Contribution of Vasopressin and the Sympathetic Nervous System in the Early Phase of High Sodium One-Kidney Renal Hypertension

CARMEN HINOJOSA AND JOSEPH R. HAYWOOD

SUMMARY This study assessed the contributions of the sympathetic nervous system and arginine vasopressin to the onset of one-kidney, one-wrap (1K1W) renal hypertension in rats fed a high sodium diet. Two weeks before renal wrap or sham wrap, rats were given a high sodium diet and water ad libitum. At 3 days postwrap, resting mean arterial pressure (MAP) was significantly greater in renal-wraped rats. The contributions of the sympathetic nervous system and vasopressin to blood pressure (BP) were assessed by ganglionic blockade and vascular vasopressin receptor antagonism, respectively. Depressor responses to ganglionic blockade were significantly greater in the normotensive rats compared to the hypertensive rats. Administration of vasopressin antagonist caused a significant fall in pressure only in wrapped rats. In addition, enhanced pressor responses to bolus injections of vasopressin were observed in hypertensive rats. These results indicate that during this phase of the hypertension there is an activation of the vasopressin pressor system without an increase in neurogenic function. Equalization of arterial pressure occurred only when both systems were blocked, regardless of the order of blockade, which indicated that the sympathetic nervous system and vasopressin interact to maintain the hypertension. Comparison of depressor responses to the blocking agents revealed that the interaction is compensatory in nature since the contributions of the sympathetic nervous system and vasopressin to the maintenance of arterial pressure were greater when the other system was blocked. (Hypertension 6: 848-854, 1984)

KEY WORDS • arterial pressure • vasopressin antagonist • ganglionic blockade • one-kidney, one-wrap hypertension

The harmful effects of dietary sodium in the pathogenesis of hypertension are under considerable debate, but evidence favors the concept that predisposing factors are necessary for the level of arterial pressure to be sensitive to salt intake. To study the contribution of sodium to hypertension, several experimental models have been developed that require a high sodium intake for the expression of increased arterial pressure. These high sodium models of hypertension include the deoxycorticosterone-saline (DOC-saline) model, the Dahl salt-sensitive strain of rats, and the reduced renal mass-saline model. Mohring et al. and Korner et al. have studied the effects of increases in sodium intake in chronically renal hypertensive animals. Their results indicated that elevated levels of sodium intake correlated with increases in arterial pressure.

Several mechanisms have been suggested as critical factors in the development of sodium-sensitive hypertension. Volume expansion and increased cardiac output leading to “whole body autoregulatory vasoconstriction” have been suggested as one explanation; however, other investigations have not been able to support this concept. The release of a sodium transport inhibitor has also been implicated in high sodium hypertension. Other possible mechanisms contributing to the increased arterial pressure are the sympathetic nervous system and arginine vasopressin. Increased sympathetic nervous system function has been observed in the Dahl salt-sensitive rats and DOC-saline animals. An enhanced plasma level of vasopressin has been measured in DOC-saline animals and rats made hypertensive by reduced renal mass and saline administration.

In the present study, we determined the increase in arterial pressure during the onset of one-kidney, one-wrap (1K1W) hypertension in rats with a high dietary sodium intake. In addition, we investigated the contributions of the sympathetic nervous system and vasopressin to the hypertension.

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Methods

Preparation of Rats

Studies were carried out on 29 male Sprague-Dawley rats weighing 250 to 300 g. The rats were fed a sodium-supplemented rat chow containing 1.2 mEq sodium/g of food, an amount equivalent to 10 times normal in rat chow. Food and tap water were given ad libitum. Two weeks after initiation of the high sodium intake, one group of rats was anesthetized with methoxyflurane and was subjected to figure 8 renal wrap and contralateral nephrectomy as described by Grollman. The other group of rats was sham-wrapped, which consisted of unilateral nephrectomy only. Both groups of animals returned to normal food and water intake within 3 days. On the 3rd day, the rats were anesthetized with methoxyflurane and had a Tygon catheter with 28-gauge Teflon tip placed in the femoral artery for mean arterial pressure (MAP) and heart rate (HR) measurements. A polyethylene catheter was inserted in the femoral vein for drug injections. The catheters were tunneled subcutaneously and exteriorized at the back of the neck. Approximately 4 hours after implantation of the catheters, when the animals had fully recovered from the anesthesia, the arterial catheter was attached to a pressure transducer through a length of polyethylene tubing. The rats were placed in an open container and studied in a conscious, unrestrained state. Once acclimatized to their environment, they were subjected to the following protocols.

Experimental Protocols

Study 1

In the first experimental protocol, the role of the sympathetic nervous system in the maintenance of blood pressure (BP) was assessed. After a 60-minute measurement of baseline MAP and HR, hexamethonium bromide (25 mg/kg, base) was injected intravenously to determine the functional contribution of the sympathetic nervous system. Approximately 15 minutes after the hexamethonium injection, during ganglionic blockade, 10 /xg/kg of the specific vascular receptor antagonist of vasopressin [L-(Q3-mercapto-3, 3-cyclopentamethylene propionic acid),2-(O-methyl)tyrosine]Arg5-vasopressin (d(CH2)5Tyr(Me)AVP) was administered intravenously to block the vasoconstrictor action of vasopressin. The doses of blocking agents used in this study were tested for completeness of blockade in other groups of animals. Hexamethonium (25 mg/kg) was found to block the reflex tachycardia due to the depressor effect of acetylcholine (0.2 /tig/kg), and d(CH2)5Tyr(Me)AVP (10 /xg/kg) blocked the pressor response to 15 mU/kg arginine-vasopressin (unpublished data).

Study 2

The second experimental protocol, performed in another group of animals, was designed to test the contribution of vasopressin to arterial pressure while the autonomic nervous system was intact. Again, after measurement of baseline MAP and HR, 10 /tig/kg of d(CH2)5Tyr(Me)AVP was administered intravenously. Then, in the absence of the vasopressor effects of vasopressin, the involvement of the sympathetic nervous system in the maintenance of arterial pressure was assessed. Hexamethonium (25 mg/kg) was given to block autonomic ganglia 20 minutes after vasopressin blockade.

Study 3

In the third protocol, dose-response curves were generated for arginine vasopressin and angiotensin II (ANG II) to determine intact, whole-animal responsiveness to these pressor stimuli. Bolus intravenous injections of aqueous solutions of arginine vasopressin (5, 15, and 50 mU/kg administered in a volume less than 100 /xl) and ANG II (30, 100, and 300 ng/kg administered in a volume less than 100 /ul) were made in wrapped and sham-operated rats. Baroreflex sensitivity was expressed as the slope of the change in MAP vs the change in HR, as determined by linear regression analysis.

Statistical Methods

All values presented are means ± standard error of the mean (SEM). Baseline MAP and HR measurements for the sham-operated and renal-wrapped groups were evaluated by using a Student’s t test for unpaired samples. Two-way analysis of variance (ANOVA) with repeated measures on one factor was used to evaluate the MAP and HR responses to the sequentially administered blocking agents and the dose-response curves in wrapped and sham-operated rats. One-way ANOVA for repeated measures was used to compare the responses within each group, and the Newman-Kuels multiple range test was used to determine which of the comparisons was significantly different. Individual treatment differences between the two experimental groups were evaluated by one-way ANOVA.

Results

Baseline Data

The baseline MAP for the sham-wrapped group was 124 ± 2 mm Hg and for the wrapped group, 144 ± 2 mm Hg. This 20 mm Hg difference in arterial pressure was statistically significant (p < 0.05). The baseline HR was not different for the sham-operated group (434 ± 7 bpm) and renal-wrapped animals (424 ± 9 bpm).

Study 1: Canglionic Blockade Followed by Vasopressin Antagonist

The effects of hexamethonium followed by d(CH2)5Tyr(Me)AVP on MAP and HR are shown in Figure 1. Administration of hexamethonium alone caused the BP to fall significantly in both sham-operated and renal-wrapped animals. In the sham-operated group, arterial pressure decreased from 121 ± 4 to 78 ± 2 mm Hg. In the renal-wrapped group, arterial pressure decreased from 133 ± 4 to 99 ± 6 mm Hg.
During ganglionic blockade, arterial pressure remained statistically greater \((p < 0.05)\) in the renal-wrapped rats as compared to sham-operated controls. Hexamethonium also caused significant decreases in HR in both groups of rats. In the sham-operated group, ganglionic blockade caused the HR to fall from 430 ± 6 to 389 ± 9 bpm, and in the renal-wrapped group, the HR fell from 432 ± 11 to 401 ±9 bpm.

When the vasopressin antagonist was administered during ganglionic blockade, there was no significant change in MAP and HR in the sham-operated group; however, in the wrapped animals, the addition of \(d(CH_2)_3Tyr(Me)AVP\) caused a significant fall in BP. The final arterial pressures after ganglionic blockade and vasopressin receptor blockade of 79 ± 4 mm Hg in the sham-operated animals and 78 ± 4 mm Hg in the renal-wrapped group were not different. The results of the two-way ANOVA indicated that there were no significant differences in HR responses to vasopressin antagonist between the two groups of animals.

Study 2: Vasopressin Antagonist Followed by Ganglionic Blockade

The results obtained when the vasopressin antagonist was given before hexamethonium are illustrated in Figure 2. Administration of \(d(CH_2)_3Tyr(Me)AVP\) had no effect on the arterial pressure of the sham-operated group. In the renal-wrapped group, vasopressin-receptor blockade resulted in a significant fall in arterial pressure from 146 ± 2 to 137 ± 3 mm Hg. The vascular vasopressin antagonist, \(d(CH_2)_3Tyr(Me)\) AVP, caused the HR to increase in both groups of animals; however, these changes in HR were not statistically significant.

When hexamethonium was given during vasopressin-receptor blockade, arterial pressure decreased significantly \((p < 0.05)\) in both groups of animals. The final BPs after vasopressin-receptor blockade combined with ganglionic blockade were not different (70 ± 3 mm Hg in the sham-operated group vs 73 ± 3 mm Hg in the renal-wrapped group). The effects of the administration of hexamethonium on HR are also shown in Figure 2. The combination of vasopressin-receptor blockade and ganglionic blockade caused a decrease in HR in both sham-operated and renal-wrapped animals; however, the decrease was statistically significant only in the renal-wrapped group.

Study 3: Response to Exogenous Vasopressin and Angiotensin II

Bolus injection of ANG II produced a dose-related increase in MAP in both sham-operated and renal-wrapped rats (Table 1). There was no difference in the pressor responses at any of the doses tested. In addition, the HR baroreflex sensitivity was similar in the normotensive and hypertensive rats. Vasopressin also caused a dose-related increase in arterial pressure in both the hypertensive and normotensive animals (Table 1). ANOVA revealed that the wrapped rats responded with significantly greater increases in arterial pressure at each dose of vasopressin. Again, there was no significant difference in the baroreflex sensitivity to vasopressin between the wrapped and sham-operated rats.
FIGURE 2. Mean arterial pressure and heart rate measurements during the control period. 15 minutes after intravenous injection of d(CH$_2$)$_5$Tyr(Me)AVP (10 fig/kg), and 15 minutes after intravenous injection of hexamethonium (25 mg/kg) in conscious sham-operated (n = 7) or renal-wrapped (n = 8) rats. * indicates a significant difference between sham and wrap groups (p < 0.05). $|$ denotes a significant difference from control (p < 0.05); $\triangle$ indicates a significant difference from the d(CH$_2$)$_5$Tyr(Me)AVP response (p < 0.05). Results are expressed as means ± SEM.

TABLE 1. Changes in Mean Arterial Pressure (MAP) and Baroreflex Sensitivity (BS) Following Bolus Injections of Angiotensin II and Arginine Vasopressin in Sham-Operated and Renal-Wrapped Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Change in MAP (mm Hg)</th>
<th>BS (bpm • mm Hg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Angiotensin II</td>
<td></td>
</tr>
<tr>
<td>Sham (n=10)</td>
<td>30 ng/kg</td>
<td>19.0±1.7</td>
</tr>
<tr>
<td></td>
<td>100 ng/kg</td>
<td>34.8±3.1</td>
</tr>
<tr>
<td></td>
<td>300 ng/kg</td>
<td>47.1±3.0</td>
</tr>
<tr>
<td>Wrap (n=10)</td>
<td></td>
<td>20.7±2.4</td>
</tr>
<tr>
<td></td>
<td>33.4±2.4</td>
<td>44.3±3.6</td>
</tr>
<tr>
<td></td>
<td>2.38±0.40</td>
<td>2.36±0.60</td>
</tr>
<tr>
<td></td>
<td>Arginine vasopressin</td>
<td></td>
</tr>
<tr>
<td>Sham (n=7)</td>
<td>5 mU/kg</td>
<td>6.7±1.4</td>
</tr>
<tr>
<td></td>
<td>15 mU/kg</td>
<td>18.0±2.9</td>
</tr>
<tr>
<td></td>
<td>50 mU/kg</td>
<td>35.7±4.0</td>
</tr>
<tr>
<td>Wrap (n=10)</td>
<td></td>
<td>14.1±2.1*</td>
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<tr>
<td></td>
<td>30.6±3.5*</td>
<td>46.8±2.7*</td>
</tr>
<tr>
<td></td>
<td>2.77±0.14</td>
<td>2.38±0.37</td>
</tr>
</tbody>
</table>

Baroreflex sensitivity was determined as the slope of AHR/AMAP in each animal. Values are expressed as means ± SEM.
*Significantly different from the sham-operated group; p < 0.05.

Discussion

The goals of this study were to assess the magnitude of the difference in arterial pressure between renal-wrapped and sham-operated rats when given a high dietary sodium intake and to determine the roles of the sympathetic nervous system and vasopressin in maintaining the difference in BP between the two groups of rats. Baseline MAP measurements indicated that there was a significant difference of 20 mm Hg between the normotensive and hypertensive rats. Other investigators have also examined the effects of high sodium intake on renal hypertension; however, in those studies sodium was given to animals with preexisting hypertension. Möhring et al.$^5$ observed an additional increase in BP after two-kidney, one clip animals were offered saline drinking fluid. Kornet al.$^6$ gave a high sodium diet to rabbits with established cellophane-wrap hypertension and observed only a modest additional elevation in BP. As these observations indicate, there is some debate as to whether sodium augments the rise in arterial pressure in renal hypertension. In malignant hypertension, Romero et al.$^{20}$ and Möhring et al.$^{21}$ found decreases in arterial pressure with high sodium intake, presumably because of the suppression of the renin-angiotensin system.
Several studies have suggested that the sympathetic nervous system and vasopressin are responsible for sodium-dependent hypertension. In DOC-saline hypertensive animals, the release of noradrenalin and turnover of noradrenalin were increased. An increase in the neurogenic vasoconstrictor tone of the isolated hindlimb has also been demonstrated. Dahl salt-sensitive rats and DOC-saline-treated animals have been shown to have exaggerated depressor responses to ganglionic blockade, which suggested an increased functional contribution of the sympathetic nervous system. As a result, an increased neurogenic component that maintains arterial pressure was predicted in the Grollman-wrapped rats given a high sodium diet.

Analysis of our data yielded unexpected results. The effects of hexamethonium (Figure 1) on MAP in the sham-operated group and the renal-wrapped group indicated that the sympathetic nervous system is involved in the maintenance of BP in both groups of rats. However, the reduction in arterial pressure in the renal-wrapped animals was significantly less than that of the sham-operated group. These results indicated that an enhanced functional sympathetic tone was not the mechanism by which an elevated BP was maintained in the renal-wrapped animals.

Plasma vasopressin is increased in DOC-saline hypertensive rats and Dahl salt-sensitive animals. A role for vasopressin in DOC-saline hypertension has been demonstrated in vasopressin-deficient, diabetes insipidus rats, which are protected against the rise in arterial pressure that is observed in normal animals. A vasoconstrictor action of vasopressin has also been demonstrated in DOC-saline hypertension.

![Graph](image)

**FIGURE 3.** Changes in mean arterial pressure caused by 25 mg/kg hexamethonium (open bars) and by the same dose of hexamethonium after pretreatment with 10 (Jg/kg d(CH2)5Tyr(Me)AVP (shaded bars) in sham (n = 7) and wrap (n = 8) groups. *indicates a significant difference between sham and wrap groups (p < 0.05). The response to hexamethonium after d(CH2)5Tyr(Me)AVP was significantly greater than the response to hexamethonium alone in the renal-wrapped group (p < 0.001). Values are means ± SEM.

In the present study, the vasopressin antagonist caused a significant decrease in BP which indicates that vasopressin was contributing to the maintenance of the elevated arterial pressure in the renal-wrapped rats. The presence of a vasopressin contribution to the hypertension may be due to an increase in circulating levels of vasopressin, an enhanced vascular responsiveness to vasopressin, or to a combination of both mechanisms. In these studies, the renal-wrapped group demonstrated an enhanced responsiveness to vasopressin as compared to the sham-operated group, while no difference between the two groups was observed when ANG II was administered. An enhanced responsiveness to vasopressin has also been observed in DOC-saline animals. Thus, the vasopressin component of the hypertension may be partially due to an increased sensitivity to vasopressin in the renal-wrapped rats.

Although this study provides evidence that vasopressin is contributing to the maintenance of the hypertension, blockade of the vasopressin receptors did not reduce BP in the hypertensive rats to the level of the normotensive animals (Figure 2). The significant difference in arterial pressure between the sham-operated group and the renal-wrapped group after vasopressin antagonist indicated that another mechanism was also involved in the maintenance of the hypertension. It is evident from Figures 1 and 2 that neither blockade of the sympathetic nervous system nor vasopressin receptors alone resulted in equalization of arterial pressure. However, both groups of animals did reach the same arterial pressure when both pressor systems were blocked, regardless of the order of blockade. To evaluate the interactions between the sympathetic nervous system and vasopressin, we compared each system in the presence and absence of the other pressor system in wrapped and sham-operated rats.

First, we compared the changes in arterial pressure caused by hexamethonium alone with the changes produced by hexamethonium in the presence of the vasopressin antagonist (Figure 3). The responses of the sham-operated group indicated that there was a slightly greater decrease in BP with hexamethonium after vasopressin antagonist as compared to the decrease with hexamethonium alone. However, the difference between the two responses was not significant. The responses of the renal-wrapped animals showed that the response to hexamethonium after vasopressin antagonist was significantly (p < 0.001) enhanced in comparison to the response to hexamethonium alone.

$\text{MAP} = \text{av. Grollman hypertension using bolus injections of the vascular vasopressin antagonist d(CH2)5Tyr(Me) AVP. Administration of the vasopressin antagonist had no effect on the MAP in the sham-operated group (Figure 2). In the renal-wrapped group, however, the vasopressin antagonist caused a significant decrease in BP which indicates that vasopressin was contributing to the maintenance of the elevated arterial pressure in the renal-wrapped rats. The presence of a vasopressin contribution to the hypertension may be due to an increase in circulating levels of vasopressin, an enhanced vascular responsiveness to vasopressin, or to a combination of both mechanisms. In these studies, the renal-wrapped group demonstrated an enhanced responsiveness to vasopressin as compared to the sham-operated group, while no difference between the two groups was observed when ANG II was administered. An enhanced responsiveness to vasopressin has also been observed in DOC-saline animals. Thus, the vasopressin component of the hypertension may be partially due to an increased sensitivity to vasopressin in the renal-wrapped rats.
We compared the changes in MAP in response to the vasopressin antagonist alone to the responses produced by the vasopressin antagonist following hexamethonium (Figure 4). The responses in the sham-operated groups were not significantly different (0 ± 1 vs 2 ± 3 mm Hg). However, in the renal-wrapped groups, the change in BP caused by the administration of the vasopressin antagonist in the presence of hexamethonium was greater than the response to vasopressin antagonist alone (21 ± 7 vs 10 ± 2 mm Hg, p < 0.01). Based on these results, we propose that there is an interaction between the sympathetic nervous system and vasopressin in maintaining high sodium 1K1W Grollman hypertension. The nature of this interaction appears to be compensatory, since the neural and vasopressin contributions are greater when the other mechanism is blocked.

A similar interaction between the sympathetic nervous system and vasopressin has been observed in other models of sodium-dependent hypertension. Matsuguchi and Schmid showed that both vasopressin and the sympathetic nervous system were contributing to the elevated vascular resistance in DOC-salt hypertensive rats. Using a blood-perfused hindlimb preparation, these authors found that a vascular vasopressin antagonist produced a greater vasodilator effect in the denervated hindlimb compared to an innervated preparation. Hatzinikolaou et al. have also demonstrated the phenomenon in anephric animals administered hypertonic saline to increase arterial pressure. In these animals, arterial pressure was reduced by a specific pressor antagonist of vasopressin; however, it did not return to the prehypertensive infusion level. If alpha- and beta-adrenergic blockade was given prior to the hypertonic saline infusion, the rise in BP was not blocked, but it was completely reversed when the vasopressin antagonist was given. This observation again indicated the ability of the two systems to compensate for the absence of the other.

A possible mechanism of this compensatory action may be a result of an activation of the baroreflex stimulated by a drop in arterial pressure by hexamethonium or the vasopressin antagonist. Thus, blockade of either system individually may cause a reflex activation of the remaining system in order to maintain arterial pressure at a resting level. This concept is supported by the observations of Rascher et al. in DOC-saline hypertensive rats. In their studies, the administration of a vascular vasopressin antagonist caused a small reduction in arterial pressure and in total peripheral resistance and an increase in cardiac output. However, after denervation of the baroreceptors, there was a decrease in BP and resistance without any change in cardiac output, which suggests that the reflexes antagonized the reduction in arterial pressure by the vasopressin antagonist in the intact rats.

In summary, our studies indicated that the vasopressin system and the sympathetic nervous system were important for the onset of high sodium 1K1W Grollman hypertension. It was also shown that there was a compensatory interaction between the sympathetic nervous system and vasopressin mechanisms to maintain arterial pressure when one of the pressor systems was blocked individually. In addition, an enhanced pressor response to vasopressin was demonstrated in the renal-wrapped group, which was selective for vasopressin. In conclusion, the elevated pressure in this model of high sodium hypertension is due to an activation of the vasopressin system combined with a preservation of a functional sympathetic nervous system.

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