Angiotensin II in Rat Brain Comigrates with Authentic Angiotensin II in High Pressure Liquid Chromatography

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SUMMARY. Indirect evidence has implicated a role for central angiotensin II in blood pressure control. To answer directly the question of whether angiotensin II exists in the brain, independent of blood-borne angiotensin, and to quantify the amounts in different parts of the central nervous system, a sensitive radioimmunoassay was used to measure extracts of male adult rat brain hypothalamus and cortex after purification with high pressure liquid chromatography with a high recovery. The fractions coeluted with authentic angiotensin. Rats were nephrectomized bilaterally, and 24 hours later the brains were extracted in acetic acid and boiled. SepPak C-18 purification preceded reverse phase high pressure liquid chromatography. High pressure liquid chromatography revealed two peaks, one which comigrated precisely with [Ile] angiotensin II, and another smaller peak which overlapped with [Des] angiotensin III. The highest levels were found in the hypothalamus (125 pg/g tissue), pituitary (190 pg/g tissue), spinal cord (199 pg/g tissue), and lower levels were found in cortex (60 pg/g tissue). The results demonstrate that the antibody which was previously used in the immunocytochemical localization of angiotensin in the hypothalamus detects authentic angiotensin. However, the study did not depend on just one antibody. A second antibody which we developed gave the same results. Molecular sieving using Sephadex G-25 with acetic acid revealed a distinct peak in the 1000 MW range and a smaller, higher molecular weight peak which needs further investigation. Spontaneously hypertensive rats did not have higher concentrations of hypothalamic angiotensin II than normotensive rats. The present data provide evidence for angiotensin in the central nervous system, and the implications of an independent brain angiotensin for control of blood pressure and water balance can be explored with this procedure. (Circ Res 56: 212–219 1985)
the hypothalamus, pituitary, and cortex of rat brains that comigrate with authentic [Ile^8] All in using high pressure liquid liquid chromatography (HPLC). We have further characterized the extract by molecular sieving and found the purified extract was the same molecular weight as All. Since the rats were previously nephrectomized and blood levels of All were at or below measurable levels, the results offer proof of All being present in the brain. A preliminary report of this finding was presented (Phillips and Stenstrom, 1983). The levels of this brain All in spontaneously hypertensive rats (SHR) were similar to those of normotensive rats, and this leads to further consideration of the action of brain All in hypertension.

Methods

Subjects

Adult male Sprague-Dawley rats, spontaneously hypertensive rats of the Okomoto strain (SHR), and Wistar-Kyoto rats (WKY) (250–350 g) were used. Each rat was housed with ad libitum food and water available. The animal room was on a 6 a.m. to 6 p.m. light and 6 p.m. to a.m. dark cycle at least 1 week before the experiment.

Surgery

Bilateral nephrectomy was performed under chloral hydrate anesthesia. All animals were in good condition after surgery, as judged by their coats and behavioral activity. Twenty-two to 24 hours later, each rat was swiftly decapitated and the brain rapidly dissected. An area including the hypothalamus with parts of the septum, thalamus, and midbrain (but not the pituitary) was dissected out, and a piece of cortex was removed from the same brain.

Extraction

Brain Tissue

The samples were either extracted immediately or first frozen on dry ice and then stored at −20°C for no more than 1 week. Brain parts were dissected out, weighed, and put in 8–10 volumes of 1 M acetic acid (containing 15 mM EDTA and 1.25 mM o-phenanthroline) and put in a boiling water bath for 15 minutes to denature proteins and prevent hydrolysis. For the fresh tissue, the maximum time between decapitation and boiling was 4 minutes. Frozen pieces were dropped into boiling 1 M acetic acid. A series of pilot experiments showed that there was no difference in All levels with the two procedures.

Hypothalamic blocks weighed an average of 146.0 mg, and cortex, 190.0 mg. Two samples of these tissues were pooled together. Pituitary tissue weighed <10 mg per rat, and 20 pituitaries had to be pooled. Two samples from 40 rats were assayed. All samples were homogenized on a Potter-Elvehjem homogenizer at 4°C. The homogenates were spun for 20 minutes at 3,000 g at 4°C. The supernatant was collected and the pellet was reextracted with half of the original volume of 1 M acetic acid and spun 20 minutes at 10,000 g.

Supernatant extracts were pooled and defatted with equal volumes of petroleum ether. The aqueous phase was dried under an airstream on a hot plate (60°C) in bovine serum albumin (BSA)-coated polyethylene scintillation vials.

Blood

One milliliter of blood containing 15 mM EDTA and 1.25 mM o-phenanthroline was extracted with 6.4 ml 85% acetone and spun for 15 minutes at 1,000 g at 4°C. The supernatant was collected and the pellet reextracted with 3.6 ml 65% acetone and spun for 15 minutes at 1,000 g. The supernatants were pooled and dried under an airstream on a hot plate in BSA-coated polyethylene scintillation vials, and later purified on an ion exchange column, as described under Chromatography.

Chromatography

For preparation of blood samples for radioimmunoassay, SP-Sephadex C-25 was used. SP-Sephadex was swollen and washed five times, first with 0.5 M sodium acetate, pH 5.0, and second, in 0.05 M sodium acetate, pH 5.0. Both buffers had 0.01% sodium azide added. Gel (7 cm (3 ml)) was packed in Kontes disposable columns, 0.8 × 20 cm, and stopped with cotton wool. Columns were washed with 30 ml 0.05 M sodium acetate, pH 5.0. Each sample was dissolved in 2 ml 0.05 M sodium acetate, pH 5.0, vortexed, left 15 minutes, and applied to the column, and the vial was rinsed with 3 ml of the same buffer. Three milliliters of 0.05 M sodium acetate, pH 5.0, were added to the column, and then All was eluted with 5 ml of 0.05 M sodium acetate, pH 7.4, with 0.005% sodium azide. All was collected in BSA-coated scintillation vials and dried under air, as before. Recovery for All in this chromatography procedure was 93.2%. This was used for the blood samples, since it provided a more rapid method without involving HPLC.

SepPak C-18 Purification

For preparation of tissue for HPLC, SepPak C-18 cartridges were used for initial purification. SepPak cartridges (Waters Associates) were moistened with 3 ml of methanol and washed with 10 ml of 1% trifluoroacetic acid (TFA) in water, then coated with 1 ml of 1% Polypep solution (Sigma) in water to protect against nonspecific absorption of angiotensin on the cartridge, and washed with 10 ml of methanol, water, and TFA (80/19/1, vol/vol), followed by 10 ml of 1% TFA in water. Dried brain extract was dissolved in 5 ml of 0.001 M HCl and applied to the SepPak cartridge, and the vial was rinsed with 5 ml of 0.001 M HCl, which was also applied. The cartridge was washed with 2 × 5 ml of 1% TFA/1% NaCl (1/1, vol/vol), and 2 ml of methanol/water/TFA (30/69/1, vol/vol). Peptides retained in the cartridge were eluted with methanol/water/TFA (70/29/1, vol/vol). The eluate was dried under air in BSA-coated polypropylene tubes on a warm plate.

High Pressure Liquid Chromatography

Angiotensins were further purified on a Beckman HPLC model 332 (2 110A pumps, 420 controller, coupled to a variable wavelength detector) with a reverse phase column (Brown Lee Spheri-5 C-8 column, 4.6 mm inner diameter × 250 mm length, filled with 5-µm beads) was used. The elution solutions were 10 mM ammonium acetate (pH 4.15) and methanol. Two gradients were used: (a) a linear gradient 30–80% methanol in 35 minutes, and (b) a gradient of 30–50% methanol for 8 minutes, staying at 50% for 20 minutes, and 50–80% methanol in 10 minutes. This was found to give a better separation of All and AllI than gradient (a). Another elution system using acetonitrile and
0.26 M formic acid with triethylamine, pH 6.5, was also tried. This system started with 16% acetonitrile for 12 minutes, followed by a gradient of 16–50% acetonitrile for 30 minutes. The flow rate in each case was 1 ml/min. One-milliliter fractions were collected in BSA-coated tubes and dried under air on a warm plate for radioimmuno-assay.

Recovery
To measure the recovery of All with this purification procedure, unlabeled All (30–200 pg) was added to brain tissue homogenates. After extraction, SepPac purification, and high pressure liquid chromatography, the amounts were measured by radioimmunoassay and the levels in pure brain tissue were subtracted from the levels obtained in brain tissue with exogenous All added.

Radioimmunoassay
Samples were dissolved in 0.05 M Tris-buffer, pH 7.5. 125I-All and antibody were dissolved in 0.05 M Tris buffer, pH 7.5, containing 1% bovine γ-globulin.

Two antibodies were used. One was the antibody Celine, a gift from Dr. Ganten. It cross-reacts 100% with AllI and with All (3–8) hexapeptide, <1% with AI, and less than 0.01% with rat angiotensinogen and saralasin. Cross-reactivity to peptides unrelated to angiotensin is <0.001% (Hermann et al., 1982). The second was an antibody developed in our laboratory which has high sensitivity (ED50 9 pg). This antibody (Ab-2) was tested for cross-reactivity to the following peptides: AIII, 100%; All, 0.5%; saralasin, 0.004%; All tetrapeptide, 0.003%; All tripeptide, 0.0007%; oxytocin, <0.0005%; bradykinin, <0.0005%; somatostatin, <0.0005%; metenkephalin, <0.0005%; substance P, <0.0005%; BSA, <0.0005%; and vasopressin, 0.003%. Measurements of the same samples by two assays with the different antibodies showed close similarity in results.

125I-All was prepared by iodination of All, using the chloramine-T method, and purified on a DEAE-Sephadex A-25 column, by a slight modification of the method of Dusterdieck and McElwae (1971). The specific activity was 1600 μCi/μg. Aliquots of 10 μCi/ml were stored at −80°C and diluted immediately before each assay.

Standard curves were made with 3.9–250 pg unlabeled [Ile] All (Peninsula). A stock solution of 1 ng/μl was stored in aliquots at −80°C and diluted immediately before each assay. The buffer solution used was 0.05 M Tris buffer, pH 7.5.

Duplicates of the samples and of the standard solutions were mixed with antibody (Celine) at a final dilution of 1:450,000 and Ab-2 to 1:300,000 and 6000 counts/min 125I-All, and incubated 20–24 hours at 4°C.

To separate the unbound All from the All which was bound to antibody, polyethylene glycol in a final concentration of 12.5% was added to all tubes. The tubes were spun for 45 minutes at 1000 g, the supernatant aspirated, and the pellet counted in a Beckman 5500 γ-counter at an efficiency of 79% for 125I.

Gel Filtration
Sephadex G-25 was used to determine the approximate molecular weight of the immunoreactive angiotensin. Two elution systems were used with Sephadex G-25: (A) 0.012 M Tris-HCl, pH 8.0, column dimension 2 × 90 cm, flow rate 0.5 ml/min, fraction size 2 ml, and (B) 0.2 M acetic acid, column dimension 1.7 × 75 cm, flow rate 0.5 ml/min, fraction size 2 ml. Eluted fractions were collected in BSA-coated tubes.

Nonpurified hypothalamic extracts were dissolved in 2 ml of the respective elution buffer and applied to the column. The eluted fractions from the column utilizing system A were assayed directly (measured for All content by RIA), whereas the fractions from system B, which could not be directly assayed until the acid was removed, were first dried under air on a warm plate.

Finally, the All-containing peak after HPLC purification of hypothalamic extract was also subjected to gel filtration using system B with Sephadex G-25.

Statistics
Least squares linear regression analysis was applied to the data for determination of slopes and their degree of parallelity.

Results
Preliminary experiments, using an ion exchanger for purification as described for blood samples in methods, showed that hypothalamic blocks were higher in All content than any other part of the brain studied. This was anticipated from immunohistochemical studies, and, therefore, we concentrated on the hypothalamic area with the cortex for comparison and purified the extracts with SepPak C18 and high pressure liquid chromatography to see whether the immunoreactive material was indeed authentic All. Shown in Figure 1 is a typical elution pattern of purified hypothalamic area extract. There is a distinct peak of immunoreactive extract eluting with the same fractions as synthetic angiotensin AllI, and there seems to be a peak in the area of AIII. However, the levels in the AII area are very low and might not be significant.

Hypothalamus contained 158 ± 29 pg/g tissue in 14 experiments with SepPak C18 and HPLC (or 139

![Figure 1](http://circres.ahajournals.org/content/56/2/214.full)

**FIGURE 1.** HPLC elution profile of brain extract. This figure shows the result of extracting All from blocks of hypothalamus from the brains of rats nephrectomized 24 hours previously. The tissue was extracted in acetic acid and passed through a SepPak C18 cartridge for initial purification followed by reversed phase HPLC. Solvent system (b) was used. The results show a distinct peak of a substance coeluting with AllI (and a second peak in the area of AII). The amount extracted from the tissue as measured by the radioimmunoassay from 14 different experiments was 158 ± 29 pg/g tissue.
± 20 pg/g when SP-Sephadex was used in 20 separate experiments), and cortex 61 ± 15 pg/g in 12 separate experiments. Another brain site containing high All levels was the pituitary (116 and 265 pg/g in two experiments with 20 pooled pituitaries in each sample). Spinal cord contained 199 ± 110 pg/g in six experiments. Synthetic All, \(^{3}\)H]All, and synthetic All were used to calibrate the HPLC system. Gradient system (b) gave good separation between All and All in the HPLC system. Gradient system (a) gave less separation. With the acetotrile elutions system, no All was recovered. All was collected from a peak, but the peak shifted in successive replications. The methanol gradient (2) was stable over many replications. Adding \(^{3}\)H]All cortex homogenate gave the elution pattern in Figure 2. This shows that co-elution of \(^{3}\)H]All and endogenous All comigrate to the same fractions and have exactly the same peak. Since cortex had smaller amounts of peptide, no peak for All was detected.

In a series of six recovery experiments performed as described in Methods, we found the recovery of our purification procedure to be 95 ± 5% (n = 6) or 95 ± 2 SE. As the recovery of All is virtually complete, we do not adjust our values for recovery.

The radioimmunoassay developed to measure the All has a high sensitivity and good reproducibility. The standard deviations are small over 16 replications (Fig. 3), and the ED\(_{50}\) is 27.9 ± 2.5 pg and the slope = 1.06 ± 0.05. The useful range of the assay is between 3.9 and 250 pg/incubate. Blood samples of the nephrectomized rats were <4 pg/ml. Extracts from the HPLC and purification procedures did not interfere with the radioimmunoassay. Different All concentrations of a sample were compared to the standard curve. From a 1-ml extract of

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**Figure 2.** HPLC elution profile of \(^{3}\)H]angiotensin. To confirm the coelution of an angiotensin-like peptide in the brain with synthetic All, cortical tissue was extracted and purified with and without the addition of tritiated All. The figure demonstrates that the All-like material coeluted with labeled All on the reverse phase HPLC.

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**Figure 3.** Standard curve for All radioimmunoassay. To make sure there was no nonspecific interference from the brain tissue, a purified extract was serially diluted and, after a log-logit transformation, the slope of the sample was compared to that of the standard curve. Serial dilution of a purified sample gives a slope parallel to that of the standard curve. (Least squares linear regression analysis of the slope for the standard curve is -2.38 for the dilution curve -2.36.) The standard deviations at each point in the standard curve represent 16 replications indicating reproducibility.
HPLC-purified neural tissue, three samples were tested: one was 400 μl, the second 200 μl plus 200 μl buffer, and the third 100 μl plus 300 μl buffer. Shown in Figure 3 is the result of such a serial dilution curve compared to the log-log transformed standard curve of the assay. As can be seen, it is parallel with the standard curve. This parallelism was confirmed by comparing the slopes after least square analysis. The slope of the standard curve was -2.38 and the slope of the dilution curve was -2.36.

There was no interference between the peptide and the tissue. We determined if a fixed quantity of exogenous All added to different amounts of tissue would give a line parallel to that of tissue without exogenous All. The levels were measured after HPLC purification and are shown in Figure 4. The results showed that after adding 62.5 pg exogenous All to tissue, there is linearity of concentration of All up to 240 mg tissue, but with higher amounts of tissue there was a decline in recovery of the peptide. The slope of the upper curve was 0.11, and of the lower curve, 0.10, up to 240 mg tissue. For the experiments, we were therefore constrained to 200-mg tissue sample series, and we did not investigate this problem further.

Table 1 shows that, in the different strains of rats used, there was not a significant difference between Sprague-Dawley, SHR, or WKY. In SHR, hypothalamic levels were 125 ± 25 pg/g, and in normotensive Sprague-Dawley, 126 ± 23 pg/g. Although the WKY appeared to have higher levels, there was much wider variation than with the other two strains.

To examine the molecular weight of the All in the present study, we investigated both crude and HPLC-purified hypothalamic extract with Sephadex G-25 gel filtration, using two different buffers. The elution profile of a crude extract in Tris-buffer showed all the immunoreactive material elutes in the void volume, suggesting a molecular weight above 5000. However, elution of crude extract in acetic acid reveals a completely different profile, and unmasks the 1000 MW All peak. There is a distinct peak in the same fractions in which the [3H]All eluted. There was a much reduced peak in the higher molecular weight fractions (Fig. 5). However, the peak remained, even after passing the extract from the Sephadex G-25 through the HPLC (Fig. 6). It should be noted that the All was within the fractionation range, as opposed to [3H]tyrosine which was in the fully retained volume. Further tests with 125I-AI (MW 1420) and 125I-AII (MW 1170) showed a complete separation of peaks. [3H]AII (MW 932) had a peak that was distinct from [3H]All, but with overlap of the curves. These results indicate that the Sephadex G-25 in these studies was able to fractionate All (MW 1026).

Discussion

The results clearly demonstrate that there are detectable levels of All in the hypothalamus, cortex, and pituitary of rat brain. The All extracted from hypothalamic and cortical tissue appears in the same fraction as the peak of radioimmunoactivity for authentic All. Control tubes which were tested without the presence of tissue contained no All. Cortex which was treated in the same way as the hypothalamic tissue showed a reduced amount of angioten-

![Sample size limitation curve.](http://circres.ahajournals.org/figure/4)

**Figure 4.** Sample size limitation curve. To test whether the tissue contains substances that would interfere with the assay, a fixed amount of All (62.5 pg) was added to different amounts of cortex and hypothalamic tissue homogenates. The samples were then extracted and purified with HPLC. The squares indicate the amount of All found in the tissue. The circles indicate the amount of All detected after 62.5 pg exogenous All were added. The results show that, up to 240 mg tissue, the curves were parallel, indicating that there was no interference from the tissue. Above 240 mg tissue, an interference was detected by the lower amount of All recovered. Slope of the lower curve = 0.10 with an intercept of 3 pg/All, and the slope of the upper curve = 0.11 with an intercept of 63 pg/All. (The curves were fitted by least squares fit.)
one peak in the 1000 MW range coelutes with \( ^3\text{H} \)AU. After it had been passed through Sephadex G-25. The distribution of these results were confirmed later by two

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tic All was being stained exclusively. In these

staining with a Varian Micropak TSK 200 column at pH

acetic acid. In this figure, it can be seen that there may

be a small peak for a high molecular weight substance, but a very
clear peak for a peptide with the same molecular weight as All. These

data were based on a crude hypothalamic extract. The result with a

HPLC-purified hypothalamic extract is shown in Figure 6.

We have previously shown the distribution of immunoreactive All, using the immunohistochemi-

cal method in brain cells and fibers of the rat, monkey, and human, and found the greatest levels

in the hypothalamus (Phillips et al., 1979, 1980; Quinlan and Phillips, 1981). However, the immu-
nocytology technique shows only immunoreactivity, and does not allow one to state that

authentic All was being stained exclusively. In these

three species, the distribution is very similar, and

the major cell localization is in the supraoptic and

periventricular nuclei of the hypothalamus. The

most concentrated fiber bundles are in the median
enfinence. These results were confirmed later by two

independent studies (Zimmerman et al., 1980; Brownfield et al., 1982), one using a different anti-

body (Zimmerman et al., 1980). For this reason, we

chose the hypothalamus as the tissue in which to measure All on HPLC. In addition, we measured
cortex, pituitary, and spinal cord.

Immunohistochemical staining of cells in the an-
terior pituitary has also been reported (Steele et al.,
1982). The present results lead us to conclude that in

the immunohistochemical studies, authentic All was being labeled.

The question of whether this All is formed by a

renin-angiotensin system or not is still an open one. Although all the components for the renin angioten-
sin system appear to exist in the brain, their distri-
bution does not overlap in all respects, as one might
expect if the presence of angiotensin depended on the
activity of renin and angiotensinogen to form AI
and its conversion by converting enzyme to form

All (Reid and Brownfield, 1982).

It may be that brain All is made "on demand" and
does not reach high levels, or that it reaches high
levels only at certain times because of circadian rhythms. Having described a procedure to measure

All by HPLC, it should be possible to study the physiology of brain All by experiments with renin

and converting enzyme inhibitors, by incorporation of the labeled amino acids, isoleucine and valine,

and by inhibiting synthesis at various stages. Prelimi-

nary results in brain cell cultures indicate that there

is synthesis of brain angiotensin involving at least conversion of All to AI (Raizada et al., 1983).

Earlier, we found All levels of up to 76 ± 5 pg/g

wet weight of tissue when extracted by 2 M acetic

acid, but only 10% coeluted with All on HPLC

(Meyer et al., 1982). The elution system used ace-

tonitrile. When we repeated the use of acetonitrile

in the present study, we were unable to obtain All.

However, by using methanol, we found measurable

quantities of AI. Therefore, the reason for the small

yield reported previously may have been the use of

acetonitrile. Acetonitrile produces too much lability

in the system, and in the Meyer et al. (1982) report,

the peaks for synthetic All were in different frac-
tions, depending on pH. Hermann and Ganten

(1982) also found acetonitrile to be inconsistent and,
like us, used methanol (Ganten et al., 1983). For the

Meyer et al. study, two pools of five whole brains

were pooled and homogenized. We have now found

that more than 240 mg tissue interferes with the All

assay and, therefore, those five whole brains may

have also interfered with the assay. Molecular siev-
ing with a Varian Micropak TSK 200 column at pH

7.0 indicated that only a high molecular weight

substance was present (Meyer et al., 1982). We now

find that with acetic acid as an elution buffer in

Sephadex G-25, a clear peak at low molecular

weight is revealed. We felt that the methodology of

the Meyer et al. (1982) report was useful in establish-
ing an extraction procedure, but required further
development and more replications because, with
each new method of extraction, the amount of an-
giotensin detected increased. In the acetic acid ex-

![Figure 5](https://example.com/fig5.png)

**Figure 5.** Molecular sieving on brain extract. The result of passing hypothalamic extract through the Sephadex G-25 column equili-

brated with acetic acid. In this figure, it can be seen that there may

be a small peak for a high molecular weight substance, but a very
clear peak for a peptide with the same molecular weight as All. These

data were based on a crude hypothalamic extract. The result with a

HPLC-purified hypothalamic extract is shown in Figure 6.

![Figure 6](https://example.com/fig6.png)

**Figure 6.** The elution profile of HPLC-purified hypothalamic tissue after it had been passed through Sephadex G-25. The distribution of

one peak in the 1000 MW range coelutes with \(^3\text{H}\)All.
traction procedure, the recovery was 48 ± 4%, and with methanol, recovery was 60 ± 1%. The present report supercedes this previous report, and the current report represent the strongest supporting evidence for the existence of endogenous brain angiotensin. Our data and those obtained independently by Hermann et al. (1982) on rat brain tissue and cerebrospinal fluid (CSF) angiotensin support this conclusion. They used similar methods and one of the two antibodies that we have used. They did not, however, test for molecular weight of the All. Recently, Husain et al. (1983) have found a family of higher molecular weight All in dog CSF. As yet, we have not completely studied the molecular weight of the higher molecular weight All found in the void volume of the Sephadex G-25 fractions, and further tests are necessary. The clinical significance of the present finding has been alluded to in studies of spontaneously hypertensive rats where blood pressure can be lowered by blockade of All in the brain with saralasin or captopril, even though the blood levels of angiotensin or renin are normal or low (Phillips et al., 1977; McDonald et al., 1980). Therefore, an important action of brain angiotensin may be in maintaining blood pressure. Excess amounts of All in SHR were not found in this study, as shown in Table 1. Highest levels occurred in WKY brains, but the variation was too large to make this significant. Only a part of the brain has been analyzed so far, and further studies are necessary to define total levels, but the more important factor may be turnover of All and the interaction of brain angiotensin levels and receptor sensitivity.

Increased angiotensin receptor sensitivity to existing amounts may contribute to hypertension. Alternatively, lack of down-regulation of receptors to constant amounts of All in the brain could equally maintain high blood pressure. Ganten et al. (1983) have recently reported that icv captopril significantly decreased All brain levels in SHR stroke prone (SHRSP), but not in WKY, and concluded from this that in SHRSP, there is a higher activity of brain renin angiotensin.

We have used bilateral nephrectomy throughout this experiment to avoid contamination with blood-borne angiotensin. A method for calculating the contamination of plasma-borne substances has been proposed by Gregory et al. (1982) utilizing tritiated angiotensin to evaluate angiotensinogen. A method for calculating the contamination of plasma-borne substances has been proposed by Gregory et al. (1982) utilizing tritiated angiotensin to evaluate angiotensinogen. We have recently reported that icv captopril significantly decreased All brain levels in SHR stroke prone (SHRSP), but not in WKY, and concluded from this that in SHRSP, there is a higher activity of brain renin angiotensin.

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However, nephrectomy by itself may have caused some action on the brain angiotensin system. Earlier, we found that angiotensin injected into the ventricles has a prolonged pressor action after nephrectomy (Hoffman and Phillips, 1976). Therefore, further experiments will be needed to separate the action of nephrectomy in lowering renin levels from any action that it has on brain angiotensin levels. This does not detract, however, from the main result of this paper, which is a demonstration that angiotensin exists in the brain with the same retention times on HPLC as authentic All, and that there are measurable differences between the content of cortex, hypothalamus, pituitary, and spinal cord. The levels found are within a range that is effective when injected directly into specific brain areas to produce drinking and a blood pressure increase (Phillips, 1978; Simpson, 1981). Finally, since the levels of All in the blood were at or below detectable levels of the radioimmunoassay, we may conclude that the brain levels of All represent an independent source of the peptide.

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