Regional distribution of angiotensinogen in the central nervous system of the rat: effect of DOC–salt treatment

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Summary
1. The distribution of angiotensinogen and endogenous renin-like activity were analysed in different areas of the central nervous system in normal and DOC–salt-treated hypertensive rats.
2. Angiotensinogen concentration and renin-like activity were significantly increased in the cerebral cortex, cerebellum, hypothalamus and brain stem of the DOC–salt-treated rats 30 days after the initiation of the experiment.
3. Influence of plasma contamination on the former results was evaluated by the determination of (a) plasma angiotensinogen concentration in control and treated animals and (b) blood content remaining in the different regions of the central nervous system, after saline perfusion of the brain, in a group of normal rats.
4. Plasma angiotensinogen concentration was significantly decreased in DOC–salt-treated rats, therefore blood contamination would tend to diminish the magnitude of increase in central nervous system angiotensinogen in these animals.
5. Present results have shown an increased concentration of angiotensinogen in some areas of the central nervous system in DOC–salt-treated rats. The results have also confirmed an enhanced activity of the endogenous renin-like enzyme in the same regions; this change seems to be mainly due to the increment in angiotensinogen. Increased formation of central angiotensin could be involved in the development of DOC–salt hypertension. The biosynthetic pathways of renin substrate as well as its endogenous regulation remain undetermined.

Key words: angiotensinogen, brain, central nervous system, renin-like activity.

Abbreviation: RA, renin-like activity.

Introduction
The presence and physiological role of an endogenous renin–angiotensin system in the brain has been the subject of considerable debate in the past few years [1–8]. Recently, renin has been immunochemically identified in the brain of man [9] and the rat [10, 11]. In a previous report [12] we have also shown the presence of a renin-like enzyme active at the physiological pH in different areas of the central nervous system of the rat. Moreover, we have observed an increment in the endogenous renin-like activity in some regions of the central nervous system during the development of DOC–salt hypertension in the rat. This change in angiotensin I (ANG I) formation was not related to an increased concentration of the enzyme, suggesting that endogenous brain angiotensinogen could be the limiting factor in the activity of the enzymatic system.

Based on these findings the present work was undertaken to analyse simultaneously the distribution of angiotensinogen concentration and of the endogenous activity of the renin-like system in the central nervous system of control and DOC–salt-treated rats 4 weeks after the initiation of the treatment.

Methods
Male rats of the Wistar strain in the weight range 200–300 g were used. Twenty-four rats without any manipulation were used as control and 35 were used as experimental animals. Rats were kept, treated, killed and processed and tissues were extracted and prepared as described in a previous report [12].

Angiotensinogen concentration and renin-like activity (RA) were evaluated in the supernatant of central nervous system tissue homogenates as follows:
(a) Angiotensinogen: a portion (25 μl) of the supernatant was incubated in the presence of 0.01 GU purified hog renin (General Biochemicals) and adequate concentrations of angiotensinase inhibitors [12] at pH 7.2.

(b) RA: a portion (50 μl) of each supernatant was incubated under the conditions of (a) without the addition of renin. Incubations were conducted with continuous shaking at 37°C during 1 h for angiotensinogen and during 3 h for RA. Enzymatic reaction was stopped by freezing the samples at -20°C. Samples were kept frozen until assay. ANG I present in all the samples was evaluated by radioimmunoassay (RIA) (Becton-Dickinson). Plasma angiotensinogen concentration was determined by incubating 5 μl of a 1/10 dilution of each sample in the presence of 0.01 GU of purified hog renin during 1 h in similar conditions to PRA [13]. ANG I formed was evaluated by radioimmunoassay.

The amount of blood remaining in the central nervous system even after thorough rinsing with 20 ml of sodium chloride solution (154 mmol/l: saline) was determined in five intact rats. The animals were anaesthetized with sodium pentobarbital (35 mg/kg). To allow adequate equilibration 121I-labelled serum albumin was given intravenously saline) was determined in five intact rats. The blood sample was obtained immediately before killing and processing the animals as described before [12]. Blood and tissue samples were placed in weighed glass tubes, reweighed and radioactivity was determined in a well-type gamma counter.

Data are expressed as means ± sem. Significance of differences was calculated by the Student's t-test.

### Results

#### Blood pressure and plasma angiotensinogen concentration

The DOC-salt treated animals increased their blood pressure from the first week of treatment; increments were significant during the second week (+24%; P < 0.001) and continued to increase until the end of the experiment (+37%; P < 0.0005). Angiotensinogen decreased significantly after the second week of the experimental period (control: 834.62 ± 79.66 ng of ANG I/ml; 13 days of treatment: 629.96 ± 41.14 ng of ANG I/ml; P < 0.02). The difference from control animals was even more significant at the end of the experiment (control: 1054.47 ± 77.25 ng of ANG I/ml; DOC-salt: 604.29 ± 47.23 ng of ANG I/ml; P < 0.0005).

#### Angiotensinogen concentration, endogenous renin-like activity and blood content in the central nervous system

The results are presented in Table 1. DOC-salt treatment produced a significant increase in angiotensinogen and RA in the cerebral cortex, cerebellum, hypothalamus and brain stem, but no significant changes were observed in the remaining regions; higher blood content was detected in these regions than in the former parts.

### Discussion

Present results confirm our previous report on the endogenous formation of ANG I when homogenates of brain tissue were incubated at physiological pH [12]. These data support the wide

### Table 1. Effect of DOC-salt treatment on angiotensinogen concentration and renin-like activity in areas of the rat central nervous system

<table>
<thead>
<tr>
<th>Area</th>
<th>Control Blood content (mg/g) (n = 5)</th>
<th>Control Angiotensinogen (ng of ANG I/g) (n = 24)</th>
<th>Control RA (ng of ANG I h⁻¹ g⁻¹)</th>
<th>DOC–salt Blood content (mg/g) (n = 5)</th>
<th>DOC–salt Angiotensinogen (ng of ANG I/g) (n = 35)</th>
<th>DOC–salt RA (ng of ANG I h⁻¹ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>11.36 ± 1.46</td>
<td>27.30 ± 1.02</td>
<td>1.08 ± 0.06</td>
<td>34.38 ± 1.32***</td>
<td>1.24 ± 0.06*</td>
<td>64.77 ± 2.43***</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>14.99 ± 2.41</td>
<td>45.21 ± 1.98</td>
<td>1.30 ± 0.06</td>
<td>64.77 ± 2.43***</td>
<td>1.61 ± 0.08**</td>
<td>85.41 ± 6.60*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>9.19 ± 2.51</td>
<td>69.03 ± 3.39</td>
<td>2.45 ± 0.11</td>
<td>85.41 ± 6.60*</td>
<td>2.91 ± 0.13**</td>
<td>51.42 ± 1.83**</td>
</tr>
<tr>
<td>Brain stem</td>
<td>10.67 ± 2.09</td>
<td>43.50 ± 1.59</td>
<td>1.95 ± 0.06</td>
<td>51.42 ± 1.83**</td>
<td>2.39 ± 0.09***</td>
<td>68.10 ± 3.78</td>
</tr>
<tr>
<td>Medulla oblongata</td>
<td>18.44 ± 6.27</td>
<td>61.53 ± 3.60</td>
<td>3.08 ± 0.22</td>
<td>46.32 ± 4.86</td>
<td>2.96 ± 0.16*</td>
<td>4.86 ± 1.38</td>
</tr>
<tr>
<td>Pineal gland</td>
<td>18.66 ± 9.07</td>
<td>59.52 ± 9.66</td>
<td>8.59 ± 1.19</td>
<td>4.86 ± 1.38</td>
<td>10.72 ± 1.38*</td>
<td>4.86 ± 1.38</td>
</tr>
<tr>
<td>Anterior hypophysis</td>
<td>33.74 ± 5.28</td>
<td>56.79 ± 5.31</td>
<td>4.70 ± 0.34</td>
<td>49.44 ± 3.80</td>
<td>4.50 ± 0.34</td>
<td>9.18 ± 0.95</td>
</tr>
<tr>
<td>Posterior hypophysis</td>
<td>32.88 ± 6.12</td>
<td>61.92 ± 4.29</td>
<td>8.25 ± 0.78</td>
<td>61.23 ± 5.82</td>
<td>9.05 ± 0.96</td>
<td>61.23 ± 5.82</td>
</tr>
</tbody>
</table>
distribution of angiotensinogen in all the areas of the central nervous system of the rat, as has been previously described [14–16]. Angiotensinogen was evaluated under adequate incubation conditions to prevent inactivation of ANG I by angiotensinases. This method differs from those reported by Lewicki et al. [14] and Sernia & Reid [16], and no comparison between their results and our data can therefore be made.

Increased RA in cerebral cortex, cerebellum, hypothalamus and brain stem of DOC–salt-treated rats has also been confirmed. The increments seem to be mainly due to an increase in angiotensinogen, induced by the experimental treatment. According to one report [17] some brain regions respond to adrenalectomy with a decreased angiotensinogen, and corticosterone replacement therapy reversed this effect. Mineralocorticoids might have a similar effect; thus they could be involved in central angiotensinogen regulation. Alternatively DOC may have glucocorticoid activity.

The degree of blood contamination was small (1–3%), but, owing to the high plasma angiotensinogen, large enough to alter the concentration of the prohormone in the central nervous system. On the other hand, blood contamination was unequally distributed in the different areas. Since DOC–salt treatment produced a significant decrease in plasma angiotensinogen, blood contamination could only diminish the real magnitude of the increment in central angiotensinogen and, even more, could conceal changes in some of the studied areas. We think that angiotensinogen was increased all over the central nervous system of the DOC–salt-treated rats but, owing to blood contamination, the changes in some areas proved to be undetectable.

Morris & Reid [18] have postulated that most of the angiotensinogen in the brain is of cerebrospinal fluid origin and is present in the extracellular space. Supporting this possibility, preliminary data obtained in our laboratory indicate a significant increase in cerebrospinal fluid angiotensinogen in DOC–salt-treated rats. Nevertheless, the significant differences in angiotensinogen observed among the analysed areas could hardly be explained by the diverse magnitude of the extracellular space in these regions.

In conclusion, present data indicate that saturating levels of angiotensinogen are not attained in the brain; therefore angiotensinogen may be a rate-limiting and regulatory factor in production of ANG I [19]. Increased brain angiotensinogen in DOC–salt hypertensive rats might be involved in the pathogenesis of this type of hypertension, since ANG II liberated in the region of the cerebral ventricles, through its central pressor and vasopressin-releasing effects [20], may play some role in sustaining high blood pressure.

Acknowledgment

We acknowledge the excellent technical assistance of Miss Graciela Dotta.

References


