Changes in Central and Peripheral Renin-Angiotensin System after Furosemide Injection

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Abstract

To examine the effects of acute stimulation on the peripheral and central renin-angiotensin system, simultaneous sampling of blood and cerebrospinal fluid (CSF) for measurements of plasma renin activity (PRA), plasma angiotensin I-immunoreactivity (PAng I-ir), plasma angiotensin II-immunoreactivity (PAng II-ir), plasma angiotensinogen and cerebrospinal fluid angiotensin II-ir (CSF Ang II-ir) and CSF angiotensinogen was carried out following intravenous injection of furosemide (5 mg/kg) in conscious dogs. Administration of furosemide induced marked increases in PRA, Ang I-ir, PAng II-ir and CSF Ang II-ir, however, neither plasma nor CSF angiotensinogen was changed. Furthermore, a relatively large dose (20 mg/kg/min) of intravenously infused synthetic Ang II for 20 min produced a five-fold increase in PAng II-ir compared with no significant increase in CSF Ang II-ir. In spite of significant suppression of PRA and PAng I-ir, there were no significant changes in either plasma or CSF angiotensinogen.

These results primarily suggest that the peripheral and the brain renin-angiotensin systems may be linked and that acute changes in the peripheral renin-angiotensin system do not alter either plasma or CSF angiotensinogen.

Since an endogenous brain renin-angiotensin system was proposed by Ganten et al. (1971) and Fisher-Ferraro et al. (1971), several investigations have been performed in order to clarify its contribution to blood pressure regulation and water-electrolyte balance under physiological and pathological conditions (Fuxe et al., 1976, Schelling et al., 1980). Several studies have tried to elucidate the relationship between the brain and the peripheral renin-angiotensin system by simultaneous measurements of plasma and cerebrospinal fluid (CSF) renin (Broniham et al., 1982), angiotensin II (Suzuki et al., 1983) and angiotensinogen (Finkielman et al., 1972, Ruiz et al., 1983, Schelling et al., 1983). Accumulated evidence from such studies has indicated that there may be a close relationship between these two compartments.
In the present study, in order to characterize the components of the renin-angiotensin system in CSF and plasma, plasma renin activity (PRA), plasma angiotensinogen, angiotensin I-immunoreactivity (Ang I-ir) and angiotensin II-immunoreactivity (Ang II-ir) and CSF angiotensinogen and CSF-Ang II-ir were simultaneously evaluated in conscious dogs under conditions which influence the peripheral renin-angiotensin system.

Materials and Methods

Animal surgery

Eleven male mongrel dogs (average body weight: 18±2 kg) were kept on a diet of 60 mEq/day sodium throughout the experiment. Iliac artery and cisterna magna catheters were implanted two weeks prior to the experiment as described previously (Suzuki et al., 1983).

Experimental Protocol: All experiments were performed in conscious animals.

Experiment 1. Acute stimulation of the renin-angiotensin system by intravenous injection of furosemide.

Furosemide (5 mg/kg) was intravenously (iv) administered in order to stimulate renin secretion in 6 conscious dogs. Blood and CSF samples were taken before, and at 10, 30, 60, and 120 min after iv injection of furosemide.

Experiment 2. Exogenous administration of angiotensin II.

Ile5 angiotensin II (synthesized by M. C. Khosla, Cleveland Clinic Foundation) was infused intravenously at a rate of 20 ng/kg/min for 20 min into 5 conscious dogs. This experiment was intended to exclude the possibility of leakage of Ang II from the plasma to the CSF.

Chemical analysis

Blood samples for assay of angiotensinogen and Ang II were collected with 15% NH4 EDTA (50 µl/ml). Angiotensinogen was measured by incubating the samples with excess partially purified dog kidney renin to convert all angiotensinogen to Ang I which was then measured by RIA using commercial reagents (New England Nuclear, Boston, MA, USA). Plasma samples were diluted 1:10 with 0.1 M phosphate buffer (pH 6.5) and 25 µl of diluted plasma was incubated for 1 h at 37°C with 50 µl dog renin solution and 325 µl of 0.1 M phosphate buffer (pH 6.5) containing 10 mM EDTA, 5 mM N-ethylmaleimide and 0.5 mM phenylmethylsulfonyl fluoride. Following incubation, the tubes were placed in an ice-bath, 100 µl of saline added and then placed in a boiling waterbath for 10 min. After centrifugation, the supernatant was assayed for Ang II. CSF samples were assayed without dilution.

Ang I was extracted from plasma by absorption onto a C-18 Sep-Pak prewashed with methanol (5 ml), tetrahydrofuran (5 ml), distilled water (10 ml) and Tris-EDTA buffer. After addition of phenylmethylsulfonyl fluoride (1 mg/ml) in ethanol (50 µl), the sample was applied to the Sep-Pak and washed with distilled water (10 ml). Ang I was eluted with 10 ml ethanol: acetic acid: water (80:4:20). The apparent pH of the eluent was adjusted to between 6.0 to 6.5 with NH4OH and ethanol was removed by evaporation. The samples were lyophilized, reconstituted to half the initial extraction volume with Tris-acetate buffer and assayed using a commercial RIA kit for Ang I (New England Nuclear, Boston, MA, USA). The minimum detection limit for this assay was 2 pg. The intra- and inter-assay coefficients of variation were 6.6% and 9.6%, respectively. Immunological recovery of Ang I was 87.8±3.8% and radioactive recovery of I-Ang I was 75.5±3.5%. Cross-reactivity of Ang I antibody with Ang II was less than 0.01%, and with angiotensinogen less than 0.05%.

Blood and CSF samples for RIA determination of Ang II-ir were collected in pre-chilled tubes with 15% NH4 EDTA and 9.25 mM 0-phenanthroline; the assay is described elsewhere (Suzuki et al., 1983). Plasma samples were assayed after elution from a C-18 Sep-Pak, whereas CSF samples were assayed directly. The lowest detectable value for Ang II-ir averaged 2 pg. The intra- and inter-assay variations were 5.1% and 6.8%, respectively. Both Ang III (heptapeptide) and Ang II (3–8 hexapeptide) showed 100% cross-reactivity with Ang II antiserum; Ang I cross-reacted less than 0.01%. PRA was measured as previously described (Haber et al., 1969).

Data analysis. All data were expressed as means±SEM. Comparisons between groups were analyzed by the Student’s t-test for unpaired data.
Changes were considered to be significant at \( p<0.05 \). Linear regression analyses were determined by the least squares method.

**Results**

**Experiment 1.**

Effects of administration of furosemide on plasma and CSF components of the renin-angiotensin system (Fig. 1).

Intravenous injection of furosemide increased PRA (1.0±0.3—10.0±2.8 ng/ml/hr), Ang I-ir (280±35—610±45 pg/ml), and Ang II-ir (15±2—55±5 pg/ml) and CSF Ang II-ir (6±2—22±3 pg/ml) significantly (\( p<0.01 \)). PRA and plasma and CSF Ang II-ir reached peak values at 10 min after injection, however, plasma Ang I-ir values were elevated at 30 min after injection. All elevated components gradually decreased during 120 min following injec-
tion. In contrast to marked elevations of PRA, plasma Ang I-ir and Ang II-ir, and CSF Ang II-ir, neither plasma nor CSF angiotensinogen showed any significant changes after furosemide injection. Correlations among PRA, plasma Ang I-ir and plasma Ang II-ir were as follows; PRA vs Ang I-ir, r = .632; PRA vs Ang II-ir, r = .578; Ang II-ir vs Ang I-ir, r = .610. These were all significantly correlated. Correlations between plasma and CSF Ang II-ir are shown in Fig. 2 (r = .64).

Experiment 2.

Intravenous infusion of Synthetic Ang II (Fig. 3).

A large dose of synthetic Ang II did increase plasma Ang II-ir but not CSF Ang II-ir, accompanied by suppression of PRA and plasma Ang I-ir. During the infusion, plasma and CSF angiotensinogen were not changed.

Discussion

In the present study, not only peripheral but also central Ang II was influenced by the maneuver to stimulate peripheral renin secretion. This finding indicates that CSF Ang II is not derived from the peripheral circulation and raises a question about what role CSF Ang II plays in the pathogenesis of acute stimulation of the peripheral renin-angiotensin system, since a relatively large dose of infused Ang II showed that high plasma levels of the peptide do not account for the presence of Ang II-ir in the CSF of normal conscious dogs. In previous studies, CSF Ang II-ir was elevated in renal hypertensive dogs (Suzuki et al., 1983) and a continuous intravenous infusion of Ang II failed to increase CSF Ang II-ir (Schelling et al., 1976, Mikami et al., 1985). If brain Ang II contributes to the regulation of water balance, an increase in the activity of this brain peptidergic system may depend upon factors which are not related to either the formation of the peptide in the blood or its action in the peripheral circulation. This important problem which is raised by the present study, requires further investigation. Further, since Ang I in CSF was not examined in the present study because of technical difficulties, it is uncertain whether the elevated CSF
Ang II-ir originated from the brain or not. Plasma and CSF angiotensinogen levels were not influenced by furosemide injection, which showed a stimulatory effect on the renin-angiotensin system. Changes in plasma angiotensinogen due to salt restriction or water deprivation remain controversial (Ruiz et al., 1983, Schelling et al., 1983, Genain et al., 1983). Rosset and Vergrat (1971) have shown that in normal human subjects sodium restriction results in a decrease in plasma angiotensinogen and also an increase in PRA, while Chen et al. (1983) have reported increased plasma angiotensinogen and significant elevation of the prohormone in some brain regions of rats deprived of water for 72 h. Further, Ruiz et al. (1983) have found that water deprivation induces a significant increase in PRA and no significant changes in plasma or CSF angiotensinogen concentration. At present, it is difficult to explain why these differences exist. Also, there have been no conclusive studies examining the effects of diuretics on plasma angiotensinogen. However, since many previous studies examined only PRA and plasma angiotensinogen, the relationships existing among the components of the
Renin-angiotensin system have not been clarified. In the present study, all components of the renin-angiotensin system except the converting enzyme were measured in the blood and there were strong correlations among the PRA, plasma Ang I-ir and Ang II-ir. In spite of marked changes in PRA, plasma Ang I-ir and Ang II-ir, there was no significant relationship between plasma angiotensinogen and the other components. These findings suggest that the level of plasma angiotensinogen may not correctly indicate the role for regulation of water-electrolyte balance during such acute stimulation of the renin-angiotensin system.

As well as plasma angiotensinogen, the levels of CSF angiotensinogen were not altered by furosemide injection. The roles of the CSF angiotensinogen under physiological and pathological conditions have been investigated and the results remain contradictory. Healy & Printz (1985) proposed that the brain tissue levels of angiotensinogen were of greater importance than CSF angiotensinogen, the former correlating better with the blood-pressure levels of the spontaneously hypertensive rats than the latter. However, other workers (Ruiz et al., 1983; Schelling et al., 1983) have demonstrated that the amount of CSF angiotensinogen was changed under several conditions. We have previously observed an increase in CSF angiotensinogen in bilaterally nephrectomized animals (Suzuki et al., 1986). These findings are in good accordance with those of other investigations. Therefore, it is expected that CSF angiotensinogen reflects some part of the brain renin-angiotensin system. Further, since CSF Ang II-ir was increased by the stimulation of renin secretion by the kidney, the brain renin-angiotensin system may be activated without increasing CSF angiotensinogen.

In conclusion, firstly the brain renin-angiotensin system may be activated by the same maneuver which stimulates the peripheral renin-angiotensin system with furosemide, judging from the simultaneous elevations of both plasma and CSF Ang II-ir. Secondly, neither plasma nor CSF angiotensinogen was altered in spite of a marked increase in PRA, plasma Ang I-ir and Ang II-ir and CSF Ang II. These results suggest that angiotensinogen in both plasma and CSF remains constant in spite of acute changes in the renin-angiotensin system.

References


