SUMMARY To examine the role of vasopressin (VP) in DOCA-salt hypertension, arterial pressure and renal vascular reactivity were studied in control Long-Evans (LE) rats and in Brattleboro rats homozygous for diabetes insipidus (DI rats). Vascular reactivity to norepinephrine, VP and angiotensin II was assessed in isolated kidneys perfused at constant flow. LE rats showed an increase in arterial pressure (AP) which was significant at 2 weeks post DOCA and averaged 180 mm Hg at 4 weeks. DI rats lacking VP showed no rise in AP after DOCA; however, DI rats given VP and DOCA developed hypertension with a course and magnitude similar to that observed in LE rats. At 6 to 10 weeks post DOCA, renal vascular reactivity to all agents was increased in LE rats and DI rats replaced with VP. Nonhypertensive DI rats lacking VP showed depressed reactivity. Assessment of changes in reactivity at 3 days post DOCA showed that changes preceded the rise in AP. These data suggest that VP may play a primary role in the pathogenesis of DOCA hypertension and that its mechanism may involve an induction of increased vascular reactivity. (Hypertension 4: 3-12, 1982)

KEY WORDS • vasopressin • hereditary diabetes insipidus

The mechanism by which VP might participate in DOCA-salt hypertension is not understood. In earlier experiments, however, it was observed that renal vascular reactivity to norepinephrine (NE), VP, and angiotensin II (AI1) was increased in DOCA hypertensive rats not only in the chronic stage but in the pre-hypertensive stage, suggesting that changes in vascular reactivity, per se, participate in the development of DOCA hypertension.

The purposes of the present studies were to determine: 1) whether VP replacement in DI rats restores the hypertensive properties of DOCA-salt; and 2) to define the nature of changes in vascular reactivity in these rats after DOCA to determine whether restoration by VP of hypertension in VP-deficient rats might be associated with an induction of increased vascular reactivity.

Methods

Experimental Animals

Studies were carried out on 12-week-old male Brattleboro rats homozygous for diabetes insipidus (DI rats) and Long Evans rats (LE rats) obtained from Blue Spruce Farms. At this age the average weight of the DI rats was 255 ± 9 g while that of the LE rats was 346 ± 7 g. Rats were housed individually in metabolic cages and fed a standard laboratory rat chow (Purina,
100 μEq NaCl/g). All rats were unilaterally nephrectomized (left kidney) under light ether anesthesia and postoperatively, received a single intramuscular injection of procaine penicillin (100,000 μg per rat).

After nephrectomy the DI rats were divided into three groups: 1) DI rats treated with DOCA-salt and replaced with VP (DI + DOCA-salt + VP); 2) DI rats treated with DOCA-salt (DI + DOCA-salt); and 3) untreated DI rats (DI control) and the LE rats divided into two subgroups: a) LE rats treated with DOCA-salt (LE + DOCA-salt) and b) untreated LE rats (LE control). Both DI and LE rats treated with DOCA-salt received a single subcutaneous implant of Silastic strips (silicone rubber, Dow-Corning Company) impregnated with deoxycorticosterone (DOCA, Sigma Chemical Company) at a dose of 100 mg/kg. Control DI and LE rats received silastic implants without DOCA. All rats were given a 0.3% NaCl solution to drink. Daily fluid intake was monitored in the DOCA-treated rats. Systolic blood pressure was determined twice weekly using an indirect tail cuff method in conscious, restrained rats prewarmed at 37°C for 5-10 minutes (automated cuff-inflator pulse detector, IITC, Inc.). The rats were conditioned to the restraining devices and cuff inflations prior to the determination of baseline parameters. Perfusion of the kidney was performed approximately 8 weeks after DOCA or Silastic implantation. Data from a group of similarly aged DI rats treated with VP alone for 6-8 weeks, studied prior to the current protocol, were also included. This was done to evaluate the effects of VP alone on arterial pressure and renal vascular reactivity and compare these effects with DOCA or Silastic treatment.

In an additional study, kidneys from DI rats treated with DOCA-salt + VP and DI rats treated with DOCA-salt alone were perfused 3-4 days post DOCA. Responses from these groups were compared to DI control rats to determine whether changes in renal vascular reactivity preceded the rise in arterial pressure. In these rats systolic pressure was monitored three times prior to the implantation of DOCA and daily for 3-4 days following implantation.

**Vasopressin (VP) Replacement**

DI rats replaced with VP were given the pituitary extract VP tannate (Pitressin tannate, Parke Davis) which contains both arginine and lysine VP suspended in peanut oil. Rats were given daily subcutaneous injections of VP at a dose of 100 mU/100 g rat. To ensure injection of a well-suspended solution, each ampule of VP was warmed at 37°C for 10 minutes and continuously mixed up to the time of injection. Replacement was begun 2 weeks prior to DOCA implantation and continued for the duration of the experiment. Injections were always given between 4-5 p.m. The rats rapidly became accustomed to the daily injections and tolerated them well. The dose of VP was selected on the basis of a previous report showing that treatment of DI rats with 50-100 mU/100 g rat produced normal levels of plasma arginine VP within 9 days of onset of treatment and that water intake, hematocrit, serum Na⁺, serum K⁺, plasma renin activity, and plasma aldosterone were restored to normal but serum osmolality remained slightly (15 mOsm) above that of heterozygous DI rats.

**Renal Perfusion**

Renal perfusion experiments were carried out 6-8 weeks post DOCA implantation under sodium pentobarbital (Nembutal, Abbott Labs) anesthesia (50 mg/kg i.p.). The right kidney was surgically isolated and perfused using previously described techniques modified for the current study. Briefly, the kidney was perfused through a catheter placed into the distal aorta and advanced to the origin of the renal artery. The superior mesenteric and suprarenal branch of the right renal artery were ligated. Following intravenous injection of heparin (250 IU/kg) arterial pressure was measured through the aortic cannula then the artificial perfusion was begun. Following the start of perfusion, the aorta was ligated proximal to the renal artery and a catheter was introduced into the renal vein to drain renal venous effluent; the inferior vena cava was ligated distally. The kidney was perfused at constant flow using a peristaltic perfusion pump which was pressure independent to pressures in excess of 200 mm Hg. The perfusate was a modified Krebs-Henseleit solution containing Ficoll (Pharmacia AB), 0.35 g/liter. The perfusate was not recirculated. The pH of the perfusate was 7.4, it was maintained at a temperature of 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. Perfusion pressure, measured from the side arm of the aortic cannula (Century EP-01 transducer) was recorded on a Beckman Type RM dynograph recorder. Perfusion flow was periodically measured from the renal venous catheter. An equilibration period of 60 minutes was allowed before the experimental protocol was started.

**Experimental Protocol**

Vasoconstrictors employed for the reactivity studies were norepinephrine (NE, Levophed bitartrate, Breon Laboratories, Inc.), VP (Pitressin, Parke-Davis) and angiotensin II (Hypertensin, CIBA-Geigy). The same Pitressin preparation was used for all reactivity studies. Perfusion flow was approximately 5.5 ml/g/min. At this flow rate the renal vascular bed appeared to be completely dilated as bolus injections of papaverine HCl (5-10 mg) into the arterial bed produced no further fall in perfusion pressure. Baseline perfusion pressure was stable for the duration of the experiment (150-170 min). After the equilibration period, cumulative dose responses to the vasoconstrictors were obtained in the following order: NE, VP, and AII. A period of 20-25 minutes allowed between drug trials. The drugs were injected into the perfusion system through a multiple puncture site just proximal to the arterial inflow catheter. Drugs were injected in bolus amounts from subthreshold to maximum doses.
Statistical Methods

All values presented in the text and in the figures are means ± standard errors (SE). Data from DI groups and LE groups were analyzed separately. One way analysis of variance was used to evaluate weekly measurements of arterial pressure, daily measurement of fluid intake and hemodynamic characteristics of the rats at perfusion.

The dose-response curves for each constrictor drug were compared among the DI and LE groups using a 3 X 3 pt parallel line bioassay. This test provides a relative potency ratio with confidence limits. In all cases of multiple comparisons when a significant (p < 0.05) F ratio was obtained the Newman Keuls test was used to determine which of the comparisons were significantly different.

Results

LE rats treated with DOCA-salt showed an increase in systolic blood pressure which was significant (p < 0.005) at 2 weeks post DOCA and averaged 180 mm Hg at 4 weeks (fig. 1). DI rats lacking VP showed no rise in arterial pressure after DOCA. DI rats replaced with VP, however, developed hypertension after DOCA; the rise in arterial pressure followed a course and obtained a magnitude similar to that obtained in LE rats (fig. 1). Although not shown here, chronic VP replacement alone in DI rats for 6-8 weeks produced no effect on arterial pressure. The average systolic pressure prior to treatment was 124.6 ± 4.0 and that after 8 weeks of chronic VP replacement was 129.8 ± 5.0 mm Hg.

Effectiveness of Vasopressin Replacement

Effectiveness of prolonged VP replacement was evaluated by monitoring daily fluid intake in the DI rats. It can be seen from fig. 2 that DI + DOCA-salt + VP rats (fig. 2 C) had an average daily intake of 0.3% NaCl of 345 ml/rat prior to the initiation of VP replacement. Within 24 hours after the first injection, intake in these rats fell significantly (p < 0.005) to an average intake of 90 ml/rat. This intake was significantly (p < 0.005) less than that in DI rats treated with DOCA-salt alone (fig. 2 B), was comparable to that seen in LE rats (fig. 2 A), and was maintained for the duration of the experiment.

Renal Vascular Resistance

Vascular resistance of kidneys from DI rats treated with VP alone and DI rats treated with DOCA-salt alone was not significantly different from untreated DI rats (table 1). In contrast, kidneys from DI rats treated with both VP and DOCA, which did develop hypertension, demonstrated a significantly (p < 0.01) increased renal vascular resistance (table 1). Kidneys from LE rats treated with DOCA-salt that became hypertensive also showed increased vascular resistance when compared to LE control rats (table 1). The magnitude of the increase in renal vascular resistance was similar for the hypertensive DI and LE rats.

Renal Vascular Reactivity

At 8 weeks post DOCA, the renal vasculature of hypertensive LE rats showed a markedly enhanced reactivity to NE (fig. 3 A). Dose-response curves were characterized by a leftward shift, a decrease in threshold, and a greater maximal response. Comparison by means of a parallel line bioassay of the response curves of DOCA-treated LE rats with those from LE rats.
control demonstrated that the leftward shift in the curve was a parallel one. Moreover, the potency ratio (table 2) derived from the bioassay showed that renal vascular beds from LE + DOCA-salt rats responded as if the dose of norepinephrine was 7 times greater than that in LE controls ($p < 0.01$).

**FIGURE 3.** Dose-response curves in isolated perfused kidneys from: A) untreated Long-Evans (LE) rats (LE control) and LE rats treated with DOCA-salt (LE + DOCA-salt); and B) untreated DI rats (DI control), DI rats treated with vasopressin (VP) alone (DI + VP replacement), DI rats treated with DOCA-salt alone (DI + DOCA-salt), and DI rats treated with both VP and DOCA (DI + DOCA-salt + vasopressin replacement). Kidneys were perfused 6–10 weeks post DOCA. Response (ordinate) is given as renal vascular resistance (mm Hg/ml.g⁻¹.min⁻¹) which was calculated as the quotient of the recorded perfusion pressure (mm Hg) divided by the constant perfusate flow (ml/g.min). Responses are expressed as group means ± SE; $n =$ number of rats in each group; abscissa = dose of norepinephrine (g/10 μl injection volume). Statistical analyses of the dose-response curves are indicated in the text (see Results, table 2).

**TABLE 1. Characteristics of the Rats at the Time of Renal Perfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (g)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Renal perfusion pressure (mm Hg)</th>
<th>Renal perfusate flow (ml/g.min⁻¹)</th>
<th>Renal vascular resistance (mm Hg/ml.g⁻¹.min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic DI rats</strong></td>
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<tr>
<td>DI + DOCA-salt + VP (n = 7)</td>
<td>392.1 ± 24*</td>
<td>171.4 ± 6.3*</td>
<td>46.1 ± 1.4*</td>
<td>5.29 ± 0.07</td>
<td>8.5 ± 0.23*</td>
</tr>
<tr>
<td>DI + DOCA-salt (n = 7)</td>
<td>314.3 ± 15</td>
<td>109.3 ± 5.2</td>
<td>31.4 ± 1.9</td>
<td>5.39 ± 0.05</td>
<td>5.83 ± 0.34</td>
</tr>
<tr>
<td>DI + VP (n = 7)</td>
<td>296.3 ± 11.6</td>
<td>103.7 ± 5.4</td>
<td>34.0 ± 1.5</td>
<td>5.5 ± 0.10</td>
<td>6.2 ± 0.22</td>
</tr>
<tr>
<td>DI control (n = 7)</td>
<td>296.4 ± 6.3</td>
<td>110.6 ± 4.0</td>
<td>36.3 ± 1.2</td>
<td>5.41 ± 0.12</td>
<td>6.6 ± 0.26</td>
</tr>
<tr>
<td><strong>Chronic LE rats</strong></td>
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<tr>
<td>LE + DOCA-salt (n = 6)</td>
<td>573 ± 16.6</td>
<td>174.2 ± 6.8*</td>
<td>46.2 ± 1.5*</td>
<td>5.33 ± 0.08</td>
<td>8.7 ± 0.26*</td>
</tr>
<tr>
<td>LE control (n = 8)</td>
<td>545.5 ± 10.5</td>
<td>109.1 ± 3.2</td>
<td>34.0 ± 2.3</td>
<td>5.4 ± 0.07</td>
<td>6.16 ± 0.23</td>
</tr>
<tr>
<td><strong>DI Rats - 3 days post DOCA</strong></td>
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<tr>
<td>DI + DOCA-salt + VP (n = 6)</td>
<td>328.7 ± 6.9</td>
<td>113.7 ± 2.9</td>
<td>34.2 ± 2.4</td>
<td>5.45 ± 0.06</td>
<td>6.3 ± 0.43</td>
</tr>
<tr>
<td>DI + DOCA-salt (n = 6)</td>
<td>287.0 ± 9.8</td>
<td>115 ± 4.0</td>
<td>32.5 ± 2.1</td>
<td>5.5 ± 0.05</td>
<td>5.9 ± 0.38</td>
</tr>
<tr>
<td>DI control (n = 6)</td>
<td>280 ± 12.8</td>
<td>111 ± 4.5</td>
<td>36.7 ± 1.3</td>
<td>5.45 ± 0.13</td>
<td>6.7 ± 0.32</td>
</tr>
</tbody>
</table>

Responses are expressed as groups means ± standard error (SE); statistical analysis by one-way analysis of variance and Newman-Keuls ranking test. VP = vasopressin; DI = diabetes insipidus rats; LE = Long-Evans rats.

*6–8 weeks post DOCA implantation.
†Direct recording from aorta, under pentobarbital anesthesia.
 Different from all other chronic DI groups, $p < 0.05$.
 Different from all other chronic DI groups, $p < 0.01$.
 Different from chronic LE control, $p < 0.005$.
 Different from all other DI – 3 day groups, $p < 0.01$. 
DI rats treated with VP alone and DI rats treated with DOCA alone showed a depression in renal vascular responsiveness to NE when compared to untreated DI rats (fig. 3 B). Only in DI rats treated with both VP and DOCA that developed hypertension, was reactivity to NE found to be elevated. There was a parallel shift in the dose-response curve to the left, threshold was lower and maximum response greater (fig. 3 B). Furthermore, renal vascular beds from hypertensive DI rats responded as if the dose of NE used was 7 times greater than that in DI control, 15 times greater than DI + VP rats, and 25 times greater than DI rats treated with DOCA-salt alone (table 2). The magnitude of the increase in reactivity to NE was the same in hypertensive LE and DI rats.

The pattern of changes in reactivity in hypertensive LE and DI rats in response to vasopressin (fig. 4 and table 2) and angiotensin II (fig. 5 and table 2) was similar to that seen with norepinephrine. In contrast

**Figure 4.** Dose-response curves of the same kidneys as in figure 3. Ordinate as in figure 3; abscissa = dose of vasopressin (g/10 μl injection volume).

**Table 2.** Vascular Reactivity in the Perfused Kidneys of LE and DI Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Norepinephrine</th>
<th>Vasopressin</th>
<th>Angiotensin II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic DI rats</strong></td>
<td></td>
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<tr>
<td>Groups vs DI control (potency = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI + DOCA-salt + VP</td>
<td>7.32†</td>
<td>5.88†</td>
<td>1.8 × 10⁶†</td>
</tr>
<tr>
<td>(4.32 - 13.3)</td>
<td>(3.5 - 9.96)</td>
<td>(1.3 × 10⁴ - 1.1 × 10⁶)</td>
<td></td>
</tr>
<tr>
<td>DI + DOCA-salt</td>
<td>0.28†</td>
<td>0.27†</td>
<td>0.03†</td>
</tr>
<tr>
<td>(0.16 - 0.46)</td>
<td>(0.17 - 0.43)</td>
<td>(0.004 - 0.15)</td>
<td></td>
</tr>
<tr>
<td>DI + VP</td>
<td>0.47†</td>
<td>1.1</td>
<td>0.37†</td>
</tr>
<tr>
<td>(0.28 - 0.75)</td>
<td>(0.17 - 1.17)</td>
<td></td>
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<tr>
<td>Groups vs DI + DOCA-salt (potency = 1)</td>
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<td></td>
</tr>
<tr>
<td>DI + DOCA-salt + VP</td>
<td>25.6†</td>
<td>17.0†</td>
<td>1.4 × 10⁵†</td>
</tr>
<tr>
<td>(12.5 - 61.4)</td>
<td>(10.0 - 34.0)</td>
<td>(4.8 × 10⁴ - 4.0 × 10⁵)</td>
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<tr>
<td>DI + VP</td>
<td>1.61</td>
<td>1.1</td>
<td>0.37</td>
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<tr>
<td>(0.91 - 2.87)</td>
<td>(0.095 - 1.49)</td>
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<tr>
<td>Groups vs DI + VP (potency = 1)</td>
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<td></td>
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<tr>
<td>DI + DOCA-salt + VP</td>
<td>14.7†</td>
<td>5.22†</td>
<td>8.2 × 10⁴†</td>
</tr>
<tr>
<td>(7.86 - 30.9)</td>
<td>(2.9 - 9.5)</td>
<td>(3.5 × 10³ - 1.8 × 10⁴)</td>
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<tr>
<td><strong>Chronic LE rats</strong></td>
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<tr>
<td>LE + DOCA-salt vs LE control (potency = 1)</td>
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<tr>
<td>(4.4 - 11.7)</td>
<td>(6.1 - 24)</td>
<td>135†</td>
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<tr>
<td>DI rats 3 Days Post DOCA</td>
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<tr>
<td>Groups vs DI control (potency = 1)</td>
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<tr>
<td>DI + DOCA-salt + VP</td>
<td>2.6†</td>
<td>2.5†</td>
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<tr>
<td>(1.4 - 5.1)</td>
<td>(1.6 - 3.9)</td>
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<tr>
<td>DI + DOCA-salt</td>
<td>0.30†</td>
<td>0.35†</td>
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<tr>
<td>(0.15 - 0.66)</td>
<td>(0.19 - 0.62)</td>
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<tr>
<td>Groups vs DI + DOCA-salt (potency = 1)</td>
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<td></td>
<td></td>
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<tr>
<td>DI + DOCA-salt + VP</td>
<td>8.0</td>
<td>6.15</td>
<td></td>
</tr>
<tr>
<td>(4.4 - 16.0)</td>
<td>(1.2 - 3.4)</td>
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*Data expressed as potency ratios determined by 3 × 3 pt parallel line bioassay (see text); figures in parentheses under each ratio represent the 95% confidence limits. DI = diabetes insipidus rats; VP = vasopressin; LE = Long-Evans rats.
†p < 0.01.
to the findings with NE, VP replacement alone in DI rats had no effect on renal vascular reactivity to VP (fig. 4B, table 2). The increase in reactivity to VP was also significantly greater \((p < 0.01)\) in hypertensive LE as compared to hypertensive DI rats.

The greatest differences in renal vascular reactivity between hypertensive and normotensive rats were found with angiotensin II as the stimulus (fig. 5 and table 2). Although the dose-response curves were somewhat flattened, probably due to the development of tachyphylaxis in both types of rats, the hypertensive rats showed a larger increase in reactivity compared to controls. The magnitude of the increase in reactivity for hypertensive LE rats was not as great as that seen for hypertensive DI rats (table 2).

Changes in Reactivity Prior to the Development of Hypertension

Changes in renal vascular reactivity were assessed in DI rats and DI rats replaced with VP 3 days after beginning DOCA-salt treatment. Responses in these vascular beds were compared to those obtained in DI control rats. In the prehypertensive stage, the blood pressures of the three DI groups did not differ from one another (table 1). Renal vascular resistance was also similar (table 1). However, changes in reactivity were already present at 3 days. Dose-response curves of renal vascular beds of DI + DOCA-salt + VP rats to both NE and VP (fig. 6) were characterized by a parallel shift to the left and increased potency ratios (table 2). In contrast, the dose-response curves from

![Renal Vascular Resistance vs. Dose](image)

**Figure 5.** Dose-response curves of same kidneys as in figure 3. Ordinate as in figure 3; abscissa = dose of angiotensin II \((g/10 \mu l\) injection volume).

renal vascular beds of DI rats treated with DOCA-salt alone were characterized by parallel shifts to the right (fig. 6) and significantly lower potency ratios (table 2). Comparison of the potency ratios showed that the changes in renal vascular reactivity in response to NE and VP were significantly \((p < 0.01)\) greater in the chronically hypertensive DI + DOCA-salt + VP rats than in the prehypertensive DI + DOCA-salt + VP rats (potency ratios: 3.2 for NE and 2.3 for VP).

**Discussion**

Previous work with cerebroventricular administration of 6-hydroxy-dopamine has shown that destruction of central adrenergic structures in rats prior to DOCA treatment totally prevents the development of hypertension.\(^{10}\) Furthermore, electrolytic destruction
of the anteroventral region of the third ventricle (AV3V), an area thought to be involved in central actions of angiotensin and the elaboration of VP also prevented the development of DOCA hypertension in rats. These findings have led to the hypothesis that there is a centrally located "trigger" mechanism for the initiation of DOCA hypertension. It has been speculated that a central mechanism participates in the development of hypertension by increasing peripheral sympathetic outflow. However, several investigators have provided evidence suggesting that the onset and magnitude of hypertension may not be entirely related to peripheral sympathetic nerve function. The central mechanism for initiation of DOCA hypertension could well be related in part to processes which are endocrine, rather than neural in nature.

DOCA hypertension has also been associated with enhanced vascular reactivity. In both rats and pigs, enhanced reactivity was observed prior to a significant rise in arterial pressure. An intact peripheral sympathetic system was not a necessary requirement for the development of enhanced reactivity; however sodium deficiency and cerebroventricular administration of 6-OHDA, both factors known to prevent the development of hypertension also prevented the development of enhanced vascular reactivity. These various findings suggest that the development of DOCA hypertension is related to an interaction of several factors. Early central stimulation of peripheral sympathetic activity and/or release of hormones from the brain could lead to increased vascular reactivity, a potential primary mechanism for the rise in arterial pressure.

Our current studies suggest that VP is a candidate for the proposed central "trigger" mechanism for DOCA-salt hypertension. In confirmation of the findings of Crofton et al. and Saito et al. we have found that DI rats totally lacking VP do not become hypertensive with DOCA. We also confirm the preliminary report of Wiserhof et al., who found that VP (Pitressin) replacement restored the hypertensive properties of DOCA-salt. Moreover, restoration by VP of hypertension in VP-deficient rats was associated with an induction of increased renal vascular reactivity and that this induction occurred rapidly, proceeding the rise in arterial pressure. The pattern of changes in reactivity found in DI rats treated with VP and DOCA was similar to that seen in DOCA-treated LE rats with normal hypophyseal function.

A primary role for VP in the pathogenesis of DOCA-salt hypertension was first suggested by Friedman et al. who found that surgical ablation of the median eminence (a procedure which produced diabetes insipidus) prevented the development of DOCA hypertension while treatment of rats receiving DOCA-salt with large doses of Pitressin shortened the onset of development of hypertension. Subsequently, Möhring et al. demonstrated that plasma concentrations of VP were increased 3- to 10-fold in chronic DOCA-treated rats and that acute administration of a specific VP antiserum resulted in a transient, large reduction in mean arterial pressure in these rats. Saito et al. also showed a blood pressure reduction in DOCA hypertensive rats after administration of VP antiserum which preceded the appearance of diuresis, suggesting involvement of a vasoconstrictor action of VP in the maintenance of DOCA-salt hypertension.

The results obtained from experiments employing antagonists of the pressor effect of VP are less clear-cut. Crofton et al. used two antagonists of the pressor effect of VP [1-deaminopencillamine, 4-valine, 8-D-arginine] vasopressin (dPVDAVP) and [1-(β-Mercapto-β-cyclopentamethylene-propionic acid), 4-valine, 8-D-arginine] vasopressin (cylo-dVDAVP). They found that both antagonists produced a substantial reduction in arterial pressure of DOCA hypertensive Long Evans rats. In contrast, Rabito et al. found that these same antagonists did not induce significant changes in mean arterial pressure in DOCA hypertensive Sprague Dawley rats. Furthermore, increased plasma concentration and urinary excretion of VP have been seen in early stages of development of DOCA hypertension. While evidence strongly suggests participation of VP in the development of DOCA hypertension, its exact mechanism of action is not clear. Saito et al. were able to induce DOCA-salt hypertension in DI rats by replacing them with deaminio 8-D-arginine vasopressin (DDAVP) which has an antidiuretic effect but no vasoconstrictor effect. This finding suggests that either both the vasoconstrictor and antidiuretic actions of endogenous VP may be involved in DOCA-salt hypertension in rats or that there is an indirect action common to both VP and DDAVP. Plasma concentrations of VP are elevated in DOCA hypertensive rats, however, the range is below that of plasma concentrations which will induce a rise in arterial pressure, in vivo, in the normal rat. The concentration of VP needed to produce a pressor response is very high and is in excess of plasma concentrations found after 48 hrs of water deprivation when maximal urinary concentration is obtained. Interestingly, it has been demonstrated, in vitro, that VP at concentrations similar to those in vivo (10^{-12}-10^{-14}M) will produce vasoconstriction of resistance vessels. A paradox arises in that while in vitro studies indicate that normal plasma concentrations of VP are capable of constricting resistance vessels, the plasma concentrations, in vivo, have to be increased to supraphysiological levels before a pressor response can be detected. A marked increase in pressor sensitivity to VP has been demonstrated in baroreceptor denervated dogs, in decapitated spinal anesthetized dogs and in humans with primary autonomic insufficiency (orthostatic hypotension) which was well in excess of an increase in pressor sensitivity to norepinephrine and angiotensin II. These data suggest that under normal circumstances the direct pressor effect of VP is buffered by these reflexes; however, in conditions where the function of these reflexes is altered physiological concentrations of VP may become pressor. Recent studies of Montani, et al. suggest an explanation for potentiation of the pressor response to VP after baroreceptor denerva-
tion. These workers found that infusion of VP at physiological concentrations in dogs produced a peripherally-mediated increase in total peripheral resistance and a centrally-mediated decrease in cardiac output resulting in an unchanged arterial pressure. Removal of baroreceptor afferents was found to suppress central cardiac inhibition of VP leaving the peripheral resistance effect. Hence, the response to physiological concentrations of VP was now found to be an increase in arterial pressure. In the state of hypertension VP could become pressor if the peripheral vasculature became more sensitive to VP, if VP modulated the function of vascular smooth muscle cell, or if there was a diminution of the ability of the baroreceptors and/or other reflex systems to buffer the pressor effects of VP.

There is evidence that vascular reactivity to VP is increased in isolated perfused vascular beds and in the systemic circulation of DOCA-treated animals. In these present studies we have shown that the augmentation in the renal vascular response to VP was slightly greater than that for NE but less than that for AII in hypertensive LE rats. In hypertensive DI rats there was a comparable augmentation in renal vascular reactivity to NE and VP. These studies suggest that changes in sensitivity to exogenous VP may be a secondary result of a primary, non-specific increase in sensitivity of vascular smooth muscle. However, changes in the reactivity of the isolated renal vascular bed may not reflect the sensitivity of whole animal to the pressor effects of VP. Crofton et al.[] observed an increase in systemic pressor sensitivity to VP in DOCA-treated LE rats 6–8 weeks post DOCA but this was not a constant observation in all rats nor was increased pressor sensitivity seen in rats 3–5 weeks post DOCA.

Vasopressin may participate in the development of DOCA hypertension by modulating vascular smooth muscle response to other vasoconstrictors. Vasopressin modifies the cardiovascular effects of catecholamines. Suppressor doses of VP increase the blood pressure responses to epinephrine and norepinephrine and potentiate catecholamine-induced contraction in the microvasculature. The exact mechanisms by which VP constricts vascular smooth muscle and potentiates the response to catecholamines are not known. Our studies suggest that the presence of VP in DI rats does modulate the function of vascular smooth muscle by inducing an increase in vascular reactivity and that the ability of VP to increase vascular reactivity occurs rapidly preceding the onset of the rise in arterial pressure. Furthermore, our studies suggest that there is a unique interaction between VP and DOCA since vascular reactivity was either unchanged or reduced in DI rats treated with DOCA or VP alone.

There has been increasing evidence that VP and the baroreceptor reflex are intricately linked. The paraventricular (PVN) and supraoptic nuclei (SON) are associated with the production of VP and oxytocin.[] Neuroanatomical studies have shown that the PVN receives neural projections from the region of the nucleus tractus solitarius (NTS) the primary site of termination of afferent nerve fibers from the baroreceptor nerves and in turn, sends projections to cardiovascular centers in the medulla and to the thoracic spinal cord. Moreover, pathways identified immunohistochemically as vasopressinergic have been found to project from the anterior hypothalamus to the NTS.[] Functional studies have shown that the baroreceptor reflex is a potent stimulus for VP secretion. It is well established that hemorrhage causes a release of VP from the neurohypophysis; bilateral carotid occlusion will do so as well. Moreover, recent electrophysiological studies have shown that single units in the PVN and SON alter their discharge rate in response to the level of activity of the baroreceptors with excitation of the baroreceptors producing a decrease in activity and carotid occlusion and hemorrhage, producing an increase in single unit activity.

The effect of VP or VP-containing neurons on baroreceptor function is less certain. Recently, Matsuguchi et al.[] have shown that micro injections of VP into the area of the nucleus tractus solitarius of rats produced significant dose related increases in blood pressure and heart rate suggesting that VP depresses the baroreflex. Ciriello and Calaresu[] have shown that stimulation of the PVN and SON produces an increase in heart rate and blood pressure and attenuates the bradycardia produced by stimulation of the carotid sinus reflex. With ablation of these nuclei, the magnitude of reflex bradycardia was found to be increased. These data suggest that VP-containing nuclei may exert tonic influence on at least, the cardiac component of the carotid sinus reflex. A hallmark sign of hypertension is a "resetting" or attenuation of the baroreceptor reflex.[] The findings that increased activity of the PVN and SON produces an attenuation of the response to carotid sinus nerve stimulation suggest that an alteration in the function of these nuclei and/or VP secretion may be responsible, in part, for resetting of the baroreflex in hypertension.

The stimulus for increased vasopressin secretion appears to be increased plasma osmolality, which occurs as a consequence of DOCA-induced increase in plasma sodium concentration.[] Recent evidence has suggested that the anteroventral region of the third ventricle (AV3V), an area involved in central actions of angiotensin II, changes in osmolality or sodium concentrations as well as arterial pressure regulation may have direct control over pituitary release of VP. Johnson et al.[] found that AV3V-lesioned rats 24 hours after the lesion did not show increased plasma VP levels in spite of the fact that they were adipsic or severely hypodipsic. Moreover, in chronic AV3V lesioned animals pressor response to central angiotensin II and hyperosmotic stimulants, thought to be due in large part to VP release, is markedly attenuated, suggesting that with ablation of the AV3V region rats cannot increment VP secretion in response to humoral stimuli. In addition to functional studies on the relation between the AV3V region and VP secretion, neuroanatomical connections between the AV3V region and the PVN and SON have been.
demonstrated. Of great importance is the fact that ablation of this region in rats prevents the development of DOCA hypertension. The data suggest that DOCA-induced increased plasma osmolality may stimulate the AV3V region which, in turn, stimulates increased release of vasopressin. The ability of the AV3V lesion to prevent the development of hypertension may be due in part to a failure of the lesioned rats to increase vasopressin secretion in response to increased plasma osmolality.

From our findings it is evident that vasopressin plays a primary role in DOCA hypertension and that the mechanism may involve a peripheral effect of inducing an increase in vascular reactivity to endogenous vasoconstrictor agents and/or a central neural effect involving an alteration in reflexes whose normal function is to buffer changes in arterial pressure.

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