Independence of the Central Nervous and the Peripheral Renin-Angiotensin Systems in the Dog

M. G. Nicholls, M.D., F.R.A.C.P.

SUMMARY What regulates the activity of the central nervous renin-angiotensin system is not known. To define whether control of this central system is linked to that in the periphery, simultaneous blood and cerebrospinal fluid (CSF) samples for measurement of immunoreactive angiotensin II were drawn from anesthetized dogs during hemorrhage, furosemide-induced volume depletion, insulin-hypoglycemia, beta-adrenergic blockade and saline infusion. Despite rigorous increments or decrements in plasma immunoreactive angiotensin II, CSF levels remained stable. Since immunoreactive angiotensin II in dog CSF is claimed to be mainly the heptapeptide des-Asp^angiotensin II (angiotensin III), the possibility that the level of this peptide within CSF simply reflects plasma concentrations was assessed by infusing angiotensin III (2.5 and 25 ng/kg/min intravenously, each for 60 minutes) and monitoring plasma and CSF peptide levels. Whereas plasma immunoreactive angiotensin II levels increased appropriately across the infusions, no change in CSF levels was observed. These studies indicate that angiotensin III does not cross the blood-CSF barrier, at least in the short term. (Hypertension 1: 228-234, 1979)

KEY WORDS • angiotensin II • renin regulation • hypertension • cerebrospinal fluid

CONSIDERABLE attention has recently been focused on the presence of the renin-angiotensin system within the central nervous system. Although its physiological functions are not fully clarified, this system may contribute to the regulation of thirst,1-8 antidiuretic hormone secretion,5, 6, 7 ACTH production,6 and to the control of blood pressure.8-13 There is evidence that the renin-angiotensin system within the central nervous system might be involved in the pathogenesis of some forms of hypertension in animals13-17 and in man.18

While the physiological and pathophysiological importance of this system is being vigorously researched, the question of what regulates its activity has received less attention. The current study therefore set out to determine whether there was any parallelism in the control of the peripheral and the central nervous renin-angiotensin systems. Further, since immunoreactive angiotensin II in dog cerebrospinal fluid (CSF) may in fact consist largely of the heptapeptide des-Asp^angiotensin II, the possibility that this peptide within CSF might simply reflect concurrent plasma levels was explored.

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Supported in part by the Michigan Memorial Phoenix Project.

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Materials and Methods

All experiments were carried out in male mongrel dogs weighing 19.1-35 kg. Each dog was used on only one occasion. Fluid and dietary intake were not restricted before the experimental day. Anesthesia was induced with sodium pentobarbital (30 mg/kg I.V.) and maintained with additional amounts of the barbiturate as required (7.5 mg/kg every 2 hours in most cases). Two cannulae were inserted into peripheral leg veins, one for infusion, the other for blood withdrawal. With the dog's head held in flexion, a 17-gauge needle (Portex Epidural Minipacks) was introduced into the cisternal space under strictly sterile conditions. Clear CSF, uncontaminated by blood, could be aspirated rapidly, and in many cases streamed under pressure from the cisternal needle. In the few instances that blood-contaminated CSF was obtained, the dog was excluded from the study. A 45-minute stabilization period prior to blood and CSF sampling was observed after insertion of the cisternal needle. As a routine, two "basal" CSF and blood samples were obtained for analysis before the experimental intervention. At each CSF sampling time the needle dead space was cleared, 0.95 ml of cisternal fluid was drawn over 3-5 seconds into 0.05 ml enzyme inhibitor solution20 in a pre-chilled 1-ml syringe, and the contents were mixed and stored at —20°C. The cisternal needle was plugged after each sample was obtained. Simultaneous blood samples for the measurement of immunoreactive angiotensin II were drawn into pre-
chilled 5-ml syringes containing 0.25 ml enzyme inhibitor solution, the plasma separated by centrifugation at +4°C and stored at −20°C. In some experiments blood for the measurement of plasma renin activity (PRA) and serum sugar was drawn along with that for immunoreactive angiotensin II.

Angiotensins II and III

Angiotensins II and III were administered intravenously to determine whether either peptide was capable of penetrating into the CSF from plasma.

Two dogs received an incremental I.V. infusion of angiotensin II (Val^8-angiotensin II, Hypertensin, Ciba) at 2.5, 5, 20, and 80 ng/kg/min, each rate lasting 1 hour. Simultaneous blood and CSF samples for measurement of immunoreactive angiotensin II were drawn before peptide administration (two samples), at the completion of each infusion rate, and 30 and 60 minutes after the final infusion.

Four animals were infused with angiotensin III (Pearce, Rockford, Illinois) at rates of 2.5 and 25 ng/kg/min each for 1 hour, with simultaneous blood and CSF sampling as described for the angiotensin II studies except that only one post-infusion sample was drawn (at 30 minutes).

Manipulations of Peripheral Renin Production

Manipulations of peripheral renin production were carried out to document whether the peripheral and central nervous renin-angiotensin systems were under parallel control.

Furosemide stimulation, then propranolol and saline inhibition of peripheral renin was performed in five animals with measurements of CSF and plasma immunoreactive angiotensin II and PRA responses. After a 45-minute stabilization period following insertion of the cisternal needle, a bolus of furosemide (10 mg/kg body weight, I.V.) was administered and simultaneous blood and CSF samples withdrawn at 20, 30 and 60 minutes. Propranolol 0.6 mg/kg over 2-3 minutes and 500 ml of 0.9 normal saline over 15 minutes were then infused intravenously, and further CSF and blood samples obtained at 30, 60 and 120 minutes.

Hemorrhage was induced in three dogs by removal of venous blood. The blood volume withdrawn (20 ml/kg) over a 10-15-minute interval was well in excess of that required to stimulate plasma renin levels in pentobarbital-anesthetized dogs. A smaller hemorrhage (5 ml/kg) was induced at 2 or 3 hours after the major bleed to ensure extreme activation of the peripheral renin-angiotensin system. Blood and CSF samples were obtained prior to the initial hemorrhage (two basal samples), then at 30 minutes, 1, 2, 3, 4 and 5 hours.

Hypoglycemia was induced in five dogs with the bolus injection of regular insulin (0.2 units/kg body weight, I.V.). Blood for measurement of serum sugar, PRA, and immunoreactive angiotensin II, and CSF samples were drawn before the insulin injection and again at 15- to 60-minute intervals for 4 hours.

Immunoreactive angiotensin II was measured in plasma and CSF by radioimmunoassay after ethanol precipitation of proteins. For both plasma and CSF, 0.4 ml in duplicate were analyzed, and all samples from any one animal were run in a single assay. Although the antibody was raised to Val^8-angiotensin II, standard curves (5-500 pg/tube) utilizing Ileu^5-angiotensin II, and the heptapeptide (des-Asp^1-angiotensin II) and hexapeptide (des-Asp^1, Arg^2 angiotensin II) fragments were virtually superimposable with the standard curve (5-500 pg/tube) for angiotensin II, indicating 100% cross-reactivity with the peptides. Results reported using this assay for both CSF and plasma, reflect “immunoreactive angiotensin II” values (the octapeptide along with the above peptides) rather than levels of angiotensin II alone. The possibility that other unknown lipophilic peptides in plasma and CSF might also cross-react with the antibody and thus contribute to immunoreactive angiotensin II levels cannot be excluded. The immunoassay standard curve utilized 5-500 pg/tube Ileu^5-angiotensin II (International Research Standard number 70/302 provided by the Medical Research Council, Holly Hill, London) which was spiked into an artificial plasma solution and carried through all steps of the assay, and I^125-labeled angiotensin II purchased from New England Nuclear, Boston, Massachusetts.

Plasma renin activity was measured by radioimmunoassay using New England Nuclear kits, and serum sugar was analyzed by a glucose oxidase method.

Results

From a total of 19 experiments in separate dogs, “basal” (pre-experimental) plasma immunoreactive angiotensin II levels were higher (70.1 ± 11.2 pg/ml, mean ± sem) than in simultaneously drawn CSF samples (40.5 ± 5.7 pg/ml). In only two dogs was the level higher in CSF than in plasma.

Angiotensin Infusions

Angiotensin II infusion in two dogs at 2.5, 5, 20 and 80 ng/kg/min resulted in stepwise increments in plasma immunoreactive angiotensin II but no change in CSF levels (fig. 1).

Angiotensin III administration likewise did not result in significant alterations in CSF immunoreactive angiotensin II, whereas the expected increases in plasma levels were observed (fig. 2).

Manipulations of Peripheral Renin Production

Furosemide stimulation (10 mg/kg) of PRA and plasma immunoreactive angiotensin II was followed by suppression with propranolol (0.6 mg/kg I.V.) and saline (500 ml) in five animals. On average, PRA values increased more than threefold and plasma immunoreactive angiotensin II rose fourfold after furosemide, then returned to or below baseline levels with propranolol-saline suppression (fig. 3). In con-
trast to these wide fluctuations in the activity of the peripheral renin-angiotensin system, CSF immunoreactive angiotensin II varied little in individual animals, and no pattern of response was observed in the group as a whole (fig. 3).

Hemorrhage of 20 ml/kg initially and 5 ml/kg 3 or 4 hours later induced vigorous PRA and plasma immunoreactive angiotensin II responses in each of the three dogs, but CSF immunoreactive angiotensin II levels remained generally steady throughout (table 1). One animal (Dog 3, table 1) had higher immunoreactive angiotensin II levels in CSF than in plasma for the prehemorrhage (basal) samples.

Hypoglycemia evoked a clear-cut increment in PRA and plasma immunoreactive angiotensin II in all of five dogs, but only minor fluctuations in CSF immunoreactive angiotensin II levels without discernible pattern were observed in four animals (fig. 4). The fifth animal, however, exhibited a fourfold increase in CSF immunoreactive angiotensin II in response to
TABLE 1. Plasma Renin Activity (PRA, ng/ml/hr) and Plasma and CSF Immunoreactive Angiotensin II (AII, pg/ml) Levels Before (Basal) and After Hemorrhage in Three Dogs

<table>
<thead>
<tr>
<th>Time after hemorrhage</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRA</td>
<td>Plasma AII</td>
<td>CSF AII</td>
</tr>
<tr>
<td>Basal 1</td>
<td>3.5</td>
<td>68</td>
<td>38</td>
</tr>
<tr>
<td>Basal 2</td>
<td>3.6</td>
<td>60</td>
<td>29</td>
</tr>
<tr>
<td>30 mins</td>
<td>8.7</td>
<td>231</td>
<td>37</td>
</tr>
<tr>
<td>1 hr</td>
<td>7.4</td>
<td>217</td>
<td>15</td>
</tr>
<tr>
<td>2 hrs</td>
<td>9.0</td>
<td>252</td>
<td>24</td>
</tr>
<tr>
<td>3 hrs</td>
<td>7.2</td>
<td>151</td>
<td>26</td>
</tr>
<tr>
<td>4 hrs</td>
<td>9.7</td>
<td>326</td>
<td>32</td>
</tr>
<tr>
<td>5 hrs</td>
<td>7.7</td>
<td>271</td>
<td>43</td>
</tr>
</tbody>
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hypoglycemia (fig. 5). The peptide response in CSF appeared to precede that in plasma. It is of possible importance that serum sugar levels fell to a lower nadir (32 mg/100 ml) in this latter experiment than in the other dogs, although no convulsive movements were induced.

In all but one of the above experiments, immunoreactive angiotensin II levels in CSF were altered minimally or not at all by maneuvers that resulted in major changes in the activity of the peripheral renin-angiotensin system. The possibility that angiotensin III was being formed within the central nervous system in response to some or all of the above experiments or was penetrating the blood-CSF barrier but was rapidly destroyed by angiotensinases within the CSF, was tested. For this purpose 10 ml of CSF were obtained by cisternal puncture from each of four dogs, angiotensin III (150 or 200 pg/ml) was added, and the samples placed in a 37°C water bath. At 30- or 60-minute intervals over the next 4 hours and again at 18 or 24 hours, 1-ml aliquots were withdrawn into the enzyme inhibitor solution and stored at −20°C prior to assay for immunoreactive angiotensin II. In three of the four experiments immunoreactive angiotensin II levels fell gradually over 4 hours from a mean of 298 pg/ml to 231 pg/ml, and finally after 18–24 hours of 37°C incubation, to 170 pg/ml. In the fourth experiment the levels increased slowly but consistently from a basal value of 203 pg/ml to 270 pg/ml after 4 hours of incubation, and 295 pg/ml at 24 hours. It was concluded that any angiotensin III formed within the central nervous system or which might have crossed the blood-CSF barrier was not rapidly destroyed by angiotensinases within CSF.

Another possible reason why more obvious changes in immunoreactive angiotensin II levels were not observed in CSF, is that the radioimmunoassay used may not have been sufficiently sensitive to detect such fluctuations, or the liquor might have contained some interfering substance. To test this possibility, standard amounts of angiotensin II and angiotensin III ranging from 5–500 pg were added to dog CSF which had been drawn into the enzyme inhibitor solution, and run in

Figure 4. Immunoreactive angiotensin II (open circles, broken line = plasma; closed circles, solid line = cerebrospinal fluid), plasma renin activity and serum sugar responses to insulin injection in four dogs. The data are shown as mean ± SEM.
parallel with a routine standard curve of angiotensin II. The resultant three curves were virtually superimposable. As shown in figure 6, when angiotensin III was added to dog CSF, the radioimmunoassay could readily detect small (5 and 10 pg) amounts of the heptapeptide. Clearly, therefore, the assay was not a limiting factor in detecting even minor fluctuations of immunoreactive angiotensin II in CSF.

A final possible complicating factor is that pentobarbital anesthesia might have impaired CSF formation or flow so that angiotensin produced within the central nervous system (or having crossed the blood-CSF barrier) may not have been readily detected in CSF sampled by cisternal puncture. However, tritiated water appeared rapidly (within 2 minutes) in cisternal CSF after a 10 μCi I.V. bolus in a dog anesthetized with pentobarbital (fig. 7).

Discussion

There is a large body of evidence pointing to an endogenous brain renin-angiotensin system, although some workers consider it is premature to conclude that such a system is functional. If there is indeed a functional brain renin-angiotensin system, one must presume that its activity is controlled by a
number of regulating factors. The main thrust of the current study was to assess whether regulation of the peripheral and the central nervous renin-angiotensin systems are similar or whether the two are controlled independently. For this purpose immunoreactive angiotensin II was monitored in both plasma and CSF during procedures which are well known to alter the activity of the peripheral renin-angiotensin system. It is pertinent to note that the radioimmunoassay used in these studies utilized an antibody raised against angiotensin II but which cross-reacted 100% with the heptapeptide angiotensin III; therefore, the observations in CSF are valid whichever of the two peptides is the important end product of this renin-angiotensin system. 19

It appears from the above data that immunoreactive angiotensin II levels in CSF and plasma have separate regulatory systems. However, there are a number of other possible explanations or interpretations. First, the immunoassay used might not have been capable of detecting immunoreactive angiotensin II in dog CSF, perhaps due to some interfering factor. That this was not so was shown by the construction of standard curves for both angiotensin II and angiotensin III added to dog CSF. The assay was clearly capable of detecting small amounts of either peptide in dog CSF. Second, angiotensin secreted into CSF might have been broken down. However when angiotensin III was added to dog CSF and incubated at 37°C, the levels as measured by radioimmunoassay were altered little over 4 hours and in some instances up to 24 hours. These findings are in agreement with those of Hutchinson et al., 19 who also observed minimal angiotensinase activity in the CSF of dogs. Third, it is conceivable that sodium pentobarbital anesthesia slowed or otherwise altered CSF production or circulation so that angiotensin levels within the cerebral ventricles were not accurately reflected by cisternal sampling. This seems unlikely since tritiated water appeared in CSF obtained by cisternal puncture within 2 minutes of its injection intravenously.

Other explanations for the apparent unresponsiveness of the central nervous renin-angiotensin system are possible but are more difficult to evaluate. For example, angiotensin levels in CSF may not reflect accurately the activity of the renin-angiotensin system within the brain. Pentobarbital anesthesia may paralyze or blunt its activity within the central nervous system — in contrast to its stimulating action on renal renin release in dogs. 51 Angiotensin formed within the central nervous system might largely be taken up by specific receptors close to the cerebral ventricles, 52 although this seems unlikely unless extremely small amounts of peptide are involved. These reservations notwithstanding, it appears that the two renin-angiotensin systems have separate control systems at least for some stimuli. What regulates the activity of the brain renin-angiotensin system remains to be uncovered.

There is controversy in the literature as to whether angiotensin II from plasma can enter CSF. Volcier and Loew 41 in mice, and Buckley and Vollmer 42 in cats reported that the octapeptide penetrated to the CSF if large enough amounts were administered into the circulation. Hoffman and Phillips 43 concluded from studies in rats that saralasin (Sar1-Ala4-angiotensin II) crossed the blood-brain barrier if given in sufficiently large doses, and similar data from Johnson and Schwob 44 led them to conclude that angiotensin II was capable of penetrating into CSF from plasma. On the contrary, Simpson et al. 45 observed a dramatic fall in CSF angiotensin II levels in rats across a 30-minute intravenous octapeptide infusion. Finally, other workers using radiolabeled 1.12 or unlabeled octapeptide 54 have concluded that little or no intact angiotensin II crosses the blood-CSF barrier. The results from the present work agree with the latter viewpoint since neither an increase nor a decrease in CSF immunoreactive angiotensin II was observed even when the peptide was infused at 80 ng/kg/min — when the blood-brain barrier was likely to have been more permeable as a result of a rapid rise in blood pressure. 57, 58

The possibility that the heptapeptide des-Asp1-angiotensin II within CSF 59 originates from plasma has not been tested. From the current studies it is evident that this peptide, like angiotensin II, does not cross the blood-CSF barrier. It must be emphasized however that the peptide was infused for only 2 hours in each dog. It is conceivable that over hours or days there is a slow equilibration and that CSF levels of immunoreactive angiotensin II simply reflect plasma concentrations. This is currently held to be so in the case of prolactin, 60 which is of much larger molecular weight than angiotensin II or III (21,000 versus 1,046 and 931).

In conclusion, the data presented demonstrate that neither angiotensin II nor angiotensin III crosses the blood-CSF barrier in anesthetized dogs, at least in the short term. What factors control the activity of the brain renin-angiotensin system remains to be defined.

Acknowledgments

I am grateful to Dr. S. Julius for his helpful advice and guidance during these studies. I wish to thank Judith Mertens who prepared the figures and typed the manuscript, and Kathy Cooley, Jenny Hackforth-Jones and Mary Elkins for technician assistance.

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Independence of the central nervous and the peripheral renin-angiotensin systems in the dog.
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Hypertension. 1979;1:228-234
doi: 10.1161/01.HYP.1.3.228

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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