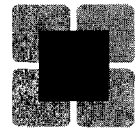

The renin angiotensin system: importance in physiology and pathology

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■ Angiotensin II has long been recognized as a key factor in cardiovascular regulation. The effectiveness of angiotensin-converting enzyme (ACE) inhibitors in controlling essential hypertension suggests that angiotensin II plays a key role in its pathology. The tools of molecular biology have provided the means for a critical reassessment of the renin-antiotensin-aldosterone system in physiology and pathology. The analysis has shown that angiotensin peptides are also synthesized and processed locally in a variety of tissues, including the vascular wall, adrenal glands, heart, and brain. Since angiotensin II is a potent modulator of cardiovascular control centers in the brain, the hypothesis is now advanced that a defect in the brain renin-angiotensin-aldosterone system contributes to the development of hypertensive disease.

□INDEX TERMS: ANGIOTENSIN II; RENIN-ANGIOTENSIN SYSTEM □CLEVE CLIN J MED 1989; 56:439–446

ANGIOTENSIN II is a potent vasoconstrictor and a humoral stimulus for the secretion of aldosterone. Recent insights into the cellular biochemistry of the renin-angiotensin-aldosterone system (RAS) suggest that angiotensin II has other equally important actions in the regulation of renal function, central endocrine secretions, and neural mechanisms governing the activity of the heart and the blood vessels. Clinical and laboratory studies done in the last 15 years have expanded knowledge of the function of the RAS in the control of blood pressure and body fluid volume. This progress has generated a more appropriate understanding of the mechanisms that both initiate and contribute to the pathogenesis of arterial hypertension.

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At the dawn of this century, physicians did not recognize high blood pressure as a disease.[1] According to Page,[2] many believed that increased blood pressure was a consequence of decreased distensibility of aging blood vessels. The situation is quite different today. Hypertension is now an object of intense study both in the basic laboratory and at the bedside. Indeed, what we have learned about this subject in the last 25 years may be viewed by future generations as a stupendous example of medical research into a major health problem.

The 1989 edition of Heart Facts estimates that more than 60 million people in the USA have systolic and diastolic blood pressures higher than 140/90 mmHg. Helfand[3] estimates that, in 1982, 271 million prescriptions were written for hypertensive drugs in 11 Western countries. Epidemiological studies show that both atherosclerosis and essential (primary) hypertension rank among the most common causes of cerebrovascular, cardiac, and renal pathology. Therefore, study of the underlying causes of high blood pressure is no longer of only academic interest. If we accept that hypertension is a community health problem, learning about hyperten-

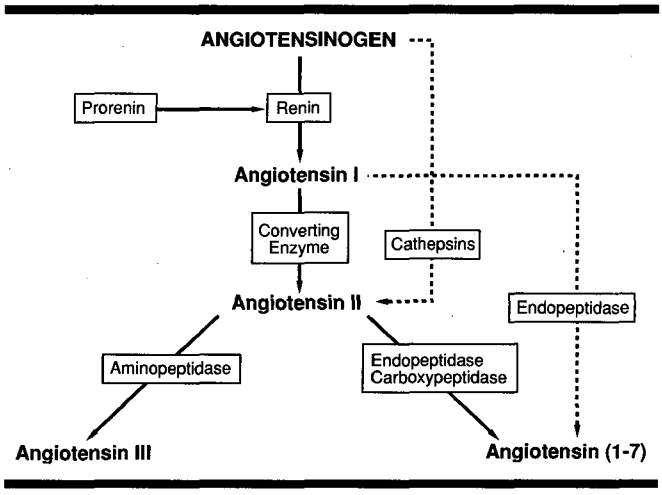

FIGURE 1. The current concept of the biochemical cascade of the renin-angiotensin-aldosterone system includes alternate pathways (broken lines) for the generation of angiotensin II and the heptapeptide angiotensin-(1–7).

sion is a necessity of current medical practice.

This article examines our understanding of probable factors contributing to the pathogenesis of arterial hypertension. On the basis of new findings, scientists are corroborating that the explanation for hypertension may lie in a molecular or cellular fault in the RAS. The emphasis of the research on the RAS is shifting to the brain, because studies of other organ systems, including the heart and the kidneys, do not adequately explain the dependence of hypertension on salt balance and the sympathetic nervous system.

ROLE OF ANGIOTENSIN II IN HYPERTENSION

A resurgence of interest in the role of the RAS in the pathogenesis of hypertensive disorders was triggered by two recent medical developments. First, a new class of antihypertensive agents has shown broad application to the treatment of high blood pressure. These drugs (ACE inhibitors) act by inhibiting the activity of angiotensin-converting enzyme (ACE) and are rapidly altering the prescribing patterns of physicians treating hypertensive patients.[4] Second, applying the techniques of molecular biology to the genes that code for the proteins that direct synthesis of angiotensin II and its processing enzymes showed that the RAS operates in organs other than the kidneys.[5,6] This progress provides a new perspective on the role of the RAS in the regulation of blood pressure and in the pathogenesis of essential hypertension. The new work also suggests that the RAS is a participant in the mechanisms underlying congestive heart failure and peripheral vascular disease.

BIOCHEMISTRY OF THE RENIN-ANGIOTENSIN SYSTEM

The pressor action of angiotensin II is a final event in a series of biochemical steps that are initiated by the proteolytic action of the enzyme renin on angiotensinogen, a glycoprotein produced in the liver. The product formed by the action of renin on its substrate is a 10-amino-acid peptide named angiotensin I. This decapeptide has no known biological activity. ACE converts the initial product of the reaction, angiotensin I, to angiotensin II. ACE (E.C. 3.4.15.1) is a dipeptidyl-carboxypeptidase that circulates in blood but is also present in tissues. ACE is found in the vascular endothelium of the lungs, heart, kidneys, adrenal cortex, testes, and brain.[7] The gene for human ACE has been cloned recently by Soubrier et al.[8] These studies suggest that ACE has only one active site and that the enzyme circulating in the plasma may be derived from post-translational modification of vascular endothelial ACE.[9,10] Angiotensin II is inactivated by aminopeptidases into several fragments. With the notable exception of angiotensin III, it was believed that other products of angiotensin II metabolism have little intrinsic biological action.

New work has led to a revision of this model in several key aspects (*Figure 1*). First, most of the renin circulating in the blood occurs in the inactive (prorenin) rather than the active form. In the kidney and other cell systems, it has been found that prorenin may be secreted by a constitutive pathway, whereas active renin is released from secretory granules.[11] In plasma, active renin represents between 10% and 30% of total circulating renin. This finding provides a potential explanation for the absence of a strong correlation between plasma renin activity and blood pressure.[12] As reviewed by Laragh,[12] normal laboratory processing techniques can cause conversion of prorenin into active renin.

Second, there exist close homologies between the gene structure of renin and that of other aspartyl proteases, such as the cathepsins. Under certain circumstances, aspartyl proteases other than renin can cleave angiotensinogen to produce either angiotensin I or angiotensin II directly. In extrarenal tissues, cathepsin D has been found to cleave angiotensin I.[13] Both cathepsin G and the serine protease tonin may generate angiotensin II directly from angiotensinogen.[14,15] Membrane-bound cathepsins are found in the azurophil granules of polymorphonuclear leucocytes, membranes of red blood

cells, and intracellular granules.[16] Thus, these findings further limit the value of plasma renin activity assays as a marker of the participation of the RAS in hypertension. The hypothetical presence of circulating activators or inhibitors of the conversion of prorenin into active renin might also account for the broad range of renin values recorded in hypertensive subjects.[2]

Third, new data suggest that ACE is not the sole enzyme able to produce angiotensin II from the cleavage of angiotensin precursors. Alternate pathways for the production of angiotensin peptide have been proposed.[17] In addition, ACE metabolizes a variety of vasoactive peptides such as bradykinin, enkephalins, neurotensin, and substance P.[18] Thus, it has been proposed that the therapeutic effects of ACE inhibitors are explained, in part, by blockade of the metabolism of other peptide hormones. This interpretation is substantiated in part by reports suggesting that chronic treatment with ACE inhibitors does not lower plasma angiotensin II.[19] The possibility that the hypotensive effects of ACE inhibitors are due to prevention of the metabolism of bradykinin has been explored by several research groups.[20–22] The evidence to date, however, is not conclusive.

On the basis of investigations that relate the structure of angiotensin II to its physiological actions, researchers discarded prematurely the idea that other congeners of angiotensin II are bioactive.[23] Recent insights into the cellular actions of angiotensins and the molecular identity of angiotensin receptors do not conform with this model. Fragments of angiotensin II, such as the C-terminal heptapeptide angiotensin III and the N-terminal heptapeptide angiotensin-(1–7), show potent biological activity in nonvascular tissues. Angiotensin III is comparable to angiotensin II in augmenting the secretion of aldosterone, eliciting thirst, and exciting the electrical activity of hypothalamic neurons.[24] Studies initiated by Schiavone et al[25] disclosed that there is another angiotensin congener that specifically mimics a fraction of the actions of angiotensin II in the brain. Angiotensin-(1–7) is as potent as angiotensin II in stimulating the secretion of vasopressin but lacks direct pressor or dipsogenic actions.[25,26] The angiotensin-(1–7) peptide contains the first seven of the eight amino acids that constitute the molecular sequence of angiotensin II. The finding that angiotensin-(1–7) is bioactive invalidates the concept that angiotensin receptors have an absolute requirement for an amino acid in the eight-position of angiotensin II.[27] The new data suggest that in the brain both angiotensin I and angiotensin II may constitute precursor molecules for highly selective and potent neuropeptides.[26]

The implication of these findings for the function of other local tissue angiotensin systems has not yet been evaluated. Nevertheless, it is clear that future research in hypertension requires re-examining the biochemical nature of the enzymes in extrarenal tissues that lead to the formation of bioactive angiotensin products. The proposal by Ferrario et al[26] that the angiotensin system comprises a family of peptides derived from the activity of parallel tissue-specific enzymic systems is a concept that merits further investigation. Angiotensin II and smaller angiotensin II fragments may act as neurohumoral regulators in areas of the brain that control blood pressure. Because researchers acknowledge that the phenotypic variation in blood pressure is polygenic, the new work has a potential for enhancing understanding of the pathogenesis of high blood pressure. The underlying mechanism of hypertension may in part reside in the abnormal expression of the genes that code for the enzymes that process angiotensinogen into angiotensin peptides, or alternatively, in the genes that may code for tissue-specific subtypes of angiotensin receptors.

REGULATION OF LOCAL TISSUE FUNCTION BY THE RENIN-ANGIOTENSIN SYSTEM

The RAS has long been considered an exception to other endocrine systems in that the active product is generated in the plasma.[28] Molecular probes have now revealed that most organs contain the genes that encode the proteins of the RAS.[5,6] These discoveries confirm that tissues produce and may even secrete angiotensin peptides. Local tissue RAS may regulate the function of the cell producing the peptide (autocrine function), the activity of neighboring cells (paracrine function), or may be released to affect cells at target sites (endocrine function). Thus, local angiotensin systems now are known to fulfill many of the criteria of classic endocrine systems.[28]

Findings from studies using techniques of molecular biology support the hypothesis that angiotensin II acts as a paracrine hormone. For example, analysis of mRNA extracts from various organs showed that the renin mRNA is present in blood vessels, the myocardium, the adrenals, the brain, and reproductive organs.[5] The potential role of a tissue RAS in the modulation of organ function is a topic of current study in many laboratories.

Cardiac and vascular wall renin-angiotensin system

Both the heart and the blood vessels provide interesting examples of a paracrine RAS. In the isolated rabbit

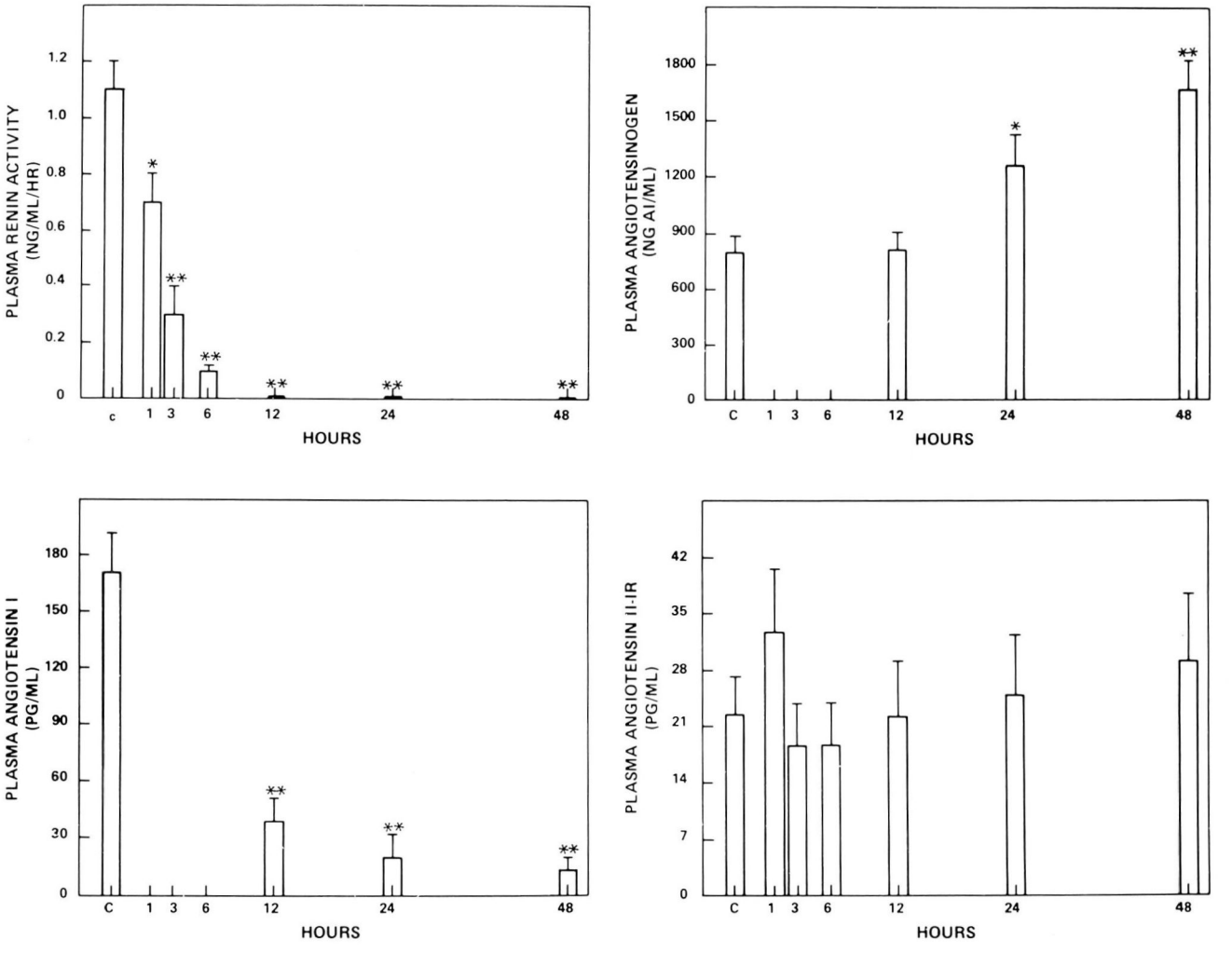

FIGURE 2. Study performed in conscious dogs between 1 and 48 hr after bilateral removal of the kidneys. Values are means ± SE; C = control measurements before nephrectomy. Longitudinal measures of plasma renin activity and concentrations of plasma immunoreactive angiotensin I, angiotensin II, and angiotensinogen reveal that tissues other than the kidneys contribute to circulating levels of these peptides. (From Suzuki et al[30] by permission of the American Heart Association, Inc.)

heart, evidence of a paracrine RAS is demonstrated by the mechanical response associated with ACE inhibition. Sympathetic nerve stimulation increased the heart rate and decreased coronary flow; both of these effects were diminished by pretreatment with the ACE inhibitor Ramipril (HOE 498). Related studies showed that angiotensin I infused through the isolated rat heart was converted to angiotensin II, and that pretreatment with the ACE inhibitor captopril blocked the conversion of angiotensin I to angiotensin II.[29] The functional importance of vascular and tissue-RAS to blood pressure regulation is underscored by the observation that bilateral nephrectomy does not reduce circulating levels of angiotensin II but markedly reduces plasma levels of angiotensin I and renin activity.[30]

As illustrated in *Figures 2* and *3*, the concentrations of angiotensin II in both the plasma and the cerebrospinal fluid (CSF) of the conscious dog remained unchanged 48 hours after nephrectomy. These data underscore the importance of nonrenal RAS in contributing to the circulating levels of angiotensin II in the blood. Nuclear angiotensin II receptors have been identified in vascular

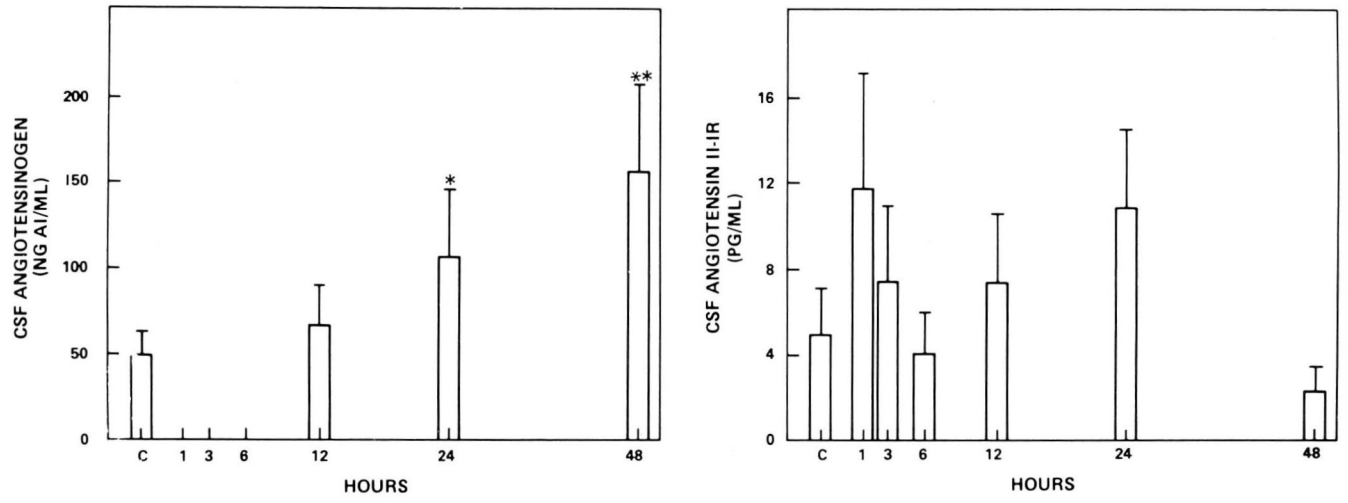

FIGURE 3. Same study as *Figure 2*. CSF angiotensin II in the same dogs remained unchanged from prenephrectomy values, consistent with an independent brain renin-angiotensin system. (From Suzuki et al[30] by permission of the American Heart Association, Inc.)

tissue. These data suggest that angiotensin II may play an important function intracellularly by acting as a growth factor to stimulate synthesis of RNA and proteins.[31] Thus angiotensin II produced intracellularly or incorporated by receptor-mediated endocytosis may be critically involved in the mechanisms that underlie cardiac and vascular restructuring in hypertension.

Renin-angiotenin system in the brain

Because of the blood-brain barrier (BBB), the brain is relatively impervious to the actions of circulating angiotensin II.[32] Nevertheless, angiotensin II circulating in the blood has important neurogenic actions both in the peripheral sympathetic nervous system[33] and at sites of the brain where the BBB is permeable.[32]

In the brain, the endothelium lining the cerebral microvessels does not contain tight junctions in regions that appose periventricular spaces. By virtue of their specific anatomical characteristics, these regions are known as circumventricular organs. Examples are the organum vasculosum of the lamina terminalis, the subfornical organ, and the median eminence in the forebrain. In the lower brain stem, the area postrema is another circumventricular organ that has attracted attention because of its close proximity to the neuronal circuits of the medulla oblongata that subserve cardiovascular function.[32] Angiotensin II receptors exist within circumventricular organs that are intimately involved in the control of blood pressure and fluid volume. Other angiotensin II receptors are contained inside the BBB and are thus not exposed to changes in the circulating levels of angiotensin II.

Recent insights into brain chemistry and advanced imaging techniques demonstrate the presence of angiotensin II binding sites in the region of the medulla oblongata that controls baroreceptor reflexes, breathing, and the neuronal outflow of the bulbar vasomotor neurons.[34] It is not surprising, then, that the existence of a brain RAS has attracted much attention. Moreover, the importance of angiotensin II in the brain as a mechanism for the pathogenesis of hypertension has gained support from several types of experiments. CSF levels of angiotensin II are raised in the renal-hypertensive dog.[35] In the rat with spontaneous hypertension, blood pressure is normalized by CSF infusions of either angiotensin II blockers or ACE inhibitors.[36] Lastly, experimental hypertension is attenuated or prevented by lesions of the brain in areas that contain angiotensin II receptors.[37–39]

The components of the brain RAS have been studied extensively in recent years, and angiotensin peptides have been a particular focus of interest. The renin-substrate angiotensinogen is the principal protein constituent of CSF. Recent molecular biological studies have documented that glial cells contain abundant quantities of mRNA coding for angiotensinogen.[40] The mechanism for the processing and storage of angiotensin II by

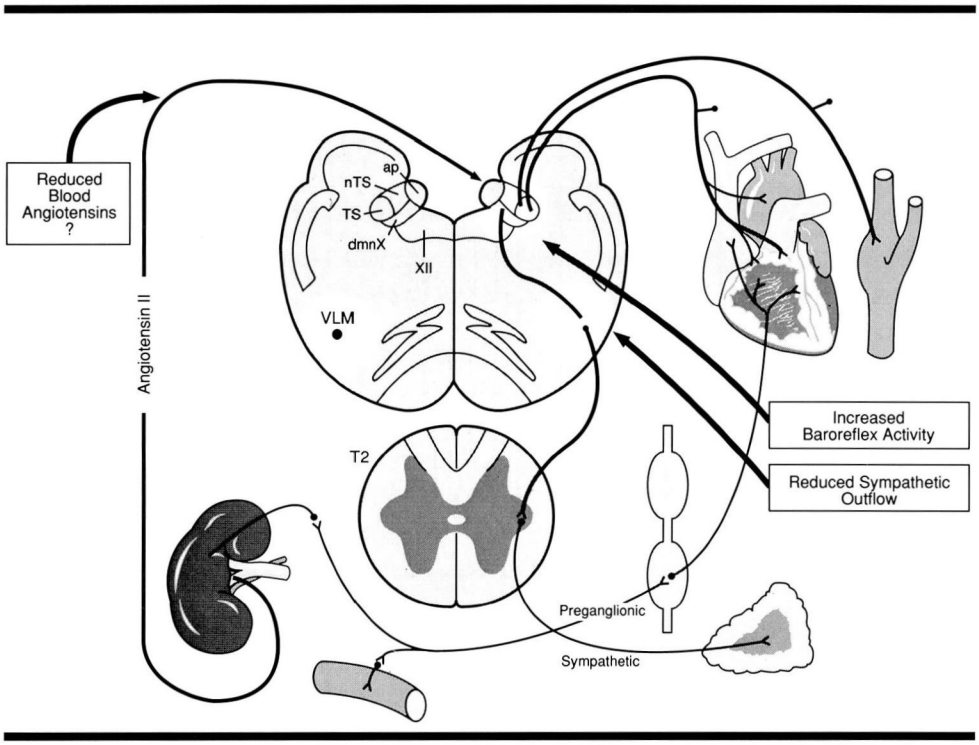

FIGURE 4. Alternative hypothesis to explain the mechanism of action of orally active angiotensin converting enzyme (*ACE*) inhibitors. Blockade of angiotensin converting enzyme activity may lead to normalization of the gain of the baroreceptor reflexes and a decrease in the facilitatory action of angiotensin II on the vasomotor neurons situated in the ventral medulla (*VLM*). Nerve afferent pathways arising from baroreceptor endings in the carotid sinus and aortic arch and performing multisynaptic connections through the nucleus tractus solitarius (*nTS*) into the ventral medulla contain receptors for angiotensin II.[34] Other abbreviations are: *ap* = area postrema; *TS* = tractus solitarius; *dmnX* = dorsal motor nucleus of the vagus; *XII* = hypoglossal nucleus; *T2* = spinal cord at level of second thoracic vertebra.

neurons, however, remains a topic of speculation. Although angiotensinogen mRNA is located primarily in glial cells, immunohistochemical studies show angiotensin II within neurons of various cardiovascular regulatory sites in the brain.[41] In addition, we have found that angiotensin II is released from the brain during application of a solution of CSF enriched with potassium.[42] These data imply that in certain areas of the brain angiotensin II acts as a neurotransmitter.

ROLE OF ANGIOTENSIN II IN HYPERTENSIVE DISEASE

Recent work has significantly broadened our understanding of the physiological functions of angiotensin II and its part in the pathogenesis of essential hypertension. Angiotensin II may have two classes of function. Circulating angiotensin II serves as an endocrine hormone for rapid regulation of cardiac and renal hemodynamics. In the tissues, local production of angiotensin peptides acts as a modulator of cell function, cell growth, or both.

Excesses in the renin-angiotensin-aldosterone axis easily explain the pathogenesis of malignant hypertension and many of the renin-dependent forms of renovascular disease.[12] Less predictable is the connection between the RAS and other forms of hypertension such as primary aldosteronism, hypertension due to oral contraceptives, and essential hypertension. Laragh[12] believes, however, that "all human hypertension fits into a spectrum of abnormal vasoconstriction-volume interactions." Analysis of the RAS suggests that this hormonal system is a long-term regulator of arterial pressure and sodium balance. But it may have a causal or active role in the pathogenesis of a more limited spectrum of hypertension. This idea fits well with the understanding that essential hypertension is not a single entity but is caused by a spectrum of operating mechanisms.

But recent insights into brain chemistry and the molecular biology of the RAS provide a potential avenue for the exploration of new ideas regarding the relation of essential hypertension to abnormalities of central neuronal and neuroendocrine pathways that control regulation of sodium balance, vascular reactivity, and peripheral sympathetic drive.[26]

WHAT IS THE ROLE OF THE BRAIN ANGIOTENSIN SYSTEM IN HYPERTENSION?

We are still far from knowing how angiotensin peptides function in the brain. However, physiological and biochemical studies suggest that one explanation for hypertension lies in a chronic but subtle abnormality of the

brain RAS. This concept is supported by the effects of treatment with inhibitors of the RAS, investigations of the neurogenic actions of angiotensin II in normal and hypertensive animals, and studies of the processes that alter the expression of the peptide in the brain. Converting enzyme inhibition therapy is a good example. Clinical experience with these inhibitors shows that control of blood pressure is not directly related either to the prevailing level of plasma renin activity or the lowering of plasma levels of angiotensin II.[19] Since ACE has few absolute restrictions on its substrate specificity[43], the therapeutic effect of ACE inhibitors may be related to the inhibition of the metabolism of other vasoactive peptides. The role of ACE in the metabolism of bradykinin is just one example. On the other hand, the therapeutic control of blood pressure by ACE inhibitors in hypertensive subjects may be related to their ability to reach the critical sites at which excess production of angiotensin peptides has either a causal or active role in the evolution of hypertension. We favor this possibility.

Many studies indicate that increased sympathetic drive participates in the pathogenesis of high blood pressure.[44] Over the past 18 years investigations at The Cleveland Clinic Foundation have documented and characterized the multiple actions of angiotensin II in the peripheral sympathetic nervous system[33] and in the brain.[45] Accordingly, analysis of the function of angiotensin II in the brain and of the actions of ACE inhibitors in the sympathetic nervous system gives a new insight into the interplay between the RAS and high blood pressure.

The diagram shown in *Figure 4* summarizes the concept. We believe that in hypertension a primary abnormality exists within the neuronal centers of the medulla oblongata that control blood pressure and process the signals for long-term control of body fluid volume and sodium balance. In the dorsal aspect of the medulla oblongata, the structures adjacent to the fourth ventricle integrate neural and hormonal signals that relate to blood pressure. The vasomotor centers reside in the ventral region of the medulla oblongata (VLM).[46]

Angiotensin II acts at neuroreceptors in the area postrema to stimulate sympathetic nerve activity, the secretion of norepineprhine and epinephrine from the adrenal medulla, and the release of neurohypophysial vasopressin.[47] The nucleus of the tractus solitarius borders the area postrema. This nucleus contains the primary synapse of the baroreceptor fibers that inhibit the tonic activity of the vasomotor neurons. Angiotensin II has a dual action in the area postrema-solitarii nuclear complex; it causes increased peripheral sympathetic drive by direct stimulation of vasomotor neurons and inhibition of the baroreceptors.[47] Application of angiotensin II directly into the neuronal vasomotor structures of the VLM also causes increases in blood pressure that are mediated by enhanced peripheral sympathetic drive.[48] Thus, neuronal actions of angiotensin II that augment peripheral sympathetic drive may be related to the neurogenic facet of essential hypertension.[49]

Since the regions of the brain most sensitive to the actions of either blood-borne or intracranial angiotensin II are highly vascularized, this represents a relatively unrestricted route for the antihypertensive action of drugs that interfere with the systemic or local production of the peptide.

Recent studies suggest that ACE inhibitors gain access to circumventricular organs of the brain and bind to neural elements that contain ACE.[50] Santos et al[51] found that a single intravenous dose of enalaprilate results in the selective inhibition of ACE activity in the nucleus of the tractus solitarius. In humans, Ibsen et al[52] have shown that ACE inhibitors increase the sensitivity of the baroreceptor reflex. Therefore, one way ACE inhibitors may control blood pressure is by their effects at the site where angiotensin II modulates baroreflex transmission and regulates activity of the bulbar vasomotor neurons that command sympathetic efferent drive.[48]

It is thus possible that further exploration of the metabolic pathways and physiological actions of angiotensin peptides in the brain may answer the central question of participation by angiotensin II in the pathogenesis of essential hypertension.

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