SUMMARY  High salt diet alters neural cardiovascular control. This influence has been attributed to central neural or efferent mechanisms. To test the hypothesis that a high salt diet might alter afferent baroreceptor function, Dahl salt-resistant (DR) and salt-sensitive rats (DS) were fed a high or a low salt diet. Blood pressure was measured intra-arterially in unanesthetized animals. Aortic baroreceptor function was then evaluated during urethane anesthesia by recording multifiber aortic depressor nerve activity during a phenylephrine-induced blood pressure ramp. Mean arterial pressure in the conscious state was elevated (155 ± 5 [SE] mm Hg) in DS fed a high salt diet but was normal in DS fed a low salt diet and in DR. Slopes of linear regressions relating aortic nerve discharge to mean arterial pressure were 71% higher in DR fed a high salt diet than in DR fed a low salt diet (p<0.025), indicating that high salt potentiated baroreceptor function in DR. In contrast, high salt diet produced no significant effects on baroreceptor function in DS. No salt-induced changes in dynamic or static aortic distensibility (assessed from pressure-volume curves of the in situ isolated arch) were detectable in either rat strain. Absence of salt-induced baroreceptor sensitization in DS was not due to the hypertensive state because the sensitization also failed to occur in separate groups of DS in which salt-induced hypertension had been prevented by chemical sympathectomy with 6-OH-dopamine. Thus, high dietary salt 1) potentiates afferent arterial baroreceptor function in DR, a phenomenon unrelated to aortic distensibility and probably resulting from sensitization of baroreceptors by high salt diet, and 2) fails to sensitize baroreceptors in DS, probably because of a primary abnormality in baroreceptor function in DS. (Hypertension 10: 55-60, 1987)

KEY WORDS  • salt  • baroreceptors  • Dahl rats  • cardiovascular reflexes  • hypertension

Dahl salt-sensitive rats (DS) become hypertensive when fed a high salt (HS) diet, whereas Dahl salt-resistant rats (DR) remain normotensive. There are profound differences in the effects of an HS diet on neural control of sympathetic nerve activity in DS and DR.1,2 These alterations in neurogenic control involve peripheral adrenergic1-2 and central neural3,4 mechanisms, but recent observations also indicate that between-strain differences exist in arterial baroreceptor function even during a rigorously low salt (LS) diet. The possibility that afferent neural mechanisms might be affected by dietary salt intake has been suggested by work from several laboratories. For instance, Takishita and Ferrario5 found that the ability of the arterial baroreceptor reflex to modulate renal sympathetic nerve activity is reduced in salt-depleted dogs. Rocchini et al.6 showed that the hypertensive response to bilateral carotid occlusion is blunted in sodium-depleted dogs. Thus, several studies suggest that chronic changes in dietary sodium can influence arterial baroreceptor reflex function, but these changes usually have been attributed to alterations in efferent mechanisms or to interactions with other reflexes, such as vagal afferents. In contrast, Andresen, Brown, and co-workers7,8 showed that ionic influences have important effects on afferent mechanisms. These investigators demonstrated that the discharge properties of
baroreceptors of an in vitro perfused aortic arch preparation and the reflex effects originating from the vascularily isolated carotid sinus are markedly affected by changing the concentration of sodium in the perfusion fluid.\textsuperscript{7,4}

In this study, we tested the concept that an HS diet might alter baroreceptor discharge properties and that this response might differ in DR and DS. Specifically, we hypothesized that DR adapt to HS diet by potentiating arterial baroreceptor discharge, whereas DS fail to exhibit salt-induced sensitization of arterial baroreceptors.

**Materials and Methods**

A total of 83 female DR and DS were studied. Rats were shipped from Brookhaven National Laboratories (Upton, NY, USA) 3 to 4 days after weaning, fed a diet with LS (0.4% NaCl) or HS (8% NaCl) content for 4 weeks, and studied during the fifth week of diet. Tap water was provided ad libitum throughout the study.

**Blood Pressure in Conscious State**

With the rats under light ether anesthesia, a cannula was introduced into the femoral artery, tunneled subcutaneously, and exteriorized at the interscapular region. After 24 hours, the rat was placed in a wide box, the arterial cannula was connected to a transducer whose zero reference level was 3 cm above the floor of the box, and the animal’s blood pressure was recorded for 40 minutes in a quiet environment. Blood pressure in the conscious state was defined as the average of the blood pressure values observed during the last 15 minutes of recording.

**Serum Electrolyte Sampling**

After the blood pressure recording was completed, 0.5 ml of blood was withdrawn from the arterial cannula and an equal amount of saline was reinfused. Serum sodium and potassium were measured spectrophotometrically.

**Aortic Baroreceptor Function**

On the same or the following day, the animal was anesthetized with urethane (1.25 g/kg i.p.). A cannula was introduced into the left femoral vein for drug injection. Through a midline ventral neck incision, the trachea was cannulated and the left aortic depressor nerve (ADN) was identified at its junction with the superior laryngeal nerve, dissected free of connective tissue, cut centrally, and placed on an Ag-AgCl recording electrode in a pool of mineral oil. Multifiber afferent neural activity was amplified using a Grass P511 bandpass amplifier (Quincy, MA, USA) with high and low frequency cutoff at 3000 and 30 Hz, respectively. The output of the amplifier was visualized on a Tektronix storage oscilloscope (Beaverton, OR, USA) and heard from an audioamplifier and loudspeaker. The output was also sent to a nerve traffic analyzer, which counted spikes exceeding a bias voltage set just above noise level. The counter’s time bin was set at 1 second so that impulse frequency was displayed in hertz. The ganglionic blocker chlorisondamine, 5 mg/kg, was then administered intravenously; the rationale for inducing ganglionic blockade was twofold: first, mean arterial pressure (MAP) was lowered in all groups of rats to similar levels of about 50 mm Hg; second, autonomic reflexes that could affect afferent baroreceptor function, such as changes in heart rate and in effenter sympathetic nerve activity, were abolished.

To assess the characteristics of aortic baroreceptor discharge, MAP was raised from baseline to 160–200 mm Hg over 2 minutes by an i.v. infusion of phenylephrine at progressively increasing doses (from 5 to 120 μg/kg/min), and ADN activity was simultaneously recorded. Values of ADN discharge frequency were calculated before starting the vasoconstrictor drug infusion, and at 20 mm Hg steps, MAP rose from 80 to 160–200 mm Hg during the drug-induced rise in pressure. Absolute values of ADN discharge during drug infusion were converted to percentage changes from baseline ADN discharge frequency before the infusion.

**Studies on the Mechanical Properties of the Aortic Arch**

To determine if HS diet influenced aortic wall mechanical properties (which in turn could have influenced baroreceptor discharge), we measured distensibility (both static and dynamic), weight, and un-stressed volume of the aortic arch. To measure distensibility, pressure-volume relationships of the in situ isolated aortic arch were analyzed. Two PE-50 saline-filled cannulas were introduced into the aortic arch, one through the right common carotid artery and the other one through the thoracic aorta. The tips of the two cannulas were positioned and tied at the origin of the innominate artery and at the level of the left subclavian artery, respectively. The left carotid and subclavian arteries and the aortic root were ligated. The right carotid cannula was connected to a pressure transducer; the other cannula was first opened to drain the aortic arch down to zero pressure volume and then connected to a saline-filled microsyringe by which stepwise distentions of the aortic arch could be performed.

To assess static distensibilities in DS and DR, slow distentions were made by manually operating a 100-μl syringe in seven to 10 steps of 10-μl each over 40 to 60 seconds.

To assess dynamic as well as static distensibility in DR, we performed separate experiments in which a 100-μl syringe was connected to a speed-adjustable step injector (5.5-μl per step) so that the distention could be performed very quickly (in about 1.5 seconds).

After the pressure-volume curves were obtained, the aortic arch was drained to lower intra-aortic pressure to zero. The aortic arch was then sealed, excised, and weighed before and after all its fluid content was extruded, so that the un-stressed fluid volume could be calculated by assessing the difference in weight before and after extruding the fluid.
Aortic Baroreceptor Function in 6-OH-Dopamine-Treated DS

Unlike DR, DS become hypertensive when fed an HS diet. To separate the effects of HS diet from the effects of the hypertension in DS, baroreceptor function was evaluated in a separate group of DS in which development of salt-induced hypertension was prevented by chemical sympathectomy with 6-OH-dopamine (75-150 mg/kg i.p. twice a week) administered for the entire period of diet feeding. A concurrent control group of vehicle-treated animals was also studied to allow discrimination between the effects of 6-OH-dopamine and the effects of vehicle injections.

Statistical Analysis

The statistical analysis compared the effects of HS and LS diets within each rat strain, since the goal of this study was to determine effects of HS diet on baroreceptor function. We did not focus on differences between rat strains on the LS diet, since this had been analyzed specifically in a previous study.9 Data on blood pressure in the conscious state, on aortic arch unstressed volume, and on aortic arch weight were analyzed by t test for unpaired observations. Data on baroreceptor function and on aortic distensibility were compared by factorial analysis of the linear regressions relating 1) percentage increases in ADN activity with increases in MAP and 2) increases in aortic arch pressure with increases in aortic arch volume. For all comparisons the level of statistical significance was set at a p level below 0.05.

Results

Blood Pressure and Serum Electrolytes

No significant difference in MAP was observed between conscious DR fed the HS diet and DR fed the LS diet (109 ± 5 vs 105 ± 3 mm Hg). On the other hand, MAP was significantly higher in DS fed the HS diet than in DS fed the LS diet (155 ± 3 vs 117 ± 4 mm Hg; p < 0.01).

As shown in Table 1, no significant differences in Na+ and K+ serum levels were observed in either rat strain in relation to salt intake.

Aortic Baroreceptor Function

Figure 1 is a representative example of the filtered neurograms recorded from the aortic nerve at various aortic distending pressures. The average data showing the effects of HS diet on ADN discharge—aortic pressure relationships are reported in Figure 2. In DR, the slopes for increases in ADN activity divided by MAP were significantly higher in the HS compared with the LS diet group (i.e., a salt-induced potentiation of baroreceptor function was observed). In DS, on the other hand, the slopes were not significantly different under the two diets; indeed, a tendency toward decreased rather than increased slopes was observed in DS fed the HS diet compared with DS fed the LS diet.

TABLE 1. Serum Na+ and K+ Levels in DR and DS Fed a High or Low Salt Diet

<table>
<thead>
<tr>
<th>Ion</th>
<th>LS (n = 7)</th>
<th>HS (n = 6)</th>
<th>LS (n = 5)</th>
<th>HS (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+ (mEq/L)</td>
<td>141.3 ± 1.2</td>
<td>142.5 ± 1.1</td>
<td>143.2 ± 0.9</td>
<td>141.6 ± 0.7</td>
</tr>
<tr>
<td>K+ (mEq/L)</td>
<td>4.1 ± 0.2</td>
<td>4.7 ± 0.4</td>
<td>4.2 ± 0.4</td>
<td>4.2 ± 0.3</td>
</tr>
</tbody>
</table>

Entries are means ± SE. In no case was the HS vs LS difference statistically significant. LS = low salt; HS = high salt.
Studies on the Mechanical Properties of the Aortic Arch

Figure 3 is an example of one dynamic and one static aortic arch distention. Data on the effects of HS diet on static pressure-volume relationships are shown in Figure 4. There was no significant effect of HS diet on static pressure-volume curves in either strain.

Dynamic aortic distensibility was also examined in a separate group of DR. Average data from these experiments are reported in Figure 5. There were no significant salt-induced differences in the dynamic or static aortic distensibility in DR.

The unstressed volume (9.7 ± 0.2 vs 9.1 ± 0.7 μl/100g; n = 6 per group) and weight of the aortic arch (11.1 ± 0.3 vs 10.7 ± 0.7 mg/100g; n = 7 per group) were also not significantly different between DR fed the HS or the LS diet.

Aortic Baroreceptor Function in 6-OH-Dopamine-Treated DS

As expected,1 salt-induced hypertension failed to develop in 6-OH-dopamine–treated but not in vehicle-treated DS. In 6-OH-dopamine–treated DS, MAP was 115 ± 6 mm Hg on the LS diet and 121 ± 6 mm Hg on the HS diet. In vehicle-treated DS, MAP was 116 ± 4 mm Hg on the LS diet and 146 ± 2 mm Hg on the HS diet (p < 0.02). Despite prevention of hypertension, studies of ADN discharge–aortic pressure relationships did not unmask salt-induced potentiation of baroreceptor discharge in 6-OH-dopamine–treated DS (Figure 6).

Discussion

The principal finding in this study is that a long-term HS diet sensitizes aortic baroreceptor afferents in DR but not in DS. Three methodological points need some comment. First, ADN activity was recorded from a multifiber preparation, which prevents us from determining the number of active fibers and the proportion of medullated versus nonmedullated fibers in each recording. Therefore, based on these data we cannot state whether the increased discharge of ADN in DR fed the HS diet as compared with DR fed the LS diet results from recruitment of more fibers, from a difference in relative population of medullated versus nonmedullated fibers, or from a greater discharge of a given population of active fibers. Thus, our observations relate to overall aortic baroreceptor afferent activity.

A second methodological point is related to the use of phenylephrine as a vasoconstrictor to produce the MAP ramps. This agent, especially at high doses, has two effects that could have influenced our results: a direct sensitizing effect on baroreceptors10 and a prominent constrictor effect on arterial muscle, which could indirectly influence firing of baroreceptors.11 In our opinion, it is unlikely that these effects of phenylephrine importantly affected our comparisons because there is no salt-induced difference in response to α-adrenergic receptor stimulation in either DR or DS,2 and in the present experiments we observed no salt-related differences in the blood pressure rise induced by phenylephrine in either rat strain. Moreover, in studies of small groups of DR fed HS or LS diet, in which angiotensin II rather than phenylephrine was used to produce the pressor ramp, the slopes of the ADN discharge–aortic pressure relationship were 48% larger in the HS than in the LS diet group (i.e., salt-induced potentiation of ADN discharge was still present). The third point deals with the possibility that the marked blood pressure fall produced by ganglionic
blockade may have been accompanied by short-term changes in vasopressin and angiotensin II that potentially were able to respectively enhance and depress baroreceptor function. The magnitude of such changes may have been different in DR and DS, thus complicating the interpretation of our results. However, at either LS or HS intake vasopressin is higher and angiotensin is lower in DS than in DR; this probably would theoretically be more prone to produce baroreceptor sensitization in DS than in DR, but this was not the case. This suggests that short-term changes in vasopressin or angiotensin, or both, are unlikely to have influenced our results. It also suggests that even long-term changes in these humoral factors (i.e., those induced by HS diet) may not have been involved in the changes in baroreceptor function we observed.

The mechanisms underlying the salt-induced augmentation of aortic baroreceptor function in DR are unsettled, but several possibilities can be considered. In DR, salt-induced baroreceptor sensitization was not accompanied by alterations in static or dynamic distensibility curves or in unstressed volume of the aortic arch. This finding suggests that salt-induced differences in the mechanical properties of the aortic wall were not the cause of the augmented aortic baroreceptor afferent activity.

Diet-related changes in the ionic concentration at the receptor sites is another mechanism that has the potential for altering baroreceptor function. We measured Na⁺ and K⁺ in the serum in our experimental animals and found no diet-related differences. Moreover, intracellular Na⁺ and K⁺ in arterial smooth muscle cells have also been found to be unaffected by Na⁺ intake in Dahl rats. Salt-induced changes in serum or arterial wall Ca²⁺, or both, may have played a role in modifying baroreceptor function; we do not have data on serum Ca²⁺ in Dahl rats fed LS and HS diets.

A third possibility, which is speculative, is that humoral factors involved in the adjustments to HS intake might sensitize arterial baroreceptors to their mechanical stimulus. For example, in DR HS diet is purported to promote secretion of a natriuretic hormone that inhibits Na⁺,K⁺-adenosine triphosphatase (ATPase) has been shown to sensitize arterial and cardiac baroreceptors both acutely and chronically in animals and humans.

Why was the salt-induced augmentation of baroreceptor function not observed in the DS? In DS, lack of salt-induced baroreceptor sensitization might have been due to baroreceptor resetting or to impairment secondary to the development of hypertension, or to both. This possibility is unlikely, however, because salt-induced baroreceptor sensitization was also absent in the group of DS in which development of salt-induced hypertension was prevented by chronic chemosympathectomy.

A more likely explanation can be found in our previous studies on mechanoreceptors in DS and DR. We found that DS have impaired reflex responses arising from both arterial and cardiopulmonary baroreceptors even in the prehypertensive stage during LS diet. For the arterial baroreceptors, the defect was localized at the afferent level. Since this defect was detected in DS fed LS diet before any blood pressure rise, it is presumably primary rather than secondary and most likely due to genetic factors. We propose that the absence of salt-induced augmentation of baroreceptor function in the present study may be explained by a genetically determined defect in DS.

The importance of the findings of this study is two-fold. First, they indicate that changes in neural cardiovascular control mechanisms in response to high sodium intake may encompass afferent mechanisms in addition to central and efferent mechanisms. Second, we speculate that salt-induced sensitization of baroreceptor function might help protect DR against salt-induced hypertension by restraining sympathetic nerve activity. Conversely, lack of salt-induced augmentation of arterial baroreceptor function might contribute to salt-induced hypertension in DS.

In conclusion, we demonstrated that HS diet potentiates afferent arterial baroreceptor function in DR. This potentiation is not explained by changes in aortic distensibility. The mechanism underlying the potentiation is uncertain, but we speculate that a humoral substance with baroreceptor-sensitizing properties might be implicated. HS diet failed to augment afferent baroreceptor function in DS, even when salt-induced hypertension was prevented by chemical sympathectomy. The absence of salt-induced augmentation of
baroreceptor function in DS probably is related to an underlying primary abnormality in baroreceptor function in DS.

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