Renin in dog brain

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RENIN SYNTHESIS has been demonstrated in organs other than the kidney, for example, the uterus (21, 28). Renin occurs also in brain tissue (12, 15, 16). Whenever renin activity has been detected in extrarenal tissues, however, (17, 20) it has not been clear whether this tissue renin activity was renin liberated from the kidney and absorbed by the tissues from the blood, or was locally synthesized, or was a combination of both absorbed and synthesized renin. Renin activity in mesenteric artery branches has recently been shown to respond to hemorrhage and clamping independently of renal and plasma renin activity (13, 14).

The blood-brain barrier has been studied extensively (7). Though anatomically different parts of this barrier system between blood and brain tissue have to be distinguished (2, 7), there is general agreement that if proteins and peptides do not pass from the blood via the choroid plexus into the cerebrospinal fluid (CSF), a similar selectivity and hindrance can be expected for the passage from blood to brain tissue (5, 8). As has been shown for other proteins than the kidney, for example, the uterus (21, 28). Renin substrate and substrate which is dissolved in 0.25 M trisphosphate buffer, of 20-80 g) was homogenized in 5 volumes of 0.0% ethanol and after bilateral nephrectomy, and b) nine dogs underwent peritoneal dialysis and were kept alive for 3-12 days after nephrectomy. The technique used for intermittent peritoneal dialysis was that described by Grotlmann et al. (18). Imperinol (Abbott Laboratories, 2-4 liters) with 1.5% glucose was used as dialysate, which was changed after 2 hr. Tube feeding, of the same diet as for dogs of group I, was necessary in most of the dogs since vomiting was rather frequent. The dogs were in rather good physical condition when they were killed. Dogs were operated on and killed under pentothal anesthesia (Nembutal, Abbott; 30 mg/kg), but no anesthesia was necessary for dialysis. For the first 2 days after bilateral nephrectomy, meperidine hydrochloride (Demerol, Winthrop) (1 ml, 2 times/day) was given intramuscularly.

Renin activity was measured in plasma and brain tissue of six 3-day-old puppies (group III). The animals were separated from the mother only a few minutes before the experiment.

Renin activity and renin substrate were measured simultaneously in plasma from the cubital vein and in cerebrospinal fluid, obtained by suboccipital cisternal puncture, in 29 other dogs. This group (group IV) was subdivided into a) 13 dogs maintained under control conditions as described in group I for 4 weeks, b) four dogs with elevated plasma renin activity after 14 days of severe sodium restriction, c) eight 2- to 6-month-old dogs with high plasma renin activity, d) three Goldblatt-hypertensive dogs, and e) one dog infused with homologous renin for 1 hr.

Synthetic (5-valine)-angiotensin II amide (Hypertensin, Ciba) was infused intravenously (200 ng/kg per min) for 1 hr to four dogs (group V) kept under the control conditions described for group I.

Determination of renin activity and angiotensin. For renin activity measurement, 100 mg of brain tissue homogenized in 3 ml of 0.9% NaCl, or in 1 ml of plasma or CSF, are incubated with an excess of homologous dog plasma substrate which is dissolved in 0.25 M trisphosphate buffer, of pH 5.5. Dowex 50W-X2 NHz resin (1 ml) is added to the incubation mixture. The angiotensin which is formed during the 12-hr incubation period at 37° C is eluted from the resin with diethylamine and ammonium hydroxide as described previously (4, 17) and then measured in the rat bioassay. Angiotensin I and II levels in CSF and tissue have been measured both by radioimmunoassay (Wilson et al., unpublished observations) and by bioassay in the nephrectomized rat.

Extraction of angiotensin from brain tissue. Brain tissue (20-80 g) was homogenized in 5 volumes of 0.0% ethanol and...
stirred continuously for 12 hr at 4 C. The homogenate was then centrifuged, the precipitate was discarded, and 4 ml of Dowex 50W-X2 NH₄⁺ was added to each 10 ml of the supernate. After being stirred for 1 hr in the cold at 4 C, the mixture was transferred to a column that contained 1 ml of Dowex resin. After washing with 30 ml of ammonium acetate (0.2 M, pH 6) and 30 ml of 10 % acetic acid, angiotensin was eluted from the column with 14 ml of 0.1 N diethylamine and 14 ml of 0.2 N ammonium hydroxide according to the method of Boucher et al (4).

Renin substrate. All determinations of renin activity in plasma, CSF, and brain tissue have been done with partially purified substrate from dog plasma according to the method of Haas et al (19).

Renin substrate content in brain tissue was then assayed as described from plasma (4), with an excess of hog renin (2 units, Nutritional Biochemicals Corporation, Cleveland, Ohio), and in separate experiments with an excess of dog renin (kidney homogenate, 1 ml corresponding to 1 g of renal cortex).

Assays for renin specificity. Brain tissue renin is similar to renal renin. It shows all the classical characteristics of an enzyme as it is nondialyzable, heat labile, and precipitable by (2.3 M) ammonium sulfate. Angiotensin formation from the renin-angiotensinogen reaction increases linearly with the amount of brain tissue incubated and with the increasing incubation time. Without addition of renin substrate, pressor material is not formed during the incubation. Kidney and brain enzyme both form angiotensin from the commercially available synthetic tetradecapeptide renin substrate (amino acid sequence: Asp-Arg-Val-Ile-His-Pro-Phc-His-Leu-Leu-Val-Tyr-Ser) (Schwarz/Mann, Inc. Orangeburg, N. Y.). Renin activity persists at pH 3 and 37 C for several hours. Soybean-trypsin inhibitor has no effect on renin activity. Brain and kidney renin can both be inhibited by hog antirenin in dog plasma.

There was no interference by dopamine, epinephrine, norepinephrine, serotonin, acetylcholine, γ-aminobutyric acid, and histamine when they were added to the incubation in the concentration of their approximate natural occurrence in brain tissue and 10 times this amount.

Differences between brain and kidney renin. a) The pH optimum for brain renin activity is 5 whereas kidney renin in dog plasma shows an activity plateau from pH 5.5 to 6.5, under the incubation conditions of Boucher's micromethod (Fig. 1). b) Brain tissue renin requires much lower concentrations of tetradecapeptide to form angiotensin than kidney renin. Furthermore, if equal amounts of brain and kidney renin, as judged by their activity on homologous dog plasma substrate, are allowed to act on synthetic tetradecapeptide, the brain renin activity is much greater (Fig 2). c) Hog antirenin is less effective against the canine brain enzyme than against canine kidney and plasma renin (15).

Characterization of the pressor material formed by brain tissue renin and homologous dog substrate. 1) Injection of the material formed during the incubation of brain homogenate and substrate prepared from plasma into the nephrectomized rat gives a blood pressure rise of short duration indistinguishable from that of angiotensin. 2) Heating of the material for 2 hr at 90 C in 0.97 % NaCl does not destroy its pressor activity. 3) The pressor effect in the rat bioassay is completely abolished after incubation with 2 % trypsin in phosphate buffer, pH 7.5, for 1 hr. 4) The pressor material is dialyzable. 5) Dowex 50W-X2 NH₄⁺ adsors the pressor material which can be eluted with diethylamine and ammonium hydroxide. 6) Immunological studies with specific angiotensin I and angiotensin II antibodies reveal that the pressor material consists of about 90 % angiotensin I and 10 % angiotensin II.
The recovery of 30-300 ng of angiotensin II standard, added to the incubation with brain homogenate, was 93.7% (11.6 ± 1.5, n = 8).

RESULTS

As shown in Table 1 no RA could be detected in the CSF in normal dogs, in dogs with high PRA after renin infusion or sodium depletion, in puppies with still high PRA, or in Goldblatt hypertensive dogs. Angiotensin also could not be detected in the CSF after infusion of Hypertensin in normal dogs. In neither normal nor experimental dogs could renin substrate be detected in the CSF. These findings indicate that neither renin nor angiotensin crosses the blood-brain barrier into the CSF in measurable amounts.

Brain tissue from normal dogs formed about 20 ng angiotensin/g wet wt per hr of incubation at 37°C. No significant differences could be found between various brain regions such as frontal cortex, caudate nucleus, thalamus, hypothalamus, midbrain, cerebellum, pons, and medulla oblongata, the range was 12-20 ng/g tissue per hr. In contrast, the mean value for mesenteric arteries was 2.4 ng/ml wet wt per hr.

In 3-day-old puppies PRA was significantly higher than in adult control dogs and brain tissue RA was markedly lower (Table 2).

Effect of nephrectomy. As shown in Table 2, RA in the caudate nucleus and frontal cortex did not change 24 hr after nephrectomy. Nephrectomized dogs, maintained alive by peritoneal dialysis for up to 12 days, still had significant concentrations of RA in brain tissue, with values which dipped and then rose to slightly above normal. There are marked variations of RA in brain tissue in these dogs. This may be partly due to the severe metabolic disorder secondary to nephrectomy and dialysis that is reflected in a mean increase of plasma potassium (from 4.8 mEq/liter before nephrectomy to 6.4 mEq/liter on the day of sacrifice), urea (from 33 to 340 mg/100 ml), and creatinine (from 1.2 to 10.5 mg/100 ml).

Renin substrate in brain. Addition of 1 ml dog kidney extract providing renin in excess for the substrate assay of 2 ml of purified brain substrate, yielded 25 ng angiotensin/g wet wt brain tissue. The blood pressure rise in the rat could be suppressed by angiotensin I antibodies and not by angiotensin II antibodies. Addition of angiotensin II antibodies to the mixture of brain extract and antiangiotensin I I had no further effect (Fig. 4). Acidification of the antigen-antibody complex to pH 2 with 1 xv HCl restored full pressor activity of the brain extract.

DISCUSSION

The classical scheme of exclusive renin production in the kidney, and angiotensin formation in plasma, by action of the proteolytic enzyme on angiotensinogen, has been challenged, since it has been shown that renin activity can persist in the nephrectomized human (6), that uterine tissue is able to synthesize renin in cell cultures (28), and that arterial tissue renin can be stimulated in nephrectomized dogs (13).

There is strong evidence that the blood-brain barrier is impermeable to polypeptides and proteins such as angiotensin, renin, and renin substrate. In the area postrema however, within the caudal half of the medulla and close to
the vagal nuclei, the blood-brain barrier seems to be deficient, and Joy and Lowe (22) have demonstrated that some of the central effects of angiotensin may be located in this area. We did not detect any angiotensin in the CSF after intravenous angiotensin infusion. Intravenous infusions of 14C-labeled angiotensin II indicate too that it does not cross the blood-brain barrier (30). Dickinson concludes that the central effect of angiotensin on the blood pressure when infused in small suppressor doses in the vertebral artery is due to vasoconstriction of the vascular supply of the vasomotor centers in the hindbrain (9).

Since neither angiotensin nor renin seem to pass the blood-brain barrier readily, renin and angiotensin found in brain tissue of normal dogs can be expected to be synthesized in this organ itself. Further evidence that PRA did not interfere in our measurements of brain tissue RA is given by the results with 3-day-old puppies, where we found very high plasma RA and significantly lower brain tissue RA than in adult control dogs. Parallel measurements in unrinsed and rinsed brain showed that there is no difference in the brain tissue RA due to the blood-brain barrier (30). Dickinson concludes that the central effect of angiotensin on the blood pressure when infused in small suppressor doses in the vertebral artery is due to vasoconstriction of the vascular supply of the vasomotor centers in the hindbrain (9).

In our experiments in nephrectomized dogs, superposition of brain RA and plasma RA is excluded, since the latter does not form angiotensin with homologous substrate occurring naturally in the plasma, while our brain tissue RA determinations have all been done with angiotensinogen, prepared from dog plasma.

Measurement of renin substrate in tissue is difficult. After partial purification and concentration we were able to demonstrate its presence in brain tissue. No quantitative figures can be given as yet. The fact, however, that in nephrectomized animals angiotensin is present in brain tissue shows that angiotensinogen must be available too for brain-tissue renin to form angiotensin. Angiotensin and renin have similar effects on water intake when injected directly into the brain (11), which is in agreement with our finding of the presence of renin substrate in brain.

Immunological characterization of the angiotensin extracted from brain tissue of dogs that had been nephrectomized 12 days previously and that showed no detectable plasma angiotensin revealed that the brain extract contained mostly angiotensin I. It is possible, however, that angiotensin II is lost during the procedure. Converting enzyme is present in brain tissue (24). At this stage it is more important to know that any form of angiotensin at all, the deca- or octapeptide, is present in brain tissue. This means that the brain renin-angiotensin system is functioning in anephric dogs.

As we have shown earlier by ultracentrifugation, part of the brain tissue renin is located intracellularly. Aldosterone administration decreases significantly the enzyme activity and progesterone markedly enhances it (15, 16).

The recently demonstrated strong effects of angiotensin on catecholamine synthesis (3), release of acetylcholine (10), and antidiuretic hormone (23), cellular ionic composition, are to be added to the increasing evidence for the central role of angiotensin in the control of fluid and electrolyte metabolism.

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