Cardiovascular Hemodynamics and Vasopressin Blockade in DOCA-Salt Hypertensive Rats

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SUMMARY In conscious rats with near-malignant phases of DOCA-salt (DS) hypertension, hemodynamics were studied with microspheres before and after administration of a vasopressin (VP) vasopressor antagonist in relation to plasma VP levels (pVP). Compared to the controls, the DS rats showed significant elevations in mean arterial pressure (MAP), total vascular resistance (TVR), and pVP, and a flow redistribution from kidney and spleen to skeletal muscles and heart, with increased vascular resistance in almost all organs. The antagonist elicited no significant systemic hemodynamic effects in DS rats as a whole; however, two subgroups, responders vs nonresponders, were identified according to the effects on MAP. In responders with a pVP of 29.2 ± 2.7 (SE) pg/ml, the antagonist lowered MAP (— 24.9 ± 5.9 mm Hg) and TVR significantly, while in nonresponders with a pVP of 15.2 ± 3.4 pg/ml, there were no effects. The major antagonist-induced regional responses were increased flow and decreased vascular resistance in skeletal muscles and skin in whole DS rats, and additionally in the gastrointestinal tract, portal organs, and testes in the responders. Significant correlations were observed between pVP, MAP, TVR, and depressor responses to the antagonist only when all data for DS and control rats were pooled. Thus, the systemic hemodynamic effects of VP are important only in responders with exceedingly elevated pVP. VP contributes significantly to the regional hemodynamic abnormalities in skeletal muscles and skin in whole DS rats, and also in several other organs in the responders. (Hypertension 6: 397-407, 1984)

KEY WORDS • systemic and regional hemodynamics • plasma vasopressin level • vasopressin vasopressor antagonist

ARGININE vasopressin (AVP) has been implicated in the pathogenesis of deoxycorticosterone (DOCA)-salt hypertension in the rat. Supporting evidence includes: 1) increased plasma concentrations1-3 and urinary excretions4 of AVP; 2) an acute, substantial fall in blood pressure after administration of either specific AVP antiserum1,3 or specific antagonists6 of the vasopressor action of AVP; and 3) the necessity of AVP replacement for the production of DOCA-salt hypertension in hypothyroidic diabetes insipidus rats lacking AVP.1-6 AVP effects may involve antidiuretic activity9 and other mechanisms,8 but the influence of AVP on the vascular system is considered to be an important factor, since the blood pressure was lowered before or without induction of diuresis when AVP antiserum or AVP vasopressor antagonists were used.

However, Burnier et al.10 noted that there was no decrease in blood pressure following administration of an antagonist in DOCA-salt hypertensive rats and no elevated plasma AVP after 4 weeks of treatment. Rabito et al.11 and Rascher et al.12 experienced similar failures with antagonists in rats with malignant hypertension (plasma AVP level not given), and in rats with established hypertension and concomitantly elevated plasma AVP levels. Controversy thus exists as to the effectiveness of AVP blockade, and hence the role of AVP vascular action in the maintenance of this form of hypertension.

For a better comprehension of hemodynamic alterations, simultaneous determinations of systemic and regional circulatory variables need to be made. There are a few available data on the regional distribution of blood flow and vascular resistance in this model of hypertension. Even when measuring cardiac output (CO) distribution only in a limited number of rats, Yates and Hiley13 found particular changes. Also, noteworthy are recent observations that exogenous administration of near-physiological amounts of AVP14-16...
or endogenous stimulation of AVP can exert significant influences on not just systemic, but regional circulatory variables, most of which can be attenuated by AVP vasopressor antagonists.

In our present work, we assessed: 1) systemic and regional hemodynamics; 2) their responses to acute treatments with an AVP vasopressor antagonist; and 3) the relationships between plasma AVP levels and hemodynamic variables, in conscious rats with advanced near-malignant phases of DOCA-salt hypertension and elevated levels of plasma AVP.

**Methods**

**Experimental Protocol**

Male Wistar rats initially weighing 180 to 230 g were used. The left kidney was excised under ether anesthesia. Seven days postoperatively, either DOCA-salt or sham treatment was instituted. DOCA-salt treatment consisted of weekly subcutaneous (s.c.) injections of DOCA (30 mg/kg) in sesame oil and substitution of 1% saline for drinking solution. Sham treatment consisted of similar injections of an equal volume of sesame oil and provision of tap water for drinking. The DOCA-salt regimen resulted in a progressive rise in mean arterial pressure (MAP), reaching values of 150 mm Hg or more at 5 weeks, and values of 170 mm Hg or more at 8 weeks, in our laboratory. At 8 weeks, the rats showed features suggestive of malignant hypertension, including weight loss, contracted plasma volume, and elevated hematocrit.

All experiments were carried out after 8 weeks of DOCA-salt or sham treatment. DOCA-salt hypertensive rats were submitted to one of four experiments, while sham-treated control rats were used exclusively in Experiment 4.

One day before the experiments, at between 6 and 8 a.m., the rats were anesthetized with ether. Tip-tapered cannulas (PE-50 tubing) were inserted into the left femoral artery and vein for pressure monitoring and intravenous (i.v.) administration of agents, respectively. In addition, in Experiments 2, 3 and 4, a similar cannula was also introduced into the left ventricle (LV) via the right carotid artery. Three identically treated rats were usually prepared; one was used as an experimental animal, and the other two as donors for blood transfusions, as described below. The wounds were treated with 1% lidocaine during surgery, and the incisions closed. All catheters were brought out through a subcutaneous tunnel between the shoulder blades.

The next morning at 9 a.m., after a 14- to 15-hour recovery period, each rat was put in a nonconfining small cage and studied in a conscious, unrestrained state. Great care was taken to minimize blood loss or dilution during experimental manipulations. Initially, 200 units of heparin were given in a volume of 0.2 ml for anticoagulation. Femoral arterial and LV pressures were recorded through catheters with Statham transducers and a polygraph (Sanei, Tokyo, Japan). The MAP was obtained by electric analog integration, and heart rate (HR) by a cardiotachometer triggered with arterial pressure signals. The room temperature was kept constant throughout the entire course of this study.

**Experiment 1**

Pressure responses to exogenous AVP before and after AVP blockade were examined in six hypertensive rats. Synthetic AVP (Sigma Chemical Company, St. Louis, Missouri), 12.5, 25, 50, and 100 ng/kg, was given i.v. in graded sequences to each rat at approximately 10-minute intervals. Subsequently, a synthetic analog of AVP, [1-(β-mercaptop-β, β-cyclopentamethylene-propionic acid)-2-(0-methyl) tyrosine] argininevasopressin or d(CH)₂Tyr(Me)AVP (Peninsula Laboratories, San Carlos, California), was given in a dose of 100 μg/kg as a bolus injection for AVP inhibition. This compound is a potent and long-acting competitive antagonist of the vascular action of AVP with virtually no diuretic potency. The dose of 100 μg/kg was based on our preliminary findings and previous studies. Following administration of the antagonist, the pressor responses to similar incremental doses of exogenous AVP were tested again, and after 1 hour, 50 ng/kg of AVP was given.

The compound diluted with physiological saline and containing 1% bovine serum albumin was stocked for use. All injections with flushing were given in a volume of 0.2 ml with microsyringes (Hamilton, Reno, Nevada).

**Experiment 2**

Influences of hemodynamic measurements with radioactive microspheres on plasma AVP concentrations were assessed in eight hypertensive rats. After recording baseline pressure and HR data, the femoral venous catheter from an experimental rat was connected through a peristaltic pump (Gilson, Viliers Le Bel, France) to the femoral arterial catheter from a similarly prepared donor rat. The femoral arterial catheter from the experimental rat was introduced through the pump into a plastic tube placed in ice. While transfusing blood from the donor into the experimental rat at a rate of 0.5 ml/min, a blood sample (2 ml) was simultaneously withdrawn into a tube at the same rate and submitted to AVP assay, as described below. During this procedure, the LV pressure showed minor fluctuations in the experimental recipient rats. After a 20-minute stabilization period, radioactive microspheres were given into the LV with the method reported elsewhere, as described below. About 20 minutes after application of microspheres, a blood sample for AVP assay was again taken in an identical manner except that a different donor rat was used.

**Experiment 3**

The reproducibility of hemodynamic measurements with radioactive microspheres was assessed in five hypertensive rats (Experiment 3A). Initially, a blood
sampling was made as described above, and then systemic and regional hemodynamic variables were measured twice 25 minutes apart. In addition, influences of 2 ml blood sampling with volume replacement on hemodynamics were assessed in nine hypertensive rats (Experiment 3B). Hemodynamics were measured 20 minutes before and 20 minutes after a blood sampling procedure.

Experiment 4

Systemic and regional hemodynamics, plasma AVP concentrations, and possible hemodynamic changes following AVP blockade were studied in DOCA-salt hypertensive (n = 19) and sham-treated control (n = 16) rats. After collecting baseline pressure and HR data, a sampling of 2 ml blood for AVP assay was made with volume replacement using group-matched donor rats. After 20 minutes of stabilization, systemic and regional circulatory variables were determined with radioactive microspheres. Approximately 20 minutes later, 100 μg/kg of the AVP antagonist was given i.v. and flushed with 0.2 ml physiological saline, and 5 minutes later a second series of hemodynamic measurements were carried out with different radioactive microspheres. After another 20 minutes, terminal MAP and HR were read, after which 50 ng/kg of synthetic AVP was given to assess the effectiveness of AVP inhibition.

Arginine Vasopressin Assay

Plasma AVP concentration was determined by radioimmunoassay (Yamane Y et al., unpublished data). The high-titer rabbit AVP antiserum, generously provided by Dr. T. Saito (Jichi Medical College, Tochigi, Japan), was used in a final dilution of 1:180,000. The cross-reaction was less than 4% with lysine-vasopressin, less than 4.2% with oxytocin, and nonexistent with desmopressin. Synthetic AVP was labeled with 125I with the use of a modified chloramine-T method, without the addition of metabisulfite. The reaction mixture was purified on a Sephadex G-25 fine column (0.9 x 20 cm) and eluted with 0.01 M acetic acid containing bovine serum albumin. Moniodinated AVP obtained from the third peak had a mean specific activity of 699 μCi/μg.

The blood samples were taken between 9:30 and 10:30 a.m. The plasma was immediately separated at 4°C and stored at -20°C until extraction with acetone and petroleum ether. A 0.5 or 1.0 ml plasma sample was used for the extraction procedure. The mean recovery of known amounts of synthetic AVP added to the plasma was 75.7% ± 6.9%. The smallest measurable level of AVP was 1.6 pg per tube. The inter- and intraassay coefficients of variation were, on an average, 7.4% and 6.0%, respectively.

Hemodynamic Measurements with Radioactive Microspheres

The previously reported method35 was used with slight modifications. In brief, blood (reference sample) was taken via the arterial catheter into a calibrated syringe at a rate of 0.93 ml/min, on an average, with a pump (Model 944D, Harvard Apparatus, South Natick, Massachusetts). The withdrawal rate was adjusted to the animal's body weight. At 10 seconds after the start of the blood withdrawal, approximately 50,000 to 100,000 radioactive microspheres (15 ± 3 μ in diameter, labeled with 85Sr, 51Cr, or 14Ce, Minnesota Mining and Manufacturing Company [3M], St. Paul, Minnesota), suspended in 0.05 ml of physiological saline, were injected into the LV and flushed with 0.4 ml of freshly prepared group-matched donor's blood over a 20-second period. Volume loss (reference sample volume minus 0.45 ml) was restored immediately with similar blood. After termination of the experiments, the rats were exsanguinated and major organs or tissues were weighed. As for skeletal muscles and skin, more than 20 g of samples were collected. Sample radioactivity was measured in a computerized scintillation analyzer (Model 1282, LKB-Wallac, Stockholm, Sweden). Adequacy of mixing and distribution of microspheres were evaluated by observing similar blood flow per weight of bilateral hemispheres of brain or testicles.

Cardiac output (CO) was calculated as: CO = cpm of microspheres injected ÷ cpm of reference sample x sampling rate (ml/min). The percentage of distribution of CO (%CO) was calculated as: %CO = cpm of each organ or tissue ÷ cpm of microspheres injected x 100. Blood flow to each organ was calculated as: flow = CO (ml/min) x %CO x 1/100. Total vascular resistance (TVR) was obtained as the quotient of MAP divided by CO, and regional vascular resistance in each organ as that of MAP divided by its flow. The CO and TVR are given in terms of both absolute and body-weight-related values. The regional circulatory data are given in terms of per sample weight.

Data Analysis

All data were processed with a computer (Model 9845B, Hewlett Packard, Fort Collins, Colorado). We used analysis of variance followed by Dunnett's procedure and paired and unpaired Student's t test. All values were presented as means ± se. Differences of p < 0.05 were considered statistically significant.

Results

DOCA-salt hypertensive rats used in Experiments 1, 2, and 3 weighed 363 ± 7 g (n = 28).

Experiment 1

This series of DOCA-salt hypertensive rats had a MAP of 174 ± 4 mm Hg. As illustrated in Figure 1, dose-response curves before and after AVP blockade were markedly different. A 100 μg/kg dose of the antagonist virtually abolished the pressor effects of exogenous AVP given to these rats. There were no pressor effects seen with 50 ng/kg of synthetic AVP given 1 hour after the antagonist.
Experiment 2

In this group of DOCA-salt rats, there were no differences in MAP (175 ± 4 vs 176 ± 6 mm Hg) and HR (446 ± 12 vs 448 ± 12 bpm) before and after application of the radioactive microspheres. Plasma AVP concentrations were not significantly different before and after injecting microspheres (19.2 ± 4.0 vs 18.7 ± 2.8 pg/ml).

Experiment 3A

There were no significant changes in MAP (176 ± 4 vs 173 ± 4 mm Hg), HR (450 ± 10 vs 455 ± 10 bpm), and CO (110 ± 15 vs 114 ± 8 ml/min or 304 ± 22 vs 308 ± 24 ml/min/kg) between the first and second hemodynamic measurements. The regional circulatory variables were reproducible in all organs (data not shown) except in the spleen and liver. At the second determination, blood flow was significantly increased by 44.8% in the spleen and 32.5% in the liver, while resistance was significantly decreased by 31.8% in the spleen and 20.5% in the liver.

Experiment 3B

No significant differences were observed in MAP (174 ± 6 vs 169 ± 4 mm Hg), HR (441 ± 18 vs 441 ± 17 bpm), and CO (105 ± 5 vs 101 ± 4 ml/min, or 286 ± 16 vs 278 ± 13 ml/min/kg) before and after a blood sampling with simultaneous volume replacement. The regional circulatory variables pre- and post-sampling were similar in all organs (data not shown) except in the spleen and the liver. After sampling, blood flow was significantly increased by 36.0% in the spleen and 33.0% in the liver, while resistance was significantly decreased by 37.8% in the spleen and 31.4% in the liver.

Experiment 4

Body weight (BW) was significantly less (356 ± 11 vs 407 ± 11 g, p < 0.01) in DOCA-salt hypertensive rats (n = 19) than in control rats (n = 16).

Systemic Hemodynamics and Plasma Levels of Arginine Vasopressin

Compared with control rats, baseline data for systemic hemodynamics (Table 1) indicated significant increases in both MAP and HR in DOCA-salt rats. The absolute CO value remained unchanged, while CO per

<table>
<thead>
<tr>
<th>Rat</th>
<th>Baseline</th>
<th>Post-sampling</th>
<th>Pre-antagonist</th>
<th>Post-antagonist</th>
<th>Terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>Control</td>
<td>122 ± 3</td>
<td>123 ± 3</td>
<td>122 ± 3</td>
<td>121 ± 3</td>
</tr>
<tr>
<td>DOCA</td>
<td>186 ± 2*</td>
<td>187 ± 5*</td>
<td>187 ± 6*</td>
<td>178 ± 5*</td>
<td>178 ± 5*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>Control</td>
<td>407 ± 6</td>
<td>414 ± 8</td>
<td>407 ± 10</td>
<td>409 ± 10</td>
</tr>
<tr>
<td>DOCA</td>
<td>436 ± 7*</td>
<td>440 ± 8*</td>
<td>438 ± 9*</td>
<td>441 ± 10*</td>
<td>445 ± 10*</td>
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<tr>
<td>Cardiac output (ml/min)</td>
<td>Control</td>
<td>104 ± 5</td>
<td>101 ± 4</td>
<td>104 ± 5</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>DOCA</td>
<td>103 ± 4</td>
<td>102 ± 4</td>
<td>103 ± 4</td>
<td>102 ± 4</td>
<td></td>
</tr>
<tr>
<td>Cardiac output (ml/min/kg)</td>
<td>Control</td>
<td>257 ± 12</td>
<td>250 ± 9</td>
<td>257 ± 12</td>
<td>250 ± 9</td>
</tr>
<tr>
<td>DOCA</td>
<td>291 ± 8*</td>
<td>290 ± 11*</td>
<td>291 ± 8*</td>
<td>290 ± 11*</td>
<td></td>
</tr>
<tr>
<td>TVR (mm Hg/ml/min)</td>
<td>Control</td>
<td>1.21 ± 0.06</td>
<td>1.23 ± 0.05</td>
<td>1.21 ± 0.06</td>
<td>1.23 ± 0.05</td>
</tr>
<tr>
<td>DOCA</td>
<td>1.85 ± 0.11*</td>
<td>1.74 ± 0.73*</td>
<td>1.85 ± 0.11*</td>
<td>1.74 ± 0.73*</td>
<td></td>
</tr>
<tr>
<td>TVR (mm Hg/ml/min/kg)</td>
<td>Control</td>
<td>3.05 ± 0.21</td>
<td>3.09 ± 0.19</td>
<td>3.05 ± 0.21</td>
<td>3.09 ± 0.19</td>
</tr>
<tr>
<td>DOCA</td>
<td>5.51 ± 0.51*</td>
<td>5.09 ± 0.34*</td>
<td>5.51 ± 0.51*</td>
<td>5.09 ± 0.34*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of rats: n = 19 for the DOCA-salt group; n = 16 for the control group. TVR = total vascular resistance. *p < 0.05; †p < 0.01; ‡p < 0.001; in comparison to control rats. No significant differences were observed among or between values in each group at different timepoints (see text).
kg was significantly increased in DOCA-salt rats. The TVR, either absolute value or expressed as per kg, was significantly elevated. Plasma AVP concentrations were significantly higher (21.4 ± 2.8 vs 5.8 ± 1.3 pg/ml, p < 0.01) in hypertensive than in control rats (Figure 2). Plasma AVP levels were undetectably low in three of 16 control rats and in one of 19 hypertensive rats.

**Systemic Hemodynamic Responses to Blockade of Arginine Vasopressin**

No significant differences were observed among or between any of the baseline, postsampling baseline, preantagonist, postantagonist, and terminal values for MAP, HR, CO, and TVR in each of the hypertensive or control groups (Table 1). In individual data, however, DOCA-salt rats were not homogenous in responses to the antagonist; in some there were considerable depressor responses. Based on the response to the antagonist, two distinct groups could be identified. The rats with a depressor response of 10 mm Hg or more were classified as responders (n = 8), while those with a depressor response of less than 10 mm Hg, as nonresponders (n = 11). In none of control rats was there a depressor response of 5 mm Hg or more.

As shown in Table 2, the antagonist lowered MAP from 204 ± 10 mm Hg to 179 ± 11 mm Hg in responders (p < 0.01), whereas it had no effect on MAP in nonresponders. Several characteristic differences were noted between the two subgroups. Responders had a smaller BW, a greater baseline MAP, a higher plasma AVP level (29.2 ± 2.7 vs 15.2 ± 3.4 pg/ml, p < 0.01), a smaller absolute CO, and a greater absolute TVR. In responders, the antagonist reduced TVR significantly, while it tended to raise CO.

In responders, the MAP declined immediately after injection of the antagonist, reached a low level at 3 to 4 minutes after injection, and remained so for at least 20 minutes. A 50 ng/kg dose of AVP given 20 minutes after injection elicited no pressor effects in either hypertensive or control rats.

**Correlation of Plasma Levels of Arginine Vasopressin to Hemodynamic Variables**

As shown in Table 3 and in Figure 2, there were significant correlations of plasma AVP levels to MAP (r = 0.653, p < 0.01), the responses to the antagonist (r = −0.445, p < 0.05), TVR (r = 0.624, p < 0.001) and BW (r = −0.530, p < 0.01) when all rats from DOCA-salt hypertensive and control groups were combined (n = 31). Plasma AVP levels correlated significantly with HR (r = 0.733, p < 0.01, n = 8) in responders. However, there were no significant correlations between plasma AVP levels and other hemodynamic variables within each of DOCA-salt or control rats, or within each of DOCA-salt subgroups.

**Regional Hemodynamics**

As compared with control rats, DOCA-salt rats displayed significantly different patterns in baseline hemodynamics (Table 4). Weight was significantly increased in the heart (1.48 ± 0.04 vs 1.25 ± 0.04 g, p < 0.001) and kidney (2.82 ± 0.12 vs 2.04 ± 0.04 g, p < 0.001). Absolute weight differences were nil in other organs (data not shown). As shown in Table 4, %CO was decreased in the kidney and spleen, while it was increased in heart. Blood flow was decreased in the kidney and spleen, yet was increased in skeletal muscles and heart. Regional vascular resistance was elevated in almost all organs except the heart. The
changes in stomach and small and large intestines ran parallel with those in the gastrointestinal tract (data not shown).

In the responders, the following data on regional circulation differed from findings in nonresponders (Table 5). The percentage of CO was increased in the muscles and decreased in the kidney, blood flow was decreased in the kidney and spleen, and resistance was increased in the spleen, pancreas, heart, and brain.

**Regional Hemodynamic Responses to Blockade of Arginine Vasopressin**

The significant responses in DOCA-salt rats were increased, namely, %CO to heart, spleen, and portal vessels; increased flows to heart, skeletal muscles, skin, and spleen; and decreased resistances in heart, lungs, skeletal muscles, skin, and spleen (Figure 3). In responders vs nonresponders, there were different responses in most vascular beds (Figure 4). In responders, blood flow and resistance in gastrointestinal tract, pancreas, portal vessels, heart, lungs, testes, muscles, and skin responded significantly to the antagonist. However, the postantagonist values of flow and resistance seen in the whole and responder DOCA-salt rats were not normalized to those postantagonist values seen in control rats.

In control rats, significant responses included increased %CO to spleen, increased flow to spleen, and decreased resistance in skeletal muscles and spleen (Figure 3).

**Table 2. Baseline, Pre- and Postarginine Vasopressin (AVP) Antagonist Variables in Responders vs Nonresponders**

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Baseline</th>
<th>Preantagonist</th>
<th>Postantagonist</th>
<th>Pre vs Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>NR</td>
<td>176 ± 5</td>
<td>175 ± 3</td>
<td>176 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>199 ± 7</td>
<td>204 ± 10</td>
<td>179 ± 11</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>NR</td>
<td>435 ± 5</td>
<td>428 ± 9</td>
<td>429 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>436 ± 10</td>
<td>450 ± 14</td>
<td>455 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>NR</td>
<td>112 ± 4</td>
<td>105 ± 4</td>
<td>29.2 ± 7.6</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>91.6 ± 6.1</td>
<td>98.4 ± 7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output (ml/min/kg)</td>
<td>NR</td>
<td>297 ± 11</td>
<td>279 ± 10</td>
<td>297 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>282 ± 14</td>
<td>304 ± 21</td>
<td>304 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>TVR (mm Hg/ml/min)</td>
<td>NR</td>
<td>1.59 ± 0.06</td>
<td>1.70 ± 0.75</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.34 ± 0.16†</td>
<td>1.81 ± 0.14</td>
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<td>p &lt; 0.01</td>
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<tr>
<td>TVR (mm Hg/ml/min/kg)</td>
<td>NR</td>
<td>4.31 ± 0.24</td>
<td>4.61 ± 0.25</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>7.33 ± 0.89†</td>
<td>5.76 ± 0.69</td>
<td></td>
<td>p &lt; 0.01</td>
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<tr>
<td>Plasma AVP (pg/ml)</td>
<td>NR</td>
<td>15.2 ± 3.4 (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>29.2 ± 2.7* (n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>NR</td>
<td>378 ± 11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>326 ± 15*</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± se. Number of rats: n = 11 for nonresponders (NR), n = 8 for responders (R). MAP = mean arterial pressure; TVR = total vascular resistance. NS = not significant. *p < 0.05; †p < 0.01; ‡p < 0.001; compared to nonresponders.

**Table 3. Correlations Between Plasma AVP Levels and Hemodynamic Variables**

<table>
<thead>
<tr>
<th></th>
<th>DOCA-salt plus control</th>
<th>Control</th>
<th>DOCA</th>
<th>Responders</th>
<th>Nonresponders</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>31</td>
<td>13</td>
<td>18</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Baseline MAP</td>
<td>0.653</td>
<td>0.131</td>
<td>0.238</td>
<td>-0.124</td>
<td>-0.379</td>
</tr>
<tr>
<td>Responses to antagonist</td>
<td>-0.445*</td>
<td>0.014</td>
<td>-0.339</td>
<td>-0.459</td>
<td>-0.143</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>-0.305</td>
<td>-0.004</td>
<td>-0.452</td>
<td>0.185</td>
<td>-0.446</td>
</tr>
<tr>
<td>Cardiac output (ml/min/kg)</td>
<td>0.155</td>
<td>-0.092</td>
<td>-0.179</td>
<td>0.042</td>
<td>-0.224</td>
</tr>
<tr>
<td>TVR (mm Hg/ml/min)</td>
<td>0.624‡</td>
<td>0.020</td>
<td>0.391</td>
<td>-0.390</td>
<td>0.273</td>
</tr>
<tr>
<td>TVR (mm Hg/ml/min/kg)</td>
<td>0.568†</td>
<td>0.054</td>
<td>0.260</td>
<td>-0.504</td>
<td>-0.036</td>
</tr>
<tr>
<td>Heart rate</td>
<td>0.204</td>
<td>-0.236</td>
<td>-0.066</td>
<td>0.733†</td>
<td>-0.477</td>
</tr>
<tr>
<td>Body weight</td>
<td>-0.530†</td>
<td>0.066</td>
<td>-0.419</td>
<td>0.248</td>
<td>-0.328</td>
</tr>
</tbody>
</table>

Values represent coefficients of correlation. MAP = mean arterial pressure; TVR = total vascular resistance. *p < 0.05; †p < 0.01; ‡p < 0.001. See text for details.
Hemodynamic and Vasopressin in DOCA-Salt Rats

**Discussion**

Experiment 1 shows that d(CH₂)₉Tyr(Me)AVP possesses a specific AVP vasopressor antagonist activity of at least 1 hour's duration in DOCA-salt hypertensive rats, thus confirming previous observations. One of the analogs acts as a partial agonist in dogs, but not in rats. No such observations were made with the antagonist that we and other investigators used. Also, this antagonist appears to have no intrinsic vasodilator action of its own, independent of the AVP blocking action.

Experiment 2 indicates the lack of any significant effects from the application of the microsphere technique on plasma AVP concentrations, thereby providing a basis for relating plasma AVP levels to hemodynamic variables.

Along with earlier studies, Experiment 3A demonstrates that the consecutive use of microspheres produces significant regional hemodynamic effects only in the spleen and liver. The changes we observed in these organs are probably so related, as are the blood sampling procedures.

Experiment 3B shows that blood sampling with volume replacement alters neither systemic nor regional hemodynamics, except in some variables in the spleen or liver.

Based on the validation experiments above, the hemodynamic alterations following administration of the antagonist can be reasonably ascribed to the inhibition of the AVP vascular activity, except in the spleen and liver. Herein, we must consider that systemic and regional effects of AVP blockade may be partially compensated for by such mechanisms as the sympathetic nervous system and the renin angiotensin system and metabolic adjustments. In animals with these mechanisms intact, the contributions of AVP may be underestimated. Thus, what we evaluated in our study is the gross effect of acute AVP inhibition.

**Main Experiment**

**Systemic Hemodynamics, Levels of Plasma Arginine Vasopressin and Plasma Arginine Vasopressin Blockade (Experiment 4)**

The finding of a marked increase in MAP and decrease in BW in the DOCA-salt rats (Table 1), together with our previous data, suggests that the hypertension may have been entering a malignant phase. Other hemodynamic characteristics included a more rapid HR, increased CO per kg with no change in absolute CO, and a clearly elevated TVR. The rapid HR, possibly due to augmented sympathetic activity, may have been responsible for this increased CO per kg.

The significant elevation of plasma AVP concentrations (21.4 pg/ml) in DOCA-salt rats over the control
The finding of responders appears to be in accord with that of Crofton et al., which demonstrates in similar hypertensive rats a 27 mm Hg fall in MAP after an antagonist. The plasma AVP levels in the hypertensive rats studied by Rascher et al., averaged 8.4 pg/ml, 2 to 3 times over control. This magnitude of elevation may not be pressor, as is the case in our nonresponders. Significant pressor levels of circulating AVP, assessed by antagonists, were found to be 22 pg/ml in water-deprived rats, and 42 pg/ml in partially nephrectomized, salt-induced hypertensive rats.

With AVP inhibition, CO tended to be elevated, and TVR was significantly reduced in responders, whereas there was an opposite trend in nonresponders (Table 2). The vascular effects of small amounts of AVP are considered to be offset by activated baroreflexes, and the resultant CO reduction is probably due to the central cardioinhibitory actions of AVP in neurogenically intact animals. Rascher et al. found increased CO and decreased TVR with no change in MAP in DOCA-salt hypertensive rats (after 4 weeks of treatment) 30 seconds after an antagonist. They also found that, in sinoaortic-denervated rats, this antagonist lowered MAP, with a decrease in TVR and no change in CO.

There was an opposite trend in nonresponders (Table 2). The vascular effects of small amounts of AVP are considered to be offset by activated baroreflexes, and the resultant CO reduction is probably due to the central cardioinhibitory actions of AVP in neurogenically intact animals. Rascher et al. found increased CO and decreased TVR with no change in MAP in DOCA-salt hypertensive rats (after 4 weeks of treatment) 30 seconds after an antagonist. They also found that, in sinoaortic-denervated rats, this antagonist lowered MAP, with a decrease in TVR and no change in CO. However, our observations in DOCA-salt rats (after 8 weeks of treatment), either in the group as a whole or in the subgroup, are not always consonant with this notion. Baroreflex function becomes impaired with a continuation of DOCA-salt hypertension. Possible occurrence of various extents of the baroreflex dysfunction may account for the different patterns of hemody-

### Table 5. Baseline Regional Hemodynamics in Responders vs Nonresponders

<table>
<thead>
<tr>
<th>Organs</th>
<th>%CO (%)</th>
<th>Blood flow</th>
<th>Vascular resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Gl tract</td>
<td>1.55 ± 0.16</td>
<td>1.22 ± 0.10</td>
<td>1.37 ± 0.07</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.36 ± 0.27</td>
<td>1.87 ± 0.24</td>
<td>1.22* ± 0.23</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.29 ± 0.21</td>
<td>1.35 ± 0.17</td>
<td>1.15 ± 0.18</td>
</tr>
<tr>
<td>Portal</td>
<td>1.53 ± 0.14</td>
<td>1.28 ± 0.10</td>
<td>1.35 ± 0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>0.30 ± 0.06</td>
<td>0.29 ± 0.04</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.88* ± 0.37</td>
<td>4.47 ± 0.58</td>
<td>2.66* ± 0.39</td>
</tr>
<tr>
<td>Testes</td>
<td>0.27 ± 0.03</td>
<td>0.24 ± 0.04</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>Heart</td>
<td>5.35 ± 0.39</td>
<td>5.10 ± 0.45</td>
<td>4.85 ± 0.38</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.44 ± 0.38</td>
<td>1.19 ± 0.14</td>
<td>1.36 ± 0.42</td>
</tr>
<tr>
<td>Brain</td>
<td>0.89 ± 0.10</td>
<td>0.90 ± 0.06</td>
<td>0.81 ± 0.11</td>
</tr>
<tr>
<td>Muscles</td>
<td>1.47* ± 0.25</td>
<td>0.86 ± 0.07</td>
<td>1.29 ± 0.16</td>
</tr>
<tr>
<td>Skin</td>
<td>0.87 ± 0.18</td>
<td>0.84 ± 0.08</td>
<td>0.76 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± se. Number of rats: n = 8 for responders (R); n = 11 for nonresponders (NR). Abbreviations and units are the same as shown in Table 4. *p < 0.05; †p < 0.01; compared to nonresponders.

Despite the finding of no significant systemic circulatory effects of the AVP antagonist in DOCA-salt rats (Table 1), our approach with consideration to individual depressor responses identified responders with more severe hemodynamic abnormalities and a lesser BW among these rats (Table 2). Plasma AVP of an average of 29.2 pg/ml (5 times above control) apparently participated in maintaining the elevated MAP in responders, as assessed from the response of −24.9 mm Hg to the antagonist, while plasma AVP of an average of 15.2 pg/ml (2.6 times above control) did not seem to play a role in maintaining the elevated MAP in nonresponders.

These results suggest that systemic pressor effects of circulating AVP are important only in the DOCA-salt hypertensive rats with plasma AVP levels elevated above a certain point. This finding, together with convincing evidence of the necessity of AVP replacement for the development of DOCA-salt hypertension in diabetes insipidus rats, suggests that other actions of AVP including antidiuretic activity may be involved. Another interpretation is that AVP must be present during the initiating phase in order for hypertension to develop, but once hypertension has been achieved, AVP plays only a minor role in maintaining the elevated MAP.

The finding of acute AVP blockade to induce significant MAP changes in whole DOCA-salt rats is compatible with the observations of Rabito et al. and Rascher et al. in rats with this form of hypertension.
HEMODYNAMICS AND VASOPRESSIN IN DOCA-SALT RATS/Yamamoto et al.

FIGURE 3. Percentage of changes in %CO₂, blood flow, and vascular resistance following an AVP vasopressor antagonist in conscious DOCA-salt hypertensive and sham-treated control rats. Hatched bars represent DOCA-salt rats (n = 19) and open bars represent control rats (n = 13). GI tract = gastrointestinal tract: portal = GI tract + spleen + pancreas. *p < 0.05; **p < 0.01; ***p < 0.001; compared to the preantagonist values of each group.

FIGURE 4. Percentage of changes in %CO₂, blood flow, and vascular resistance following an AVP vasopressor antagonist in responders and nonresponders. Dotted bars represent responders (n = 8) and hatched dark bars represent nonresponders (n = 11). Abbreviations are the same as shown in Figure 3. *p < 0.05; **p < 0.01; ***p < 0.001; compared to the preantagonist values of each group.
Correlations Between the Levels of Plasma Arginine Vasopressin and Circulatory Variables

The coefficients of correlation obtained between the plasma AVP levels and several variables in the DOCA-salt plus control rats, albeit significant, were relatively small (Table 3). This suggests, on the one hand, a low degree of direct relationship and, on the other hand, complicated interrelationships. We consider that AVP's pressor and the antagonist's depressor actions are influenced by vascular reactivity, and interactions with neurohormonal mechanisms. The lack of significant correlations within DOCA-salt or control rats, or within DOCA-salt subgroups, suggests no direct relationships. In addition to these complex factors, a relatively small variation in the hemodynamic data within each group may also explain this negative finding.

Regional Hemodynamics

The finding of changes in %CO and regional flow (Table 4) suggests that blood flow was redistributed away from kidney and spleen to skeletal muscles and heart in DOCA-salt rats. Yates and Hiley, measuring only flow distribution in rats with this form of established hypertension (bilateral kidneys intact), found that the %CO per g tissue was increased in forelimb muscles, unchanged in heart and skin, and decreased in kidneys. Despite differences in the experimental conditions, directional changes in %CO in kidney(s) and muscles are consistent in both studies.

Although vascular resistance was elevated in almost all organs, the relative contribution of each vascular bed to the elevated TVR was not equal (Table 4). In view of voluminous skeletal muscle tissues constituting almost 40% of the body composition, the relatively small increase in resistance seen in the muscles may contribute most importantly to the elevated TVR. Normal resistance in the heart seen in the whole DOCA-salt rats may be related to increased flow demands owing to increased afterload and HR. Consistent with this is a similar observation on the coronary vasculature of spontaneously hypertensive rats.

The more marked and extensive alterations in regional hemodynamics in responders (Table 5) may represent a manifestation of extreme hypertensive vascular abnormalities that results from the DOCA-salt treatment. For example, in contrast to nonresponders, the responders had increased coronary resistance.

Regional Hemodynamic Responses to Blockade of Arginine Vasopressin

Our study seems to be the first evaluation of the regional effects of AVP blockade in DOCA-salt hypertension. In light of the importance of the contribution to the TVR, the most noteworthy responses were increased flow to skin and decreased vascular resistance in the skeletal muscles and skin in DOCA-salt rats (Figure 3). The responses were more striking and extensive in responders (Figure 4). However, acute AVP blockade did not completely reverse toward the values seen in control rats the basic abnormalities in the regional vasculatures seen in DOCA-salt rats and even in responders. These observations suggest that AVP maintained a vasoconstriction in the skeletal muscles and skin in DOCA-salt rats, and also in the gastrointestinal tract, pancreas, testes, and so forth, in the responders, but that AVP effects were responsible only partially for these regional hemodynamic abnormalities seen in these rats. Our findings in the skeletal muscles and skin are in accord with previous reports on the high AVP sensitivity of these vascular beds. The significant response of the gastrointestinal tract seen in the responders is also important in view of its relative contribution to TVR. This result seems to be consistent with data on the medium high-AVP sensitivity of this vasculature.

Unexpected was the significant response of the muscles in the control rats. The controls underwent uninephrectomy, sham treatment, and vessel cannulation, and the plasma AVP levels in these rats were somewhat higher than the values reported by others. It thus seems that, in this particular, near-normal condition, AVP exerted a significant circulatory effect on skeletal muscle vessels.

Other intriguing responses to AVP blockade were the increases in %CO and flow and the decrease in vascular resistance in the heart seen in DOCA-salt rats. Our MAP and HR data following AVP blockade in the DOCA-salt rats as a whole and in responders do not suggest increases in cardiac oxygen and, hence, metabolic requirements. However, as we did not evaluate indices of cardiac contractility, preload, and diastolic coronary resistance, these data should be interpreted with reservations if they are to imply that AVP may have contributed to coronary vasoconstriction in these rats. AVP caused a direct coronary constriction and negative inotropism in dogs, whereas it had no effects on coronary flow and resistance in rats, despite causing a declined cardiac performance.

The decrease in resistance, but not increase in flow, seen in the spleen following AVP blockade in whole and responder DOCA-salt rats (Figure 4) outweighed in a relative magnitude those seen in DOCA-salt rats that received no AVP antagonist (Experiment 3A). Accordingly, AVP may contribute in some way to elevated splenic resistance in these rats. The splenic responses in control rats, although significant, appear to result from repeated microsphere injections, since relative changes are comparable to those observed in normal rats by some researchers when microspheres are repetitively used. Because of the finding of no significant hemodynamic responses, the basal abnormalities in the kidney and brain seen in whole and responder DOCA-salt rats cannot be ascribed to AVP effects. In support of this notion are earlier reports on the relative low AVP sensitivity of the renai and cerebral vasculatures in dogs and rats. The remarkable abnormalities in renal and splenic vessels that we observed in the preblockade may relate in some degree to sympathetic overactivity.
sensitivity to sympathetic amine. The responses in flow and vascular resistance in lungs are not readily attributable to bronchial vasodilation, because arteriovenous shunt flow is also reflected in the lungs.

Conclusions
In conscious rats with near-malignant DOCA-salt hypertension, AVP probably plays a minimal role in the elevations of MAP and TVR. In responders with exceedingly elevated levels of plasma AVP, however, AVP contributes substantially to these systemic hemodynamic changes. The regional hemodynamic changes in the muscles, skin, and perhaps spleen seen in DOCA-salt rats as a whole, and those in the muscles, skin, gastrointestinal tract, spleen, pancreas, and tested seen in responders, can be ascribed partly to AVP effects. The renal and cerebral circulatory changes do not seem to be AVP-related.

Acknowledgments
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