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Organum vasculosum laminae terminalis contributes to increased sympathetic nerve activity induced by central hyperosmolality

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Abstract

The contribution of the organum vasculosum laminae terminalis (OVLT) in mediating central hyperosmolality-induced increases of sympathetic nerve activity (SNA) and arterial blood pressure (ABP) was assessed in anesthetized rats. Solutions of graded NaCl concentration (150, 375, and 750 mM) were injected (150 μ l) into the forebrain vascular supply via an internal carotid artery (ICA). Time-control experiments ($n = 6$) established that ICA NaCl injections produced short-latency, transient increases of renal SNA (RSNA) and mean ABP (MAP) ($P < 0.05$ –0.001). Responses were graded, highly reproducible, and unaltered by systemic blockade of vasopressin V1 receptors ($n = 4$). In subsequent studies, stimulus-triggered averaging of RSNA was used to accurately locate the OVLT. Involvement of OVLT in responses to ICA NaCl was assessed by recording RSNA and MAP responses before and 15 min after electrolytic lesion of the OVLT ($n = 6$). Before lesion, NaCl injections increased RSNA and MAP ($P < 0.05$ –0.001), similar to time control experiments. After lesion, RSNA responses were significantly reduced ($P < 0.05$ –0.001), but MAP responses were unaltered. To exclude a role for fibers of passage, the inhibitory GABA-A receptor agonist muscimol was microinjected into the OVLT (50 pmol in 50 nl) ($n = 6$). Before muscimol, hypertonic NaCl increased RSNA, lumbar SNA (LSNA), and MAP ($P < 0.05$ –0.001). After muscimol, both RSNA and LSNA were significantly reduced in response to 375 and 750 mM NaCl ($P < 0.05$). MAP responses were again unaffected. Injections of vehicle (saline) into OVLT ($n = 6$) and muscimol lateral to OVLT ($n = 5$) each failed to alter responses to ICA NaCl. We conclude that OVLT neurons contribute to sympathoexcitation by central hyperosmolality.

Keywords

sympathetic nerve discharge; body fluid homeostasis; blood pressure; osmolality

Over the past several years, studies have established that acute hyperosmotic stimulation of the brain increases sympathetic nerve activity (SNA) (3, 12, 15, 24, 29). Indeed, equal osmolar loads of hypertonic NaCl, mannitol, and sucrose have each been shown to increase renal sympathetic nerve activity (RSNA) and ABP when injected into an internal carotid artery (ICA) that perfuses the forebrain (12). Sympathetic activation by central hyperosmolality appears to serve important homeostatic functions since acutely lowering plasma osmolality in the forebrain arterial supply promptly lowers arterial blood pressure

(ABP) in water-deprived rats (10) and in rats with salt-sensitive arterial hypertension (32, 33).

It should be emphasized that osmosensory mechanisms that initiate sympathoexcitatory responses to hyperosmolality have yet to be determined. What has been demonstrated, however, is that hyperosmotic stimulation of SNA involves activation of excitatory synaptic inputs to the hypothalamic paraventricular nucleus (PVN). Indeed, hyperosmolality-induced increases of SNA and ABP require activation of ANG II AT1 receptors (12) and ionotropic glutamate receptors (3) in the PVN, as well as recruitment of a vasopressinergic PVN-spinal pathway (3). The possibility that responses might involve intrinsic osmosensitivity of PVN neurons seems unlikely, given data showing that microinjection of hypertonic NaCl directly into the PVN has no effect on either LSNA or ABP (3). In contrast, osmosensitive inputs to the PVN appear essential, since transection of neural pathways immediately rostral to the PVN effectively eliminates hyperosmolality-induced increases of LSNA and ABP (3).

On the basis of available evidence, osmosensory neurons of the forebrain lamina terminalis were postulated to contribute to hyperosmolality-induced sympathetic activation. This concept is consistent with data showing that regions of the lamina terminalis known as circumventricular organs (CVOs) lack an effective blood-brain barrier (23, 29, 49). Consequently, blood-borne solutes can gain free access to the extracellular fluid surrounding CVO neurons (23, 28, 29). Here, we tested the hypothesis that sympathetic activation by central hyperosmolality critically depends on neuronal activity within the most ventral forebrain CVO, the organum vasculosum laminae terminalis (OVLT). Like other CVOs (e.g., subfornical organ, area postrema, median eminence), the OVLT is perfused by a dense network of fenestrated capillaries (28, 29) and contains neurons that exhibit intrinsic osmosensitivity (14). Indeed, OVLT neurons are already known to contribute significantly to hyperosmolality-induced thirst (27, 28, 44, 48), as well as pituitary release of vasopressin (30, 48) and oxytocin (44). Moreover, OVLT neurons innervate the PVN through mono-synaptic (25, 36) and polysynaptic (21, 29, 49) pathways. These features make OVLT neurons excellent candidates for mediation of sympathoexcitation in response to acute central hyperosmotic stimulation.

In this study, ICA injections of graded concentrations of hypertonic NaCl were performed, and effects on RSNA, LSNA, and ABP were determined in anesthetized rats. Responses under control conditions were compared with those obtained after electrolytic lesion or chemical inhibition of OVLT neurons. Consistent with our previous findings (12), ICA injections of graded concentrations of hypertonic NaCl elicited graded increases in RSNA, LSNA, and ABP. Although ICA injections have the potential to broadly impact CNS structures, even those within the blood-brain barrier (28, 29, 48), we found that ablation of OVLT tissue and inhibition of OVLT neurons each significantly attenuated sympathoexcitatory responses to central hypertonic NaCl. These findings indicate that OVLT neurons play a prominent role in mediating sympathetic activation by central hyperosmolality.

METHODS

Animals

Experiments were performed on male Sprague-Dawley rats ($n = 27$) that weighed 350 – 450 g (Charles River Laboratories, Wilmington, MA). Rats were housed in a temperature-controlled room (22–23°C) with a 14:10-h light-dark cycle (lights on at 7 AM). Tap water and laboratory chow (Harlan Teklad LM-485, 0.3% NaCl) were available ad libitum. All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Surgical and Experimental Procedures

Rats were anesthetized with isoflurane (2–3% in oxygen) and catheters [polyethylene (PE)-50 tubing] were placed in a femoral artery and vein to record ABP and administer drugs, respectively. Heart rate (HR) was obtained from an ECG (lead I). To stimulate osmotic-sensitive regions of the forebrain, a nonoccluding flame-pulled PE-50 catheter was implanted into the left ICA and advanced 1.0–1.3 cm beyond the carotid sinus to avoid activation of arterial baroreceptors. Rats were then removed from isoflurane and anesthesia was maintained with a mixture of α -chloralose (80 mg/kg iv) and urethane (800 mg/kg iv). An adequate depth of anesthesia was assessed by the absence of limb withdrawal or a pressor response to foot pinch. Supplemental anesthetic (10% of initial dose) was given as necessary. After tracheal cannulation, rats were paralyzed with gallamine triethiodide (25 mg/kg/h iv) and artificially ventilated with oxygen-enriched room air. End-tidal PCO₂ was monitored and maintained between 40 and 50 mmHg during experiments by adjusting ventilation rate (80–100 breaths/min) and/or tidal volume (2.0–3.0 ml). Body temperature was measured with a rectal thermometer and maintained at $37 \pm 1^\circ\text{C}$ with a water-circulating pad. In these studies, RSNA was recorded alone or, in the case of microinjection experiments, together with LSNA, as previously described (39).

OVLT Mapping

To precisely locate the OVLT, stimulus-triggered averaging of RSNA was used. Rats were placed in a stereotaxic apparatus, and the skull was leveled between bregma and lambda. A concentric bipolar electrode (500 μm OD, tip tapered at 60° ; FHC, Bowdoin, ME) was lowered into the ventral forebrain at an angle of 8° toward the midline using a piezoelectric micropositioner (EXFO-Burleigh, Quebec, Canada). The electrode was initially placed ~ 2 mm rostral to the estimated location of the OVLT using the following stereotaxic coordinates: 2 mm rostral to bregma, 1 mm lateral to the midline, 8 mm ventral to dura (26). Next, RSNA responses to 30 single-pulse electrical stimuli (1.0 ms, $\sim 50 \mu\text{A}$, 0.5 Hz) were averaged. Stimulus pulses were then delivered at sites progressively caudal to the initial electrode placement using steps that averaged ~ 0.1 to 0.5 mm. The mediolateral position was similarly adjusted until a maximum RSNA response was recorded. Histological examination confirmed that the site yielding the maximum increase in RSNA consistently corresponded to the OVLT (see Fig. 3).

Experimental Protocols

Time-control experiments—To establish the reproducibility of RSNA and ABP responses to repeated ICA injections of graded concentrations of NaCl, we first performed time-control experiments. After surgery was complete, rats were allowed to stabilize for 30 min; OVLT mapping was not performed and the OVLT remained undisturbed. Next, changes in RSNA, HR, and ABP were recorded in response to ICA injection (150 μl) of graded concentrations (150, 375, and 750 mM) of NaCl. Injections were made over a period of ~ 10 s in a random sequence at least 5 min apart, so that recorded variables had returned to baseline between each injection. After 30 min had elapsed, ICA injections of NaCl were repeated, again in a random sequence, to confirm that the first and second series of injection elicited similar RSNA and ABP responses.

Vasopressin V1 receptor blockade—In a separate group of rats ($n = 4$), experiments were performed to assess the influence of vasopressin on responses to ICA NaCl. This was accomplished by recording RSNA and mean arterial pressure (MAP) responses to repeated ICA injections of graded concentrations (150, 375, and 750 mM) of NaCl before and during systemic blockade of V1 receptors with “Manning compound” {[d(CH₂)₅Tyr²(ME)Arg₈]-vasopressin, 10 $\mu\text{g/kg}$ iv}. This dose of Manning compound was similar to that used in

previous studies (32, 33, 50) and was confirmed to effectively block pressor responses induced by vasopressin (20 ng iv).

OVLT lesion experiments—After mapping the location of the OVLT by stimulus-triggered RSNA averaging, rats were allowed to stabilize for 30 min before obtaining responses to the first series of ICA NaCl injections. As in time-control experiments, graded concentrations (150, 375, and 750 mM) of NaCl were injected (150 μ l) via the ICA in random order and RSNA, HR, and ABP responses were recorded. The OVLT was then lesioned by passing direct current (500 μ A, 45 s) through the same electrode used for OVLT mapping. To match the timing of control experiments, 15 min was allowed to elapse after OVLT lesion before performing a second series of identical ICA NaCl injections, again in a random sequence.

OVLT inhibition experiments—In separate experiments, effects of OVLT neuronal inhibition on responses to ICA hypertonic NaCl were tested. The location of OVLT was mapped as described above and control responses of RSNA, LSNA, HR, and ABP to ICA injections (150 μ l) of NaCl (150, 375, and 750 mM) were recorded. Fifteen minutes later the GABA-A receptor agonist muscimol (Sigma) was pressure ejected (50 pmol, 50 nl, 30 s) into the OVLT from a single-barreled glass micropipette (30–50 μ m OD). After another 15 min had elapsed, responses to a second series of ICA NaCl injections were recorded. To verify that microinjections targeted the OVLT, Chicago Sky Blue dye (2% in saline) was microinjected (50 nl) at the same coordinates. Vehicle controls were performed by microinjecting normal saline. A separate group was used for anatomical control experiments in which muscimol injections were purposely delivered ~200 μ m lateral to the mapped location of the OVLT.

Hematology measurements—In muscimol microinjection experiments, plasma osmolality (Posm), hematocrit (Hct), and plasma protein concentration (Pprotein) were measured from blood samples taken from the femoral vein prior to the first and second series of ICA NaCl injections. Hematocrit was determined from duplicate capillary tubes using standard methods. Blood samples (0.4 ml) were centrifuged (10,000 rpm, 3 min), and Posm was measured using a freezing point depression osmometer (Advanced Instruments, model 3320, Norwood, MA). Pprotein was determined by refractometry (VWR International, Buffalo Grove, IL).

Histology—At the end of lesion experiments, transcardiac perfusion was performed, first with 100 ml of chilled (4°C) 0.1 M PBS containing heparin (20 units/ml) followed by 300 ml of chilled PBS containing 4% paraformaldehyde. Brains were removed, postfixed overnight at 4°C in 4% paraformaldehyde, and cryoprotected (30% sucrose-PBS) for at least 2 days. Brains were then cut into 50- μ m-thick coronal sections, mounted on gelatin-coated slides, and cover-slipped. Lesion sites were identified under bright-field microscopy.

For microinjection studies, brains were removed without transcardiac perfusion and postfixed for 24 h. Brains were cryoprotected, sectioned at 50 μ m, mounted on slides, and coverslipped. The tissue distribution of microinjected dye was mapped as previously described (12, 13, 40, 41). Briefly, the outermost dye boundary in each OVLT section was drawn onto a digitized image of the stereotaxic plate corresponding to the same rostral-caudal plane. Sections from similar rostral-caudal levels from each animal were then overlaid, and the outermost dye distribution was drawn to indicate the farthest extent of injectate diffusion for the entire group.

Data analysis—Signals were acquired and analyzed using Spike2 software (ver. 4.21, Cambridge Electronic Design, Cambridge, UK). Baseline values of MAP, HR, RSNA, and LSNA were measured as the average of each variable over a 60-s period immediately before each treatment. Responses of all variables to central osmotic stimulation were measured as the average of a 5-s period centered on the peak response. RSNA and LSNA responses were expressed as a percentage of baseline after subtracting electrical noise from each signal. The latter was determined at the end of each experiment as the average of each voltage recorded 5 min after injection of the ganglionic blocker hexamethonium (30 mg/kg iv). MAP, HR, and SNA responses to the first and second series of ICA NaCl injections were measured and compared in time-control, systemic vasopressin V1 receptor blockade, OVLT lesion, and vehicle/muscimol microinjection experiments. Data were compared using two-way ANOVA with a Bonferroni post hoc test for pairwise comparisons (Prism, ver. 4.0, GraphPad Software, San Diego, CA). Hematology data obtained prior to the first and second series of ICA NaCl injections were compared using a paired *t*-test. *P* values less than 0.05 were considered significant.

RESULTS

Effects of ICA NaCl Injections on MAP, HR, and RSNA

In time-control experiments ($n = 6$), MAP and HR (Table 1), as well as RSNA (Table 2) values recorded prior to the first and second series of ICA NaCl injections were not different, indicating that stimuli had no significant effect on resting hemodynamics. Consistent with our previous findings (12), ICA injections of isotonic NaCl were without effect on all recorded variables (Fig. 1*A*). In contrast, hypertonic NaCl (375 and 750 mM) caused concentration-dependent increases in RSNA ($P < 0.05$ – 0.01 ; Fig. 1*B, left*) and MAP ($P < 0.05$ – 0.001 ; Fig. 1*B, right*). HR was unaffected. The onset latency of RSNA and ABP responses to 375 mM and 750 mM NaCl were similar, beginning 3.0 ± 0.4 s and 4.7 ± 0.5 s after completing each injection. The duration of ABP responses to ICA injection 375 mM and 750 mM NaCl was different ($P < 0.05$), averaging 19.9 ± 1.5 s and 29.1 ± 2.2 s, respectively. Group data demonstrate that the magnitude (Fig. 1*B*) of RSNA and pressor responses to the first and second series of ICA NaCl injections were highly reproducible. ICA injections of NaCl produced a small increase of Posm ($<1\%$) accompanied by reductions of Hct ($\sim 2.5\%$) and Pprotein ($\sim 3.5\%$) (Table 3).

Effect of Systemic Vasopressin Receptor Blockade

Experiments were performed in a separate group of rats ($n = 4$) to determine the influence of systemic vasopressin on responses to ICA hypertonic NaCl. Prior to blockade of vasopressin (V1) receptors, changes of MAP, HR, and RSNA to the first series of ICA injections of NaCl (150, 375, and 750 mM) were similar to those in time-control experiments (compare Figs. 1 and 2). Preliminary studies established that a dose of vasopressin (20 ng iv) raised MAP by an average of 23 ± 6 mmHg ($n = 3$, data not shown). This pressor effect was similar to that evoked by the 750 mM ICA injection of NaCl (see Figs. 1 and 2) and was effectively blocked by the Manning compound (10 μ g/kg iv). During systemic blockade of vasopressin V1 receptors, resting MAP and RSNA were unchanged from baseline (Tables 1 and 2). Peak increases of MAP and RSNA to the second series of ICA NaCl injections were also unchanged compared with the first. These data are summarized in Fig. 2*B* and indicate that systemic vasopressin does not influence short latency pressor and renal sympathoexcitatory responses to ICA hypertonic NaCl.

OVLT mapping—The OVLT was located by constructing stimulus-triggered averages of RSNA at sites within the ventral forebrain. Fig. 3 shows a representative series of RSNA averages. The first was generated from midline ~ 2 mm rostral to the stereotaxic coordinates

for the OVLT (*middle*, top trace). Stimuli directed at this site had little or no observable effect on RSNA. Stimulating at more caudal sites along the midline evoked progressively larger RSNA responses (*middle*, 2nd trace), with the maximum occurring at a site later determined from histological examination to correspond to the OVLT (*middle*, 3rd trace). Stimuli delivered ventral (*middle*, bottom trace) and lateral (*left* and *right*) to the OVLT produced noticeably smaller RSNA responses. The latency to peak RSNA averaged 157 ± 9 ms in 16 experiments.

Effects of OVLT Lesions

Similar to time-control experiments, ICA injections of NaCl produced short-latency, graded increases in RSNA and ABP prior to lesioning the OVLT (Fig. 4*A*, *left*). While passing direct current to lesion the OVLT, HR was unchanged, but MAP fell by an average of ~ 11 mmHg, from 97 ± 3 mmHg to 86 ± 3 ($P < 0.05$; $n = 6$). After the lesion, MAP immediately began to rise and returned to the prelesion baseline within 1–3 min. RSNA also fell while passing direct current to a value $94 \pm 1.5\%$ of baseline but recovered in pace with MAP (data not shown). Indeed, resting RSNA and MAP recorded prior to the first and second series of ICA NaCl injections were not different (Tables 1 and 2). The lack of persistent change in either MAP or RSNA suggests little contribution of OVLT to the maintenance of ongoing ABP under these experimental conditions. After lesioning the OVLT, RSNA responses to hypertonic NaCl were attenuated (Fig. 4*A*, *left* vs. *right*). Group data ($n = 6$) indicate that the magnitude of RSNA responses to ICA hypertonic NaCl were significantly (375 mM, $P < 0.01$, 750 mM, $P < 0.05$) reduced 15 min after OVLT lesion (Fig. 4*B*). In contrast, lesions did not significantly alter the magnitude of pressor responses or the time course of RSNA and ABP responses. Histological examination of forebrain tissue showed that lesions effectively destroyed the OVLT with minimal damage to the surrounding neuropil. A representative example of the OVLT lesion is shown in Fig. 4*C*. Note that tissue destruction was mostly rostral to the optic recess of the third cerebral ventricle at a level mostly anterior to the ventral subdivision of the median preoptic nucleus.

Effects of OVLT Inhibition

To ascertain whether effects of OVLT lesions were attributable to destruction of local neurons or interruption of fibers of passage, RSNA and ABP responses to ICA hypertonic NaCl were recorded before microinjection of muscimol and were compared with responses obtained 15 min thereafter. In these experiments, LSNA was also recorded to assess how ICA hyperosmolality influences sympathetic outflow to different end organs and to determine the role of OVLT in controlling regional SNA responses. Interestingly, LSNA responses were significantly larger than simultaneously recorded RSNA responses ($P < 0.001$; Fig. 5, *A* and *B*). Resting values of MAP, HR, RSNA, and LSNA were maintained after vehicle and muscimol microinjections into the OVLT (Tables 1 and 2). Likewise, responses to ICA hypertonic NaCl were unaffected by vehicle microinjections ($n = 6$) into the OVLT (Fig. 5*A*, *top*, *left* vs. *right* and 5*B*, *left*). In contrast, injections of muscimol into the OVLT had effects similar to those of OVLT lesion—pressor responses were not affected, but significant reductions were observed in RSNA (375 mM, $P < 0.05$, 750 mM, $P < 0.01$) and LSNA (375 mM, $P < 0.01$, 750 mM, $P < 0.01$) responses to hypertonic NaCl (Fig. 5*A*, *bottom*, *left* vs. *right* and 5*B*, *right*).

To verify that effects of muscimol were due to actions within the OVLT, additional control experiments were performed in which muscimol was purposely microinjected ~ 200 μ m lateral to the OVLT. Results again show that LSNA responses to ICA hypertonic NaCl were significantly larger than RSNA responses under control conditions ($P < 0.01$). Laterally injected muscimol was without effect on sympathoexcitatory or pressor responses to ICA hypertonic NaCl (Fig. 6*A*, *top* vs. *bottom* and 6*B*).

As in previous studies (12, 37–39), the distribution of microinjected muscimol was estimated by the spread of microinjected dye. Histological examination revealed that dye was largely confined to the OVLT, with little spread to the ventral portion of the median preoptic nucleus (Fig. 7, black area). This was evident in dye being restricted to tissue ventral to the anterior commissure and rostral to the optic recess of the third ventricle. At this plane, the anterior commissure appears as two distinct fiber bundles each ~300 μ m lateral to the midline. Anatomical control microinjections directed lateral to midline did not encroach significantly on the OVLT (Fig. 7, gray area).

DISCUSSION

The OVLT plays a major role in hyperosmolality-stimulated thirst (19, 27, 28, 44, 48) and pituitary release of vasopressin (30, 44, 48) and oxytocin (17, 35). To date, however, involvement of OVLT in mediating hyperosmolality-induced increases of SNA has not been directly assessed. In this study, electrolytic ablation and chemical inhibition of OVLT neurons each attenuated the acute rise of SNA following ICA injections of hypertonic NaCl without affecting resting ABP or SNA. These findings indicate that OVLT neurons play a significant role in mediating sympathoexcitation induced by central hyperosmotic challenge but do not appear to contribute significantly to maintenance of resting ABP or SNA in urethane-chloralose anesthetized rats.

The present findings and those recently provided by Antunes et al. (3) are consistent with our earlier report, which showed that graded concentrations of hyperosmotic NaCl evoke graded increases of SNA when injected into the forebrain arterial supply via an ICA (12). Using this approach, equivalent osmotic loads of glucose and mannitol were shown to also elicit increases of SNA (12), thereby indicating that the “adequate stimulus” for evoking an acute rise of SNA is central hyperosmolality, not elevated NaCl per se.

It should be emphasized that peak increases of Posm produced by ICA injections of hypertonic NaCl are estimated to exceed levels observed under physiological or pathophysiological conditions. We acknowledge that a far more important estimate of the strength of our osmotic stimuli is their effect on osmolality of extracellular fluid surrounding OVLT and other forebrain neurons. Unfortunately, accuracy of such an estimate is difficult to evaluate. This is the case because injected NaCl solutions would mix with blood in the vasculature and undergo an uncertain degree of dilution due to osmotic draw of water from red blood cells and the interstitium. In addition, because ICA injections were transient (~10 s), it is unlikely that a steady-state level of hyperosmolality was achieved in the vicinity of individual OVLT neurons. This is in contrast to other recent studies in which longer-term carotid artery infusions were used (8, 10, 32, 33). We further acknowledge that our osmotic stimuli do not mimic physiological changes in body fluid osmolality, which are normally much more gradual and do not occur selectively in the brain. Nevertheless, ICA injections provide key experimental advantages in studies intended to identify neural substrates and possibly cellular mechanisms that mediate biological responses to hyperosmolality, such as increases of SNA. This is the case because stimuli can be repeated in a single subject before and after defined experimental manipulations, e.g., tissue lesions or microinjections, with little concurrent impact on systemic body fluid osmolality.

Given that Posm increased by an average of ~2 mosmol/l over the course of these experiments (Table 3), an influence of peripheral sodium/osmoreceptors on acute responses to hypertonic NaCl should be considered (4, 20). Although our studies did not directly assess this possibility, we suggest that it seems unlikely. This is because the volume of each ICA injection was only 150 μ l and the onset latency (<5 s) and duration (<30 s) of responses were also quite short. Therefore, dilution in the peripheral circulation would likely have

prevented a “spike” of osmolality sufficient to activate hepatic-portal sodium/osmoreceptors in this time frame (4, 20). In addition, the osmotic threshold for activation of hepatportal sodium/osmoreceptors is higher than the level of Posm achieved by our NaCl injections (20). The latter is consistent with our observation that the ~2 mosmol/l increase of resting Posm after the first series of ICA NaCl injections was not associated with a change of ongoing MAP, HR, (Table 1) RSNA, or LSNA (Table 2).

Although ablation and inhibition of the OVLT each reduced the rise of SNA produced by ICA hypertonic NaCl, neither treatment significantly reduced the associated pressor responses. Reasons for this are not presently known, but a direct vasoconstrictor role for vasopressin appears unlikely. This is the case because systemic blockade of V1 receptors in this study had no effect on pressor or SNA responses to ICA NaCl. This observation is fully consistent with evidence that pressor responses to electrical stimulation of the OVLT are prevented by ganglionic blockade, indicating dependence on sympathetic activation (26). Finally, because increases of SNA were attenuated after OVLT lesion/inhibition, it would seem that vasoconstrictor actions of vasopressin would have had to undergo enhancement after OVLT manipulations in order for pressor responses to be fully maintained. This is contrary to evidence in the literature (30, 44, 48).

An alternative explanation for maintenance of pressor responses after OVLT lesion/inhibition is that the pressor effectiveness of SNA could have increased during our experiments. Several lines of evidence support this possibility. Increased vascular wall tension can increase vasoconstrictor efficacy of norepinephrine (51), and blood volume expansion increases calcium channel activity in vascular smooth muscle (22). Whether such effects could have been achieved in the present study given the modest increase of blood volume (see Table 3) is not presently known. Even without a frank increase of blood volume, an effective increase of arterial volume may have occurred if NaCl administration acutely increased cardiac output, thereby transferring blood from the venous to the arterial circulation (45, 52).

After completing OVLT lesion experiments and noticing that pressor responses to ICA NaCl were maintained, we chose to simultaneously record RSNA and LSNA in subsequent microinjection studies. This was done in part to assess whether maintenance of pressor responses to ICA NaCl after OVLT disruption might involve enhanced sympathetic outflow to end organs other than the kidneys. This was not observed. Instead, ICA hypertonic NaCl promptly increased both RSNA and LSNA prior to the rise of ABP. This is consistent with earlier studies that recorded only RSNA (12, 16, 37) or LSNA (3). Interestingly, responses of LSNA were significantly greater than responses of RSNA. Although the neural mechanisms underlying this differential effect are not presently known, there is precedent for such an observation. For example, acute chemical inhibition of the PVN has been shown to reduce LSNA significantly more than RSNA in rats deprived of water for 48 h (40). The extent to which larger LSNA responses to hyperosmolality reflects greater activation of circuits controlling LSNA vs. RSNA or less potent reflex buffering remains to be determined (5, 34, 43).

According to our hypothesis, osmosensitive neurons in blood-brain barrier-deficient CVOs of the lamina terminalis are likely to contribute to central hyperosmolality-induced sympathetic activation. This is consistent with evidence that forebrain CVOs form direct and indirect synaptic connections with downstream sympathetic regulatory neurons in the hypothalamus (21, 25, 31, 36, 46, 47, 49). Indeed, sympathetic activation by central hypertonic NaCl has been linked to activation of the hypothalamic PVN. Importantly, the latter does not depend on intrinsic osmosensitivity of PVN neurons since knife cuts directed rostral to the PVN eliminate hyperosmolality-evoked increases in LSNA (3). Moreover,

blockade of PVN ANG II AT1 receptors (13) or ionotropic glutamate receptors (3) attenuates hyperosmolality-evoked SNA responses, thereby suggesting that hyperosmolality activates an angiotensinergic and/or glutamatergic pathway to the PVN that originates from the lamina terminalis. A major role for descending sympathetic control pathways of the PVN has also been demonstrated as a blockade of spinal vasopressin V1 receptors dramatically attenuated the increase of lumbar SNA evoked during vascular perfusion of the CNS with hypertonic NaCl (3).

An important consideration for the current study is that lesion and inhibition of the OVLT each attenuated but did not prevent increases of SNA evoked by ICA hypertonic NaCl. This is reminiscent of evidence by McKinley et al. (28) and Thrasher et al. (48), indicating that OVLT lesions in sheep and dogs, respectively, also failed to fully block hyperosmolality-stimulated drinking and vasopressin release. Histological analysis of lesion and muscimol microinjection sites indicates that the OVLT was consistently destroyed and fully covered by the diffusion of an injected drug. Thus, incomplete ablation/inhibition of the OVLT in the present study seems unlikely to explain our failure to abolish acute increases of SNA. An alternative explanation is that forebrain regions in addition to OVLT contribute to SNA responses. Support for this possibility comes from recent evidence that transection of the brain caudal to the lamina terminalis largely prevents lumbar sympathetic activation by central hyperosmotic challenge (3). One forebrain region that could contribute is the subfornical organ (SFO). Like the OVLT, the SFO is a highly vascular circum-ventricular organ that lacks a blood-brain barrier (29–31, 49). Moreover, SFO neurons project to hypothalamic and brain stem regions that control SNA (31, 36, 46, 47, 49) and, importantly, exhibit intrinsic osmosensitivity (2). Direct evidence for (or against) a contribution of the SFO in mediating hyperosmotic activation of SNA, however, must await further investigation. It should also be noted that vascular administration of hypertonic NaCl can induce osmotic dehydration of brain regions that possess a complete blood-brain barrier (28, 48). Consequently, it is possible that sites other than forebrain CVOs could also contribute to sympathetic activation by central hyperosmolality.

Although the present study indicates that OVLT neurons contribute to sympathoexcitatory effects of central hypertonic NaCl, the mechanism of osmoreception by OVLT neurons remains to be fully elucidated. On the basis of available evidence, osmotic transduction likely involves activation of stretch-inactivated cation (SIC) channels (6, 11). The molecular identity of SIC channels is not known with certainty, but N-terminal variant transient receptor potential vanilloid type 1 channels (TRPV1) have been put forth as a likely candidate (38). Indeed, OVLT neurons from mice that lack TRPV1 channel expression fail to respond to hyperosmotic stimulation (14). Moreover, TRPV1-null mice exhibit a basal increase of plasma osmolality and a significantly blunted drinking response to acute hyperosmotic stimulation (14). Future studies that modulate the activity and/or expression of TRPV1 channels to determine how this impacts SNA responses to acute and chronic hyperosmolality should help to clarify the cellular mechanism of osmotic transduction among neurons comprising sympathetic-regulatory pathways.

Perspectives

The present study has demonstrated a significant role for OVLT neurons in mediating increases of renal and lumbar SNA evoked by acute central hyperosmotic challenge. These findings have potentially important implications for understanding sympathetic control under physiological and pathological conditions. Regarding the former, osmotic regulation of SNA is important for maintenance of ABP during water deprivation (10, 40, 41). The mechanism has been shown to involve ANG II (18) and glutamatergic (3, 18) inputs to the PVN that activate neurons projecting to the rostral ventrolateral medulla and spinal intermediolateral cell column (39, 42). The PVN-RVLM pathway is almost certainly

excitatory, as the vast majority of these neurons are glutamatergic, on the basis of their expression of mRNA encoding the type 2 vesicular glutamate transporter (42). This is consistent with evidence that blockade of ionotropic glutamate receptors in the RVLM significantly reduces ABP in water-deprived but not euhydrated rats (7, 8). It has also been demonstrated recently that elevated NaCl intake for 14 days enhances the sympathoexcitatory actions of glutamate in the RVLM. The latter form of plasticity is reversed within 7 days of resuming normal NaCl intake (1). Importantly, neural mechanisms activated by hyperosmolality may be a key to the development of certain forms of salt-sensitive neurogenic hypertension (9, 32, 33). The latter observations highlight the importance of understanding neuronal osmotransduction at the cellular and molecular levels, as well as the need to develop a detailed map of osmosensitive sympathetic regulatory circuits in the CNS. With such information in hand, it will become increasingly likely that new and potentially important targets for antihypertensive drugs will be identified.

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Fig. 1.

Time-control experiments in untreated rats showing effects on arterial blood pressure (ABP), renal sympathetic nerve activity (RSNA), and integrated RSNA of repeated internal carotid artery (ICA) injections of graded concentrations of NaCl. *A*: ICA NaCl injections elicited graded increases of RSNA and ABP (*left*). Note that responses to the first series (*series 1*) and second series (*series 2*) of ICA injections were highly reproducible. *B*: group data ($n = 6$) showing that *series 1* (open bars) and *series 2* (solid bars) ICA injections each produced similar graded increases of RSNA (*left*) and mean arterial pressure (MAP) (*right*). * $P < 0.001$ vs. 150 mM NaCl, † $P < 0.01$ vs. 375 mM NaCl, ‡ $P < 0.001$ vs. 375 mM NaCl.

Fig. 2. Effects on ABP, RSNA, and integrated RSNA of repeated ICA injections of graded concentrations of NaCl before and during systemic blockade of vasopressin V1 receptors. *A:* prior to blockade of V1 receptors, the first series of ICA NaCl injections elicited graded increases of RSNA and ABP (*left*). Responses to the second series of ICA injections during V1 receptor blockade were unaltered (*right*). *B:* group data ($n = 4$) showing that RSNA (*left*) and MAP (*right*) responses were similar before (*series 1*, open bars) and during (*series 2*, solid bars) systemic V1 receptor blockade * $P < 0.001$ vs. 150 mM NaCl, [†] $P < 0.05$ vs. 375 mM NaCl, [‡] $P < 0.01$ vs. 150 mM NaCl, [§] $P < 0.001$ vs. 375 mM NaCl.

Fig. 3.

Stimulus-triggered averages of RSNA used to determine the location of the OVL. Stimulating sites >0.2 mm rostral to the organum vasculosum laminae terminalis (OVL) caused no obvious peak in the RSNA average (*middle*, top trace). Stimulating within ~0.1 mm rostral of OVL (*middle*, second trace) produced a constant latency increase of RSNA with a maximal response (*middle*, third trace) being evoked from a site corresponding to the OVL (see Fig. 7 for histology). Stimuli directed 0.1 mm lateral (*right* and *left*) and ~0.1 mm ventral (*middle*, bottom trace) to the site of maximum response evoked little or no RSNA response. The latency to the peak RSNA average response following OVL stimulation averaged 157 ± 9 ms ($n = 16$).

Fig. 4.

Effects on ABP, RSNA, and integrated RSNA of ICA injections of graded concentrations of NaCl before and after electrolytic lesion of the OVLT. *A*: before lesion (*left*), injection of isotonic NaCl had no effect on recorded variables, whereas hypertonic NaCl (375 and 750 mM) produced graded increases in ABP and RSNA. After lesioning the OVLT (*right*), RSNA responses to hypertonic NaCl were attenuated. Pressor responses were unaffected. *B*: group data ($n = 6$) comparing effects of OVLT lesions on MAP and RSNA responses to ICA NaCl. Compared with control responses before OVLT lesion (open bars), increases in RSNA evoked by 375 mM and 750 mM NaCl were significantly reduced afterward (solid bars). Increases in MAP were not affected. *C*: histological section (50 μ m) through the OVLT region showing a typical lesion. LV, lateral cerebral ventricle; AC, anterior commissure. * $P < 0.05$ vs. control, ** $P < 0.01$ vs. control.

Fig. 5.

Effects on ABP, integrated RSNA, and integrated LSNA of ICA injections of graded concentrations of NaCl before and after chemical inhibition of the OVLT. *A*: microinjection of vehicle into the OVLT had no effect on responses to ICA hypertonic NaCl (*top, left vs. right*). In contrast, responses were blunted following microinjection of the GABA-A receptor agonist muscimol into the OVLT (*bottom, left vs. right*). *B*: summary data show that increases in LSNA were significantly greater than increases in RSNA. For the group, vehicle injections did not alter responses to ICA NaCl ($n = 6$), whereas muscimol significantly reduced both LSNA and RSNA responses without altering pressor responses ($n = 6$). * $P < 0.05$ vs. control, ** $P < 0.01$ vs. control. † $P < 0.001$ vs. corresponding RSNA response.

Fig. 6.

Effects on ABP, integrated RSNA, and integrated LSNA of ICA injections of graded concentrations of NaCl before and after microinjecting muscimol ~200 μ m lateral to the OVLT. *A*: prior to muscimol microinjection (*top*), ICA hypertonic NaCl (375 and 750 mM) injections caused graded increases in ABP, RSNA, and LSNA. Isotonic NaCl had no effect. Responses 15 min after microinjection of muscimol lateral to the OVLT were not different from control. *B*: group data ($n = 5$) reveal that muscimol microinjected outside the OVLT had no effect on MAP, RSNA, or LSNA responses to ICA hypertonic NaCl. † $P < 0.01$ vs. corresponding RSNA response.

Fig. 7.

Histological verification of OVLT microinjection sites. *A*: section through the forebrain showing a typical microinjection site. Note that the track of tissue damage caused by the stimulating electrode/glass microinjector ends just dorsal to the OVLT and toward its rostral-most extent. *B*: schematic drawings of microinjection sites targeting the ventral lamina terminalis/OVLT region. Black regions represent the overlapping distributions of Chicago sky blue dye for all rats receiving muscimol microinjections targeting the OVLT. Vehicle injections had a similar distribution (see Fig. 5 for response data). Gray regions indicate the distribution of muscimol injected lateral to the OVLT (see Fig. 6 for response

data). The 0.0 mm plane (*middle*) represents the site at which OVLT stimulation evoked the peak RSNA response.

Table 1

Values of MAP and HR prior to the first and second series of ICA NaCl injections

Group	n	Series 1		Series 2	
		MAP, mmHg	HR, BPM	MAP, mmHg	HR, bpm
Time control	6	100±3	402±12	100±4	407±13
VIR blockade	4	99±1	405±5	101±1	404±7
OVLt lesion	6	102±6	394±15	100±7	412±22
Vehicle in OVLT	6	97±4	426±12	99±4	439±12
Muscimol in OVLT	6	99±4	401±15	98±8	402±15
Muscimol outside OVLT	5	100±4	406±15	95±4	405±16

Values are expressed as means ± SE. ICA, internal carotid artery; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; OVLT, organum vasculosum laminae terminalis; VIR, V1 receptor.

Table 2

Baseline RSNA and LSNA values prior to the first and second series of ICA injections of NaCl

Group	n	Series 1		Series 2		RSNA, % Change	LSNA, % Change
		RSNA, V	LSNA, V	RSNA, V	LSNA, V		
Time control	6	0.35±0.04		0.37±0.02		5.7±0.3	
VIR blockade	4	0.24±0.06		0.26±0.03		8.5±0.3	
OVLt lesion	6	0.29±0.04		0.32±0.07		10.3±0.5	
Vehicle in OVLT	6	0.34±0.04	0.18±0.04	0.39±0.07	0.20±0.04	14.7±0.5	11.1±0.8
Muscimol in OVLT	6	0.25±0.04	0.21±0.05	0.28±0.04	0.22±0.04	12.0±0.6	4.8±0.6
Muscimol outside OVLT	5	0.33±0.04	0.21±0.07	0.35±0.04	0.23±0.05	6.1±0.5	9.5±0.4

Values are expressed as means ± SE. RSNA, renal sympathetic nerve activity; LSNA, lumbar sympathetic nerve activity. V, volts.

Table 3

Values of Posm, Hct, and Pprotein prior to the first and second series of ICA injections of NaCl in rats that received muscimol microinjections into the OVLT

Variable	<i>n</i>	Series 1	Series 2
Posm, mosmol/l	6	303±1	305±1 *
Hct, %	6	42±1	40±1 *
Pprotein, g/dl	6	5.7±0.1	5.5±0.1 *

Values are expressed as means ± SE. Posm, plasma osmolality; Hct, hematocrit; Pprotein, plasma protein concentration.

* Significant difference from Series 1 value ($P < 0.05$).