Salt-Dependent Hypertension in the Sinoaortic-Denervated Rat

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To determine the extent to which baroreceptor function is a determinant of salt-dependent hypertension, we studied the cardiovascular and renal responses to increasing dietary sodium chloride in sinoaortic-denervated (n=9) and sham-denervated (n=9) Sprague-Dawley rats. Rats were instrumented with an arterial catheter for measurement of arterial pressure and were individually housed for daily measurements of water intake, sodium intake, urinary output, and urinary sodium excretion. Arterial pressure was monitored daily over a 30-minute period by computer. After 3 days of control measurements (0.4% sodium chloride diet), dietary sodium chloride was increased to 8.0% for 21 days, followed by a 3-day recovery period (0.4% sodium chloride). Ingestion of an 8.0% sodium chloride diet resulted in a 20- to 25-fold increase in sodium intake and a fivefold increase in water intake in both groups. In sinoaortic-denervated rats, arterial pressure increased approximately 10 mm Hg on days 5-10, 20 mm Hg on days 11-18, and 30 mm Hg on days 19-21 of 8.0% sodium chloride. Arterial pressure returned to control levels within the first 24 hours of the recovery period. Elevated sodium intake had no significant effect on arterial pressure in the sham-denervated group. Finally, there were no significant differences between groups in urine output or urinary sodium excretion at any time during the study. We conclude that a primary impairment in the afferent limb of the arterial baroreceptor reflex results in salt-dependent hypertension in the Sprague-Dawley rat.

KEY WORDS • baroreceptors • sodium-dependent hypertension • sympathetic nervous system

Despite the established relation between dietary sodium and hypertension, the mechanisms whereby increased salt intake chronically elevates arterial pressure are not well understood. One hypothesis is that some forms of salt-sensitive hypertension result from impairment of cardiovascular sympathetic reflexes. This hypothesis is consistent with reports of attenuated arterial baroreceptor and cardiopulmonary reflexes in the Dahl salt-sensitive (DS) rat compared with Dahl salt-resistant (DR) controls. However, two arguments can be made against this hypothesis. First, DS rats have attenuated pressure-natriuretic responses before developing hypertension, which may explain their predisposition to salt-induced elevations of arterial pressure. Second, the conclusion that impaired reflex control of the circulation leads to salt-sensitive hypertension infers that, under normal conditions, hypertension is prevented by baroreceptor reflexes. This is not compatible with the idea that baroreceptors adapt to chronic changes in pressure and are therefore unable to chronically regulate arterial pressure.

The present study was conducted to directly test the hypothesis that a primary dysfunction of the arterial baroreceptor reflex results in salt-sensitive hypertension. We examined the effect of increased dietary intake of sodium chloride (NaCl) on arterial pressure in Sprague-Dawley rats with normal and impaired arterial baroreceptor reflexes. Impaired baroreceptor function was produced by surgical interruption of the afferent projections of the sinoaortic baroreceptors. Our results support the hypothesis that failure to observe salt-induced increases in arterial pressure in normal rats is, in part, dependent on a normally operating arterial baroreceptor reflex.

Methods

Experimental Procedures

Surgical procedures. One week before instrumentation for cardiovascular studies, male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) were randomly selected to undergo either sinoaortic denervation (SAD, n=9) or sham SAD (SHAM, n=9). Rats were anesthetized with pentobarbital (50 mg/kg) and atropinized (0.4 mg/kg) with a single intraperitoneal injection. SAD and SHAM surgeries were performed as previously described using the method of Krieger. A 0.4% NaCl diet (Research Diets, New Brunswick, N.J.) and distilled water were provided ad libitum throughout the recovery period. One week later, rats were anesthetized as above and instrumented with a chronic indwelling Silastic arterial catheter as previously described. On recovery from anesthesia, rats were placed in individual stainless steel metabolic cages. A 0.4% NaCl powdered diet and distilled water were provided ad libitum.

Experimental protocol. All variables were measured in unrestrained, chronically instrumented rats at rest in their home cage. Three days after catheter implanta-
tion, daily measurements of mean arterial pressure (MAP), heart rate, food intake, water intake, and urine output were begun. Measurements were made for 27 consecutive days, which were divided into three experimental periods: 1) control (days 1–3, 0.4% NaCl), 2) high NaCl (days 4–24, 8.0% NaCl), and 3) recovery (days 25–27, 0.4% NaCl). Variables were measured between the hours of 8:30 AM and 2:00 PM. MAP was measured by connecting the arterial catheter to a pressure transducer coupled to a polygraph (Grass Instruments, Inc., Quincy, Mass.). The MAP signal was monitored for 30 minutes by computer at a sampling rate of 1 Hz as previously described. The average and standard deviation of MAP was calculated from the 1,800 data points for each recording period. The standard deviation of MAP was used as an index of the lability of MAP. Heart rate was measured by increasing the chart speed and counting peaks on the pulsatile pressure tracing. Twenty-four-hour food and water intakes and urine output were measured using standard methods. Sodium intake was calculated from food intake (grams per day) and sodium content of the diet (0.4% NaCl, 0.07 meq/g; 8.0% NaCl, 1.00 meq/g). Urinary sodium concentration was measured with an ion-specific electrode (Nova Biomedical, Waltham, Mass.). Urinary sodium excretion was calculated as the product of urine flow rate and urinary ion concentration. Finally, in a subset of SHAM (n=5) and SAD rats (n=6), 200-μl samples of arterial blood were obtained for determination of total plasma protein concentration. Samples were obtained on the third control day and days 1, 7, 14, and 21 of increased dietary NaCl. Samples were immediately centrifuged, and protein concentration was determined with a Reichert refractometer (Cambridge Instruments, Buffalo, N.Y.).

**Statistical Analysis**

Values for the 3 control days were averaged to obtain a single control value for each group. Between-group comparisons of these control values were made using the unpaired Student's t test. To compare the effects of increased dietary NaCl between SHAM and SAD groups, we first normalized data by calculating the change from control for each group. Statistical analysis of variables over time was performed only for rats in which MAP was successfully measured. The number of SHAM rats included in the analysis was: days 1–15, n=9; days 16–18, n=8; days 19–21, n=7; days 22–27, n=6. The number of SAD rats included in the analysis was: days 1–15, n=9; days 16–20, n=8; days 21–25, n=7; days 26–27, n=5. Between-group comparisons were made by analysis of variance. A significant F ratio was followed by Duncan's multiple range test. The relation between arterial pressure lability and hypertensive responses to increased dietary NaCl was determined by linear correlation analysis.

**Results**

During the control period, MAP was slightly but significantly lower in SAD (95±1 mm Hg) compared with SHAM (102±2 mm Hg) rats. More importantly, increasing dietary NaCl significantly increased MAP in SAD but not SHAM rats (Figure 1). MAP rose gradually in SAD rats beginning 5 days after dietary NaCl was increased and had increased significantly more than in SHAM rats by day 11. With the exception of an unexplainable drop on days 17 and 18, MAP continued to increase in SAD rats, reaching a level of 32±7 mm Hg above control the 21st day of 8.0% NaCl. MAP returned to control levels within the first 24 hours of the recovery period.

Lability was twice as high in SAD (11.6±1.4 mm Hg) compared with SHAM rats (6.11±0.4 mm Hg; p<0.05) as a group. Furthermore, there was a significant correlation between the lability of MAP in SAD rats and the change of MAP observed on the last day of 8.0% NaCl (Figure 1). Although there was a range of lability in SHAM rats (3.5–8.9 mm Hg), there was no correlation with changes in MAP.

Hypertension in SAD rats was not due to increased renal retention of sodium and water relative to SHAM rats. Before increased dietary NaCl, there were no differences between SHAM and SAD rats for sodium intake (0.71±0.07 versus 0.69±0.03 meq/day), sodium excretion (0.61±0.08 versus 0.56±0.07 meq/day), or water intake (15.3±2.12 versus 12.7±0.9 ml/day). Urine output was only slightly less (p<0.05) in SAD rats (4.4±0.5 ml/day) than in the SHAM group (6.3±0.6 ml/day) during the control period. Furthermore, NaCl induced changes in sodium and water intake; urine output and urinary sodium excretion were not significantly different between SHAM and SAD groups (Figure 2). Failure to observe differences between groups...
for sodium and water handling is supported by measurements of total plasma protein concentration (g/100 ml). There were no differences in total plasma protein concentration for SHAM versus SAD groups during the control period (5.4±0.2 versus 5.3±0.1) or days 1 (5.4±0.2 versus 5.3±0.1), 7 (5.7±0.1 versus 5.3±0.1), 14 (6.0±0.2 versus 6.2±0.1), or 21 (6.3±0.1 versus 6.4±0.1) of 8.0% NaCl.

Discussion

Reports of impaired arterial1–3 and cardiopulmonary4 baroreceptor reflexes before salt-induced elevations of arterial pressure in the DS rat have led to the hypothesis that neurogenic mechanisms are at least partly responsible for salt-sensitive hypertension in this model.11 It is difficult, however, to implicate a single mechanism, because numerous other differences between the DS and DR rat have been found. For example, DS rats have established renal dysfunction before the development of hypertension,4 which also may explain their hypertensive responses to increases in dietary salt.

In the present investigation, we studied the cardiovascular effects of increased dietary NaCl in Sprague-Dawley rats with intact or denervated arterial baroreceptors. This approach allowed us to isolate and study the effect of a primary dysfunction of the baroreceptor reflex, independent of renal dysfunction, on salt sensitivity of arterial pressure. The contradictory results of previous studies using this approach12,13 may be due to the fact that arterial pressure was measured in restrained rats with the use of indirect methods. In the present study, arterial pressure was monitored by computer in unrestrained rats. Our results clearly demonstrate that, in rats with an impaired baroreceptor reflex, large increases in sodium and water intake result in a slowly developing but rapidly reversible hypertension. Furthermore, there was a direct relation between the degree of baroreceptor reflex impairment and the hypertensive response to increased salt and water intake. This is suggested by the correlation between a quantitative measure of baroreceptor function, arterial pressure lability, and the hypertensive response to sodium in SAD rats. These results suggest that a normally operating arterial baroreceptor reflex is required to prevent chronic salt-induced increases in arterial pressure. This idea is not compatible with previous studies that have shown that SAD did not affect chronic regulation of arterial pressure in experimental14,15 or spontaneous16 hypertension. The implication of these previous findings is that, although the baroreceptor reflex actually buffers changes in blood pressure, chronic regulation of pressure is not dependent on this reflex because of the resetting properties of arterial baroreceptors.7

If baroreceptors reset to chronic changes in arterial pressure, then what is the underlying mechanism of salt-dependent hypertension in the SAD rat? There are at least three possibilities. First, SAD may in some way reduce renal excretory capacity. This would result in plasma volume expansion in SAD rats on a high salt diet and ultimately in hypertension.7 However, there were no differences in urinary sodium and water excretion between the two groups, despite equivalent increases in sodium and water intake. Moreover, if volume expansion had occurred in SAD rats, this should have resulted in a dilution of plasma proteins. However, plasma...
protein concentrations were virtually identical in both groups. It is unlikely that SAD would significantly impair volume regulatory mechanisms, because systems that chronically maintain body fluid homeostasis, such as the renin-angiotensin-aldosterone system, arginine vasopressin (AVP), atrial natriuretic peptide, and intrinsic renal mechanisms, are presumably unaltered by SAD. Indeed, plasma levels of renin, aldosterone, AVP, and atrial natriuretic peptide are not chronically affected by SAD. Based on this logic and our results, it is unlikely that salt-sensitive hypertension in the SAD rat was the result of a greater degree of plasma volume expansion relative to SHAM rats. However, this possibility cannot be ruled out until precise measurements of body fluid volumes are made.

A second possibility is that increased salt and water intake results in an equivalent degree of volume expansion in SAD and SHAM rats, but the hypertensive response to increased plasma volume is enhanced by SAD. This would explain the failure to find significant differences between SAD and SHAM rats in indicators of sodium and water balance or plasma protein concentration. Theoretically, such a volume expansion would lead to increased cardiac output. Presumably, the pressor response would be offset by reductions of peripheral vascular resistance in intact but not denervated rats. Indeed, this hemodynamic pattern has been observed in conscious DR and DS rats. Increased salt and water intake resulted in similar increases in blood volume and cardiac output in the DS and DR strains. Failure to observe hypertension in DR rats was the result of a decrease in peripheral vascular resistance, which did not occur in the DS rat. That observation, combined with the results of the present study, suggests that the hypertensive response to increased dietary NaCl in the DS rat may be partly the result of a dysfunction of the baroreceptor reflex.1-3

A third possibility is that salt-dependent hypertension in the SAD rat occurs in the absence of plasma volume expansion. Recent studies have shown that intragastric administration of a hypertonic NaCl solution elicits marked increases in circulating AVP in conscious rats via stimulation of splanchnic osmoreceptors.10 Hence, it is possible that AVP release occurs independent of changes in extracellular fluid osmolality or volume. Based on 24-hour intakes of sodium and water in the present study, the solution ingested on the 0.4% NaCl diet was hypotonic (approximately 50 meq of sodium per liter). However, on the 8.0% NaCl diet, the increase in water intake was small relative to the increase in sodium intake, resulting in the ingestion of a hypertonic solution (approximately 300 meq of sodium per liter). This raises the possibility that rats on an 8.0% NaCl diet have elevated plasma AVP levels. Indeed, increasing dietary NaCl intake from 0.4% to 8.0% increases plasma AVP in the Dahl rat.20 Whereas AVP has minimal pressor activity in the conscious normal rat, hypertensive responses to acute infusions of AVP are enhanced by SAD.17 It remains to be demonstrated whether salt-sensitive hypertension in the SAD rat is dependent on chronic elevations of plasma AVP.

In addition to its vasoconstrictor actions, the centrally mediated sympathoinhibitory effects of AVP may play a role in this model of hypertension. Compared with equipressor doses of other circulating vasoconstrictors, AVP results in a greater inhibition of sympathetic vasoconstrictor activity in conscious rats.21-22 Although the mechanism of this effect has not been elucidated in the rat, studies in the rabbit strongly suggest that circulating AVP decreases sympathetic outflow via interactions with the area postrema.23 It is of interest that the sympathoinhibitory response to electrical and chemical stimulation of the area postrema is dependent on input from both arterial and cardiopulmonary baroreceptors.24 Thus, it is possible that arterial baroreceptors play a permissive role in AVP-mediated sympathoinhibition. The relevance of this observation to salt-dependent hypertension is that, under conditions in which plasma AVP is elevated, sympathetic activity may be chronically suppressed via AVP interactions with the area postrema. Theoretically, this would occur even when baroreceptor resetting has occurred, because tonic baroreceptor activity would still be present. AVP-mediated sympathoinhibition would not occur in the SAD animal, however. The response of plasma AVP to increased salt may explain contradictions between studies regarding the effect of SAD on salt-loading hypertension. Although it has been reported that SAD had no effect on the steady-state level of arterial pressure in dogs with reduced renal mass hypertension, plasma volume was expanded by intravenous infusion of isotonic saline, a manipulation that would not stimulate AVP release. This contrasts with a recent report that intravenous administration of hypertonic saline produces hypertension in uninephrectomized SAD rabbits but not in nephrectomized rabbits with intact baroreceptors.16 Hypertension in SAD rabbits was associated with elevated plasma concentrations of AVP and norepinephrine compared with baroreceptor-intact rabbits. Chronic administration of a V1 antagonist prevented the increase in both plasma norepinephrine and hypertension. These observations suggest that AVP–baroreceptor reflex interactions are a critical component of this model of hypertension. Such interactions may play a key role in the differing response of normal animals and baroreceptor-denervated animals to chronic increases in dietary NaCl intake.

References
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