The Role of the Central Nervous System in NaCl-Sensitive Hypertension in Spontaneously Hypertensive Rats

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The central and peripheral nervous system is typically considered to be a short-term modifier of sympathetic nervous system activity, but several lines of evidence suggest that they contribute to chronic elevation of arterial pressure in at least some forms of hypertension. Our studies focus on the mechanisms underlying NaCl-sensitive hypertension in the spontaneously hypertensive rat (SHR). When these rats are fed a high NaCl diet, their arterial pressure rapidly increases and is maintained about 30 mm Hg higher than those of pair fed controls. The increase in arterial pressure is associated with a decrease in norepinephrine release, specifically in the anterior hypothalamic nucleus (AHN), resulting in increased sympathetic nervous system activity, peripheral vasoconstriction, and arterial pressure. Furthermore, administration of an α2-adrenergic receptor agonist in this area blocks the NaCl-sensitive increase in arterial pressure in the SHR but has no significant effect on arterial pressure in normotensive controls. We have identified three intermediary steps by which dietary NaCl reduces AHN norepinephrine release. First, dietary NaCl causes an increase in plasma NaCl and a blunting of the plasma NaCl circadian rhythm. Second, alterations in plasma NaCl activate osmosensitive neurons in the organum vasculosum of the lamina terminalis (OVLT). Third, OVLT input to the AHN appears to increase the release of atrial natriuretic peptide with a resultant decrease in the local release of norepinephrine. Finally, our evidence demonstrates that these factors lead to an increased rise in sympathetic nervous system activity during the early wake phase in SHR on a high NaCl diet, contributing to NaCl-sensitive hypertension in SHR. Am J Hypertens 2001;14:155S–162S © 2001 American Journal of Hypertension, Ltd.

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nervous system activity, blood pressure regulation, and dietary NaCl excess in NaCl sensitive humans remains an enigma, in part, due to the lack of direct measures of sympathetic nervous system activity in most human studies. The mechanisms of NaCl sensitive hypertension can be studied more fully in animal models, in which the role of the nervous system can be assessed directly by invasive measures, thus potentially elucidating the mechanisms underlying NaCl sensitive hypertension in humans.

The Spontaneously Hypertensive Rat of the Okamoto Strain (SHR) as a Model for Neural Control of Hypertension

Hypertension in spontaneously hypertensive rat (SHR) bears many similarities to primary hypertension in humans,9,10 and studies in SHR suggest that neural mechanisms play a role in the SHR hypertension.11 Research indicates that in SHR alterations of brain catecholamine concentrations contribute to NaCl sensitive hypertension. Such changes in catecholamine stores and activity are first observed during the developmental phase of hypertension in SHR.12-14 In 4-week-old SHR compared to normotensive Wistar-Kyoto (WKY) rats, sodium stores are lower in several areas, including the anterior, paraventricular, and dorsomedial hypothalamic nuclei.13-15 Decreases in dopamine-β-hydroxylase (DBH) activity parallel decreases in sodium content, suggesting that catecholamine synthesis is reduced in these regions. In addition, activity of the adrenaline-synthesizing enzyme phenylethanolamine-N-methyltransferase (PNMT) is elevated in the A1 and A2 areas of brainstem in 4-week-old SHR compared to WKY controls.16 Parallel to these PNMT activity changes, plasma sodium concentration increases during the onset of hypertension in the SHR. Furthermore, destruction of central noradrenergic neurons by intracerebroventricular injection of 6-hydroxydopamine prevents the development of hypertension in SHR fed a basal NaCl diet.17,18 These findings indicate that altered activity of catecholaminergic terminals in the hypothalamus may be related to increased sympathetic nervous system activity and hypertension.

The Role of Central Sodium in NaCl Exacerbated Hypertension in SHR

Our primary focus has been to define the mechanisms by which a high NaCl diet exacerbates hypertension in SHR, thus potentially providing insight into mechanisms that contribute to NaCl sensitive hypertension in humans.19 Whereas transplantation studies suggest that primary renal dysfunction contributes to NaCl sensitive hypertension in SHR, the nervous system also appears to play a primary role, either through the maladaptive regulation of the kid-

![FIG. 1. Average extracellular concentration of the sodium metabolite MOPEG in anterior hypothalamic nucleus of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) fed either a basal or high NaCl diet. MOPEG is a stable metabolite of norepinephrine, and serves as an accurate surrogate for measurement of norepinephrine release. *P < .05 versus WKY fed a similar diet; †P < .05 versus SHR fed the 1% NaCl diet.](https://academic.oup.com/ajh/article-abstract/14/S3/155S/205242/548)
NaCl sensitive hypertension in SHR, and it was therefore somewhat unexpected to discover that AHN (primarily a sympathoinhibitory center) was important in this disease. Several lines of evidence confirm the predominately sympathoinhibitory function of AHN. Electrical stimulation of AHN reduces blood pressure and heart rate in both normotensive and hypertensive rats, whereas electrical stimulation of surrounding nuclei, such as the paraventricular, ventromedial, and posterior hypothalamic nuclei, increases blood pressure and heart rate. Large lesions of the anterior hypothalamic region produce fulminating hypertension in normotensive rats, and in SHR fed a basal NaCl diet, bilateral neurotoxin-induced lesions that are restricted to the AHN result in blood pressure increases equal to those induced by high NaCl diets. This is in marked contrast to posterior hypothalamic area lesions or lesions in the anteroventral third ventricle area (AV3V), both of which tend to decrease blood pressure. The AHN sympathoinhibitory response is mediated by input from two sensory systems, including the baroreceptors. Work of Miyajima and Bunag indicates that the AHN modifies both vagal and sympathetic nerve activity in response to acute pharmacologic manipulation of arterial pressure, as destruction of this area results in increased arterial pressure and decreased bradycardic responses to intravenous injection of phenylephrine. Furthermore, the discharge rate of AHN neurons is altered by carotid sinus nerve stimulation or baroreceptor activation in isolated carotid sinus preparations. Anterior hypothalamic nucleus neurons respond to atrial stretch, and sodium release is increased in the AHN during baroreceptor activation. Osmotic feedback also modifies AHN activity, as intracerebroventricular infusion of NaCl reduces the sympathoinhibitory responses elicited by anterior AHN stimulation. Thus, the AHN plays a role in the inhibition of sympathetic nervous system control, and any blunting of this function could predispose an individual to increased sympathetic nervous system activity and a resulting increase in arterial pressure.

**Role of Sodium in the AHN**

Several studies have demonstrated the importance of sodium release in the AHN. Microinjections of AHN with sodium or α2-adrenergic receptor agonists (eg, clonidine or guanabenz) reduce blood pressure and heart rate in normotensive and hypertensive rats, and these responses are blocked by coadministration of the appropriate antagonist. In contrast, microinjections of the α2-adrenergic receptor agonist into the lateral or posterior hypothalamic areas increase arterial pressure and heart rate, responses that are also blocked by coadministration of the antagonists. Furthermore, studies in the rabbit indicate that sodium turnover in the AHN is increased during baroreflex activation and decreased during baroreceptor unloading. Thus, local infusion of sodium increases the sympathoinhibitory function of AHN neurons, and sodium is released into the AHN at an appropriate time for sympathoinhibition (ie, when the baroreceptor reflex is attempting to decrease arterial pressure).

To further test the role of α2-adrenergic receptors in AHN-mediated cardiovascular control, α2-adrenergic receptor agonists were injected into the AHN of conscious C57BL/6 mice in which α2-adrenergic receptors were functionally deleted by a single point mutation. In control mice, microinjection of 50 nL of either clonidine or guanabenz (10−7 mol/L) caused a rapid decrease in mean arterial pressure that lasted for up to 2 min. In the knockout mice there was no response to the injection. The responses in the control mice were very similar in magnitude to the responses to similar AHN microinjections in WKY. These experiments confirm that AHN α2-adrenergic receptors mediate the sympathoinhibitory response to AHN sodium infusion.

To define the AHN, α2-adrenergic receptor mechanisms underlying NaCl sensitive hypertension, microinjections of an α2-adrenergic receptor agonist were made into the AHN of SHR. The microinjection of the agonists caused a greater dose-related reduction in arterial pressure in SHR fed a high (compared to basal) NaCl diet. Furthermore, the reductions were greater in SHR on either diet than in NaCl resistant SHR (SHR-R) or WKY fed a comparable diet. Correlated with this potentiated response was an increase in α2-adrenergic receptors in SHR fed a high NaCl compared to basal NaCl diet and in SHR compared to SHR-R and WKY fed either NaCl diet. Thus, the enhanced responses in SHR on a high NaCl diet reflect the upregulation of α2-adrenergic receptors after the decrease in sodium release in the AHN of SHR on a high NaCl diet. Interestingly, although the adrenergic receptors upregulate in the AHN of SHR on the high compared to basal NaCl diet, the increase is only about 15% in contrast to a decrease in sodium release of about 66%. Thus, the upregulation of receptors does not fully compensate for the reduction in sodium levels. Two additional experiments have been conducted to demonstrate that the sodium decrease in the AHN of SHR on a high NaCl diet contributes to NaCl sensitive hypertension in this model. First, continuous direct infusion of the AHN with an α2-adrenergic receptor agonist (via an osmotic pump connected to a 32-gauge cannula) abolishes the hypertensive response of SHR to a high NaCl diet, but does not alter arterial pressure in SHR on a basal NaCl diet or in WKY on either diet. Second, neurotoxin-induced lesions of the AHN elicits a chronic increase in arterial pressure in SHR on a basal NaCl diet, but in SHR on a high NaCl diet the lesion causes no additional increase in arterial pressure.
Mechanisms That Alter Sodium Release in SHR AHN

Although the increase in dietary NaCl reduces sodium release in AHN and increases arterial pressure in SHR, it appears the NaCl-induced decrease in sodium release is mediated by an additional factor. Our studies suggest that atrial natriuretic peptide (ANP) acts as a neuromodulator to modify sodium release in this nucleus. We examined both circulating and brain ANP concentrations in SHR, and found that both circulating ANP and ANP content of the anterior hypothalamic region was elevated in SHR compared to WKY. Subsequent studies demonstrated that ANP inhibits neuronal sodium release in the brain and reduces the activity of AHN neurons. Because a high NaCl diet elevates plasma ANP concentration, thereby reducing the volume load on the cardiovascular system, we theorized that ANP levels were also increased in the AHN in response to the high NaCl diet. If this were the case, then ANP would be expected to inhibit sodium release in AHN and reduce the sympathoinhibitory drive of AHN neurons, thereby contributing to NaCl sensitivity in SHR. To test this hypothesis, we microinjected an endogenous ANP antibody into AHN and measured changes in arterial pressure. Atrial natriuretic peptide microinjection into the AHN caused significant acute, dose-related decreases in mean arterial pressure and heart rate in SHR but not in WKY. These data provided the first demonstration that endogenous ANP in the brain can influence cardiovascular function in the rat.

To confirm that the cardiovascular effects of ANP in the AHN are mediated through α2-adrenergic receptors, the AHN of α2 knockout mice was microinjected with ANP (20 nL; 10⁻⁷ mol/L). These microinjections caused a rapid increase in mean arterial pressure in the control mice, but a rapid decrease in mean arterial pressure in the knockout mice. The responses in the control mice were similar to responses in WKY, albeit somewhat smaller in magnitude. These experiments support the role of α2-adrenergic receptors in mediating the cardiovascular action of ANP in AHN.

In addition to ANP’s effects in the AHN, we also observed that ANP stores were significantly decreased in the nucleus tractus solitarii (NTS) of SHR fed a high NaCl diet. Previous studies from both our and other laboratories have demonstrated that baroreflex control of heart rate are significantly blunted in SHR compared to WKY. We hypothesized that altered levels of ANP in the NTS may contribute to the blunting of baroreceptors in SHR. To test this, ANP was microinjected into the caudal NTS and the data demonstrated a further blunting of baroreflex control of heart rate in SHR but not in WKY rats. In contrast, microinjection of the monoclonal ANP antibody enhanced the sensitivity of baroreflex control of heart rate in SHR but not in WKY. The results suggested that ANP acts in the NTS to inhibit baroreflex control of sympathetic activity.

Acute Challenges Alter AHN Sodium

Although the above studies demonstrated that AHN sodium release was regulated by ANP, we hypothesized that AHN sodium release was also modified by both baroreceptor inputs and plasma NaCl concentration. To test the arterial baroreceptor regulation, the release of sodium in AHN was measured after acute increases in arterial pressure in SHR on basal and high NaCl diets. The high NaCl diet elevated mean arterial pressure and greatly reduced basal sodium levels in AHN of SHR but not in WKY. Infusion of tramazoline (an imidazoline that causes long-lasting hypertension) that increased arterial pressure by 25 mm Hg elevated AHN sodium concentrations significantly more in SHR on the basal compared to high NaCl diet. In contrast, in WKY a similar increase in arterial pressure elevated AHN sodium concentrations slightly more in rats on the 8% NaCl diet than in those on the 1% NaCl diet. Thus, elevated arterial pressure increases AHN sodium release through baroreceptor feedback, and this release is blunted in SHR on a high compared to basal NaCl diet.

We also hypothesized that dietary NaCl excess alters AHN sodium release by way of increases in plasma sodium, which is slightly elevated after ingestion of a meal and that these changes may provide a signal that triggers the reduction in release of sodium in AHN. Awake, freely moving rats were challenged with an intravenous infusion (75 μL/min) of hypertonic (2.7%) saline for 20 min. In SHR fed a basal NaCl diet, the hypertonic saline infusion elevated mean arterial pressure by 12% and reduced the concentration of sodium in AHN by 19%; these alterations persisted after termination of the hypertonic saline infusion. In contrast, SHR maintained on the high NaCl diet showed greatly reduced arterial pressure and AHN sodium responses, as would be predicted, as dietary NaCl would have already reduced AHN sodium by >60%. Thus, a small elevation in plasma NaCl can reduce the release of sodium in the AHN of SHR, but in normotensive rats these effects are greatly reduced.

Plasma Sodium is Elevated in Rats on a High NaCl Diet

The above studies suggested that increases in plasma sodium might trigger NaCl sensitive hypertension in SHR. Therefore, we tested the hypothesis that dietary NaCl excess elevates plasma sodium concentration in rats. On a basal NaCl diet the plasma sodium rhythms of SHR and WKY were nearly identical, but plasma sodium concentration was significantly higher in SHR at almost every time point during the 24-h period (Fig. 2). The high NaCl diet increased plasma sodium concentration to a similar extent in SHR and WKY, but the high NaCl diet significantly blunted the plasma sodium rhythm only in SHR (ie, it remained high throughout the full cycle; Fig. 2). These
results demonstrate that a high NaCl diet elevates plasma sodium concentration in both SHR and WKY, but blunts plasma sodium rhythm only in SHR. We hypothesize that the loss of this circadian rhythm may cause a chronic activation of the sodium sensing pathway in the brain and a related elevation of sympathetic nervous system activity.

**Role of the Organum Vasculosum Lamina Terminals (OVLT) in Monitoring Plasma Sodium**

Although baroreceptors convey pressure information to the AHN, organum vasculosum lamina terminals (OVLT) is likely the primary sensor of acute changes in plasma NaCl levels, although it is also possible that there is a direct alteration in extracellular sodium in the brain. However, we have not found cerebrospinal fluid sodium levels to be altered by a high NaCl diet. The OVLT is a circumventricular organ that contains osmoreceptors or sodium receptors, and it regulates the release of the neurotransmitters and hormones from the hypothalamus to maintain plasma sodium and water homeostasis. We hypothesized that the efferent OVLT pathway is a major link between the detection of plasma NaCl concentration and the release of noradrenaline in AHN. To test this hypothesis, the axons that carry information from the OVLT were unilaterally severed, and the rat was challenged with an increase in plasma NaCl (which also increased arterial pressure). We observed the “normal” response to the challenge on the intact side of the brain (ie, the infusion of NaCl caused a reduction in AHN sodium). In contrast, on the lesioned side, the increase in plasma NaCl resulted in an increase in sodium concentration in AHN, apparently in response to an elevation in arterial pressure. Increasing arterial pressure using a phenylephrine infusion resulted in the normal increase in sodium levels both ipsilateral and contralateral to the lesion. Taken together, these data suggest that there are two independent pathways (ie, the baroreceptor and OVLT pathways) that normally regulate sodium release in AHN. Although the baroreceptor pathway stimulates sodium release and thereby activates sympatoexcitatory neurons in AHN, the OVLT pathway reduces sodium release and inhibits sympatoexcitatory neurons in AHN. We hypothesize that these two pathways are normally in opposition to each other, preventing any change in arterial pressure in response to NaCl ingestion. However, in SHR in the presence of high plasma NaCl the OVLT pathway appears to dominate, potentially contributing to NaCl sensitive hypertension in SHR.

**Circadian Rhythm of Sympathetic Nervous System Activity is Exaggerated in SHR by a High NaCl Diet**

Our hypothesis suggests that the net result of the dietary NaCl excess is an increase in sympathetic nervous system activity in SHR but not in WKY. Our plasma sodium data suggested this was the case; however, we wanted to have a more direct method for assessing these features. Early in the progression of NaCl sensitive hypertension in SHR, nighttime arterial pressure is significantly elevated but daytime (sleep period) arterial pressure remains unchanged. If sympathetic nervous system overactivity was responsible for these changes, the increase in sympathetic nervous system activity from daytime to nighttime should be greater in SHR on a high compared to basal NaCl diet.
for 5 days. Direct lumbar nerve traffic recording demonstrated that this prediction was valid. In SHR on a basal NaCl diet, lumbar sympathetic nervous system activity increased by about 17% between 16:00 and 18:00 (Fig. 3). In contrast, in SHR on a high NaCl diet for 5 days, lumbar sympathetic nervous system activity increased by nearly 70% over the same time period (Fig. 3). This large differential is supportive of the hypothesis that sympathetic nervous system activity contributes to the hypertensive response of SHR to a high NaCl diet.

Hypothesis

These and other data suggest that the following cascade of events normally occur in response to dietary NaCl intake (Fig. 4). First, elevated plasma NaCl results increases vascular volume as fluid shifts from the intracellular to the extracellular compartment. This volume expansion is sensed by the baroreceptors, which activate sympathoinhibitory neurons in several central nuclei including the AHN. Second, elevated plasma NaCl activates osmosen-sitive neurons in the OVLT, which decrease the activity of sympathoinhibitory AHN neurons. Thus, the two pathways are in a push–pull relationship and prevent changes in arterial pressure in response to dietary NaCl intake. In contrast, alterations in the “tone” of each pathway contributes to NaCl sensitive hypertension in SHR. In SHR, the ingestion of excess NaCl leads to an elevation of plasma NaCl and a blunting of the normal decrease in plasma NaCl that occurs at the beginning of the dark period (Fig. 4). This causes an increased activation of sodium receptors in the OVLT, altering the release of ANP in the AHN, which in turn inhibits the release of sodium and a resulting decrease in sympathoinhibition by AHN neurons. Furthermore, decreased baroreceptor sensitivity in SHR results in reduced baroreceptor input to AHN sympathoinhibitory neurons. The net result is that OVLT input is higher

**FIG. 3.** Continuous recording of lumbar sympathetic nervous system activity in spontaneously hypertensive rats (SHR) fed either a 0.6% or 8% NaCl diet demonstrated that chronic exposure to the high NaCl diet greatly increased the rise in sympathetic nervous system activity that occurred during the period in which the rats are awakening. The shaded bar reflects the nighttime (lights off) period.

**FIG. 4.** Our hypothesis suggests that the organum vasculosum lamina terminalis (OVLT) and baroreceptor input to anterior hypothalamic nucleus (AHN) sympathoinhibitory neurons are dynamically balanced in NaCl-resistant Wistar-Kyoto rats or Sprague-Dawley rats (top). In contrast when spontaneously hypertensive rats are fed a high NaCl diet (bottom), the OVLT input is dominant, that is, the OVLT input increases (dark arrow) and the baroreceptor input decreases (light gray arrow), leading to a withdrawal of sympathoinhibition and an increase in sympathetic nervous system activity.
compared to baroreceptor input, leading to increased sympathetic nervous system activity and a resulting increase in arterial pressure. Although other mechanisms may contribute to these effects, these and other studies demonstrate that these pathways are important to NaCl sensitive hypertension in SHR.

References


