Vasopressin in Salt-Induced Hypertension of Experimental Renal Insufficiency

DONALD J. DiPETTE, M.D., IRENE GAVRAS, M.D., WILLIAM G. NORTH, PH.D., HANS R. BRUNNER, M.D., AND HARALAMBOS GAVRAS, M.D.

SUMMARY The purpose of these experiments was to determine if arginine vasopressin (AVP) contributes to the blood pressure elevation induced by salt excess in renal insufficiency. Conscious rats were studied 6 days after surgical removal of 85% renal mass, which resulted in renal insufficiency as evidenced by an elevation in BUN and creatinine. Four groups of subtotally nephrectomized animals were studied; two at time zero and two following a 24-hour 0.9% NaCl intravenous (i.v.) infusion. One group at each time had plasma AVP determined, while the other was given 30 μg i.t. of a specific inhibitor of the pressor effect of AVP. A fifth subtotally nephrectomized and a sixth normal nonsurgical group were observed without infusion for 24 hours, at which time plasma AVP was determined. Subtotal nephrectomy alone increased baseline BP and tended to increase plasma AVP levels over those of normal control animals. Twenty-four hour saline infusion increased BP in both infused groups by 29 ± 4 and 30 ± 3 mm Hg respectively. No change in BP occurred in either the subtotally nephrectomized or normal animals observed without infusion for 24 hours. Following saline infusion, BP decreased by 20.2 ± 1.5 in response to the AVP inhibitor as compared to 13 ± 1.5 mm Hg at time zero (p < 0.001). AVP was 42 ± 9.6 following saline infusion as compared to 10.5 ± 1.5 pg/ml at time zero (p < 0.001). Six of the saline-infused animals had urine collected over the 24 hours. The percent of Na excreted/Na infused was 83%, while the percent of volume excreted/volume infused was 91.5% (p < 0.05), demonstrating to an extent selective retention of Na as opposed to volume. Taken together, the elevation of plasma AVP and decrease in BP in response to AVP inhibitor in the saline-infused animals demonstrate that AVP plays an important role in the BP elevation acutely induced by salt excess in renal insufficiency.

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KEY WORDS • subtotal nephrectomy • saline infusion • vasopressin inhibitor

RECENT studies have focused on the role of vasopressin in various types of experimental hypertension. First, Mohring, et al.,1 in 1978, found elevated vasopressin levels in rats with malignant hypertension of the two-kidney, one clip type, and that an antiserum to vasopressin could decrease blood pressure. Crofton, et al.,2,3 in 1978 and 1979, found that spontaneously hypertensive rats and rats with mineralocorticoid and salt-induced hypertension had increased secretion of vasopressin.2,3 In the latter, the blood pressure decreased after inhibition of vasopressin with a specific antagonist of its pressor action. Other investigators, however, failed to reproduce these findings. Thus, Rabito, et al.,4 in 1981, found that neither animals with renin-dependent two-kidney renovascular hypertension nor the chronic sodium-dependent hypertension of mineralocorticoid and sodium excess would have decreased blood pressure in response to vasopressin inhibition. In a more recent study, Lee-Kwon et al.,5 found that another model of chronic sodium-dependent hypertension, the partially nephrectomized salt-fed rat, failed to respond with an important decrease in blood pressure after vasopressin inhibition. These studies cast doubt on the participation of vasopressin in experimental hypertension.

Recently, Hatzinikolaou et al.,6 while investigating the causes of blood pressure rise after infusion of hypertonic saline, found that in the anephric rat, vasopressin was responsible for at least half of the observed increment. Those experiments raise the possibility that vasopressin can be implicated in the elevation of blood pressure produced under less extreme conditions, namely, by the reduction of renal mass combined with infusion of normal saline, a procedure known to cause hypertension with elevated peripheral vascular resistance. The increased vascular resistance had previously been attributed to autoregulation of total body blood flow.7

From the Thorndike Memorial Laboratories, Boston City Hospital, and the Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, and the Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire.

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Address for reprints to: Dr. Haralambos Gavras, 80 East Concord Street, Boston, Massachusetts 02118.
Methods

Male Wistar rats (Charles River Breeding Laboratories) initially weighing between 250 and 275 g were studied. Subtotal nephrectomy was performed on 32 animals under ether anesthesia in the following manner. Through bilateral flank incisions, a right nephrectomy was performed as well as removal of both poles of the left kidney. Additional renal mass was carefully removed so as not to damage the hilar vessels and ureter. This left a small residual (10%-15%) of renal tissue around the left hilum. Bleeding from cut surfaces was controlled by application of topical thrombin (0.1 ml) and gauze. Tetracycline was added to tap water for 48 hours postoperatively. All animals were maintained on a regular Purina rat chow diet.

Six days later all animals were anesthetized with ether. The right external iliac artery was cannulated with a PE 50 catheter for direct aortic pressure recording and the right femoral vein with a PE 10 catheter for i.v. infusion and/or drug administration. Arterial pressure was monitored with a Statham transducer and recorded on a Hewlett-Packard recorder (Model 7702 B). Upon awakening, the animals were maintained in a semirestrained position on a light mesh screen for at least 60-90 minutes until a stable baseline blood pressure was obtained, at which time the experiment was begun. Mean blood pressure and heart rate were recorded directly during the experiment.

A solution of 0.9% NaCl was used for intravenous infusion in some of the animals. It was administered via a Harvard pump at a rate of 0.056 ml/min for 24 hours for a total of 12.4 mEq Na in 80.6 ml of fluid. The peptide 1-[(β-mercapto-β, β-cyclopentamethylenepropionic acid) 2-(0-methyl) tyrosine] arginine vasopressin (AVP), which is a competitive antagonist of AVP at the vascular receptor level, was used as an inhibitor of the pressor effect of the antidiuretic hormone. A 2 mg amount of this compound was dissolved in a solution made from 10 ml of 0.9% saline, 10 mg bovine serum albumin, and 3 μl acetic acid, brought to a pH 6.4 with NaOH. A dose of 0.15 ml of this solution containing 30 μg of AVP inhibitor was injected intravenously where indicated.

Samples of 2 ml blood were drawn very rapidly through the arterial catheter for plasma vasopressin determination as previously described. Following this blood sample, an additional 2 ml of blood were drawn for serum electrolytes, BUN, and creatinine which were measured via autoanalyzer, and for microhematocrit which was determined by centrifugation in heparinized microcapillary tubes. Plasma osmolality was calculated from the serum sodium determinations.

Results are expressed as means ± SEM. Student's t test for paired and unpaired data and one-way analysis of variance followed by the Scheffé multiple comparison procedure were used where appropriate. A probability level of less than 0.05 was considered significant.

Six groups of animals were used: Groups 1 through 5 all had previously received subtotal nephrectomy, while Group 6 consisted of normal intact animals receiving no surgical manipulation.

Group 1

Group 1 rats (n = 6) had blood samples drawn for AVP, electrolytes, BUN, creatinine, and hematocrit 1 hour after blood pressure stabilization.

Group 2

Group 2 rats (n = 6), following blood pressure stabilization, were given intravenously the AVP inhibitor and had blood pressure and heart rate (HR) monitored at 15-minute intervals for 1 hour. At this time blood was drawn for electrolytes, BUN, creatinine, and hematocrit.

Group 3

Group 3 rats (n = 8) received a saline infusion for 24 hours, without access to drinking water, at the end of which time blood was drawn for AVP, electrolytes, BUN, creatinine, and hematocrit.

Group 4

Group 4 rats (n = 6) received a saline infusion for 24 hours, without access to drinking water, followed by administration of AVP inhibitor. After observation for 1 hour, blood was drawn for electrolytes, BUN, creatinine, and hematocrit determination.

Group 5

Group 5 rats (n = 6) were observed for 24 hours without i.v. infusion, and were allowed to drink water ad libitum. At the end of 24 hours, blood was obtained for AVP, electrolytes, BUN, creatinine, and hematocrit analysis.

Group 6

Group 6 (n = 7) consisted of intact rats catheterized as the previous groups and followed as Group 5 for 24 hours, at the end of which time blood samples were drawn as above.

Urine Collection

In six saline-infused subtotally nephrectomized animals, urine was collected for 24 hours under paraffin oil to prevent evaporation. The collection was started after blood pressure and HR had stabilized and the animals were voiding spontaneously. Samples were obtained separately during the initial period of rapid blood pressure rise (U1) and later during the period of gradual blood pressure elevation (U2). Blood pressure, HR, and urine volume were recorded at the time of each sample. Urine sodium concentration was determined in each sample by flame photometry.

Results

The blood chemistries of all six groups of animals are shown in table 1. As can be seen, BUN and creatinine are elevated approximately three- to four-
There was no significant difference in BUN or creatinine between the five subtotally-nephrectomized groups. Plasma sodium and chloride were significantly elevated in the two saline-infused groups (Groups 3 and 4) as compared to the noninfused animals (p < 0.001). No difference in plasma potassium was seen among any of the groups.

Table 2 presents the comparison of blood chemistries among groups. The results are expressed as means ± SEM. BUN and Cr of Group 6 were significantly lower than in all five subtotally nephrectomized groups. Na and Cl of Groups 3 and 4 (saline-infused) were significantly higher than in the other four groups. SN = subtotally nephrectomized, studied on Day 1. SN-NaCl = subtotally nephrectomized, saline-infused for 24 hours. SN-H2O = subtotally nephrectomized, water ad libitum for 24 hours. N = normal intact rats, water ad libitum for 24 hours.

AVP inhibitor caused no decrease in MAP in subtotally nephrectomized animals (Group 2), which had received no intravenous saline infusion (132 ± 9 to 131 ± 8 mm Hg). Following 24 hours of saline infusion in subtotally nephrectomized animals (Group 4), AVP inhibitor caused an immediate fall of MAP from 158 ± 4.5 to 139 ± 4.5 mm Hg (p < 0.001) (fig. 1), leaving a residual MAP elevation of 9.5 mm Hg, which was significantly higher than baseline (p < 0.05). The HR increased significantly following AVP inhibitor in the same animals (fig. 1).

Plasma AVP levels are shown in figure 2. Subtotal nephrectomy alone tended to elevate plasma AVP when compared to normal rats kept under similar conditions (Group 5 vs 6); however, the difference did not reach significance. Keeping the animals in a semi-restrained position with catheters in place for 24 hours did not change plasma AVP levels in subtotally-nephrectomized animals (Group 5 vs 1; 8.6 ± 1.9 vs 7.8 ± 1.3 pg/ml). However, 24 hours of saline infusion in the subtotally-nephrectomized animals significantly elevated plasma AVP levels approximately fourfold to 42 ± 9.6 pg/ml over that of subtotally-nephrectomized animals without saline infusion.

The saline-infused animals demonstrated an average increase in serum sodium of 12 mEq/liter,
which led to an increase in plasma osmolality by an average of 24 mOsm/liter.

Urine volume measurements and chemistries during the continuous saline infusion were collected in two separate samples: one during the initial rapid phase of blood pressure elevation ($U_1$) and the second during the later phase of gradual blood pressure plateau ($U_2$), as shown in Table 3. As can be seen, 47% ± 5% of intravenously-infused sodium is excreted in $U_1$ as compared with 82% ± 5% at the time of $U_2$ ($p < 0.01$). Over 24 hours, subtotally nephrectomized animals excreted 92% ± 2% of the infused volume vs 83% ± 3% of the infused sodium ($p < 0.05$).

**Discussion**

Animals with a reduction in renal mass of 70% or greater rapidly increase their blood pressure when given a diet with excessive salt content. In the present experiments, we estimated that renal mass was surgically reduced by 80%-85%. Loss of this amount of renal mass resulted in renal insufficiency as demonstrated by a three- to fourfold rise in BUN and creatinine as compared to the normal intact animals. There was no significant difference in either BUN or creatinine among the groups of subtotally-nephrectomized animals, thus excluding the possibility that a difference in the amount of residual renal mass could be responsible for any difference in the observed blood pressure levels or the mechanisms involved.

Subtotal nephrectomy alone caused a blood pressure elevation of 9-14 mm Hg as compared with the intact rats, which is in agreement with previous observations. It also caused a decrease in HR, probably due to baroreceptor reflex activity. Maintenance on water ad libitum for 24 hours, without saline infusion, caused no blood pressure change from baselines in either subtotally-nephrectomized or intact animals. Infusion of normal saline for 24 hours produced a further elevation of pressure by an average of 30 mm Hg above the baseline. Intravenous administration of the AVP antagonist in the saline-infused animals at that point produced an immediate fall of blood pressure by an average of 19 mm Hg, with concomitant increase in HR. Thus, approximately two-thirds of the blood pressure increment induced by the infusion of saline in these animals was due to stimulation of endogenous vasopressin and could be abolished by inhibition of AVP. In rats that had received no saline infusion, the AVP antagonist produced no change in pressure, indicating that this compound had no depressor effect of its own and that the chronic small pressure increase induced by the loss of renal mass alone was not attributable to vasopressin.

Subtotal nephrectomy per se was accompanied by a small tendency to elevated plasma AVP levels, as shown in Figure 2, which is also in accordance with observations by other investigators and probably reflects decreased elimination of AVP through the kidneys. Maintenance on water ad libitum for 24 hours did not change AVP levels, whereas infusion of normal saline increased them by over fourfold. There are two possible explanations for the increased AVP seen in the subtotally-nephrectomized saline-infused animals. First, increased osmolality is a potent stimulus to AVP. The saline-infused animals demonstrated an increase in plasma sodium by an average of 12 mEq/liter, which would lead to an increase in os-
Table 3. Comparison of Urine Obtained during the Initiation of Rapid Mean Blood Pressure (MAP) Rise ($u_1$) and during the Later Gradual MAP Elevation ($u_2$) during 24 hours i.v. Saline Infusion in Subtotally Nephrectomized Rats

<table>
<thead>
<tr>
<th>MAP increase (mm Hg)</th>
<th>Time (hrs)</th>
<th>Vol (ml)</th>
<th>Na (i.v.) (mEq)</th>
<th>Na(u) (mEq)</th>
<th>% Na excreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>$u_1$</td>
<td>23 ± 4</td>
<td>5.25 ± 0.6</td>
<td>16 ± 2</td>
<td>2.75 ± 0.3</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>$u_2$</td>
<td>38 ± 5*</td>
<td>18.75 ± 0.6</td>
<td>58 ± 2</td>
<td>9.75 ± 0.3</td>
<td>8.1 ± 0.4</td>
</tr>
</tbody>
</table>

During the latter period ($u_2$), a significantly higher percentage of infused sodium was excreted than during the period of rapid MAP increase ($u_1$). Increase in MAP measured from baseline. Results expressed as means ± SEM.

*p < 0.05; $u_2$ vs $u_1$.
†p < 0.01; $u_2$ vs $u_1$.

pressure elevation. Two likely explanations may account for this difference. First, in our study we estimated an approximate 80%-85% decrease in renal mass, as opposed to the 70% reported by these authors, who did not specify the levels of BUN and creatinine in their animals. It is therefore possible that the degree of renal insufficiency is an important factor in this model since the greater the loss of renal function, the greater the retention of sodium, which in turn may stimulate AVP secretion. Second, differences in time course between the two studies prohibit a direct comparison. Our animals were studied 24 hours after i.v. saline infusion, while those of Lee-Kwon et al. were studied at 48 hours and 8-10 days following administration of saline as drinking water. In fact, the two studies may be complementary: these authors reported higher urinary and plasma AVP levels at an earlier phase of administration of saline as drinking water, with a subsequent decline in AVP levels. Since we are studying the animals acutely after 24 hours of saline administration, we may be observing the period of maximal contribution of AVP to blood pressure elevation. That is, AVP may play a greater role in the development of the blood pressure elevation than in the maintenance or chronic phase of hypertension. It is probable that the phase of established hypertension in this model is mostly sustained by a different mechanism. Preliminary data from another series of experiments in our laboratory suggest that this may be a predominantly neurogenic, adrenergically-mediated mechanism.

It is also likely that such a neurogenic mechanism may account for the residual one-third of blood pressure elevation remaining after the administration of AVP inhibitor to the subtotally nephrectomized saline-infused animals (fig. 1). Alternatively, a deficiency of renomedullary interstitial cells after subtotal nephrectomy, ensuing in a loss of an endogenous antihypertensive substance, may also account for part of this blood pressure elevation.

The increase in blood pressure and peripheral resistance induced by salt loading in partially nephrectomized animals has been studied extensively in the past, particularly by Coleman and Guyton and Cowley. These investigators, noting that changes in cardiac output and intravascular fluid volume were not an adequate explanation for the maintenance of
chronically increased blood pressure, which was characterized by elevated peripheral resistance, attributed the elevation of peripheral resistance to autoregulation of regional blood flows; that is, an intrinsic tendency of all organs to initiate local vasoconstriction, thus maintaining high regional resistances.

Our present data describe an acute vasoconstrictor mechanism stimulated by sodium loading. Taken in conjunction with our other recent studies in which we explored different models of sodium-dependent experimental hypertension either acutely induced, or chronically maintained over several days or weeks, our data suggest that this rise in peripheral resistance may not be due to intrinsic local autoregulatory mechanisms but rather to stimulation by sodium of at least two systemic vasoconstrictor principles in parallel or in sequence: vasopressin and the sympathetic nervous system. Vasopressin seems to play a role in the early phase, whereas the sympathetic nervous system contributes to both the initiation and the chronic maintenance of salt-induced hypertension.

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