Neurogenic Actions of Angiotensin II

CARLOS M. FERRARIO, M.D.

SUMMARY It has become increasingly evident that blood-borne angiotensin II has major effects upon brain cardiovascular centers. With the discovery of an angiotensin I-forming enzyme or isoenzymes in the central nervous system of mammals, alternative concepts have emerged regarding the role of this peptide in the regulation of central adrenoreceptor activity, pituitary function, and hydromineral metabolism. These concepts are reviewed, and a framework for future research is suggested by the author. (Hypertension 5 (supp V): V-73-V-79, 1983)

KEY WORDS • arterial hypertension • angiotensin II • cerebrospinal fluid • neuropeptides • blood/brain barrier • baroreceptor reflexes

In the search for primary mechanisms of chronic hypertension, the focus has been on identifying possible humoral stimuli for progressive arterial constriction. Until recently, neurogenic factors have been thought to play a permissive role, at most. This is surprising, if we consider that the site of action of commonly used antihypertensive agents is to be found in the autonomic nervous system. In addition, an ever increasing number of studies indicate that enhanced neurogenic activity may be more critical than has been appreciated in the pathogenesis of some forms of hypertension. For example, the concentration of norepinephrine (NE) in the plasma and cerebrospinal fluid (CSF) of patients with primary hypertension may be above normal levels. Other investigators have shown that the height of the systolic blood pressure correlates with the concentration of NE in the CSF compartment. From experiments in animals, we have learned that surgical or chemical interruption of cardiovascular pathways either effectively prevents the development of high blood pressure, or alternatively, causes a remission of the hypertensive process. Last, but not least, delivery of either adrenolytic compounds or blockers of the renin-angiotensin system into a brain ventricle produces antihypertensive effects. These studies therefore indicate that autonomic mechanisms of blood pressure regulation may either coparticipate or be the cause of elevated vascular resistance, a hallmark of the hypertensive process. Although the disregulation of the neurogenic control mechanism in hypertension may have various causes and occur at multiple sites of the neuroaxis, converging evidence from several disciplines suggests that the increase in sympathetic activity could in part be related to the known properties of the hormone angiotensin II to facilitate the function of the neurogenic control system.

Aside from the lucid and convincing demonstration by Braun-Menendez et al. that angiotensin II can augment the release of catecholamines by the adrenal medulla, other effects of the parent hormone upon the autonomic nervous system remained unrecognized for another 20 years. In 1962, an interrelationship between the peripheral autonomic nervous system and angiotensin II (AII) was found by the observations that the vasoconstrictor action of the peptide was reduced by sympathectomy. Later experiments indicated that the most probable explanation of this phenomenon was that angiotensin II acts on the peripheral sympathetic...
nervous system to intensify cardiovascular responsiveness to efferent sympathetic discharges. It is now known that the facilitation of adrenergic neurotransmission by All at presynaptic sites is by way of increasing the release of NE molecules per nerve impulse, by inhibition of uptake and by a combination of both mechanisms.

Although the effects of All alone and in conjunction with changes in the concentration of the sodium ion are important in modulating the adrenergic dynamics at the neuroeffector site, this is not the only way by which All can regulate the adrenergically mediated effects on vascular smooth muscle. In 1961, Bickerton and Buckley noticed that injections of All into the isolated circulation of a dog's brain caused increased central sympathetic vasmotor discharge. Because the technique employed by these investigators has certain perversities and the rises in arterial pressure were elicited at concentrations higher than those that could be achieved under "condition" of disease, their early work remained relatively ignored for almost a decade. Several laboratories have now provided proof for multiple actions of All upon central nervous system (CNS) structures. These discoveries indicate that the CNS actions of All may comprise an important facet of the neuronal and neuroendocrine regulatory mechanisms engaged in the control of cardiovascular function and sodium and water homeostasis. The actions of All upon CNS structures also spurred interest into the possibility that brain All receptors may be normally exposed to the peptide formed locally. The occurrence of either renin or an isorenin in the brain and of angiotensinogen and AI converting enzyme in the brain and CSF is now well established, although questions remain as to the source of their biosyntheses. From what has been learned thus far, we cannot say, however, whether the principal physiological effects of All are due to either the generation of the peptide in brain parenchyma, or the passage of the circulating hormone into the extracellular spaces of the brain via the blood brain barrier (BBB) or the CSF, or a combination of both. Convincing arguments for and against each of these two possibilities are presented here and elsewhere. This evidence will be summarized, alternative explanations outlined, and new research directions suggested.

Cardiovascular Actions of All Administered into the Central Nervous System

In theory, putative brain receptors involved in the expression of the cardiovascular action of All may be stimulated by the arrival of the peptide to the receptive site via either the CSF or the bloodstream. In the first case, the hormone enters the intercellular spaces of the brain parenchyma through the endothelial lining constituting the CSF brain barrier; in the other case, All reaches receptors via functional "gaps" in the BBB. While there is evidence for both routes of approach, the second rather than the first may be of physiological significance.

Infusion or injection of All into either a brain ventri-
angiotensin into the vertebral arteries; in parallel studies, we were able to block the All pressor response reversibly by local cooling of the structure.46, 48 Our recent studies have shown that the area postrema contains an All-sensitive pressor mechanism.51, 52 that the magnitude of the pressor response is modulated by the endogenous brain opioid system,53 and that this structure acts to increase the intrinsic activity of bulbospinal sympathetic vasomotor neurons via a neuronal pathway originating in the area postrema itself.52, 54, 55 The demonstration that, in conscious trained dogs, surgical inactivation of the area postrema caused mild hypotension and reduced "spontaneous hemodynamic variability" established that this area of the lower brain stem is involved in the regulation of cardiovascular function.52 In these experiments, Ferrario et al.52 also found that the removal of the area postrema caused a significant suppression of the pressor response due to intravenous injection of the peptide, an indication that the vasoconstrictor effects of circulating All are in part mediated by activation of central neurogenic mechanisms. While it is not yet known what is the importance of the area postrema pressor pathway with regard to the development of experimental hypertension, preliminary studies in conscious dogs during the development of the one-kidney, one clip hypertension model are encouraging.5 Confirmation of these initial findings would provide strong support to the concept of increased central neurogenic drive as a factor important to the development of experimental renal hypertension.4 Taking into account that Suzuki et al.56 have detected for the first time in conscious dogs that there are significant and parallel increases in the concentrations of NE and All immunoreactivity in both blood and CSF during the early stages of two-kidney, one clip hypertension, pertinent evidence for an interaction between the renal pressor system and CNS mechanisms of cardiovascular control has now been obtained.1, 14

The selective sensitivity of the vertebral artery circulation to All has been confirmed in conscious dogs.43 These investigations also showed that the increase in sympathetic nerve activity produced by intravertebral All was associated with suppression of plasma renin activity (PRA) but no change in the plasma concentration of vasopressin, corticosterone, or ACTH.43 There is suggestive evidence in rats that removal of the area postrema elicits a greater consumption of a NaCl solution.57 Although similar studies have not been performed in the dog as yet, Wise and Ganong48 observed that lesions of the area postrema did not cause electrolyte abnormalities or changes in urine flow and/or concentrating ability in this species. On the other hand, removal of the area postrema interfered with the ability of the kidneys to increase urinary sodium excretion following an intravenous load of hypertonic sodium chloride.58

Via the Carotid Circulation

The few studies that have investigated whether All can elicit a central neurogenic response via its administra-

tration into the circulation of the dog's carotid artery showed that the elevation in arterial pressure is of a magnitude not greater than that elicited via similar amounts of the peptide given intravenously.53 In the rat, however, the pressor response produced by intracarotid administration of All is significantly greater than that produced intravenously,26 and the application of an electrolytic lesion in the periventricular tissue of the anteroventral third ventricle (AV3V) abolished the enhanced sensitivity.59 There appear to be significant differences between rats and dogs, since in one recent study we have found that, in AV3V-lesioned dogs, the pressor response due to intracarotid infusion of the peptide remains unaffected while the pressor response due to injection of All directly into the third ventricle was significantly blunted.60 In conscious dogs, intracarotid administration of All suppressed PRA, and in contrast to the effects obtained via the vertebral artery route, large doses of All stimulated release of vasopressin (VP) and ACTH.43

From the evidence discussed in the preceding paragraphs it can be concluded that the predominant effect of the All pressor sensitive pathway at the level of the area postrema is to cause changes in heart rate and vascular tone via an action upon sympathetic bulbospinal preganglionic neurons. On the other hand, the stimulatory effects of the peptide upon forebrain regions supplied by the carotid arteries appear to elicit thirst and also cause the release of pituitary hormones into plasma. There is reason to believe that either the subfornical organ41, 42 or another structure (median eminence43) contained within or in the vicinity of the AV3V region is a receptive site for the central action of All following its administration via the carotid arteries. Because All causes a preferential activation of sympathoneuronal mechanisms at the level of the area postrema while at sites in the forebrain the predominant effects are to stimulate neurosecretion and the release of pituitary hormones,44 changes in the blood concentration of the peptide may assume a powerful role in exerting both short-term (sympathoneuronal) and long-term (hormonal) effects upon the blood pressure regulatory mechanism.

Via Cerebrolateral Ventricles

Many studies have employed this approach to evaluate the functions of All upon neuronal and hormonal mechanisms. The rat has been used most commonly, and the findings are reviewed extensively elsewhere.26, 29, 31, 40, 59 We shall briefly discuss here what has been found in other species; differences rather than similarities will be highlighted. In the midbrain of both cats41 and dogs,25, 42 there are other areas which respond to angiotensin by causing a rise in blood pressure. The phenomenon can be elicited by direct application of the peptide into either the lateral cerebral ventricle or by bathing the third ventricle or the cisterna magna with All. In contrast with the response elicited via the vertebral arteries, larger doses are needed to cause pressor responses via this route. In the dog,25, 65 100 to 200 ng of All are required to produce rises in arterial pressure.
comparable to those caused by intravertebral All (average dose: 3.0 ng/kg/min). Characteristically the rises in pressure are slow in onset, have a short lasting plateau, and the afterresponse is very prolonged. Increases in pressure are due to a rise in peripheral resistance, but, in contrast with the pressor response following intravertebral infusion, cardiac rate may be reduced. These hemodynamic characteristics suggest that increased sympathetic nerve activity is not the only factor accounting for the rise in arterial pressure. In rats, combined pharmacological sympathectomy and adrenalectomy affect the early but not the late component of the pressor response; the latter component may be due to increased release of vasopressin, perhaps via an action of All upon brain prostaglandins. Moreover, in rats with hereditary hypothalamic diabetes insipidus, the increases in pressure due to i.v.t. All are markedly blunted. Additional experiments have shown that central adrenoceptor mechanisms are involved in mediating the cardiovascular response to All given via a brain ventricle since central depletion of catecholamines causes a significant attenuation of the pressor effects and, conversely, i.v.t. All alters brain catecholamine activity. It is also pertinent to note that similar responses are produced by the i.v.t. administration of renin.

It is not clear at present whether the site at which All acts to cause a rise in blood pressure via the i.v.t. route involves the same neural structure responsible for increased thirst and/or release of hypophysial hormones. Both the SFO and the organum vasculosum of the laminae terminalis (OVLT) are favored. The studies of Brody and colleagues and Miselis et al. indicate that a variety of important elements are contained within the boundaries of the AV3V region, namely, the OVLT, the subnucleus medianus (NM), projections to and from the SFO, and even the most rostral part of the anterior hypothalamus. Many of these nuclei appear to be involved in the regulation of water and salt metabolism as well as having a modulatory influence upon central vasopressinergic pathways.

Considering that these periventricular nuclei subserve key functions as regulators of neuroendocrine hormones, we are not surprised that angiotensin could have a multiplicity of effects at this level. Comparison of current findings in rats and dogs again suggests species differences. In the rat, the OVLT may be a receptive site for the centrally mediated effects of All while in the dog the effects of blood-borne All upon water intake are mediated by the SFO; the OVLT may act as a site for the stimulatory action of the peptide upon hypothalamic neurosecretory pathways that activate release of ACTH and VP.

Our experiments and also those reviewed by Schoelkens et al. indicate that the NM and the OVLT are receptor sites that can be stimulated by All released and/or secreted into the CSF. Because CSF may serve as a route of transport for peptides from one area of brain to another, the effects of CSF All may differ drastically from those recorded via the bloodstream. Many studies from the field of neurobiology have shown that there is a marked difference in effects produced by intraventricular as opposed to intravenous administration of peptides. Since it is known that peptides injected into the CSF may rapidly enter blood via absorption into the superior sagittal sinus at the arachnoid villi (see fig. 1), extreme precaution ought to be taken in interpreting data via this route. Pardridge has reviewed the limitations of this ap-

**FIGURE 1.** Distribution of horseradish peroxidase (0.5% in 0.1 ml) given into either a lateral ventricle (i.v.t.) (top) or the cisterna magna (CM) (bottom). Horseradish peroxidase (HRP) given i.v.t. showed some penetration into the parenchyma immediately adjacent to the ventricular surface, and the HRP product was also observed to penetrate the brain stem from its ventral surface. Heavy labelling, present in the walls of the third ventricle, can also be seen to follow the perivascular spaces in the ventral aspects of the brain stem. More intensive labelling of the ventral brain stem and perivascular spaces follows injection of HRP into the CM. However, in the ventricular system labelling was restricted to the ependymal layer. Note that cortical tissue was minimally labeled after i.v.t. injection and showed no label with CM administration. Data from Chernick et al. Not shown here, HRP was not seen to penetrate into the area postrema when given into the CSF by either route (see ref. 88). Arrow in the top figure shows part of the i.v.t. cannula track. Horizontal bar in bottom figure = 5 mm.
proach and also concluded that the rapid clearance of 
CSF should be evaluated in experiments in which ex-
tra-CNS peptide action is studied following ventricular 
peptide administration. Passaro and co-workers97 have 
shown that cholecystokinin (CCK) and other peptides 
enter plasma in rabbits with a half-time of 13 minutes 
following ventricular administration via a bolus injec-
tion. Kawano and I (unpublished observations) ob-
served that the vasculotropic actions of arginine va-
sopressin given intravenously to anesthetized dogs 
were blocked 40 to 60 minutes after intraventricular 
administration of a competitive vasopressin antagonist 
\[d(CH_2)_5Tyr(Me) AVP\]. These studies emphasize the 
importance of measuring plasma peptide levels in any 
study that seeks to evaluate the central actions of a 
peptide after central administration. Until these pre-
cautions are taken the interpretation of results obtained 
via delivery of All into the CSF remains suspect.

Framework to Evaluate Future Research

The evidence presented thus far indicates that blood-
borne All does affect CNS autonomic and endocrine 
functions. In our view, most but not necessarily all of 
the known central effects of this peptide can be ex-
plained by the penetration of All into the fluid matrix 
of the brain via specialized conduits either the BBB or 
the blood-CSF barrier. It is possible, however, that 
some of the effects observed after the acute introduc-
tion of the peptide into the CSF only mimic the intrinsic 
action of locally synthesized All in the brain. The 
following observations support this contention: 1) the 
biochemical identification of the key proteins needed 
to form All in brain tissue and CSF;31, 36, 80, 81 2) the 
localization of specific All binding sites in various 
parts of the CNS;82 3) the histological visualization of 
nervous fibers and cells displaying All-like immuno-
reactivity;87 and 4) the effects of cerebroventricular 
injection of renin, converting enzyme inhibitors, or 
All competitive antagonists upon the arterial pressure 
and the sympathetic and endocrine functions of normal 
animals and those with either acquired or genetic forms 
of hypertension. Of these various lines of evidence, the 
biochemical findings argue strongly but not conclusi-
vely for the existence of an extrarenal isorenin angio-
tensin system in the brain of mammals, including hu-
mans. In this respect, the observations that: 1) brain 
angiotensinogen shows molecular heterogeneity with 
respect to the plasma counterpart,37 2) in the dog, the 
immunohistochemical characteristics of the isorenin en-
zyme are distinct from the enzyme of kidney origin,36, 83 
and 3) extended molecular forms of AI can be 
formed in the CSF are decisive, but not yet conclu-
sive, steps toward proving the existence of a biosyn-
thetic pathway for the formation of angiotensin pep-
tides in the CNS.

Other approaches have significant, albeit not always 
recognized limitations. An appreciation of this prob-
lem requires an understanding of some basic aspects of 
the transport of substances into the microenvironment 
of the brain itself. Because the BBB acts as a second 
order cell membrane, molecules gain access to the 
brain interstitium by only one of two mechanisms: 1) 
lipid-mediation or free diffusion through the endotheli-
al plasma membrane; and 2) carrier mediation or trans-
port via specific, enzyme-like carriers located within 
the endothelial lumen and antiluminal membranes.76 The 
second mechanism may be pertinent for the transport 
of All through the BBB. Peptide transport between 
blood and brain can also occur via circumventricu-
lar organs that are structurally characterized by 
low resistance tight junctions.85 According to Crone,66 
the surface area of capillaries perfusing the CVOs is at 
least 5000-fold smaller than the surface area of the 
vessels comprising the BBB. Thus, diffusion of pep-
tides into the interstitium of the brain via CVO is both 
nonspecific and slow, with a half-time on the order of 
several hours.78 In other words, the absence of a BBB 
at a CVO only allows circulating peptides to quickly 
reach the plasma membrane of neuronal elements 
located at, or terminating in, the structure but also 
permits the peptides to slowly efflux into the CSF after 
diffusing into either adjacent or distal brain regions. 
Convection due to bulk flow of CSF, and the perme-
ability of the blood CSF barrier, may determine the site 
at which a peptide entering the interstitium of the brain 
via CVO effluxes into the CSF. According to Par-
dridge,86 this is how all substances in plasma eventually 
gain access to CSF. The mechanics of the formation, 
absorption, and circulation of CSF are now understood; 
further work in this area can help to disen-
tangle the riddle about the nature of the cardiovascular 
and neuroendocrine function of cerebroventricular 
All.

If blood All can diffuse via CVO into the intersti-
tium of the brain and then into the CSF, histochemical 
and pharmacological evidence of recognition and 
binding sites cannot be used as a proof for the presence 
of an endogenous brain renin-angiotensin system. The 
occurrence of neuroreceptors in the CNS provides a 
solid tenet to the contention that All acts in the brain 
to modulate cardiovascular and neuroendocrine function, 
but it does not exclude the possibility that these recep-
tors are there to couple with the peptide transported 
from blood to the brain parenchyma. Rapidly evoked 
responses due to increases in the blood concentration 
of All indicate clearly that the peptide is generating a 
metabolic response by its action upon either the CVO 
or, alternatively, upon specific receptors located in the 
luminal side of the BBB. The latter mechanism has 
been shown to account for the sequestration of insulin 
by brain capillary endothelia receptors.87 On the other 
hand, slowly produced biological responses may re-
fect the process of entrance and diffusion into recep-
tive sites either adjacent or distal to the anatomical 
location of the CVO.

This information does not belittle the plausibility for 
the local formation of the hormone by an independent 
brain renin-angiotensin system. It suggests, however, 
that further work will be needed before we can disen-
tangle those brain receptive sites and mechanisms that 
are influenced by the release of All into the blood, 
from those that will be stimulated only by the local
formation of the peptide within the brain parenchyma. While we continue to search for a meaning to this riddle, it cannot be forgotten that the accrued information argues strongly for a role of blood-borne AII as a modulator of CNS regulatory mechanisms of cardiovascular and neuroendocrine functions.

Acknowledgments
This review is dedicated to Dr. James W. McCubbin who, during his professional life, provided the most penetrating early experimental analysis of the neurogenic actions of AII.

References
42. Ferrario CM, Barnes KL, Berto GA: Cardiovascular Effects on Angiotensin II Mediated by the Central Nervous System. Amsterdam: Excerpta Medica, 1977, p 50
ANGIOTENSIN II AND BRAIN MECHANISMS/Ferrario

52. Ferrario CM, Barnes KL, Sztalgyi JE, Brosnihan KB: Physiological and pharmacological characterization of the area postrema pressor pathways in the normal dog. Hypertension 1: 235, 1979
65. Chernicky CL, Barnes KL, Michelini L, Ferrario CM: Horse-dash peroxidase (HRP) injected in the cerebrospinal fluid (CSF) of the dog shows a penetration in the ventral brain stem (abstr). Neurosci Abstr 8: 325, 1982
71. Reid IA, Ramsay DJ: The effects of intracerebroventricular administration of renin on drinking and blood pressure. Endocrinology 97: 536, 1975
86. Stone C: The blood-brain barrier — facts and questions. In Ion Homeostasis of the Brain, edited by Sieges JK, Sorensen SC. Copenhagen: Munksgaard, 1971, p 52
88. Chernicky CL, Barnes KL, Michelini L, Ferrario CM: Horse-dash peroxidase (HRP) injected in the cerebrospinal fluid (CSF) of the dog shows a penetration in the ventral brain stem (abstr). Neurosci Abstr 8: 325, 1982
Neurogenic actions of angiotensin II.
C M Ferrario

*Hypertension*. 1983;5:V73
doi: 10.1161/01.HYP.5.6_Pt_3.V73

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/5/6_Pt_3/V73

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org//subscriptions/