Distribution of renin activity and angiotensinogen in rat brain. Effects of dietary sodium chloride intake on brain renin.

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Research Article

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Distribution of Renin Activity and Angiotensinogen in Rat Brain

Effects of Dietary Sodium Chloride Intake on Brain Renin

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Abstract

The purpose of this study was to investigate the biochemistry and the regulation of the brain renin-angiotensin system in the Sprague-Dawley rat. Renin activity and angiotensinogen concentrations (direct and indirect radioimmunoassays) were measured in several brain areas and in neuroendocrine glands. Regional renin activities were measured in separate groups of rats on high and low NaCl diets. Mean tissue renin activities ranged from 2.2±0.6 to 54.4±19.7 fmol/mg protein per h (mean of 7±SD), with the highest amounts in pineal, pituitary, and pons-medulla. NaCl depletion increased renin activity in selected regions; based on estimates of residual plasma contamination (despite perfusion of brains with saline), increased renin activity of pineal gland and posterior pituitary was attributed to higher plasma renin. To eliminate contamination by plasma renin, 16-h-nephrectomized rats were also studied. In anephric rats, NaCl depletion increased renin activity by 92% in olfactory bulbs and by 97% in anterior pituitary compared with NaCl-replete state. These elevations could not be accounted for by hyperreninemia. Brain renin activity was low and was unaffected by dietary NaCl in amygdala, hypothalamus, striatum, frontal cortex, and cerebellum. In contrast to renin, highest angiotensinogen concentrations were measured in hypothalamus and cerebellum. Overall, angiotensinogen measurements with the direct and the indirect assays were highly correlated ($n = 56$, $r = 0.96$, $P < 0.001$). We conclude that (*a*) NaCl deprivation increases renin in olfactory bulbs and anterior pituitary of the rat, unrelated to contamination by plasma renin; and (*b*) the existence of angiotensinogen, the precursor of angiotensins, is demonstrated by direct radioimmunoassay throughout the brain and in neuroendocrine glands.

Introduction

Angiotensin II has several effects on the brain, including facilitation of adrenergic transmission and elevation of arterial pressure (1), stimulation of thirst, and release of pituitary hormones (2). It is tempting to postulate that a renin-angiotensin system operates in the central nervous system independently from its analogue in the peripheral circulation. An endogenous brain renin-angiotensin system was initially proposed by Ganten et al. (3) and Fisher-Ferraro et al. (4). However, the existence of a central angiotensin-forming pathway in vivo is controversial (5). Specific measurements of renin activity in brain are difficult, because brain tissue contains an acid protease similar to cathepsin D, a lysosomal enzyme that cleaves angiotensinogen to produce angiotensin I at acidic pH (6). Recently, independent investigators have shown the existence in brain and in neuroendocrine glands of a protease resembling kidney renin that acts on renin substrate at neutral pH (7, 8). Compared with plasma renin, this enzyme has a lower isoelectric point (9), and its concentration in brain tissue is low.

Although the regulation of renin release by the kidney has been studied extensively, there is little available information concerning the regulation of renin in the central nervous system. One approach to this problem is to determine if stimuli that affect renal renin also affect central renin. Renin release by the kidney is suppressed by dietary NaCl loading and stimulated by dietary NaCl deprivation. One purpose of the present investigation was to characterize the changes in tissue renin activity induced by variations in dietary NaCl intake, both in specific areas of the rat brain and in neuroendocrine glands.

Angiotensinogen, the substrate for renin and the precursor of angiotensins in the circulation, is another important component of a putative central renin-angiotensin system. To date, evidence for the existence of renin substrate in the brain has been based on its detection by indirect methods of angiotensin I generation followed by radioimmunoassay of angiotensin I (10). Using a highly specific antibody, Bouhnik et al. (11) have recently developed a direct radioimmunoassay for the measurement of angiotensinogen in rat plasma. To determine if brain renin substrate is a protein having the same antigenic structure as plasma angiotensinogen, in the present study, the distribution of angiotensinogen in rat brain was characterized using a direct radioimmunoassay and compared with measurements obtained by indirect assay.

Methods

Animal groups. Male Sprague-Dawley rats weighing 250–300 g were housed in individual cages under a 12-h dark–light cycle. To study the influence of dietary NaCl intake upon brain renin, two groups of seven rats each were maintained on either a low (<0.01 meq Na^+/g of chow) or a high (1.70 meq Na^+/g of chow) NaCl diet for 10 d. All rats drank distilled water. Daily urinary sodium excretion was measured on the final 2 d of the diets, and was 0.16±0.16 meq Na^+/24 h (mean±SD) in the low NaCl group and 15.20±3.48 meq Na^+/24 h in the high NaCl group. Brain angiotensinogen was measured in a separate group of eight rats on a normal NaCl diet (0.19 meq Na^+/g of chow, urinary Na^+ = 3.01±0.94 meq/24 h).

Rats were killed by exsanguination under anesthesia (Inactin, 100 mg/kg i.p.; Andrew Lockwood Assoc., Lansing, MI), and plasma was stored frozen at −28°C for the determination of plasma renin concentration. To remove plasma proteins trapped inside the brain vasculature, brains were perfused in situ via a retrograde aortic catheter with 200 ml of cold isotonic saline before removal from the skull and dissection on dry-ice–cooled plates. The following brain regions and neuroendocrine glands were isolated: pineal gland, posterior and anterior pituitary, olfactory bulbs, hypothalamus, amygdala, frontal cortex, striatum, cerebellum, and pons-medulla (12). The hypothalamus was divided into two

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sections: the anterior portion was dissected from the posterior limit of the olfactory tubercule to the posterior edge of the optic chiasm; the posterior part, from the posterior edge of the optic chiasm to the mammillary bodies. Tissue was weighed and immediately processed for protein extraction.

In an additional group of 12 animals, potential contamination of brain tissue with plasma was estimated on the basis of recovering counts in brain after intravenous injection of ^{125}I-albumin. Rats were anesthetized and a catheter was inserted into a femoral vein. 20 μCi of ^{125}I-labeled bovine serum albumin (New England Nuclear, Boston, MA) was injected through the catheter and the rats were killed 20 min later by exsanguination. Brains were perfused with 200 ml saline in six animals, but not in the other six; thus it was possible to evaluate the effect of perfusion on the plasma volume trapped in the brain vessels. Brains were dissected as above, and the tissue blocks were weighed and placed in counting vials along with aliquots of plasma. The volume of plasma in a brain area was determined from the amount of radioactivity present in this area, by the formula: nanoliters plasma/milligram brain tissue = (counts per minute/milligram tissue)/(counts per minute/nanoliter plasma). In brains not perfused with saline, the calculated amount of plasma in brain ranged from 5.6 to 152 nl plasma/milligram tissue, depending on the region analyzed. In contrast, after perfusion of brains with saline, plasma contamination in brain ranged from 0.7 to 27 nl plasma/milligram tissue. In both nonperfused and perfused brain, the greatest amount of plasma contamination was observed in the pineal gland and the anterior and posterior pituitary.

All brain renin measurements were obtained after saline perfusion. For each region, the contribution of plasma renin to the measurement of brain renin activity was calculated on the basis of the mean value for regional contamination of brain with plasma and the value of plasma renin concentration by the formula:

$$\frac{(\text{mean regional contamination} + 3\ \text{SD}) \times (\text{weight of tissue}) \times (\text{plasma renin concentration})}{(\text{tissue protein content})},$$

with variables expressed in the following units: regional contamination, nanoliters plasma/milligram tissue; weight of tissue, milligrams; plasma renin concentration, femtomoles angiotensin I/nanoliter plasma per hour; protein content, milligrams protein.

To further address the problem of plasma contamination, the influence of high and low NaCl diets on brain renin was also studied in two groups of seven rats each which were bilaterally nephrectomized under light ether anesthesia 16 h before being killed. The brains of these rats were also perfused with saline.

Biochemical measurements. Protein extraction from brain tissue was performed at 4°C to minimize enzymatic protein degradation. Tissue was homogenized by sonication in 10–20 vol of 100 mM phosphate buffer, pH 7.00, centrifuged for 30 min at 5,000 *g*, and the supernatants collected. Pellets were re-extracted in the same volume of buffer, and the combined supernatants were centrifuged at 30,000 *g* for 45 min. The resulting supernatant was freeze-dried after addition of EDTA (final concentration, 15 mM). Samples were reconstituted to the volume of the initial homogenate with distilled water and the extracts were stored at −28°C until assayed for renin activity, acid protease activity, angiotensinogen, and total protein concentration.

For measurement of brain renin activity, 150 μl of brain extract was incubated in phosphate buffer 100 mM, pH 7.00, in the presence of angiotensinase inhibitors, neomycin sulfate (1.5 mM), and exogenous renin-substrate. Incubations were carried out at 37°C in a total volume of 300 μl over a 24-h period. They were stopped by placing the tubes in boiling water for 10 min. They were then centrifuged, and supernatants were assayed for angiotensin I. The angiotensinase-inhibiting mixture contained 15 mM EDTA (final concentration), 0.2 mM phenylmercuric acetate (PMA), and 2.9 mM phenylmethyl-sulfonyl fluoride (PMSF).[1] These inhibitors completely suppressed the activity of brain angiotensinases for 24 h, as shown in preliminary experiments where the recovery of 1–100 ng of ^{1}Asp-^{5}Ile-angiotensin I (Sigma Chemical Co., St. Louis, MO) added to incubations was measured (Fig. 1). The kinetics of rat plasma renin were unaffected by these inhibitors. Nephrectomized rat plasma, devoid of renin activity, was used as the source of substrate for the measurement of tissue renin activity. For each incubation a volume of 50 μl, containing 16 μg of angiotensinogen, was used.

In these conditions of incubation, the activity of brain acid protease (cathepsin) upon angiotensinogen is totally inhibited, as shown previously (13). Using a commercial preparation of cathepsin D purified from bovine spleen (Sigma Chemical Co.), we confirmed that cathepsin failed to generate angiotensin I under the incubation conditions used to measure brain renin activity.

Plasma renin "concentration" was measured through the amount of angiotensin I generated in 200 μl of plasma for 1 h at pH 7.00 and at 37°C, in phosphate buffer (100 mM) containing plasma angiotensinase inhibitors and an excess of rat plasma angiotensinogen. For measurement of both brain renin and plasma renin, radioimmunoassay of angiotensin I was performed according to the method of Menard et al. (14), using their antibody for angiotensin I. ^{125}I-Ile angiotensin I obtained from New England Nuclear was used as a tracer. No blank value was detected in brain extracts before incubation, indicating that the angiotensin I antibody did not cross-react with other brain peptides. This antibody also has no cross-reactivity with angiotensins II and III (14). Cross-reactivity with synthetic tetradecapeptide (Lot A 12403, Bachem, Switzerland) was 0.1%.

Cathepsin-like activity was measured in some brain areas, to assess the specificity of the changes in brain renin activity induced by dietary salt intake. This was done according to the procedure of Anson (15). 25 μl of brain extracts was incubated with 250 μl of a 4% solution of freshly denaturated bovine hemoglobin (Sigma Chemical Co.) in 0.4 N acetic acid, pH 3.50, for 3 h and at 37°C. Incubations were stopped by the addition of 200 μl of 0.3 N TCA and the TCA-nonprecipitable peptide was measured (16). Blanks were made by adding TCA to the mixture before starting the incubation.

Brain angiotensinogen concentration was measured in brain extracts both by a direct radioimmunoassay and by the method of angiotensin I generation followed by radioimmunoassay of angiotensin I (indirect assay). For the indirect assay, 25 μl of brain extract was incubated for 4 h at 37°C in 200 mM phosphate buffer, pH 6.50, containing EDTA (15 mM), PMA (0.2 mM), and PMSF (2.9 mM), with 60 ng of mouse submaxillary renin purified on pepstatin-aminohexyl-agarose (17). Preliminary experiments showed that these conditions were adequate for complete hydrolysis of the angiotensinogen contained in the brain extracts in 2 h. Incubations were stopped by cooling the tubes in ice and assayed for angiotensin I.

The direct assay for angiotensinogen was carried out according to the procedure described for plasma angiotensinogen (11). The rabbit anti-angiotensinogen antiserum was provided by Dr. J. Bouhnik, Dr. P. Corvol, and Dr. J. Menard (Paris, France). This antibody was developed

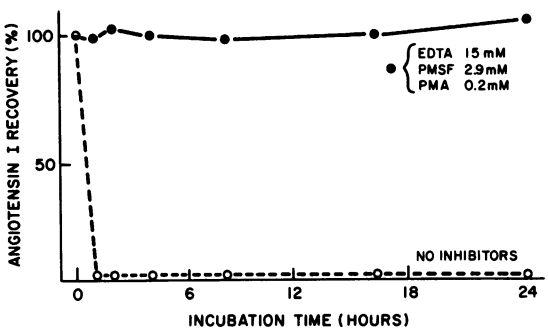

Figure 1. Recoveries of exogenous angiotensin I added to incubations of brain extracts for periods up to 24 h in the presence (solid line) or absence (dashed line) of angiotensinase inhibitors.

1. *Abbreviations used in this paper:* PMA, phenylmercuric acetate; PMSF, phenylmethyl-sulfonyl fluoride.

against pure rat angiotensinogen and does not cross-react with angiotensin I, II, or III. However, it fully recognizes des-angiotensin I-angiotensinogen, which is the protein residue remaining after cleavage of angiotensin I from angiotensinogen. Tracer was produced by iodination of pure angiotensinogen (molecular weight, 57,000) by the chloramine T method. For the measurement of brain angiotensinogen, 1–10 μl of brain extract (50–100 μl for pineal and pituitary) was incubated at 4°C for 24 h in the presence of ^{125}I-labeled rat angiotensinogen (10,000 cpm/tube), and rabbit angiotensinogen antiserum (final dilution, 1/50,000), in a total volume of 500 μl of phosphate buffer (100 mM, pH 7.50). After incubation, bound angiotensinogen was precipitated with 1 ml of 20% polyethylene-glycol (molecular weight, 6,000; Eastman Kodak, Rochester, NY) in the presence of 1 mg of bovine gamma-globulin (Sigma Chemical Co.). The tubes were then centrifuged, and the pellets were counted for radioactivity. The standard curve was established with purified rat plasma angiotensinogen. The radioimmunoassay has a sensitivity of 5 fmol (or 280 pg) of angiotensinogen. Serial dilutions of brain extracts were assayed in comparison with dilutions of plasma in order to validate the radioimmunoassay for brain angiotensinogen. The coefficients of variation of this assay for measurements in brain were determined in the midportion of the standard curve and were, respectively, 5.9% (intraassay) and 16.4% (interassay).

Protein concentration was measured in brain extracts by the method of Lowry et al. (16). The standard curve was constructed with bovine serum albumin (Sigma Chemical Co.). Results of renin activity, acid protease activity, and angiotensinogen concentration in brain were expressed in units per milligram of protein. This was done to minimize the experimental error due to variable recovery of protein after extraction of the brain samples.

Statistical comparisons were made by one-way analysis of variance (effects of diets and of nephrectomy on brain renin activity and on brain acid protease activity in each brain area, comparison of indirect assay and direct assay of brain angiotensinogen). Correlation and regression coefficient between direct and indirect assay of brain angiotensinogen was computed by the least squares method (18).

Results

Brain renin activity. Renin activity was widely distributed in the central nervous system, and it was detected in every area analyzed (Table I). However, regional differences were present in the brain, and neuroendocrine glands (pineal, anterior, and posterior pituitary) contained the highest activities. Dietary NaCl intake affected tissue renin activity in selected regions, both in intact and in anephric rats. In rats with kidneys, compared with respective values on a high NaCl intake, NaCl deprivation significantly increased renin activity in pineal gland, posterior and anterior pituitary, and brain tissue of olfactory bulbs. NaCl intake did not affect brain renin activity in any of the other areas studied.

Plasma renin concentration was significantly increased by NaCl deprivation in animals with intact kidneys (Table I). Based on estimates of plasma contamination, we could not exclude the possibility that the elevations of tissue renin on a low NaCl diet reflected contamination by high concentrations of plasma renin. Consequently, the effect of dietary NaCl on brain renin was also studied in anephric animals. 16 h after nephrectomy, plasma renin was still detectable. Furthermore, in the anephric rat, plasma renin concentration was higher in NaCl-depleted than in NaCl-repleted animals; tissue renin activity was increased by the low NaCl diet only in anterior pituitary and olfactory bulbs (Table I). However, in anephric rats with extremely low levels of plasma renin, the elevation of renin content of anterior pituitary and olfactory bulbs on the low NaCl diet cannot be explained by plasma contamination. Contamination of anterior pituitary and olfactory bulbs by plasma after brain perfusion was 11.2±8.2 (SD) nl/mg tissue and 2.5±1.0 nl plasma/mg tissue, respectively. Adjusting for the maximum potential contamination (based on these volumes of plasma and on plasma renin concentration in the animals on the high and low NaCl diets), a significant effect of dietary NaCl on central renin persisted in these two regions: in the anterior pituitary, adjusted renin activities on a high NaCl and low NaCl diet were 3.8±0.6 and 7.2±1.9 fmol/mg protein per h, respectively ($P < 0.01$); in the olfactory bulbs, adjusted brain renin activities on the two diets were 3.8±0.8 and 7.2±3.8 fmol/mg protein per h, respectively ($P < 0.05$).

Comparing animals with and without kidneys, renin activity in pineal gland, and anterior and posterior pituitary was lower in anephric animals. These differences were observed on both

Table I. Influence of Dietary NaCl Intake on Brain Renin Activity in Intact and Nephrectomized Rats

	Intact		Nephrectomized	
	High NaCl	Low NaCl	High NaCl	Low NaCl
Pineal gland	54.4±19.7	111.0±36.9*	27.4±12.9‡	25.3±10.7‡
Olfactory bulbs	3.1±1.7	10.6±4.4*	3.8±0.8	7.3±3.8*
Posterior pituitary	35.0±7.8	99.5±36.4*	19.8±6.9‡	22.7±12.0‡
Anterior pituitary	21.6±15.1	60.5±20.3*	3.8±0.6‡	7.5±1.8*‡
Anterior hypothalamus	5.8±1.8	9.1±2.1	6.2±1.7	7.3±2.7
Posterior hyothalamus	9.1±2.6	6.6±2.4	8.5±2.4	8.4±1.3
Amygdala	2.2±0.6	3.3±2.3	2.2±0.8	3.2±1.6
Frontal cortex	2.8±1.6	2.6±1.2	2.5±1.3	3.2±2.6
Striatum	6.2±2.9	4.8±1.5	6.7±5.0	4.2±1.6
Cerebellum	3.5±2.2	5.0±1.8	2.4±1.5	3.4±1.5
Medulla	25.7±8.4	26.0±6.3	24.7±4.9	19.5±3.1
Plasma	5.8±2.5	23.0±10.8*	0.08±0.06‡	0.9±0.2*‡

Brain renin activity is expressed as femtomoles angiotensin I/milligram protein per hour, and plasma renin concentration in picomoles/milliliter per hour. Mean±SD (n = 7/group). Statistics were performed after logarithmic transformation of the data, as it is apparent that the variances increased in proportion to the means. However, the original means and SD are shown in the table. * $P < 0.05$, low NaCl vs. high NaCl. ‡ $P < 0.05$, nephrectomized vs. intact.

Table II. Influence of Dietary NaCl Intake on Brain Acid Protease Activity in Intact and Nephrectomized Rats

	Intact		Nephrectomized	
	High NaCl	Low NaCl	High NaCl	Low NaCl
Pineal gland	90.3±20.4	118.0±17.0	103.5±22.9	103.8±20.8
Olfactory bulbs	15.4±1.5	16.5±2.3	16.4±1.9	16.4±0.8
Posterior pituitary	25.5±4.2	24.0±4.2	24.1±4.6	25.6±7.0
Anterior pituitary	27.7±2.1	28.1±2.6	28.1±2.0	27.6±2.2
Anterior hypothalamus	20.9±4.5	22.4±4.5	25.5±4.9	20.2±4.8

Data are expressed as nanomoles bovine serum albumin/milligram protein per hour. Mean±SD, n = 7/group.

the high and low NaCl diets. Because of the high degree of plasma contamination, the higher renin content of these glands in animals with kidneys is largely accounted for by contamination with plasma renin.

Table II shows the results for acid protease activity analyzed in the regions where renin activity was affected by dietary NaCl intake. Acid protease activity was higher in the pineal gland than in all other areas. In all areas studied, acid protease activity was not affected by either nephrectomy or variations in NaCl intake.

Brain angiotensinogen content. Fig. 2 shows the standard curve for the direct radioimmunoassay of angiotensinogen and the curves obtained with serial dilutions of rat plasma and rat brain extracts. The parallelism of these curves demonstrates the immunological identity between plasma and brain angiotensinogen. The analysis of regional brain angiotensinogen content, both by the direct assay and by the indirect assay, revealed that

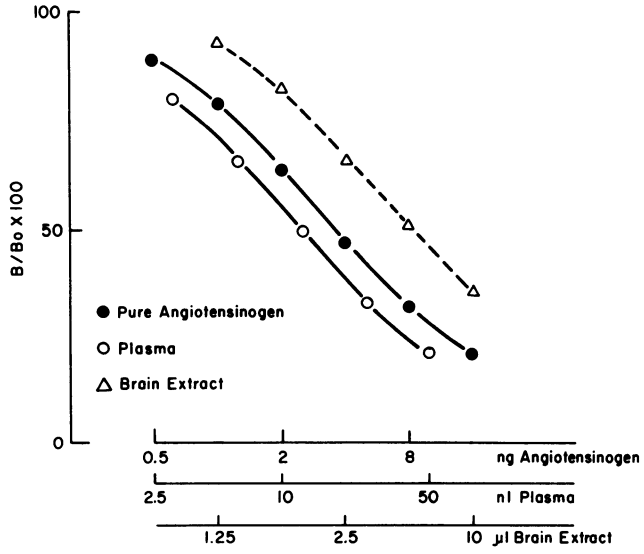

Figure 2. Direct radioimmunoassay of angiotensinogen (semilog plot). Bo, maximum binding of ^{125}I-labeled angiotensinogen in the assay conditions. B, binding in the presence of increasing amount of pure angiotensinogen (standard curve), plasma angiotensinogen, or brain angiotensinogen.

Table III. Angiotensinogen Concentration in the Rat Brain Analyzed by Direct Assay and by Indirect Assay

	Direct assay	Indirect assay
	fmol/mg protein	*fmol/mg protein*
Pineal gland	1,560±553	—
Olfactory bulbs	629±223	579±80
Posterior pituitary	882±404	—
Anterior pituitary	458±186	—
Anterior hypothalamus	3,958±1,178	3,631±1,082
Posterior hypothalamus	4,621±892	4,404±1,146
Amygdala	1,269±385	990±405
Frontal cortex	635±136	526±147
Striatum	703±104	597±86
Cerebellum	2,821±533	2,159±376*

Mean±SD, obtained from eight brains. Sufficient material from pineal, anterior, and posterior pituitary was not available for indirect assays.
* Statistics: $P < 0.05$, indirect assay vs. direct assay.

angiotensinogen was widely present in the central nervous system but, like renin activity, heterogeneously distributed. The highest amounts were measured in the hypothalamus (posterior and anterior parts) and the cerebellum (Table III). When compared with the indirect assay, values obtained with the direct assay were higher, although the difference between the two assays was not statistically significant except in cerebellum. Overall, the results of the two methods were highly correlated (r = 0.96, P < 0.001) (Fig. 3).

Discussion

The present study reports the distribution of tissue renin activity and angiotensinogen in the central nervous system of the rat, and the effects of dietary NaCl intake upon regional tissue renin activity. Renin activity was widely present in brain, although

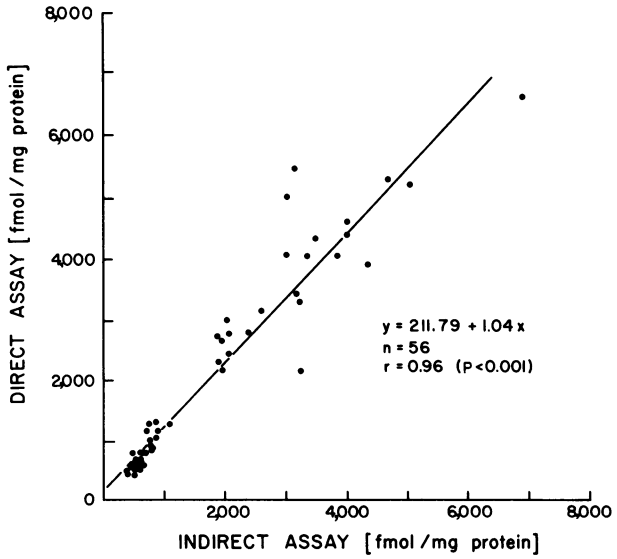

Figure 3. Correlation between indirect and direct assays for brain angiotensinogen.

regional differences were observed with highest levels in pons-medulla. High renin activities were also present in pineal gland and pituitary. In contrast, angiotensinogen was primarily located in hypothalamus and cerebellum. Similar to kidney renin, brain renin was responsive to dietary NaCl intake. Low dietary NaCl induced a 5–10-fold increase in plasma renin concentration and increased renin in the olfactory bulbs, the pineal gland, and in the anterior and posterior pituitary. Dietary NaCl intake did not affect tissue cathepsin activity. This confirms that our method of measurement was selective for measuring true renin activity in brain extracts.

Similar to our results, other investigators have also reported significantly higher amounts of renin in the pineal and the anterior pituitary than in brain tissue in rats (19–21) and hogs (9). The presence of renin activity in the posterior pituitary has been reported in rats (19, 20), but not in hogs. Quantitatively, our results are difficult to compare with those of other studies because of differences in species and in the technique of measurement. The use of certain angiotensinase inhibitors, and also the nature of the substrate used for angiotensin I generation, can modify the rate of the reaction and thus the apparent enzymatic activity of renin.

Our study is the first report showing the effects of dietary NaCl deprivation on brain renin activity in selected regions of the rat brain. The results are partly in agreement with data reported by Haulica et al. (21), who found that an acute intravenous NaCl load reduced renin activity in the pineal gland and in the pituitary. They also reported that this maneuver stimulated renin activity in cortex, hypothalamus, and brain stem, a result inconsistent with our findings. However, the latter study was done in intact rats and the brains were not perfused. Our results are also consistent with Slaven's (22) observation that NaCl deprivation increases angiotensin I concentrations in the brain stem of rats. In apparent contrast, Brosnihan et al. (23) reported that chronic salt depletion in the dog decreased renin activity in brain, particularly in the brain stem. They analyzed brain renin activity in larger brain areas than we did. Nevertheless, our results show that this is not the case in rats.

In the present study, we did not include a group of animals on an intermediate or "normal" NaCl intake. However, in a preliminary study reported in abstract form (24), in rats with intact kidneys, we have found that renin activity of olfactory bulbs, pineal gland, and anterior and posterior pituitary was significantly higher in animals on a low NaCl diet than in animals on an intermediate NaCl intake (0.19 meq Na^+/g of chow) or a high NaCl intake; values in the latter two groups did not differ. Thus, in the present study, we conclude that different tissue contents of renin on high and low NaCl diets are primarily related to an increase of tissue renin induced by NaCl deprivation rather than to a reduction of tissue renin by NaCl loading.

Some of the areas with elevated tissue renin activity on the low NaCl diet were highly contaminated by plasma trapped in the brain vessels, even after extensive perfusion of the brain. Plasma contamination was estimated with ^{125}I-albumin, a molecule that is larger than renin but that does not cross the blood-brain or the capillary barriers (25). The relatively high amount of contamination in nonperfused brains is similar to that reported by Gregory et al. (26) with [^{3}H]inulin. Perfusion of the brain with saline ameliorated, but did not completely eliminate, the problem of contamination of brain with plasma proteins. Using an estimate of residual plasma contamination obtained from a separate experiment and the values of plasma renin, we calculated the extent to which this contamination could have contributed to brain renin activity. We could not exclude the possibility that higher brain renin in NaCl-deprived rats with kidneys reflected higher plasma renin. Consequently, nephrectomized rats were also studied. In these rats, plasma renin levels were considerably reduced but were still higher in the low NaCl group than in the high NaCl group. The higher plasma renin measured in NaCl-depleted, 16-h–nephrectomized rats is likely attributable to small amounts of kidney renin present in the circulation after removal of the kidneys; we have observed that plasma renin concentration is undetectable 48 h after nephrectomy in the rat. Tissue renin activity in the 16-h–nephrectomized rats was increased by the low NaCl diet in olfactory bulbs, and in anterior pituitary. In these anephric rats, the computed contamination by plasma renin was too low to account for these elevations of tissue renin activity. Additionally, we confirmed that renin activity was undetectable in the minute volume of plasma that was calculated to be present in brain or glandular tissue. Furthermore, the amount of contamination by plasma was not higher in olfactory bulbs than in any other brain region; however, the olfactory bulbs were the only areas in brain where renin activity was affected by variations in NaCl intake. Thus, we conclude that elevation of tissue renin activity in olfactory bulbs and anterior pituitary induced by NaCl deprivation reflects changes in the renin endogenous to these tissues.

Alternatively, it is possible that renin measured in brain and in neuroendocrine glands reflects the activity of circulating renin bound to the endothelium of blood vessels, even in 16-h–nephrectomized rats. Although the present study does not directly address this potential concern, the existence of a true brain renin has been demonstrated on the basis of immunohistochemical studies and studies with cultured cells (27, 28). In ultrastructural studies, rat brain renin activity is associated with synaptosomal fractions, suggesting that it is intracellular (29). In the rat, renin activity is present in the brain, but not in plasma, 48 h after nephrectomy (unpublished observation, and reference 13). Furthermore, brain renin and kidney renin have different physiochemical characteristics (7, 9, 13, 28). Additionally, the renin-like enzyme contained in the cerebral microvessels of the rat is not detectable at neutral pH but, like cathepsin-related enzymes, exhibits a maximum of activity at a pH of 4.5 (30).

Previous reports suggest that nephrectomy does not affect renin activity in the rat brain (19, 31). We confirmed this finding for most regions that were analyzed. However, apparent decreases of renin activity were induced by nephrectomy in the pineal and the pituitary. In these highly vacularized regions, contamination by plasma renin largely accounted for the measurement of tissue renin activity. Consequently, the potential effects of nephrectomy on endogenous pineal or pituitary renin cannot be assessed.

We observed that angiotensinogen, the only known substrate for renin, is also widely distributed in the rat brain. The data previously obtained by an indirect measurement (10) were confirmed by direct radioimmunoassay. The problem of contamination by plasma for the measurement of brain angiotensinogen is less critical than for brain renin, because brain angiotensinogen concentrations are relatively high compared with plasma angiotensinogen concentrations (18.1±2.6 pmol/ml in the present study). Computed contamination of brain tissue and glandular tissue with plasma angiotensinogen is negligible (<2%). In addition, as we used a direct radioimmunoassay unaltered by the activity of tissue angiotensinases, the technique of measurement was reliable and simplified. When compared with the indirect

assay, results with the direct assay are almost identical, demonstrating the existence in brain of a precursor molecule for angiotensin I that is immunologically identical to plasma angiotensinogen. This finding supports what has been suggested by in vitro incubations of brain slices and physicochemical characterization of angiotensinogen in brain (32), and by studies of translation products of angiotensinogen mRNA purified from rat brain (33). The highest concentrations of angiotensinogen were measured in the hypothalamus. The distribution that we describe confirms the immunofluorescence data obtained with the same angiotensinogen antiserum that we used for direct measurement of brain angiotensinogen content (34).

The distribution of angiotensinogen and renin activity in the brain differs. This raises the questions of whether and how these proteins interact to produce angiotensin I in situ in the brain. Nevertheless, our results indicate that substantial amounts of renin and angiotensinogen are present in areas such as olfactory bulbs and hypothalamus. Further studies will be necessary to determine whether the brain renin angiotensin system is functional through local production of angiotensin II.

The functional significance of the brain renin angiotensin system is unclear. Our study indicates that, similar to kidney renin, brain renin activity in specific brain areas is stimulated by NaCl deprivation. Sodium chloride depletion is also associated with increased sympathetic nervous system activity (35), and conceivably this may be mediated by a central effect of angiotensin II on adrenergic transmission. Since NaCl-induced changes in brain renin are confined to specific regions, it might be hypothesized that brain renin has different functions in different locations. A recent report by Chen et al. (36) suggests that olfactory bulbs, which in the rat contain a high density of receptors for angiotensin II (37), might be involved in the control of fluid and food intake. Evidence also suggests that pineal renin may interfere with the metabolism of serotonin and enkephalins in brain (38). Pituitary renin may modulate secretion of pituitary hormones through actions of locally formed angiotensin II (2, 39). Thus, alteration of brain renin activity may contribute to a number of physiologic responses to dietary NaCl deprivation.

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