Importance of Chloride for Deoxycorticosterone Acetate–Salt Hypertension in the Rat

JOHN C. PASSMORE, SHIRLEY A. WHITESCARVER, COBERN E. OTT, AND THEODORE A. KOTCHEN

SUMMARY Selective dietary sodium loading (without chloride) fails to produce hypertension in the Dahl salt-sensitive rat. This study attempted to evaluate the effect of selective sodium loading on blood pressure in another NaCl-dependent model of hypertension — deoxycorticosterone acetate (DOCA)-salt hypertension. Three groups of uninephrectomized rats were studied for 32 days on one of the following regimens: (1) high NaCl diet plus DOCA, (2) high dietary sodium intake without chloride plus DOCA, and (3) high NaCl diet without DOCA. Both indirect and direct arterial pressure were higher (p < 0.01) in the DOCA-NaCl group than in the other two groups. In the two DOCA-treated groups, net sodium and potassium balance and total carcass sodium and potassium content did not differ. In the DOCA-NaCl group, higher blood pressures were associated with a more positive chloride balance and total carcass chloride content (p < 0.01), an expanded extracellular fluid volume (p < 0.05), and increased renal vascular resistance (p < 0.01). Higher renal vascular resistance in DOCA-NaCl animals suggests that chloride contributes to NaCl-induced vasoconstriction.

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KEY WORDS: extracellular fluid volume • renal vascular resistance • sodium chloride

The mechanism by which a high dietary sodium chloride intake produces hypertension in the susceptible host has not been defined, although sodium chloride induced hypertension is associated with increased peripheral resistance.1,2 This has been attributed to a transient increase of plasma volume,3 neurogenic vasoconstriction,4 increased responsiveness to vasoconstrictor substances,3 and alterations of sodium and calcium transport in vascular smooth muscle.6 In one model of sodium chloride sensitive hypertension, the Dahl salt-sensitive rat, we have recently demonstrated that selective dietary sodium loading, without chloride, fails to produce hypertension.7 This observation provides a novel opportunity to evaluate mechanisms of sodium chloride induced hypertension.

The purpose of this study was to evaluate the effect of selective dietary sodium loading on the development of hypertension in another sodium chloride sensitive model, the deoxycorticosterone acetate (DOCA)-salt rat. To obtain information about mechanisms of sodium chloride induced hypertension, we compared hemodynamic responses to sodium chloride loading and to selective sodium loading. In addition, to evaluate the possibility that failure of selective sodium loading to produce hypertension might be related to a hypertensive effect of the diet itself, blood pressure responses to sodium chloride and selective sodium loading also were compared in Sprague-Dawley rats with one-kidney, one-clip hypertension.

Methods

Long-Term Dietary Loading

Sequential blood pressure measurements were obtained in separate groups of male Sprague-Dawley rats on the following regimens: (1) high sodium chloride diet plus DOCA (Sigma Chemical Company, St. Louis, MO), (2) high dietary sodium intake, without chloride, plus DOCA, and (3) high sodium chloride diet without DOCA. All animals underwent unilateral nephrectomy 10 to 14 days before the institution of the diets and DOCA.

For all groups, diets were prepared by adding electrolytes to powdered Purina rat chow (Ralston Purina Co., Richmond, IN) and the final electrolyte content was determined by direct measurement after nitric acid extraction (Table 1). Six percent sodium chloride was added to obtain a high sodium chloride diet. As we have previously described,7 selective sodium loading was achieved by adding an equivalent amount of sodium with assorted anions (NaAA) other than chloride (phosphate, bicarbonate, aspartate, glutamate, and...
glycinate). In both groups treated with DOCA, diets were also supplemented with non-chloride-containing potassium salts (KH$_2$PO$_4$, KHCO$_3$, KSO$_4$). The DOCA (30 mg/kg) was administered subcutaneously twice weekly in sesame oil, and animals not treated with DOCA were injected with the vehicle alone twice weekly. All diets and distilled drinking water were provided ad libitum.

All animals were housed in a light-controlled and temperature-controlled small animal facility. Animals were housed in individual metabolic cages to determine net electrolyte balances, computed by subtracting urinary and fecal losses (after nitric acid extraction) from dietary intake. Weight and systolic blood pressure (tail plethysmograph) were measured periodically in unanesthetized animals up to 32 days after initiation of the diets and DOCA. Subsequently, each rat was subjected to a terminal protocol to measure glomerular filtration rate, extracellular fluid volume, total renal blood flow, and plasma and total carcass electrolyte concentrations.

**Terminal Experimental Protocol**

Each rat was anesthetized with intraperitoneal sodium pentobarbital (40 mg/kg). A 10-ml syringe was filled with 2.0% inulin solution, weighed accurately to 0.1 mg, and then placed in a Harvard infusion pump (Harvard Instruments, Millis, MA). Inulin was infused into a jugular vein through a polyethylene catheter at a rate of 0.5 ml/minute for 1 minute and then 0.05 ml/minute for the duration of the experiment. Before starting the inulin infusion, a catheter was placed in the bladder to collect all urine excreted from the rat's only remaining kidney. An arterial catheter was placed for direct measurement of arterial pressure and for collection of blood. A left flank incision was made, and a Statham electromagnetic flow probe (Statham Instruments Inc., Oxnard, CA) was placed on the renal artery for measurement of renal blood flow. For each animal, zero flow was confirmed by totally occluding the renal artery distal to the probe. After 1 hour of inulin infusion, a timed, 30-minute urine sample was taken to measure glomerular filtration rate. At the end of this urine collection, an arterial blood sample was taken for the measurement of pH, plasma inulin, sodium, potassium, chloride, and ionized blood calcium concentrations, and plasma renin activity (PRA). The rat was then killed, and the heart and kidney were removed for weighing. The inulin infusion catheter was carefully removed, and the syringe was weighed to determine the quantity of inulin that had been infused. Urinary excreted inulin was subtracted from the quantity of inulin infused. The remainder was then divided by the plasma inulin concentration to calculate extracellular fluid volume. To determine carcass electrolyte content, the skin, head, and tail were removed from the rat; the carcass was then weighed and blended (Sears blender, Sears Roebuck and Co., Chicago, IL) into a uniform slurry with approximately 500 ml of 0.75 N nitric acid. The total weight of the slurry in the blender was determined by weighing the blender when filled and subtracting the weight of the blender when empty. An aliquot of the blender contents was analyzed for sodium, potassium, and chloride concentrations. Each electrolyte concentration was multiplied by the total blender volume and then calculated as milliequivalent per gram of carcass.

**Renal Clip Hypertension**

In a separate experiment, either sodium chloride or NaAA was added to Purina chow of 10 Sprague-Dawley rats (5 rats per group) immediately following unilateral nephrectomy and placement of a 0.20-mm silver clip on the remaining renal artery. Indirect arterial pressures were measured periodically for 17 days. Animals were then killed by decapitation, and blood emanating from the trunk was collected for measurement of PRA and plasma creatinine concentration.

**Analytic Methods**

Sodium and potassium concentrations were measured with an IL (Instrumentation Laboratory, Morris Plains, NJ) flame photometer. Chloride was measured with a chloridometer (Buchler Instruments, Division Nuclear Chicago, Fort Lee, NJ). Plasma ionized calcium concentration was measured with an Orion electrode (Cambridge, MA). Inulin level was measured by an anthrone method, and arterial pH was measured with an IL blood gas analyzer. Serum creatinine level was measured by the method of Kennedy et al., and PRA was measured by the method of Haber et al. To test statistical significance, two-group comparisons were made with an unpaired Student's t test. For three-group comparisons, statistical significance was determined using the Bonferroni modification of the t test. Results are given as means ± se.

**Results**

**Doxycorticosterone-Salt Hypertension**

Beginning on Day 11 of the diets, and for the duration of the study, blood pressure of DOCA-NaCl animals was significantly higher (p < 0.05) than that of both DOCA-NaAA animals and sodium chloride fed animals not treated with DOCA (Figure 1). At the end of the balance protocol, blood pressure of the DOCA-NaAA animals was also higher (p < 0.001) than that of sodium chloride controls. On Day 32, direct intraarterial pressure of the DOCA-NaCl animals (160 ± 9
mm Hg) was also higher ($p < 0.01$) than that of DOCA-NaAA animals (135 ± 3 mm Hg) and animals not treated with DOCA (115 ± 7 mm Hg); arterial pressure of DOCA-NaAA animals was higher ($p < 0.05$) than that of animals not treated with DOCA.

There were no group differences of starting body weight or weight gain during the balance study (Table 2). Both DOCA-treated groups gained weight at the same rate throughout the 32-day period. In animals fed a high sodium chloride diet but not treated with DOCA, weight gain during the first 12 days of study tended to be less than that of both groups of DOCA-treated animals; however, the difference was not statistically significant. Mean terminal heart weight of both DOCA-treated groups was greater ($p < 0.01$) than that of animals not treated with DOCA. Heart weight of DOCA-NaCl animals tended to be greater than that of DOCA-NaAA animals, although this difference was not statistically significant. Kidney weight of animals not treated with DOCA was less than that of both DOCA-treated groups ($p < 0.01$); kidney weights of DOCA-NaCl and DOCA-NaAA animals did not differ. Comparing the two DOCA-treated groups, there were no differences in dietary sodium intake, urinary sodium excretion, net sodium or potassium content, or total carcass sodium or potassium content (Table 3). Dietary sodium intakes and net sodium balances also did not differ during the first 12 days of the balance study. Net chloride balance and carcass chloride were lower ($p < 0.01$) in the DOCA-NaAA group than in the DOCA-NaCl group. Electrolyte balances were not determined in animals not treated with DOCA. Carcass sodium content did not differ in the two DOCA-treated groups and was higher ($p < 0.05$) than that in animals not treated with DOCA. Carcass chloride content of DOCA-NaAA animals was lower ($p < 0.01$) than that of the other two groups. Carcass chloride of DOCA-NaCl animals tended to be higher than that of sodium chloride fed animals not treated with DOCA, although this difference was not statistically significant. Extracellular fluid volume was greater ($p < 0.05$) in DOCA-NaCl animals than in the other two groups and did not differ between DOCA-NaAA animals and animals not treated with DOCA.

There were no significant group differences of plasma sodium, potassium, or ionized calcium concentrations (Table 4). Plasma chloride concentration was lower ($p < 0.01$) in the DOCA-NaAA group than in the other two groups. Both DOCA-treated groups had comparable elevations of arterial pH ($p < 0.05$) compared with animals not treated with DOCA. The PRA tended to be lower in both DOCA-treated groups than in animals treated with sodium chloride alone; however, these differences were not statistically significant. The PRA did not differ in the DOCA-NaCl and DOCA-NaAA groups.

The glomerular filtration rate did not differ among the three groups (Figure 2). Expressed in relation to total body weight, the renal blood flow of DOCA-NaAA treated animals was higher ($p < 0.01$) than that of DOCA-NaCl treated and sodium chloride fed animals not treated with DOCA; renal blood flow did not differ in the latter two groups. Computed renal vascular resistance was higher ($p < 0.001$) in DOCA-NaCl animals than in the other two groups. Because of lower kidney weights of animals not treated with DOCA, renal blood flow also was expressed per gram of kidney. In this instance, renal blood flow in animals not receiving DOCA was higher ($p < 0.01$) and renal

![Figure 1. Mean (± se) sequential systolic blood pressure measurements (tail plethysmography) in the three groups of rats.](image)

**Table 3. Net 32-Day Electrolyte Balance, Carcass Electrolyte Content, and Extracellular Fluid Volume**

<table>
<thead>
<tr>
<th>Group</th>
<th>Na(^+) (mEq/day)</th>
<th>K(^+) (mEq/day)</th>
<th>Cl(^-) (mEq/day)</th>
<th>Carcass Na(^+) (mEq/g)</th>
<th>K(^+) (mEq/g)</th>
<th>Cl(^-) (mEq/g)</th>
<th>ECFV (ml/g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1.3 ± 0.4</td>
<td>0.7 ± 0.1</td>
<td>1.4 ± 0.4</td>
<td>0.044 ± 0.002*</td>
<td>0.091 ± 0.002</td>
<td>0.040 ± 0.002</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>DOCA-NaCl</td>
<td></td>
<td></td>
<td></td>
<td>0.053 ± 0.002</td>
<td>0.090 ± 0.004</td>
<td>0.046 ± 0.002</td>
<td>0.23 ± 0.02*</td>
</tr>
<tr>
<td>DOCA-NaAA</td>
<td></td>
<td></td>
<td></td>
<td>0.053 ± 0.002</td>
<td>0.085 ± 0.004</td>
<td>0.033 ± 0.002*</td>
<td>0.15 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± se.

**Table 2. Mean Body Weight at the Beginning of the Balance Study and Body Weight, Heart Weight, and Kidney Weight at Death**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (Day 0) (g)</th>
<th>Heart weight (Day 32) (g)</th>
<th>Kidney weight (Day 32) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (n = 9)</td>
<td>245 ± 1.3</td>
<td>306 ± 1.6</td>
<td>1.08 ± 0.3</td>
</tr>
<tr>
<td>DOCA-NaCl</td>
<td>270 ± 1.3</td>
<td>314 ± 1.9</td>
<td>1.33 ± 0.05</td>
</tr>
<tr>
<td>DOCA-NaAA</td>
<td>263 ± 1.1</td>
<td>318 ± 1.2</td>
<td>1.21 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± se.

DOCA = deoxycorticosterone acetate; NaAA = sodium with assorted anions

*p < 0.05 compared with the other two groups.
vascular resistance was lower \( (p < 0.01) \) than respective values in both DOCA-treated groups.

**Renal Clip Hypertension**

Blood pressure increased to comparable levels in animals with one-kidney, one-clip hypertension, fed either sodium chloride or NaAA (Table 5). Net sodium and potassium balances did not differ in the two groups; positive chloride balance was greater \( (p < 0.01) \) in sodium chloride fed animals. The PRA was lower \( (p < 0.01) \) in sodium chloride fed animals than in NaAA fed animals.

**Discussion**

Blood pressure of DOCA-treated uninephrectomized Sprague-Dawley rats fed sodium chloride was considerably higher than that of comparably treated rats fed sodium without chloride. Similarly, Kurtz and Morris have reported that dietary sodium bicarbonate also fails to produce hypertension in this model, although bicarbonate fed animals were potassium depleted (mean plasma potassium level was 2.1 mEq/L). Potassium depletion itself may lower blood pressure. In the present study, dietary potassium was supplemented in both groups of DOCA-treated animals (slightly more in DOCA-NaAA than in DOCA-NaCl groups), and plasma potassium concentrations, net potassium balances, and total carcass potassium contents did not differ significantly in the two groups. Although plasma potassium concentration tended to be lower in DOCA-NaAA animals, other investigators have observed severe hypertension in DOCA-NaCl treated rats with mean plasma potassium concentrations either lower than or identical to that in our DOCA-NaAA groups. In our previous study with the Dahl salt-sensitive rat, in contrast to NaCl fed animals, hypertension did not develop in NaAA fed animals, although plasma potassium concentration in both groups was identical. Thus, we conclude that failure of NaAA fed animals to develop hypertension is not related to potassium deficiency in these two models of salt-sensitive hypertension. Additionally, in both the Dahl salt-sensitive rat and the DOCA-salt treated rat, higher blood pressures in sodium chloride fed animals than in NaAA fed animals were not associated with differences of arterial pH or plasma ionized calcium concentrations.

To evaluate the possibility that the NaAA diet might prevent hypertension by a hypotensive effect of the diet, we compared the effects of high dietary sodium chloride and high NaAA on blood pressure in Sprague-Dawley rats with one-kidney, one-clip hypertension. In contrast to the Dahl salt-sensitive model and the DOCA-salt hypertensive rat, NaAA feeding did not prevent hypertension in the one-kidney, one-clip model. This finding suggests that failure of NaAA fed Dahl salt-sensitive rats and DOCA-treated rats to develop hypertension is specifically related to a deficiency of dietary chloride rather than to some other effect of the NaAA diet itself.

**Table 4** Plasmas Electrolyte Concentrations, Blood Calcium, Arterial pH, and Plasma Renin Activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Na(^+) (mEq/L)</th>
<th>K(^+) (mEq/L)</th>
<th>Cl(^-) (mEq/L)</th>
<th>Ca(^{2+}) (mEq/L)</th>
<th>pH</th>
<th>PRA (ng/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>142 ± 1</td>
<td>4.4 ± 0.3</td>
<td>115 ± 1</td>
<td>2.2 ± 0.1</td>
<td>7.42 ± 0.02*</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>DOCA-NaCl</td>
<td>142 ± 1</td>
<td>4.5 ± 0.5</td>
<td>117 ± 2</td>
<td>1.9 ± 0.1</td>
<td>7.52 ± 0.03</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>DOCA-NaAA</td>
<td>145 ± 2</td>
<td>3.3 ± 0.2</td>
<td>102 ± 3†</td>
<td>2.0 ± 0.1</td>
<td>7.54 ± 0.03</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE.

DOCA = deoxycorticosterone acetate; NaAA = sodium with assorted anions

\*p < 0.05 compared with the other two groups.

†p < 0.01 compared with the other two groups.

**Table 5. Animals with One-Kidney, One-Clip Hypertension Fed High NaCl or High NaAA Diets**

<table>
<thead>
<tr>
<th>Group (n = 5)</th>
<th>Weight (g)</th>
<th>Blood pressure (mm Hg)</th>
<th>Creatinine (mg/dl)</th>
<th>PRA (ng/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>Day 0 187 ± 5</td>
<td>Day 17 217 ± 6</td>
<td>Day 0 108 ± 2</td>
<td>Day 7 157 ± 4</td>
</tr>
<tr>
<td>NaAA</td>
<td>Day 0 191 ± 6</td>
<td>Day 17 214 ± 7</td>
<td>Day 0 109 ± 2</td>
<td>Day 7 149 ± 8</td>
</tr>
</tbody>
</table>

PRA = plasma renin activity; NaAA = sodium with assorted anions

\*p < 0.01 compared with high NaCl group

Values are means ± SE
The DOCA-salt hypertension has previously been shown to be associated with sodium and water retention and expansion of the extracellular fluid volume. In the present study, dietary sodium intake, net sodium balance, and total carcass sodium content of DOCA-treated rats fed sodium chloride and NaAA did not differ. The development of hypertension in DOCA-treated, sodium chloride fed animals was not associated with sodium but with a more positive chloride balance, increased total carcass chloride, and an expanded total extracellular fluid volume. These results provide evidence that expansion of extracellular fluid volume and development of hypertension are dependent on provision of sodium as chloride.

Renal blood flow was lower and renal vascular resistance was higher in DOCA-NaCl than in DOCA-NaAA animals, although glomerular filtration rate and PRA did not differ. These results are consistent with the observation of Iversen et al. that DOCA-salt hypertension in the rat constrains the renal afferent arteriole by an angiotensin-independent mechanism. Kidney weight in animals not receiving DOCA was less than that in DOCA-treated animals, which confirms an earlier observation that mineralocorticoids stimulate renal hypertrophy following unilateral nephrectomy. Adjusted for kidney weight, renal blood flow was higher and renal vascular resistance was lower than that in both DOCA-treated groups, although expressed in terms of total body weight renal blood flows in DOCA-NaCl animals and animals not treated with DOCA did not differ. In addition, plasma chloride concentration was higher in the DOCA-NaCl group than in the DOCA-NaAA group. Wilcox has recently reported that hyperchloremia produces progressive renal vasoconstriction in the intact dog. Thus differences of plasma chloride concentrations may contribute to differences of renal blood flow in the DOCA-NaCl and DOCA-NaAA animals.

Renal vascular reactivity has been found to be increased in DOCA-salt animals even before the appearance of hypertension. Reduced arteriolar diameters also have been observed in other vascular beds in the DOCA-salt rat. Increased vascular reactivity has been shown to be dependent on a high sodium (presumably provided as sodium chloride) intake. Increased vascular reactivity is also associated with increases in turnover of vascular potassium and chloride and is dependent on central stimulation of sympathetic nervous system activity. Renal denervation also has been reported to prevent sodium retention and delay the onset of hypertension in this model. Our results are consistent with the hypothesis that increased vascular activity is related to chloride rather than to sodium in the DOCA-treated rat. In the presence of DOCA and a high sodium intake, the development of hypertension may be associated with neurally mediated, chloride-dependent renal vasoconstriction.

In the intact, sodium chloride deprived rat, we have previously reported that inhibition of renin release by sodium chloride is specifically related to increased absorptive chloride transport in the thick ascending limb of the loop of Henle. Consistent with these results, in the present study of one-kidney, one-clip hypertension, PRA of animals fed sodium chloride was lower than that of animals fed NaAA. In apparent contrast, in the DOCA-salt model, PRA was comparably suppressed in animals fed sodium chloride and NaAA. Suppression of renin in the NaAA fed animals may in part be related to an effect of DOCA itself. Additionally, we have previously observed that the effect of chloride on renin release was more apparent in animals on a low sodium chloride intake, presumably because of the different kinetics of sodium and chloride transport in the loop of Henle. Increasing dietary chloride from a low to a “normal” level may result in sufficient chloride uptake in the thick ascending limb of the loop of Henle such that large increases of dietary sodium inhibit renin release, regardless of the accompanying anion.

In summary, similar to our earlier results in the Dahl salt-sensitive rat, we found that the complete expression of DOCA-salt hypertension was dependent on provision of dietary sodium as sodium chloride. In the absence of concomitant chloride administration, dietary sodium loading had relatively little or no effect on blood pressure in these two salt-sensitive models. The DOCA-salt hypertension was associated with an expanded extracellular fluid volume, decreased renal blood flow, and increased renal arterial resistance. These results suggest that salt sensitivity may be related to the combined effects of sodium and chloride on vascular reactivity.

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