The Importance of Vasopressin in the Development and Maintenance of DOC-Salt Hypertension in the Rat

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SUMMARY Experiments were performed to determine the role of vasopressin in deoxycorticosterone (DOC)-salt hypertension. In order to determine if vasopressin is necessary for the development of DOC-salt hypertension, rats with hereditary diabetes insipidus (DI) and normal Long-Evans rats (LE) were unilaterally nephrectomized, treated with DOC Pivalate (30 mg/kg • week) and given saline to drink for 8 weeks. A second group of DI rats were unilaterally nephrectomized, but received no treatment. Systolic blood pressure (SBP) increased 40 mm Hg in the LE group \((p < 0.01)\) but failed to increase significantly in either DI group. Urinary excretion of vasopressin \((\text{U}_{\text{ADH}})\) and SBP were measured in unilaterally nephrectomized LE rats treated with DOC and salt (DOC-LE), salt alone (NaCl-LE) and untreated rats (H2O-LE). The \(\text{U}_{\text{ADH}}\) was elevated in DOC-LE \((p < 0.01)\) and NaCl-LE \((p < 0.05)\) rats, but only the DOC-LE rats became hypertensive. Finally, the I.V. injection of analogs of vasopressin, which block its pressor but not antidiuretic activity, lowered mean arterial blood pressure \(27 \pm 5\) mm Hg in 11 conscious DOC-salt hypertensive rats. It is concluded that vasopressin plays a major role as a pressor agent in both the onset and maintenance of DOC-salt hypertension. (Hypertension 1: 31-38, 1979)

KEY WORDS • vasopressin • DOC-salt hypertension • antidiuretic hormone • Brattleboro rat • diabetes insipidus • vasopressin competitive inhibitor

ELEVATED levels of vasopressin have been reported recently in several forms of hypertension. Khokhar and Slater\(^1\) found that the urinary excretion of vasopressin was elevated in patients with essential hypertension when compared to normotensive controls. We have reported\(^2\) similar findings in the Okamoto-Aoki spontaneously hypertensive rat, a model of hypertension resembling essential hypertension. Urinary excretion of vasopressin, plasma vasopressin concentration, and total pituitary content of vasopressin were substantially elevated above levels found in the Wistar-Kyoto hypertensive control rat. When the plasma concentration of vasopressin in patients with malignant hypertension was compared to that in patients with essential hypertension or in normotensive controls, vasopressin was again found to be elevated.\(^3\) Rats subjected to removal of 70% of the renal mass, and given 1% NaCl to drink showed a marked increase in urinary excretion of vasopressin.\(^4\) Möhring et al.\(^8\) have reported an elevated plasma vasopressin concentration in rats with two-kidney Goldblatt hypertension. Plasma vasopressin concentration was also elevated in rats made hypertensive by unilateral nephrectomy, treatment with deoxycorticosterone (DOC) and substitution of 1% NaCl for drinking water.\(^6\) The plasma vasopressin concentration was elevated 10-fold above control values in the malignant phase of hypertension, and threefold above control in the benign phase. The severity of the hypertension appeared to be correlated with circulating levels of vasopressin. Furthermore, an I.V. injection of a specific antiserum to vasopressin lowered arterial pressure in these rats, most
dramatically in the rats in the malignant phase of hypertension. Möhring et al. suggested that vasopressin is involved as a pressor agent in DOC-salt hypertension.

In order to determine whether vasopressin is essential in the onset and/or maintenance of DOC-salt hypertension, we have attempted to produce this form of hypertension in rats with hereditary hypothalamic diabetes insipidus. An increased plasma concentration of vasopressin may be reflected by an increased excretion of this hormone in the urine. Therefore, in rats that do not have diabetes insipidus, DOC-salt hypertension was created and excretion of vasopressin was measured to determine when vasopressin levels are increased with respect to the developing hypertension. With the recent development of analogs of vasopressin that block its pressor, but not its antidiuretic action, it was possible to determine whether vasopressin exerts a direct pressor action in the DOC-salt hypertensive rat.

Methods

Experiment I

Nine male rats with hereditary hypothalamic diabetes insipidus (DI) derived from the Brattleboro strain and six normal male hooded Long-Evans rats (LE) obtained from Blue Spruce Farms, Inc. were used in the present study. The animals were housed in individual cages in a room where temperature (23°-24° C) and the lighting cycle (12 hours on-12 hours off) were constant. Purina laboratory chow and tap water were given ad libitum. The DI and LE rats were age-matched (3 to 4 months old at the beginning of the study).

Systolic blood pressure (SBP) was measured once a week under light ether anesthesia by tail plethysmography. A detailed description of this method is given elsewhere. Control SBP measurements were obtained for all rats 2 weeks before unilateral nephrectomy. At the beginning of the third week, the rats were unilaterally nephrectomized under ether anesthesia. No SBP measurements were made during this week. At the beginning of the fourth week, SBP measurements were resumed. Following the measurement of SBP on the fourth week, five of the DI rats (DOC-DI) and all of the LE rats (DOC-LE) were given DOC (Percorten Pivalate, 30 mg/kg body weight) subcutaneously and salt solution replaced the drinking water. The remaining four DI rats (CON-DI) served as a separate group at a later time. The conditions of housing, nephrectomy and SBP measurement were virtually identical to those described for the DOC-DI and DOC-LE groups.

In the 2 weeks preceding unilateral nephrectomy, the DOC-DI and the DOC-LE rats were trained to drink salt solution, since the DI rat will not readily do so. However, when given a choice between water and salt during the first week of study, the DI rat began to show a preference for the salt solution. Because the DI rat normally consumes its body weight in water daily, the DI group was given 0.3% NaCl to drink, whereas the LE group was given 1% NaCl to drink. This was done in an attempt to provide similar NaCl intake in the two groups. It should be noted that it was impossible to induce these DI rats to drink salt solutions much greater than 0.3%. At the beginning of the second week of the experiment, both groups of rats were given only salt solution to drink. All of the animals made this transition with no difficulty. In the week during recovery from unilateral nephrectomy, free choice between water and salt was resumed. The day before resuming SBP measurement and initiation of treatment with DOC the water was discontinued and replaced with salt solution. The CON-DI group received only tap water to drink.

Body weight was measured three times a week in the DOC-DI and the DOC-LE groups and once a week in the CON-DI group. Water and NaCl intake were measured daily. For simplicity in presentation of the data, measurements within a week were averaged to provide weekly means. The DOC-DI and the DOC-LE rats were followed for 9 weeks after nephrectomy; the CON-DI group was followed for 5 weeks after nephrectomy.

Experiment II

Seventeen LE rats (Blue Spruce) were housed individually in stainless steel metabolism cages. At age 6 weeks, all were unilaterally nephrectomized under ether anesthesia, and allowed to recover for 1 week. They were then separated into three groups. One group of seven rats (DOC-LE) received DOC (Percorten Pivalate, 30 mg/kg body weight) subcutaneously at weekly intervals, and 1% NaCl solution replaced the drinking water. To assess possible effects of the high NaCl diet on vasopressin excretion, the second group of four rats (NaCl-LE) was given 1% NaCl to drink, but were not treated with DOC. A third group of 6 rats (H2O-LE) was given tap water to drink and served as a non-treated control group. The NaCl-LE and H2O-LE rats were injected subcutaneously with 0.9% NaCl (1.2 ml/kg body weight) each week. Beginning at age 7 weeks, and once a week thereafter, urine was collected for the measurement of 24-hour excretion of vasopressin (UADH/V). The SBP was determined as described above. Immediately after the 24-hour excretion of vasopressin and SBP measurements at age 7 weeks, treatment with DOC and salt or salt alone was begun and continued for the remainder of the study. Body weight was measured three times a week and fluid intake was measured daily. The animals were followed for 5 weeks.

The urine used for the measurement of 24-hour excretion of vasopressin was collected under mineral oil into 50-ml plastic centrifuge tubes containing 0.2 ml of glacial acetic acid and was stored at -14° C. Vasopressin was extracted from the urine by a method involving adsorption of vasopressin onto a column of Amberlite CG-50 ion exchange resin and elution with acidified ethanol. Vasopressin was measured by

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radioimmunoassay using a highly specific arginine vasopressin (AVP) antiserum. This antiserum does not cross react with lysine vasopressin, oxytocin, arginine vasotocin or angiotensins I and II. The standard used was USP Posterior Pituitary Reference Standard. Recovery of vasopressin from urine obtained from DOC-LE, NaCl-LE and H₂O-LE rats was determined as follows. On the day of extraction, the samples were thawed and 2–3 ml aliquots were taken from each sample in each of the three groups and pooled within groups. Each pooled sample was divided into two 7-ml aliquots; 1050 μU of vasopressin were added to one aliquot, while the other aliquot served as a blank. This was done each time urine was extracted and assayed. Average recovery for the DOC-LE group was 82.8 ± 2.2% (n = 5); 82.2 ± 2.8% for the NaCl-LE group (n = 5); and 84.5 ± 3.9% for the H₂O-LE group (n = 5). The U_ADH V was not corrected for incomplete recovery.

Experiment III

On completion of Experiments I and II, four of the DOC-LE rats from Experiment I and seven of the DOC-LE rats from Experiment II were anesthetized with ether, and catheters were placed into a femoral artery (SVE 6 polypropylene expanded, Dural Plastics) for blood pressure measurement, and into a femoral vein (PE 20) for injections. After the animals had regained consciousness, they were allowed to recover for at least 3 hours. They were partially restrained, but had backward and forward mobility. Mean arterial blood pressure (MABP) was recorded from the femoral artery by a Statham P23Gb pressure transducer and a Brush 2600 recorder. After MABP had stabilized for at least 1 hour, the rats from Experiment I were injected with 30 μg of a synthetic analog of arginine vasopressin [1-(β-mercaptop-β, β-cyclopentamethylepropionic acid), 4-valine, 8-D-arginine] vasopressin (cyclo-dVDAVP). The DOC-LE rats from Experiment II were given 33 μg of a second synthetic analog of AVP [1-deaminopenicillamine, 4-valine, 8-D-arginine] vasopressin (dPVDAVP). The DOC-LE rats from Experiment II were given 33 μg of a second synthetic analog of AVP [1-deaminopenicillamine, 4-valine, 8-D-arginine] vasopressin (dPVDAVP). The synthesis and pharmacological properties of these analogs are reported elsewhere. Both analogs antagonize the vasopressor action of AVP, and both have low to moderate antidiuretic activity, but neither inhibits the antidiuretic response to AVP. In the normal animal, dPVDAVP has a slightly greater potency than cyclo-dVDAVP in blocking the pressor response to exogenous AVP. Seven of the rats were also injected with vehicle alone; 0.05 M glacial acetic acid-0.5% chlorobutanol-0.9% NaCl for the rats from Experiment I, and 0.9% NaCl-0.1% bovine serum albumin-0.03% acetic acid for the rats from Experiment II.

Six H₂O-LE rats from Experiment II and three untreated age-matched LE rats were prepared for recording of blood pressure as described above. We injected 30 μg of cyclo-dVDAVP into the LE group and 33 μg of dPVDAVP into the H₂O-LE animals, to determine if either analog had a nonspecific blood-pressure-lowering effect. Vehicle alone was also injected into all the rats. One hour after the injection of the analog, the rats were given 20 mU AVP i.v. (Bachem). After MABP had returned to basal levels, the rats were injected with either 30 μg of cyclo-dVDAVP or 33 μg of dPVDAVP immediately followed by 20 mU AVP. After blood pressure stabilized, this last procedure was repeated with 20 ng of angiotensin II substituted for the AVP. Four of the NaCl-LE rats from Experiment II were also prepared for blood pressure recording and were injected with 33 μg of dPVDAVP and with vehicle alone. The volume of each I.V. injection in this group of experiments was 0.25 ml.

Statistics

A one- or two-factor analysis of variance for repeated measures was carried out where appropriate. Either Newman-Keuls a posteriori tests or multiple t tests were made to isolate within and between group differences. In the latter analysis, a p < 0.01 was set for individual comparisons to ensure that the overall probability for the experiment was less than 0.05. Some of the data were tested by t statistic alone. Means are given ± one standard error in figures, tables and text, but these standard errors are not used in calculating statistical significance.

Results

In addition to an inability to synthesize vasopressin, it has been established that the DI rat also has a growth defect. Since the DOC-LE and DOC-DI animals in Experiment I were age-matched rather than weight-matched, the body weights of the DOC-DI rats were half those of the DOC-LE rats at the beginning of the study (table 1). In the DOC-LE rats, body weight increased (p < 0.01) through Week 7 of the study, but fell progressively from Weeks 8 through 11. By Week 11, body weight had fallen to initial control levels, a loss of 54 ± 9 g compared to maximum body weight at Week 7. The DOC-DI (table 1) and CON-DI (table 2) rats, except for the week following surgery, gained weight at a rate similar to that reported for DI rats. When NaCl consumption (mmoles/24 hr • 100 g body weight) was compared between the DOC-LE and DOC-DI rats (table 1), differences (p < 0.005) were found at Weeks 4, 5 and 6 when NaCl consumption by the DOC-DI rats was greater, and at Week 11 when NaCl intake by the DOC-LE rats was greater. The DOC-LE rats steadily increased NaCl intake throughout the experiment (p < 0.01), whereas NaCl intake by the DOC-DI rats did not change.

Although there was a slight fall in SBP (fig. 1) in both the DOC-LE and DOC-DI rats in the week following unilateral nephrectomy (Week 4), these changes were not statistically significant. In the DOC-LE group, SBP increased progressively from Weeks 7 through 11. At the conclusion of the experiment, Week 11, SBP in the DOC-LE group was 40 mm Hg.
higher \( p < 0.01 \) than that observed before unilateral nephrectomy. Yet, there was no statistically significant change in SBP in the DOC-DI rats. Systolic blood pressure in the DOC-LE group was significantly higher than in the DOC-DI group \( p < 0.005 \) during the last 3 weeks of the experiment. In the CON-DI group SBP did not change significantly (table 2).

If an increased secretion of vasopressin plays a role in the development of DOC-salt hypertension, we felt that this would be reflected by an increased urinary excretion of vasopressin as the hypertension develops. This increase could be related to either substitution of saline for drinking water alone or the combination of DOC treatment and the drinking of saline. These possibilities were tested by measuring vasopressin excretion in unilaterally nephrectomized LE rats with normal hypothalamic function treated with DOC and salt (DOC-LE), salt alone (NaCl-LE), and neither DOC nor salt (H\(_2\)O-LE).

Systolic blood pressure in the DOC-LE rats increased \( p < 0.01 \) progressively throughout the 5 weeks of treatment to a level 70 mm Hg above the initial value (fig. 2A). In the NaCl-LE and H\(_2\)O-LE rats SBP increased 30 and 20 mm Hg, respectively, during the first 3 weeks of the experiment and then either plateaued (NaCl-LE) or fell (H\(_2\)O-LE) in the remaining 2 weeks of study. These increases were statistically significant \( p < 0.05 \) only in the H\(_2\)O-LE group at Weeks 3 and 4. There were no differences in SBP between the NaCl-LE and H\(_2\)O-LE groups at any time. However, SBP in the DOC-DI rats was greater \( p < 0.01 \) than in the other two groups for the third week of the experiment on, with the exception of Week 4, when the DOC-LE and H\(_2\)O-LE groups were not different.

Substitution of 1% NaCl for drinking water resulted in a two- to threefold increase in the 24-hour urinary excretion of vasopressin in the NaCl-LE rats \( p < 0.05 \) and a three- to fourfold increase in the DOC-LE rats \( p < 0.01 \); fig. 2B). Although the 24-hour urinary excretion of vasopressin was consistently greater in the DOC-LE than in the NaCl-LE rats, the differences between these two groups were not statistically significant. Twenty-four-hour urinary excretion of vasopressin remained relatively constant in the H\(_2\)O-LE rats throughout the experiment.

Consumption of NaCl (table 3) did not change with time in either the DOC-LE or the NaCl-LE rats of Ex-

**Table 1.** Body Weight and NaCl Intake During the Course of Experiment I in Normal Long-Evans (DOC-LE) and DI (DOC-DI) Rats*

<table>
<thead>
<tr>
<th>Type of rat</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC-LE (n = 6)</td>
<td>503</td>
<td>520</td>
<td>528</td>
<td>526</td>
<td>540</td>
<td>557</td>
<td>584</td>
<td>566</td>
<td>541</td>
<td>520</td>
<td>510</td>
</tr>
<tr>
<td>SE</td>
<td>± 9</td>
<td>± 10</td>
<td>± 11</td>
<td>± 10</td>
<td>± 11</td>
<td>± 12</td>
<td>± 12</td>
<td>± 8</td>
<td>± 6</td>
<td>± 6</td>
<td>± 7</td>
</tr>
<tr>
<td>DOC-DI (n = 5)</td>
<td>253</td>
<td>248</td>
<td>262</td>
<td>265</td>
<td>275</td>
<td>286</td>
<td>284</td>
<td>284</td>
<td>284</td>
<td>288</td>
<td>296</td>
</tr>
<tr>
<td>SE</td>
<td>± 10</td>
<td>± 8</td>
<td>± 9</td>
<td>± 10</td>
<td>± 11</td>
<td>± 11</td>
<td>± 10</td>
<td>± 12</td>
<td>± 11</td>
<td>± 11</td>
<td>± 9</td>
</tr>
<tr>
<td>NaCl intake (mmole/24 hr · 100 g body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC-LE (n = 6)</td>
<td>3.49</td>
<td>4.54</td>
<td>5.52</td>
<td>6.43</td>
<td>6.76</td>
<td>7.61</td>
<td>8.81</td>
<td>7.70</td>
<td>9.41</td>
<td>9.41</td>
<td>9.41</td>
</tr>
<tr>
<td>SE</td>
<td>± 0.22</td>
<td>± 0.15</td>
<td>± 0.20</td>
<td>± 0.24</td>
<td>± 0.29</td>
<td>± 0.33</td>
<td>± 0.36</td>
<td>± 0.51</td>
<td>± 0.51</td>
<td>± 0.51</td>
<td>± 0.51</td>
</tr>
<tr>
<td>DOC-DI (n = 5)</td>
<td>8.17</td>
<td>8.10</td>
<td>7.45</td>
<td>7.74</td>
<td>7.63</td>
<td>7.53</td>
<td>7.10</td>
<td>6.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>± 0.35</td>
<td>± 0.29</td>
<td>± 0.34</td>
<td>± 0.36</td>
<td>± 0.25</td>
<td>± 0.40</td>
<td>± 0.39</td>
<td>± 0.34</td>
<td>± 0.34</td>
<td>± 0.34</td>
<td>± 0.34</td>
</tr>
</tbody>
</table>

*Rats were unilaterally nephrectomized after measurements on Week 3; DOC treatment was begun after measurements on Week 4.

\( *p < 0.01 \) when compared to Week 1.

Abbreviations: DOC = deoxycorticosterone; DI = diabetes insipidus.

**Table 2.** Systolic Blood Pressure (SBP) and Body Weight in Unilaterally Nephrectomized DI (CON-DI) Rats from Experiment I*

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>122</td>
<td>126</td>
<td>140</td>
<td>133</td>
<td>124</td>
<td>128</td>
<td>133</td>
</tr>
<tr>
<td>SE</td>
<td>± 4</td>
<td>± 4</td>
<td>± 9</td>
<td>± 5</td>
<td>± 5</td>
<td>± 11</td>
<td>± 1</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>247</td>
<td>249</td>
<td>233</td>
<td>242</td>
<td>250</td>
<td>256</td>
<td>255</td>
</tr>
<tr>
<td>SE</td>
<td>± 13</td>
<td>± 14</td>
<td>± 10</td>
<td>± 14</td>
<td>± 15</td>
<td>± 12</td>
<td>± 12</td>
</tr>
</tbody>
</table>

*These rats were unilaterally nephrectomized after observations on Week 2 and received neither NaCl nor deoxycorticosterone; DI = diabetes insipidus.

\( \ast p < 0.05 \) when compared to the first week of observation.
### Table 3. Sodium Chloride Intake and Body Weight of Rats from Experiment II*

<table>
<thead>
<tr>
<th>Type of rat</th>
<th>Body weight (g)</th>
<th>NaCl intake (mmoles/24 hr • 100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DOC-LE (n = 7)</td>
<td>149 ± 5</td>
<td>184†± 7</td>
</tr>
<tr>
<td>NaCl-LE (n = 4)</td>
<td>151 ± 12</td>
<td>200†± 14</td>
</tr>
<tr>
<td>H₂O-LE (n = 6)</td>
<td>154 ± 6</td>
<td>200†± 5</td>
</tr>
<tr>
<td>NaCl intake (mmole)</td>
<td>6.00</td>
<td>6.38</td>
</tr>
<tr>
<td>DOC-LE (n = 7)</td>
<td>± 0.79</td>
<td>± 0.57</td>
</tr>
<tr>
<td>NaCl-LE (n = 4)</td>
<td>± 0.68</td>
<td>± 0.59</td>
</tr>
</tbody>
</table>

*All rats were unilaterally nephrectomized before Week 1. Treatment with deoxycorticosterone (DOC-LE) and saline (DOC-LE and NaCl-LE) was begun after measurements on Week 1.

tp < 0.01 when compared to the first week of observation.

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**Figure 1.** Systolic blood pressure (SBP) in Long-Evans (DOC-LE) and diabetes insipidus (DOC-DI) rats subjected to unilateral nephrectomy and treated with deoxycorticosterone and salt. Asterisks above or below the lines indicate statistically significant differences within the group when compared to Week 1. Asterisks between the lines indicate significant differences between groups.
Experiment II, but was 30% to 70% greater in the former group \( (p < 0.005) \) throughout the experiment. Body weight increased significantly with time \( (p < 0.01) \) for all three groups of Experiment II (table 3). No significant differences were found among any of the three groups at any time.

Four of the DOC-LE rats from Experiment I and seven of the DOC-LE rats from Experiment II were injected with one of two analogs of vasopressin that block the pressor activity of that hormone (fig. 3A). There was a reduction in MABP of at least 14 mm Hg. The DOC-LE rats from Experiment I, injected with 30 \( \mu \)g of cyclo-dVDAVP, showed a greater average decrease in MABP \( (39 \pm 9 \) mm Hg) than the animals from Experiment II injected with 33 \( \mu \)g dPVDAVP \( (21 \pm 4 \) mm Hg). However, this difference was not statistically significant \((t \) test). In none of these animals was there a decrease in MABP when vehicle alone was injected.

The injection of AVP blocker, either cyclo-dVDAVP \( (n = 3) \) or dPVDAVP \( (n = 6) \) into nine normotensive LE rats resulted in a reduction in MABP of \( 4 \pm 1 \) mm Hg (fig. 3B). When vehicle alone \( (n = 9) \) was injected, MABP fell \( 1 \pm 0.7 \) mm Hg. Similarly, the response of the NaCl-LE rats \( (n = 4) \) to the AVP analog (MABP decreased \( 6 \pm 2 \) mm Hg) was not different from the response to vehicle (MABP decreased \( 4 \pm 1 \) mm Hg (fig. 3C). The pressor response to 20 mU AVP was reduced \( 70 \pm 3\% \) by cyclo-dVDAVP and \( 73 \pm 4\% \) by dPVDAVP. Neither blocker had any effect on the pressor response to 20 ng of angiotensin II in eight animals tested.
Discussion

A central role for vasopressin in the pathogenesis of DOC-salt hypertension was first suggested by Freidman et al. When unilaterally nephrectomized rats treated with DOC and salt were given large doses of Pitressin, the latent period before the onset of hypertension was shortened by 1 week, and blood pressure tended to be slightly higher in the vasopressin-treated group. When rats with surgically induced diabetes insipidus were unilaterally nephrectomized and treated with DOC and salt, blood pressure failed to rise during the 3-week period of observation; treatment with Pitressin did not restore the ability of these DI rats to become hypertensive. Since the DI rats were followed for only 3 weeks, the possibility that they had a prolonged latent period before the development of hypertension could not be ruled out. In addition, the failure of the DI rats treated with vasopressin to become hypertensive may have been due to an inadequate dose of Pitressin, as these workers pointed out. The possibility that vasopressin plays a major role in the pathogenesis of DOC-salt hypertension in rats is considerably strengthened by the recent report of Möhring et al. who found a threefold elevation of the plasma vasopressin concentration in the benign form of this hypertension and a 10-fold elevation in the malignant phase. Furthermore, the I.V. injection of a specific vasopressin antiserum resulted in a substantial reduction in MABP in both phases of this model of hypertension. These reductions in blood pressure were transient. Whether a sustained reduction in blood pressure could have been achieved by long-term blockade of vasopressin was not determined.

Thus, the present experiments in conjunction with the work of Friedman et al. and Möhring et al. appear to establish that vasopressin is essential for the development and maintenance of hypertension in the unilaterally nephrectomized rat treated with DOC and salt.

First, we were unable to produce DOC-salt hypertension in the rat with hypothalamic diabetes insipidus. We feel that we can rule out several possible explanations, other than a lack of vasopressin, for this failure, such as inadequate sodium intake, poor tolerance for the saline that replaced drinking water, or impaired adrenocortical function. Although, on the basis of our own experience, as well as that of others, it is difficult to induce rats with diabetes insipidus to drink salt solutions, we were able to achieve this. Salt consumption by the DI group on a body weight basis remained high throughout the experiment, exceeding that by the LE group in all weeks except the last 2 of the experiment. Illness due to poor tolerance for the salt solution that replaced water for drinking can also be ruled out. The DI rats gained weight throughout most of the experiment at a rate normal for Brattleboro rats and showed no signs of illness. The LE group became either lethargic or increasingly difficult to handle. Indeed, several of the LE rats died in stroke-like seizures before the termination of the experiment; data from these rats were not included in the paper. In addition to being unable to synthesize vasopressin, there is evidence that the Brattleboro rat also has some impairment of adrenocortical function. However, we think that this is unlikely to be responsible for the failure of the DI rats in these experiments to become hypertensive, in view of their high salt intake and treatment with large amounts of DOC. Furthermore, blood pressure does increase when DI rats subjected to the DOC-salt regimen are treated with Pitressin. Since this work is reported in abstract form only, there are insufficient details to evaluate it properly. However, this type of study should be repeated, but arginine vasopressin should be used rather than Pitressin (a mixture of lysine and arginine vasopressins), and criteria should be established to ensure that an appropriate dose of vasopressin is used.

Second, we found that the urinary excretion of vasopressin was elevated within 1 week after the start of treatment with DOC and salt in rats with normal hypothalamic function, when SBP was increased 26 mm Hg. To the extent that a long-term increase in the urinary excretion of vasopressin reflects an increased release of this hormone from the neurohypophysis, these data suggest that plasma levels of vasopressin are elevated at an early stage in the development of DOC-salt hypertension. It should be noted that the earliest that plasma levels of antidiuretic hormone (ADH) have been measured was 4 to 7 weeks after the start of treatment with DOC.

Third, our observation that two different analogs of vasopressin, which block its pressor action, lowered blood pressure in rats with DOC-salt hypertension confirm similar findings by Möhring et al. with a vasopressin antiserum. These observations support the view that vasopressin plays an important role as a pressor agent in the maintenance of the elevated blood pressure in DOC-salt hypertension.

Although vasopressin is a very potent pressor agent, at least as potent as angiotensin II, a key question is whether the plasma concentrations of vasopressin that are achieved in DOC-salt hypertension are high enough to exert a direct pressor action. Indeed, the plasma vasopressin levels reported by Möhring et al. in this form of hypertension in the rat (1.2 μU/ml in the benign stage and 5.2 μU/ml in the malignant stage) seem too low for this to be the case. Padfield et al. reported that, although plasma vasopressin levels were elevated in patients with malignant hypertension, the achievement of similar concentrations of vasopressin in normal subjects by the infusion of vasopressin was without effect on MABP. It is well known clinically that, in patients in whom the plasma vasopressin concentration is elevated as a result of an inappropriate secretion of this hormone, blood pressure is not higher than normal. Szczepanska-Sadowska found that MABP was increased by only about 15 mm Hg in conscious dogs when the plasma vasopressin concentration was increased to 22 μU/ml by the infusion of Pitressin. Finally, Möhring et al.
found that when DI rats were infused with vasopressin at a rate sufficient to elevate MABP to levels seen in the benign DOC-salt hypertensive rats, plasma vasopressin concentrations were 40 times those found in the hypertensive rats. It seems necessary, therefore, to postulate that in DOC-salt hypertension there is, in addition to elevated plasma levels of vasopressin, an increased sensitivity to the pressor action of this hormone. This is supported by the report by Hinke et al. that the isolated ventral caudal artery from rats with DOC-salt hypertension is hyper-responsive to vasopressin. This enhanced responsiveness is apparently generalized, rather than confined to vasopressin, since there are a number of reports of increased vascular reactivity to sympathetic stimulation and catecholamines in animals with DOC-salt hypertension.

The stimulus for the increased secretion of vasopressin in the DOC-salt hypertensive rat may be primarily an increased plasma osmolality. Thus, Möhring et al. found that the plasma osmolality was elevated in the benign stage of DOC-salt hypertension and to a greater extent in the malignant stage. In addition, in the present experiments, the ingestion of 1% NaCl, with or without DOC, was sufficient to produce an increased urinary excretion of vasopressin. The hypovolemia in the malignant phase of DOC-salt hypertension should serve as an additional stimulus for vasopressin release. Of course, the possibility cannot be ruled out that DOC, either alone or in combination with sodium chloride, increases ADH release by some mechanism other than increased plasma osmolality and decreased blood volume.

In conclusion and summary, there is now a considerable body of evidence that vasopressin plays a central role in the onset and maintenance of DOC-salt hypertension in the rat. First, rats with hereditary hypothalamic diabetes insipidus do not become hypertensive when subjected to the protocol used for inducing this form of hypertension. Second, there is evidence for an increased secretion of vasopressin from the neurohypophysis in DOC-salt hypertension, beginning at the time when blood pressure first rises. Third, the injection of an antiserum to vasopressin or analogs of vasopressin, that specifically block its pressor action, lowers blood pressure in rats with established DOC-salt hypertension.

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