[70–72]. This pentapeptide is produced by some actinomycetes strains[73] and strongly binds to a number of acid proteases such as pepsin, cathepsins and renin from kidney and extrarenal sources. These enzymes copurify when pepstatin is used as a ligand and even the highly purified enzyme preparations from kidney and brain were still found to contain acid protease activity after pepstatin affinity chromatography[70]. This has been misinterpreted by some authors to indicate identity of brain renin and "cathepsin D-like" acid protease[22,74]. However, it is possible to differentially elute acid protease activity and renin activity from pepstatin affinity columns. This has been shown for kidney renin by Murakami *et al.*[75] and has been demonstrated for human brain renin by Speck and Ganten[76] (Fig. 2). The human brain enzyme obtained after differential elution from the pepstatinyl–Sepharose affinity column satisfies all criteria of specificity *in vitro* and *in vivo* (Table 1). Separation of acid protease activity which forms an unidentified product from denatured hemoglobin substrate, from renin activity which specifically cleaves ANG I from angiotensinogen, has now been accomplished from brain tissue of dog, sheep and man[60, 76–79].

The pH activity profiles of the purified enzymes indicate optima of enzyme activity between pH 4.0 and 6.5, depending on species, purity and incubation conditions[17, 19, 22, 60, 76, 77, 79]. An optimum of pH 7.0 to 7.5 for rat brain renin has also been reported[65]. Since enzyme characteristics depend on the *in vitro* incubation conditions (purity of the components, type of substrate, buffers, etc.) they have little consequence as to the enzyme activity *in vivo*. Activity of brain renin with endogenous brain angiotensinogen under *in vivo* conditions has been demonstrated (Table 1). The presence of inactive precursors of renin in brain is suggested by the fact that enzyme activity increased following acidification of brain tissue. Preliminary results with Sephadex gel filtration indicate the presence of a high molecular zymogen of renin in human brain[76] as has recently been shown for kidney renin[78].

BRAIN ANGIOTENSIN

ANG I and ANG II have been extracted from brain tissue of dog, rat and rabbit[17, 19, 22, 65, 79, 82]. The physico-chemical characterization has revealed a high degree of similarity with synthetic angiotensins. Quantitative measurements of angiotensin have been attempted[17, 19, 65, 82, 83] but must be interpreted with caution, since characterization of the peptide has not been possible in the small tissue samples and the high angiotensinase activity in brain[84, 85] may falsify results. A recent report indicates that binding of angiotensin to larger protein molecules similar to the binding of pituitary peptides to neurophysins may occur[81]. This could explain the failure of

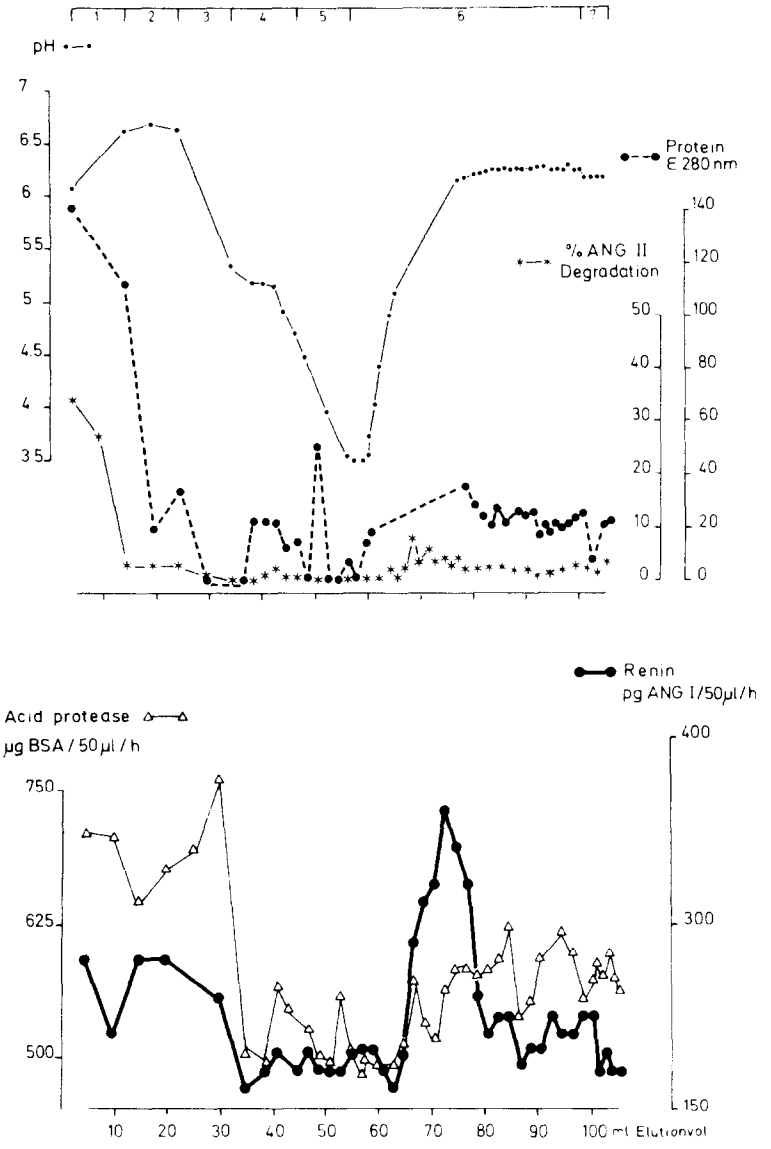

Fig. 2. Purification of human brain renin by a pepstatin–aminohexyl–Sepharose column. The brain was extracted with cold acetone and the remaining powder containing the enzyme prepurified on Sephadex G100 gel filtration. Numbers on top indicate elution buffers: (1) 0.02 M phosphate buffer, pH 6.8; (2) 0.1 M phosphate buffer, pH 6.8; (3) 0.1 M acetate buffer, pH 5.2, containing 1 M NaCl; (4) gradient 0.1 M acetate buffer, pH 5.2→0.1 M acetic acid, pH 3.4, (5) 0.1 M acetic acid; (6) gradient 0.1 M acetic acid, pH 3.4→0.1 M acetic buffer containing 3 M urea, pH 6.3; (7) 0.1 M acetic buffer with 3 M urea. All buffers contained 5 mM EDTA and 0.01 mM PMSF.

some authors to extract ANG II from brain[22, 86]. ANG II concentrations are in the fmol to pmol range per g brain tissue[17, 19, 65, 82, 83]; however, local concentrations in specific areas may exceed this value. Immunocytochemical studies have indicated particularly high ANG II-positive immunofluorescence in the medial external layer of the median eminence, *nucleus amygdaloideus centralis*, *nucleus tractus spinalis nervi trigemini* and in the sympathetic lateral column and *substantia gelatinosa* of the spinal cord[19, 83, 87, 88]. Cell bodies in the paraventricular nucleus and perifornical area contain ANG II immunoreactivity[19]. ANG I immunofluorescence has been reported in the *area postrema*, subfornical organ, *pars intermedia* and median eminence[83].

The presence of ANG II in CSF has been reported[52, 89]. The levels were high in spontaneously hypertensive rats[17] but have been found to be too low to be detected by RIA in several strains of normotensive rats. The presence of ANG-(2–8)heptapeptide (ANG III) in CSF has been described[80]. Renin activity is extremely low in CSF so that despite high angiotensinogen concentration there is probably little if any ANG I formation in CSF *in vivo*. Converting enzyme is present, but angiotensinase activity is absent from CSF; both enzymes are present in the ependymal lining of the brain ventricles and in the choroid

Table 1. Criteria for specificity and *in vivo* activity of the brain RAS

1. Brain renin forms angiotensin I from naturally occurring homologous and heterologous angiotensinogen[19, 70].
2. Specific active-site directed peptide enzyme inhibitors inhibit brain renin in a competitive manner[70].
3. Purified human brain renin does not hydrolyse the following substrates: ANG I (converting enzyme), ANG II (angiotensinase), denatured hemoglobin (cathepsin D-like), β-endorphin[70, 76].
4. Injection of purified human brain renin into the brain ventricles of conscious rats entails angiotensin I formation from CSF angiotensinogen and blood pressure increase. The pressor effect is reversed by specific angiotensin II blockade[70].
5. Injection of angiotensinogen into brain tissue elicits drinking behaviour. This effect is reversed by inhibition of brain ANG I-converting enzyme and by angiotensin II blockade[31–33, 63].
6. Angiotensin I and angiotensin II can be extracted from brain tissue and have been localized by immunohistochemical techniques in neural structures and specific brain areas[19, 83, 87, 88].
7. Blockade of endogenous brain angiotensin II at the receptor level results in altered biological function: decrease of electrical firing of single neurons[20], decrease of blood pressure in spontaneously hypertensive rats[17, 19, 20, 107, 109] and reduction of water uptake[105, 106].

plexus[17, 19]. Upon injection of exogenous renin into the brain ventricles, large amounts of ANG I and ANG II are formed[62, 70].

ANG I-CONVERTING ENZYME (E.C. 3.4.15) AND ANGIOTENSINASE ACTIVITY

ANG I-converting enzyme is a peptidyldipeptide carboxyhydrolase which converts the ANG I (1–10) decapeptide to the ANG II (1–8) octapeptide. It is present in high concentrations in brain tissue[84, 90, 91]. Peptide inhibitors such as SQ 20 881 or SQ 14 225 reduce ANG II formation both *in vitro* and *in vivo*. Biological effects such as drinking elicited by ANG I or angiotensinogen injection into the brain are decreased in the presence of ANG I-converting enzyme inhibitors. A regulatory role of the converting enzyme for local ANG II formation is possible[90]. ANG I-converting enzyme also degrades kinins into inactive fragments and it may be active on other peptides.

A group of peptidases are summarized under the term angiotensinases. They have broad substrate specificity and are probably involved in the metabolism of many other brain peptides[84, 85]. Angiotensinases degrade ANG I, ANG II and ANG III into inactive peptide fragments and play an important role in the termination of angiotensin effects. They may be carboxy-, amino-, as well as endo-peptidases. Angiotensinases have not been sufficiently purified and characterized from brain tissue. Their optimum enzyme activity is at pH 7.5. All brain regions studied so far have been found to contain higher angiotensinase activity than plasma. The periventricular organs, subfornical organ and *area postrema* contain the highest angiotensinase activity[92]. Interestingly, ANG II- (1–8) octapeptide can be converted into ANG- (2–8) heptapeptide (ANG III) by an aminopeptidase (Fig. 1).

INTERRELATIONSHIP BETWEEN THE PLASMA RAS AND THE BRAIN RAS

Extrarenal sources can contribute to plasma renin levels (for literature see:[18, 21]). The regulation of renin release from the kidney has been more thoroughly studied than the regulation of renin release from organs such as uterus, submaxillary gland and arteries. Hence, it can be assumed that in most conditions renin secretion from the kidney is the major determinant of plasma renin concentration. Moreover, brain renin appears not to leak into the periphery. Since both the kidney-plasma RAS (vasoconstriction, aldosterone stimulation) and the brain RAS (ADH release, increase of sympathetic tone, drinking) participate in salt and volume homeostasis and blood pressure regulation, a coupling between the central and peripheral system can be suspected.

To study this question parallel measurements of plasma renin and brain renin concentrations under various pathophysiological conditions have been performed[93]. In rats with experimental hypertension and high plasma renin, brain renin was found to be suppressed while it was unchanged in another model of renal hypertension with normal plasma renin. In desert rats which were subjected to 10 days of complete water deprivation, plasma renin increased while brain renin decreased in *hypothalamus, hypophysis* and frontal cortex. Puppies with high plasma renin exhibited low brain renin while brain enzyme concentrations were high in adult dogs with lower plasma renin[93]. Great variations of brain renin were observed in bilaterally nephrectomized dogs, but values above control were frequently measured[59]. If rats were injected subcutaneously with doses of 48 pmol to 480 nmol ANG II over a period of 7 days, a significant decrease of brain renin in the *adenohypophysis* but not in other brain areas was measured. A suppression of brain renin was also observed when saralasin was administered at high doses (550 nmol subcutaneously over 7 days). At the dose of 55 pmol, saralasin had no effect. In the latter experiments the brain renin suppressing effect was therefore probably due to ANG II-like agonistic activity of saralasin at the high dose[94]. Sen *et al.*[68] reported a suppression of kidney renin and an increase of brain renin by the ANG II-receptor antagonist [Sar^1, Val^5, Ile^8] ANG-(1–8) octapeptide at a dose of 100 μg per day. In spontaneously hypertensive rats we have found increased renin concentrations in the *posterior hypothalamus, despite low plasma renin levels*[76]. Printz *et al.*[60] reported an inverse relationship of angio-

tensinogen levels in brain and plasma of spontaneously hypertensive rats. A negative feedback of circulating plasma ANG II and ANG II levels in CSF was reported by Simpson *et al.*[95]. In collaboration with these authors we have not been able to confirm their results and no increased ANG II levels were found in CSF after nephrectomy[96].

The bulk of evidence indicates that increased plasma ANG II can lead to a suppression of the brain RAS. Conversely, elevation of brain ANG II, e.g. by injection of renin into the brain ventricles, can lead to a suppression of plasma renin[62]. Thus, there appears to be a coupling between the brain RAS and plasma renin. To assess this more precisely, a method has been devised in our laboratory to investigate renin, angiotensinogen, ANG I-converting enzyme and ANG II in small functional nuclei of rat brain which are obtained by the punching technique[97]. Such a detailed study of the RAS in brain and plasma will probably lead to a better understanding of the interrelationship between the plasma RAS and the brain RAS.

The choroid plexus is of interest in this context. It has been named a "miniature kidney" because of its capability to control fluid and electrolytes in CSF. Renin and ANG I-converting enzyme concentrations are extremely high in this tissue[17, 19]. We have obtained preliminary evidence that circulating plasma ANG II may influence electrolyte handling by the choroid plexus. High plasma ANG II levels were accompanied by increased renin content in the choroid plexus. Acute unilateral carotid artery stenosis decreased and chronic unilateral occlusion increased choroid plexus renin. These changes are similar to the ones expected in the kidney after renal artery stenosis[98]. It may be speculated that the feedback of circulating ANG II on the brain RAS occurs via changes of electrolytes across membranes such as the choroid plexus. Direct contact of the peripheral and central RAS would be possible via tanycytes or via brain sites where the blood–brain barrier is deficient.

BIOSYNTHESIS AND FUNCTION OF THE BRAIN RAS

The expanded APUD concept (acronym for amine content and precursor uptake and decarboxylation)[99] postulates that all peptide hormone-producing cells are derived from the neural ectoderm. The presence of ANG II in brain and gut seems to fit this concept. The renin-producing cells of the juxtaglomerular apparatus in the kidney, however, are probably not of neuro-ectodermal origin. Considering the omnipotency of all cells originally, it is not surprising that other cells not derived from neuro-ectoderm do not suppress their renin- and peptide-synthesizing capacity at certain stages of differentiation and specialization[15].

The kidney RAS can be considered a hormonal system with a single source of secretion and direct ingress of the secretory product, renin, into the blood stream for distribution to a distant receptor tissue. However, the kidney RAS has special aspects since it is not the hormone itself which is secreted by the kidney but an enzyme, which in turn must act on a large protein (angiotensinogen) from yet another distant organ, the liver, to produce not the hormone itself but a hormone precursor, ANG I. This may represent a phylogenetically special development of an originally cellular enzyme system with newly acquired hormonal function to accomplish more widespread effects. A local tissue function of the RAS within the kidney has been postulated[100]. Extrarenal tissues which contain large amounts of renin (uterus, adrenal gland, salivary gland, arteries, brain) seem to have more local function and contribute little to circulating renin under physiological circumstances[15, 18, 21].

The subcellular localization of brain renin in the mitochondrial fraction and in purified synaptosomes as well as the intraneuronal occurrence of angiotensin suggest that formation of ANG II can occur intracellularly. It is possible that the enzyme–prohormone reaction occurs in intracellular compartments e.g. the renin-containing granules[19]. Another possibility of angiotensin synthesis in brain tissue is indicated by the report of Kreutzberg *et al.*[101] that large molecules such as the enzyme acetylcholine esterase, after being released into extracellular compartments, may be bound to the outer surface of a dendritic membrane to be later taken up again by the dendrite. An interesting hypothesis and logical sequence of events would be that renin is released at dendritic points, bound to the membrane, cleaves angiotensin from angiotensinogen which may be available in extracellular compartments or in the axoplasm. Angiotensin would then either be active locally or may be taken up by the dendrite and transported to a more distant site of action. This concept has been proposed for the biosynthesis of opioid peptides[7] and allows for the action of several enzymes (renin, ANG I-converting enzyme, angiotensinase) which could be bound in series to the membrane. The stepwise catalysis of angiotensinogen to ANG I and conversion to ANG II and ANG III and possibly the activation of an inactive renin precursor (pro-renin) could thus be explained. This model would be in harmony with the concept of intragranular hormone synthesis such as insulin, where several trypsin-like enzymes are bound to the inner surface of the granule membrane to produce insulin from proinsulin through multiple enzymatic steps.

Membrane binding also appears to influence enzyme specificity. This could explain how such a multitude of specific peptides and peptide fragments can be achieved by relatively few sets of different enzymes. Limited proteolysis appears to be the major mechanism for the control of hormone activity and conversion of prohormones into the active component, e.g. insulin, gastrin, glucagon, parathyroid hormone, ACTH, opioid peptides and angiotensin. A set of acid proteases such as renin or cathepsins could acquire specificity *in vivo* by membrane binding or by the presence of high substrate concentrations at the strictly localized site of enzyme activity (dendrites, granules), irrespective of whether the reactions are favoured *in vitro* or not.

The kininogenase–kinin enzyme system has striking similarities with the RAS. All components of the kininogenase–kinin system are present in various tissues and in plasma. It is generally believed that the decapeptide-bradykinin (kallidin) is the tissue hormone while the nonapeptide bradykinin is the circulating effector peptide. The tissue hormone correlate of kallidin in the RAS may be the ANG-(2–8) heptapeptide (ANG III) which is active in the adrenal gland and in brain, and which has been identified in CSF of dogs[80]. Such a dual function of a true hormone, circulating in the blood, and a local tissue role not distant from the site of synthesis is probable for several other peptide hormones besides angiotensin and kinins, for example ACTH, ADH, prolactin, opioid peptides, various releasing factors and also for catecholamines. In the brain some of these peptides are believed to have neurotransmitter function. This makes their classification as a hormone, neurohormone or neurotransmitter difficult. Since also invertebrates as well as vertebrates make multiple use of the same few classes of peptides for a variety of functions, a broader definition of these terms appears adequate. A neurohormone would not necessarily have to circulate in blood to affect nervous tissue function, but could be synthesized by specialized cells to affect the function of other cells. Using this definition, brain angiotensin could be considered a neurohormone.

The differences between neurohormonal communication and synaptic transmission have been pointed out by Barker and Smith[102]. The effective neurohormone concentrations are lower than those of transmitters and neurohormone receptors may be localized extrasynaptically and remote from the sites of peptide synthesis.

Neurohormonal communication may not use the same mechanisms as known in classical synaptic transmission. In the latter only rapid kinetics are described while rapid and slow kinetics appear to occur in neurohumoral communication. The possible direct chemical interaction of peptides with membranes[103, 104] is noteworthy. This could result in a change of membrane structure and could influence the excitability of neurons in a manner different from that of amine synaptic transmission. The known effects of ANG II on contractile elements such as actin–myosin and tubulin would be in harmony with this theory. The possibly important role of dendrites for the synthesis of peptides has been mentioned above. Their role in "low voltage processing of information" has been discussed by Guillemin[7] and peptides may play an important role in this type of information traffic.

Due to widespread distribution of the neurohormone upon release, a sustained influence on multiple nerve cell aggregates is achieved. These result in complex and concerted output which, teleologically, may have been elaborated because one molecule can thereby effectively and rapidly, at different levels of the body, command and organize the long-lasting activity of a set of neurons, controlling multiple endocrine functions and behaviour of survival value. For example, dehydration, loss of electrolytes and decrease of blood pressure will stimulate the humoral plasma RAS. Plasma angiotensin stimulates vasoconstriction, release of aldosterone and catecholamines from the adrenal gland. Effects of central angiotensin such as drinking, release of antidiuretic hormone, increased sympathetic tone and elevation of blood pressure equally provide a concerted answer against the circulatory volume deficit, providing a better chance of survival.

Catatonia, which can be produced by opioid peptides, is another example of concerted output by neurohormonal communication, representing survival value in those species not using flight or fight, but rather motionlessness for defense. Interestingly, the synthesis of these peptides is related to the synthesis of β-melanocyte-stimulating hormone, which is involved in the regulation of skin color and camouflage and is also related to the synthesis of ACTH which is a circulating stress hormone. ACTH also influences the activity of the RAS via its effects on the adrenal gland.

The neurohormonal brain RAS appears not to be operative under normal physiological conditions, since central angiotensin blockade has no effect on water uptake in hydrated animals, nor is it effective on blood pressure in normotensive rats. However, after dehydration water uptake can be inhibited by angiotensin blockade[63, 105, 106] and blood pressure can be decreased in spontaneously hypertensive rats and even more so in stressed hypertensive animals[17, 19, 107–109]. The observation that neurohormones may not be involved in the physiological moment to moment regulation, but may only be operative following pathological stimulation, appears to be true also for other brain peptides. Opioid peptides can produce analgesia. Naloxone, the receptor antagonist, does not alter pain perception unless the opioid peptide system is stimulated or patients are extremely pain-sensitive[110].

The different components of the RAS leading to the synthesis of angiotensin are well studied. Pharmacological interferences with a high degree of specificity are possible at each step of this enzymatic cascade[111] (Fig. 1). These different possibilities of interference have been used to assess the role of the kidney and brain RAS in various pathophysiological states. This gives the RAS an advantage over other brain peptide systems and should make rapid progress possible as to the understanding of its pathophysiological role.

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