A high-salt diet does not influence renal sympathetic nerve activity: a direct telemetric investigation

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McBryde FD, Malpas SC, Guild SJ, Barrett CJ. A high-salt diet does not influence renal sympathetic nerve activity: a direct telemetric investigation. Am J Physiol Regul Integr Comp Physiol 297: R396–R402, 2009. First published June 3, 2009; doi:10.1152/ajpregu.90741.2008.—The importance of dietary salt in the development of hypertension has long been a source of controversy. Recent studies suggest a combination of high-salt and ANG II infusion may increase sympathetic drive; however, the effect of a change in dietary salt alone is unclear. Using telemetry, we recorded renal sympathetic nerve activity (RSNA), arterial pressure (MAP), and heart rate (HR) in seven New Zealand white rabbits before and during a 6-day period of increased salt intake (normal NaCl 0.5 g·kg⁻¹·day⁻¹, high NaCl 2.5 g·kg⁻¹·day⁻¹) and a second group of seven rabbits with normal salt intake throughout. The responses to stressful stimuli encountered in the laboratory were recorded and compared with rest in control and high-salt groups. Resting MAP, HR, and RSNA were not significantly altered with high salt intake [88 ± 5 vs. 91 ± 6 mmHg; 251 ± 8 vs. 244 ± 9 beats per minute (bpm); 9.7 ± 1.2 vs. 10.8 ± 1.7 normalized units (nu)] despite significant reductions in plasma renin activity (1.88 ± 0.18 vs. 1.27 ± 0.15 nmol ANG I·1⁻¹·h⁻¹; P < 0.05) and ANG II (7.5 ± 1.2 vs. 4.3 ± 0.8 pmol/l). Increasing levels of stressful stimuli (resting in home cage, containment in box, handling, and nasopharyngeal activation) in animals on a normal salt diet caused graded increases in MAP (89 ± 2 mmHg, 95 ± 2 mmHg, 107 ± 4 mmHg, and 122 ± 5 mmHg, respectively) and RSNA (9.7 ± 0.9 nu; 11.8 ± 2.7 nu; 31.4 ± 3.7 nu; 100 nu) but not HR (245 ± 8 bpm; 234 ± 8 bpm; 262 ± 9 bpm; 36 ± 5 bpm). High dietary salt did not significantly alter the responses to stress. We conclude that a 6-day period of high salt intake does not alter the level of RSNA, with non-neural mechanisms primarily responsible for the observed renin-angiotensin system suppression.

blood pressure; dietary salt; renin-angiotensin system

The importance of dietary salt intake in the development of hypertension has been a source of controversy for many years, with evidence both for and against changes in dietary salt intake influencing the level of blood pressure. It is generally accepted that salt homeostasis within the body is regulated by renal levels. Changes in salt balance influence the regulation of renin secretion via a number of potential mechanisms, including renal baroreceptor activity, renal sympathetic nerve activity, and the macula densa pathway, but the relative importance of these pathways is not clear (6). In the case of dietary salt influencing arterial pressure levels, a major point of controversy is the effect that dietary salt is having on the sympathetic nerve activity. Many of the studies that have found no change in mean arterial pressure (MAP) with dietary salt have also indicated suppression of the sympathetic nervous system with high salt intake (2, 16, 19, 36). Conversely, where MAP has been found to be salt sensitive, concurrent activation of the sympathetic nervous system has been shown to occur (11, 12, 16, 35, 53). Brooks et al. (7) propose that in salt-sensitive hypertension a high-salt diet may result in a centrally driven sympathetic activation through small changes in plasma sodium chloride and osmolarity. However, there is a lack of definitive data on the effects of dietary salt on sympathetic nerve activity, even in normotensive animals, because of the absence of direct within-animal long-term recordings.

Work from our own laboratory has shown that rabbits on a high-salt diet do not have a higher baseline level of arterial pressure or any change in global sympathetic tone (as indicated by the depressor responses to ganglionic blockade) compared with control animals (38). In addition, recent evidence suggests that the natriuretic response to volume expansion in the absence of a change in arterial pressure may be independent of the renal nerves, with the adrenergic β1-receptor antagonist metoprolol having no effect on the decreases in renin, ANG II, and aldosterone caused by a hypertonic saline infusion in dogs (6). In contrast, renal denervation studies that have assessed the contribution of the renal sympathetic nerves in modulating the response to an increases in salt intake have produced conflicting results; renal denervation has been shown to attenuate (26) or have no effect (23, 41) on the return to sodium balance following a change in salt intake. In the case of the hypertension induced by the combination of high salt and elevated ANG II levels, inappropriate sympathetic nerve activity levels are thought to contribute. We have previously shown that in response to a combination of low-dose ANG II and a high-salt diet, there is a sustained depressor response to ganglionic blockade, despite the increase in arterial pressure (38). Similarly, King et al. (32) showed an increase in whole body norepinephrine spillover in rats treated with the combination of ANG II and high dietary salt, but not ANG II alone (32). Thus, the combination of dietary salt and ANG II appears to chronically alter the neural control of arterial pressure. Given the vital role of the kidneys in regulating fluid and electrolyte homeostasis, it is important to understand how the neural control of the kidneys will respond to an increase in dietary salt intake.

As well as chronic influences, elevated salt intake has also been shown to heighten the arterial pressure response to acute stress in spontaneously hypertensive rats (20, 22). Human studies have suggested enhanced pressor and HR responses to both emotional and startle stress in salt-sensitive patients (8, 10). Ito et al. (31) and Pawloski-Dahm and Gordon (45) have shown excitation of brain stem RVLM neurons to produce augmented pressor responses in rats on a high-salt diet. Similarly, Adams et al. (1) showed enhanced sympathoexcitatory...
and sympathoinhibitory responses from the RVLM in rats on a high-salt diet. Importantly, arterial pressure in these rats was not increased by high dietary salt, suggesting that even when baseline arterial pressure is not altered, a high-salt diet may sensitize the sympathetic nervous system response to stressful stimuli. However, the effects of high dietary salt on the acute sympathetic responses to stress have not been tested directly in the conscious setting.

We have used telemetry to record renal sympathetic nerve activity and arterial pressure and measured plasma hormonal markers, in rabbits during the transition from a normal to a high-salt diet. Also, the possibility that increased dietary salt intake may be increasing the cardiovascular and sympathetic responsiveness to acute stress is investigated. We hypothesize that an increase in dietary salt intake will be associated with a sustained decrease in renal sympathetic drive, contributing to the expected suppression of the renin-angiotensin-system as the body excretes the excess salt load.

MATERIALS AND METHODS

Experiments were conducted on 16 male and female New Zealand White rabbits with initial weights of 2.4–3.5 kg and were approved by the University of Auckland Animal Ethics Committee. The rabbits were housed individually in cages (height 45 cm, width 65 cm, and depth 65 cm) with a telemetry receiver (model RLA2000; Data Sciences International, St. Paul, MN), positioned on the top or bottom of each cage. The rabbits were fed daily (100 g standard rabbit pellets with 0.5% NaCl content, supplemented with hay, carrot, and apple) at 0900, and water was available ad libitum. Food and fluid intake were monitored daily. The room was kept at a constant temperature (18°C) and dark-light cycle (lights on from 0600 to 1800).

Telemetry devices to measure arterial blood pressure (model PAD70, Data Sciences International, St. Paul, MN) and renal sympathetic nerve activity (RSNA) (model TR76S, Telemetry Research Ltd, Auckland, New Zealand) were surgically implanted into all animals, as has been described in detail previously (3, 46). Briefly, the blood pressure transmitter catheter was inserted into a branch of the left iliac artery and advanced so that the tip of the catheter lay in the abdominal aorta. The cannula was tied into position, and the body of the transmitter was placed in the abdominal cavity, and the incision was closed. A retroperitoneal incision was then made to expose the left kidney, and the renal nerve identified and placed within a pair of coiled electrodes. The electrode wires and nerve were then coated in a silicone elastomer (Kwik-sil, World Precision Instruments, Sarasota, FL), the body of the nerve transmitter was placed subcutaneously, and the incision was closed.

After surgery, the rabbits were treated prophylactically with an antibiotic (5 mg/kg scenfloroxacin, Baytril; Bayer, Auckland, New Zealand) and analgesic (2 mg/kg sc daily for 3 days; ketoprofen, Ketofen; Rhone Merieux, Essex, UK). As soon as the rabbits regained consciousness, they were returned to their home cages. A heating pad was placed in the cage for 24 h after the surgery.

Data collection. Animals were allowed to recover from surgery (5–7 days) before a 5-day baseline period was recorded. Recovery from surgery was characterized by the rabbits eating and drinking normally and recording an observable circadian variation in heart rate. Fourteen of the sixteen animals instrumented were found to have viable RSNA signals following surgery to implant telemetry devices, and these were used for the protocol described below. Nerve viability was assessed by examining the entrainment of sympathetic bursts to the cardiac cycle, and the increase in RSNA with nasopharyngeal reflex activation (see Chronic effects of high dietary salt). Arterial pressure and RSNA signals were sampled at 500 Hz using an analog-digital data acquisition card (PCI 6024E, National Instruments, Austin, TX) and continuously displayed by a data acquisition program (Universal Acquisition 11, Telemetry Research, Auckland, New Zealand). Heart rate (HR) was derived from the arterial pressure waveform. The original RSNA signal was amplified, filtered between 50–5,000 Hz, full-wave rectified, and integrated using a low-pass filter with a 20-ms time constant.

RSNA recording was scheduled for 15 min every 2 h, a protocol that has been shown to accurately represent the underlying average (27). The averaged data presented for all variables were taken only from those periods when RSNA was actively being recorded. For the calculation of the overall mean levels of MAP and RSNA, the 500-Hz sampled signals were averaged every 2 s and saved to disk along with HR, with additional sample periods of the original 500-Hz data saved also. All subsequent data collection and analysis were performed using a data analysis program (Universal Analysis 11, Telemetry Research, Auckland, New Zealand).

Experimental protocol. Animals were divided into two groups: control (n = 7) and high salt (n = 7). After recording MAP, HR, and RSNA for a 5-day baseline period (baseline), animals in the high-salt group were placed on a high-salt diet (0.9% NaCl in drinking water), while control animals remained on a normal salt diet. MAP, HR, and RSNA were recorded for a further 6 days.

On the first day of data recording, 5 days before the high-salt group were placed on a high salt intake (day −5) and on the sixth day of the high-salt period (day 6), the animals were removed from their cage and placed in a containment box. A catheter was inserted into the medial ear artery under topical local anesthetic, and arterial blood samples were taken for the measurement of plasma ANG II, plasma renin activity (PRA), hematocrit, osmolality, and sodium and potassium concentrations. This required 5 ml of blood to be taken; this volume was replaced with haemocel solution (DeltaSelect, Dreieich, Germany) via a venous catheter. Samples were collected in the afternoon ~6 h after feeding. At this time, maximum RSNA was evaluated by activation of the nasopharyngeal reflex using cigarette smoke delivered to the nasal area with a 50-ml syringe. The baseline noise level was established following a subcutaneous injection of the ganglionic blocking agent pentolinium tartrate (5 mg/kg). Data obtained for the duration of containment in the box and for 2 h following ganglionic blockade were not included in daily average analysis.

In the second part of this study, a group of animals, including animals from both the above protocol and other projects was used. Rabbits were grouped according to the level of dietary salt: normal (n = 11) or high salt (n = 6). Animals in the high-salt group had been on a high salt diet for 6 days prior. On the day of the experiment, the responses to the following acute stressful stimuli were recorded: 1) handling, in the form of being wrapped up and restrained in a towel for 20 s (handling), 2) placement in a containment box, a standard procedure in the lab used when performing short-term interventions (15–30 min), such as taking blood samples (in box), 3) activation of nasopharyngeal reflex with cigarette smoke (nasopharyngeal). The responses to stressful stimuli were compared with a 15-min control period taken overnight between 1 and 4 AM overnight when the rabbit was resting undisturbed in its home cage (home cage). The responses to acute stress were compared between animals on a normal (n = 11) and a high-salt diet (n = 6).

Data analysis. RSNA was normalized with 0% set as the level following ganglionic blockade and 100% being the maximal response to nasopharyngeal activation. Values for normalization were taken from day −5 of the study. To ensure that high dietary salt was not altering the magnitude of the RSNA nasopharyngeal response and thus impacting the normalized results, the unscaled response to nasopharyngeal activation was compared within-animals before (day −5) and after (day 6) the increase in dietary salt intake. MAP, HR, and RSNA were collected as 2-s averages for the duration of the experiment and converted to daily averages to examine the chronic effects of dietary salt, with comparisons made between the 5-day baseline and 6-day high-salt periods. Measurements of plasma osmolality, hematocrit, sodium, and potassium concentrations, ANG II, and PRA were compared between
experimental days −5 and 6. Within-animal statistical comparisons were made using an ANOVA with repeated measures, with comparisons between baseline and high-salt periods (high salt) and the equivalent time control (control).

To evaluate the effects of high salt on the acute responses to stress, data were averaged for the periods of control, handling, and containment in box, and maximal MAP and minimum HR were measured following activation of the nasopharyngeal reflex. ANOVA with Bonferroni post hoc comparisons was used to compare the responses to the various stimuli between groups. Changes were considered significant when \( P < 0.05 \).

**RESULTS**

**Chronic effects of high dietary salt.** To ensure the level of dietary salt was not having an effect on the RSNA response to activation of the nasopharyngeal reflex, the unscaled absolute RSNA data were examined prior to normalization. In the high-salt group, RSNA increased with nasopharyngeal activation to similar levels on day −5 of the baseline period and day 6 of high dietary salt (4.6 ± 1.1 μV vs. 4.3 ± 0.8 μV; nonsignificant (ns)). Control animals displayed similar increases in RSNA with nasopharyngeal activation, which did not change between day −5 and day 6 of normal dietary salt (4.3 ± 1.8 μV vs. 3.7 ± 0.5 μV; ns). These results demonstrate that a high-salt diet does not alter the unscaled level of RSNA either at rest or during activation of the nasopharyngeal reflex, and thus confirms the validity of using the nasopharyngeal response to normalize RSNA.

The change from normal drinking water to a 0.9% salt solution was not associated with a significant increase in fluid intake, with rabbits in the high-salt group drinking a daily average of 205 ± 18 ml during the 5-day baseline period and 225 ± 28 ml over the 6 days of high salt intake (Fig. 1). Animals on a high-salt diet also continued eating normally and did not show any subjective signs of a change in general condition, such as listlessness or decreased activity. Dietary salt intake was 2.0 ± 0.3 g/day higher in the group receiving 0.9% NaCl in their drinking water. This resulted in a total daily salt intake of 2.5 g/day in high-salt animals compared with a control salt intake of 0.5 g/day.

Group data showing the daily averages of HR, MAP, and RSNA before and during an increase in dietary salt intake can be seen in Fig. 2. No statistically significant differences from baseline or between groups were found in any variables. A high-salt diet did not cause any significant change in the average level of RSNA (9.7 ± 1.2 nu during the baseline period vs. 10.8 ± 1.6 nu during high salt; ns).

Animals receiving high dietary salt did not show any significant changes in plasma concentrations of sodium or potassium, plasma osmolality, or plasma hematocrit after 6 days of high salt intake (ns compared with day −5) (Table 1). A high-salt diet was, however, associated with significant sup-

![Fig. 2. - Daily averages of renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP), and heart rate (HR) in rabbits on normal and high-salt diets. Data shown are 5 days prior to and 6 days following a transition from a normal salt (0.6 g·kg⁻¹·day⁻¹) to a high-salt (3g·kg⁻¹·day⁻¹) diet (○, n = 7) or continuance on a normal salt diet (●, n = 7). RSNA is expressed as normalized units, with 100 representing the maximal response to nasopharyngeal activation with cigarette smoke, and zero representing the level following ganglionic blockade. Data are shown as means ± SE.](http://ajpregu.physiology.org/)
expression of the renin-angiotensin system. In the high-salt group, both PRA (1.88 ± 0.18 nmol ANG I-1^{-1}·h^{-1}) before the high-salt diet on day -5 vs. 1.27 ± 0.15 nmol ANG I-1^{-1}·h^{-1} on day 6, P < 0.05) and ANG II were markedly decreased (baseline value of 7.5 ± 1.2 pmol/l on day -5 vs. 4.3 ± 0.8 pmol/l on day 6, P < 0.05). In control animals, both PRA (1.72 ± 0.35 vs. 1.69 ± 0.20 nmol ANG I-1^{-1}·h^{-1}) and plasma ANG II (8.0 ± 1.0 vs. 7.8 ± 0.9 pmol/l) remained steady.

Effects of dietary salt on acute responses to stress. On day -5 during the baseline period, stress induced by being constrained in a containment box resulted in small, but not significant, rises in RSNA compared with resting undisturbed in the home cage (9.7 ± 0.9 nu vs. 11.8 ± 2.7 nu; ns) and MAP (89 ± 2 mmHg vs. 95 ± 2 mmHg; ns), and a small, but not significant, decrease in HR (245 ± 8 bpm vs. 233 ± 8 bpm, ns) (Fig. 3). Handling and restraint in a towel resulted in significant increases in RSNA (31.4 ± 3.7 nu; P < 0.05) and MAP (107 ± 4 mmHg; P < 0.05) but not HR (262 ± 9 bpm; ns) from home cage levels. Finally, activation of the nasopharyngeal reflex with cigarette smoke caused the largest increases in MAP (to 122 ± 6 mmHg; P < 0.05) and RSNA. Because the RSNA response to nasopharyngeal activation has been used for normalization, all animals show 100% (Fig. 3). Thus, RSNA was increased 11.3 ± 1.2 times above baseline levels with nasopharyngeal activation (P < 0.05). HR showed a profound transient decrease during nasopharyngeal activation to 36 ± 5 bpm (P < 0.05).

The stress responses in animals on a high-salt diet did not differ significantly from those seen in animals on a normal salt diet in any of the variables examined. The pattern of responses to different levels of stress mirrored those observed in control animals (Fig. 3).

DISCUSSION

Using direct recordings of RSNA, we have shown that a high-salt diet did not result in renal sympathoinhibition in rabbits, despite a significant suppression of the renin-angiotensin-system (RAS). This was contrary to our hypothesis, where it was proposed that renal sympathetic activity would be decreased during a high-salt diet contributing to the inhibition of the RAS, thus aiding excretion of the excess salt load. The lack of suppression of RSNA during high dietary salt in the present study suggests that either there has been a decrease in responsiveness of the renin-secreting cells of the juxtaglomerular apparatus to adrenergic stimuli, or nonneural mechanisms are wholly responsible for the inhibition of the RAS under the condition of elevated salt intake in the rabbit.

This finding is consistent with our previous work using indirect measures of global sympathetic tone, which showed no difference between animals on normal and high-salt diets (38). Recent work from Osborn et al. (43) shows that disruption of peripheral sympathetic pathways does not cause salt-sensitive hypertension in the rat, suggesting that the ability to inhibit the sympathetic nervous system during high salt intake is not critical. Our results are also consistent with recent studies in dogs by Bie et al. (6) where β1-adrenoreceptor blockade did not prevent suppression of the RAS following an acute intravenous sodium load. Holmer et al. (29) also showed that the ability to modulate renin gene expression in response to dietary salt intake of denervated and innervated kidneys was similar. Our results provide further evidence that alterations in renal nerve activity are not critical in mediating the physiological response to high salt.

An important question is that because neither RSNA nor arterial pressure is altered during high salt intake, what physiological factors are responsible for the observed suppression of the RAS? Renin release from the juxtaglomerular apparatus may be suppressed in response to many factors, including increases in perfusion pressure, decreases in direct or indirect sympathetic drive, or increases in plasma or tubular sodium concentrations. Osborn and Kinstetter (42) showed in anesthetized dogs that high dietary salt intake altered the renal release of renin in response to electrical stimulation of the renal nerves. Animals on a high-salt diet appeared to show a blunted renal PRA response to renal nerve stimulation compared with animals on a normal and low-salt diet (42). Thus, the possibility remains that although RSNA is unchanged during high salt intake, the relationship between sympathetic activity and renin release may have been altered.
It seems unlikely that the suppression of the RAAS can be explained by an increase in plasma sodium levels. We found that plasma concentrations of sodium and potassium, as well as hematocrit and plasma osmolarity, did not change in animals on a high-salt diet. This finding is in agreement with several earlier studies and suggests that a high-salt diet does not change plasma sodium concentrations or osmolarity. Crofton et al. (15) found no change in plasma sodium or osmolarity in rats given 1% NaCl water to drink, with or without concurrent treatment with deoxy-corticosterone acetate. Similarly, Tajima et al. (49) examined the effects of low, normal, and high-salt diets in unilaterally nephrectomized Wistar rats and found no changes in plasma sodium, potassium, hematocrit, plasma volume, or extracellular fluid volume between normal salt and high-salt rat groups (49). In contrast, it has been shown in conscious dogs, in which renal mass had been reduced to one-third of normal that plasma [Na⁺] increased from 144 to 150 mM with intravenous saline loading (~3.5 l/day), with no change in plasma [K⁺] (13). However, the impact of such a large nephrectomy combined with the difference in the administration of the high salt load in these animals (intravenous vs. dietary) makes direct comparisons difficult. A further study in chronically instrumented Sprague-Dawley rats found that a high-salt diet (4% chow) was associated with elevations in plasma sodium especially during the dark phase of the daily light-dark cycle (28). It should be noted that the size of the observed changes in plasma sodium were very small (1–3%), and much less than the changes in [Na⁺] necessary to induce alterations in norepinephrine uptake or transporter expression in cultured sympathetic neurons in vitro (28). Our finding that plasma sodium concentration and osmolarity do not change measurably with a high-salt diet in the rabbit suggests these factors are not likely to be directly responsible for the observed suppression of the RAAS with salt loading. However, this does not rule out intrarenal changes in sodium delivery to the macula densa, which could play a key role in mediating the response to salt load in the absence of changes in MAP and RSNA (5).

A second important finding is that increased salt intake for 5 or 6 days was not associated with an increased sympathetic or cardiovascular response to stress. These results suggest that, in the rabbit at least, a high-salt diet is not sensitizing or enhancing the reactivity of central sites to stressful inputs, as has been suggested previously (8, 9, 20, 31, 45). A possible reason that may explain these discrepancies is that the rabbits used in this study do not appear to exhibit salt sensitivity over the time frame examined, whereas previous studies demonstrating an increase in the stress response with high dietary salt have generally done so in salt-sensitive human subjects or salt-sensitive animal models of hypertension (8, 9, 20). This is not always the case, as Pawloski-Dahm and Gordon (45) showed an increased reactivity of brain stem RVLM vasomotor neurons in Sprague-Dawley rats, which showed no increase in MAP after 10 days of high dietary salt intake. These experiments, however, were conducted using central microinjections of the excitatory neurotransmitter glutamate into anesthetized rats; one potential area of great interest would be to assess the acute responses to stress in these rats in the intact conscious state to investigate whether the increased sensitivity of the RVLM translates into an augmentation of stress responses in these animals. Our results are consistent with those of Bucholz et al. (8), who found that salt-resistant human subjects (defined by a lack of an MAP response after changing from a high- to a low-salt diet) did not show an exaggerated stress response with an increase in dietary salt intake (8). One possibility is that exposure to a high-salt diet for longer periods may reveal an effect, as suggested by the recent work of Adams et al. (1), in which a high-salt diet was associated with exaggerated blood pressure responses to RVLM stimulation after 21 but not 7 days. This would be an area of great interest for further study.

A finding unrelated to changes in salt intake was the observation that handling caused considerable elevations in RSNA and MAP, indicating that the rabbit was in a stressed state, although after the rabbit had been left quietly in a containment box for a short period of time, these values were not significantly different to baseline values recorded when the rabbit was resting undisturbed in its home cage. This is important because although we believe it important to record data remotely with as little handling or disturbance of the animal as possible, this is occasionally necessary for some interventions commonly performed such as taking blood samples or investigating baroreflex function (4, 47). Our findings give us confidence that a low level of disturbance such as containment in a box is unlikely to have an adverse effect on the results obtained, but it does emphasize that situations in which an animal is being handled intensively and under stress do not accurately reflect the baseline state of the animal.

Limitations. Previous studies have increased dietary salt intake using a variety of approaches, including increasing the salt content of food (18, 37, 44), adding salt to drinking water (24, 25, 38), and by continuous intravenous infusions of saline (17, 36). By giving isotonic saline in the drinking water in the current study, we increased salt intake of the rabbits from a baseline of 0.5 g/day to 2.5 g/day. This increase of about 5 times normal is similar to that used in recent studies by King and colleagues (33, 34), who increased salt intake with a 2% NaCl diet from a control level of 0.4%, but contrasts past studies in the rat in which rats were given diets with a salt content of 4% or 8% to increase salt intake by up to 20 times normal (31, 48, 50). In humans, a low salt intake is considered to be anything less than 3 g/day, so a 20 times increase would require a salt intake of up to 60 g/day, a level that is well outside that observed even in populations with extreme salt intakes (30). Humans have been suggested to have evolved and adapted to a level of salt intake of 1–2 g/day (40), with current levels of salt intake in Western populations averaging 10 g/day. Thus, the proportional difference in salt intake between normal and high-salt diets in animals from the current study is comparable to the human setting, and we believe that in our current study, we have given a physiologically relevant salt load.

One potentially confounding factor could be the timeframe examined; many human and animal studies that have shown salt sensitivity of arterial pressure have examined populations after a lifetime of differences in salt intake (30) or experimentally investigated the effect of a high-salt diet over extremely long time periods ranging from several months (14, 51) to 2 years (21). A study by Van Vliet et al. (52) found there to be two phases in the hypertension induced by a high-salt diet (4%) in Dahl rats: the first, a rapid and reversible increase in arterial pressure developing rapidly within 4 days, and the second, a continuing and progressively more irreversible rise in arterial pressure occurring over 6 wk (52). Thus, it may be that the time...
course of the current study was simply too short to capture the sort of slow, progressive cardiovascular changes associated with high salt intake that have been observed in other species previously. Interestingly it has recently been shown that under anesthesia, the RSNA and blood pressure responses to glutamate and GABA injections into the RVLM are enhanced in rats that have been drinking 1% NaCl for 21 days, but not 1 or 7 days (1). Clearly, it would be of interest to extend the time course of our current study to evaluate much longer time periods. In addition, although the focus of this study was on the long-term level of RSNA, it is possible that transient decreases contributing to the excretion of the salt load may have occurred and not been detected by the analysis of daily averages or else may have occurred between the 15-min recording periods performed at 2 hourly intervals.

A further issue to be considered is the ability, or statistical power, to measure small changes in the variables being assessed. The impact of sample size on the power of the experiment to detect statistically significant changes has been previously described (39), and these principles can be used to evaluate the precision of our study. With 80% power, we can estimate that we could have detected an effect of a high-salt diet of +4.5 normalized RSNA units (1.4 standard deviations) from our baseline level of 9.7 nu, given our baseline variability (standard deviation 3.2 nu). In reality, however, this will tend to underestimate the precision, as this analysis does not take into account the within-subject design. The possibility remains that small, but physiologically relevant, changes in RSNA may have occurred that were beneath our ability to detect.

Perspectives and Significance

With an increasing number of studies examining the effects of dietary salt on various experimental models of hypertension, it is important to distinguish between the effects of dietary salt alone on the cardiovascular and neural systems, and the combined effects of dietary salt and the hypertensive model itself. Our findings, here, show that in the healthy adult rabbit, a high salt intake caused inhibition of the renin-ANG II system, as expected; however, it did not result in a decrease in renal sympathetic drive. We conclude that renal sympathetic activity is not altered during the transition to a high-salt diet, and thus, suppression of the renin-angiotensin system during high salt intake is due to other mechanisms. Previous work has suggested that salt sensitivity of arterial pressure in human subjects or animal models may be due to an inappropriate activation of the sympathetic nervous system in response to an increase in dietary salt (11, 12, 16, 35, 53). Given that we have shown in the current study that the normal physiological response to an increase in dietary salt does not require a change in renal sympathetic nerve activity, it would be of great interest in future studies to identify factors that may disrupt or alter the response of the sympathetic nervous system to dietary salt and investigate the role of the sympathetic nervous system during salt-sensitive hypertension.

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