Arterial baroreceptor denervation impairs long-term regulation of arterial pressure during dietary salt loading

JOHN W. OSBORN AND BARBARA J. HORNFELDT
Departments of Physiology and Animal Science and the Graduate Program in Neuroscience, University of Minnesota, St. Paul, Minnesota 55108

Osborn, John W., and Barbara J. Hornfeldt. Arterial baroreceptor denervation impairs long-term regulation of arterial pressure during dietary salt loading. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1558–H1566, 1998.—Experiments were performed to examine the contribution of arterial baroreceptors to long-term regulation of mean arterial pressure (MAP) during changes in dietary salt intake. Normotensive Sprague-Dawley rats were subjected to either sinoaortic denervation (SAD; n = 8) or Sham surgery (n = 6) and instrumented 1 wk later with radiotelemetry transmitters for continuous minute-to-minute monitoring of MAP and heart rate (HR) over the 8-wk protocol. Rats consumed three levels of dietary NaCl: 0.4% NaCl (week 1), 4.0% NaCl (weeks 2–4), and 8.0% NaCl (weeks 5–7). Rats returned to a 0.4% NaCl diet during the eighth week of the experiment. During week 1 (0.4% NaCl), there were no differences between Sham and SAD groups for 24-h averages of MAP or HR. However, by the third week of 4.0% NaCl, 24-h MAP was elevated significantly from baseline in SAD (10 ± 2 mmHg) but not Sham (1 ± 1 mmHg) rats. By the end of the third week of 8.0% NaCl diet, 24-h MAP was elevated 15 ± 2 mmHg above control in SAD rats compared with a 4 ± 1 mmHg increase in Sham rats (P < 0.05). Hourly analysis of the final 72 h of each level of dietary salt revealed a marked effect of dietary NaCl on MAP in SAD rats, particularly during the dark cycle. MAP increased ~20 and 30 mmHg in SAD rats over the 12-h dark cycle for 4.0 and 8.0% NaCl diets, respectively. In contrast, increased dietary NaCl had no effect on MAP during any phase of the light or dark period in Sham rats. These data support the hypothesis that arterial baroreceptors play a critical role in long-term regulation of arterial pressure. SAD animals had no evidence of hypertensive responses and obscured the magnitude of the differences between groups. Furthermore, because of the extreme lability of MAP in SAD animals, it is critical to use computerized monitoring methods to average a large number of MAP samples to obtain an accurate measurement of pressure.

Contrary to this logic, recent studies suggest that arterial baroreceptors may be important in long-term regulation of MAP under conditions of increased salt intake. Howe and colleagues (17) reported that increasing dietary salt intake resulted in hypertension in sinoaortic denervated (SAD) but not baroreceptor-intact rats. However, in that study, MAP was monitored in restrained rats using indirect methods that may have resulted in acute stress-induced pressor responses and obscured the magnitude of the differences between groups. Furthermore, because of the extreme lability of MAP in SAD animals, it is critical to use computerized monitoring methods to average a large number of MAP samples to obtain an accurate measurement of pressure.

We recently confirmed the finding of Howe et al. (17) using daily direct computerized measurements of MAP in tethered, unrestrained rats (36). We found that increasing dietary NaCl from 0.4% to 8.0% for a period of 3 wk resulted in a gradually developing hypertension, which was reversible within 24 h of the rats being returned to a 0.4% NaCl diet. Although that study supports the idea that arterial baroreceptors may play an important role in regulation of MAP during long-term changes in dietary salt, it was limited by the fact that MAP was monitored for only 30 min each morning during the rats’ inactive phase. Hence, the role of arterial baroreceptors during the active phase (i.e., nighttime), when the animals actually ingest the salt, remains unknown.

The role of arterial baroreceptors in long-term regulation of arterial pressure has been the subject of considerable controversy. In general, there are two lines of evidence that baroreceptors play little to no role in day-to-day control of blood pressure. First, following an acute phase of sympathetically mediated hypertension, several studies have shown that surgical denervation of baroreceptor afferent projections has no long-term effect on the basal level of MAP (9, 12, 31, 34, 39). Other studies have demonstrated only a modest level of hypertension (19, 41). Second, baroreceptors do not appear to chronically buffer the MAP response to induction of experimental (10, 11, 13, 26) and spontaneous (32) hypertension. This conclusion is based on the observation that the steady-state phase of hypertension in these models is similar in intact and baroreceptor-denervated animals (13). The physiological explanation of this finding is based on the well-established phenomenon of baroreceptor adaptation. Studies have shown that the stimulus-response curve for baroreceptors and the baroreceptor reflex shifts 15–40% of the pressure increase within 5–15 min (acute resetting) (2, 7, 23, 25) and resets completely within 48 h. The end result is that the “set point” of the baroreceptor reflex is chronically shifted to a higher operating point such that the baroreceptor reflex controls MAP around a higher level. Taken together, these observations support the hypothesis that arterial baroreceptors do not provide a long-term feedback signal to the central nervous system and therefore are not important in the chronic regulation of MAP.

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The objective of the present study was to examine more precisely the role of arterial baroreceptors in regulating MAP during long-term alterations in NaCl intake. To perform these studies, intact and baroreceptor-denervated rats were instrumented with radiotelemetry transducers. This made it possible to continuously monitor mean MAP and heart rate (HR) in the same rats over an 8-wk period, during which time the rats ingested different levels of dietary salt. Moreover, cardiovascular variables in nontethered rats were monitored during both the inactive (daytime) and active (nighttime) phases of the day. Our results confirm our earlier report (36) and in fact suggest that arterial baroreceptors play a much greater role in the regulation of MAP during changes in dietary salt than previously believed.

METHODS

General Procedures

Male Sprague-Dawley rats (250–275 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN) and housed for 1 wk in the College of Veterinary Medicine animal housing facility with controlled temperature and lighting. Subsequently, rats were brought to the laboratory for surgical procedures as described below. Rats were then individually housed in a chronic cardiovascular monitoring laboratory with a 12-h day/night cycle for the duration of the experiment (~60 days). All procedures were approved by the institutional Animal Care and Use Committee and were conducted in accordance with institutional and National Institutes of Health guidelines.

Surgical Procedures

Sinoaortic denervation. One week before instrumentation for cardiovascular studies, rats were randomly selected to undergo either SAD (n = 8) or sham SAD (Sham; n = 6). Rats were anesthetized with pentobarbital sodium (50 mg/kg) and administered atropine (0.4 mg/kg) with a single intraperitoneal injection. SAD and Sham surgeries were performed as previously described (36) using the method of Krieger (24). A 0.4% NaCl diet (Research Diets, New Brunswick, NJ) and distilled water were provided ad libitum throughout the recovery period.

Implantation of telemetry transmitter. Cardiovascular data acquisition and analysis were performed using a commercially available telemetry system (Data Sciences International, St. Paul, MN). Sham and SAD rats were anesthetized as described above, and the abdominal aorta was exposed via a midline abdominal incision for implantation of the telemetry transmitter unit (model TA11PA-C40). The unit consisted of a fluid-filled catheter attached to a transducer/transmitter. The catheter was inserted directly into the terminal aorta using a 21-gauge needle as a catheter introducer and advanced rostrally so that the tip was distal to the renal arteries. The catheter was then glued into place with cyanoacrylate adhesive. The transmitter was sutured to the abdominal wall during closure of the wound. Penicillin (100,000 U) was administered intramuscularly. On recovery from anesthesia, rats were returned to their home cage.

Experimental Protocol

Rats were given a 1-wk recovery period after instrumentation and before we started the protocol. MAP and HR were continuously monitored in Sham and SAD rats over the next 8 wk. During the first week (control period), the rats ingested a 0.4% NaCl diet (Research Diets) and distilled water ad libitum. Dietary NaCl was then increased to 4.0% during weeks 2–4 and further increased to 8.0% during weeks 5–7. Finally, dietary NaCl was returned to 0.4% during week 8 (recovery period).

The transmitter signal was monitored by a receiver (model RLA1010) beneath the cage that was connected to a BCM 100 consolidation matrix, which was connected to an IBM compatible computer (Compaq Presario 850). Data acquisition and analysis were performed using Dataquest IV software (Data Sciences International). The MAP signal was sampled for 10 s each minute, and HR was determined from the pressure waveform. MAP and HR data were then calculated and stored for later analysis.

Data Analysis and Statistics

Twenty-four-hour averages for MAP and HR were calculated from the 1,440 data points sampled each day. The standard deviations of the 24-h averages were also determined as an index of pressure and HR variability. In addition, to examine the effect of dietary salt on circadian rhythms, hourly averages of MAP and HR were determined for the final 72 h of each level of salt. Statistical analysis for within- and between-group differences was performed using an analysis of variance followed by linear contrast analysis (30) for comparisons between Sham and SAD rats at specific time points. All values are reported as means ± SE, and P < 0.05 was considered statistically significant.

RESULTS

Baseline MAP and HR

Shown in Fig. 1 are representative tracings of MAP and HR for individual Sham and SAD rats. Also shown are 24-h averages for MAP and HR for individual Sham and SAD rats over a 24-h recording period during control period (0.4% NaCl diet). Mean arterial pressure (MAP) and heart rate (HR) were sampled for 10 s of each minute beginning at 12:00 AM (hour 0). Values for the 24-h mean and standard deviation of the mean calculated from 1,440 data points are shown beneath each tracing. Note marked decrease in HR in Sham rats at the beginning of the light cycle (hour 7) and the transient increase between hours 12 and 13. This was routinely observed in all Sham rats but not in SAD rats.

Fig. 1. Individual examples of data obtained from a sinoaortic-denervated (SAD) rat and Sham rat over a 24-h recording period during control period (0.4% NaCl diet). Mean arterial pressure (MAP) and heart rate (HR) were sampled for 10 s of each minute beginning at 12:00 AM (hour 0). Values for the 24-h mean and standard deviation of the mean calculated from 1,440 data points are shown beneath each tracing. Note marked decrease in HR in Sham rats at the beginning of the light cycle (hour 7) and the transient increase between hours 12 and 13. This was routinely observed in all Sham rats but not in SAD rats.
in Fig. 1 are minute-to-minute values for a 24-h-period (time interval begins at 12:00 AM) during the first week of the study while the rats were consuming a 0.4% NaCl diet. Despite similar 24-h averages of MAP and HR for the Sham and SAD rat, lability of MAP, defined as the standard deviation of the 1,440 data points over the 24-h recording period, was noticeably greater in the SAD rat, whereas the lability of HR was lower. Indeed, statistical comparison of the groups showed no difference between the Sham and SAD groups for basal MAP or HR (Fig. 2). However, MAP lability was significantly higher in SAD rats, whereas HR lability was significantly lower than Sham rats. A reduction in the variability of HR is consistent with the loss of baroreceptor control of the heart, since modulation of HR is one of the buffering mechanisms of the baroreceptor reflex. Similarly, loss of baroreceptor control results in an increase in MAP lability.

**Effect of Dietary Salt on 24-h Averages of MAP and Heart Rate**

Twenty-four hour averages for MAP and HR for all three levels of salt (0.4, 4.0, and 8.0% NaCl) are shown in Fig. 3. As mentioned above, there were no differences between groups for MAP or HR during the first week of the protocol (0.4% NaCl). Although MAP increased similarly in both groups during the first 4 days of the 4.0% NaCl period, MAP then returned to control levels in Sham rats, whereas SAD rats remained hypertensive. Statistically significant differences between the groups did not occur until day 9 of 4.0% NaCl (protocol day 16) and were maintained for days 11–21 of the 4.0% NaCl period. During the last week of 4.0% NaCl, MAP remained unchanged at $\pm 1$ mmHg above control (7-day average) in Sham rats compared with a $\pm 2$ mmHg elevation in SAD rats. A similar response pattern was observed when salt intake was increased further during the subsequent 3-wk 8.0% NaCl period. MAP increased similarly in both groups during the initial 4 days of 8.0% NaCl, but by the last week MAP was elevated only $\pm 1$ mmHg above control in Sham rats compared with a $\pm 2$ mmHg increase in SAD rats. Finally, when dietary NaCl was returned to 0.4%, MAP returned to control within 24 h in SAD rats. Interestingly, MAP also decreased in Sham rats such that it was below control levels for the duration of the 1-wk recovery period.

There were also differences in HR between the groups (Fig. 3). Beginning 14 days after NaCl was increased to 4.0% and continuing until the end of the 8.0% NaCl period, HR was significantly lower in Sham rats compared with SAD rats. Both groups showed a transient tachycardic response when NaCl was returned to 0.4%, but the differences between the groups remained over the final 4 days of the protocol.

Fig. 2. Group averages of MAP and HR obtained during 7-day control period (0.4% NaCl diet) for Sham and SAD rats. MAP lability and HR lability represent standard deviations of 1,440 data points of each 24-h period. This value was calculated for each rat, and means $\pm$ SE were then determined for Sham and SAD rats for statistical comparison between groups (*$P < 0.05$ unpaired t-test).
The lability of MAP, a quantitative index of baroreceptor reflex function, remained significantly different between groups for the duration of the experiment. During the first week of the protocol, MAP lability averaged 17.5 ± 1.6 mmHg in SAD rats compared with 8.4 ± 0.4 mmHg in the Sham group (P < 0.05). Lability of MAP was not affected by dietary salt in either group (data not shown). Finally, MAP lability averaged 19.0 ± 1.9 and 9.7 ± 0.4 mmHg for SAD and Sham groups, respectively, during the final week of the protocol, indicating that baroreceptor reflex control of MAP did not improve in SAD rats over the 60-day study.

**Effect of Dietary Salt on Circadian Rhythms of MAP and HR**

To examine more precisely the steady-state relationship among dietary salt, MAP, and HR, the final 72-h period of each level of salt intake was examined on an hourly basis. When consuming 0.4% NaCl, Sham and SAD rats exhibited similar oscillations in MAP approximating 10 mmHg over a 24-h period with pressure increasing during the nighttime hours (Fig. 4, shaded bars) and decreasing during the daytime. However, in contrast to Sham rats, in which increasing dietary NaCl intake to 4.0 and 8.0% had no affect on this circadian rhythm, high-salt intake had marked affects on nighttime MAPs in SAD rats. For example, at the beginning of the dark cycle, MAP was similar for all three levels of salt intake in SAD rats. However, over the subsequent 12-h dark period, MAP steadily rose 20 and 30 mmHg when these rats ingested 4.0 and 8.0% NaCl, respectively. MAP then decreased just as rapidly over the subsequent light period that such that normotensive levels were reached by midafternoon. MAP levels for 0.4, 4.0, and 8.0% were statistically different from each other in SAD but not Sham rats.

A similar analysis of HR is shown in Fig. 5. Similar to MAP, nighttime HR levels were elevated above daytime levels, oscillating ~100 beats/min over a 24-h period in both groups. Finally, HR levels for 0.4, 4.0, and 8.0% NaCl were statistically different from each other over the 72-h period in both SAD and Sham groups.

**DISCUSSION**

This study confirms and extends previous investigations in several ways. First and foremost, these experiments firmly establish that arterial baroreceptors play a major role in long-term control of MAP under conditions of increased dietary salt intake. This is in contrast to the concept that arterial baroreceptors are important only in the moment-to-moment stabilization of MAP (12, 13). Second, this study demonstrates the importance of 24-h monitoring of MAP in studies examining the effects of oscillatory inputs (e.g., salt and water ingestion) on the cardiovascular system. Indeed, previous investigations from our laboratory (36) and Howe and colleagues (17), in which MAP was monitored intermittently during daytime hours, markedly underestimated the magnitude of chronic, salt-induced increases in MAP in SAD rats, since MAP steadily falls during the daytime hours from its highest level at the end of the dark cycle. Finally, the results of this study support the conclusion of earlier investigations that primary dysfunction of arterial baroreceptors may play a role in some genetic forms of salt-dependent hypertension such as the Dahl-S (14, 15, 27) and salt-sensitive spontaneously hypertensive rat (42). Further support of these conclusions is presented below.

**Effect of SAD on Salt-Sensitivity of MAP in Normal Rats**

Despite intense interest in the role of neural reflexes in salt-sensitive hypertension, there are few studies
that have directly examined the effect of increased dietary salt on MAP in animals with experimentally induced baroreceptor reflex dysfunction. Two earlier studies in which MAP was measured by the tail-cuff method in restrained Sham and SAD rats came to opposing conclusions. Velasquez and Alexander (43) reported that under conditions of a normal salt intake, SAD rats were hypertensive and increasing salt intake had no effect on MAP. Soon after, Howe and colleagues (17) reported that SAD rats were normotensive and exhibited salt-dependent hypertension. More recently, using direct computerized measurements of MAP in freely moving tethered rats, we showed that increasing dietary salt resulted in a slowly developing, but rapidly reversible, salt-dependent hypertension in SAD rats (36). In that study, MAP was measured over a 30-min period sometime between the hours of 8:30 AM and 2:00 PM.

The present study, in which MAP and HR were continuously monitored in unrestrained, nontethered rats, confirms that SAD rats are indeed salt sensitive. From 24-h averages, we found that MAP was elevated by 15 mmHg above control levels in SAD rats consuming an 8.0% NaCl diet. More importantly, analysis of MAP on an hour-by-hour basis revealed that MAP increased during the dark cycle and fell during the light cycle at a rate of 2–3 mmHg/h in SAD rats consuming an 8.0% NaCl diet. Although a 10-mmHg circadian rhythm was observed in Sham rats, it was not influenced by dietary salt content. This is an important observation, since intermittent measurements of MAP made at 10:00 AM would clearly yield markedly different results than measurements made at 3:00 PM. The importance of this observation is that comparisons of previous studies from different laboratories in which intermittent measurements of MAP were made are impossible, since it is highly unlikely that measurements were made at the same time of day within and across studies. Moreover, other variables that impact on MAP regulation, such as blood volume, neurohumoral factors, and sympathetic nerve activity, must be measured at night to better understand the mechanisms of salt-dependent hypertension. The results of previous studies in which such measurements were made during the day may have underestimated the contribution of these factors to salt-dependent hypertension.

Mechanism of Salt-Sensitive Hypertension in SAD Rats

The mechanism of salt-sensitive hypertension in SAD rats remains to be established. One possibility is that SAD impairs volume homeostasis, resulting in a greater degree of blood volume expansion when salt and water intake are increased compared with intact
rats. This could be the result of either an enhanced thirst drive and water intake or reduced renal excretory capacity in SAD rats. However, we have reported that both sodium and water intakes of SAD rats consuming 8.0% NaCl food are not different from intakes of the intact rats (36). Additionally, daily urinary excretion of sodium and water was not different between groups (36). Although balance measurements were not made in the present study, we did monitor water intake by weighing the water bottles every 3 days to estimate water intake and did not observe any difference between SAD and intact rats (data not reported). Additionally, studies in other species have demonstrated that hormonal controllers of renal function such as the renin-angiotensin system (26, 38), aldosterone (26), vasopressin (38, 44), and atrial natriuretic peptide (38) are not chronically affected by SAD. Taken together, these observations suggest that the hypertension induced by increased dietary salt in SAD rats was not the result of impaired regulation of body fluid volume. However, a definitive conclusion cannot be made until measurements of blood volume are made.

Is salt-dependent hypertension in the SAD rat caused by central resetting of sympathetic activity to a higher level such that it cannot be suppressed by a high-salt diet? Certainly, loss of baroreceptor afferent nerve activity elevates sympathetic nerve activity acutely, but many studies have demonstrated, both directly and indirectly, that it returns to normal levels within days of baroreceptor denervation. On the basis of the acute and chronic responses of MAP, HR, and renal sodium excretion to SAD, we concluded that sympathetic activity to the heart, vasculature, and kidneys returned to normal within 7 days after SAD in the rat (34). The acute depressor response to ganglionic blockade, which has been used to assess neurogenic pressor activity in the conscious rat (40), supports the idea that sympathetic vasoconstrictor activity is elevated acutely but not chronically in SAD rats (1, 35). Finally, direct measurement of renal sympathetic nerve activity with chronically implanted electrodes also suggests that the sympathetic drive to the kidney returns to normal within days after SAD (3, 18). On the basis of these studies from several laboratories, using a variety of approaches to assess sympathetic activity, it is quite clear that SAD does not chronically elevate sympathetic nerve activity. Therefore, salt-sensitive hypertension in the SAD rat is most likely not the result of a high basal level of sympathetic activity.

Although the mechanism whereby interruption of arterial baroreceptor afferent nerve activity results in chronic salt-dependent hypertension remains to be investigated, we previously hypothesized that the underlying cause is related to the inability of SAD animals to chronically suppress sympathetic activity when dietary salt is elevated (4, 33). Indeed, there is abundant experimental and clinical evidence to support the idea that many forms of neurogenic salt-sensitive hypertension are the result of an impaired sympathoinhibitory response to increased dietary salt independent of the basal level of sympathetic activity (4). The most logical explanation is that the absence of baroreceptor input in SAD rats disables the arterial baroreceptor reflex, which prevents the normal sympathoinhibitory response to increased arterial blood volume and pressure. However, it is important to keep in mind that baroreceptors also modulate sympathetic responses to some circulating hormones, many of which are linked to sodium and water homeostasis (4, 33). Hence, increased salt sensitivity in SAD rats may also be due to removal of baroreceptor modulation of hormonal afferent pathways. This is a subtle but important distinction, since it means that salt-dependent hypertension in SAD rats may not be simply due to interruption of the baroreceptor reflex per se. In other words, baroreceptor afferents are important, but this does not necessarily mean that the baroreceptor reflex is the primary neural controller of MAP under conditions of increased dietary salt intake. As we have discussed previously, it is possible that hormonal responses are more important, but they require baroreceptor afferent activity to operate effectively (32, 33).

Effect of SAD on Salt-Sensitivity in Experimental Hypertension

The ability of baroreceptors to chronically buffer MAP responses to hypertensive stimuli has been studied in several experimental models (11, 13, 26, 32). The consistent finding has been that the rate of the development of hypertension is accelerated in SAD dogs, but the steady-state level of MAP is not different from that observed in baroreceptor-intact dogs. This has been reported for several hypertensive models, including the Goldblatt (26), reduced renal mass (11), and the angiotensin-infusion models (10).

Why did SAD result in salt-sensitive hypertension in this study but not in these earlier investigations? The explanation may be related to how the sodium and water load was administered. There are two important differences that need to be considered. The first difference is the temporal pattern of the salt and water load. In the current study, hour-by-hour analysis of MAP responses to hypertensive stimuli has been studied in experimental models (11, 13, 26, 32). The consistent finding has been that the rate of the development of hypertension is accelerated in SAD dogs, but the steady-state level of MAP is not different from that observed in baroreceptor-intact dogs. This has been reported for several hypertensive models, including the Goldblatt (26), reduced renal mass (11), and the angiotensin-infusion models (10).

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effect in models employing a continuous intravenous isotonic saline load but would unmask the hypertensive response to an intermittent salt load.

The second difference between dietary salt-loading studies in baroreceptor-denervated rats and earlier studies in dogs is the route of administration of the salt and water. Intravenous infusion of isotonic saline results in a volume load of the extracellular fluid compartment with no osmotic stimulus to the central nervous system. In contrast, rats ingesting an 8% NaCl diet increase sodium intake 20-fold above control levels compared with only a 5-fold increase in water intake (i.e., hypertonic salt load) (36). Additionally, since this hypertonomic load is taken into the gastrointestinal tract rather than the cardiovascular compartment, it may stimulate hepatoportal osmoreceptors (6, 8, 16, 28, 37), whereas intravenous isotonic saline infusion would not. We have recently reported that intragastric infusion of a modest hypotonomic saline load (2.5 ml of 600 mosmol/kg) in the rat increases portal venous but not systemic arterial osmolality (6). Moreover, utilizing Fos immunocytochemistry, our laboratory and others have shown that intragastric (6, 22) or portal venous infusion (29) of hypertonic saline activates cells in the medial nucleus tractus solitarius, area postrema, lateral parabrahial, and paraventricular and supraoptic nuclei. These studies are consistent with earlier electrophysiological demonstrations of central projections of hepatoportal osmoreceptors (20–22). Moreover, the renal sympathetic nerve activity and natriuretic responses to oral salt loading in conscious dogs have been shown to be dependent on hepatoportal afferent nerves (28). These findings have important implications regarding the difference between the response to dietary versus intravenous salt loading in baroreceptor-denervated animals. We propose that a gastrointestinal salt load activates central autonomic nuclei by stimulation of hepatoportal osmoreceptors, which also receive baroreceptor input. Hence, the autonomic response pattern to dietary salt loading may depend on integration of baroreceptor and hepatoportal osmoreceptor inputs to the brain stem. Under these conditions, the absence of arterial baroreceptor input in SAD animals may alter the long-term autonomic response to salt loading. This is not the case in studies in which the salt load was administered by intravenous isotonic saline infusion, since salt-induced afferent signals from the gastrointestinal tract, or the hepatoportal circulation, would not be stimulated.

At this point it is important to state that an 8.0% NaCl diet most likely results in a chronic osmotic stress, which exceeds the normal physiological range. Unfortunately, this has become the standard dietary salt load, since many salt-sensitive models in the rat were initially characterized using an 8.0% NaCl diet. In the present study we chose to examine the effect of increasing dietary salt to both 4.0% and 8.0% NaCl. Because normal rat chow is 1.0% NaCl, we reasoned that a 4.0% NaCl diet is a more physiological salt load than 8.0% NaCl. Clearly, salt-dependent hypertension was observed in SAD rats consuming a 4.0% as well as 8.0% NaCl food. Hence, we conclude that the increased salt sensitivity observed in SAD rats occurs over a physiological range of salt intake.

In summary, dietary salt loading provides an oscillatory input to arterial and cardiopulmonary baroreceptors in addition to activation of gastrointestinal afferent pathways. In contrast, continuous intravenous infusion of isotonic saline results in a static input to baroreceptors and does not stimulate hepatoportal osmoreceptors. We propose that dietary salt loading provides a more physiological stimulus to the autonomic nervous system compared with intravenous administration. As such, failure to observe a long-term increase in salt sensitivity of MAP in baroreceptor-denervated animals in earlier studies (10, 11, 13, 26) may be the result of a using a nonphysiological method of salt loading.

**Baroreceptor Reflex Dysfunction and Salt-Sensitive Hypertension in Genetic Models**

Neurogenic mechanisms have been proposed to explain salt-dependent hypertension in the Dahl-S rat (14, 15, 27) and the salt-sensitive strain of the spontaneously hypertensive rat (42). Although autonomic dysfunction has been demonstrated in these models, the coexistence of vascular and renal dysfunction makes it difficult to definitively conclude that impaired neural control mechanisms are the primary cause of salt-dependent hypertension. The present study was designed to study the effect of baroreceptor dysfunction on salt sensitivity of MAP in the presence of normal intrinsic renal and vascular control. The present study suggests the idea that baroreceptor dysfunction alone may be a key component of salt sensitivity in these genetic models. This is particularly evident when the results of this study are compared with reports in genetic strains in which MAP was monitored 24 h per day, and elevations in salt intake were achieved by increasing the NaCl content of the diet. Similar to the present study, high-salt food elevated nighttime MAP in the salt-sensitive spontaneously hypertensive rat but not in the salt-resistant Wistar-Kyoto rat (5). Indeed, the magnitude of the salt-induced changes in the MAP circadian rhythms were almost identical to those observed in the present study.

There are limitations to the experimental approach employed for these studies. The experimental design relies on the assumption that changes in the response to high-sodium diet after baroreceptor denervation reflect a physiological role of the baroreceptor reflex in mediating responses to a high-sodium diet in intact animals. Clearly, this is an indirect approach to address the physiological role of arterial baroreceptors. Nevertheless, it is the most common approach in studies of this nature and provides further support that arterial baroreceptors are indeed important in opposing the development of salt sensitive hypertension.

In summary, the results of this study demonstrate a critical role for arterial baroreceptors in the long-term control of MAP under conditions of elevated dietary salt intake. Measurement of MAP by telemetry in unre-
strained rats revealed that the magnitude of the hypertensive response to increased salt in baroreceptor-denervated rats was underestimated in previous investigations in which MAP was measured intermittently during the inactive phase (daytime) of the rat.

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