Modulation of Brain Angiotensin-Converting Enzyme by Dietary Sodium and Chronic Intravenous and Intracerebroventricular Infusion of Angiotensin II

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SUMMARY Angiotensin-converting enzyme (ACE) in rat brain closely resembled that in lung in its kinetics with the substrate Hip-His Leu, the inhibitors SQ 20,881 and SQ 14,225, and in its Cl activation profile. Modification of dietary NaCl intake was associated with marked changes in brain ACE activity. Sodium-loaded rats had lower activity of ACE in hypothalamus, striatum, and midbrain, and higher activity in spinal cord compared to controls. In sodium-restricted rats, ACE was elevated in pituitary and depressed in spinal cord. Chronic intravenous infusion of angiotensin (AI) was associated with a pattern of changes partly resembling sodium loading: ACE was depressed in hypothalamus and striatum but elevated in midbrain. After chronic intracerebroventricular infusion of AI, ACE was elevated in striatum and hippocampus, and depressed in spinal cord; a pattern of changes quite different from those associated with intravenous AI. These results show that ACE in several brain regions is sensitive to dietary sodium intake and support the hypothesis that angiotensin-containing neurons in these areas might be responsive to NaCl status of the animal. The observed changes in brain ACE do not seem to be explained in any simple manner by changes in circulating or central angiotensin II. (Hypertension 4: 590-596, 1982)

KEY WORDS • sodium status • kininase II • osmotic minipumps • renin-angiotensin system • thirst • blood pressure

THERE is strong evidence that the renin-angiotensin system may exist in the brain independently of the circulating system. Renin, renin substrate, angiotensin-converting enzyme, and specific high affinity receptors for angiotensin II (AI) have been demonstrated in brain tissue. Several groups have identified AI-containing nerve terminals and neurons in the CNS by immunohistochemistry. When injected into the cerebral ventricles or applied to specific brain regions, AI produces a marked rise in blood pressure, polydypsia, halophilia, and release of the pituitary hormones ADH and ACTH. These actions of centrally-administered AI are all related to NaCl and water homeostasis and blood pressure control. Since brain AI-binding properties are influenced by NaCl intake, it is of interest to determine if other components of the renin angiotensin system in the CNS change in states of altered sodium balance.

We therefore undertook experiments to evaluate the effect of dietary NaCl on brain ACE activity. We have previously reported that angiotensin-converting enzyme (ACE) occurs in lower activity in three brain regions of spontaneously hypertensive rats when compared to normotensive rats. Since there is some evidence for increased activity of the brain angiotensin system in these animals, the possibility arose that there might be an inverse regulation of brain ACE by central AI. To test this hypothesis we infused AI into the lateral cerebral ventricles of conscious rats using chronically implanted osmotic minipumps. As a control, and to evaluate the role of circulating AI in possible effects of altered dietary NaCl, similar experiments were performed using intravenous infusion of AI.
Dietary Sodium Manipulation

Male Sprague Dawley rats (300-500 g) were fed a sodium-deficient diet (6 mmoles Na⁺/kg) for 4 weeks. The sodium-deficient group was given distilled water to drink. A control group ate the same diet and was given a choice of distilled water or 0.9% sodium chloride to drink, whereas a sodium loaded group had only 0.9% sodium chloride solution to drink.

Intracerebroventricular and Intravenous Infusion of Angiotensin II

Male Wistar-Kyoto (WKY) rats weighing 250-350 g were fed a standard lab chow (Clark King Company pellets, GR 2+; 60-80 mmole Na⁺/kg) and given tap water to drink. The animals were lightly anesthetized with ether, and a cannula was implanted into either the left lateral cerebral ventricle or the left jugular vein as follows: for intracerebroventricular (ICV) cannulation, a 27-gauge stainless steel cannula attached by PE-60 tubing to an Alzet osmotic minipump (Model 2002) was implanted into the left lateral ventricle at coordinates AP1.0, L1.5, H4.0 using the bregma as reference. The minipumps contained either Asn1 Val5 All (Hypertensin, Ciba) at 12.5 mg/ml in artificial CSF or artificial CSF was homoephysed in an identical manner except that 1 mM EDTA was added to block converting enzyme. Fluorescence intensity in the sample incubation was corrected for that in the blank incubation (always less than 5% of the sample) and read off a standard curve prepared using His-Leu (Miles Laboratories, Indiana) in the range 2.5 to 10 nmol. In all cases less than 1% of the substrate was consumed during the incubations.

Since this assay depends on stability of the product His-Leu, this was checked by adding the dipeptide (5 nmoles) to incubation mixtures prepared in the same manner as above except that the substrate Hip-His-Leu was omitted.

Protein was measured using standards of crystalline bovine serum albumin (Calbiochem, grade A).

Characterization of the Brain Enzyme

Effect of Inhibitors

In some experiments the effect of the inhibitors of converting enzyme: teprotide SQ 20,881 (Squibb Laboratories, Princeton, New Jersey Batch No. NN 012ND), captopril SQ 14,225 (Batch No. NN 011 NB, Squibb Laboratories), or Na₂ EDTA (1 mM) were evaluated. Using low-speed supernatants of cerebellum.

Chloride Dependency

Hypothalamus and rat lung were homogenized as described above except that the phosphate buffer contained no NaCl. Incubation mixtures were made up in duplicate with a range of NaCl concentrations from 0 to 400 mM and incubated for 1 hour. Blank incubations containing EDTA (1 mM) were included at each NaCl concentration.

Thin Layer Chromatography of the Cleavage Products

Cerebellum homogenate (130 μg protein) was incubated with 5 mM Hip-His-Leu as described above. After incubation at 37°C for 4 hours, 5 μl of the incubation mixture was applied to a Merck silica gel thin layer plate and developed with 1-butanol-acetic acid: water (18:2:5) for 5 hours at 22°C. The plate was dried in a stream of air and sprayed successively with ninhydrin (BDH Ltd., Poole, England) and Pauly’s reagent. Standards of histidine, leucine, His-Leu, and...
Hip-His-Leu (1 mg/ml) were run on the same plate and had Rf's of 0.02, 0.26, 0.07, 0.31 respectively.

Comparison of Kinetics of Brain and Lung Enzymes

Enzyme velocity vs substrate curves were obtained for low-speed supernatants of both lung and cerebellum and kinetic constants calculated after fitting the curves directly to hyperbolae by a least squares iterative technique as previously described.22 Plasma renin activity (PRA) was measured using a radioimmunoassay for AI.23

Statistical Methods

The significance of changes in brain ACE in the nine regions was assessed simultaneously using a multivariate technique, Hotellings T2 test.24 Univariate unpaired Student's t tests were used to test the significance of changes in one region between a treatment and control group.

Results

Validation of the Assay and Properties of the Enzyme

The generation of His-Leu was linear with both time of incubation (5–20 min) and concentration of homogenate added to the assay (20–60 μg protein). Thin layer chromatography of the assay incubation mixture revealed the appearance of a ninhydrin positive, Pauly positive spot migrating with the same Rf as His-Leu with no evidence of either histidine or leucine liberation. No cleavage was observed using incubation mixtures containing EDTA (1 mM) or SQ 20,881 (25 μM) or boiled enzyme. These results indicate the action of a dipeptidyl carboxypeptidase.

Stability of the cleavage product was tested by incubating His-Leu with homogenates from each of the 8 brain regions. Degradation of the dipeptide was readily detectable by this method since leucine gave no detectable fluorescence in the assay and histidine only 17% that of His-Leu on a molar basis. There was no significant loss of His-Leu during incubation with any of the brain regions, the mean recovery of the dipeptide being 101.6% (SEM 2.0, n = 8, p > 0.4). Between-assay variability as assessed by 40 consecutive measurements on a pool of serum gave a coefficient of variation of 10.8%. Between-assay variability was also assessed by measuring ACE in 108 different brain homogenates on two separate occasions involving 11 different assays. The correlation coefficient between the results of the two assays was r = 0.9829 (p << 0.001); the slope was not significantly different from 1.0 and the intercept not different from zero.

EDTA (1 mM) blocked His-Leu generation by greater than 90% in all cases. Converting enzyme values reported represent the EDTA-sensitive component of generation of His-Leu.

In the presence of SQ 20,881 (25 μM), there was no detectable generation of His-Leu. K, for the inhibitory effect of SQ 20,881 was 2.20 ± 0.06 (SEM, n = 3) × 10^-4 M, which is similar to the value obtained with the rat lung enzyme of 2.12 ± 0.15, n = 3, p > 0.6. SQ 14,225 inhibited generation of His-Leu by > 97% at 10^-3 M, with a K, of 1.83 ± 0.24 × 10^-4 M (n = 3), which is not different from the K, observed using rat lung microsomes of 1.66 ± 0.25 × 10^-3 M (n = 4) (p > 0.6).

His-Leu generation in the absence of Cl⁻ was not different from that in incubations with EDTA and represented less than 4% of the maximal activity. There was a marked activation of ACE activity with increased Cl⁻ with a half maximal effect near 75 mM Cl⁻. The Cl⁻ activation curves for the brain and lung enzymes were indistinguishable (fig. 1).

The K_m of the enzyme from cerebellum acting on Hip-His-Leu was 1.19 ± 0.02 mM (n = 3), which is similar to that observed with the lung enzyme of 1.91 ± 0.16 mM (n = 19), (p = 0.1).

Maximal velocity occurred with Hip-His-Leu concentrations of 5 mM and substrate inhibition was observed at higher concentrations of 10 and 20 mM Hip-His-Leu. For the routine assay a substrate concentration of 5 mM was therefore chosen.

Effects of Manipulations of Dietary Sodium Intake

As shown in figure 2, alterations of sodium intake produced marked changes in brain ACE. When changes in all nine regions were considered simultaneously using Hotellings T2 test, the sodium-restricted group showed a borderline difference (F 9 2 = 2.58, p = 0.06) from the control rats, whereas the pattern of
ACE levels in the sodium-loaded rats was highly significantly different from controls ($F_{2,15} = 78.1$, $p < 0.0003$). In sodium-restricted rats, ACE was elevated in pituitary ($p < 0.005$) and depressed in spinal cord ($p < 0.001$) when compared to the control group. Sodium-loaded rats had lower levels of ACE in hypothalamus ($p < 0.025$), striatum ($p < 0.001$) and midbrain ($p < 0.001$) and higher levels in the spinal cord ($p < 0.025$).

Serum ACE was $90.4 \pm 6.8$ nmoles/ml/min (SEM, $n = 6$) in controls and not different between the three groups (one-way ANOVA, $F_{2,15} = 2.1$, $p > 0.15$). PRA was significantly different in the three groups ($F_{2,15} = 7.9$, $p < 0.005$), being increased in the sodium-restricted group at $3.57 \pm 0.47$ ng/ml/hr (SEM, $n = 6$) and decreased in the sodium-loaded group at $1.54 \pm 0.32$ when compared to the control values of $2.56 \pm 0.26$ ng/ml/hr, as expected.

Intravenous Infusion of Angiotensin II

Animals infused with All intravenously for 6 days showed changes in the overall pattern of brain ACE which was highly significant as assessed by Hotellings $T^2$ test ($F_{9,6} = 12.8$, $p < 0.005$). There were lower levels of ACE in hypothalamus and striatum and higher levels in the spinal cord when compared to the vehicle-infused group (fig. 3), ($p < 0.05$ in each case).

Serum ACE did not differ between the two groups ($t = 0.85$, $p > 0.4$). PRA was suppressed in the AII-infused group, being $0.60 \pm 0.14$ ng/ml/hr (mean $\pm$ SEM, $n = 8$) compared to controls of $1.17 \pm 0.20$ ($p < 0.05$).

Intracerebroventricular Infusion of Angiotensin II

Animals infused with All ICV had higher levels of ACE in striatum ($p < 0.05$) and hippocampus ($p < 0.01$) and depressed ACE in spinal cord ($p < 0.05$) as assessed by univariate $t$ tests (fig. 4). However, the pattern of levels in all nine regions taken simultaneously was not significantly different from the vehicle-infused group as assessed by Hotellings $T^2$ test ($F_{9,6} = 2.40$, $p = 0.15$). Serum ACE was not different between animals infused ICV with All or vehicle ($t = 0.70$, $p > 0.5$). PRA was suppressed at $0.37 \pm 0.17$ ng/ml/hr (mean $\pm$ SEM, $n = 8$) in the All-infused group compared to controls of $0.88 \pm 0.16$ ($p < 0.05$).
Discussion

ACE in rat brain occurs in high concentrations in the striatum, cerebellum and pituitary, as confirmed here, and in human brain in the striatum and substantia nigra. In all tissues, ACE occurs at the luminal surface of vascular endothelial cells and has been found in brain vessels and choroid plexus. However, in some sites the enzyme appears to be associated with nerve terminals since it is enriched in synaptosomes. On the basis of experiments using intrastratal injection of kainic acid and observations in Huntington’s disease, it seems likely that the majority of ACE in the substantia nigra of both rat and man is localized in terminals of neurons whose cell bodies originate in the striatum. Currently, it is uncertain whether ACE in other brain regions is of vascular or neuronal origin.

Angiotensin receptors have been measured in various regions of rat brain and found to be highest in the midbrain, hypothalamus, and medulla. This pattern is
clearly different from the distribution of ACE found in this and other studies. The problem of assigning brain ACE to either neural or vascular structures is also true for angiotensin receptors and might account for the regional dissociation of these two factors. In addition, the complexity of the brain opioid system which has at least three endogenous ligands and multiple receptor types indicates that neuropeptide systems need not show a simple central action between the distributions of the peptide and its receptor.

All has potent central actions on blood pressure, drinking, and salt appetite. ACE seems to be involved in many of these actions since inhibition of the enzyme has been shown to attenuate drinking due to water deprivation, hypovolemia, renin given by peripheral or central routes, central administration of tetradecapeptide or plasma-renin substrates and intracranial AI. Similarly, converting enzyme inhibition attenuated pressor responses to centrally administered AI. These reports make it probable that ACE is involved in the physiological function of central angiotensin-containing neurons. It is likely that the activity of these neurons might be altered in states of altered sodium appetite or fluid homeostasis as such occurs with changes in dietary sodium intake.

The current finding that ACE in striatum, hypothalamus, midbrain, and spinal cord is responsive to dietary sodium raises the possibility that the activity of angiotensin-containing neurons, or their terminals, in these areas are modified by dietary sodium. The alternative possibility is that ACE localized to the cerebral vascular endothelium might change with altered NaCl balance. However, in many brain areas, ACE did not change with manipulation of dietary salt. Furthermore, lung and serum ACE were not responsive to dietary salt in these experiments. Any such changes in vascular ACE would, therefore, need to be highly region-specific to explain the observed changes in ACE. The mediator of the change in brain ACE during dietary NaCl modification does not appear to be circulating or CSF AI: during sodium depletion, circulating renin is increased and plasma AI elevated but infusion of AI did not mimic this effect on brain ACE. In contrast, the effect of intravenous AI infusion more closely resembled that of sodium loading. It is possible that the change in brain ACE after AI infusion might be explained by sodium retention induced by the direct renal or aldosterone-mediated effects of the peptide.

Intracerebroventricular infusion of AI led to increases in ACE in striatum, hippocampus, and depression of ACE in spinal cord. This pattern is quite different from that induced by intravenous AI and is almost the converse of the pattern after sodium loading. The effects of ICV AI might be explained by direct effects of the peptide or alternatively could be secondary to sodium depletion which was observed in these animals. These data do not support the hypothesis that brain ACE activity might be inhibited by local AI.

The mechanism whereby changes in sodium chloride balance lead to changes in ACE in hypothalamus, striatum, midbrain, and spinal cord is unclear. Either humoral or nervous pathways are possible mediators but, on the basis of the chronic infusion experiments, changes in plasma or CSF angiotensin II are unlikely to be the explanation. It is possible that some other unknown humoral factor, whose levels were responsive to salt status, could exert a highly region-specific modulation of vascular ACE in the brain. This hypothesis seems less likely than the alternative explanation that nervous pathways are involved in modulating brain ACE. Vagal afferents arising from distension receptors in the heart and capacitance vessels are known to exert an inhibitory tonus on ADH release probably via inhibition of neuronal activity in the supraoptic and/or paraventricular nuclei. It is possible that similar pathways might modulate the activity of central angiotensin-containing neurons but confirmation of this would require a more complete knowledge of central pathways involved in cerebral regulation of body fluids.

These results support the concept that central nervous AI might be involved in NaCl homeostasis.

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