Cerebrospinal Fluid Hypernatremia Elevates Sympathetic Nerve Activity and Blood Pressure via the Rostral Ventrolateral Medulla

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Abstract—Elevated NaCl concentrations of the cerebrospinal fluid increase sympathetic nerve activity (SNA) in salt-sensitive hypertension. Neurons of the rostral ventrolateral medulla (RVLM) play a pivotal role in the regulation of SNA and receive mono- or polysynaptic inputs from several hypothalamic structures responsive to hypernatremia. Therefore, the present study investigated the contribution of RVLM neurons to the SNA and pressor response to cerebrospinal fluid hypernatremia. Lateral ventricle infusion of 0.15 mol/L, 0.6 mol/L, and 1.0 mol/L NaCl (5 μL/10 minutes) produced concentration-dependent increases in lumbar SNA, adrenal SNA, and arterial blood pressure, despite no change in splanchnic SNA and a decrease in renal SNA. Ganglionic blockade with chlorisondamine or acute lesion of the lamina terminalis blocked or significantly attenuated these responses, respectively. RVLM microinjection of the gamma-aminobutyric acid (GABA) agonist muscimol abolished the sympathoexcitatory response to intracerebroventricular infusion of 1 mol/L NaCl. Furthermore, blockade of ionotropic glutamate, but not angiotensin II type 1, receptors significantly attenuated the increase in lumbar SNA, adrenal SNA, and arterial blood pressure. Finally, single-unit recordings of spinally projecting RVLM neurons revealed 3 distinct populations based on discharge responses to intracerebroventricular infusion of 1 mol/L NaCl: type I excited (46%; 11/24), type II inhibited (37%; 9/24), and type III no change (17%; 4/24). All neurons with slow conduction velocities were type I cells. Collectively, these findings suggest that acute increases in cerebrospinal fluid NaCl concentrations selectively activate a discrete population of RVLM neurons through glutamate receptor activation to increase SNA and arterial blood pressure. (Hypertension. 2015;66:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.05936.)

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Salt-sensitive hypertension is mediated, in part, by an increase in cerebrospinal fluid (CSF) [NaCl] and elevated sympathetic nerve activity (SNA).1,2 Experimental models of salt-sensitive hypertension such as the Dahl salt-sensitive and Spontaneously hypertensive rats are associated with significant increases in CSF [Na+] during high-salt diet.3,4 The increase in CSF [Na+] parallels or even precedes the increase in arterial blood pressure (ABP). To our knowledge, only 1 study is available in salt-sensitive humans and has reported that a chronic high-salt diet increased CSF [Na+] and ABP.5 Importantly, these changes in CSF [Na+] do not occur in salt-resistant counterparts.3,4,6 However, it is not known whether CSF [Na+] of salt-sensitive subjects fluctuate from meal to meal or vary across the circadian cycle. Consistent with the above notion, acute or chronic intracerebroventricular infusion of hypertonic NaCl increases ABP in rodents.7–11 Such responses are enhanced by a high-salt diet12 or exaggerated in salt-sensitive strains such as the Dahl salt-sensitive rat.7,9 Finally, lesion or interruption of neurotransmission in various hypothalamic structures including the lamina terminalis and hypothalamic paraventricular nucleus (PVH) attenuates the increase in ABP produced by acute or chronic central infusions of hypertonic NaCl12–14 and also antagonizes the development of salt-sensitive hypertension in several experimental models.15–18 These hypothalamic circuits raise SNA and ABP through a pathway that involves the epithelial sodium channel and ouabain signaling.1,2

Despite evidence for a central NaCl-driven increase in SNA and ABP initiated by the forebrain hypothalamus, the downstream circuitry and signaling mechanisms are unknown. Antunes et al.19 reported that acute increases in circulating NaCl concentrations activate a spinal vasopressinergic pathway originating in the PVH. In contrast, salt-sensitive hypertension including the Dahl salt-sensitive rat depends on neurotransmission in the PVH and rostral ventrolateral medulla (RVLM).19,20 RVLM neurons play a pivotal role in the regulation of SNA and ABP during various physiological and
pathophysiologival conditions. These neurons are barosensitive and tonically active and support basal SNA through direct projections to preganglionic neurons of the intermediolateral cell column. Moreover, RVLM neurons receive direct mono- or polysynaptic inputs from several hypothalamic structures activated by hypernatremia. Therefore, these observations prompted us to perform a series of experiments to address the extent by which RVLM neurons mediate changes in ABP and SNA to various end organs during acute increases in CSF NaCl concentration.

Methods
All of the experimental procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State College of Medicine. All experiments were performed in male Sprague–Dawley rats (250–400 g; Charles River Laboratories), anesthetized with Inactin (120 mg/kg, IV), and prepared for simultaneous recordings of ABP and SNA (lumbar, renal, splanchnic, and adrenal) as described previously. A brain cannula was implanted into the lateral ventricle for intracerebroventricular infusion of artificial CSF, 0.6 mol/L NaCl, or 1.0 mol/L NaCl (5 µL/10 minutes). The vasopressin antagonist Manning compound (10 µg/kg, IV) was also administered before intracerebroventricular infusions to eliminate the contribution of vasopressin to the NaCl-induced responses. In preliminary experiments, vasopressin receptor blockade attenuated the pressor response by 2±1 mm Hg but did not alter SNA responses to intracerebroventricular infusion of 1 mol/L NaCl. A detailed methods section is available in the online-only Data Supplement.

Results
There were no differences in baseline mean ABP or heart rate for the various experimental groups (Table S1 in the online-only Data Supplement).

Central Components of the NaCl-Induced Pressor Response

Experiment 1
Initial experiments were performed to determine how changes in CSF NaCl concentration altered SNA to various end organs. Intracerebroventricular infusion of 0.15 mol/L, 0.6 mol/L, and 1.0 mol/L NaCl produced concentration-dependent increases in lumbar SNA, adrenal SNA, heart rate, and mean ABP (Figure 1). However, the increase in adrenal SNA and heart rate was delayed and increased ≈8 to 10 minutes after start of the intracerebroventricular infusion. Intracerebroventricular infusion of 1.0 mol/L NaCl decreased renal SNA but did not alter splanchnic SNA. All variables returned to baseline values within 60 minutes (Figure 1).

Experiment 2
In a separate group of animals, CSF was collected from the fourth ventricle to measure changes in Na+ and Cl− during infusion of aCSF or 1.0 mol/L NaCl. Infusion of 1.0 mol/L increased fourth ventricular CSF [Na+] (baseline, 155.3±0.3 mmol/L versus 15 minutes, 159.0±1.6 mmol/L; n=6; P<0.05) and [Cl−] (baseline, 116.4±0.5 mmol/L versus 15 minutes, 119.6±0.7 mmol/L; n=6; P<0.05). Infusion of 0.15 mol/L CSF did not alter CSF [Na+] (baseline, 155.7±0.3 mmol/L versus 15 minutes, 155.0±0.6 mmol/L; n=6; P<0.05) and [Cl−] (baseline, 116.7±0.3 mmol/L versus 15 minutes, 117.0±0.2 mmol/L; n=6; P<0.05).

Experiment 3
To assess the contribution of elevated SNA to the NaCl-induced pressor responses, intracerebroventricular infusions were performed after ganglionic blockade. Intravenous injection of chlorisondamine promptly decreased baseline SNA and mean ABP and abolished the SNA, tachycardic, and pressor response to 0.6 mol/L and 1.0 mol/L NaCl (Figure 2).

Experiment 4
To demonstrate that these SNA responses depend on the lamina terminalis, intracerebroventricular infusions were performed in a fourth set of animals with acute electrolytic lesion of the AV3V. AV3V lesions significantly attenuated the increase in lumbar SNA, adrenal SNA, mean ABP, and heart rate to infusion of 0.6 mol/L and 1.0 mol/L NaCl (Figure 3; Figure S2 for histology). The lesion also significantly attenuated the renal sympathoinhibitory response to 0.6 mol/L and 1.0 mol/L NaCl.

RVLM Neurons Mediate Sympathoexcitatory and Pressor Responses to Intracerebroventricular NaCl

Experiment 5
To test whether RVLM neurons mediate the sympathoexcitatory response to CSF NaCl, intracerebroventricular infusions were performed after inhibition of the RVLM. Bilateral injection of the GABAa agonist muscimol promptly reduced lumbar SNA (−76±6%; P<0.01), renal SNA (−53±10%; P<0.01), adrenal SNA (−62±6%; P<0.01), splanchnic SNA (−35±9%; P<0.01), mean ABP (88±6–57±7 mm Hg; P<0.01), and heart rate (405±12–365±19 bpm; P<0.01). Importantly, bilateral injection of muscimol abolished the lumbar sympathoexcitatory and pressor response to intracerebroventricular infusion of 1.0 mol/L NaCl (Figure 4). The adrenal sympathoexcitatory response was partially attenuated. Bilateral injection of aCSF into the RVLM did not significantly alter any baseline variable (data not shown) or alter the responses to intracerebroventricular infusion of NaCl (Figure 4).

Experiment 6
To identify the neurotransmitter receptor in the RVLM that mediates the above responses, intracerebroventricular infusions were performed after blockade of ionotropic glutamate or angiotensin type 1 receptors in the RVLM. Bilateral injection of kynurenic acid into the RVLM produced a small reduction in lumbar SNA (−6±1%; P<0.05) but did not alter renal SNA (−4±5%; P>0.01), adrenal SNA (−4±7%; P>0.05), splanchnic SNA (−1±1%; P>0.05), mean ABP (85±5–84±4 mm Hg; P>0.05), and heart rate (350±18–346±13 bpm; P>0.05). Blockade of RVLM glutamate receptors with kynurenic acid significantly attenuated the increase in lumbar SNA, adrenal SNA, heart rate, and mean ABP to intracerebroventricular infusion of 1.0 mol/L NaCl (Figure 5). In addition, kynurenic acid also reduced the renal sympathoinhibitory response. In contrast, bilateral injection of the angiotensin type 1 receptor antagonist losartan did not alter the sympathetic, tachycardic, and pressor responses to intracerebroventricular infusion of 1.0 mol/L NaCl (Figure 5). RVLM injection of losartan did not alter baseline lumbar SNA (10±7%; P>0.05), renal SNA (2±3%; P>0.05), adrenal SNA (−1±3%; P>0.05), splanchnic
SNA (3±2%; P>0.05), mean ABP (90±4 to 92±5 mm Hg; P>0.05), and heart rate (371±15 to 368±14 bpm; P>0.05).

CSF Hypernatremia Differentially Affects the Discharge of RVLM Neurons

Experiment 7
A final set of experiments was performed to establish that CSF hypernatremia alters the activity of barosensitive, spinally projecting RVLM neurons. Neurons were divided into 3 types based on the discharge responses to intracerebroventricular infusion of 1 mol/L NaCl. Type I neurons (11/24; 46%) had a baseline discharge of 11±2 Hz and conduction velocity of 2.1±0.4 m/s. Intracerebroventricular infusion of 1.0 mol/L NaCl increased cell discharge within 2 minutes after onset of the infusion, and the activity remained elevated throughout the infusion despite an increased mean ABP (Figure 6). Type II neurons (9/24; 37%) had a baseline discharge and conduction velocity of 11±3 Hz and 2.6±0.3 m/s, respectively. However, infusion of 1 mol/L NaCl decreased the discharge rate of these neurons at 4 minutes after the onset of the intracerebroventricular infusion (Figure 6; Figure S3). Type III neurons (4/24; 17%) displayed a baseline discharge and conduction velocity of 17±8 Hz and 2.3±0.5 m/s, respectively. Infusion of 1 mol/L NaCl did not alter discharge rate in these neurons (Figure 6; Figure S4). A previous study suggested that RVLM neurons may be distinguished between C1 and non-C1 cells based on the conduction velocity because slowly conducting neurons (<1 m/s) were consistently identified as C1 neurons. Therefore, we performed a retrospective analysis of RVLM neurons and the respective conduction velocities using a criteria of <1 m/s to identify putative C1 neurons. This analysis identified 4 RVLM neurons with a conduction velocity of 0.8±0.1 m/s and baseline discharge rate of 7.5±3.0 Hz. Interestingly, all 4 neurons displayed an increased discharge response to intracerebroventricular infusion of 1 mol/L NaCl (Figure 6).

Discussion
Previous studies have documented that increased CSF [Na+] elevates ABP, but the neural pathways and contribution of RVLM neurons have not been determined previously. The present findings provide several novel observations: (1) acute intracerebroventricular infusion of NaCl differentially increases lumbar and adrenal SNA, decreases renal SNA, and does not change splanchnic SNA, (2) acute AV3V lesion prevents these changes, (3) inhibition of RVLM neurons with the GABA A agonist muscimol or blockade of RVLM ionotropic glutamate receptors significantly attenuates the sympathetic and ABP responses to intracerebroventricular infusion of hypertonic NaCl, and (4) acute intracerebroventricular infusion of hypertonic NaCl differentially affected the discharge frequency of
spinally projecting RVLM neurons. Collectively, these findings suggest that the sympathoexcitatory response to CSF hypernatremia depends on AV3V neurons to increase glutamatergic drive onto a selective population of RVLM neurons.

Plasma or CSF hypernatremia increases SNA and ABP in both rodents and humans.1,33 However, the sympathoexcitatory response is likely end-organ dependent. Indeed, our current findings document, for the first time, that acute intracerebroventricular infusion of hypertonic NaCl produced a differential activation of lumbar and adrenal SNA but inhibition of renal SNA and no change in splanchnic SNA. In agreement, several studies have acutely raised NaCl concentrations in different species and through different routes to produce qualitatively similar responses and include (1) intravenous infusion in rodents produce a similar differential SNA response,34 (2) intracarotid infusion of NaCl to produce physiological changes decreases renal SNA,35 and (3) intravenous infusion in humans increases muscle SNA.36,37 This SNA pattern may promote increased sodium excretion through a pressure-natriuresis mechanism and concurrent inhibition of renal SNA through a direct Na+-sympathoinhibitory pathway or a baroreceptor-mediated inhibition of RVLM discharge. The latter may be particularly evident under Inactin-anesthesia; however, unpublished findings in our laboratory indicate a similar pattern of SNA to intracerebroventricular infusion of hypertonic NaCl across several anesthetics (urethane, chloralose and isoflurane).

The forebrain lamina terminalis is a pivotal site for the interaction between NaCl and SNA.1,33,39 A V3V lesions blunt osmotically dependent responses including thirst, vasopressin secretion, and natriuresis.40 These same lesions attenuate or reverse several experimental models of salt-sensitive models associated with elevated CSF NaCl concentration.

Figure 3. A, Arterial blood pressure (ABP); mean ABP (gray line); and lumbar, adrenal, and renal sympathetic nerve activity (SNA) during intracerebroventricular (ICV) infusion of artificial cerebrospinal fluid (aCSF) or 1.0 mol/L NaCl in AV3V-lesioned rat. B, Mean±SEM peak changes of control and AV3V-lesioned animals during ICV infusion of aCSF, 0.6 mol/L NaCl, and 1.0 mol/L NaCl. *P<0.05 vs control, #P<0.05 between NaCl concentrations (aCSF vs 0.6 mol/L vs 1.0 mol/L), rSNA indicates raw SNA.

Figure 4. A, Arterial blood pressure (ABP); mean ABP (gray line); and lumbar, adrenal, and renal sympathetic nerve activity (SNA) during intracerebroventricular (ICV) infusion of 1.0 mol/L NaCl after bilateral rostral ventrolateral medulla (RVLM) injection of artificial cerebrospinal fluid (aCSF) or the GABA_A agonist muscimol. B, Mean±SEM peak changes and schematic illustration of RVLM injection sites. *P<0.05 vs 0.15 mol/L NaCl, #P<0.05, aCSF vs muscimol. HR indicates heart rate; and rSNA, raw SNA.
Our findings extend these observations and demonstrate that both sympathoexcitatory (lumbar and adrenal) and sympathoinhibitory (renal) responses to increased CSF [NaCl] are prevented by AV3V lesions. The manner by which AV3V neurons contribute to these responses likely reflect a Na+-specific versus osmosensitive mechanism as intracerebroventricular or intracarotid infusions of NaCl versus other solutes produce greater increases in SNA or ABP. In this regard, Blaustein et al. have postulated that NaCl activates an epithelial sodium channel-ouabain pathway in the hypothalamus to increase SNA and ABP; however, future studies are needed to directly link this signaling pathway to excitatory responses of Na+-sensing neurons to hypertonic NaCl.

A major goal of these experiments was to identify whether RVLM neurons contribute to the sympathetic and pressor effects of central hypernatremia. Indeed, our findings demonstrate that inhibition of RVLM neurons or blockade of ionotropic glutamate receptors on RVLM neurons prevents or attenuates, respectively, the sympathetic and pressor responses to intracerebroventricular NaCl. Interestingly, blockade of glutamate receptors in the RVLM lowers ABP in Dahl salt-sensitive rats. Although we did not identify the origin of glutamatergic drive to the RVLM, it likely involves a polysynaptic pathway from the AV3V region as injection of retrograde tracers into the RVLM does not produce robust labeling in these nuclei. Although all sources of glutamatergic drive to the RVLM have not been identified, the majority of PVH neurons projecting to the RVLM express vesicular glutamate transporter-2 mRNA, a marker of glutamatergic neurons. Furthermore, the PVH contributes to the sympathetic and pressor responses to acute intracerebroventricular NaCl infusions and elevated ABP in Dahl salt-sensitive rats. Altogether, these findings suggest that central hypernatremia activates hypothalamic pathways to increase glutamatergic drive onto RVLM neurons to increase SNA and ABP in salt-sensitive hypertension.

The single-unit recordings of RVLM neurons demonstrate 3 populations distinguished by the discharge response to intracerebroventricular infusion of NaCl. Approximately one half of these neurons increased discharge to central NaCl infusion, a response consistent with the increase in lumbar and adrenal SNA. The other populations (types II and III) may reflect neurons that regulate renal and splanchnic SNA, respectively. It is unclear whether the discharge responses reflect the integration of glutamatergic, GABAergic, and other synaptic inputs to the RVLM.
at the level of RVLM versus a selective increase in glutamatergic drive onto RVLM neurons regulating lumbar and adrenal SNA. Finally, RVLM neurons have been neurochemically distinguished by the presence or absence of PNMT and considered C1 versus non-C1 neurons, respectively. A previous electrophysiological study reported that all neurons with a conduction velocity <1 m/s were C1 neurons. Here, a retrospective analysis identified 4 neurons with a conduction velocity <1 m/s, and all 4 neurons displayed an increase in discharge during intracerebroventricular infusion of hypertonic NaCl. Although the immunocytochemical processing for tyrosine hydroxylase in our study was unsuccessful, these findings raise the interesting possibility that increased CSF NaCl concentrations may increase SNA and ABP through a selective activation of C1 neurons in the RVLM.

The current study used an acute intracerebroventricular infusion of hypertonic NaCl to gain insight into the pathways and mechanisms that may be activated in salt-sensitive hypertension. A clinical study and various experimental animal models have reported that a high-salt diet increases CSF Na+ concentrations in salt-sensitive subjects or animals but not in salt-resistant counterparts. Evidence suggests that a tonic activation of forebrain Na+ sensitive mechanisms persist in salt-sensitive hypertension as acute intracarotid infusion of hypotonic fluid lowers SNA and ABP in DOCA-salt hypertension rats. However, there are likely additional neuroplastic changes that occur during chronic increases in CSF Na+ concentrations or dietary salt intake to further elevate SNA and ABP. For example, a high-salt diet exaggerates SNA and ABP responses of Sprague–Dawley, Dahl salt-resistant, and Dahl salt-sensitive rats to various stimuli including subsequent intracerebroventricular infusion of hypertonic NaCl, thereby suggesting that salt sensitizes central sympathetic networks. Independent of salt intake, salt-sensitive individuals and animal strains, display exaggerated SNA and ABP responses to various stressors or activation of RVLM neurons. Additional studies are needed to investigate whether these differences reflect altered neuronal function within discrete populations of RVLM neurons that are affected by acute or chronic changes in CSF [Na+] or dietary salt.

Perspectives
Salt-sensitive hypertension is mediated, in part, by elevated SNA driven by a central hypernatremia. The present findings highlight the accumulating evidence for the ability of the central nervous system to differentially control SNA and end-organ function. Such control mechanisms have important implications for nerve denervation studies. For example, acute intracerebroventricular infusion of hypertonic NaCl decreased renal SNA. Moreover, renal denervation has little effect on the development of hypertension in salt-sensitive models associated with a central hypernatremia (ie, Dahl salt-sensitive rats). In addition, the findings also highlight the presence of distinct population of neurons in the RVLM that provide the cellular basis for the differential control of SNA. The identity and cellular phenotype of these cells may represent a future therapeutic target for the treatment of salt-sensitive hypertension.

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Disclosures
None.

References
Novelty and Significance

What Is New?

- An acute increase in cerebrospinal fluid NaCl concentration elevates lumbar and adrenal sympathetic nerve activity, decreases renal sympathetic nerve activity, and does not affect splanchnic sympathetic nerve activity.
- The sympathoexcitatory response depends on glutamatergic receptor activation in the rostral ventrolateral medulla (RVLM).
- Acute intracebroventricular infusion of hypertonic NaCl differentially affects the activity of bulbospinal RVLM neurons including an increased discharge frequency.
- Every slowly conducting RVLM neuron (<1 m/s) displayed an increased firing rate to intracerebroventricular infusion of hypertonic NaCl.

What Is Relevant?

- Increased cerebrospinal fluid NaCl concentrations differentially alter sympathetic outflow to increase arterial blood pressure.
- The sympathetic and pressor responses depend on glutamatergic inputs onto a select population of RVLM neurons.

Summary

The findings suggest that acute increases in cerebrospinal fluid NaCl concentrations differentially elevate sympathetic nerve activity and ABP through increased glutamatergic inputs onto bulbospinal RVLM neurons that originates from the AV3V region.