Deoxycorticosterone Acetate–Salt Rats
Hypertension and Sympathoexcitation Driven by Increased NaCl Levels

Theresa L. O’Donoughy, Virginia L. Brooks

Abstract—Using deoxycorticosterone acetate (DOCA)–salt rats, we tested the hypothesis that increased plasma NaCl concentration supports sympathetic activity and blood pressure (BP) during salt-sensitive hypertension. One day before experimentation, femoral catheters and an electrode for measurement of lumbar sympathetic nerve activity (LSNA) probe were surgically positioned in DOCA-salt and Sham-salt rats. DOCA-salt rats exhibited increased ($P<0.05$) BP and NaCl concentration (BP, 163±8 mm Hg; NaCl, 260.8±3.3 mEq/L [DOCA-salt]; BP, 106.3±4.2 mm Hg; NaCl, 254.3±1.7 mEq/L [Sham-salt]). After V$_1$ vasopressin blockade (Manning compound, 5 μg IV), infusion (0.12 mL/min) of 5% dextrose in water decreased NaCl concentrations, BP (−28±7 mm Hg), and LSNA (−39±5%) in DOCA-salt but not Sham-salt rats. To explain how such small (∼2%) increases in plasma NaCl could underlie the hypertension, we hypothesized that DOCA augments the pressor and sympathoexcitatory actions of NaCl. To address this hypothesis, animals with equally elevated NaCl but no DOCA (Sham-1.7% salt) and animals with increased DOCA but normal NaCl levels (DOCA-water) were prepared and administered the infusion of 5% dextrose in water. BP and LSNA were not altered in DOCA-water rats. In the Sham-1.7% salt rats, BP fell ($P<0.05$), but not LSNA, and the responses were significantly smaller than that observed in the DOCA-salt animals. Collectively, these data suggest that increased NaCl levels contribute to sympathoexcitation and hypertension in DOCA-salt rats because of amplification of the NaCl signal by DOCA. (Hypertension. 2006;47:680-685.)

Key Words: hypertension, sodium-dependent

In individuals with salt-sensitive hypertension (SSH), an increase in dietary salt increases arterial blood pressure (BP). Renal fluid retention and brain-initiated sympathoexcitation may both be involved; however, the mechanisms by which increased salt intake triggers sympathoexcitation in SSH are unknown (reviewed in References 1–3). Use of an SSH animal model, like the deoxycorticosterone acetate (DOCA)–salt model of hypertension, allows this question to be addressed. In addition to volume expansion and increased vasopressin levels, DOCA-salt hypertension is associated with enhanced sympathetic activity of unknown etiology.4–7 Moreover, because the factors causing hypertension in this SSH model are not genetic and can be independently manipulated, it is ideal for uncovering the origin of the sympathoexcitation.

One proposed mechanism is that increases in NaCl levels and/or an increase in plasma osmolality trigger sympathoexcitation.1–3 Indirect evidence to support this hypothesis is that increased salt intake, a major component of DOCA-salt hypertension, causes a small but significant elevation of plasma NaCl and osmolality.8–12 Moreover, acute and chronic increases in osmolality in normal animals activate the sympathetic nervous system in a regionally specific manner; although renal sympathetic nerve activity is unaffected or suppressed, lumbar sympathetic nerve activity (LSNA) and adrenal nerve activity are increased.13–16 However, whether increased NaCl levels contribute to the hypertension and sympathoexcitation in SSH has not been directly investigated. Therefore, a goal of this study was to test the hypothesis that, during DOCA-salt hypertension, increased plasma NaCl levels support LSNA and BP. We reasoned that, if elevated NaCl is tonically stimulating LSNA, then acute normalization of NaCl levels by IV infusion of water should decrease LSNA and BP.

If NaCl does contribute to tonic sympathoexcitation, then a question that arises is this: how can such small increases in NaCl impart significant and chronic activation of the sympathetic nervous system? One possibility is that the sympathoexcitation induced by increased plasma NaCl levels is augmented by other factors. In DOCA-salt rats, evidence indirectly suggests that excess mineralocorticoids (MCs), specifically DOCA, may be an amplifying factor. First, MC receptors are present in osmosensitive brain regions, such as the organum vasculosum of the lamina terminalis and median preoptic nucleus.5,7,17–19 Second, increased MCs can enhance the pressor and sympathoexcitatory effects of acute increases in NaCl.19,20 However, whether such a cooperative interaction between salt intake and MCs can be sustained in a SSH model, such as DOCA-salt, is unknown. Therefore, we hypothesized that, during DOCA-salt hypertension, increased DOCA enhances the effects of elevated NaCl.
levels to support BP and LSNA. To test this hypothesis, it was determined whether acute decreases in plasma NaCl, by IV infusion of water, decreases BP and LSNA more in DOCA-salt animals than in animals with either high NaCl levels alone (rats drinking hypertonic 1.7% saline; Sham-1.7% salt) or high DOCA alone (DOCA-water).

Methods

Animals

Male Sprague-Dawley rats weighing between 275 and 375 g (Sasco, Wilmington, MA) were housed in pairs in Plexiglas cages in the Animal Care Unit with ad libitum access to water and a 0.4% NaCl diet (Harlan Teklad) for ≥1 week before any surgeries were performed. The facility was maintained at a constant temperature of 22±2°C with a 12:12-hour light-dark cycle. All of the procedures were conducted in accordance with the National Institutes of Health Guide for the Health and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University.

Surgery

Four groups of rats were prepared for experimentation as described. Animals were first anesthetized with 2% isoflurane in oxygen. A 1.5-cm incision was made in the skin above the right kidney. After blunt-dissection through the muscle layer, the kidney was exteriorized, tied off, and removed. The muscle layer was sutured closed. Before suturing the skin, a thin silicone pellet (~3-cm diameter) with (DOCA) or without (Sham) 65 mg of DOCA was placed dorsally under the skin. For the duration of the experiment, the DOCA-salt–hypertensive group and its control (Sham-salt) drank salt water (1% NaCl and 0.2% KCl). The group with increased DOCA in the absence of hypertonicity (DOCA-water) drank distilled water. The final group, Sham-1.7% salt, drank a 1.7% NaCl solution, which produced hypertonicity in the absence of increased DOCA. The DOCA-salt, Sham-salt, and DOCA-water rats were all studied 2 to 3 weeks (19.8±0.5 days; range, 15 to 26 days; n=27) after pellet implant and nephrectomy. The Sham-1.7% salt animals were studied after optimal hypertonicity had been achieved, which resulted in a slightly shorter recovery period (13.9±1.7 days; range, 4 to 19 days; n=8). One day before experimentation, animals in all of the groups were instrumented with femoral catheters and an LSNA electrode as described elsewhere.3 Briefly, femoral arterial and venous catheters, filled with heparinized saline (200 U/mL), were implanted for measurement of arterial pressure and for infusions, respectively. A bipolar electrode lead was gently secured around the lumbar nerves with lightweight dental silicone (Bisico). Distal ends of the catheters and electrode were tunneled subcutaneously to exit at the nape of the neck. Animals were allowed to recover overnight in their home cage.

Data Acquisition

During all of the experiments, BP, heart rate (HR), and LSNA were continuously recorded. The arterial catheter was connected to a Grass bridge amplifier (7P1) for measurement of pulsatile and mean BP. The pulsatile signal was directed to a Grass tachograph amplifier (7P4) for determination of HR. Raw LSNA was band-pass filtered to transmit frequencies between 100 and 3000 Hz, amplified (20 000 to 50 000×), whole-wave rectified, and integrated (Grass 7P10) over 1-s intervals. BP, HR, and LSNA signals were recorded on a Grass polygraph (7D). At the end of the experiment, background noise was quantified after ganglionic blockade with hexamethonium (30 mg/kg IV) and increases in arterial pressure by IV infusion of phenylephrine. This background level was subtracted from values of LSNA recorded during the experiment. LSNA was normalized to baseline nerve activity before the experimental infusions were initiated (% baseline).

Experimental Protocols

Between 9:00 AM and 10:00 AM the day after surgery, vascular catheters and nerve electrode leads were connected to the recording equipment, while the rats rested, unrestrained in their home cages. Animals were allowed at least a 2-hour habituation before the start of a protocol. During this time, a bolus of sodium nitroprusside (70 μg, IV) was administered to assess nerve viability, and a baseline blood sample (350 μL) was drawn for measurement of basal hematocrit and plasma protein, Na, K, and Cl levels. Blood was replaced with an equal volume of isotonic saline. At least 30 minutes after the blood draw, while the animals were resting quietly, 1 of the following protocols was performed.

Protocol 1

The purpose of this protocol was to test the hypothesis that, during DOCA-salt hypertension, increased plasma NaCl supports LSNA by determining whether normalization of NaCl levels decreases BP and LSNA in DOCA-salt, but not Sham-salt, rats. To remove the confounding influence of changes in vasopressin levels on BP, HR, and LSNA during decreases in NaCl levels, a V1 vasopressin antagonist was first administered (Manning Compound; 5 μg, IV, Bachem). At least 20 minutes after V1 blockade, when the rats were resting quietly, baseline hemodynamic parameters were established, and a 2-hour IV infusion (0.12 mL/min) of 5% dextrose in water (5DW) was begun. Blood samples were drawn and replaced, as described above, after 60 and 120 minutes of infusion.

Protocol 2

This experiment was designed to determine whether the volume load or other nonspecific effects contributed to changes in measured hemodynamic parameters and LSNA during infusion of 5DW. This protocol was identical to protocol 1, except that isotonic 0.9% saline was administered IV in V1-blocked DOCA-salt animals at a rate (0.09 mL/min) demonstrated previously to deliver an equivalent volume load.22

Protocol 3

To test the hypothesis that DOCA enhances the pressor and sympathoexcitatory effects of NaCl during DOCA-salt hypertension, 2 additional groups of animals were prepared. Preliminary experiments indicated that Sham rats consuming 1.7% saline (Sham-1.7% salt) achieved similar increases in plasma NaCl levels to those seen in the DOCA-salt animals, but without the increase in DOCA. DOCA-water rats were also prepared; these rats received DOCA implants to increase DOCA levels as in DOCA-salt rats, but were given water to drink. If excess DOCA enhances the sympathoexcitatory effect of increased plasma NaCl during DOCA-salt hypertension, then acute decreases in NaCl in animals with either high plasma NaCl only (Sham-1.7% salt) or high DOCA only (DOCA-water) should result in smaller decreases in BP and LSNA compared with DOCA-salt animals. Thus, these additional groups of rats underwent V1 blockade and received a 2-hour IV infusion of 5DW as in protocol 1.

Analytical Analysis

Plasma electrolytes were determined from whole blood with a Nova CRT electrolyte analyzer (Nova Biomedical Corporation). Duplicate hematocrit tubes were filled with ~30 μL of arterial blood and spun. Hematocrit was determined with an Adams microhematocrit reader. Tubes were then broken, and the plasma was used for determination of plasma protein with a SUR-Ne hand held protein refractometer (Atago Instrumentation).

Statistical Analysis

All of the data are presented as mean±SE. Statistical analyses were performed using GB Stat v 7.0 software (Dynamic Microsystems). Baseline blood values were compared using a 1-way ANOVA followed by a Bonferroni post hoc test. Changes in BP, HR, LSNA, and blood chemistries, because of fluid infusion or administration of V1 antagonist, were tested with 2-way ANOVA (repeated measures) on all 5 of the groups. In cases in which significant interactions were evident, a Bonferroni post hoc test was performed to determine
**Results**

**Baseline Values**

Treatment of animals with either DOCA or salt alone had no effect on BP, but together significantly increased it (Table 1). HR was not different between groups (Table 1). Individually, neither plasma Na nor CI levels were significantly elevated in all of the DOCA-salt animals compared with Sham-salt rats (Table 1). However, Na plus Cl (NaCl) levels were increased in DOCA-salt and Sham-1.7% salt groups receiving the hypotonic infusion compared with the DOCA-water and Sham-salt groups (Figure 1). Plasma K levels were slightly but significantly suppressed in DOCA-salt animals compared with the Sham-salt group. Plasma Cl levels were lower in DOCA-water animals compared with the Sham-1.7% salt group (Table 1).

**Effects of Administration of V1 Antagonist**

Table 2 summarizes the changes in HR, BP, and LSNA 10 minutes after IV injection of the V1 vasopressin antagonist and 25 minutes later when preinfusion baseline data were collected (35.0±2.4 minutes after antagonist injection). In DOCA-salt animals, administration of the V1 vasopressin antagonist decreased BP. LSNA increased transiently in both DOCA-salt and DOCA-water animals, and HR increased initially in the Sham-1.7% salt rats (Table 2).

**Effects of Fluid Infusions on Plasma Protein and Hematocrit**

Fluid infusion decreased plasma protein and hematocrit (ANOVA, time \( P<0.05 \)) indirectly suggesting volume expansion (Table 3). However, between-group differences were not observed (ANOVA, group and interaction, \( P>0.10; \) Table 3).

**Effects of 5DW Infusion in DOCA-Salt and Sham-Salt Rats**

Infusion of 5DW lowered plasma NaCl levels (Figure 1 and Table 3) in both DOCA-salt and Sham-salt groups. The decreases in NaCl did not alter BP or LSNA in Sham-salt rats (Figure 2); however, in stark contrast, in DOCA-salt rats, BP (−27±8 mm Hg) and LSNA (−39%±5%) were decreased by 120 minutes after the start of the infusion (Figure 2). No significant changes in HR were observed during the infusion within either group; nevertheless, on completion of the 2-hour infusion, HR was higher in DOCA-salt rats compared with Sham-salt rats (Figure 2).

**Time Control: Effects of 0.9% Saline Infusion in DOCA-Salt Rats**

In DOCA-salt animals, plasma NaCl levels were not altered by saline infusion (Table 3). HR did not change during the 2-hour infusion, nor was it significantly suppressed relative to levels in rats receiving the 5DW infusion (Figure 2). No changes in BP or LSNA were observed (Figure 2).

**DOCA-NaCl Synergism: Effects of 5DW Infusion in DOCA-Water and Sham-1.7% Salt Rats**

In DOCA-water animals, infusion of 5DW decreased plasma NaCl concentrations to the same level as in the DOCA-salt group (Figure 1). HR, BP, and LSNA did not change with the infusion (Figure 3). In Sham-1.7% salt rats, 5DW infusion decreased NaCl concentrations as in the DOCA-salt group (Figure 1). HR transiently increased but returned to control levels by the end of the hypotonic infusion (Figure 3). Fluid infusion reduced BP, and the absolute decrease was significantly smaller than in DOCA-salt rats (Figure 3); nevertheless, as a percentage of control, the depressor responses were not different (\( P=0.09 \)). In 5 of 6 Sham-1.7% salt rats, LSNA decreased (to 80%±3% of baseline); however, overall, this suppression did not achieve statistical significance (Figure 3). Importantly, the percentage change in LSNA was significantly less than in the DOCA-salt group. Indeed, although there was a significant relationship between the fall in plasma NaCl levels and the decrease in LSNA in both DOCA-salt and Sham-1.7% salt groups, the slope of this relationship was significantly greater in DOCA-salt rats (Figure 4).

**Discussion**

The purpose of this study was to investigate mechanisms underlying the sympathoexcitation associated with DOCA-salt...
hypertension. The novel findings are as follows: (1) acute normalization of plasma NaCl levels in DOCA-salt hypertension, but not Sham-salt, rats markedly decreases BP and LSNA; and (2) the depressor and sympatholytic effects are greater in DOCA-salt animals than in groups with either elevated DOCA or NaCl levels alone. These data are the first to directly demonstrate a causal relationship between elevated plasma NaCl levels and enhanced sympathetic tone in any SSH model.

Although it has long been know that sympathetic activity is increased during DOCA-salt hypertension,\(^5\) the mechanism has not been delineated. The hypothesis that increased sympathetic activity could be mediated by an increase in plasma NaCl levels is a logical extension of existing research. During DOCA-salt hypertension, systemic NaCl levels are significantly increased, as demonstrated in our study and others.\(^6\) Moreover, both acute and chronic increases in NaCl or osmolality can increase sympathetic activity to some vascular beds.\(^11\) However, whether increases in NaCl levels contribute to increased sympathetic activity in SSH has not been directly investigated. In support of our hypothesis, we found that an acute decrease in plasma NaCl in DOCA-salt animals rapidly and profoundly decreased LSNA and BP. These responses were not observed in Sham-treated animals, nor were they secondary to increases in plasma volume subsequent to 5DW infusion, because isotonic saline infusion was without effect. Indeed, the magnitude of the depressor and sympatholytic effects of water infusion, although large, may be an underestimate of the effect of NaCl, because at least the BP response had not reached a steady state after 2 hours of infusion. Moreover, it is possible that chronically elevated NaCl levels induced changes in gene expression or synaptic plasticity,\(^2^4\) which would take longer to be reversed. Nevertheless, the results strongly suggest that, in DOCA-salt–hypertensive rats, increased plasma NaCl supports BP and sympathetic activity.

In the present study, neither Na nor Cl levels individually were statistically different between groups; however, the sum of Na and Cl concentrations was significantly elevated in the DOCA-salt and Sham-1.7% salt rats, albeit by only \(\approx 2\%\). Moreover, significant decreases in BP and LSNA were observed in the DOCA-salt group, although 2 of 8 of these animals had plasma NaCl levels that were within the 95% CI of the NaCl levels measured in the Sham-salt group. A question, then, is how could such small increases in NaCl be mediating such profound changes in nerve activity and BP? Indeed, as we illustrate in Figure 4, only a 1% decrease in plasma NaCl (\(\approx 2.5\) mEq/L) results in a 6% decrease in LSNA. We hypothesized that DOCA amplifies the sympathoexcitatory effects of osmotic inputs. To address our

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Change in HR (bpm)</th>
<th>Change in BP (mm Hg)</th>
<th>Change in LSNA (% baseline)</th>
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<tr>
<td>DOCA-salt (n=13)</td>
<td>10 min</td>
<td>20.6±5.4</td>
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<td></td>
<td>Preinfusion baseline</td>
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<td>Preinfusion baseline</td>
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<td>-5.2±0.5</td>
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<tr>
<td>DOCA-water (n=5)</td>
<td>10 min</td>
<td>10.0±10.9</td>
<td>1.5±4.6</td>
<td>27.5±6.0*</td>
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<tr>
<td></td>
<td>Preinfusion baseline</td>
<td>15.0±3.8</td>
<td>1.6±0.7</td>
<td>11.1±6.6</td>
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<td>Sham-1.7% salt (n=9)</td>
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<td>26.3±8.7*</td>
<td>-7.8±2.0</td>
<td>2.9±5.2</td>
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<tr>
<td></td>
<td>Preinfusion baseline</td>
<td>13.3±7.3</td>
<td>-7.4±2.1</td>
<td>-5.1±4.8</td>
</tr>
</tbody>
</table>

Values are mean±SE of data taken 10 minutes after injection of the V\(_1\) antagonist (10 minutes) and again just before fluid infusion (preinfusion baseline) 35.0±2.4 minutes after antagonist injection.

* Difference from baseline values taken immediately before administration of V\(_1\) antagonist.

Data are mean±SE. Min indicates minutes after start of infusion.

* Different from baseline. Plasma protein and hematocrit decreased with infusion of fluid (ANOVA, time P<0.05); however, because these values lacked a significant interaction, Bonferroni post hoc tests were not performed.
hypothesis, the depressor and sympathoinhibitory responses to acutely decreased plasma NaCl levels in rats with elevated DOCA or NaCl alone were compared with DOCA-salt rats; we found that the responses were considerably larger in DOCA-salt rats. The blunted depressor and sympathoinhibitory responses in the Sham-1.7% salt rats were especially telling, because these animals had plasma NaCl levels similar to those observed in the DOCA-salt animals, but these rats lacked elevated DOCA levels. Therefore, we conclude that the presence of excess DOCA augments the sustained sympathoexcitatory and pressor actions of hypertonicity. The finding that the slope of the relationship between NaCl concentration and LSNA is increased in DOCA-salt rats, compared with rats with high-plasma NaCl alone (Figure 4), additionally supports this conclusion.

The site(s) at which increases in circulating NaCl are sensed remains to be unequivocally defined. Osmoreceptors are located peripherally in the liver, kidney, and adrenals and are also located in the brain. Several whole-animal studies suggest that activation of the central osmoreceptors is key. Pennington and McKinley²⁵ demonstrated that intracerebroventricular (ICV) infusion of mannitol, to reduce brain osmolality, in aldosterone-treated sheep slowly decreases BP. Furthermore, studies by Wang et al²⁰ indicate that ICV aldosterone increases the sympathoexcitatory effect of ICV hypertonic saline. In addition, bilateral intracarotid infusions of a hypotonic fluid, to lower the osmolality of blood perfusing the brain, decreases BP and LSNA in conscious, water-deprived rats,²⁶ suggesting that increased osmolality acts centrally to support arterial pressure in these animals; similar results have been observed in DOCA-salt rats.²⁷ Importantly, the carotid arteries do not perfuse the hind-

Figure 2. Changes in BP, HR, and LSNA in DOCA-salt (DS; n=9; ■) and Sham-salt (SS; n=6; ○) rats receiving 2-hour IV infusions of 5DW (0.12 mL/min) and in DS rats receiving 2-hour IV infusions of saline (n=5; 0.09 mL/min; □). *Significant difference over time; †Difference between groups at ≥1 time point.

Figure 3. Changes in BP, HR, and LSNA in DOCA-salt (DS; n=9; ■), DOCA-water (DW; n=6; ▲), and Sham-1.7% salt (1.7%S; n=9; ●) rats receiving 2-hour IV infusions of 5DW (0.12 mL/min). *Significant difference over time; †Difference between groups at ≥1 time point.

Figure 4. Linear regressions comparing LSNA vs change in plasma NaCl levels in DOCA-salt (n=6; ■; P<0.0001) and Sham-1.7% salt (n=9; ○; P=0.005). *Significant difference between slopes of these relationships as determined by ANCOVA. The equation for the DOCA-salt regression is %LSNA=99.3+2.3 NaCl and for Sham-1.7% salt is %LSNA=99.6+0.7 NaCl.
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brain,28,29 suggesting that sites in the forebrain are involved. Sensitive osmoreceptors are located in multiple circumventricular organs,30 brain regions lacking a blood-brain barrier, including the organum vasculosum of the lamina terminalis, the subfornical organ, and also in the median preoptic nucleus. Interestingly, MC receptors have been identified in each of these regions,17 suggesting that the amplification of the NaCl-induced sympathoexcitation by DOCA could occur in these forebrain sites. However, additional study is required to test this hypothesis. In conclusion, these data show for the first time that chronically increased plasma NaCl and, consequently, osmolality can increase tonic sympathoexcitatory inputs to the lumbar sympathetic bed, thereby elevating BP in a salt-sensitive model of hypertension.

Perspectives

The current study demonstrates that, in the DOCA-salt model of hypertension, elevated NaCl levels increase sympathetic input to the lumbar bed to elevate BP. However, whether our findings related to LSNAs are indicative of actions in other or all sympathetic beds remains to be determined. Several previous studies demonstrate that intact renal sympathetic innervation is required for complete development of DOCA-salt hypertension.28,29 Moreover, a recent study by Jacob et al34 suggests that the renal nerve contributes to the hypertension, in part, by promoting salt and water retention, indirectly suggesting that renal sympathetic nerves are activated. However, the mechanism underlying this activation has not been studied. Given that hypertonicity supports the lumbar nerve, it is tempting to speculate that a similar signal also activates renal nerve activity. Future experiments are required to test this hypothesis.

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References


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