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Activation of the hypothalamic paraventricular nucleus by forebrain hypertonicity selectively increases tonic vasomotor sympathetic nerve activity

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Holbein WW, Toney GM. Activation of the hypothalamic paraventricular nucleus by forebrain hypertonicity selectively increases tonic vasomotor sympathetic nerve activity. Am J Physiol Regul Integr Comp Physiol 308: R351–R359, 2015. First published December 17, 2014; doi:10.1152/ajpregu.00460.2014.—We recently reported that mean arterial pressure (MAP) is maintained in water-deprived rats by an irregular tonic component of vasomotor sympathetic nerve activity (SNA) that is driven by neuronal activity in the hypothalamic paraventricular nucleus (PVN). To establish whether generation of tonic SNA requires time-dependent (i.e., hours or days of dehydration) neuroadaptive responses or can be abruptly generated by even acute circuit activation, forebrain sympathoexcitatory osmosensory inputs to PVN were stimulated by infusion (0.1 ml/min, 10 min) of hypertonic saline (HTS; 1.5 M NaCl) through an internal carotid artery (ICA). Whereas isotonic saline (ITS; 0.15 M NaCl) had no effect (n = 5), HTS increased (P < 0.001; n = 6) splanchic SNA (sSNA), phrenic nerve activity (PNA), and MAP. Bilateral PVN injections of muscimol (n = 6) prevented HTS-evoked increases of integrated sSNA and PNA (P < 0.001) and attenuated the accompanyingpressor response (P < 0.01). Blockade of PVN NMDA receptors with D-(2R)-amino-5-phosphonovaleric acid (AP5; n = 6) had similar effects. Analysis of respiratory rhythmic bursting of sSNA revealed that ICA HTS increased mean voltage (P < 0.001) without affecting the amplitude of inspiratory or expiratory bursts. Analysis of cardiac rhythmic sSNA likewise revealed that ICA HTS increased mean voltage. Cardiac rhythmic sSNA oscillation amplitude was also increased, which is consistent with activation of arterial baroreceptor during the accompanyingpressor response. Increased mean sSNA oscillation voltage by HTS was blocked by prior PVN inhibition (muscimol) and blockade of PVN NMDA receptors (AP5). We conclude that even acute glutamatergic activation of PVN (i.e., by hypertonicity) is sufficient to selectively increase a tonic component of vasomotor SNA.

hypertension; dehydration; osmolality; rostral ventrolateral medulla; respiratory network; arterial baroreceptor reflex

ONGOING VASOMOTOR SYMPATHETIC nerve activity (SNA) normally consists of bursts of action potentials synchronized with the respiratory and cardiac cycles (1, 24, 26). Numerous studies in rats have established that the overall level of integrated SNA to multiple vascular beds increases when body fluid homeostasis is disrupted by dehydration (water deprivation) or acute/chronic salt loading (3, 4, 8, 9, 11, 14–17, 21, 41, 42, 44, 46). Using an in situ arterially perfused rat preparation, Colomberti et al. (16) have further demonstrated that the increase of integrated thoracic SNA after 72 h of dehydration involves a significant increase in the amplitude of inspiratory, but not expiratory, burst discharge. Enhancement of the inspiratory burst of SNA appears to be a relatively late circuit adaptation because our recent findings indicate that dehydration for 48 h selectively activates an irregular component of splanchnic SNA without changing either respiratory or cardiac sympathetic bursting (29, 30). Two features of this tonic portion of SNA are especially noteworthy. First, it is required for support of mean arterial pressure (MAP) in the 48-h dehydrated rat, which indicates that it subserves vasomotor function. Second, it depends on activity of presympathetic neurons of the hypothalamic paraventricular nucleus (PVN) (30).

Presympathetic PVN neurons are not intrinsically osmosensitive (3), but become active during dehydration due largely to excitatory input from osmosensory neurons of the forebrain lamina terminalis (7, 11, 15, 31, 36, 45, 49). Local ionotropic L-glutamate receptors (3, 21) and ANG II AT1 receptors (14, 17, 21) mediate hyperosmotic activation of presympathetic PVN neurons (8, 9, 46). Presympathetic PVN neurons, in turn, transmit sympathoexcitatory signals downstream via glutamatergic inputs to the rostral ventrolateral medulla (8, 9, 46) and vasopressinergic inputs to the spinal cord (3). Tonic SNA driven by the PVN has so far been observed only after progressive dehydration. Therefore, it could be that tonic SNA is generated as an adaptive response to progressive or prolonged circuit activation. Alternatively, tonic SNA could be an emergent property of a normally quiescent PVN-inclusive sympathetic network, such that even acute activation of sympathoexcitatory inputs to PVN could elicit increased tonic SNA.

To resolve these issues, experiments were performed to acutely activate sympathoexcitatory osmosensory inputs to PVN and determine the extent to which the resulting increase of integrated SNA involves increased respiratory or cardiac rhythmic bursting or activation of tonic SNA. Hypertonic saline (HTS) was infused for 10 min at a constant rate through the internal carotid artery (ICA) to stably activate forebrain osmosensory inputs to PVN. Whereas isotonic saline (ITS) infusion had no effect, HTS elicited a sustained increase of splanchnic SNA (sSNA). Further analysis revealed that HTS induced an irregular tonic component of sSNA without changing the amplitude of its respiratory or cardiac rhythmic bursts.

In a separate group of rats, the GABA₄ receptor agonist muscimol or the selective NMDA receptor antagonist D-(2R)-amino-5-phosphonovaleric acid (AP5) was injected bilaterally...
into PVN prior to ICA infusion of HTS. These pretreatments prevented the increase of tonic sSNA elicited by HTS infusion. The pressor response was attenuated by muscimol and blocked by AP5. We interpret these findings to indicate that increased arterial pressure during acute forebrain hypertonic stimulation involves selective activation of a tonic component of sSNA driven by an NMDA receptor-dependent mechanism in the hypothalamic PVN.

**METHODS**

**Animals, housing, and use approval.** Adult male Sprague-Dawley rats (250–400 g) (Charles River Laboratories) were housed in a temperature-controlled room (22–23°C) with a 14:10-h light-dark cycle (lights on at 0700). Rats had ad libitum access to tap water and laboratory chow (Harlan Teklad LM-485, 0.3% NaCl). All experimental and surgical procedures complied with guidelines set forth by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

**Experimental Procedures**

**Surgery.** Rats were anesthetized by intraperitoneal injection of a mixture of α-chloralose (80 mg/kg) and urethane (800 mg/kg) (Sigma-Aldrich). Catheters [polyethylene (PE)-50 tubing] were implanted in the left femoral artery and both femoral veins for recording ABP and the administration of drugs, respectively. To stimulate osmosensory regions of the forebrain, a nonoccluding catheter made of flame-pulled PE-10 tubing was inserted into the left ICA and advanced ~12 mm beyond the carotid sinus to avoid activation of arterial baroreceptors. A lead 1 ECG was recorded, and heart rate (HR) was derived from the R-R interval. After tracheal cannulation, rats were artificially ventilated with oxygen-enriched room air and paralyzed with gallamine triethiodide (25 mg·kg⁻¹·h⁻¹ iv). An adequate depth of anesthesia was assessed by the absence of a withdrawal reflex before paralysis and lack of a pressor response to foot pinch thereafter. Supplemental doses of anesthetic (10% of initial dose) were given as necessary. Cervical vagus nerves were transected to eliminate transmission of pulmonary stretch receptor inputs and, thereby, prevent entrainment of the central respiratory network to the frequency of artificial venti-

**Phrenic and sympathetic nerve recording.** To record phrenic nerve activity (PNA), tissue overlying the left scapula was incised and retracted laterally. The phrenic nerve was isolated near the brachial plexus and transected; its proximal end was then placed on a bipolar silver wire electrode (A-M Systems; 0.005 in. OD). To record splanchnic SNA (sSNA), the greater splanchnic nerve was exposed through a retroperitoneal incision, isolated proximal to the adrenal gland, and placed on a bipolar stainless-steel wire electrode (A-M Systems; 0.005-in. OD). To isolate recordings from body fluid, each nerve-electrode interface was covered with a silicon-based impression material (Super-Dent Light, Carlisle Laboratories). Signals were obtained through high-impedance probes connected to AC amplifiers that were equipped with half-amplitude frequency filters (band pass: 30–1,000 Hz) and a 60-Hz notch filter. Nerve signals were amplified (20–50 ks), full-wave rectified, RC integrated (τ = 10 ms), and digitized at 1.5 kHz using a Micro 1401MK II analog-to-digital converter and Spike2 software (v7.1, Cambridge Electronic Design). Noise in sSNA recordings was determined as a 3-min average of the signal recorded 5 min after bolus injection of the ganglion blocker hexamethonium (30 mg/kg iv).

**Data Analysis**

Values of sSNA, PNA, and MAP were quantified from 5-min segments of stable data immediately before and 15 min after PVN injections. Effects of ICA ITS and HTS were quantified during the last 5 min of infusion, while recorded variables were stable. Values of sSNA are expressed in microvolt units (µV) determined after subtracting voltage due to noise, which was determined from a 3-min average of signal remaining 5 min after administration of hexamethonium. MAP was calculated as the sum of the average diastolic pressure and one-third of the average pulse pressure.

**Histology**

Hematoctrit (Hct) was measured from duplicate capillary tubes using a Lancer microhematocrit reader (Brunswick). Plasma osmolality (PosM) was determined from the average of duplicate plasma samples using a freezing-point depression osmometer (model 3320, Advanced Instruments). Refractometry (VWR International) was used to determine plasma protein concentration (Pprotein).
Hypertonic excitation of PVN increases tonic SNA

Table 1. Baseline values of recorded variables

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, bpm</th>
<th>sSNA, μV</th>
<th>PNA Ampl., μV</th>
<th>PNA AUC, μV·s</th>
<th>PNA Freq., BPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertonic Saline ICA</td>
<td>6</td>
<td>104 ± 4</td>
<td>415 ± 12</td>
<td>4.1 ± 0.6</td>
<td>9.1 ± 1.3</td>
<td>4.5 ± 0.4</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Isotonic Saline ICA</td>
<td>5</td>
<td>105 ± 2</td>
<td>420 ± 12</td>
<td>4.3 ± 0.5</td>
<td>11.3 ± 3.2</td>
<td>4.4 ± 0.6</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Hypertonic Saline IV</td>
<td>4</td>
<td>98 ± 5</td>
<td>407 ± 16</td>
<td>5.0 ± 1.0</td>
<td>14.0 ± 3.7</td>
<td>4.8 ± 0.8</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>Hypertonic Saline ICA and PVN Muscimol</td>
<td>6</td>
<td>103 ± 3</td>
<td>419 ± 12</td>
<td>5.0 ± 0.7</td>
<td>14.2 ± 2.3</td>
<td>5.2 ± 0.6</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Hypertonic Saline ICA and PVN AP5</td>
<td>6</td>
<td>100 ± 4</td>
<td>426 ± 8</td>
<td>4.8 ± 0.4</td>
<td>11.7 ± 2.3</td>
<td>4.5 ± 0.6</td>
<td>39 ± 3</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = number of rats. MAP, mean arterial pressure; HR, heart rate; sSNA, splanchnic sympathetic nerve activity; PNA Ampl., phrenic nerve activity burst amplitude; PNA AUC, PNA area under the curve; PNA Freq., PNA frequency.

Table 2. Hematologic values before and 5 min after saline infusions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Hct, %</th>
<th>PosM, mosmol/kg</th>
<th>Pprotein, g/dl</th>
<th>Hct, %</th>
<th>PosM, mosmol/kg</th>
<th>Pprotein, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertonic NaCl ICA</td>
<td>6</td>
<td>46 ± 1</td>
<td>326 ± 4</td>
<td>4.2 ± 0.2</td>
<td>44 ± 1</td>
<td>342 ± 4*</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>Isotonic NaCl ICA</td>
<td>5</td>
<td>45 ± 2</td>
<td>332 ± 4</td>
<td>4.3 ± 0.2</td>
<td>44 ± 2</td>
<td>329 ± 3</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>Hypertonic NaCl IV</td>
<td>4</td>
<td>46 ± 1</td>
<td>329 ± 4</td>
<td>4.7 ± 0.1</td>
<td>43 ± 2</td>
<td>349 ± 5*</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Hypertonic NaCl ICA and PVN muscimol</td>
<td>6</td>
<td>44 ± 1</td>
<td>332 ± 2</td>
<td>4.6 ± 0.2</td>
<td>42 ± 2</td>
<td>348 ± 2*</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Hypertonic NaCl ICA and PVN AP5</td>
<td>6</td>
<td>45 ± 1</td>
<td>334 ± 3</td>
<td>4.5 ± 0.2</td>
<td>43 ± 1</td>
<td>345 ± 4*</td>
<td>4.4 ± 0.2</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = number of rats; Hct, hematocrit; PosM, plasma osmolality; Pprotein, plasma protein concentration. As previously reported (12, 18), PosM values are elevated by ~30 mOsmol H2O due to the osmotic effect of urethane-chloralose anesthesia. *P < 0.01 vs. before infusion.

RESULTS

Recorded Variables at Baseline

Table 1 shows baseline values of MAP, HR, sSNA, and PNA in all experimental groups. Values of each variable were similar across groups. Note that values of PNA amplitude, AUC, and frequency indicate that central respiratory drive at baseline was also similar across groups.

Effects of ICA Infusions on Hct, PosM, and Pprotein

Table 2 shows values of Hct, PosM, and Pprotein before and after infusions of ITS and HTS. Values before infusions were similar across groups, although, as previously reported (14, 42, 46), baseline PosM was elevated as a result of urethane anesthesia, which acts as a uniformly distributed osmolyte (18). Five minutes after completing each 10-min infusion of HTS, Hct and Pprotein were unchanged. As expected (41, 42), PosM increased similarly in all groups by an average of ~17 mosmol/kg H2O (P < 0.01).

Effects of Hypertonic Saline on sSNA, MAP, and PNA

Figure 1A shows representative responses to ICA infusion of ITS (left) and HTS (right). As expected, ITS had no effect on recorded variables. Consistent with our previous studies (14, 41, 42, 47, 48), HTS promptly increased sSNA and MAP without affecting HR (data not shown). ICA infusion of HTS also promptly increased the amplitude of PNA bursts. A small increase of burst frequency was also noted. Responses of recorded variables peaked within 1–2 min and remained elevated during the remainder of the 10-min ICA infusion period and during the postinfusion period prior to administration of hexamethonium.

Statistical analysis

Baseline sSNA, PNA (amplitude, AUC, and frequency), MAP, HR, and ETCO₂, hematocrit, PosM, and Pprotein were compared across groups with one-way ANOVA. Effects of ICA infusions and PVN injections on MAP, HR, sSNA, and PNA within a group were compared with paired Student’s t-tests. Effects across groups were compared by two-way ANOVA. When significant F values were obtained, independent t-tests with layered Bonferroni corrections were performed for pair-wise intergroup comparisons. Statistical tests were performed using Prism software (v5.0, GraphPad). A critical value of P < 0.05 was considered statistically significant.
To confirm the role of PVN neuronal activity in sympathoexcitatory and pressor responses to ICA HTS, the GABA_A receptor agonist muscimol (100 pmol·50 nl⁻¹·side⁻¹) was bilaterally injected prior to the start of ICA HTS infusion. Consistent with previous reports (4, 29, 44, 46), PVN muscimol had no effect on resting sSNA, MAP, or PNA amplitude (data not shown). However, as Fig. 1B (left) shows, sympathoexcitatory and pressor responses to ICA HTS were nearly abolished compared with those recorded in untreated control rats (Fig. 1A, right). As previously reported (14, 41, 42), control infusions of intravenous HTS (n = 5) were without effect (sSNA: +0.1 ± 0.1 μV; MAP: −0.3 ± 1 mmHg), indicating that the response to ICA HTS was likely due to forebrain stimulation.

**Fig. 1.** Effects of forebrain hypertonic saline on splanchnic sympathetic nerve activity (SNA), arterial blood pressure (ABP), and phrenic nerve activity (PNA). A: representative responses to internal carotid artery (ICA) infusion of isotonic (left) and hypertonic (right) saline. Whereas isotonic saline (ITS; 0.15 M NaCl, 0.1 ml/min, 10 min) was without effect, hypertonic saline (HTS; 1.5 M NaCl, 0.1 ml/min, 10 min) promptly (1–2 min) increased sSNA, PNA (burst amplitude), and ABP. Variables remained stably elevated throughout the infusion period. B: bilateral inhibition of PVN with muscimol (100 pmol·50 nl⁻¹·side⁻¹; left) and blockade of NMDA receptors with NMDA receptor antagonist D-(2R)-amino-5-phosphonovaleric acid (AP5) (6 nmol in 50 nl/side; right) each attenuated sSNA, PNA, and ABP responses to HTS. C: summary data indicate that ICA infusion of ITS was consistently without effect on recorded variables (open bars). By contrast, HTS (solid bars) significantly (P < 0.01) increased integrated sSNA (top left), mean arterial pressure (MAP; top right), and PNA burst amplitude (bottom left). PNA burst frequency was unaltered (bottom right). Paraventricular nucleus (PVN) pretreatment with muscimol (n = 6) or AP5 (n = 6) effectively abolished HTS-evoked increases of integrated sSNA and PNA burst amplitude. PVN muscimol attenuated and AP5 prevented pressor responses to ICA HTS. Summary data are expressed as means ± SE. *P < 0.001 vs. before infusion. †P < 0.05 vs. HTS only.
Nonselective blockade of ionotropic glutamate receptors in PVN is known to attenuate sympathoexcitatory and pressor responses to intravascular HTS infusion (3). Given the robust expression (27, 51) and functional significance (4, 13, 20, 34) of NMDA receptors in PVN, additional experiments were performed to determine their specific role in mediating responses to ICA HTS. Figure 1B (right) shows representative responses to HTS after PVN pretreatment with the selective NMDA receptor antagonist AP5. Consistent with previous studies (4, 13, 34), blockade of PVN NMDA receptors alone had no effect on resting sSNA, ABP, or PNA (data not shown), but effectively abolished sustained increases elicited by HTS infusion.

Summary data in Fig. 1C show that whereas ITS (n = 5) had no effect on recorded variables, HTS (n = 6) significantly increased sSNA (+1.9 ± 0.3 µV; P < 0.001) and MAP (+43 ± 7 mmHg; P < 0.001). HTS did not affect PNA burst frequency (+1.0 ± 1.0 BPM) or duration (−0.03 ± 0.02 s, data not shown), but did increase PNA burst amplitude (+4.2 ± 1.0 µV; P < 0.001) and AUC (+0.9 ± 0.1 µV·s; P < 0.01, data not shown).

Pretreatment of PVN with muscimol effectively prevented sSNA (∼0.1 ± 0.4 µV) and PNA burst amplitude (+0.09 ± 0.24 µV) responses to HTS, while also blunting the pressor response (+16 ± 4 mmHg; P < 0.01) compared with controls (P < 0.05). Like muscimol, PVN AP5 also blocked sympathoexcitatory (+0.1 ± 0.2 µV), pressor (+0 ± 2 mmHg), and neural inspiratory (+0.09 ± 0.18 µV) responses to ICA HTS infusion.

Effects of Hypertonic Saline on Respiratory Rhythmic sSNA

In keeping with its lack of effect on resting variables, IV HTS infusion had no effect on amplitude of the inspiratory peak (IP: +0.1 ± 0.1 µV), expiratory trough (ET: +0.01 ± 0.1 µV), or expiratory peak (EP: 0.0 ± 0.03 µV) of sSNA. To determine effects of forebrain HTS stimulation on the pattern of respiratory rhythmic SNA, PNA burst-triggered averages of sSNA were constructed before and during the stable period of responses to ICA infusion. Figure 2A shows representative responses to ICA HTS in an untreated control rat (left) and rats receiving PVN pretreatment with muscimol (middle) and AP5 (right). Data were analyzed as illustrated in the inset of the muscimol pretreatment experiment. Before ICA infusion (black lines), PNA-triggered sSNA averages showed a clear IP in all groups, which was followed by a small ET and a somewhat variably sized EP. Infusion of HTS (gray lines) increased mean voltage in the control rat (left) without altering the overall pattern of respiratory rhythmic SNA. Pretreatment of the PVN with muscimol or AP5 blocked the increase of mean sSNA voltage, again without changing the amplitude of the IP, ET, or EP.

Group data in Fig. 2B indicate that HTS infusion (n = 6) significantly increased mean sSNA voltage (+1.9 ± 0.5 µV; P < 0.01) without affecting the amplitude of its IP, ET, or EP.

![Graphs showing effects of HTS on sSNA](image_url)

Fig. 2. Effects of hypertonic hyperosmotic saline on respiratory rhythmic sSNA. A: representative PNA burst-triggered averages of sSNA constructed before (black trace) and during (gray trace) internal carotid artery (ICA) infusion of hypertonic saline (HTS; 1.5 M NaCl, 0.1 ml/min, 10 min). The inset in the muscimol panel (middle) shows quantified parameters of respiratory rhythmic sSNA. In a control rat (left), HTS increased mean voltage of sSNA without affecting its inspiratory peak (IP), expiratory trough (ET), or expiratory peak (EP). PVN inhibition by pretreatment with muscimol (middle) and blockade of PVN NMDA receptors with AP5 (right) selectively prevented the HTS-evoked increase of mean sSNA voltage without altering the IP, ET, or EP. B: summary data (n = 6/group) confirm that HTS selectively increased mean voltage of sSNA, which was prevented both by PVN inhibition (muscimol) and blockade of NMDA receptors (AP5). Triggered sSNA averages were constructed from the onset of 150 consecutive PNA bursts. Summary data are expressed as means ± SE. *P < 0.01 vs. before infusion.
ICA HTS also did not affect the AUC or duration of the IP, ET, or EP (data not shown). As previously reported (29), PVN injections of muscimol (n = 6) or AP5 (n = 6) prior to ICA infusions had no effect on mean sSNA voltage (muscimol: +0.0 ± 0.2 µV; AP5: −0.2 ± 0.2 µV) or its respiratory rhythmic peaks (amplitude, AUC, or duration). However, PVN pretreatments prevented HTS-evoked increases of mean sSNA voltage (muscimol: −0.7 ± 0.5 µV; AP5: −0.4 ± 0.3 µV). ICA infusion of ITS had no effect on either the mean sSNA voltage or its respiratory rhythmic bursting (data not shown). These data indicate that in urethane-and-chloralose-aneustedized rats, acute hypertonic stimulation of the forebrain increases MAP by activating a tonic component of sSNA that is not respiratory rhythmic. Data further indicate that increased tonic sSNA during ICA HTS infusion depends on PVN neuronal activity and activation of PVN NMDA receptors.

**Effects of Hypertonic Saline on Cardiac Rhythmic sSNA**

Consistent with its lack of effect on resting variables, intravenous infusion of HTS had no effect on the amplitude or periodicity of the cardiac rhythmic sSNA oscillation (+0.1 ± 0.1 µV). To determine effects of forebrain HTS stimulation on cardiac rhythmic discharge of SNA, ECG R-wave-triggered averages of sSNA were constructed before and during the stable period of responses to ICA infusion. Figure 3A shows representative responses to ICA HTS in an untreated control rat (left) and rats receiving PVN pretreatment with muscimol (middle) or AP5 (right). Before ICA infusion (black line) in all subjects, R-wave-triggered sSNA averages showed a clear cardiac rhythmic oscillation, the period of which was equal to the average R-R interval calculated from analyzed data segments. ICA infusion of HTS (gray line) in a control rat increased the mean voltage of sSNA (left) and the amplitude of its cardiac oscillation. We previously demonstrated that the latter is due to activation of arterial baroreceptor inputs, i.e., during the pressor response to ICA HTS. Pretreatment of PVN with muscimol prevented the HTS-evoked increase of mean sSNA voltage and blunted the increased cardiac oscillation amplitude. The latter is consistent with the effect of muscimol to blunt the pressor response to HTS (see Fig. 1C). Like muscimol, PVN pretreatment with AP5 largely prevented effects of HTS on cardiac rhythmic sSNA.

Summary data in Fig. 3B indicate that ICA HTS infusion (n = 6) significantly increased the mean voltage of sSNA (+3.0 ± 0.8 µV; P < 0.01) and its cardiac rhythmic oscillation amplitude (+3.7 ± 0.7 µV; P < 0.01). Mean voltage (−0.2 ± 0.4 µV) and cardiac oscillation amplitude (−0.2 ± 0.2 µV) were not affected by ICA ITS infusion (data not shown). PVN pretreatment with muscimol or AP5 blocked HTS-evoked increases of mean sSNA voltage (muscimol: −0.1 ± 0.2 µV; AP5: −0.3 ± 0.3 µV) and its cardiac oscillation amplitude (muscimol: +0.8 ± 0.4 µV; AP5: +0.5 ± 0.2 µV). Similar to observations made from PNA burst-triggered averages of sSNA, these data from R-wave-triggered sSNA averages indicate that HTS acutely increases a tonic component of sSNA that depends on PVN neuronal discharge driven by NMDA receptor activation.

**Histology**

Figure 4 shows the location of muscimol (left) and AP5 (right) injection sites in the PVN. As indexed by the distribution of coinjected fluorescent microspheres, injection sites consistently targeted the dorsal and lateral parvocellular subregions throughout the rostrocaudal extent of PVN bilaterally. Note that the central magnocellular region on each side of the PVN was also unavoidably targeted.

**DISCUSSION**

Studies in rats have established that acute intravascular infusion of HTS or an equivalent hyperosmotic (nonelectro-
lyte) solution increases renal SNA (13, 42), lumbar/thoracic SNA (3, 16, 17, 42), and MAP without affecting HR (3, 14, 16, 17, 42). It is important to note that similar hypertonic NaCl stimuli in conscious sheep elicit a somewhat different response pattern. Similar to rats, MAP is increased with little change of HR (22, 23, 35). Integrated cardiac SNA is largely unchanged (23) and, unlike rats, renal SNA is strongly reduced (22, 23, 35). Whether in rats or sheep, these responses largely result from activation of osmosensory inputs to the hypothalamic PVN that originate in the forebrain lamina terminalis (22, 23, 31, 35, 36, 45, 49). Here, we report that the pressor response to ICA infusion of HTS in anesthetized rats is also accompanied by increased sSNA. Because ICA ITS and intravenous HTS were both without effect, sympathoexcitatory and pressor responses to ICA HTS appear to reflect selective activation of the osmosensory forebrain.

Consistent with previous reports (32, 33, 43, 50), we observed that ICA HTS increased the burst amplitude of PNA. The frequency of PNA bursts also tended to rise, but this effect did not reach significance. Given that ICA HTS increased the strength of neural inspiration, i.e., PNA burst amplitude, we expected that the inspiratory burst of sSNA might also increase with ICA HTS stimulation. However, we observed no change in either inspiratory or expiratory bursting of sSNA. Instead, forebrain HTS stimulation increased only the mean voltage of sSNA, which we interpret to reflect the emergence of an irregular tonic component of sSNA, as previously described in 48-h water-deprived rats (29, 30). Of note is that a similar increase of tonic sSNA was also apparent in cardiac rhythmic sSNA analyzed using R-wave-triggered averages. In addition, Colombari et al. (16) reported a similar upward shift of voltage in PNA-triggered averages of thoracic SNA in water-deprived rats (16). Unlike water deprivation, which causes a progressive rise of plasma osmolality, ICA HTS increases osmolality abruptly. Hence, it seems that increased tonic sSNA is a stereotyped response to forebrain osmosensory activation whether stimulation occurs acutely or progressively. From this, we conclude that generation of tonic SNA does not require a time-dependent forebrain/hypothalamic neuroadaptive response.

As in our recent studies in water-deprived rats, increased MAP and increased tonic sSNA by ICA HTS were both nearly abolished by PVN inhibition with muscimol. Here, we also determined that PVN blockade of NMDA receptors similarly prevented both the increase of tonic sSNA and MAP by ICA HTS. Collectively, available evidence indicates that increased MAP during forebrain osmosensory stimulation largely results from a selective increase of tonic SNA that is dependent on activation of PVN NMDA receptors. Of note is that increased forebrain tissue osmolality is considered the adequate stimulus for increased integrated SNA elicited by ICA HTS infusion (14, 41, 42, 47) or water deprivation (11). Here, increases of plasma osmolality measured 5 min after ICA HTS infusion averaged $\sim$17 mosmol/kg, which is only slightly greater than plasma hyperosmolality measured after water deprivation for 48 h (15, 44, 46). It should be emphasized that, as previously estimated (42), the effective osmolality within osmosensory regions of the forebrain was almost certainly higher in the present study than would be achieved during 48-h water deprivation. Another caveat worth noting is that urethane anesthesia increases baseline body fluid osmolality. That being the case in the present study, increased plasma osmolality caused by ICA infusion of hypertonic NaCl was superimposed upon a prevailing level of hyperosmolality. Of special note, however, is that urethane is highly lipophilic and, thus, distributes uniformly across extracellular and intracellular compartments. As a result, urethane induces only a transient osmotic gradient, such that cellular dehydration subsides within $\sim$1–2 h of administration (18, 19). Because our experiments commenced several hours after rats were first anesthetized with urethane, it is unlikely that urethane-induced hyperosmolality had a significant impact on pressor and sympathoexcitatory responses to ICA hypertonic NaCl. This conclusion is supported by evidence that pressor and sympathoexcitatory responses to acute intracerebroventricular (17) and intravascular (3) hypertonic NaCl in unanesthetized arterially perfused rats are similar in magnitude to those reported here.

It should be emphasized that previous studies in water-deprived rats sought to investigate physiological mechanisms regulating SNA (4, 8, 9, 11, 16, 44, 46). Here, our goal was to simply drive osmosensory regions of the forebrain acutely to elicit a rapid onset increase of sSNA and to achieve a stable plateau level of sympathoexcitation for a long enough period of time to allow for construction of high-resolution PNA- and R-wave-triggered sSNA averages. Accomplishing this goal reinforced the conclusion that even acute forebrain osmosensory activation is sufficient to elicit a seemingly stereotyped increase of tonic sSNA.

As in previous studies that recorded lumbar and/or renal SNA responses to ICA HTS (14, 42), here, we determined that increased sSNA is similarly dependent on PVN neuronal activity. This is also consistent with a previous report that used an arterially perfused in situ preparation to determine that nonselective blockade of PVN ionotropic glutamate receptors attenuates the lumbar SNA response to hypertonic stimulation (3). Our data extend this finding by showing that selective blockade of PVN NMDA receptors had no effect on resting sSNA but
effectively prevented sympathetic and pressor responses to ICA HTS infusion. Thus, activation of the osmosensory forebrain leads to direct or indirect synaptic activation of the PVN, which, in turn, transmits a descending signal that encodes increased tonic SNA. It is worth noting in this regard that presympathetic PVN neurons have been reported to lack intrinsic osmosensitivity (3).

As noted, analysis of respiratory and cardiac rhythmic bursting of sSNA revealed that forebrain HTS stimulation selectively increased mean voltage of sSNA without significantly altering the amplitude, duration, or AUC of respiratory rhythmic bursts. Although the cardiac rhythmic sSNA oscillation amplitude increased during forebrain HTS stimulation, this appears to reflect arterial baroreceptor activation during the accompanying pressor response (29). Because increased mean sSNA voltage during ICA HTS infusion was prevented by PVN pretreatment with muscimol or AP5, it appears that PVN presympathetic neurons play an essential role in generating and/or transmitting an irregular component of SNA to downstream elements of the extended sympathetic network. Accordingly, forebrain/hypothalamic and brain stem network elements appear to be arranged in such a fashion that respiratory and cardiac rhythmic bursts of discharge are unaltered, while also being superimposed on the increased level of tonic SNA generated during forebrain hypertonic stimulation. Numerous circuit models could potentially explain this patterning, but a likely arrangement would be one in which the osmosensitive irregular signal transmitted downstream from the PVN dominantly targets vasomotor neurons that are largely distinct from those entrained to encode respiratory and/or cardiac rhythmic bursting of SNA.

In conclusion, the present findings indicate that acute ICA infusion of HTS activates a direct or indirect glutamatergic input to the PVN from osmosensory regions of the forebrain lamina terminalis that increases sSNA and MAP. Acute forebrain hypertonic stimulation has a negligible effect on respiratory or cardiac rhythmic bursting of sSNA. Instead, it increases the mean level of sSNA in support of elevated MAP. Taken together with previous studies in water-deprived rats, the present findings indicate that even acute body fluid hyperosmolality activates a normally quiescent tonic component of SNA that accounts for most, if not all, of the resulting increase of neurogenic vasomotor tone.

**Perspectives and Significance**

Neurons comprising circumventricular organs of the forebrain lamina terminalis (7, 31, 36, 42, 45, 47) and brain stem (15) are pivotal mediators of enhanced sympathetic vasomotor tone during salt loading or water deprivation. In the specific case of OVLT neurons, studies have shown that hyperosmolality and the peptide hormone ANG II each gate a nonselective cation current with similar kinetics, ion selectivity, and voltage dependence (6). This suggests that convergent signaling occurs early within osmotic and ANG II-sensing forebrain regions and is consistent with functional studies linking forebrain regions to sympathetic activation in various models of salt-sensitive arterial hypertension (10, 12, 38, 49). Likewise, PVN neuronal activity is critical for full manifestation of physiological (16, 21–23, 29, 35, 39, 44), as well as cardiovascular disease-related (2, 5, 25, 28, 37, 39, 40) sympathetic network activation. The present findings together with those of previous studies in water-deprived rats indicate that inhibition of PVN neuronal activity consistently and selectively reduces a tonic component of SNA without directly impacting its respiratory or cardiac rhythmic bursting patterns. Collectively, available data indicate that increased tonic SNA does not require time-dependent (48-h water deprivation) circuit adaptations but can be manifested rapidly and is, therefore, possibly an intrinsic property of SNA driven by the osmosensory forebrain and transmitted through the PVN. From this, we speculate that sympathoexcitatory states, whether acute or chronic, that depend on PVN neuronal activity could, in general, be characterized by exaggerated tonic sympathetic activity. Further study is needed to determine the extent to which this generalization holds true and, if so, to identify the specific neurons and mechanisms that govern the level of tonic SNA.

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