Role of Vasopressin in Salt-Induced Hypertension in Baroreceptor-Denervated Uninephrectomized Rabbits

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To elucidate the contributions of renal, humoral, and arterial baroreceptor reflex components to salt-induced hypertension, we administered 10% NaCl intravenously for 10 days to sinoaortic-denervated rabbits with unilateral nephrectomy (n=7), sinoaortic-denervated rabbits with intact kidneys (n=7), and sham-operated sinoaortic-denervated rabbits with unilateral nephrectomy (n=7). Serial changes in mean arterial pressure (MAP), heart rate, and blood pressure variability were recorded. In sinoaortic-denervated rabbits with unilateral nephrectomy, MAP increased significantly from 109±2 to 124±3 mm Hg (day 4) and remained elevated for the rest of the experiment. This elevation of MAP was accompanied by a reduction in the standard deviation of MAP, with significant elevations in plasma vasopressin, norepinephrine, and atrial natriuretic peptide concentrations and in sodium retention. In the other groups, there were no significant changes in these vasoactive hormones. In the sham-operated sinoaortic-denervated rabbits with unilateral nephrectomy, sodium retention was similar to that of sinoaortic-denervated rabbits with unilateral nephrectomy. Continuous infusion (1 μg/kg/hr) of a V1 antagonist prevented the elevation of blood pressure and plasma norepinephrine, the accumulation of sodium, and the reduction of blood pressure lability, whereas a bolus injection (10 μg/kg) on day 4 reduced blood pressure from 128±3 to 115±2 mm Hg (p<0.005). These results imply that vasopressin plays a crucial role in the expression of salt-induced hypertension in rabbits with compromised baroreceptor and renal function.

Sodium is an important factor in the development and maintenance of hypertension, and renal, neural, and humoral factors apparently are also involved in these mechanisms. As for neural factors, salt loading has been demonstrated to sensitize the arterial baroreceptor reflex and cardiopulmonary reflex, both of which modulate sodium handling in the kidney through the renal sympathetic nerves. Moreover, these reflexes and sodium handling have been reported to be influenced by various humoral factors. In chronic salt loading, these factors would be expected to affect each other and the regulation of the blood pressure. However, no integrated studies to characterize these relations have been reported.

The purpose of this study was to examine the contributions of renal, humoral, and neural factors to salt-induced hypertension and to determine the changes in the lability of blood pressure in this type of hypertension. To characterize these factors, we used sinoaortic denervation (SAD), which interrupts the arterial baroreceptor reflex control, and unilateral nephrectomy, which promotes the development of hypertension in salt loading. In these animals, salt loading with intravenous administration of hypertonic saline for 10 days was carried out. In addition, pharmacological studies with an arginine vasopressin (AVP) V1 receptor antagonist were performed to examine the role of AVP in this type of hypertension.

Methods

Animal Preparation

Experiments were performed on 73 female Japanese rabbits (2.5–3.5 kg). The rabbits received tap water ad libitum and were maintained on a fixed daily diet that provided 6.66 meq sodium. Rabbits were placed in individual metabolic cages in a room with a constant temperature (20±4°C) and light cycle (8:00 AM–8:00 PM). All surgical procedures were carried out through the use of an aseptic technique with rabbits under pentobarbital anesthesia (25 mg/kg i.v.). Three to 5 weeks before the initiation of hyper-
tonic saline loading, SAD was performed as described previously.\textsuperscript{10} Completeness of denervation was checked by the reflex decrease in heart rate (HR) in response to intravenous injection of phenylephrine. The response was less than 0.5 beats/min/mm Hg compared with approximately 2.0 beats/min/mm Hg of the sham-operated sinoaortic-denervated animals. Sham operation for SAD was performed by exposing both the carotid sinuses and aortic depressor nerves bilaterally with no dissection of the region. Two to 3 weeks before the initiation of hypertonic saline loading, right nephrectomy (NX) or sham operation for NX was performed through a flank incision. One week before the initiation of experiments, catheters made of silicon tubing bonded to a PE-60 catheter (Becton Dickinson, Clay Adams, Parsippany, N.J.) were implanted in the left subclavian artery and in the right atrium through the right external jugular vein. All catheters were exteriorized at the back of the neck.

**Experiments**

Experiments were performed with rabbits slightly restrained and conscious in an experimental cage. Mean arterial pressure (MAP), HR, and mean right atrial pressure (RAP) were recorded continuously on a polygraph recorder (RM-6000, Nihon Koden, Tokyo, Japan) through a transducer (TP-400T, Nihon Koden). Data on these three hemodynamic parameters were fed into an A/D converter (Dataq Instruments, Inc., Akron, Ohio). Information was extracted from these waveforms at a sampling rate of 2 Hz for 60 minutes for each parameter by the M-CODAS system (Dataq) using an IBM microcomputer. The average and standard deviation of these three parameters were analyzed with the statistical software ASYSTANT (Asyst Software Technologies, Inc., Rochester, N.Y.). Standard deviation of MAP is abbreviated as MAP-SD. Before any experimental intervention, rabbits were allowed a minimum of 60 minutes to acclimate to the laboratory environment.

Blood samples (10 ml) were taken from the arterial catheter at the end of the experiments each day. As soon as possible, the same amount of blood was transfused from another rabbit with the same treatment. With these blood samples we measured hematocrit; serum osmolality; serum sodium and potassium concentrations; serum creatinine; plasma renin activity (PRA); and AVP, atrial natriuretic peptide (ANP), and plasma norepinephrine (NE) concentrations.

Urine volume, food intake, water consumption, and body weight were recorded every day. Urinary sodium excretion was measured every day for calculation of cumulative sodium balance.

Serum osmolality was determined by a model 3MO micro-osmometer (Advanced Instruments, Inc., Needham, Mass.). Serum and urine electrolytes were measured by a flame photometer. Serum creatinine was determined by Jaffe’s method. Commercially available radioimmunassay kits were used to measure PRA (Dainabot, Tokyo, Japan), AVP (Amersham, Tokyo, Japan), and ANP (Amersham). We measured NE with the high-performance liquid chromatography trihydroxyindol method.\textsuperscript{11} For extraction of AVP and ANP, we used a Sep-Pak C18 ODS cartridge (Waters Associates, Milford, Mass.). Plasma (1 ml) was applied to an activated ODS cartridge. The material retained on the cartridge was eluted with 3 ml 80% acetonitrile in 0.1% trifluoroacetic acid.\textsuperscript{12} The eluates were lyophilized and then stored at −20°C until analysis. Average recovery of AVP from plasma was 88.1% (n=9); intra-assay and interassay variations were 5.5–7.2% and 6.7–9.2%, respectively. Average recovery of ANP from plasma was 91.8% (n=9); intra-assay and interassay variations were 4.8–6.7% and 5.6–7.9%, respectively. In the PRA assay, intra-assay and interassay variations were within the values earlier reported.\textsuperscript{13} In the NE assay, intra-assay and interassay variations were 2.4–4.4% and 6.5–7.4%, respectively.

**Experimental Protocol**

**Protocol 1:** Forty-two rabbits were divided into three groups. 1) SAD+NX group. The animals of this group underwent SAD and NX (n=14). 2) SAD group. Animals in this group underwent SAD and sham operation for NX (n=14). 3) NX group. Animals underwent NX and sham operation for SAD (n=14). Half of the rabbits in each group were used as donors for blood transfusion.

After rabbits had recovered 1 week from catheterization, we began the experiment. Two days of 0.9% NaCl loading were followed by 10 days of 10% NaCl loading (day −2 to day 0 and day 0 to day 10). We administered 0.9% or 10% NaCl intravenously (2.4 ml/kg) every 8 hours. MAP, HR, RAP, and MAP-SD were recorded on days −2, −1, 1, 4, 7, and 10. Blood (10 ml) was drawn just after hemodynamic data acquisition.

**Protocol 2:** Bolus injection of AVP antagonist. In Protocol 1, because blood pressure elevation was found to be accompanied with an increase in AVP, we administered the selective vasoconstrictive (V\textsubscript{2}) antagonist d(CH\textsubscript{2})\textsubscript{5}Tyr(Me)AVP (AVPX) (Sigm Chemical Co., St. Louis) to determine the role of AVP.

Seven rabbits underwent SAD and NX and received the same salt loading as in Protocol 1. On day 4, after a minimum of 60 minutes, sterile isotonic saline was infused in the rabbits at 4.27 μl/min for 60 minutes by an infusion pump (special model, Harvard Apparatus, South Natick, Mass.). Then a bolus injection of AVPX (10 μg/kg) was followed by the continuous infusion of AVPX (1 μg/kg/hr, 4.27 μl/min) for 100 minutes. Data from the 60 minutes of saline infusion and the last 60 minutes of AVPX infusion were stored and analyzed by the method described above.

**Protocol 3:** Continuous infusion of AVP antagonist. Twenty-four rabbits were divided into two groups. One day before the beginning of experiments, an Alzet osmotic minipump (model 2ML1, Alza Corp., Palo Alto, Calif.) was placed subcutaneously, and its cathe-
ter was implanted in the right femoral vein. With this pump, we administered vehicle or AVPX (1 μg/kg/hr for 13 days) continuously. At the end of this protocol, we administered AVP (3.0 milliunits/kg/min for 100 minutes) to determine the efficacy of AVPX. The two groups were 1) AVPX group. Animals in this group underwent SAD, NX, and implantation of the osmotic minipump containing AVPX (n=12). 2) Saline group. Animals underwent SAD, NX, and implantation of the minipump containing normal saline (n=12). These experimental methods were as described in Protocol 1.

**Statistical Analyses**

All values are presented as mean±SEM. We used two-way analysis of variance followed by one-way analysis of variance with repeated measures. Scheffe's F test was performed for multiple comparisons.

Correlations of MAP and MAP-SD between other parameters in Protocol 1 were determined by the nonparametric repeated measures Spearman's rank correlation procedure. Sampling data were determined from the values in the SAD+NX and SAD groups from day −1 to day 10 (n=70). The data on day −2 were eliminated because the cumulative sodium balance on day −2 was always 0 meq.

A value of p<0.05 was considered statistically significant.

**Results**

**Protocol 1**

Serial changes in hemodynamic parameters in Protocol 1 are depicted in Figure 1.

**Changes in MAP.** Basal values of MAP in the SAD+NX and SAD groups averaged 109±2 and 105±4 mm Hg, respectively, which were significantly elevated compared with those of the NX group. Hypertonic saline administration induced a blood pressure elevation only in the SAD+NX group. No significant elevation of MAP was observed in other groups (SAD and NX groups).

**Changes in MAP-SD.** Basal values of MAP-SD in rabbits that underwent SAD were significantly higher than those in SAD sham-operated rabbits. In the SAD+NX group, MAP-SD decreased gradually and significantly with salt loading. Other groups did not show any significant changes throughout the experiment.

**Changes in HR.** Basal values of HR in the SAD+NX and SAD groups were significantly higher than those in the NX group. Salt loading did not alter HR in all groups.

**Changes in RAP.** No significant differences were found in basal values of RAP among all groups. Only in the SAD+NX animals, immediately after the initiation of high salt loading, RAP significantly increased to 1.7±0.5 mm Hg on day 1 and then tended to decrease gradually.

**Sodium balance and endocrine responses to salt loading.** Figure 2 shows the serial changes in cumulative sodium balance and several hormones in Protocol 1.

In the SAD+NX and NX groups, sodium retention achieved significance compared with that in the SAD group from day 4, and this retention was maintained until the end of the experiment.

Basal values of NE in the SAD group were higher than those in the NX group. NE increased twofold on day 4 and gradually decreased toward the end of the experiments. In other
groups, there were no significant changes in NE throughout the experiments.

There were no significant differences in basal values of AVP among all groups. Only in the SAD+NX group, AVP increased approximately threefold from day 1 and continued to be significantly elevated.

Basal values of ANP among all groups were essentially the same. However, in the SAD+NX group, a marked increase in ANP was observed from 53±8 pg/ml on day −1 to 397±42 pg/ml on day 4 (p<0.0001) in response to salt loading. Other groups achieved significance on day 7 or 10 (99±8 pg/ml on day 7 in the NX group, p<0.0001; 86±8 pg/ml on day 7 in the SAD group, p<0.05, compared with values on day −1).

PRA decreased significantly with salt loading in all groups.

Other biochemical data. There were neither significant differences in basal values of body weight nor significant changes in response to salt loading among all groups. However, in the SAD+NX group, body weight increased mildly in response to salt loading but achieved no significant levels. Hematocrit decreased significantly only in the SAD+NX group (37.3±1.2% on day −1 versus 33.7±1.4% on day 10, p<0.05). Both serum sodium concentration and osmolality increased significantly (serum sodium, 140.6±0.8 meq/l on day −1, 143±1.0 meq/l on day 7, p<0.05; serum osmolality, 294.6±1.0 mosm/kg/H2O on day −1, 297.3±0.8 mosm/kg/H2O on day 10, p<0.05) only in the SAD+NX group. There were no significant differences in basal values of serum potassium concentration and creatinine among all groups and no changes in response to salt loading.

Correlations. Correlations between MAP and other parameters were as follows (70 samples): AVP, r=0.37, p<0.002; ANP, r=0.43, p<0.0004; NE, r=0.28, p<0.002; cumulative sodium balance, r=0.37, p<0.002.

Correlations between MAP-SD and other parameters were as follows (70 samples): AVP, r=-0.30, p<0.01; ANP, r=-0.52, p<0.0001; NE, r=-0.19, p=0.12; cumulative sodium balance, r=-0.45, p<0.0004.

Protocol 2

Bolus injection of AVPX on day 4 reduced MAP significantly (after AVPX injection, 115±2 mm Hg versus before AVPX injection, 128±3 mm Hg, p<0.005). But the value of post-AVPX injection was still higher compared with the value on day −1 (p<0.01). No significant changes in HR and MAP-SD to AVPX injection were observed.

Protocol 3

Continuous infusion of AVPX completely prevented the elevation of blood pressure and the decrease in MAP-SD in response to salt loading (Figure 3). Neither elevation of NE concentration nor sodium retention was observed (NE, 804±119 pg/ml on day 4 in Saline group versus 371±28 pg/ml on day 4 in AVPX group, p<0.005; cumulative sodium balance, 74.7±13.8 meq on day 10 in saline group versus 11.8±4.1 meq on day 10 in AVPX group, p<0.005).

AVP infusion in AVPX animals at day 10 did not induce any significant changes in MAP, HR, or MAP-SD.

Discussion

The major findings of this study are 1) salt loading produced hypertension under the conditions of impaired baroreceptor reflex function and reduced abil-
FIGURE 3. Line graphs showing serial changes in response to salt loading in mean arterial pressure (MAP), standard deviation of MAP (MAP-SD), and heart rate (HR) in conscious sinoaortic-denervated uninephrectomized rabbits receiving 1 μg/kg/hr AVP V1 antagonist (AVPX, ○, n=6) and in sinoaortic-denervated and uninephrectomized rabbits receiving vehicle (Saline, ●, n=6). Values are mean±SEM. *Significantly (p<0.05) different from AVPX on each day; #significant (p<0.05) compared with values on day -1 in each group.

Although the mechanisms of salt-induced hypertension still remain unclear, it is agreed that salt loading can cause hypertension in humans and experimental animals. Among the factors that contribute to salt-induced hypertension, neurocirculatory regulation plays an important role. When the arterial baroreceptor reflex is impaired, blood pressure increases more rapidly because of the increases in blood volume. In sinoaortic-denervated animals, the effects of salt loading have yielded conflicting data. It has been reported that natriuresis in response to a sodium load occurred in sinoaortic-denervated rats, but the blood pressure was not changed by salt loading. However, the sympathetic activation, especially the increase in renal sympathetic nerve activity induced by the removal of sinoaortic receptors (SAD), could facilitate sodium retention, inducing the development of hypertension. In the present study, in sinoaortic-denervated animals with intact kidneys with the elevation of basal levels of plasma NE, blood pressure did not increase during the administration of hypertonic saline. No direct measurements of renal sympathetic nerve activity were performed; however, plasma NE as an index of activity of the sympathetic nervous system was found to be elevated only in sinoaortic-denervated animals with uninephrectomy in response to salt loading. These data may indicate that the inability of the kidneys to excrete sodium is required for the development of hypertension in sinoaortic-denervated animals with salt loading and that the activation of the sympathetic nervous system at the initiation of salt loading cannot induce hypertension without sodium retention. Indeed, in sinoaortic-denervated animals with uninephrectomy, salt loading induced a prominent sodium retention compared with sinoaortic-denervated or uninephrectomized animals.

Although peripherally administered AVP generally is considered to decrease sympathetic nerve activity in normal animals, in salt-dependent hypertension it has been supposed to enhance the sympathetic nervous system. In sinoaortic-denervated animals with salt loading, plasma levels of AVP were increased, and bolus injection of AVPX partially reduced MAP, whereas a continuous infusion of AVPX completely blocked the elevation of blood pressure. Moreover, a continuous infusion of AVPX abolished sodium retention and the elevation of plasma levels of NE. In regression analysis between MAP and other parameters, a closer correlation between MAP and AVP was observed than between MAP and NE. These data suggest that AVP plays a crucial role in salt-induced hypertension in SAD+NX animals.

In addition to its peripheral direct vasoconstrictor action, AVP is known to exert various cardiovascular effects through peripheral and central mechanisms. Matsuguchi and Schmid demonstrated that AVP and neurogenic stimuli work together in some manner to elevate vascular resistance in salt-induced hypertension. Moreover, these interactions were not observed in normotensive animals. Several studies have demonstrated that the interaction of central AVP and the sympathetic nervous system contributes to the development of salt-induced hypertension. Furthermore, centrally administered AVP raised blood pressure by sympathetic activation and AVP release. These findings would support our results that central as well as peripheral AVP is mainly involved in the development of salt-induced hypertension in our model. However, because of the lack of appropriate methods to evaluate the role of...
AVP in central regulation of blood pressure, the precise mechanism by which AVP acts centrally to regulate blood pressure remains to be elucidated. The sites of AVP action on the central regulation of blood pressure have been suggested to be in the anteroventral region of the third ventricle (AV3V area) and the area postrema, as well as vasopressinergic pathways of the hypothalamus. Some of these areas include the circumventricular organs that lack a blood-brain barrier. One interpretation of our results is that the continuous intravenous administration of AVP exerted its effects by acting centrally. The baroreceptor-mediated release of AVP is induced by changes in blood volume and arterial pressure, that is, stretch receptors in the heart atria and the arterial baroreceptors. In addition, in sinoaortic-denervated animals, AVP release is reported to be facilitated. In sinoaortic-denervated animals, salt loading did not induce any increase in AVP as judged by plasma levels, whereas in sinoaortic-denervated animals with uninephrectomy, plasma levels of AVP significantly increased. In sinoaortic-denervated animals with uninephrectomy, sodium retention and elevation of serum sodium and osmolality were observed. By contrast, during the intravenous infusion of AVPX in sinoaortic-denervated animals with uninephrectomy, sodium retention was not found. These data raise the possibility that AVP release and sodium retention influence each other. Based on these considerations, we interpret the results to suggest that a subtle sodium retention induced a release of AVP and that elevated AVP activated the sympathetic nervous system and the vascular responses. This hypothesis is consistent with the conclusion of Gavras and Gavras that AVP acts as the ignition key in the earlier developmental stages of salt-induced hypertension.

The second major finding in this study was that blood pressure lability was reduced when blood pressure increased in SAD+NX animals with salt loading. There has been no direct evidence that explains the alterations in blood pressure lability induced by salt loading. Furthermore, although lability is the most consistent feature in sinoaortic-denervated animals, only a few studies investigating the influence of arterial pressure changes on lability in sinoaortic-denervated animals have been presented. Jacob et al demonstrated that hypertension per se does not reduce the variability of MAP. Cowley and Guyton demonstrated that in sinoaortic-denervated dogs, continuous intravenous infusion of isotonic saline induced a mild reduction in blood pressure lability, although there were no changes in normal controls. With normal arterial baroreceptor reflex function, blood pressure lability is reported to be closely related to arterial baroreceptor reflex sensitivity. In SAD, Raymundo et al have reported recently that the cardio pulmonary baroreceptor reflex (CPBR) contributes to regulation of peripheral resistance more strongly after SAD. It is therefore likely that CPBR might regulate the pressure lability in sinoaortic-denervated animals. Furthermore, there is evidence that salt loading sensitizes CPBR. Victor et al reported that high salt diet sensitized CPBR in Dahl salt-resistant rats. Similarly, high sodium intake is reported to augment forearm vaso-constrictor responses to lower body negative pressure in borderline hypertension. In both in vitro and in vivo studies, the baroreceptors were shown to respond to very small changes in sodium concentration. In our study, elevation of serum sodium was observed only in the SAD+NX rabbits in response to salt loading. It therefore is likely that salt-induced changes in ionic environment are involved in reduction of blood pressure lability under the sensitization of CPBR.

A second possibility is CPBR sensitization by humoral factors induced by salt loading. In the present study, elevations in ANP and AVP were found. ANP is known to act in the heart to stimulate cardiopulmonary vagal afferent discharge and thereby to inhibit sympathetic nerve activity. Ferrari et al reported that ANP potentiated the cardiac inhibitory arterial baroreceptor reflex. From these results, it is suggested that sensitization of the CPBR by ANP could be involved in the reduction of blood pressure lability.

Another humoral factor that is a candidate for sensitization of the CPBR is AVP. AVP is widely known to sensitize the CPBR, and intravenous injection of AVP reverses this sensitization. In our experiments, AVP increased significantly in response to salt loading, but bolus injection of AVP on day 4 did not reduce MAP-SD. Jacob et al demonstrated that AVP administration to sinoaortic-denervated rats did not alter the lability of blood pressure. Taken together, these results suggest that AVP is less likely involved in the reduction of blood pressure lability.

Last, the role of endogenous digitalislike factor should be considered, because this factor has been implicated as one of the major regulatory factors in salt-induced hypertension. Inhibition of Na+K+-ATPase with cardiac glycosides is known to sensitize both arterial and cardiopulmonary baroreceptors. Thus, endogenous digitalislike factor may be the candidate for the important regulatory factors in blood pressure lability as well as in blood pressure elevation in salt-induced hypertension.

In conclusion, these results imply that AVP plays a crucial role in the expression of salt-induced hypertension in rabbits with compromised baroreceptor and renal function.

References

1. Houston MC: Sodium and hypertension Arch Intern Med 1986;146:179-185
4. Hatzinikolaou P, Gavras H, Brunner HR, Gavras I: Role of vasopressin, catecholamines, and plasma volume in hypertonic
12. Kusuhara M, Yamaguchi K, Nagasaki K, Hayashi C, Suzaki A, Hori S, Handa S, Nakamura Y, Abe K. Production of endo-
18. Weinstock M, Scher AM, O'Leary DS, Sampson PD. Cardio-
26. Cowley AW Jr, Laird JF, Guyton AC: Role of the barorecep-
29. Raymundo H, Scher AM, O'Leary DS, Sampson PD. Cardio-
30. Mark AL, Lawton WJ, Abboud FM, Fitz AE, Connor WE, Hestad DD: Effects of high and low sodium intake on arterial pressure and forearm vascular resistance in borderline hyperten-
31. Brown AM: Receptors under pressure: An update in barore-
35. Hassel EM, Undesser KP, Bishop VS. Interaction of vasopres-

**KEY WORDS** • sodium-dependent hypertension • blood pressure • nephrectomy • vasopressin • atrial natriuretic peptides • sodium • rabbit studies
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Hypertension. 1991;17:1085-1091
doi: 10.1161/01.HYP.17.6.1085

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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