Attenuation of Spontaneous Hypertension in Rats by a Vasopressin Antagonist

CELIA D. SLADEK, MARTHA L. BLAIR, CAROL STERLING, AND MICHAEL L. MANGIAPANE

SUMMARY Although abnormalities in the vasopressin system have been reported in spontaneously hypertensive rats (SHR), neither short-term nor long-term administration of the vasopressin antagonist d(CH2)3-Tyr(Me)arginine vasopressin (AVP), which selectively blocks the action of vasopressin on vascular (V1) receptors, altered the course of hypertension in SHR. In the current study, long-term administration of a different vasopressin antagonist, d(CH2)3-d-Tyr(Me)VAVP, to SHR and Wistar-Kyoto rats (WKY) from 4 to 12 weeks of age significantly attenuated the development of systolic hypertension in SHR (p < 0.05) without altering blood pressure in normotensive WKY. The antagonist was delivered subcutaneously by osmopump at 0.1 μg/hr. Systolic blood pressure was monitored twice weekly by tail plethysmography beginning at 5 weeks of age. In a second group of SHR, the drug infusion was continued until 18 weeks of age. In this group, the attenuation of systolic hypertension by the drug was extended and became more prominent (p < 0.007). Resting mean arterial pressure measured by indwelling catheters in the conscious state at 18 weeks of age was significantly reduced in the antagonist-treated SHR (144 ± 4 vs 157 ± 4 mm Hg; p < 0.05). Heart rate also was significantly reduced by the drug (351 ± 6 vs 392 ± 7 beats/min; p < 0.001). Following measurement of mean arterial pressure in the rats at 18 weeks of age, the osmopumps were removed and systolic blood pressure, mean arterial pressure, and heart rate were observed until 22 weeks of age. All of these parameters returned to the levels observed in untreated SHR within 2 weeks after drug withdrawal. Although this antagonist has both V1 and V2 (antidiuretic) antagonist properties, the infusion protocol used in this study resulted in antagonism of the pressor action of vasopressin but incomplete antagonism of the antidiuretic action of vasopressin. Thus, the mechanism responsible for the antihypertensive action of this antagonist is not clear, but the results suggest that long-term blockade of the actions of endogenous vasopressin does alter the course of hypertension in SHR. (Hypertension 12: 506-512, 1988)

KEY WORDS • vasopressin • spontaneously hypertensive rats • vasopressin antagonist • blood pressure • vascular resistance

PERIPHERAL resistance is elevated in spontaneously hypertensive rats (SHR),1, 2 and this elevation is thought to be central to the hypertensive process. Evidence of elevated plasma and urinary vasopressin (VP) in SHR,3, 4 and the findings that the VP response to a decrease in plasma volume in vivo5 or following exposure of the hypothalamo-neurohypophyseal system to acetylcholine and angiotensin II in vitro6, 7 is exaggerated in 5- and 8-week-old SHR, led to the hypothesis that the vasoconstrictor action of VP contributes to the elevated peripheral resistance and development of hypertension in SHR.

A role for VP in spontaneous hypertension was supported by early studies in which injection of a VP antiserum lowered blood pressure in stroke-prone SHR,8 but studies employing short-term administration3 or long-term infusion of a specific antagonist of the vascular effects of VP, d(CH2)3-Tyr(Me)arginine vasopressin (AVP),9 suggested that the vasoconstrictor action of VP was not an important component of the hypertension in SHR. Short-term administration of the antagonist to 11-week-old SHR resulted in a small decrease in mean arterial pressure (9 ± 1 mm Hg),3 but long-term infusion of the antagonist from 4 to 12 weeks of age at rates that blocked the pressor actions of VP did not alter the course of systolic hypertension.9 This vascular antagonist has weak antidiuretic agonist
properties (0.3 vs 323 U/mg for VP). Thus, the failure to obtain antihypertensive effects with this antagonist comparable to those reported with VP antiserum could have reflected the opposite action of the VP analogue and the VP antiserum on the renal action of VP. Therefore, experiments were performed to assess the effect on the development of hypertension in SHR of long-term infusion of a VP analogue, d(CH₃)₂-d-Tyr(Me)AVP, which blocks the action of VP at V₁ receptors (V₁) and the renal VP receptors (V₂). This analogue has similar potency as a V₁ antagonist to the selective V₁ antagonist used in the previous study (antipressor affinity constant [pA₂] = 8.62 and 8.48 for the V₁ and V₁-V₂ antagonists, respectively). Its potency at the V₁ receptor is approximately fivefold greater than its potency at the V₂ receptor (antiantidiuretic pA₂ = 7.77). Both of these VP analogues antagonize the action of oxytocin, but the V₁ antagonist is approximately twice as potent as the V₁-V₂ antagonist (antioxytocic pA₂ = 8.13 and 7.57 for the V₁ and V₁-V₂ antagonists, respectively). Thus, the V₁-V₂ antagonist was chosen for these studies because it is the most potent of the combined V₁-V₂ antagonists.

**Materials and Methods**

Male SHR and Wistar-Kyoto (WKY) rats were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA) at 4 weeks of age. Animals were housed in the vivarium and had free access to food (Purina Laboratory Chow pellets, St. Louis, MO, USA) and tap water except as described in Experiment 1. Osmopumps (Alzet Model 2002, Alza Pharmaceuticals, Palo Alto, CA, USA) were implanted subcutaneously at 4 weeks of age and were replaced at 2-week intervals while the rats were under ether anesthesia. Two separate experiments were performed.

**Experiment 1**

Thirteen rats of each strain were used. Seven rats of each strain received the VP antagonist d(CH₃)₂-d-Tyr(Me)AVP (provided by M. Manning, Medical College of Ohio, Toledo, OH, USA) by osmopump at 0.1 µg/hr (0.5 µl/hr) from 4 to 12 weeks of age. The remaining rats of each strain received vehicle (isotonic saline) until they were killed at 12 weeks of age. Systolic blood pressure was measured twice weekly in rats from 5 to 11 weeks of age by tail plethysmography (IITC, Landing, NJ, USA). Animals were acclimated to a 27 °C, noise-attenuating chamber, and systolic pressure was determined from the mean of five separate measurements. For statistical analysis the twice weekly values for systolic pressure were averaged to give a single weekly pressure determination.

The degree of antagonism of the antidiuretic action of VP by the antagonist was assessed by monitoring water intake and urine volume and osmolality in the rats at 6 and 8 weeks of age during ad libitum water intake and following 24 hours of dehydration at 11 weeks of age.

At 12 weeks of age, animals were anesthetized with chloral hydrate pentobarbital and surgically prepared with chronic indwelling catheters (ethyl vinyl acetate) located in the abdominal aorta by way of the femoral artery for measurement of mean arterial pressure (MAP) and in the vena cava by way of the femoral vein for drug infusion. The tip of the aortic catheter was located caudal to both renal arteries. After the operation all animals were given 100,000 units of procaine penicillin G and were housed individually. Following a 48-hour recovery period, resting MAP was determined in the conscious state. The arterial cannula was connected to a low-volume pressure transducer (Model CP-02, Century Technology, Inglewood, CA, USA), and blood pressure was recorded on a Beckman Dynograph R611 (Palo Alto, CA, USA). Arterial pressure was allowed to stabilize for 45 minutes before the resting value was determined. The animals were completely unrestrained.

After resting MAP was determined, the efficacy of the analogue blockade of the pressor effects of VP was evaluated by testing the effect on MAP of an intravenous injection of 25 ng AVP/kg body weight. The animals were decapitated the following day. Trunk blood was collected for determination of hematocrit, plasma osmolality (Pₒm₀), plasma sodium (Pₙa), serum renin activity (SRA), and serum VP (SVP) concentration as described previously. The hypothalamus and posterior pituitary were dissected, homogenized, and assayed for VP. Kidneys were homogenized and assayed for renal renin content. Unlike the d(CH₃)₂-Tyr(Me)AVP analogue, the antagonist used in these studies did not cross-react with the VP radioimmunoassay. Therefore, it was possible to measure tissue and plasma levels of endogenous VP in the antagonist-treated rats.

**Experiment 2**

The same experiment was repeated in a second group of SHR, but the length of antagonist infusion was extended to 18 weeks of age. In this experiment, seven SHR received the antagonist at the same rate used in Experiment 1 and seven SHR received saline by osmopump. Systolic blood pressure was monitored in these rats, as described for Experiment 1, from 5 to 17 weeks of age. At 18 weeks of age, arterial and venous catheters were implanted in rats as already described, and MAP and heart rate were monitored 48 hours later when the rats were fully conscious and unrestrained in their home cages. V₁ antagonism was assessed by evaluating the pressor response to a 15 ng/kg body weight injection of VP.

The osmopumps were removed at the end of 18 weeks, and systolic pressure was monitored twice weekly until 22 weeks of age. MAP and heart rate...
were measured weekly as long as the arterial catheter remained patent. At 22 weeks of age, the animals were decapitated and trunk blood was collected for measurement of hematocrit, P_{O_2}, P_{Na}, SRA, and SVP. Hypothalamic and posterior pituitary VP contents were determined as already described.

Statistical Analysis

Statistical analyses were performed using Student's t test, two-way analysis of variance (ANOVA) for unbalanced group size followed by analysis of simple main effects and the Newman-Keuls multiple mean comparison analysis, or by two-way ANOVA with repeated measures. Data are presented as means ± SEM.

Results

Experiment 1

The effect of long-term infusion of the VP antagonist on systolic blood pressure is shown in Figure 1. Blood pressure was not significantly altered by the antagonist in WKY from 5 to 11 weeks of age ($F_{1,11} = 0.022$), but blood pressure was significantly lower in the antagonist-treated SHR ($F_{1,11} = 4.83, p < 0.05$). Comparison of the treatment and control SHR groups by Newman-Keuls analysis indicated significant differences at 8 and 11 weeks of age ($p < 0.05$). ANOVA across strains at each week indicated that blood pressure was significantly greater in the antagonist-treated SHR compared with the saline-treated SHR ($F_{1,18} = 4.83, p = 0.002$).

The MAP for the VP antagonist-treated and saline-infused SHR and WKY at 12 weeks of age is shown in Table 1. These values were obtained while the animals were conscious and unrestrained by direct recording through chronically indwelling catheters. MAP was significantly lower in the WKY compared with the SHR ($F_{1,18} = 11.7, p = 0.002$). MAP tended to be lower in the antagonist-treated SHR compared with the saline-treated SHR, but it did not reach statistical significance.

The pressor response to a VP injection of 25 ng/kg also is shown in Table 1. This dose caused a significant pressor response in the saline-treated animals, but the peak change in MAP was significantly attenuated in both the antagonist-treated SHR and WKY ($F_{1,18} = 12.01, p = 0.003$). The duration of the pressor response to the VP injection was significantly longer in the SHR compared with the WKY ($F_{1,18} = 26.825, p < 0.001$), but it was significantly shorter in the antagonist-treated rats of both strains ($F_{1,18} = 18.34, p < 0.001$). This finding demonstrates that the VP analogue was actively antagonizing the action of VP at the vasculature.

Antagonism of the renal actions of VP by the VP analogue was evaluated by measuring water intake and urine volume and osmolality at 6 and 8 weeks of age while the animals had free access to water and at 11 weeks of age following a 24-hour dehydration. As shown in Table 2, the WKY strain drank significantly more water than did the SHR at both 6 and 8 weeks of age (6 weeks: $F_{1,17} = 4.99, p = 0.034$; 8 weeks: $F_{1,12} = 12.15, p = 0.002$). Urine volume also was significantly reduced in the SHR compared with the WKY at 12 weeks of age ($F_{1,18} = 4.83, p = 0.002$), and there was a significant treatment effect ($F_{1,12} = 5.9, p = 0.022$) and strain-treatment interaction ($F_{1,12} = 6.8, p = 0.015$) at 11 weeks of age. This finding indicates that the effect of the antagonist treatment was limited to the SHR and became more prominent by 11 weeks of age.

Heart rate was determined from the plethysmography recordings at 11 weeks of age. Heart rate was significantly lower in the WKY than in the SHR ($F_{1,22} = 23.03, p < 0.001$), but it was not significantly altered in either strain by the antagonist infusion (saline-treated SHR, 410 ± 8 beats/min; drug-treated SHR, 405 ± 10 beats/min; saline-treated WKY, 351 ± 12 beats/min; drug-treated WKY, 367 ± 9 beats/min).

Table 1. Effect of d(CH\textsubscript{2})\textsubscript{3}-D-Tyr(Me)VAVP on MAP and the Pressor Response to Exogenous Vasopressin at 12 Weeks of Age

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>142±3</td>
<td>129±11</td>
</tr>
<tr>
<td>BP response to VP</td>
<td>24±1</td>
<td>16±2</td>
</tr>
</tbody>
</table>

Values are means ± SEM. VAVP = valine AVP; BP = blood pressure; VP = vasopressin.

*p < 0.01, t* < 0.001, strain difference.

*p < 0.003 drug effect.

Asterisk indicates week mean difference in drug-treated group (p < 0.05). Number of rats is shown in parentheses. Cont = control.
was greater in the WKY (6 weeks: $F_{1,21} = 31.99, p < 0.001$; 8 weeks: $F_{1,22} = 33.125, p < 0.001$). The strain difference in water intake is accounted for by the differences in weight, but a significant strain effect persisted when urine output was expressed as a function of body weight (see Table 2), suggesting that extrarenal (e.g., respiratory or fecal, or both) water losses were greater in SHR than in WKY. Treatment with the antagonist caused a slight increase in urine volume ($p = 0.05$) and decrease in urine osmolality ($p = 0.08$) at 6 weeks of age, but this effect was no longer evident at 8 weeks of age. At 11 weeks of age, treatment with the antagonist did not compromise urine-concentrating capabilities during 24 hours of dehydration (see Table 2).

As shown in Table 3, at the end of the 12-week infusion period, $P_{\text{Na}}$ was comparable across strain and treatment groups, but $P_{\text{osm}}$ was higher in the SHR compared with WKY ($F_{1,20} = 40.12, p < 0.001$), and the VP antagonist treatment resulted in a significant elevation in $P_{\text{osm}}$ in both strains ($F_{1,20} = 9.6, p < 0.005$). Hematocrit was comparable across strains, but it was significantly lower in the antagonist-treated groups of both strains ($F_{1,19} = 15.76, p = 0.001$). There was no effect of the antagonist on either SVP or SRA (see Table 3), but SRA was suppressed in SHR ($F_{1,22} = 6.1, p = 0.023$).

Renal renin content and concentration were also suppressed in the SHR compared with WKY (content: $F_{1,19} = 24.04, p < 0.001$; concentration: $F_{1,22} = 14.04, p = 0.001$), but they were not altered by the antagonist treatment (see Table 3). Hypothalamic and posterior pituitary VP content were not altered by the antagonist treatment, but the content remained low in the hypothalamus and high in the posterior pituitary of SHR compared with WKY, as has been reported previously3-5 (see Table 3).

**Experiment 2**

Figure 2 shows the effect of infusion of the VP antagonist on systolic blood pressure from 4 to 18 weeks of age in a second group of SHR and the

### Table 2. Effect of d(CH2)3-d-Tyr(Me)VAVP on Water Balance

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHR</th>
<th>WKY</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline $V_1V_2$</td>
<td>Saline $V_1V_2$</td>
<td>Strain</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>81±3</td>
<td>84±2</td>
<td>125±5</td>
</tr>
<tr>
<td>Water intake (ml/24 hr)</td>
<td>14±4</td>
<td>15±4</td>
<td>22±6</td>
</tr>
<tr>
<td>Water intake (ml/24 hr/100 g body wt)</td>
<td>18±6</td>
<td>19±5</td>
<td>18±5</td>
</tr>
<tr>
<td>Urine output (ml/24 hr)</td>
<td>2±0.4</td>
<td>4±1</td>
<td>9±2</td>
</tr>
<tr>
<td>Urine output (ml/24 hr/100 g body wt)</td>
<td>5±1</td>
<td>7±1</td>
<td>10±1</td>
</tr>
<tr>
<td>Urine osmolality (mosm/kg H2O)</td>
<td>1825±110</td>
<td>1444±80</td>
<td>1364±432</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Strain and Treat refer to overall strain and treatment effects. No strain-treatment interactions were significant. VAVP = valine AVP.

### Table 3. Effect of d(CH2)3-d-Tyr(Me)VAVP on Blood Parameters and Renal Renin Content at 12 Weeks of Age

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHR</th>
<th>WKY</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline $V_1V_2$</td>
<td>Saline $V_1V_2$</td>
<td>Strain</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42±2</td>
<td>39±1</td>
<td>44±4</td>
</tr>
<tr>
<td>Plasma osmolality (mosm/kg H2O)</td>
<td>299±3</td>
<td>304±3*</td>
<td>294±2</td>
</tr>
<tr>
<td>Plasma Na (mEq/L)</td>
<td>146±2</td>
<td>145±3</td>
<td>144±5</td>
</tr>
<tr>
<td>Serum VP (pg/ml)</td>
<td>6.9±3</td>
<td>8.4±0.4</td>
<td>6.9±2</td>
</tr>
<tr>
<td>Serum renin activity (ng Ang I/100 ml/h)</td>
<td>3.1±1.6</td>
<td>2.13±1.5</td>
<td>4.9±1.5</td>
</tr>
<tr>
<td>Renal renin content (pg Ang I/100 ml/h)</td>
<td>72.8±3.4</td>
<td>64.5±3.6</td>
<td>106.0±6.5</td>
</tr>
<tr>
<td>Renal renin concentration (ng Ang I/h/kg kidney)</td>
<td>77.7±2.6</td>
<td>68.3±3.6</td>
<td>100.8±6.4</td>
</tr>
<tr>
<td>Hypothalamic VP content (ng/homogenate)</td>
<td>48.8±4.3</td>
<td>55.3±14.9</td>
<td>86.5±16.9</td>
</tr>
<tr>
<td>Posterior pituitary VP content (pg/mg pituitary)</td>
<td>1.25±0.28</td>
<td>1.25±0.22</td>
<td>0.82±0.12</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Strain and Treat refer to overall strain and treatment effects. No strain-treatment interactions were significant. VAVP = valine AVP; Ang I=angiotensin I; VP=vasopressin.

*p < 0.005, treatment effect within strain.
FIGURE 2. Effect of long-term infusion of the VP antagonist d(CH_{2})_{3}-D-Tyr(Me)VAVP on systolic blood pressure (BP) in SHR from 4 to 18 weeks of age and the effect of terminating the drug infusion on systolic blood pressure. The antagonist significantly reduced blood pressure in SHR from 8 to 18 weeks of age. The effect lasted for at least a week after the infusion ended, but it was ultimately reversible, with systolic pressure rising to the level observed in untreated SHR within 2 weeks after drug removal. D = drug; C = control.

Table 4. Effect of d(CH_{2})_{3}-D-Tyr(Me)VAVP on MAP, Heart Rate, and the Pressor Response to Vasopressin in Experiment 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>18 weeks</th>
<th>19 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>V_{2}V_{1}</td>
<td>Saline</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>157±4</td>
<td>144±4*</td>
<td>179±4</td>
<td>162±5*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>392±7</td>
<td>351±6†</td>
<td>424±11</td>
</tr>
<tr>
<td>BP response to VP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak change (mm Hg)</td>
<td>37±4</td>
<td>29±4†</td>
<td>40±2</td>
</tr>
<tr>
<td>Duration (min)</td>
<td>8±1</td>
<td>3±0.5‡</td>
<td>5±1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. VAVP = valine AVP; BP = blood pressure; VP = vasopressin.

* p < 0.05, † p < 0.001, drug effect.

Discussion

The VP antagonist d(CH_{2})_{3}-D-Tyr(Me)VAVP significantly attenuated the development of hypertension in SHR, but it had no effect on blood pressure in WKY up to 12 weeks of age. The attenuation of hypertension was demonstrated in two separate experiments and became progressively more prominent with age. In SHR at 18 weeks of age, the antagonist treatment resulted in a 21 mm Hg reduction in systolic pressure. Although this reduction was not sufficient to return pressure to the level observed by this laboratory in 18-week-old WKY (142 ± 1 mm Hg; n = 82), it represents attenuation of 40% of the systolic hypertension. The antagonist treatment caused a significant decrease in MAP of 13 (18 weeks) to 17 mm Hg (19 weeks; see Table 4). The lack of a statistically significant decrease in MAP in the antagonist-treated SHR at 12 weeks of age in spite of a significant decrease in SHR compared with the saline-infused group at 18 weeks of age (p < 0.025). It remained lower in this group at 19 weeks of age, 1 week after drug withdrawal (p < 0.05), but it rose to levels comparable to those in the untreated group by 20 weeks of age.

Heart rate was significantly reduced in the antagonist-treated rats at 18 weeks of age (p < 0.001; see Table 4). It remained lower at 19 weeks of age (p < 0.05), but by 20 weeks of age it had increased to the rate observed in the untreated group (see Table 4).

As shown in Table 4, the antagonist treatment significantly attenuated both the peak pressor response and the duration of the pressor response to a VP injection of 15 ng/kg. This effect was apparent at 18 weeks of age (p < 0.001), but it was no longer apparent at 19 weeks of age. Thus, antagonism of the pressor response to exogenous VP disappeared more rapidly upon withdrawal of the VP antagonist than did the effect of the antagonist on systolic pressure, MAP, and heart rate.

The animals were killed at 22 weeks of age, 4 weeks after the antagonist infusion had been stopped. At this time, there were no significant differences in any of the following parameters (data not shown): hematocrit, P_{o_{2}}, P_{N_{2}}, SVP, SRA, hypothalamic and posterior pituitary VP content, and renal renin content and concentration.
systolic pressure at 11 weeks of age primarily reflects the group size and the variance in that group. Thus, these data suggest that endogenous VP contributes to the hypertension in SHR.

The effect of the antagonist on blood pressure was reversible. This finding supports the conclusion that the attenuation of hypertension in the antagonist-treated SHR was a result of antagonist infusion and suggests that endogenous VP participates in the hypertensive process for as long as 19 weeks of age in SHR.

The specific mechanism responsible for attenuation of hypertension by the antagonist remains to be elucidated. The d(CH$_2$)$_3$-d-Tyr(Me)AVP analogue has antagonist actions at both V$_1$ and V$_2$ receptors, but its affinity for the V$_2$ receptor is much less than its affinity for the V$_1$ receptor. In these experiments, the pressure response to an injection of exogenous VP was markedly attenuated. The amount of VP injected was selected from our previous studies as a dose that would be attenuated, but not completely blocked, by the antagonist infusion. Since serum VP concentration was not markedly elevated in the antagonist-treated group at 12 weeks of age, the rate of infusion of the antagonist was probably sufficient to block the action of endogenous VP at vascular V$_1$ receptors. However, our previous failure to observe attenuation of hypertension in SHR with long-term infusion of a specific V$_1$ VP antagonist, d(CH$_2$)$_3$-Tyr(Et)AVP, suggests that the efficacy of the antagonist used in the current experiments is not solely related to its antagonistic action at vascular V$_1$ receptors. This hypothesis is supported by the observation that, upon withdrawal of the antagonist, its antihypertensive action appeared to outlast its antagonistic action of the vascular V$_1$ receptors. Nevertheless, a contribution of V$_2$ antagonism to the antihypertensive action of the V$_1$-V$_2$ antagonist cannot be excluded, because offsetting V$_2$ agonist activity may have contributed to the absence of an antihypertensive action of the V$_1$ antagonist in our previous studies, and the antagonist treatment was only carried to 12 weeks of age in that study.

Another possible mechanism of the antihypertensive action of the V$_1$-V$_2$ antagonist is partial blockade of the renal VP receptors. A small decrease in blood volume as a result of partial renal V$_2$ antagonism may directly decrease blood pressure by decreasing stroke volume. This is a reasonable possibility since the analogue used has V$_2$ antagonist properties. Partial V$_2$ blockade is an attractive explanation for the antihypertensive action of the antagonist because the V$_2$ antagonist activity of this analogue is the obvious difference between it and the d(CH$_2$)$_3$-Tyr(Me)AVP analogue, which was an ineffective antihypertensive agent in the SHR. The measurements of water balance that we performed clearly indicated that the renal V$_2$ receptors were not completely blocked by the antagonist infusion, but the possibility of partial blockade of the antidiuretic action of VP at the infusion rate employed cannot be completely ruled out. Urine volume was increased and urine osmolality was slightly decreased in the antagonist-treated rats at 6 weeks of age. In addition, P$_{\text{osm}}$ was slightly elevated in the antagonist-treated rats at 12 weeks of age. These observations are consistent with partial antagonism of the renal actions of VP. However, the ability to concentrate urine during 24 hours of dehydration was unaffected by 7 weeks of antagonist infusion, and there was no effect of the analogue infusion on water intake and body weight.

Another problem with predicting what the renal actions of this antagonist might have been in these experiments is that another very similar VP analogue, which acts acutely as a V$_1$,V$_2$ antagonist in vivo, has V$_1$ agonist properties when infused chronically. This analogue, d(CH$_2$)$_3$-d-Tyr(Et)AVP, differs only in the size of the alkyl substituent on the d-Tyr from the one employed in our study. When infused at a rate of 72 µg/day for 7 days in Sprague-Dawley rats, the d-Tyr(Et) antagonist caused only a transient increase in water intake and excretion, which subsequently reverted to normal. In Brattleboro rats, this infusion protocol resulted in a marked decrease in water intake and urine output. Thus, during long-term infusion at high rates, the analogue behaved as an antidiuretic agonist.

Since the infusion rate used in our experiments was considerably lower and since the antagonist used was slightly different, it is unclear whether antidiuretic agonist activity could be expected. We found no evidence of increased urinary concentrating capacity of the antagonist-treated rats. Thus, the VP antagonist treatment used in this study did not appear to induce either antidiuresis or diuresis, but further analysis of the renal effects of this antagonist infusion protocol is warranted.

The significant decrease in heart rate in the antagonist-treated rats is also of interest relative to the mechanism of attenuation of the hypertension. Heart rate in untreated SHR was elevated relative to that in WKY at 12 weeks of age in this study as well as in our previous study. Thus, the ability of the antagonist to reduce heart rate could be an important component of its antihypertensive action. VP infused into the lateral cerebral ventricles or microinjected into the locus ceruleus or the nucleus tractus solitarii causes an increase in blood pressure and heart rate in rats. Thus, it is possible that the VP antagonist blocked this central action of VP, resulting in a decrease in heart rate and cardiac output.

The central effects of VP on blood pressure and heart rate are blocked by peripheral administration of phentolamine and propranolol, and therefore appear to be mediated by stimulation of sympathetic outflow. This effect has been confirmed in studies demonstrating that ventricular injections of VP cause an increase in splanchnic and renal sym-
Pathetic nerve activity. Therefore, a possible mechanism responsible for the antihypertensive action of the antagonist is a reduction in sympathetic outflow secondary to blockade of centrally released VP. This mechanism would serve to decrease peripheral resistance as well as heart rate.

A central site of action of the V₁-V₂ antagonist is difficult to reconcile with the differential efficacy of this antagonist and the V₁ antagonist used in our earlier studies because the central actions of VP on blood pressure, heart rate, and sympathetic activity are blocked by the V₁ antagonist. Thus, both antagonists should have been effective in blocking these actions of central VP. However, differential permeability of the blood-brain barrier to the two antagonists could render one (the V₁-V₂ antagonist) more effective than the other in reducing hypertension when infused peripherally and therefore account for the difference in their efficacy as antihypertensive agents. This possibility deserves further study.

The decrease in arterial pressure induced by the V₁-V₂ antagonist appears to be independent of changes in the renin-angiotensin system. Renin secretion is stimulated by reductions in arterial pressure and inhibited by V₁ VP receptor stimulation. Thus, administration of the VP antagonist might result in increased renin release, which would serve to raise arterial pressure and thereby mask the antihypertensive effects of VP blockade. In the present experiments, however, we found no effect of the antagonist on either SRA or renal renin content after 8 weeks of antagonist administration (12 weeks of age) in either SHR or WKY. The lower renal renin content in SHR as compared with WKY is consistent with our earlier observations of suppressed renal renin content in 18-week-old SHR.

In conclusion, these studies suggest a role for VP in the development and maintenance of hypertension in SHR. The ability of a VP antagonist to selectively lower blood pressure in hypertensive, but not normotensive, rats up to 12 weeks of age suggests that it acts on a component of the hypertensive process. The ability of an analogue with both V₁ and V₂ antagonist properties to attenuate the development of hypertension in SHR in a reversible manner contrasts with the ineffectiveness of a VP analogue that selectively antagonizes the action of VP at V₁ receptors. The differential effectiveness of these two analogues suggests that multiple actions of VP cooperate in its contribution to the hypertension, but further evaluation of the mechanisms underlying the antihypertensive action of the antagonist is required.

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