Role of Extracellular Volume Expansion in the Development of DOC-Salt Hypertension in the Rat

MARIO F. VILLAMIL, CARLOS AMORENA, JORGE PONCE-HORNOS, ANGÉLICA MULLER, AND ALBERTO C. TAQUINI

SUMMARY Changes in inulin space, plasma and blood volume, exchangeable and "noninulin" sodium were studied during the prehypertensive, early and late hypertensive stages of deoxycorticosterone (DOC)-salt administration in the rat. The effect of an acute water load in previously nephrectomized animals was also studied. Hypertension developed after 1 to 2 weeks of the DOC-salt regimen and was always preceded by enlargement of the inulin space and increased plasma and blood volume. Expansion of extracellular fluids receded when blood pressure started to rise but reappeared after 4 to 6 weeks of treatment. Plasma sodium was high only in the hypertensive groups. An acute water load increased blood pressure of normal rats and decreased blood pressure of DOC-salt early hypertensive rats. These findings suggest that extracellular volume expansion inhibits a vasopressor mechanism that involves vasopressin and could be stimulated by hypernatremia.

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KEY WORDS • body fluids • deoxycorticosterone • hypertension

HYPERTENSION produced by deoxycorticosterone (DOC) and salt is usually included in the "volume-dependent" variety because it is regularly associated with the expansion of the extracellular volume 1-2 at least at some stage of the disease. However, we have recently found that an acute extracellular expansion lowers rather than raises the blood pressure of early, nonexpanded DOC-salt hypertensive rats. 3 This latter effect was tentatively ascribed to the inhibition of some vasopressor volume-dependent mechanism possibly involving vasopressin.

The aim of this paper was to try to reconcile these apparently contradictory findings and to gain further insight into the problem by following the time course of changes in exchangeable sodium (Na) and extracellular body fluids during the prehypertensive, early, and late stages of hypertension. In addition we have studied the effect of an acute water load, which is the most powerful physiological inhibitor of vasopressin release on blood pressure. 4

Methods

General Procedure

We studied 105 male Wistar rats weighing approximately 250 g. We gave 71 rats a drinking fluid of 1% (w/v) saline supplemented with 0.2% KCl in water, and 25 mg of DOC enantate (Schering, Argentina) in castor oil intramuscularly twice a week during 1 to 6 weeks. As controls we used 34 male rats of similar breed, who drank tap water. All animals were given normal rat chow. Systolic blood pressure was measured in the unrestrained awake animals at 30°C by the plethysmographic tail method before starting treatment and then at weekly intervals until the time of sacrifice. Reproducibility between successive readings was better than 10 mm Hg. Therefore, rats were considered hypertensive when differences between readings performed at different days exceeded this upper limit.

Two series of experiments were conducted: in the first one the time course of changes in blood pressure, exchangeable Na, and body fluids were followed at different stages of the disease. The second series was aimed at studying the effect of an acute water load on the blood pressure of normal and DOC-salt early hypertensive rats.

First Series of Experiments

The first series consisted of 22 control and 54 experimental rats. The latter group was divided in three subgroups as follows: 1) rats that did not increase their blood pressure after 1 to 2 weeks of DOC-salt treatment (prehypertensive subgroup); 2) rats that significantly increased their blood pressure within the first 2 weeks of DOC-salt treatment (early hypertensive subgroup); and 3) rats treated with DOC-salt for 4 to 6 weeks (late hypertensive subgroup). The day before
sacrifice. 12 control and 26 experimental animals of this series were injected with 50 $\mu$C of $^{24}$Na intraperitoneally and placed in metabolic cages for 24 hours under the same fluid they were previously drinking. During this time, food was withheld and urine was carefully collected. The day of sacrifice the animals were anesthetized with ether and bilaterally nephrectomized. One jugular vein was catheterized and 1 ml of 20% inulin in Krebs solution* was injected intravenously. Seven control and 28 treated rats that had not received $^{24}$Na were injected with 10 $\mu$C of $^{131}$I-HSA dissolved in 0.2 ml of Krebs solution via jugular vein 10 minutes before the withdrawal of a blood sample which took place 3 hours after the inulin injection.

**Second Series of Experiments**

The second series of experiments consisted of 12 control and 12 nonexpanded DOC-salt early (1–2 weeks) hypertensive rats. Both groups were bilaterally nephrectomized and infused through the jugular vein with distilled water at a rate of 0.489 ml • kg$^{-1}$ for 90 minutes (44 ml/kg body weight), which proved to be sufficient to prevent hemolysis. Seven control and seven experimental animals were subjected to essentially the same procedure as those of the first series except that only inulin space was measured. After withdrawal of the first blood sample, red blood cells were resuspended in Krebs solution and reinjected. Thirty minutes after the infusion, systolic blood pressure was again recorded and a second blood sample was obtained. In five control and five DOC-salt early hypertensive rats of this series, blood pressure was continuously monitored during the infusion through a catheter placed in a carotid artery; a polygraph (Grass Model 5D) and a Statham pressure transducer P23 were used. To check the effect of time on blood pressure, blood pressure was also monitored in five additional DOC-salt rats receiving no infusion. Space measurements were not performed in these animals.

**Laboratory Determinations**

Blood samples were centrifuged and the hematocrit was recorded. The following determinations were performed in plasma: water content (refractometry), inulin, $^{3}$H tracer activity, Cl (coulombmetric titration), and Na and K (internal standard flame photometry). Inulin space was used for the estimation of extracellular volume. It was derived from the ratio of inulin injected to inulin in 1 ml of plasma water. Since plasma inulin concentration steadily falls approximately 5 hours after the injection, a final inulin space was calculated by multiplying the initial value by the ratio [Cl]/[Cl], where [Cl] and [Cl] are the initial and final Cl concentrations in plasma water. This calculation involves the assumption that most Cl is extracellular.

"Noninulin" Na was taken as an approximation of intracellular Na and was calculated by subtracting from exchangeable Na the amount dissolved in the inulin space in the same concentration as in plasma water. Exchangeable Na was derived from the ratio of cpm injected – cpm excreted in urine to the specific activity of plasma where specific activity is cpm/mEq Na.

Plasma volume was measured by the space of distribution of $^{131}$I-HSA. Previous studies by us have shown that 10-minute plasma samples in both normotensive and hypertensive groups yield the same values as those obtained by extrapolating the plasma decay curve at zero time.

Blood volume (BV) was derived from plasma volume (PV) and hematocrit (HCT) according to the equation BV = PV/1 – HCT. All values were referred to kg body weight.

**Statistics**

The effect of the DOC-salt regimen on blood pressure was evaluated by subjecting paired differences between pre- and post-treatment values to the t test. With this sole exception, each experimental group was compared with the normal group, which was used as a reference. If the F test failed to show that variances were significantly different, they were pooled together and the t test was used. Otherwise, this was replaced by the d test for small samples.

**Results**

In the first series of experiments, 21% of rats became hypertensive after 1 week of treatment. This value increased to 75% at the end of the second week and to 100% at the end of the fourth to sixth week. In none of the animals was blood pressure affected by nephrectomy. Inulin space increased by 13% and plasma volume by 20% in those animals that failed to develop hypertension after 1 to 2 weeks of treatment, and remained normal in those that became hypertensive during the same period. These changes were unrelated to duration of treatment. Both values increased again 17% and 22% after 4 to 6 weeks of treatment, in spite of continuous progression of hypertension. In contrast with these fluctuations, total exchangeable Na and the Na fraction located outside the inulin space (noninulin Na) increased by an average of 25% and 100% respectively from the first week of treatment and remained at these high levels throughout the period of study. These changes preceded the onset of hypertension (table 1).

Plasma Na was significantly higher in all hypertensive rats but did not differ significantly from normal in normotensive rats. Plasma K fell by an average of 1.25 mEq/liter from the first week of treatment. There was little or no change in plasma Cl (table 2).

In the second series of experiments, the blood pressure increased as a consequence of DOC-salt treatment ($+37 \pm 2.8$ mm Hg; $p < 0.001$). Thirty minutes after the water load, blood pressure increased in the untreated rats ($+15 \pm 4.3$ mm Hg; $p < 0.02$) and decreased

* Krebs solution contained (in mM) NaCl 118, NaHCO$_3$ 25, KCl 4, CaCl$_2$ 2, MgSO$_4$ 1, KH$_2$PO$_4$ 1, and glucose 5, and was previously equilibrated with 5% CO$_2$ in 95% O$_2$. 

TABLE 1. Changes in Systolic Blood Pressure (BP), Inulin Space (InSp), Plasma Volume (PV), Blood Volume (BV), Exchangeable (exch) Na and Noninulin Na of Untreated and DOC-Salt-Treated Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>DOC-salt 1-2 weeks normotensive</th>
<th>DOC-salt 1-2 weeks hypertensive</th>
<th>DOC-salt 4-6 weeks hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment (mm Hg)</td>
<td>110</td>
<td>107</td>
<td>105</td>
<td>105</td>
</tr>
<tr>
<td>After treatment (mm Hg)</td>
<td>217</td>
<td>110.5</td>
<td>136</td>
<td>158</td>
</tr>
<tr>
<td>InSp (ml/kg body weight)</td>
<td>36.4</td>
<td>43.3</td>
<td>50.9</td>
<td>58.1</td>
</tr>
<tr>
<td>PV (ml/kg body weight)</td>
<td>65.6</td>
<td>80.4</td>
<td>68.0</td>
<td>77.8</td>
</tr>
<tr>
<td>BV (ml/kg body weight)</td>
<td>43.0</td>
<td>53.6</td>
<td>50.9</td>
<td>58.1</td>
</tr>
<tr>
<td>Exch Na (mEq/kg body weight)</td>
<td>11.6</td>
<td>18.5</td>
<td>19.0</td>
<td>21.5</td>
</tr>
<tr>
<td>Noninulin Na (mEq/kg body weight)</td>
<td>0.99</td>
<td>1.55</td>
<td>0.70</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of observations is given in parentheses. The effect of DOC-salt treatment on blood pressure was evaluated by the t test of paired differences between pre- and posttreatment values. In all other instances each experimental group was compared against the control group.

TABLE 2. Plasma Electrolytes from Normal Rats and from Rats Treated with DOC and Salt During Variable Periods of Time

<table>
<thead>
<tr>
<th>Group</th>
<th>Na (mEq/liter)</th>
<th>K (mEq/liter)</th>
<th>Cl (mEq/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>142</td>
<td>4.96</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>0.77</td>
<td>0.15</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
<td>(23)</td>
<td>(24)</td>
</tr>
<tr>
<td>DOC-salt</td>
<td>144</td>
<td>3.57</td>
<td>97.6</td>
</tr>
<tr>
<td>1-2 weeks normotensive</td>
<td>1.08</td>
<td>0.15</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(11)</td>
<td>(10)</td>
</tr>
<tr>
<td>DOC-salt</td>
<td>147</td>
<td>3.81</td>
<td>103</td>
</tr>
<tr>
<td>1-2 weeks hypertensive</td>
<td>0.92</td>
<td>0.11</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(15)</td>
<td>(16)</td>
</tr>
<tr>
<td>DOC-salt</td>
<td>148</td>
<td>3.76</td>
<td>102</td>
</tr>
<tr>
<td>4-6 weeks hypertensive</td>
<td>1.70</td>
<td>0.27</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of observations is given in parentheses.
in the treated rats (−23 ± 2.7 mm Hg; p < 0.001). Inulin space expansion was small, 11% and 5.5%, respectively, and not significantly different in both groups. Initial plasma Na was higher in DOC-salt rats (p < 0.01) and decreased after the water load by the same extent in the control and experimental group (−7 and −9 mEq/liter, respectively) (table 3).

Direct recording of mean blood pressure during water infusion showed the same trend found with the plethysmographic method: it rose to a maximum of +20 mm Hg in the control rats and fell by −15 mm Hg in the DOC-salt rats. No change was found in blood pressure of DOC-salt rats subjected to the same procedure but without water loading (fig. 1).

**Discussion**

The onset of hypertension in the DOC-salt rats occurred between 1 to 2 weeks of treatment, and expansion of the extracellular fluid volume was invariably found before the blood pressure started to rise. At the time blood pressure elevation was detected, the volume of extracellular fluids was again normal. Since we did not have continuous measurements of these parameters we are not in a position to establish the time sequence between them.

The events observed during the prehypertensive and early hypertensive stages of hypertension could be compatible with the hypothesis that views the blood pressure elevation as a compensatory mechanism mediated by an early increase in cardiac output secondary to hypervolemia and aimed at the restoration of salt balance. This concept implies that extracellular fluid volume expansion and increased cardiac output are necessary events in the development of DOC-salt hypertension, the "escape" phenomenon being the result of a "pressure" diuresis. However, we have recently found that an acute extracellular volume expansion lowers the blood pressure of early, nonexpanded DOC-salt hypertensive rats; but it raises that of nontreated rats. Furthermore, it has been shown that the "escape" can occur in the absence of hypertension, the latter developing even when increase in cardiac output is prevented by beta-blockers.

Both the increase in blood pressure of DOC-salt rats with concurrent normalization of extracellular fluid volume and its decrease produced by an acute extracellular expansion would be reconciled if the expansion had a delaying rather than a triggering effect on the development of DOC-salt hypertension. Being so, blood pressure would start to rise whenever this inhibitory effect is removed by the escape. This interpretation is compatible with the data by Gavras et al., who found that a period of hemoconcentration marked the onset of the malignant phase of DOC-salt hypertension. As we have postulated before, it is possible that the extracellular volume expansion inhibits a volume-sensitive vasopressor mechanism involving vasopressin, thus counterbalancing the stimulation due to hypovolemia. Vasopressin has been found to play an important role in DOC-salt hypertension. Additional support to our view is provided in the present study by plasma Na levels that were significantly elevated only in the hypertensive groups. Moreover, the hypotensive effect of an acute water load in DOC-salt early hypertensive rats contrasts with its hypertensive effects in untreated rats and provides further indirect evidence to support this interpretation since it brought blood pressure and plasma Na back to normal levels. However, it must be pointed out that the findings in nephrectomized water-loaded rats may not be comparable to the "intact" DOC-salt hypertensive rats.

Increased vascular reactivity to ADH, which has been reported in the prehypertensive stage of DOC-salt hypertension in the rat, could enhance the vasopressin effect. The fact that this hypersensitivity was prevented by intraventricular injections of 6-hydroxydo-
pamine gives further support to the hypothesis that a central, neurohumoral mechanism is involved in DOC-salt hypertension.

The late reappearance of extracellular fluid volume expansion which occurred in spite of continuous progression of hypertension suggests that it was no longer effective in counterbalancing the stimulating effect of mounting hypernatremia. The early, marked, sustained elevation of noninulin Na which was found to precede the onset of hypertension may have also exerted a pathogenic role by affecting adrenergic mechanisms and/or reactivity of vascular smooth muscle. This interpretation does not exclude the late participation of other factors, such as renal lesions and structural redesign of the arterial wall, which could have contributed to the maintenance and progression of hypertension.

Acknowledgments

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References

Role of extracellular volume expansion in the development of DOC-salt hypertension in the rat.
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