Effect of Arterial Baroreceptor Denervation on Sodium Balance

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Abstract—During chronic increased dietary sodium intake, arterial baroreceptors buffer against sustained increases in arterial pressure, and renal sympathoinhibition contributes importantly to the maintenance of sodium balance by decreasing renal tubular sodium reabsorption and increasing urinary sodium excretion. The present study examined the effect of arterial baroreceptor denervation on sodium balance in conscious rats during low, normal, and high dietary sodium intake. Compared with measurements made before arterial baroreceptor denervation, arterial baroreceptor–denervated rats had similar sodium balance during normal dietary sodium intake but significantly more negative sodium balance during low dietary sodium intake and significantly more positive sodium balance during high dietary sodium intake. At the end of the high dietary sodium intake period, arterial pressure (under anesthesia) was 159 ± 5 mm Hg after arterial baroreceptor denervation and 115 ± 1 mm Hg before arterial baroreceptor denervation. Sham arterial baroreceptor denervation in time control rats had no effect on sodium balance or arterial pressure during the different dietary sodium intakes. These studies indicate that (1) arterial baroreceptor denervation impairs the ability to establish sodium balance during both low and high dietary sodium intake, and (2) arterial baroreceptor denervation leads to the development of increased arterial pressure during high dietary sodium intake in association with increased renal sodium retention. (Hypertension. 2002;40:547-551.)

Key Words: renal nerves ■ sodium ■ arteries ■ baroreceptors

Rats with arterial baroreceptor denervation (SAD) exhibit salt (NaCl)-sensitive hypertension.1–3 Several mechanisms have been considered for this finding. SAD could impair volume homeostasis so that SAD rats have a greater degree of blood volume expansion during increased dietary sodium intake than do intact rats. Although blood volume measurements are lacking, this has been thought an unlikely explanation because both sodium and water intake and excretion have been reported not to differ between SAD and intact rats during increased dietary sodium intake.2 Because of central resetting, it is unlikely that SAD would result in chronic increases in basal (renal) sympathetic nerve activity (RSNA); it is possible that SAD rats are unable to normally suppress RSNA during chronic increases in dietary sodium intake.2,3

It is well known that changes in RSNA influence renal tubular sodium reabsorption and urinary sodium excretion (reviewed in DiBona and Kopp4). Increased RSNA increases renal tubular sodium reabsorption and results in antinatriuresis, whereas decreased RSNA decreases renal tubular sodium reabsorption and results in natriuresis. In normal rats, dogs, monkeys, and sheep, renal denervation impairs the natriuretic response to acute sodium loading. In pathological conditions characterized by chronic renal sodium retention, renal denervation studies indicate that 30% to 40% of the renal sodium retention is dependent on intact RSNA. During a chronic increase in dietary sodium intake in conscious normal dogs that produced a short duration transient increase in arterial pressure, unilateral renal innervation impaired urinary sodium excretion from the ipsilateral kidney.5 In conscious dogs infused with angiotensin II to the same level of increased arterial pressure, urinary sodium excretion from the innervated kidney doubled when arterial baroreceptors were intact, whereas it halved when arterial baroreceptors were denervated.6 Taken together, these results suggest that increased dietary sodium intake results in a signal that is sensed by the arterial baroreceptors and results in inhibition of sympathetic nerve activity. Reduction in sympathetic nerve activity to the heart and resistance vasculature buffers any increase in arterial pressure, whereas reduction in RSNA facilitates urinary sodium excretion and the reestablishment of sodium balance, thus minimizing renal sodium retention and blood volume expansion. This view receives support from the finding that the sustained hypertension occurring after chronic unloading of carotid baroreceptors was associated with a decrease in urinary sodium excretion in the face of a constant sodium intake, suggesting renal sodium retention.7

The current study was undertaken to explore the effect of arterial baroreceptor denervation on the ability of the kidney...
to establish sodium balance in response to both increases and decreases in dietary sodium intake.

Methods
Male Sprague-Dawley rats (weight, 325 to 375 g; Harlan, Indianapolis, Ind) were used for all experiments. All procedures in rats were performed in compliance with the University of Iowa policies and guidelines concerning the use of animals in research and teaching and the Guide for the Care and Use of Laboratory Animals (NIH publication No. 93-23, revised 1985).

Each rat was placed in an individual metabolic cage and provided with low sodium rat pellet diet (<0.004 meq Na/gm) and 50 meq/L NaCl drinking solution ad libitum (normal sodium intake [NNa], see below). They were allowed to equilibrate for a minimum of 7 days before the onset of the study.

The experimental protocol consisted of 2 portions, with the first portion being control and the second portion being sinoaortic denervation (SAD). Each portion consisted of the provision of 3 different levels of dietary sodium intake, each for 5 days and in the following order: normal sodium (NNa, days 1 to 5), low sodium (LNa, days 8 to 12) and high sodium (HNa, days 15 to 19). The rats consumed low-sodium rat pellet diet throughout and drank 50 meq/L NaCl drinking solution during NNa, tap water (0 meq/L NaCl) during LNa, and 154 meq/L NaCl drinking solution during HNa. At the end of a dietary period (days 6 and 13), the next diet in sequence was instituted and balance measurements started 2 days later after the immediate transient changes and during more steady-state conditions (days 8 and 15). At the end of the control portion on day 20, the rats were anesthetized with methohexital sodium (50 mg/kg IP), and a catheter was inserted into a femoral artery. Arterial pressure (MAP) and heart rate (HR) were measured for a 2-hour period, after which the MAP and HR responses to intravenous injection of norepinephrine sufficient to increase MAP by a minimum of 30 mm Hg were measured. Then, SAD was performed. Thereafter, the rats were allowed to recover from surgery while consuming NNa. Ten days later in the SAD portion of the experimental protocol, the above-described scheme of dietary interventions and measurements was repeated in its entirety. At the end of the SAD portion, the rats were reanesthetized, and femoral arterial and venous catheters were inserted. A continuous infusion of 5% dextrose in water at 0.05 mL/min was begun into the femoral vein. A 2-hour recording of MAP and HR was made. Then, the MAP and HR responses to an intravenous injection of norepinephrine sufficient to increase MAP by a minimum of 30 mm Hg were measured.

A separate group of rats was subjected to the same overall experimental protocol except that sham SAD was performed.

Analytical Measurements
Sodium concentrations in drinking fluid and urine were made with flame photometry. The arterial pressure catheter was connected to an electronic strain-gauge pressure transducer (Statham P23d), of which the pulsatile output drove a cardiotachometer (Grass P44) for the measurement of HR. Both HR and MAP were recorded on a direct writing ink recorder (Grass 7D). MAP and HR were digitized at 5 Hz and averaged using 1-second time bins. The SD was used as an index of variability.

Calculations were made as follows. Daily sodium intake=mn (milliliters drinking solution consumed per day)×(sodium concentration of drinking solution). Daily sodium output=unt (urine volume per day)×(urinary sodium concentration). Daily sodium balance=daily sodium intake−daily sodium output. Cumulative sodium balance per diet period was calculated as continuous summation of daily sodium balance within each 5-day dietary period. Cumulative sodium balance overall was calculated as continuous summation of daily sodium balance across all three dietary periods. Similar calculations were made for water balance.

Statistical Analysis
Statistical analysis was performed with ANOVA with the subsequent use of Scheffé’s method for simultaneous comparisons within groups and the subsequent use of the F ratio and modified statistic for nonsimultaneous comparisons between groups. A significance level of 5% was chosen. Data in the text, tables, and figures are expressed as mean±SE.

Results
Figure 1 shows the results for daily sodium intake, daily sodium output, and daily sodium balance for the control and SAD phases of the experimental group. Daily sodium intake was not significantly different between controls and SAD during NNa, LNa, and HNa. During NNa, daily sodium output and daily sodium balance were similar between controls and SAD. During LNa, daily sodium output was significantly greater and daily sodium balance was significantly more negative in SAD than in controls. During HNa, daily sodium output was significantly less and daily sodium balance was significantly more positive in SAD than in controls. Figure 2 shows the daily sodium balance data on a larger scale. After SAD, daily sodium balance was similar to that of controls during NNa, more negative than that of controls during LNa (P<0.05 for days 8 to 12), and more positive than that of controls during HNa (P<0.05 for days 15 to 19).

In regard to water balance (data not shown), daily water intake was not significantly different between controls and SAD during NNa, LNa, and HNa. Daily water output, daily water balance, and cumulative water balance (per diet period, overall) were not significantly different between controls and SAD during NNa, LNa, and HNa.

Figure 3 shows the cumulative sodium balance data per diet period. During NNa, cumulative sodium balance was similar between controls and SAD. During LNa, cumulative sodium balance was more negative in SAD than in controls (P<0.05 for days 10 to 12). During HNa, cumulative sodium balance was more positive in SAD than in controls (P<0.05 for days 17 to 19).

Cumulative sodium balance data overall was similar to cumulative sodium balance data per diet period. During NNa,
cumulative sodium balance was similar between controls and SAD. During LNa, cumulative sodium balance was less positive in SAD than in controls. During HNa, cumulative sodium balance was more positive in SAD than in controls ($P<0.05$ for days 18 to 19).

Figure 4 shows the data on MAP, standard deviation of MAP (SD-MAP), and cumulative sodium balance overall at the conclusion of the HNa period. MAP ($P<0.01$), SD-MAP ($P<0.01$) and cumulative sodium balance overall ($P<0.05$) were greater in SAD than in controls. HR variability was also greater in SAD than controls (36±9 versus 16±5 bpm, $P<0.05$).

In the sham SAD experimental protocol ($n=10$), daily sodium intake, daily sodium output, daily sodium balance, cumulative sodium balance per diet period, and cumulative sodium balance overall were not different between the control and sham SAD portions. Figure 5 shows the data on MAP, SD-MAP, and cumulative sodium balance overall at the conclusion of the HNa period. MAP, SD-MAP, and cumulative sodium balance overall were similar in sham SAD and controls.

In regard to verification of SAD, norepinephrine produced a similar increase in MAP in controls (40±3 mm Hg) and SAD (38±5 mm Hg) and produced a reflex decrease in HR in controls (36±5 bpm) but not in SAD (32±7 bpm).

**Discussion**

The major findings of the current study are as follows: (1) arterial baroreceptor denervation impairs the ability to establish sodium balance during both low and high dietary sodium intake, and (2) arterial baroreceptor denervation leads to the development of increased arterial pressure during high dietary sodium intake (i.e., NaCl-sensitive hypertension) in association with increased renal sodium retention.
A prior study in SAD rats exposed to different dietary sodium intakes did not detect changes in sodium intake or excretion between SAD and intact rats. In this study, the pellet food contained nominally zero sodium, and the entire dietary sodium intake was provided via the sodium content of the drinking solution. This approach facilitates a precise measurement of both dietary sodium intake, as it is provided in an easily measurable form, and urinary sodium output is uncontaminated by sodium-containing food.

Arterial baroreceptor denervation resulted in a measurable degree of impairment in the renal responses to both decreases and increases in dietary sodium intake. During LNa, daily sodium balance was more negative owing to greater urinary sodium excretion, and during HNa, daily sodium balance was more positive owing to lesser urinary sodium excretion in SAD than in controls. Thus, during LNa, SAD rats lost more sodium than normal, and during HNa, SAD rats retained more sodium than normal. Therefore, of the multiple mechanisms thought to participate in the normal renal responses to increases and decreases in dietary sodium intake, arterial baroreceptors are of quantitative significance in that their functional absence results in measurable abnormalities in the renal regulation of sodium balance.

Given the overall importance to the organism of defending sodium balance and the fact that only one of the multiple mechanisms involved in its defense was removed, it was anticipated that the size of the sodium deficit and excess after SAD would not be large. The continued and likely increased activity of the several other mechanisms involved in the renal response to both decreases and increases in dietary sodium intake would be expected to limit the magnitude of both the sodium deficit and excess observed in this relatively short-term study. These mechanisms would also be expected to ultimately permit daily sodium balance to be achieved. For example, afferent cardiopulmonary receptor innervation remained intact, which can, in response to alterations in central cardiopulmonary volume or pressure stimuli, regulate both RSNA and the activity of the renin-angiotensin system, via its influence on renin secretion rate (reviewed in Dibona and Kopp). The resultant alterations in both RSNA and the activity of the renin-angiotensin system would contribute to the appropriate renal responses to both increases and decreases in dietary sodium intake.

As others have observed, arterial baroreceptor denervation results in NaCl-sensitive hypertension. The magnitude of the increase in MAP observed here (under anesthesia), 44±4 mm Hg, is somewhat greater than the 32±7 mm Hg increase found when daily 30-minute intraarterial recordings were made in conscious rats and is much larger than the 15±2 mm Hg increase found when continuous intraarterial telemetric recordings were made in conscious rats. In addition, SAD increased the variability of both MAP and HR.

The association between the development of hypertension and excess renal sodium retention following SAD directs attention to the hypothesis that SAD causes NaCl-sensitive hypertension because it impairs volume homeostasis. This hypothesis predicts that SAD rats undergo a greater degree of blood volume expansion (by virtue of an imbalance between intake and output) when dietary sodium intake is increased compared with control rats. On the intake side, during HNa, daily sodium intake was slightly but not significantly less (not greater) in SAD than in controls. On the output side, during HNa, daily sodium output was significantly less in SAD than in controls for each of the 5 days, resulting in significantly more positive daily sodium balance and greater cumulative sodium balance (per period, overall) in SAD than in controls. There were no significant differences in any parameter of water balance between controls and SAD during any of the dietary sodium intake periods. Thus, the current observations wherein the development of hypertension in SAD rats exposed to increased dietary sodium intake was associated with increased renal sodium retention is compatible with this hypothesis. However, it seems problematic whether the sensitivity of the currently available methods for the measurement of blood volume would permit the detection of the increment in blood volume associated with the measured amount of excess renal sodium retention. It is possible to detect significant increases in right atrial cardiac filling pressure, an indirect index of blood volume, with alterations in dietary sodium intake. In previous studies, it was found that right atrial pressure in conscious rats was 0.9±0.3 mm Hg during HNa, −0.5±0.2 mm Hg during NNa and −1.0±0.2 mm Hg during LNa, with each value being significantly different from the others. Thus, it may be suggested that the kidneys of SAD rats consuming HNa retain excess sodium, which leads to an increase in blood volume as reflected by an increase in right atrial pressure.

The mechanism(s) whereby SAD impairs renal sodium handling during LNa and HNa appear to involve the renal sympathetic nerves. First, it is known that RSNA regulates renal tubular sodium reabsorption directly via action on renal tubular epithelial sodium transport (α1-adrenoceptor mediated) and indirectly via its influence on renal renin secretion rate and the production of angiotensin, which affects renal tubular epithelial sodium transport. Second, in response to a similar increase in arterial pressure, urinary sodium excretion from an innervated kidney increased when arterial baroreceptors were intact but decreased after SAD, identifying the renal sympathetic nerves as an effector pathway contributing to the natriuretic response to the sensing of increased arterial pressure by arterial baroreceptors. Thus, it may be suggested that during HNa, intact arterial baroreceptors sense a short duration transient increase in arterial pressure (related to the increase in blood volume), which results in reflex suppression of RSNA, which contributes to the natriuresis with reestablishment of sodium balance and avoidance of renal sodium retention. After SAD, the arterial baroreceptor reflex is nonfunctional, RSNA is not appropriately suppressed, establishment of sodium balance is impaired, and renal sodium retention occurs. There is abundant experimental and clinical evidence that supports the view that many forms of neurogenic NaCl-sensitive hypertension result from an impaired (renal) sympathoinhibitory response to increased dietary sodium intake unrelated to the baseline level of (renal) sympathetic nerve activity.

Likewise, during LNa, intact arterial baroreceptors could sense a short duration transient decrease in arterial pressure (related to the decrease in blood volume), which results in
reflex stimulation of RSNA, which contributes to the antinatriuresis with reestablishment of sodium balance and avoidance of renal sodium wasting. After SAD, the arterial baroreceptor reflex is nonfunctional, RSNA is not appropriately stimulated, establishment of sodium balance is impaired, and renal sodium wasting occurs. This analysis fits with the observation that in the presence of sufficiently severe dietary sodium deprivation, renal denervation prevents the establishment of sodium balance and renal sodium wasting occurs.\textsuperscript{13}

Perspectives
A neural mechanism for the control of blood volume would involve a sensor detecting an error or offset signal related to a blood volume–related variable such as arterial pressure or mechanical distortion at the level of the carotid sinus or such as aortic baroreceptor, chamber pressure, or wall stress at the level of the left atrial volume receptor. Activity in afferent neural pathways to critical brain regulatory sites would result in regulated alterations in the activity of multiple efferent pathways involved in the control of intake (thirst, NaCl appetite) or output (RSNA, natriuretic and antinatriuretic hormonal factors). The overall goal is the adjustment of the balance between intake and output so as to restore blood volume back to its control or zero error or offset signal level. The importance of the arterial baroreceptors as a participant in such a neural mechanism for the control of blood volume is revealed by the abnormal responses of the animal to both decreases and increases in dietary sodium intake. In the case of a decreased dietary sodium intake, sodium balance is impaired and renal sodium wasting occurs. In the case of increased dietary sodium intake, sodium balance is also impaired and renal sodium retention occurs, which is associated with the development of increased arterial pressure (ie, NaCl-sensitive hypertension).

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