Evidence Against a Role of Vasopressin in the Maintenance of High Blood Pressure in Mineralocorticoid and Renovascular Hypertension

SARA F. RABITO, M.D., OSCAR A. CARRETERO, M.D., AND ALFONSO G. SCICLI, PH.D.

SUMMARY To determine the role of vasopressin in the maintenance of high blood pressure, the antihypertensive effect of the antagonists of the vasopressor effect of vasopressin, [1-deaminopenicillamine, 4-valine, 8-D-arginine] vasopressin (dPVDAVP), and [1-(β-mercapto-β, 8-cyclopentamethylene propionic acid), 4-valine, 8-D-arginine] vasopressin (cyclo dVDAVP), was studied in unanesthetized, nonsurgically stressed rats with adrenal regeneration hypertension, malignant DOCA-salt hypertension, and malignant two-kidney, one clip Goldblatt hypertension. The doses of vasopressin antagonist used blocked the blood pressure (BP) response to vasopressin almost completely, with no changes in the pressor response to norepinephrine and angiotensin II. Administration of the vasopressin antagonists did not induce significant changes in the mean BP in any of the three experimental groups studied. It is suggested that in unanesthetized, nonsurgically stressed rats with adrenal regeneration hypertension, malignant DOCA-salt hypertension, and malignant two-kidney, one clip Goldblatt hypertension, vasopressin does not have a role in the maintenance of high BP.

KEY WORDS • salt loading • vasopressin • Goldblatt rat • adrenal regeneration hypertension • malignant hypertension • mineralocorticoid activity

RECENT evidence seems to indicate that vasopressin may be important in the pathogenesis of certain forms of hypertension. The participation of vasopressin in human hypertension has been suggested by Khokar and Slater who reported that the levels of vasopressin in urine were elevated in patients with essential hypertension, and by Padfield et al. who found that patients with malignant hypertension had higher levels of vasopressin in plasma when compared to essential hypertensive or normotensive subjects. High levels of vasopressin have also been reported in spontaneously hypertensive rats, in two-kidney, one clip Goldblatt hypertensive rats, and deoxycorticosterone acetate (DOCA)-salt hypertensive rats. More direct evidence for a role of vasopressin as a pathogenetic factor in hypertension was found by Möhring et al. who reported that a specific antibody against vasopressin decreased the blood pressure (BP) in DOCA-salt and two-kidney, one clip hypertensive rats especially in those with malignant hypertension. Crofton et al., using specific vasopressin antagonists, also reported a fall in the BP in DOCA-salt hypertensive rats.

To study further the role of vasopressin in the pathogenesis of hypertension and determine whether continuous blockade of endogenous vasopressin results in a sustained decrease in BP, we studied the effect of a bolus injection followed by an infusion of the competitive vasopressin antagonist on the BP of rats with adrenal regeneration hypertension, malignant DOCA-salt hypertension, and malignant two-kidney, one clip Goldblatt hypertension. The effect of one vasopressin antagonist on the BP of adrenal regeneration hypertensive rats was studied, since the hypertension of this model has been suggested to be due to high mineralocorticoid activity and vasopressin has been implicated as a major pressor agent in the maintenance of mineralocorticoid-mediated hypertension, such as DOCA-salt hypertension.

Two competitive vasopressin inhibitors, [1-deaminopenicillamine, 4-valine, 8-D-arginine] vasopressin (dPVDAVP) and [1-(β-mercapto-β, 8-cyclopentamethylene propionic acid), 4-valine, 8-D-arginine] vasopressin (cyclo dVDAVP), were used in the present study. Both analogs are inhibitors of the pressor effect of vasopressin and do not have inhibitory effects on the antidiuretic action of the hormone.
The animals were then placed into individual cages and given 1% saline as drinking fluid.

**Adrenal Regeneration Hypertension**

Eighteen female Sprague-Dawley rats weighing 70–80 g were unilaterally adrenalectomized and nephrectomized. The contralateral adrenal was enucleated using the technique of Ingle and Higgins. The animals were then placed into individual cages and given 1% saline as drinking fluid.

**Group 2: Malignant DOCA-Salt Hypertension**

Eighteen male Wistar rats weighing 160–180 g were unilaterally nephrectomized and one strip of DOCA-silicone rubber (100 mg of DOCA per kg body weight) was inserted subcutaneously. These rats were placed into metabolic cages and received 1% saline as drinking fluid. The implants of DOCA were prepared by mixing DOCA with silicone rubber (Dow-Corning) in a ratio of 1:3.

**Group 3: Malignant Two-Kidney, One Clip Goldblatt Hypertension**

Eighteen male Sprague-Dawley rats weighing 125–150 g were made hypertensive by placing a U-shaped silver clip with an internal gap of 0.20 mm around the left renal artery. The contralateral kidney was left untouched. The animals were placed into metabolic cages and given tap water ad libitum.

**Experimental Protocol**

Systolic BP was measured once a week by the tail-cuff method. Eight adrenal regeneration hypertensive rats with systolic BP equal to or above 150 mm Hg, eight DOCA-salt hypertensive rats, and six two-kidney, one clip Goldblatt hypertensive rats with signs of malignant hypertension (systolic BP of 170 mm Hg or above, retardation of daily weight gain or weight loss, and increase in daily water intake and diuresis) were selected for the experiments. In these animals the BP response to competitive vasopressin antagonist was studied. Adrenal regeneration hypertensive and malignant two-kidney, one clip hypertensive rats received the antagonist dPVDAVP, while malignant DOCA-salt hypertensive rats received the antagonist cyclo-dPVDAVP. For this purpose, PE 10 catheters were chronically implanted into the abdominal aorta and inferior vena cava through the femoral artery and the femoral vein respectively. Both catheters were led subcutaneously to the scapular region of the rat as previously described. Twenty-four hours later, direct mean BP was recorded by a pressure transducer (Micron MP 15) and a four-channel recorder (Brush 440). During the experiment, the rats were unanesthetized and only partially restrained. After the BP had stabilized, 100 ng of angiotensin II (Hypertensin, CIBA), 100 ng of 1-norepinephrine (Levophed, Winthrop), and 1.25, 2.5, 5.0, and 10.0 mU of vasopressin (Pitressin, Parke-Davis) were administered separately to each rat as bolus injections via the venous catheter. All injections were given in a volume of 0.1 ml followed by 0.2 ml of saline. Subsequently, after the BP had stabilized, 30 µg of the vasopressin antagonist was injected as a bolus followed by the infusion of another 30 µg at the rate of 1 µg/min/rat using a Harvard pump at 19.7 µl/min. The BP was recorded continuously during the infusion and for 90 minutes afterward; 5 to 30 minutes after the end of the antagonist infusion the BP response to angiotensin II, norepinephrine, and vasopressin was determined again.

Blood (0.5 ml) was drawn from each rat for plasma renin activity (PRA) determinations before and 90–100 minutes after the antagonist infusion. The same amount of blood that was drawn was immediately replaced with blood from nephrectomized donor rats. The plasma was separated by centrifuging the blood samples at 2000 rpm at 4°C for 30 minutes. The PRA determinations were made by using a modification of the radioimmunoassay method of Haber et al., as previously described; PRA was expressed in nanograms (ng) of generated angiotensin I per milliliter (ml) of plasma per hour of incubation.

Comparisons were made between the BP measurements taken during the control period and BP measurements taken at different times during the experimental period using Bonferroni’s multiple comparisons protection levels test. Statistical significance was considered to be \( p < 0.05 \). All results are expressed as mean ± SE.

**Results**

Figure 1 shows the dose-response curve to vasopressin before and after the administration of vasopressin blockers. No significant differences were observed in the slope of the dose-response curve and in the degree of inhibition of exogenous vasopressin in the three experimental groups studied. The results from all three groups have been averaged. As shown, the pressor effect of vasopressin was almost completely abolished even when 10 mU of vasopressin, a dose sufficient to raise the BP 40 mm Hg, was injected. The average pressor activity of angiotensin II and norepinephrine in the three groups before the vasopressin antagonists was 37.3 ± 3.2 and 23.5 ± 2.4 mm Hg, and after it was 36.5 ± 2.7 and 23.5 ± 2.9 mm Hg respectively.

Table 1 shows the mean BP of the adrenal regeneration hypertensive, DOCA-salt hypertensive, and two-kidney, one clip Goldblatt hypertensive rats before, during, and after the infusion of the vasopressin antagonist. No significant changes in the mean BP were observed during and after the infusion of the antagonist in any of the three groups. In addition, no significant changes were observed in the level of PRA before and after the infusion of the blocker (table 2).
The high specificity of the vasopressin antiserum used by Möhring et al. excludes the possibility that the decrease in the BP was due to the inhibition of an endogenous vasopressor substance other than vasopressin. However, the possibility that the fall in the BP was due to a mechanism other than vasopressin blockade, such as anaphylactoid reaction, activation of the complement, and/or the plasma kallikrein-kinin system due to the antigen antibody reaction, cannot be totally excluded.

To clarify the role of vasopressin in the pathogenesis of hypertension, we have studied the effect of specific vasopressin antagonists on the BP of three different models of experimental hypertension. Two vasopressin antagonists, dPVDAVP and cyclo-dVDAVP, were used in these experiments. Adrenal regeneration hypertensive and two-kidney, one clip hypertensive rats were infused with dPVDAVP while DOCA-salt hypertensive rats were treated with cyclo-dVDAVP. Both antagonists induced a marked inhibition of vasopressor responses to vasopressin, but not to norepinephrine or angiotensin II. Although it has been reported that dPVDAVP is a slightly more potent vasopressor antagonist than cyclo-dVDAVP, we did not observe any differences in antagonist potency between them, which was probably due to the high doses (60 µg/rat) we used in these experiments.

When the vasopressin antagonist was injected and infused intravenously, no changes in the mean BP were observed in any of the three experimental groups studied. Since the infusion of the vasopressin antagonist inhibited the pressor effect of exogenous vasopressin, it was assumed that the endogenous vasopressin was also blocked. Therefore, the present results, showing that the blockade of endogenous vasopressin does not modify the BP, suggest that vasopressin does not have a role in the maintenance of high BP in the experimental models studied.

The level of PRA observed in the two-kidney, one clip Goldblatt hypertensive rats was extremely high, suggesting further that these rats were in the malignant phase of hypertension. Several studies have demonstrated that the renin-angiotensin system is the principal pathogenetic factor during the onset of malignant renovascular hypertension. However, Möhring et al. have reported that the intravenous injection of a specific vasopressin antiserum lowers the BP of these animals.

Discussion

Recent studies have indicated that slightly elevated or even normal plasma vasopressin concentrations could have a vasopressor effect in hypertensive animals, suggesting that this hormone could be involved in the pathogenesis of certain types of hypertension. This concept has been raised mainly by Möhring et al. after their finding that plasma ADH concentrations increased in rats with DOCA-salt, two-kidney, one clip Goldblatt, and spontaneous hypertension, and that the injection of a specific vasopressin antiserum lowers the BP of these animals.
with this type of malignant hypertension lowers the BP toward normotensive levels in 50% of the animals. The experiments reported here, showing that the inhibition of the pressor effect of vasopressin with dPVDAVP does not modify the mean BP of rats with malignant renovascular hypertension, do not support Möhring's hypothesis about the participation of vasopressin in BP regulation during malignant hypertension.

Several studies have also demonstrated that intravenous infusion of vasopressin decreases PRA either through a mechanism mediated by a vascular receptor in the renal afferent arteriole or by a direct influence on the juxtaglomerular cells. If physiological increases in plasma levels of vasopressin can inhibit renin secretion, it could be expected that the blockade of endogenous vasopressin would have the opposite effect. In our experiments this does not seem to be the case, since in both cases of mineralocorticoid-mediated hypertension the changes in PRA were not statistically significant. These results, however, do not rule out the possibility that the vasopressin receptor for renin release is not affected by the vasopressin antagonist used in the present experiments.

The importance of endogenous vasopressin in the development of hypertension has also been assessed by studying whether rats with hereditary hypothalamic diabetes insipidus developed hypertension. Crofton et al. reported that DOCA-salt treatment failed to significantly increase the systolic BP in rats with hypothalamic diabetes insipidus, while the same treatment induced an increase of 40 mm Hg in the systolic BP of normal Long-Evans control rats. In addition, the same group of investigators have reported that in the Long-Evans group treated with DOCA and salt the injection of a vasopressin antagonist induced a reduction in mean arterial BP of at least 14 mm Hg. These experiments, in conjunction with Möhring and Crofton's experiments, seem to indicate that vasopressin is essential for the development and maintenance of hypertension in uninephrectomized rats treated with DOCA and salt. On the other hand, Johnston et al. have reported that rats with hereditary hypothalamic diabetes insipidus develop two-kidney, one clip Goldblatt hypertension, indicating that vasopressin is not essential for the development of this type of renovascular hypertension.

This study and our present results, however, fail to confirm the findings of Möhring et al. and Crofton et al. regarding the participation of vasopressin in the maintenance of malignant DOCA-salt and two-kidney, one clip Goldblatt hypertension. One explanation of this discrepancy could be the fact that both Möhring and Crofton studied the effect of the vasopressin blockade 3 to 4 hours after the surgery to place the catheters. It has been demonstrated in the dog that surgical trauma is a potent stimulus that can increase the plasma level of vasopressin three to four times, for at least 6 hours. The infusion of a vasopressin blocker into a hypertensive animal under these circumstances could result in a decrease in the BP not related to the pathogenesis of hypertension, but to the homeostatic role of vasopressin.

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### References


### Table 2. Plasma Renin Activity in Hypertensive Rats Before and After the Infusion of a Vasopressin Antagonist

<table>
<thead>
<tr>
<th>Type of Hypertension</th>
<th>Before</th>
<th>After</th>
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<tbody>
<tr>
<td></td>
<td>ADH-antagonist</td>
<td>ADH-antagonist</td>
</tr>
<tr>
<td>Adrenal regeneration</td>
<td>0.30 ± 0.12</td>
<td>0.52 ± 0.19</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>0.42 ± 0.10</td>
<td>0.56 ± 0.17</td>
</tr>
<tr>
<td>Two-kidney, one clip Goldblatt</td>
<td>74.50 ± 15.6</td>
<td>73.30 ± 11.5</td>
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