Evidence Against a Pressor Role for Vasopressin in Spontaneous Hypertension

Celia D. Sladek, Martha L. Blair, and Michael Mangiapane

SUMMARY The hypothesis that the vasoconstrictor action of vasopressin may contribute to the development of hypertension in spontaneously hypertensive rats (SHR) was tested by chronic infusion of a specific antagonist of the vascular effects of vasopressin. From 4 to 13 weeks of age, SHR and Wistar-Kyoto rats (WKY) received subcutaneously either isotonic saline or the vasopressin pressor antagonist, d(CH2)5Tyr(Me)arginine vasopressin by osmopump. Systolic blood pressure was measured by tail cuff from 5 to 11 weeks of age. In SHR, the vasopressin analogue did not alter the rate or magnitude of increase in systolic blood pressure. In WKY, systolic blood pressure in the vasopressin analogue group was slightly reduced compared with the saline infusion values until 10 weeks of age (F10 = 10.18, p = 0.008). At 12 to 14 weeks of age, all animals were prepared with indwelling arterial and venous catheters. Resting mean arterial pressure was not altered significantly by the vasopressin analogue infusion in either strain, but the response to an acute vasopressin infusion of 5, 15, or 50 ng/kg body weight was markedly attenuated by the analogue treatment, indicating that plasma levels of the vasopressin analogue were sufficient to block pressor effects of endogenous vasopressin. A bolus injection of the angiotensin II converting enzyme inhibitor teprotide (SQ 20881) resulted in a decrease in mean arterial pressure (p< 0.05) that was comparable in all groups, and serum renin concentration was not elevated in the vasopressin analogue-treated rat. Thus, chronic blockade of the pressor effects of endogenous vasopressin does not alter the course of hypertension in SHR after 4 weeks of age, and there was no evidence for compensation by the renin-angiotensin system.

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KEY WORDS • vasopressin • spontaneously hypertensive rats • hypertension • vasopressin antagonist • teprotide

ABNORMALITIES in the circulating levels, urinary excretion rate, and secretory response of vasopressin (VP) have been observed in spontaneously hypertensive rats (SHR) of the Okamoto strain. Specifically, small but significant elevations in plasma VP were reported in SHR from 5 through 10 weeks of age,1,2 and urinary excretion of VP is significantly greater in SHR compared with age-matched normotensive Wistar-Kyoto rats (WKY).1 In addition, hypothalamic VP content is reduced and posterior pituitary VP content is elevated in SHR relative to WKY.1,2 and at 5 and 8 weeks of age VP release is exaggerated in SHR relative to WKY in response to a decrease in plasma volume in vivo3 or following exposure of the hypothalamoneurohypophyseal system to acetylcholine and angiotensin II in vitro.4,5 Thus, increased secretion of VP in response to selected stimuli may underlie the elevation of plasma and urinary VP in SHR.

Peripheral resistance is elevated in SHR,6,7 and this elevation is thought to be central to the hypertensive process. Although increased activity of the sympathetic nervous system in SHR4 represents one mechanism contributing to the elevated peripheral resistance, VP may also contribute to the elevation of peripheral resistance in this model of hypertension. In addition to the elevations in circulating VP already mentioned, the pressor potency of VP is greater in SHR than in WKY.9,10 The pressor potency of VP in SHR appears to stem from impairment of the baroreceptor reflexes that buffer against the pressor effects of VP in WKY.10,11 These observations, in conjunction with the
hyperresponsiveness of VP release, suggest that the pressor action of VP may contribute to the elevation of blood pressure in the SHR and, therefore, to the development of hypertension. The present study tested this hypothesis by evaluating the effect on blood pressure of chronic infusion of a specific VP pressor antagonist in SHR and WKY from 4 to 13 weeks of age.

**Materials and Methods**

Male SHR and WKY were obtained from Charles River Laboratories (Wilmington, MA, USA) at 4 weeks of age. Osmopumps (Alzet, Model 2002; Alza Pharmaceuticals, Palo Alto, CA, USA) were implanted subcutaneously with the rats under ether anesthesia. Half of the animals of each strain were infused with the VP pressor antagonist d(CH₂)₅Tyr(Me)arginine vasopressin (AVP) (Bachem, Torrence, CA, USA) at 0.1 μg/hr (0.5 μl/hr), and the other half were infused with vehicle (isotonic saline) until they were killed at 12 to 14 weeks of age. The osmopumps were replaced every 2 weeks with the rats under ether anesthesia. Animals were housed in the vivarium. Purina Laboratory Chow pellets (St. Louis, MO, USA) and tap water were available ad libitum.

Systolic blood pressure was measured twice weekly from 5 to 11 weeks of age by tail plethysmography (ITTC, 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 biweekly values for systolic pressure were averaged to give a single weekly pressure determination.

At 12 weeks of age, animals were anesthetized with chloralhydrate 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 and heart rate and in the vena cava by way of the femoral vein for drug infusion. After the operation all animals were given 100,000 U of procaine penicillin and were housed individually. Following a 48-hour recovery period, resting mean arterial pressure and heart rate were 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). Heart rate was monitored with a cardiotachometer. Arterial pressure was allowed to stabilize for 45 minutes before the resting value was determined. The animals were completely unrestrained.

After resting mean arterial pressure and heart rate were determined, the efficacy of the analogue blockade of the pressor effects of VP was evaluated by testing the effect on blood pressure and heart rate of intravenous injections of VP. Bolus injections of isotonic saline or AVP, 5, 15, or 50 ng/kg body weight, were given sequentially. Blood pressure was allowed to return to baseline between injections. Twenty minutes after the final VP injection, a bolus injection of the converting enzyme inhibitor teprotide (1.5 mg; Bachem) was given to assess the effect of the VP analogue treatment on the activity of the renin-angiotensin system. Blood pressure was monitored for 20 minutes after the injection, and then an injection of angiotensin I (ANG I), 50 ng/kg body weight, was administered to evaluate the efficacy of the converting enzyme inhibition achieved by the teprotide injection. The protocol could not be completed in several rats because of blocked or severed cannulas.

The following day, 0.5-ml blood samples were drawn from the arterial catheter for renin determination. Serum renin activity was determined by radioimmunoassay for ANG I as described previously.³

Statistical analyses were performed using either two-way analysis of variance (ANOVA) for unbalanced group sizes followed by analysis of simple main effects and the Newman-Keuls multiple mean comparison analysis or by two-way ANOVA with repeated measures.¹² Data are expressed as means ± SEM.

**Results**

**Systolic Blood Pressure**

As shown in Figure 1, chronic infusion of the VP pressor antagonist did not alter the course of development of hypertension in SHR. Systolic blood pressure increased progressively from 5 to 11 weeks of age in both the antagonist-treated and the saline-treated SHR (F = 183, p < 0.0001), but there was no significant drug effect (F = 1.71). In the WKY, blood pressure also increased during the experimental period (F = 25.59, p < 0.001), but to a lesser extent than in the SHR. There was a significant drug effect in the WKY (F = 10.75, p = 0.008), with drug-treated animals having lower blood pressure than the controls.
Table 1. Effect of d(CH₂)₅Tyr(Me)arginine Vasopressin on Mean Arterial Pressure and Heart Rate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood pressure (mm Hg)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Saline</td>
<td>125 ± 3</td>
<td>105 ± 4*</td>
</tr>
<tr>
<td>Tyr(Me) AVP</td>
<td>142 ± 8</td>
<td>367 ± 13</td>
</tr>
<tr>
<td></td>
<td>Heart rate (beats/min)</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>356 ± 6</td>
<td></td>
</tr>
<tr>
<td>Tyr(Me) Saline</td>
<td>367 ± 13</td>
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</tr>
</tbody>
</table>

Values are means ± SEM, AVP = arginine vasopressin. *F strain = 28.85, p < 0.001; †F strain = 8.07, p = 0.012, compared with values in SHR.

from 5 through 9 weeks of age. However, blood pressure was not determined before antagonist infusion. Blood pressure was statistically compared in the two strains by two-way ANOVA of the data obtained at 5, 8, and 11 weeks of age. Blood pressure was significantly higher in SHR compared with WKY at all three ages (5 weeks, p = 0.002; 8 weeks, p < 0.001; 11 weeks, p < 0.001). A significant drug effect was not evident in these analyses, but there was a significant interaction effect at 8 weeks (p = 0.009).

Mean Arterial Pressure

Table 1 presents the mean arterial pressure and heart rate values for the VP antagonist-treated and saline-infused SHR and WKY at 12 weeks of age. These values were obtained while the animals were conscious and unrestrained by direct recording through chronically indwelling catheters. These measurements confirm the observations made by indirect measurement of systolic blood pressure. Mean arterial pressure was significantly elevated in the SHR compared with the WKY (F = 28.81, p < 0.001), but was not significantly altered by the VP antagonist (F = 2.46). Heart rate also was elevated in the SHR (F = 8.07, p = 0.012), but it was not different in the animals receiving the pressor antagonist (F = 0.49).

Efficacy of Vasopressin Blockade

The peak change in mean arterial pressure caused by the injection of 0.2 ml of saline or VP, 5, 15, or 50 ng/kg body weight, is shown in Figure 2A, and the time required for mean arterial pressure to return to the preinjection level is shown in Figure 2B. The saline injection caused a slight increase in pressure, which rapidly returned to baseline. This response was not statistically different between strains and was not altered by chronic exposure to the VP antagonist. In the chronic saline-infused WKY and SHR, the VP injections resulted in increases in pressure that were dose-dependent in terms of both the maximal change in pressure and the time to return to baseline; however, the response in the SHR was significantly greater than that in the WKY (F = 5.99, p = 0.02).

Chronic exposure to the VP antagonist markedly

![Figure 2. The effect of intravenous injections of saline or vasopressin (VP) on mean arterial blood pressure in SHR and WKY following chronic infusion of either the VP antagonist d(CH₂)₅Tyr(Me)arginine vasopressin (AVP) or isotonic saline. A. Peak change in blood pressure as a function of the VP dose. The peak change in blood pressure occurred 30 to 60 seconds after the injection and was markedly attenuated in the antagonist-treated rats of both strains, but the SHR were more sensitive to the pressor effects of VP than the WKY. B. Duration of the pressor response was markedly attenuated by the antagonist infusion. The number in each group is shown in Table 1.](http://hyper.ahajournals.org/Download)
attenuated the pressure response to exogenous VP in both SHR and WKY (see Figure 2). The absolute magnitude of the blood pressure response to the highest dose of VP was significantly attenuated in the analogue-treated SHR relative to the response in untreated SHR (F = 62.10, p = 0.0002). In addition, treatment with the antagonist significantly reduced the length of the response in both the SHR (F = 31.51, p < 0.001) and the WKY (F = 9.62, p = 0.005) relative to values in their saline controls (see Figure 2B). The untreated SHR showed a more prolonged pressure response than the untreated WKY (F = 5.99, p = 0.02), but there was no difference in the time required for blood pressure to return to baseline in the two strains following the treatment with the antagonist (F = 0.01).

Data on the change in heart rate following the VP injections (5 and 50 ng/kg) were obtained in some of the animals and are shown in Figure 3. The 10-ng dose of VP resulted in significant decreases in heart rate in both strains of rats (p < 0.01), and the decrease in heart rate was not altered significantly by the antagonist treatment in either strain. In the untreated SHR, heart rate also was significantly decreased 1 minute after the 5-ng/kg injection of VP (p < 0.05). This response was not observed in the WKY nor in the antagonist-treated SHR. The difference in the heart rate response to the 5-ng/kg injection of VP in the antagonist-treated and untreated SHR was statistically significant (F = 13.54, p = 0.01).

Response to Teprotide

The effect of converting enzyme inhibition on mean arterial pressure was evaluated in the antagonist-treated and untreated SHR and WKY by giving a bolus intravenous injection of teprotide (1.5 mg/0.25 ml). As shown in Figure 4, the blood pressure response to the bolus injection of teprotide was characterized by an immediate biphasic decrease and subsequent increase in blood pressure that was followed by a slower and prolonged decrease in pressure. Mean arterial pressure remained below baseline up to 20 minutes after the injection. The blood pressure response to teprotide was similar in SHR and WKY, and it was not altered by chronic infusion of the VP antagonist. The efficacy of the converting enzyme inhibition was evaluated by giving ANG I, 50 ng/kg body weight, intravenously between 21 and 25 minutes after the teprotide injection. This injection of ANG I caused an increase in mean arterial pressure of 20 mm Hg in the absence of teprotide and no change in mean arterial pressure in the presence of teprotide.

Serum Renin Activity

Resting serum renin activity was not elevated by chronic infusion of the VP antagonist. In the WKY,
serum renin activity was 3.02 ± 0.84 and 4.92 ± 1.38 ng ANG I/ml/hr in the saline-treated (n = 6) and VP antagonist-infused (n = 5) animals, respectively. In SHR, serum renin activity was 3.06 ± 1.35 ng ANG I/ml/hr (n = 4) in the antagonist-infused group. Although serum renin data were not obtained for the saline-infused SHR, basal renin activity has not differed from that of age-matched WKY in previous studies from this laboratory.3

Discussion

A role for VP in the development of hypertension in SHR was suggested by previous observations that urinary VP excretion1 and VP release in response to physiological stimuli1-5 were elevated in these rats during the development of hypertension. Since VP has been shown to be a more potent vasoconstrictor agent in SHR than in WKY,9,10 the hypothesis that the vasoconstrictor actions of VP contributed to the development of hypertension was evaluated in the present experiments by chronically infusing an analogue of VP, d(CH2)5Tyr(Me)AVP, which specifically blocks the action of VP on vascular VP receptors.14 The VP antagonist was infused at a rate that resulted in plasma VP levels of 60 pg/ml in previous experiments involving VP infusions in Brattleboro rats.15 This dose of antagonist blocked the blood pressure response to all but the largest dose of exogenous VP administered. Despite markedly attenuating the vasoconstrictor actions of VP, the antagonist did not alter the development of hypertension in the SHR. Thus, these studies demonstrate that the vasoconstrictor action of VP is not required for the development of hypertension in SHR after the 4th week of age.

In spite of this evidence that the hypertension in SHR is independent of the vasoconstrictor actions of VP, VP might still play a role in the hypertensive process by acting on another type of VP receptor. At least two types of VP receptors termed the V1 and V2 receptors, are known to be present in the periphery. The vascular receptors are the prototype of V1 receptors, and the renal receptors are the prototype of V2 receptors.16 The relative specificity of VP agonists and antagonists for these two receptor types has been determined based on their relative ability to mimic or block the vasoconstrictor or antiuretic effects of VP.14 Thus, the analogue used in this study is a potent antagonist of the vasoconstrictor actions of VP but is relatively ineffective as either an agonist or antagonist of VP in the kidney. Information about its action on VP receptors in other organs is incomplete. Thus, it is possible that the antiuretic action of VP contributes to the hypertension in SHR or that the action of VP at some other site that is not blocked by the V1 antagonist is critical in the hypertensive process.

The hypothesis that VP may contribute to hypertension through mechanisms that are not blocked by the V1 antagonist is suggested by findings in other studies. Vascular antagonists of VP have proved ineffective in reducing blood pressure in several models of hypertension in which elevated plasma VP was suspected to be an underlying or contributing factor in the hypertensive process. Specifically, in the malignant phase of deoxycorticosterone acetate-salt and two-kidney, one clip Goldblatt hypertension, an acute infusion of V1 receptor antagonists did not cause a significant decrease in blood pressure,17-19 but a decrease in blood pressure was observed following an acute injection of a specific VP antiserum in both of these models of hypertension.20,21 The discrepancy in the effects of the vascular VP antagonist and the VP antiserum have been attributed to nonspecific effects of the antiserum, such as anaphylactic reactions.17-19 Another possible cause of the discrepant results is the difference in specificity of the blockers used. Presumably, the VP antiserum blocked the action of VP at all sites available to plasma VP, whereas the VP antagonist was effective at only one class of VP receptors. Thus, while the experiments with the V1 antagonist eliminate the requirement for the pressor effects of VP in the development of hypertension in SHR, malignant deoxycorticosterone acetate-salt hypertension, and malignant two-kidney, one clip Goldblatt hypertension, they do not eliminate the possibility that VP contributes to the hypertension through receptor mechanisms other than the V1 subtype.

In addition to its vascular effects, other actions of VP have been described that could alter cardiovascular function. These include its antiuretic effects, its effects on secretion of renin and atrial natriuretic factor, and its effects on the cardiovascular centers in the central nervous system. The central actions of VP include its ability to increase heart rate and blood pressure when given centrally22-24 and its ability to modulate the sensitivity of the baroreceptor reflex when given peripherally.25,26 The V1 receptor is involved in the effects of VP on secretion of renin and atrial natriuretic factor as well as the central action to increase heart rate.22,28-34 Thus, any role for these actions of VP in modulating the hypertensive process in SHR should have been blocked in the antagonist-infused animals in the current study. Specifically, renin secretion is inhibited by VP,28 but this effect is reversed in the presence of d(CH2)5Tyr(Me)AVP.29 In the current study, blockade of VP inhibition of renin release by the V1 antagonist could have contributed to maintaining the hypertensive process in the SHR, but serum renin activity was not elevated and the blood pressure response to converting enzyme inhibition (loteplide) was not altered by the VP antagonist treatment. Thus, no evidence was obtained for an increased role of the renin-angiotensin system in supporting blood pressure in the antagonist-treated SHR. VP stimulates secretion of atrial natriuretic factor, and this effect is blocked by d(CH2)5Tyr(Me)AVP.30 Atrial natriuretic factor is a potent depressor agent in SHR,35-38 and the plasma concentration of atrial natriuretic factor increases dramatically in SHR as the hypertension develops.39 Thus, it is possible that the chronic infusion of d(CH2)5Tyr(Me)AVP attenuated the secretion of atrial
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natriuretic factor as well as blocked the vasoconstrictor actions of VP and, therefore, would simultaneously have removed offsetting pressor and depressor influences, resulting in no net change in the hypertensive process. Finally, the ability of VP to increase heart rate and blood pressure when injected either into the cerebral ventricles or into the nucleus of the tractus solitarius or the locus ceruleus is blocked by central administration of d(CH₂)₃Tyr(Me)AVP²², 31-35 but not by acute intravenous administration.⁴⁰ Thus, it is possible that this site of action of VP was not blocked in the current study, although under conditions of chronic infusion, the V₁ antagonist may gain access to the central nervous system.

V₂ receptors have been implicated in the ability of VP to increase the sensitivity of the baroreceptor reflex to elevations in blood pressure.⁴¹ Thus, in the antagonist-infused rats of this study, this depressor action of VP would have remained operational. This effect may have contributed to the blood pressure-lowering effects of the antagonist in the WKY, but others¹⁰, ¹¹ have shown that this action of VP is markedly attenuated in SHR; therefore, a blood pressure-lowering effect similar to that observed in WKY would not be achieved in SHR by selectively blocking the pressor, but not one of the depressor, actions of VP with d(CH₂)₃Tyr(Me)AVP. The loss or attenuation of the VP sensitization of the baroreceptor reflex in SHR is thought to underlie the greater pressor sensitivity of SHR to VP observed in this and previous studies.⁹, ¹⁰

In conclusion, this study demonstrates that, after 4 weeks of age, the hypertensive process in SHR is not dependent on the vasoconstrictor action of VP, but the possibility remains that VM may play a role in the hypertensive process that is mediated by V₂ receptors or that involves V₂ receptors that are not blocked by the peripheral administration of d(CH₂)₃Tyr(Me)AVP. The development of a strain of SHR with diabetes insipidus does not eliminate these possibilities, because the abnormalities in the VP system in the stroke-prone strain of SHR used in the cross-breeding experiments are different from the abnormalities in the non-stroke-prone strain used in the present study. In the stroke-prone strain of SHR, VP does not contribute to the development of hypertension, but it may participate in maintaining pressure during the chronic phase.⁴³-⁴⁵

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