Renin-Like Activity in the Rat Brain During the Development of DOC-Salt Hypertension

NIDIA BASSO, PH.D., PATRICIA RUIZ, B.C., ELSA MANGIAURA, B.C., AND ALBERTO C. TAQUINI, M.D.

SUMMARY Levels and distribution of an angiotensin-forming enzyme, active at the physiological pH (isorenin), were determined in the central nervous system of 24 rats treated with 25 mg/kg of deoxycorticosterone (DOC) subcutaneously, twice a week, plus saline to drink during 30 days, and in 14 control animals. Different areas of the brain were excised and homogenized. Renin activity and concentration were determined by incubation of the supernatant of each homogenate at pH 7.2 alone and in the presence of an excess of renin substrate. The angiotensin generated was measured by radioimmunoassay. Concentration of the renin-like enzyme was significantly higher in the posterior hypophysis and in the brain stem of the experimental group; isorenin activity was higher in the hypothalamus, cerebral cortex, cerebellum, and brain stem of the DOC-salt-treated rats than in the control rats. Changes in the angiotensin-forming enzyme in the central nervous system of experimental animals, active at physiological pH, suggest that this isorenin system may play a role in the physiological response to DOC-salt in the rat. The significance of the brain isorenin system in the regulation of blood pressure requires further analysis. (Hypertension 3 (suppl II): 11-14—11-17, 1981)

KEY WORDS pineal gland • angiotensin • central nervous system • isorenin • hypothalamus •

THE existence of an independent brain reninangiotensin system, involved in the regulation of arterial pressure and water balance, has been postulated.1-4 Local production of angiotensin II (AII) requires the presence of a substrate, AI releasing enzyme, and converting enzyme.4 The presence of all these components as well as angiotensinases1 has been reported: the existence of angiotensinogen in the brain and cerebrospinal fluid is generally accepted;6, 9 renin-like activity within the central nervous system has been reported in humans,1, 6 the rat,5, 4 and dog.2, 9 However, the physiological significance of this enzymatic activity is controversial.10-18 Most investigators have described angiotensin-generating activity at pH 4.5-5.0 and almost no activity at pH 7.4.1-8, 11 Day and Reid11 have ascribed the isorenin enzymatic activity in the brain of dogs to another enzyme: cathepsin D; this view is supported by Hackenthal et al.18 On the contrary, chromatographic separation of renin-like activity has recently been achieved in the brains of dogs17 and rats.18 Although the amount of renin reported for rat brain was very low (250 pg A1/g/hr), we have been able to find a significant concentration of an angiotensin-forming enzyme, active at pH 7.2, in the central nervous system of rats and we have analyzed the regional distribution.10

The purpose of the present work was to investigate changes in the behavior of the central isorenin-angiotensin system in DOC-salt hypertensive rats. This type of hypertension was chosen because antidiuretic hormone seems to play a role in its pathogenesis,30 and because it has been demonstrated that AII given centrally enhances vasopressin secretion and increases blood pressure.31

Material and Methods

Male rats of the Wistar strain weighing 200-300 g were kept in an automatically lighted room (from 7 am to 7 pm) at a constant temperature (22° ± 1°C) and were fed both Purina rat chow and tap water ad

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libitum. Twenty-four rats were used as experimental animals and 14 as controls. Blood pressure was measured in conscious animals by the indirect plethysmographic tail method. Blood samples were obtained by cutting the tip of the tail. Experimental animals received DOC (Cortefon depot, Schering; 50 mg/kg body weight twice a week) subcutaneously, and saline replaced the drinking water. After 30 days, all animals were sacrificed by cervical dislocation, and the brain was perfused in situ with 20 ml cold saline. After craniotomy, the brain was removed and the pineal gland (PG), anterior hypophysis (AH), posterior hypophysis (PH), cerebral cortex (CC), cerebellum (Cbl), brain stem (BS), hypothalamus (Htl), and medulla oblongata (MO) were separated. Tissues were homogenized in cold 8 mM EDTA in saline and centrifuged. Samples were collected and processed below 4°C to prevent AI generation and its subsequent loss by enzymatic degradation. Renin concentration and activity were evaluated in the supernatant of tissue homogenates as follows.

Renin Concentration

One volume each of supernatant was incubated with one-half volume of partially purified rat angiotensinogen, obtained as has been described for human substrate, in the presence of adequate concentrations of angiotensinase inhibitors (8-hydroxyquinoline: 34 mM; dimercaprol: 1.6 mM; phenylmethylsulfonylfluoride: 2.8mM). Concentrated Tris chloride buffer (4.0 mM) pH 7.2 was added to the incubation medium, to obtain a final concentration of 0.4 mM. Incubation was conducted under continuous shaking for 3 hours at 37°C. Enzymatic reaction was stopped by freezing the samples to —20°C. Samples were kept frozen until assay.

Renin Activity

A similar incubation was performed but without the addition of exogenous substrate. AI present in all the samples was evaluated by radioimmunoassay (RIA). Blood samples were centrifuged at 4°C, plasma separated and kept frozen until assay, and plasma renin activity (PRA) determined by RIA. Results are expressed as means ± standard error. The data were analyzed by Student's t test.

### TABLE 1. Angiotensin-Forming Enzyme in the Central Nervous System of the Rat

<table>
<thead>
<tr>
<th>Rat group</th>
<th>PG (ng AI/g/hr)</th>
<th>AH (ng AI/g/hr)</th>
<th>PH (ng AI/g/hr)</th>
<th>CC (ng AI/g/hr)</th>
<th>Cbl (ng AI/g/hr)</th>
<th>Htl (ng AI/g/hr)</th>
<th>MO (ng AI/g/hr)</th>
<th>BS (ng AI/g/hr)</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>x</td>
<td>84.51 ± 8.33</td>
<td>75.31 ± 4.12</td>
<td>59.52 ± 9.11</td>
<td>13.27 ± 1.56</td>
<td>11.80 ± 1.41</td>
<td>20.70 ± 1.38</td>
<td>12.20 ± 0.98</td>
<td>11.08 ± 1.14</td>
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<td>SEM</td>
<td>(13)</td>
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<td>Doc-salt</td>
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<tr>
<td>x</td>
<td>94.66 ± 10.47</td>
<td>79.85 ± 4.61</td>
<td>90.37* ± 8.97</td>
<td>13.12 ± 1.15</td>
<td>13.22 ± 1.13</td>
<td>18.59 ± 1.52</td>
<td>13.61 ± 0.89</td>
<td>16.45* ± 1.68</td>
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</tbody>
</table>

PG = pineal gland, AH = anterior hypophysis, PH = posterior hypophysis, CC = cerebral cortex; Cbl = cerebellum; Htl = hypothalamus; MO = medulla oblongata; BS = brain stem.

*p < 0.025.

**p < 0.005.

†p < 0.001.
Results

Blood Pressure and Plasma Renin Activity (PRA)

All the animals developed hypertension. Increments became significant during the 3rd week of DOC-salt administration. PRA was significantly diminished by 15 days of treatment (fig. 1).

Renin-Like Concentration and Renin-Like Activity in the Central Nervous System

Distribution of renin concentration and renin activity in different areas of the central nervous system in controls and in DOC-salt treated hypertensive animals are shown in table 1. Highest values for both were found in the pineal gland, posterior hypophysis, and anterior hypophysis. An important amount was also observed in the hypothalamus. Treatment with DOC-salt was accompanied by a determined significant increase in renin concentration in the posterior hypophysis and brain stem. When the effect of DOC-salt on renin activity was analyzed, significant increments were detected in the hypothalamus, cerebral cortex, cerebellum, and brain stem.

Discussion

Previous experiments in our laboratory have shown the presence of significant amounts of an angiotensin-generating enzyme system, active at physiological pH, in the central nervous system of the rat.\(^6\) The distribution of the enzyme observed in the present work confirms our previous observation of highest levels in the pineal gland, and anterior and posterior hypophysis. Also, an important concentration was detected in the hypothalamus which might be related to the physiological function of central angiotensin and the presence of AII receptors in the periventricular system.\(^7\) Hirose et al.\(^8\) have analyzed isorenin distribution in dog brain, and found the highest concentrations in the pineal gland and anterior hypophysis; the hypothalamus and cerebellum showed intermediate levels while the medulla oblongata, brain stem, and cerebral cortex concentrations followed in descending order. Even though they used another animal, their results are similar to our findings except for the very low levels found in the posterior hypophysis.

Present results indicate an increased formation of AII in some areas of the central nervous system in the hypertensive animals. Augmented AII generation was not necessarily related to an increased concentration of the AI-forming enzyme, thus suggesting that other factors are modulating the activity of the enzyme. The presence of different amounts of inhibitors or potentiators of the enzymatic system and/or changes in angiotensinogen concentration could be involved in the increased formation of AII. If angiotensinogen was the main regulator of enzyme activity, one might expect to find a correlation in the distribution of the substrate and the activity of the isorenin. Using available data on angiotensinogen concentration,\(^9, 10\) we found that a clear correlation could be established for some areas but not for all. However, this lack of correlation could be due mainly to the different techniques employed to determine the angiotensinogen and renin activity. Further studies using the methodology described in this report should most probably afford the necessary information on the role of the substrate in the modulation of the enzymatic system.

The clear dipsogenic effect found in DOC-salt-treated rats could be related to increased central or peripheral angiotensin;\(^11\) since PRA is markedly diminished in these animals, it is difficult to accept that circulating AII is involved in the central activity.

Although present information is insufficient to establish any involvement of central A in the pathogenesis of DOC-salt hypertension, the data in this report suggest that the isorenin activity present in some areas of the central nervous system is responsive to DOC-salt treatment. Whether the changes in the central isorenin system have any role in blood pressure regulation or in the pathogenesis of DOC-salt-treated rats requires further investigation.

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