Time course of synergistic interaction between DOCA and salt on blood pressure: roles of vasopressin and hepatic osmoreceptors

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Brooks, Virginia L., Korrina L. Freeman, and Yue Qi. Time course of synergistic interaction between DOCA and salt on blood pressure: roles of vasopressin and hepatic osmoreceptors. Am J Physiol Regul Integr Comp Physiol 291: R1825–R1834, 2006. First published July 20, 2006; doi:10.1152/ajpregu.00068.2006.—In DOCA-salt rats, the time course of the synergistic interaction between osmolality and DOCA to produce hypertension is unknown. Therefore, in rats 2 wk after implantation of subcutaneous silicone pellets containing DOCA (65 mg) or no drug (sham), we determined blood pressure (BP) and heart rate (HR) responses, using telemetric pressure transducers, during 2 wk of excess salt ingestion (1% NaCl in drinking water). BP was unaltered in sham rats after increased salt, but in DOCA rats BP increased within 4 h. The initial hypertension of 30–35 mmHg stabilized within 2 days, followed ~5 days later by a further increment of ~30 mmHg. HR first decreased during the dark phase: the second phase was linked to an abrupt increase in HR and BP variability and decreased HR variability. Pressor responses to acute intravenous hypertonic saline infusion were doubled in DOCA-treated rats via vasopressin and nonvasopressin mechanisms. Only in DOCA-treated rats, portal vein hypertonic saline infusion increased BP, which was prevented by V1 vasopressin blockade. After 2 wk of DOCA-salt, oral ingestion of water rapidly decreased BP. Intraportal infusion of water did not lower BP in DOCA-salt rats, suggesting that hepatic osmoreceptors were not involved. In summary, the hypertension of DOCA-treated rats consuming excess salt exhibits multiple phases and can be rapidly reversed. Hypertonicity-induced vasopressin and nonvasopressin pressor mechanisms that are augmented by DOCA, and hepatic osmoreceptors may contribute to the initial developmental phase. With time, combined DOCA-salt induces marked changes in the regulation of the autonomic nervous system, which may favor hypertension development.

Evidence to support this hypothesis (32). First, plasma NaCl levels were increased ~2%, similarly to the increases observed in humans consuming excess salt (14, 20). Second, DOCA-salt hypertension is associated with brain-initiated activation of the sympathetic nervous system (13, 18, 37), and acute normalization of the elevated NaCl levels, achieved by intravenous infusion of water, rapidly and profoundly decreased BP and lumbar sympathetic nerve activity (32); the responses were greatly exaggerated in DOCA-salt rats compared with rats with elevated NaCl levels alone (32). These findings suggest that increased NaCl levels underlie a major component of the hypertension and that DOCA transforms a rather small increase in osmolality into a powerful pressor and sympathoexcitatory stimulus. However, the developmental time course of the synergistic interaction between DOCA and NaCl is unknown.

Acute elevations in osmolality rapidly increase BP both by triggering release of vasopressin and by activation of the sympathetic nervous system (17, 52). Therefore, if DOCA amplifies the pressor actions of a hypertonic stimulus via the same mechanisms, then the enhanced pressor response to oral salt ingestion in rats with elevated DOCA levels should develop quickly. To test this hypothesis, we compared time courses of changes in BP and heart rate (HR), measured telemetrically, in rats chronically treated with DOCA as they began consuming 1% saline with the time courses of untreated rats drinking 1% saline and of DOCA-treated rats drinking water.

Additional experiments investigated potential mechanisms of hypertension development. To determine whether elevated DOCA amplifies specifically the acute pressor effects of increased osmolality, the increase in BP in response to intravenous hypertonic saline infusion was compared in rats with and without chronically elevated DOCA levels. Because ingested salt is first perceived by osmoreceptors in the liver, and acute increases in the osmolality of portal blood increase BP, thirst, and vasopressin secretion and activate brain regions important in the control of the sympathetic nervous system (1, 9, 25, 30, 31, 43, 44), we also determined if the pressor effects of intraportal hypertonic saline infusion were enhanced in DOCA-treated rats. Rats were studied intact and after V1 vasopressin blockade, so that both vasopressin and nonvasopressin components could be quantified.

METHODS

Animals

Male Sprague-Dawley rats (275–375 g; Sasco, Wilmington, MA) with ad libitum access to fluid and a 0.4% NaCl diet (Harlan Teklad, 3181 SW Sam Jackson Park Rd., Portland, OR 97239 (e-mail: brooks@ohsu.edu)).

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Madison, WI) were used. The rats were allowed at least 1 wk of acclimatization to their environment before any surgeries were performed. The housing facility was maintained at a constant temperature of 22 ± 2°C with a 12:12-h light-dark cycle. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional (Oregon Health & Science University) Animal Care and Use Committee.

**Surgery**

Nephrectomy and Silastic implant. After induction of anesthesia (2% isoflurane in oxygen), rats received prophylactic penicillin G (30,000 units im) before initiating any surgery. A 1.5-cm flank incision was then made. After blunt dissection through the muscle layer, the right kidney was exteriorized, tied off, and removed. The muscle layer was sutured, and a thin silicone pellet (3 cm diameter) was placed dorsally under the skin. The skin was then sutured closed. Rats were allowed at least 10 days to recover before any experimentation. Rats received analgesic [acetaminophen (0.24 mg/ml) and codeine phosphate (0.024 mg/ml) in the drinking water] during the first 2–3 days after this and other surgeries.

Catheterizations. Catheters were surgically placed while the rats were anesthetized with isoflurane (2% in oxygen). Gel-filled catheters with attached telemetry transmitters (model PA-C40, with 15-cm catheter; Data Sciences International, St. Paul, MN) were implanted in the abdominal aorta via the femoral artery. The transmitter was then positioned subcutaneously on the flank.

In other rats, femoral arterial and venous catheters (PE-10 chemically welded to PE-50) were implanted for direct measurement of BP and infusions, respectively. Some rats received a nonoccluding catheter implanted in the portal vein. The catheter was constructed using Micro-Renathane tubing (0.025 in. OD; Braintree, MA) heat welded to PE-50. After a midline incision, the portal vein was exposed, and two ligatures were placed around the vessel. While briefly occluding blood flow by putting tension on the ligatures, a small hole was made in the vessel between the ligatures, and the catheter was inserted and secured in place using Vet Bond (3M Animal Care Products, St. Paul, MN). Care was taken to ensure that the tip of the catheter was upstream of the bifurcation of the portal vein as it entered the liver. The free end of the catheter was coiled once in the abdomen and stitched to the abdominal wall. Femoral and portal catheters were then tunneled subcutaneously to exit at the nape of the neck. All incisions were sutured closed in layers using 3-0 silk.

Catheters were generally flushed with sterile isotonic saline every 2–3 days. When not in use, femoral catheters were filled with sterile heparinized saline (200 U/ml) and the portal catheter with sterile isotonic saline.

**Implantation of portal vein flow probe.** To verify the magnitude of portal blood flow, in one rat under isoflurane anesthesia (2% in oxygen), an ultrasonic flow probe (2SB; Transonic) was chronically implanted around the portal vein. A midline incision was made. The flow probe was positioned around the vessel, with the leads secured to abdominal muscle and exteriorized at the nape of the neck. After 9 days recovery from the surgery, portal flow was measured for ~2 h while the rat rested quietly in its home cage.

**Experimental Protocols**

**Experiment 1.** The purpose of this protocol was to characterize hypertension development induced by increased salt intake in DOCA-treated rats by using telemetry, with a focus on the transients as salt ingestion was begun and terminated. Surgery was performed to place arterial catheters with telemetry transmitters, to remove one kidney, and to implant DOCA-filled or empty silicone subcutaneous pellets. Immediately after surgery, the rats were moved to the recording room, so they could adjust to the slightly shifted light cycle (lights on at 12:00 PM). Ten days were allowed for recovery, during which the rats drank deionized water. Three days of BP and HR measurements were then continuously recorded, sampling for 10 s every minute at 500 Hz. At the end of the active period of the third control day, 4 h before lights on, the water bottle was replaced with water containing 1% NaCl and 0.2% KCl in both sham and DOCA rats; some DOCA rats continued to drink water. The switch was timed for the active period so that the rats would begin drinking the fluid immediately, and the rapidity of the transients could be studied. Telemetric recordings of BP and HR were continued for 14 days.

To determine whether oral ingestion of water rapidly decreases BP in rats with established DOCA-salt hypertension, we monitored the changes in BP and HR when water was reintroduced on the 14th day of excess salt. The switch from salt water to pure water again occurred 4 h before lights on, at the end of the dark active phase. Telemetric measurements continued for a final 3 days.

Twenty-four-hour daily measurements of fluid intake were collected throughout the protocol in all three groups, except on the days when the fluid was switched between deionized water and salt water. On these days, fluid intake was measured for the 20 h before the switch and then for the first 4 h after the switch.

**Experiment 2.** Ingestion of salt water with salty food likely increases osmolality. Therefore, this experiment tested whether the pressor response to intravenous hypertonic saline infusion is greater in rats with chronically elevated DOCA levels. The rats underwent surgery to implant Silastic pellets, with or without DOCA, and to remove the right kidney. After ~10 days, they were instrumented with femoral arterial and venous catheters for measurement of BP and infusions, respectively. Experiments were performed ~3–10 days later while the rats remained unrestrained in their home cages. Occasionally, DOCA-treated rats exhibited high BP levels (>125 mmHg). When this occurred, experiments were not performed, and longer recovery times from surgery were allowed. Usually, basal BP would fall (~115–120 mmHg), at which time experiments were conducted. During this preparation period, rats had free access to water as their sole drinking fluid.

On the experimental day, arterial BP was measured via the arterial catheter, a pressure transducer, Grass Bridge amplifier (7P1), and Grass polygraph. HR was determined from the pulsatile signal using a Grass tachygraph (7P4). The venous catheter was connected to a syringe containing 2.5 M NaCl. After an equilibration period (~1 h), a control blood sample (350 μl) was collected for measurement of plasma osmolality and Na and Cl concentrations and was replaced with an equal volume of isotonic saline. At least 30 min after the blood draw, while the rats were resting quietly, the V1 vasopressin antagonist (5 μg Manning Compound) or vehicle was injected intravenously; the dose of antagonist in this and previous studies (6) completely blocked the pressor response to intravenous injection of vasopressin (30 ng). Later (20 min), 10 min of baseline BP and HR were recorded, and intravenous infusion of hypertonic saline (1 ml over 30 min) commenced. After termination of the infusion (15 min), a second blood sample was collected and replaced as for the first sample.

To determine whether differences in responses to hypertonic saline infusion were secondary to enhanced vascular reactivity, the pressor responses to intravenous phenylephrine injection were compared in sham- and DOCA-treated rats. Four doses of phenylephrine (1.5, 3, 15, and 30 μg/kg) were injected intravenously as a bolus in random order, with at least 15 min between injections (after baseline BP and HR levels were reestablished).

**Experiment 3.** This protocol tested the hypothesis that DOCA sensitizes pressor pathways activated by increased hepatic plasma NaCl levels. These rats were prepared as in experiment 2, except that an intraportal catheter was also implanted at the time that the rats underwent a unilateral nephrectomy and received subcutaneous Silastic pellets. After ~2 wk, femoral arterial and venous catheters were surgically placed, and experiments were conducted 2–5 days later.
After control data and a basal blood sample were collected as in experiment 2, while the rats were resting quietly, a buffered hypertonic NaCl solution (containing in mM: 415 NaCl, 2.6 KCl, 1.3 CaCl₂, 0.9 MgCl₂, 20 NaHCO₃, and 1.3 Na₂HPO₄, pH 7.4, ~900 mosmol/kgH₂O) was infused via the portal catheter or intravenously in DOCA- and sham-treated rats at increasing rates (5 min each) estimated to increase the osmolality of portal plasma by ~1.5, 3.0, and 6% (0.1, 0.2, and 0.4 ml/min). This estimate was based on a portal blood flow measurement in one rat of ~20 ml/min, in agreement with a previous report (12), and the assumption that the salt did not penetrate blood cellular elements. To determine the contribution of vasopressin release to the resulting pressor responses, in another group of DOCA rats, the intraportal saline infusion was preceded 20 min by an intravenous injection of the V₁ vasopressin antagonist (5 μg). A final blood sample was collected and replaced 15 min after the hypertonic saline infusion.

Experiment 4. The purpose of this protocol was to determine whether a decrease in the NaCl concentration of portal blood of DOCA-salt or sham-salt rats decreases BP. These rats were surgically prepared with unilateral nephrectomy, subcutaneous pellets, and portal catheters as in experiment 3. Rats began drinking 1% NaCl and 0.2% KCl solution immediately after this initial surgery. Later (2–3 wk), a second surgery was performed to insert femoral arterial and venous catheters. After 2–4 days recovery, the experiment was performed.

The experimental protocol was identical to experiment 3, except that buffered fluid without NaCl (osmolality ~40 mosmol/kgH₂O) was infused at 0.1, 0.2, and 0.4 ml/min, each for 5 min. Assuming a portal blood flow of 20 ml/min and distribution of the fluid in plasma and cellular compartments, these infusions would lower the osmolality of portal blood by ~0.5, 1, and 2%, respectively.

Analysis of Blood Samples

Plasma concentrations of Na and Cl were measured from whole blood using a Nova 5⁺ electrolyte analyzer (Nova Biomedical, Waltham, MA). Blood samples were then centrifuged (refrigerated Eppendorf 5402), and plasma osmolality was measured in two to three replicate 20-μl samples using an Advanced Micro Osmometer (model 3300).

Statistics

All data are presented as means ± SE. Differences in BP and HR responses between all groups in an experiment were determined using two- or three-way ANOVA for repeated measures. When significant (P < 0.05) interactions were found, this initial analysis was followed by two-way ANOVA that compared by pairs each experimental group with the control group. In some cases, the post hoc Bonferroni test was used to determine at which times specific within- and between-group differences occurred. Differences in basal values between DOCA- and sham-treated rats were determined using the Student’s t-test. Statistical analyses were performed using GB Stat version 7.0 software (Dynamic Microsystems, Silver Spring, MD).

RESULTS

Effect of Chronically Elevated DOCA Levels on the Changes in BP and HR in Response to Increased Oral Administration of Salt

DOCA treatment alone for 10–14 days increased BP by ~10–15 mmHg relative to shams but did not significantly alter HR (Fig. 1; days 1–3, P < 0.01). Moreover, the hypertensive effect of DOCA alone developed further (without significant changes in HR) in DOCA rats that continued to drink water during the 2.5-wk protocol (Fig. 1).

Initiation of increased salt intake (1% NaCl, 0.2% KCl as sole drinking fluid) had no effect on BP in sham rats but rapidly increased BP in DOCA-treated animals, as shown in a representative rat (Fig. 2). For example, 4 h after presenting the salty fluid, BP increased by 17 ± 3 mmHg and HR decreased by 45 ± 8 beats/min in DOCA-treated rats (P < 0.05). However, BP and HR were unaltered in shams (change in BP 2 ± 3

Fig. 1. Mean arterial pressure (MAP) and heart rate (HR) in sham rats that drank 1% NaCl and 0.2% KCl (sham salt, n = 5), DOCA-treated rats that drank 1% saline (DOCA salt, n = 5), and DOCA-treated rats that continued to drink water (DOCA water, n = 5). During both lights on (left) and lights off (right), 2-way ANOVA of MAP of all 3 groups revealed significant (P < 0.0001) group and time effects and interaction. ANOVA of HR responses indicated that, although there was no group effect (P > 0.50), significant (P < 0.0001) time effects and interaction were present. Separate ANOVAs that compared responses of DOCA salt or DOCA water with sham salt rats again revealed highly significant group, time, and interactions (P < 0.0005). Post hoc Bonferroni analysis indicated that basal BP was higher in the DOCA-treated animals compared with shams (P < 0.05). In addition, although saline consumption decreased HR during lights out for the first 5 days (up through day 8) in every DOCA-treated rat, this response was not detectable within animals using the Bonferroni post hoc test on all possible comparisons; however, HR was significantly suppressed (P < 0.05) in the DOCA salt rats on day 7 (4 days of salt) compared with both DOCA water and sham salt rats.*P < 0.05 compared with day 3 (last control day) within groups using Bonferroni post hoc test.
mmHg, change in HR $-9 \pm 12$ beats/min. The amount of fluid drunk by DOCA and sham rats during this initial 4-h period was not significantly different [50 ± 15 ml, DOCA; 22 ± 8 ml, sham; not significant (NS)].

Increased daily fluid consumption was evident in DOCA and sham rats that switched from water to salt water for 2 wk (Table 1). In both groups, the maximal level of drinking was achieved within the 1st day, but the increase was greater in the DOCA rats (Table 1). Figure 1 shows the resulting changes in BP and HR for both the dark and light phases because the changes, in particular in HR, differed between the phases. As with the initiation of increased salt intake, continued increased dietary salt failed to significantly alter BP or HR in sham animals. In contrast, in the DOCA-treated rats, excess salt induced hypertension, which exhibited two phases. The initial increase in BP reached a steady state of 140–150 mmHg after ~2 days, which was associated with a decrease in HR in the dark phase only (see legend to Fig. 1). However, beginning 7 days after initiating excess salt intake, a further abrupt increment ($P < 0.05$, compared with days 2–5 of excess salt) in BP was observed (Figs. 1 and 2). HR also increased ($P < 0.05$ compared with days 2–5 of excess salt) in both the dark and light phases after 8–9 days of increased salt intake to reach levels significantly above control when lights were off (Figs. 1 and 2). Interestingly, this second hypertensive and tachycardic phase was associated with a marked increase in BP variability and decrease in HR variability (Figs. 2 and 3). We also found that, while DOCA-treated rats drinking water exhibited a strong positive correlation between minute averages of BP and HR ($r = 0.81$, slope was $3.1 \pm 0.5$ beats·min$^{-1}$·mmHg$^{-1}$, $n = 5, P < 0.05$), suggesting changes in HR are largely “feed forward,” after 13 days of DOCA-salt treatment, the slope of this relationship was markedly suppressed ($P < 0.05; 1.0 \pm 0.6$ beats·min$^{-1}$·mmHg$^{-1}$, $n = 5$).

Although it took >1 wk for BP to reach its maximum level, the hypertension was rapidly reversed when the rats resumed water intake. As shown in a representative tracing (Fig. 2), BP began to fall within 1–2 h after rats initiated drinking and was reduced by 28 ± 7 mmHg after 4 h ($P < 0.05$). HR increased initially in most DOCA-treated rats (e.g., see Fig. 2) but after 4 h was not different from control (−13 ± 7 beats/min, $P > 0.05$). The rapid fall in BP was not because of termination of the 1% saline intake alone, because in two rats that delayed their water intake for four or more hours, BP did not decrease (until they drank water). In contrast, BP (−1 ± 4) and HR

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**Table 1. Average daily fluid intake**

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<th>Control</th>
<th>Experimental</th>
<th>Recovery</th>
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<tr>
<td>Sham salt</td>
<td>51 ± 3</td>
<td>78 ± 7*</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>DOCA water</td>
<td>87 ± 5†</td>
<td>81 ± 5</td>
<td>74 ± 6†</td>
</tr>
<tr>
<td>DOCA salt</td>
<td>67 ± 4</td>
<td>153 ± 21*†</td>
<td>76 ± 7</td>
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Daily fluid intake in milliliters was averaged from 2 control days (all rats drank water), 13 experimental days (DOCA water rats drank water; Sham salt and DOCA salt rats drank 1% NaCl, 0.2% KCl), and 3 recovery days (all rats drank water). Volumes drunk on transition days are not included. Overall ANOVA revealed significant ($P < 0.005$) group, time, and interaction. *$P < 0.05$ compared to control within group; †$P < 0.05$ compared to Sham salt rats using Bonferroni post hoc test after significant ($P < 0.05$) interaction.

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**Fig. 3.** Daily standard deviation (SD) of MAP and HR in sham- and DOCA-treated rats that drank saline for 14 days (sham salt, $n = 5$; DOCA salt, $n = 5$) and DOCA-treated rats that drank water throughout (DOCA water, $n = 5$). ANOVA of MAP and HR for all 3 groups indicated significant ($P < 0.01$) group, time, and interactions. DOCA salt was different from the other two groups ($P < 0.0001$), but DOCA water was not different from sham salt. *$P < 0.05$ within group using Bonferroni post hoc test.
(12 ± 13) were not significantly altered when sham rats switched from salt water to pure water; sham rats also did not exhibit the initial tachycardia (data not shown). Again, the volume of water consumed during this initial 4-h period was not significantly different between groups (43 ± 18 ml, DOCA; 27 ± 9 ml, sham; NS).

**Effect of Elevated DOCA Levels on Changes in BP and HR in Response to Intravenous Hypertonic Saline Infusion**

Directly measured BP levels were slightly but significantly increased in DOCA-treated rats (108 ± 2 mmHg, DOCA; 100 ± 3 mmHg, sham; *P < 0.05), as observed in rats whose BP and HR were measured by telemetry. However, basal HR was not significantly different (342 ± 11 beats/min, DOCA; 360 ± 7 beats/min, sham). No differences were observed in basal plasma levels of Na, Cl, or osmolality, and intravenous hypertonic saline infusion increased (*P < 0.0001, ANOVA, time) plasma Na and Cl levels and osmolality similarly (ANOVA, groups and interaction, NS) in both groups by ~3% (see Table 2). The infusion also increased BP, and the pressor responses were significantly greater in DOCA-treated rats compared with shams (*P < 0.0005; Fig. 4, top). The enhanced response persisted after V1 vasopressin blockade (Fig. 4, bottom), suggesting that DOCA amplifies a nonvasopressin component of the pressor response. In addition, the vasopressin component was estimated by subtracting the average maximum pressor response of V1-blocked DOCA- or sham-treated rats from the maximum pressor response of individual respective untreated rats. This estimated vasopressin component was larger in the DOCA animals (23 ± 1 mmHg) compared with shams (14 ± 3 mmHg, *P < 0.05), suggesting that DOCA may also enhance vasopressin release or actions to increases in osmolality. In contrast, the dose-related (*P < 0.0001) increments in BP after phenylephrine administration were not different between groups (pressor responses in mmHg: DOCA, *n* = 5, 23 ± 2, 34 ± 3, 58 ± 4, 67 ± 4; Sham, *n* = 4, 23 ± 2, 37 ± 4, 57 ± 3, 65 ± 5; NS).

Despite a greater pressor response in DOCA-treated rats, hypertonic saline infusion decreased HR (ANOVA, time, *P < 0.0001) similarly in both groups of rats (at end of 30 min saline infusion: −35 ± 7 beats/min, DOCA; −46 ± 9 beats/min, sham; ANOVA, group and interaction, NS). Pretreatment with the V1 antagonist attenuated the bradycardia (ANOVA, V1 blockade, *P < 0.0005), although this effect was significant only in the shams (at end of 30 min saline infusion: −23 ± 8 beats/min, DOCA; −5 ± 5 beats/min, sham; V1 blockade by group interaction, *P < 0.05).

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**Table 2. Effect of intravenous hypertonic saline infusion on plasma NaCl levels and osmolality**

<table>
<thead>
<tr>
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<th>Control</th>
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<th>Control</th>
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<tr>
<td>Plasma Na concentration, meq/l</td>
<td>143.9 ± 0.6</td>
<td>150.3 ± 0.7</td>
<td>144.0 ± 0.6</td>
<td>149.3 ± 0.6</td>
</tr>
<tr>
<td>Plasma Cl concentration, meq/l</td>
<td>108.1 ± 0.6</td>
<td>115.6 ± 0.9</td>
<td>108.9 ± 0.6</td>
<td>115.5 ± 0.7</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kgH2O</td>
<td>299 ± 1</td>
<td>310 ± 1</td>
<td>300 ± 1</td>
<td>308 ± 2</td>
</tr>
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Values are means ± SE. Two-way ANOVA for repeated measures revealed that iv hypertonic saline (HS) increased plasma Na, Cl, and osmolality (time effect, *P < 0.0001); however, the increments were not different between groups [group and interaction, not significant (NS)].

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**Fig. 4. Effect of iv hypertonic saline infusion on MAP in sham- and DOCA-treated rats.** Rats were studied without pretreatment (top: intact; DOCA, *n* = 6; sham, *n* = 5) or after V1 vasopressin blockade (bottom: V1 blocked; DOCA, *n* = 6; sham, *n* = 5). Three-way ANOVA indicated significant group, time, and V1 blockade effects (all *P < 0.0001). In addition, the ANOVA revealed significant group by time and V1 blockade by time interactions (*P < 0.0001). Pair-wise comparisons of intact or V1-blocked DOCA and sham rats with a 2-way ANOVA indicated significant (*P < 0.0005) group, time effects, and interactions. *Between-group differences, as indicated by significant interaction (*P < 0.0005).
Effect of Elevated DOCA Levels on Changes in BP and HR in Response to Intraportal Hypertonic Saline Infusion

In this experiment, again, the DOCA-treated rats (n = 7) exhibited elevated basal BP levels compared with sham rats (n = 6; DOCA, 118 ± 3 mmHg; Sham, 103 ± 2 mmHg; P < 0.005). In addition, basal HR was significantly suppressed in the DOCA rats (DOCA, 371 ± 7 beats/min; sham 396 ± 9 beats/min; P < 0.05). Initial plasma levels of Na and osmolality were not different; however, Cl was lower in the DOCA-treated rats (P < 0.005). Nevertheless, intraportal and intravenous hypertonic saline infusion produced similar (ANOVA, time; see Table 3). As shown in Fig. 5, intraportal hypertonic saline infusion increased BP in DOCA-treated but not in sham rats. Because the infusion did not alter BP when infused intravenously in DOCA (or sham) rats, the pressor response was mediated via the liver. Interestingly, the response was completely prevented by prior treatment with the V1 vasopressin antagonist (Fig. 5), suggesting that it is mediated largely by vasopressin release. Hypertonic saline infusion did not significantly alter HR in any group (ANOVA time and interaction, NS; data not shown).

Effect of Intraportal Hypotonic Fluid Infusion on BP and HR in DOCA-salt and Sham-Salt Rats

As expected, basal BP was significantly elevated (P < 0.0001) in DOCA-salt rats (159 ± 7 mmHg) compared with sham-salt rats (101 ± 3 mmHg), although HR was not different (DOCA, 369 ± 8 beats/min; sham, 372 ± 15 beats/min). Basal osmolality (in mosmol/kg H2O: 306 ± 1, DOCA; 302 ± 1, sham) and plasma Na concentration (in meq/l: 146 ± 1, DOCA; 142 ± 1, sham) were increased in the hypertensive animals (P < 0.05); plasma Cl was not significantly different (in meq/l: 109 ± 1, DOCA; 108 ± 1, sham). Intraportal hypotonic fluid infusion decreased plasma Na concentration and osmolality similarly by ~1% (ANOVA, time, P < 0.005) in both groups; Cl was unaltered (see Table 4). The infusion did not lower BP in either group (mmHg change in BP to 3 infusion rates: −1 ± 1, 2 ± 1, 2 ± 1, DOCA-salt; 1 ± 1, 4 ± 2, 4 ± 2, sham-salt; P > 0.25, time and interaction). Interestingly, however, HR increased at the highest infusion rate (P < 0.05, Bonferroni) in the DOCA-salt rats (beat/min change in HR to 3 infusion rats: 5 ± 2, 6 ± 4, 20 ± 6, DOCA-salt; −2 ± 1, 0 ± 3, −2 ± 7, sham-salt; P < 0.05, time and interaction).

DISCUSSION

This study investigated the time course of BP changes, measured by telemetry, of rats chronically treated with DOCA during initiation and termination of salt water ingestion. We focused on transients to test the hypothesis that DOCA amplifies pressor pathways activated by salt. The important new findings are as follows. First, chronic elevations in DOCA enhanced rapid increases in BP after oral, intravenous, and intraportal salt administration. The enhanced pressor response to intravenous hypertonic saline infusion involved both vasopressin and nonvasopressin components. In contrast, the pressor response to intraportal hypertonic saline infusion was prevented by V1 vasopressin blockade. Second, in rats with established DOCA-salt hypertension, oral water ingestion rapidly and profoundly decreased BP to levels indistinguishable from those in rats with chronic increases in DOCA alone. Because intraportal infusion of water did not lower BP in DOCA-salt rats, this rapid depressor response appears inde-
pendent of hepatic osmoreceptors. Third, using a novel method to induce DOCA-salt hypertension by initiating excess oral salt intake 2 wk after implantation of DOCA pellets, coupled with telemetric recordings of BP and HR, several new observations related to hypertension development were made: 1) DOCA treatment alone induced a steady increase in BP without a fall in HR; 2) excess salt did not alter BP or HR in sham-treated rats. However, in DOCA-treated rats, salt-induced hypertension exhibited two phases: a rapid increase in BP of 30–35 mmHg followed ~5 days later by a further increment of ~30 mmHg; and 3) whereas the hypertension was first associated with bradycardia during the dark active phase, the second phase was linked to an abrupt increase in BP variability, increase in HR, and a decrease in HR variability throughout the day. These data support our hypothesis that DOCA sensitizes salt-activated pressor pathways that include vasopressin and nonvasopressin mechanisms.

The present telemetric studies confirm that increased dietary salt does not significantly elevate BP or alter HR in uninephrectomized rats. Moreover, although it is well established that high DOCA levels transform excess dietary salt from a benign to a hypertensive stimulus (18, 37), we further demonstrated that, in rats chronically primed with DOCA, the hypertension develops rapidly (within hours) and dramatically. In contrast, in another recent telemetric study, in which DOCA pellets were implanted after salt intake was increased, 2–3 days elapsed before significant hypertension was produced, and BP reached a considerably lower maximal level (~140 mmHg) only after 3 wk of DOCA-salt treatment (23). Thus DOCA pretreatment appears to markedly enhance the rate at which hypertension develops after increased dietary salt.

The mechanism of the immediate hypertensive response in the present study is likely multifactorial. Within minutes after ingestion, fluid is absorbed from the gut (43), and blood volume increases. BP is normally maintained constant during an acute volume load by hormonally and neurally mediated excretion of salt and water and by baroreflex activation (3, 11). Although not specifically studied, it is probable that elevated DOCA levels attenuate both the quantity and speed of renal salt excretion by preventing the normal suppression of mineralocorticoid activity. In addition, because ANG II levels were increased in the DOCA-salt rats. *P < 0.05 between DOCA-salt and sham-salt rats. In addition, Na and osmolality were decreased after intraperitoneal infusion (ANOVA, time effects *P < 0.005); however, this effect was not different between groups (interaction, not significant).

In response to volume expansion, reflex decreases in HR and sympathetic activity also contribute to BP regulation. However, previous research indicates that increased mineralocorticoid levels alone (58), or combined DOCA-salt treatment (28, 48), depresses baroreflex function. Baroreflex activation may have occurred in the DOCA rats because, during the first 4 h and then continuing for 5 days, food and saline intake during the active dark period was associated with decreases in HR. However, in view of the sizable pressor response, baroreflex activation was apparently insufficient to maintain BP. In addition, in the DOCA-salt rats during the light phase, and in DOCA-treated rats drinking water, HR was not suppressed despite significant hypertension. Recent data suggest that the arterial baroreflex can tonically restrain hypertension for a period without complete resetting (2, 27, 49). Therefore, the present findings are consistent with previous work showing that DOCA-salt treatment impairs the arterial baroreflex. If so, this impairment may contribute not only to the acute pressor responses that accompanied food and fluid consumption but also to the more sustained elements of hypertension development.

Increases in osmolality, secondary to consumption of salty food along with isotonic saline, may also contribute to the initial hypertension. Moreover, chronic DOCA-salt treatment (32, 45, 50, 51, 53, and the present study) and even DOCA alone (16, 29, 50, 51) can produce sustained increases in plasma Na concentration and osmolality. Indeed, precise quantification of Na and water content in DOCA-salt rats recently revealed that considerably more salt than water is retained, indirectly indicating an increase in osmolality (50, 51). Therefore, another aim was to determine if DOCA amplifies the pressor effects of acute increases in osmolality.

The pressor response to intravenous infusion of hypertonic saline was doubled in DOCA-treated rats, and this difference persisted after V1 vasopressin blockade. Hypertonicity-induced increases in BP and peripheral resistance after V1 blockade are mediated by activation of the sympathetic nervous system (17). Moreover, the pressor responses to phenylephrine injection were indistinguishable, as previously reported in intact and ganglion-blocked DOCA-salt rats (28) and in rats treated with DOCA alone (47); therefore, the difference was not because of greater vascular reactivity to α-adrenergic agonists. Thus these data support the hypothesis that DOCA amplifies the sympathoexcitatory effects of increased osmolality. Interestingly, our data also indirectly suggest that the vasopressin response is enhanced by DOCA. This may be secondary to enhanced release or to increased vascular reactivity to vasopressin, since

### Table 4. Effect of intraperitoneal hypotonic fluid infusion on plasma NaCl levels and osmolality in DOCA-salt and sham-salt rats

<table>
<thead>
<tr>
<th>Osmolality, mosmol/kg H2O</th>
<th>Plasma Na Concentration, meq/l</th>
<th>Plasma Cl Concentration, meq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Fluid</td>
<td>Control Fluid</td>
</tr>
<tr>
<td>DOCA salt</td>
<td><strong>306±1</strong></td>
<td><strong>145.5±0.7</strong></td>
</tr>
<tr>
<td>Sham salt</td>
<td>301±1</td>
<td>142.1±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANOVA revealed a group effect (*P < 0.05) for both osmolality and Na. Student’s t-tests indicated that basal Na and osmolality were elevated in the DOCA-salt rats. *P < 0.05 between DOCA-salt and sham-salt rats. In addition, Na and osmolality were decreased after intraperitoneal infusion (ANOVA, time effects *P < 0.005); however, this effect was not different between groups (interaction, not significant).
intravenous vasopressin injection induces greater increases in BP in DOCA-treated rats (28, 35).

Because BP increased immediately at the onset of oral saline administration, we were interested in determining whether DOCA amplifies the sensitivity of hepatic osmoreceptors. Hypertonic saline administration in the portal vein, at rates that were ineffective intravenously, increased BP only in DOCA-treated rats, and the BP rise was eliminated by prior V₁ vasopressin blockade. These data indicate that it is secondary to vasopressin release, consistent with previous reports that hepatic osmoreceptor activation can acutely and tonically drive enhanced vasopressin secretion (1, 9, 30, 43, 44). However, the sympathoexcitatory component of the acute intravenous osmotic pressor response must therefore be initiated by osmoreceptors located elsewhere. One likely site is the brain, since Wang et al. (57) recently demonstrated that a prior 2-h intracerebroventricular infusion of aldosterone transforms a normally nonpressor 10% increment in cerebrospinal fluid osmolality into a pressor and renal sympathoexcitatory stimulus. Collectively, these data suggest that chronic elevations in DOCA amplify acute sympathoexcitatory and vasopressin responses to both oral saline ingestion and increases in plasma osmolality via actions at the liver and also the brain.

Another novel finding was that BP decreased rapidly (within 4 h) upon termination of excess salt ingestion, to nearly the same levels as in rats with high DOCA alone. Clearly, this sharp decrease occurred too quickly to be explained by renal excretion of salt and water. Because portal vein infusions of hypotonic fluid did not lower BP in DOCA-salt rats with elevated osmolality and Na levels, hepatic osmoreceptors are likely not involved. On the other hand, in parallel studies in conscious V₁ vasopressin-blocked DOCA-salt rats, we found that acute decreases in plasma NaCl levels, after intravenous infusion of 5% dextrose in water (5DW), rapidly decreased BP and lumbar sympathetic nerve activity (32). In addition, intracarotid hypotonic fluid administration also produced a prompt depressor response that included both vasopressin and sympathetic components (33). Taken together, these data suggest that the rapid fall in BP upon resumption of water intake is because of decreases in both vasopressin secretion and sympathetic activity, mediated by deactivation of central osmoreceptors.

The fall in BP that occurred when the DOCA rats began drinking water was associated initially by an increase in HR, which was followed soon thereafter by HR suppression. In humans, drinking water (but not isotonic saline) increases both sympathetic and cardiac vagal activity (HR does not change), possibly triggered via detection of the absorbed water by hepatic receptors (8, 24, 39). In DOCA-salt rats, this balanced response may shift toward cardiac sympathoexcitation. In support of this hypothesis, we found intraportal hypotonic fluid administration increased HR only in DOCA-salt rats. On the other hand, the ultimate decrease in HR to control levels suggests that salt, possibly via an osmotic signal, drives increased HR.

Although our prime interest was the transient BP changes after induction and termination of excess salt in DOCA-treated rats, the present study revealed several interesting features of more prolonged hypertension development, which has not been apparent from single day studies of DOCA-salt rats. In particular, the second developmental phase was characterized by an abrupt, vigorous increment in pressure that was accompanied by increased HR, increased BP variability, and decreased HR variability. Interestingly, these features are characteristic of hypertension and have been associated with increased end-organ damage (36, 54, 56). The mechanism of the increased BP variability and decreased HR variability was not investigated in the present study. However, the decreased baroreflex sensitivity previously documented in DOCA-salt rats may be one component (28, 48). On the other hand, “feed-forward” changes in HR that contribute to changes in pressure may also be attenuated, since the direct relationship between BP and HR became markedly flattened in DOCA-salt rats in the second phase. Future studies investigating temporal changes in feedback baroreflex sensitivity, as well as feed-forward relationships, are required to test these possibilities.

The trigger for the rapid transition to this second phase was also not investigated. Nevertheless, it is clear that the combination of DOCA and salt is required because neither alone was sufficient to significantly alter HR, HR variability, or BP variability. In addition, it appears that the elevated BP per se was not the cause of these autonomic changes, since termination of the excess salt ingestion rapidly lowered BP and HR without significantly reversing the changes in BP and HR variability. Instead, we speculate that the marked increase in HR and changes in variability may contribute to the hypertensive second phase.

We found that DOCA treatment alone modestly but progressively increased BP. A greater role for increased mineralocorticoid activity in human essential hypertension has emerged from recent results documenting significant antihypertensive actions of mineralocorticoid receptor blockers such as spironolactone or eplerenone (38, 42), but the mechanisms are incompletely understood. Interestingly, DOCA treatment can increase plasma Na concentration (16, 29, 50, 51) and has recently been shown to cause net Na retention in excess of water retention (50, 51). From this information, it might be hypothesized that DOCA-induced hypertension includes an osmotic component. However, intravenous 5DW infusion in DOCA-treated rats did not elicit the rapid decreases in BP or sympathetic activity that occur in DOCA-salt rats (32), arguing against this mechanism. Nevertheless, it remains possible that DOCA-induced salt retention raises BP in part by activating osmoreceptors, but through a genomic action with a slower reversal rate. In support of this possibility, Gomez-Sanchez (18) has documented that chronic intracerebroventricular administration of aldosterone produces a slowly developing hypertension that is prevented by coadministration of the Na channel blocker benzamil. Alternatively, the hypertensive action of increased Na may depend upon where the salt is retained. Titze et al. (50) reported that much of the excess salt of DOCA-treated rats is stored in skin and muscle. Na relative to water was also increased in the carcass (less skin, muscle, and bone); however, whether Na content or osmolality increases in specific tissues relevant to cardiovascular regulation, such brain or vascular smooth muscle (14, 41), is unknown.

In summary, the hypertension of DOCA-treated rats that begin consuming excess salt is rapidly evoked, exhibits multiple phases, and can be rapidly reversed. Because DOCA sensitizes pressor pathways activated by acute increases in osmolality that involve vasopressin and nonvasopressin pressor mechanisms and hepatic osmoreceptors, these mechanisms may contribute to the initial developmental phases. In addition,
with time, the combination of DOCA and salt induces marked changes in the regulation of the autonomic nervous system, which may favor augmentation of the hypertension. Finally, the rapid reversal of hypertension, in conjunction with earlier work showing that systemic and central normalization of elevated plasma NaCl levels decreases BP and activity of the sympathetic nervous system (32; 33), suggests that chronic DOCA-salt hypertension is maintained largely by osmotically driven activation of the sympathetic nervous system and vasopressin secretion.

Perspectives

Are the conclusions of the present study relevant to other hypertension models, in particular human SSH? Humans exhibit increased plasma Na concentrations with consumption of increased dietary salt (for review, see Ref. 14). Plasma and cerebrospinal fluid concentrations of Na also increase slightly but significantly in normal animals and in several animal models of SSH (15, 19, 21, 26). It has been proposed that these small increases in Na can become powerfully hypertensive in the presence of inappropriately elevated activity of the renin-angiotensin-aldosterone system (4, 7, 26). Recent studies of rats with increased mineralocorticoid levels and of Dahl salt-sensitive rats, as well as the present results, support this proposal (22, 32, 57). However, whether these recent findings are universally applicable, in particular to humans, requires further study.

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