Angiotensinogen Concentration in the Cerebrospinal Fluid in Different Experimental Conditions in the Rat

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SUMMARY Angiotensinogen is the most important component of the renin-angiotensin system present in the cerebrospinal fluid (CSF) of the rat. Its physiological significance as well as its origin have not been clearly elucidated. In this experiment we have examined plasma renin activity (PRA) and plasma and CSF angiotensinogen concentration under the following experimental conditions in male rats of the Wistar strain: 1) adrenalectomy (Adx) 4 days prior to sample collection; controls were sham Adx animals; 2) nephrectomy (Nx) 48 hours before blood and CSF collection; controls were sham Nx rats; 3) DOC-salt treatment (Corteron depot, 50 mg/kg.s.c. twice a week) plus saline to drink was given during 4 weeks; controls were intact rats; 4) DOC-salt plus captopril: captopril (100 mg/kg/day) in the drinking fluid was added to the treatment of experimental and control animals of Group 3; 5) two-kidney, two clip hypertension: silver clips placed in both renal arteries 8 weeks before samples collection; control: sham-operated rats; 6) water deprivation: rats deprived of water for 5 days; controls: intact rats; 7) peripheral sympathectomy: 6-hydroxydopamine (6-HODA) injected s.c. from birth until 16 weeks of age, adrenomedulllectomy and adrenal denervation performed at 8 weeks; controls were vehicle-injected animals.

Determination of angiotensinogen concentration in plasma and CSF was accomplished by incubation of the samples with excess hog renin. The angiotensin I released as well as PRA were evaluated using an specific radioimmunoassay technique. PRA was significantly increased by Adx, captopril treatment, and water deprivation, and was almost suppressed by Nx, DOC-salt, and DOC-salt plus captopril treatment. Plasma angiotensinogen concentration was significantly elevated by Nx and significantly diminished by Adx, DOC-salt, and DOC-salt plus captopril, and captopril treatment. CSF angiotensinogen concentration was significantly increased by Nx, DOC-salt, DOC-salt plus captopril, and 6-HODA treatment while it was significantly depleted by Adx. No correlation existed between the individual values of angiotensinogen concentration from plasmatic and central sources. The results of this study do not support the plasma origin of central angiotensinogen and suggest the existence of specific mechanisms for the regulation of the CSF renin-substrate.

(Hypertension 5 (supp V): V-29-V-33, 1983)

KEY WORDS • DOC-salt hypertension • captopril • plasma renin activity • renovascular hypertension • water deprivation • sympathectomy • 6-hydroxydopamine

THE presence of angiotensinogen in the brain and cerebrospinal fluid (CSF) of rabbits, dogs, rats, and humans has been clearly demonstrated. Biochemical analysis has shown a similar molecular weight and electrophoretic mobility for plasma and CSF renin substrate; moreover, the final product released by both prohormones was identical to synthetic angiotensin I (AI) with respect to isoelectric point or pressor response. Clear differences between CSF and plasma angiotensinogen were also observed. In this regard, central renin substrate was heterogeneous with respect to isoionic points; the plasma protein presented only one component. In addition, immunological differences between the central and peripheral renin substrate were also established. Although not definitively demonstrated, most of the available information supports a central origin for the angiotensinogen present in the brain and CSF of mammals and humans. Some experimental conditions determine a similar change in peripheral and central prohormone. However, in a previous report we found a clear dissociation between plasma and brain angiotensinogen in DOC-salt-treated hypertensive rats.

The purpose of our present study was to further analyze changes in peripheral (plasma) and central (CSF) prohormone in some experimental conditions that are known to induce alterations in plasma renin substrate, blood pressure and/or drinking behavior, in order to investigate the existence of some kind of regulation of the central prohormone and to add information as its origin.
Material and Methods

Male rats of the Wistar strain weighing 200 to 300 g were kept in an automatically lighted room (from 7 a.m. to 7 p.m.) at a constant temperature (22 ± 1°C) and fed Purina rat chow and tap water ad libitum. They were studied under different experimental conditions; operated or treated rats were analyzed with their adequate controls. Surgery, when indicated, was performed under ether anesthesia. Blood samples were always obtained in conscious animals by cutting the tip of the tail. CSF samples were extracted under pentobarbital anesthesia (40 mg/kg body weight, intraperitoneally). Anesthetized animals were immobilized with the head secured in extreme dorsiflexion. The atlantooccipital membrane was exposed by dissection, and a 20 μl bloodless cerebrospinal fluid (CSF) sample was collected from the cisterna magna using a 26-gauge needle connected to a 50-μl Hamilton syringe with a PE 10 polyethylene cannula.

Rats were adrenalectomized 72 hours before CSF and blood sample collection; control animals were sham-adrenalectomized. Nephrectomy and sham nephrectomy were performed 48 hours prior to sample collection. Experimental animals received DOC (Cortezon depot. Schering, 50 mg/kg twice a week) subcutaneously, and saline (0.9% NaCl solution) replaced the drinking water. Half of the experimental and control animals were treated with the converting enzyme inhibitor captopril (Squibb, Princeton, New Jersey) by its addition to the drinking fluid in a concentration that assured 100 mg/kg daily intake. Captopril treatment was begun 4 days before initiating DOC-salt administration; the latter was maintained for 30 days. Two-kidney, two clip (2K2C) renal hypertensive rats were studied 8 weeks after renal artery clipping. Silver clips 0.25 mm width were placed on both renal arteries. Control animals were sham-operated rats. Animals were deprived of water but not food for 5 days, blood and CSF samples were then obtained; intact rats were used as control. Finally, the effect of peripheral sympathectomy with 6-hydroxydopamine (6-HODA) plus adrenomedullectomy and bilateral adrenal denervation was analyzed in animals that were chronically treated with the drug since the day of birth. Rats were injected subcutaneously with 100 μg 6-HODA per g body weight on the day of birth and on the following day; 10 μl of 0.5% ascorbic acid in saline were used as vehicle. Further doses of 250 μg/g in 50 μl were given on Days 8 and 15, of 100 μg/g on Days 21, 28 and 35; thereafter weekly doses of 50 μg/g were injected until sacrifice. Control animals were injected with vehicle following the same schedule. The 6-HODA schedule presently employed was a modification of that described by Provoost et al.8 Samples were obtained at 16 weeks of age. CSF samples were processed immediately after collection. Plasma samples were kept frozen until assay. Plasma renin activity (PRA) was evaluated by radioimmunoassay technique (RIA) (Becton-Dickinson).9 Estimation of angiotensinogen concentration was performed by incubation of a 0.5 μl plasma sample or a 2 μl CSF sample with an excess hog kidney renin (25 μl of a 0.4 GU/ml renin solution in 8 mM EDTA) during 60 minutes at 37°C in the presence of 0.4 mM tris chloride buffer pH 7.2. AI released was measured with the mentioned RIA technique. Angiotensinogen concentration was expressed in ng Al/ml fluid.

Data are expressed as the means ± SEM. Analysis of differences between groups was accomplished by the Student's t test; results were considered significant when p < 0.05.

Results

The effect of the different experimental conditions on the renin substrate concentration in plasma and CSF as well as the levels of PRA in each case are presented in table 1.

Adrenalectomy decreased the plasma and central prohormone concentration, while PRA was increased significantly. Nephrectomy increased renin substrate concentration in both plasma and CSF. PRA was almost undetectable. No correlation was found between the individual values from either compartment. On the other hand, the changes of the prohormone in the plasma of adrenalectomized and nephrectomized animals were greater than those observed in CSF (fig. 1).

DOC-salt treatment induced hypertension in all animals. Blood pressure was 146.3 ± 2.7 mm Hg in the experimental group and 120.4 ± 2.4 mm Hg in the control group at the end of the study. A clear dissociation between plasma and CSF renin substrate was observed since a significant decrease in the peripheral prohormone coexisted with a significant increment in the CSF angiotensinogen concentration. PRA was almost undetectable in DOC-salt treated rats. Captopril added to the mineralocorticoid treatment did not influence the described changes. Captopril chronically given to control animals induced a strong inhibitory effect on plasma angiotensinogen concentration while CSF renin substrate concentration remained unchanged. A clear dissociation between both compartments was again observed.

Hypertension was present in all the animals after bilateral clipping of the renal arteries. Direct mean blood pressure was 151.7 ± 5.4 mm Hg in the 2K2C hypertensive rats and 113.6 ± 3.8 mm Hg in the controls at the end of the experiment. No significant changes in any of the components of the renin-angiotensin system presently studied, either in plasma or in CSF, were observed. Water deprivation produced a slight decrease in peripheral and central renin substrate that did not attain statistical significance. A fourfold increase in PRA was detected in water-deprived rats.

Chronic chemical sympathectomy with 6-HODA did not modify PRA or plasma renin substrate concentration but the concentration of the prohormone in the CSF was significantly elevated.

Percent changes in plasma and CSF angiotensinogen concentration in all the experimental groups are presented in figure 1.
**Table 1.** Plasma Renin Activity (PRA), Plasma and Cerebrospinal Fluid (CSF) Angiotensinogen Concentration in the Different Experimental Conditions Analyzed

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PRA (ng AI/ml/hr)</th>
<th>Plasma angiotensinogen concentration (ng AI/ml)</th>
<th>CSF angiotensinogen concentration (ng AI/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomy (Adx)</td>
<td>61.14±13.11 (6)*</td>
<td>512.08±84.91 (14)*</td>
<td>41.12±3.15 (10)*</td>
</tr>
<tr>
<td>Sham Adx</td>
<td>3.12±0.42 (4)</td>
<td>903.50±80.31 (8)</td>
<td>56.87±4.89 (7)</td>
</tr>
<tr>
<td>Nephrectomy (Nx)</td>
<td>0.066±0.013 (7)*</td>
<td>2361.46±146.54 (13)*</td>
<td>65.75±4.30 (12)*</td>
</tr>
<tr>
<td>Sham Nx</td>
<td>4.20±1.00 (5)</td>
<td>865.86±171.55 (7)</td>
<td>38.68±3.75 (8)</td>
</tr>
<tr>
<td>DOC-salt</td>
<td>0.065±0.006 (29)*</td>
<td>583.82±35.35 (28)*</td>
<td>62.52±3.05 (41)*</td>
</tr>
<tr>
<td>Control</td>
<td>4.82±0.48 (8)</td>
<td>933.65±64.26 (20)</td>
<td>46.98±1.63 (39)</td>
</tr>
<tr>
<td>DOC-salt + captopril</td>
<td>0.10±0.014 (32)*</td>
<td>633.52±38.65 (22)*</td>
<td>63.09±2.86 (31)*</td>
</tr>
<tr>
<td>Control + captopril</td>
<td>17.42±3.17 (15)</td>
<td>236.25±36.64 (8)</td>
<td>48.31±2.38 (14)</td>
</tr>
<tr>
<td>2K2C hypertension</td>
<td>10.27±2.43 (11)</td>
<td>871.89±80.98 (11)</td>
<td>46.24±3.72 (11)</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>7.47±1.07 (7)</td>
<td>850.74±19.36 (7)</td>
<td>46.88±4.08 (7)</td>
</tr>
<tr>
<td>Water deprivation</td>
<td>37.72±5.19 (11)*</td>
<td>743.00±68.20 (11)</td>
<td>39.83±3.10 (11)</td>
</tr>
<tr>
<td>Control</td>
<td>7.12±2.48 (7)</td>
<td>807.06±81.09 (7)</td>
<td>46.28±2.38 (7)</td>
</tr>
<tr>
<td>Peripheral sympathectomy</td>
<td>8.13±1.69 (8)</td>
<td>816.06±117.10 (8)</td>
<td>56.76±2.00 (8)*</td>
</tr>
<tr>
<td>Control</td>
<td>10.02±2.49 (10)</td>
<td>901.69±97.73 (10)</td>
<td>38.27±2.30 (10)</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM. Number of animals is in parentheses. *p < 0.05.

**Figure 1.** Angiotensinogen concentration in plasma and cerebrospinal fluid (CSF) expressed as a percentage change from control values under different experimental conditions: DOC-salt (30 days); captopril (34 days); DOC-salt + captopril (34 days); Clip: 2K2C hypertension (8 weeks); 6-HODA: sympathectomized since birth (16 weeks); water-deprived (5 days); Nx: nephrectomy (48 hrs); Adx: adrenalectomy (72 hours).
Discussion

Present results have confirmed a previous report on the effect of adrenalectomy on plasma and CSF renin substrate concentration. Adrenalectomy is also capable of diminishing the prohormone concentration in the brain tissue. Glucocorticoids seem to regulate not only the peripheral but also the central concentration of angiotensinogen. We have found a similar effect with a mineralocorticoid and salt treatment. A significant increase in renin-like activity in some regions of the rat brain was induced by chronic DOC-salt treatment. This increment was mainly due to an increased angiotensinogen concentration that seems to be a modulatory factor of the enzymatic central system. The presence of an increased concentration of angiotensin in CSF of DOC-salt-treated rats appeared to indicate a correlation between brain tissue and CSF and suggested that the central nervous system could be the major source of the angiotensinogen present in CSF.

Nephrectomy also induced parallel changes in plasma and CSF renin substrate concentration. Previously, we observed an increased renin-like activity in some regions of the brain of the rat after 48 hours following nephrectomy, therefore, also in this case the increment in angiotensinogen concentration might be related to an increased renin substrate content in brain parenchyma. In this regard, significant elevations in net angiotensinogen content have been found in selected areas of the rat brain 32 hours after bilateral nephrectomy.

Adrenalectomy and nephrectomy induced parallel changes in plasma and CSF angiotensinogen concentration while DOC-salt treatment resulted in a clear dissociation of the prohormone in both compartments. This effect differs from that produced by glucocorticoids which is one of the most effective stimuli for increased angiotensinogen secretion by the liver and at the same time produces a significant increment in the central concentration of renin substrate. On the contrary, DOC-salt treatment significantly depleted the plasma concentration of the prohormone and elevated its concentration in brain parenchyma and CSF as already discussed. The effect on plasma could be due to lack of circulating All, since this polypeptide is an important regulatory factor of the peripheral renin substrate. Chronic treatment of intact rats with captopril strongly suppressed plasma angiotensinogen and elevated renin concentration while formation of All was blocked by inhibition of converting enzyme. The decrease of plasma angiotensinogen could be the result of enhanced consumption of the substrate by increased concentration of the enzyme and the missing stimulatory action of All. In all DOC-salt rats even in those receiving captopril, although All formation was suppressed the consumption of angiotensinogen was not observed since renin secretion was strongly diminished. CSF angiotensinogen concentration was not modified by captopril treatment, resulting in a dissociation of the peripheral and central prohormone.

The development of 2K2C renal hypertension did not produce any significant change in either PRA or peripheral or central angiotensinogen concentration. Therefore, hypertension is not necessarily related to an increment in CSF angiotensinogen concentration.

Water deprivation induced a significant increase in PRA and no significant changes in plasma or CSF angiotensinogen concentration. Chen et al. have reported increased plasma renin substrate concentration and significant elevation of the prohormone in some brain regions in rats deprived of water during 72 hours. We were not able to confirm these results either in plasma or CSF. The main difference between both experiments was the period of water deprivation selected in each case. We have maintained our animals deprived of water for a longer time.

Finally, peripheral chemical sympathectomy with 6-HODA produced a significant increment in CSF angiotensinogen concentration that was not accompanied by any changes in the plasma concentration of the enzyme or its substrate; once more a clear dissociation between both compartments was present in this experimental condition. CSF renin substrate may play some role on the maintenance of blood pressure in sympathectomized animals through a possible regulatory effect on central All release at sites associated with its systemic pressor effect.

Conclusions

Although in some conditions CSF and plasma angiotensinogen seem to be interdependent or regulated by a similar mechanism, under other experimental situations they manifest a clear dissociation that does not support a plasma origin for the central prohormone. There seems to be more than one mechanism involved in the variation of central angiotensinogen: adrenal steroids, sympathetic innervation, and perhaps, peripheral levels of the renin-angiotensin system. There is not a clear link between hypertension development and increased CSF angiotensinogen concentration. More analysis is necessary to elucidate the physiological meaning of the central prohormone.

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Angiotensinogen concentration in the cerebrospinal fluid in different experimental conditions in the rat.
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Hypertension. 1983;5:V29
doi: 10.1161/01.HYP.5.6_Pt_3.V29

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