Inhibitory mechanism of the nucleus of the solitary tract involved in the control of cardiovascular, dipsogenic, hormonal, and renal responses to hyperosmolality

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1Department of Physiology and Pathology, School of Dentistry, São Paulo State University, Araçoiaba, São Paulo, Brazil; 2Henry Wellcome Laboratories for Integrative Neuroscience, University of Bristol, UK; and 3Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

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Blanch GT, Freiria-Oliveira AH, Murphy D, Paulin RF, Antunes-Rodrigues J, Colombari E, Menani JV, Colombari DS. Inhibitory mechanism of the nucleus of the solitary tract involved in the control of cardiovascular, dipsogenic, hormonal, and renal responses to hyperosmolality. Am J Physiol Regul Integr Comp Physiol 304: R531–R542, 2013. First published January 30, 2013; doi:10.1152/ajpregu.00191.2012.—The nucleus of the solitary tract (NTS) is the primary site of visceral afferents to the central nervous system. In the present study, we investigated the effects of lesions in the commissural portion of the NTS (commNTS) on the activity of vasopressinergic neurons in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei, plasma vasopressin, arterial pressure, water intake, and sodium excretion in rats with plasma hyperosmolality produced by intragastric 2 M NaCl (2 ml/rat). Male Holtzman rats with 15–20 days of sham or electrolytic lesion (1 mA; 10 s) of the commNTS were used. CommNTS lesions enhanced a 2 M NaCl intragastrically induced increase in the number of vasopressinergic neurons expressing c-Fos in the PVN (28 ± 1, vs. sham: 22 ± 2 c-Fos/AVP cells) and SON (26 ± 4, vs. sham: 11 ± 1 c-Fos/AVP cells), plasma vasopressin levels (21 ± 8, vs. sham: 6.6 ± 1.3 pg/ml), pressor responses (25 ± 7 mmHg, vs. sham: 7 ± 2 mmHg), water intake (17.5 ± 0.8, vs. sham: 11.2 ± 1.8 ml/h), and natriuresis (4.9 ± 0.8, vs. sham: 1.4 ± 0.3 meq/l/h). The pretreatment with vasopressin antagonist abolished the pressor response to intragastric 2 M NaCl in commNTS-lesioned rats (8 ± 2.4 mmHg at 10 min), suggesting that this response is dependent on vasopressin secretion. The results suggest that inhibitory mechanisms dependent on commNTS act to limit or counterbalance behavioral, hormonal, cardiovascular, and renal responses to an acute increase in plasma osmolality.

c-Fos expression; blood pressure; vasopressin; osmoreceptor; thirst
neurons in the hypothalamic PVN or supraoptic nucleus (SON) have not yet been described.

Thus, in the present study, we investigated the changes in c-Fos expression in the PVN and SON vasopressinergic neurons, plasma levels of AVP and OT, arterial pressure, water intake, and urinary excretion, resulting from hyperosmolality induced by intragastric 2 M NaCl in commNTS-lesioned rats.

METHODS

Animals

Male Holtzman rats weighing 300 to 330 g were used. The animals were housed individually in stainless-steel cages in a room with controlled temperature (23 ± 2°C) and humidity (55 ± 10%). Lights were on from 7:00 AM to 7:00 PM. Standard Purina rat chow (Paulinia, SP, Brazil) and tap water were available ad libitum. The Ethical Committee for Animal Care and Use from Dentistry School of Araraquara-UNESP approved the experimental protocols used in the present study (CEEA, protocol 08/2007).

Commissural NTS Lesions

Rats were anesthetized with ketamine (80 mg/kg body wt) combined with xylazine (7 mg/kg body wt) and placed in a stereotaxic apparatus (model 900, David Kopf Instruments). A partial craniotomy of the occipital bone was performed, and the dorsal surface of the brain stem was exposed. A tungsten electrode (0.1 mm in diameter) bared at the tip (0.5 mm) was inserted into the brain using the following coordinates: 0.4, 0.7, and 1.0 mm caudal to the calamus scriptorius, in the midline and 0.3 mm below the dorsal surface of the brain stem. The electrolytic lesion of the commNTS was performed using a cathodal current (1 mA during 5 s, for two times). A clip attached to the tail was used as the indifferent electrode. The sham-lesioned rats had the electrode placed along the same coordinates, except that no current was passed. A prophylactic dose of penicillin (50,000 IU, intramuscularly) and of the anti-inflammatory ketoprofen (1 mg/kg body wt, subcutaneously) were given postsurgically. Rats were allowed to recover for 15–20 days before the experiments.

Osmotic Stimulus

Intragastric 2 M NaCl (2 ml), which produces 4% elevation of both plasma osmolality and sodium concentration, was used as osmotic stimulus. Concurrent reduction of plasma renin activity and no alteration in plasma volume after intragastric 2 M NaCl indicate that this procedure does not induce change in the volume of extracellular compartment (40).

Immunohistochemical Procedures

The animals were deeply anesthetized with sodium thiopental (70 mg/kg body wt ip). Thereafter, all animals were transcardially perfused with 300 ml of 0.1 M PBS (pH 7.4), followed by 500 ml of 4% (wt/vol) paraformaldehyde (PFA; Sigma, St. Louis, MO) solution in 0.1 M PBS, pH 7.4. The brains were removed, fixed for 4 h in 4% (wt/vol) PFA solution, and stored at 4°C in 0.1 M PBS containing 20% (wt/vol) sucrose for no longer than 1 wk. Five sets of coronal sections (30 μm) of the hypothalamus were sectioned on a cryostat (Leica, CM1800). The free-floating sections were collected in 24-well tissue culture plates containing PBS. For the DAB staining, three of the five rat hypothalamic sections were preincubated for 10 min in 3% (vol/vol) hydrogen peroxide (Sigma) in 0.1 M PBS followed by 15-min incubation in a blocking solution comprising 10% (vol/vol) normal goat serum (NGS; Sigma) and 0.3% (vol/vol) Triton X-100 (Sigma) in 0.1 M PBS followed by rinses in PBS (3 × 10 min). Sections were then incubated in a polyclonal rabbit anti-c-Fos primary antibody (1:20,000; Ab-5; Calbiochem, San Diego, CA) in PBS containing 1% (vol/vol) NGS and 0.3% (vol/vol) Triton X-100 for 48 h at 4°C. After the primary antibody incubation the sections were rinsed in PBS (3 × 10 min) prior to 1-h incubation with biotinylated goat anti-rabbit IgG (1:500; Vector Laboratories, Burlingame, CA), followed by further rinses in PBS (3 × 10 min), and incubation with streptavidin HRP (1:500, Vector Laboratories) for 1 h. Sections were rinsed (3 × 10 min) and diaminobenzidine (DAB; Sigma) was used to produce a brown nuclear c-Fos reaction product. Sections were rinsed (3 × 5 min), mounted onto slides in 0.5% (wt/vol) gelatin and allowed to air-dry, dehydrated in a series of alcohols, cleared in xylene and cover slipped. Cells expressing positive nuclear c-Fos immunoreactivity were counted bilaterally by hand each 150 μm, in matched, representative sections of the tissue.

For the double labeling, two of the five hypothalamic sections obtained as described above, were double-labeled using a polyclonal rabbit anti-c-Fos primary antibody and either monoclonal mouse anti-neurophysin II (vasopressin-derived; PS41) or mouse anti-neurophysin I (oxytocin-derived; PS38), both kindly provided by Prof. H. Gainer, (National Institutes of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD). Sections were incubated in primary antibodies (anti-c-Fos: 1:10,000; anti-neurophysin II: 1:500 or anti-neurophysin I: 1:500) in PBS containing 1% (vol/vol) NGS and 0.3% (vol/vol) Triton X-100 for 48 h at 4°C. After the primary antibody incubation, the sections were rinsed in PBS (3 × 10 min) prior to 1-h incubation with biotinylated goat anti-rabbit IgG (1:500 Vector Laboratories). Sections were rinsed in PBS (3 × 10 min) prior to 1-h incubation in Streptavidin Alexa Fluor 488 conjugate and goat anti-mouse Alexa Fluor 594 (both 1:500; Molecular Probes, Eugene, OR). Following further rinses in PBS (3 × 5 min), sections were mounted onto slides in 0.5% (wt/vol) gelatin and allowed to air-dry for 10–15 min before being coverslipped using an antifade fluorescent mountant (VectorShield; Vector Laboratories, Peterborough, UK). The sections (each 150 μm) were visualized on a fluorescent microscope with the appropriate filter.

Radioimmunoassay for Vasopressin and Oxytocin

In animals with chronic commNTS or sham lesion, trunk blood was collected into cooled plastic tubes containing heparin (10 μl/ml) for the measurement of AVP and OT. Blood samples were centrifuged (1,940 g for 20 min at 4°C), and plasma was kept in a freezer at −20°C. For the AVP and OT determinations, samples were extracted from 1 ml of plasma with acetone and petroleum ether. Plasma levels of AVP and OT were measured by specific radioimmunoassay, as described previously (23, 35). AVP and OT antiserum (both raised in rabbit) and 125I-OT were purchased from Bachem (Belmont, CA) and 125I-APV from Duport (Duport NEN Research Products, Boston, MA). The assay sensitivity and the intra-assay and interassay coefficients of variation were 0.7 pg/ml, 7.6% and 12% for AVP, and 0.9 pg/ml, 6.8% and 12.6% for OT, respectively. All samples from an individual subject for all three tests were determined in duplicate in the same assay.

Arterial Pressure Recording

Mean arterial pressure (MAP) and heart rate (HR) were recorded in unanesthetized rats with chronic sham or commNTS lesion. Under xylazine and ketamine anesthesia, polyethylene tubing (PE-10 connected to a PE-50) was inserted into the abdominal aorta through the femoral artery on the day before the experimental procedure. At the same time, PE tubing was also inserted into the femoral vein for drug administration. Arterial and venous catheters were tunneled subcutaneously and exposed on the back of the rat to allow access in unrestrained, freely moving rats. To record pulsatile arterial pressure, MAP, and HR, the arterial catheter was connected to a Statham Gould (P23 Db) pressure transducer coupled to a preamplifier (model no. ETH-200 Bridge Bio Amplifier) that was connected to a PowerLab computer data acquisition system (model PowerLab 16SP, Dunedin, New Zealand).
Histology to Confirm commNTS Lesions

At the end of the experiments, the animals were deeply anesthetized with sodium thiopental (70 mg/kg body wt ip). Thereafter, all animals were transcardially perfused with 0.1 M PBS (pH 7.4) followed by 10% (vol/vol) formalin. The brains were removed, fixed in 10% (vol/vol) formalin, frozen and cut coronally in 50-μm sections on a cryostat (Leica, CM1800), stained with Giemsa stain, and analyzed by light microscopy to confirm the commNTS lesions.

Statistical Analysis

All data are expressed as the means ± SE. One- or two-way (as appropriate) ANOVA followed by Student-Newman-Keuls post hoc or Student’s t test were used for comparisons. Differences were taken as significant at P < 0.05.

Experimental Protocols

c-Fos expression in vasopressinergic and oxytocinergic neurons of the PVN and SON in sham or commNTS-lesioned rats treated with intragastric 2 M NaCl. Immunohistochemistry for c-Fos protein expression in the paravascular and magnocellular regions of the PVN (pPVN and mPVN, respectively) and in SON, as well as AVP or OT immunohistochemistry, was analyzed in chronic sham or commNTS-lesioned rats treated with intragastric gavage of 2 M or 0.15 M NaCl (2 ml). To minimize any possible c-Fos expression related to stress, on day 1, rats received an intragastric gavage of 2 ml of 2 M NaCl and in the subsequent 6 days an intragastric gavage of 2 ml of 0.15 M NaCl. In the day of the experiment (day 8), water and food were removed from the cages, and all rats (sham and commNTS, n = 3 or 4/group) received an intragastric gavage of 2 ml of 0.15 M NaCl or 2 M NaCl randomly. Two hours after the gavage, rats were deeply anesthetized with thiopental sodium (70 mg/kg body wt ip) and perfused with 4% (wt/vol) PFA; then, the brains were processed for immunohistochemistry only for c-Fos (DAB staining) or double labeled for c-Fos/AVP or c-Fos/OT (immunofluorescence), as described in METHODS.

Plasma AVP and OT levels in sham or commNTS-lesioned rats treated with intragastric 2 M NaCl. Chronic sham (n = 8) or commNTS-lesioned rats (n = 7–10) were decapitated 20 min after intragastric 2 M NaCl or 0.15 M NaCl (2 ml), and trunk blood was collected into cooled plastic tubes containing heparin (50 μl) for the measurement of AVP and OT.

Arterial pressure and heart rate in sham or commNTS-lesioned rats treated with intragastric 2 M NaCl. Arterial pressure and HR were recorded in conscious chronic sham or commNTS-lesioned rats. Tests started at least 20 min after connecting the arterial cannula to the recording system. Baroreflex was initially tested in all rats with intravenous injections of a pressor dose of phenylephrine (2.5 μg/kg body wt) and a depressor dose of sodium nitroprusside (30 μg/kg body wt), followed by testing chemoreflex with an intravenous injection of potassium cyanide (KCN, 40 μg/0.1 ml/rat). The interval between two intravenous injections was 10 min, and the data are expressed as peak (maximum) change.

In one group of sham (n = 10) and commNTS-lesioned rats (n = 10), 20 min after KCN injection, animals received an intragastric gavage of isotonic saline (intragastric 0.15 M NaCl, 2 ml), and MAP and HR were continuously recorded for 30 min, followed by an intragastric gavage of 2 M NaCl (2 ml) and an additional 60-min recording of MAP and HR.

In another group of sham (n = 19) or commNTS-lesioned rats (n = 20), 20 min after KCN, the role of vasopressin in the cardiovascular responses to intragastric 2 M NaCl (2 ml) was tested. Manning Compound ([β-Mercapto-β,β-cyclopentamethylenepropionyl], O-Me-Tyr², Arg³]-vasopressin; Sigma, St. Louis, MO; V1 antagonist, 10 μg/kg body wt iv) was injected 10 min before intragastric 2 M NaCl. The changes in MAP and HR after intragastric 2 M NaCl were recorded for an additional 60 min. A pressor dose of vasopressin ([Arg³]-vasopressin; Sigma, St. Louis, MO; 12.5 μg/0.1 ml/rat) was injected intravenously before and 5 and 70 min (end of experiment) after the Manning Compound to test the efficacy of vasopressin V1 receptor blockade.

In another group of chronic sham (n = 5) or commNTS-lesioned (n = 6) rats, baroreflex responses were tested with intravenous infusion of a pressor dose of phenylephrine (8.3 μg·kg⁻¹·min⁻¹) and a depressor dose of sodium nitroprusside (35 μg·kg⁻¹·min⁻¹). The infusion was maintained until MAP had raised or fallen 30 mmHg from the baseline for 30 s. The baroreflex was analyzed as previously described (17).

Water intake in sham or commNTS-lesioned rats treated with intragastric 2 M NaCl. On the day of the experiment, animals with chronic commNTS (n = 14) or sham lesions (n = 14) had water and food removed from the cages. Half of each group received an intragastric 2 M NaCl (2 ml), and the other half received intragastric 0.15 M NaCl (2 ml). One hour after the gavages, the animals had access to water in graduated (0.1 ml division) glass burettes fitted with metal spouts. Cumulative water intake was recorded at 15, 30, 60, 90, and 120 min.

Renal excretion and plasma levels of sodium and potassium in sham or commNTS-lesioned rats treated with intragastric 2 M NaCl. Another group of rats with chronic commNTS (n = 17) or sham lesions (n = 13) were housed in metabolic cages and on the day of the experiment, half of each group received either an gavage of intragastric 2 M NaCl (2 ml) or 0.15 M NaCl (2 ml), and urine was collected for 1 h in graduated tubes. Three days later, the groups were tested again in a counterbalanced manner. Immediately prior to the gavage, all animals received a suprapubic massage to induce micturition and a second suprapubic massage was done before removing the urine tubes from the cage. Urinary Na⁺ and K⁺ concentrations were measured by ion-specific electrode (NOVA 1; Nova Biomedical, Waltham, MA). Total Na⁺ and K⁺ excretions were determined by the product of urine volume and the concentration of each ion in the urine.

Three days after the last urine excretion test, the same animals were submitted to intragastric gavage of 2 ml of either 2 M NaCl or 0.15 M NaCl. After 60 min, the animals were deeply anesthetized with sodium thiopental (70 mg/kg body wt ip), and blood was collected by cardiac punch in prerrefrigerated tubes at 4°C containing a separating gel for determination of serum concentration of protein, Na⁺ and K⁺. Samples were centrifuged for 10 min and analyzed immediately. Serum Na⁺ and K⁺ concentrations were measured by ion-specific electrode (NOVA 1; Nova Biomedical), and serum protein concentration was measured in a refractometer (Atago).

RESULTS

Histological Analysis of the commNTS Lesions

The electrolytic lesions were located in the midline, above the central canal and extended from the level of the obex to ~1 mm caudal to the obex. Lesions of the commNTS (Fig. 1) did not damage the lateral portions of the NTS, such as intermediate NTS and the ventrolateral or dorsolateral NTS, the hypoglossal nucleus (n. XII), or the AP, as shown previously (5, 14, 33, 45). There was only some damage to the dorsal nucleus of the vagus. Only results from the animals that displayed the typical lesion of the commNTS were analyzed. The responses from animals with partial lesion of the commNTS were similar to sham.
c-Fos Expression in the Vasopressinergic Neurons of the PVN and SON in commNTS-Lesioned Rats Treated with Intragastric 2 M NaCl

Compared to sham rats, an increased number of neurons expressed c-Fos in the mPVN of commNTS-lesioned rats treated with intragastric 2 M NaCl (139 ± 22, vs. sham: 70 ± 12 positive cells/section, each 150 μm bilaterally) [F(3,9) = 27.28; P < 0.05] and in the SON (270 ± 11, vs. sham: 191 ± 12 positive cells/section) [F(3,9) = 307.64; P < 0.05] (Fig. 2). c-Fos expression was similar in the pPVN of sham or commNTS-lesioned rats treated with intragastric 2 M NaCl (Fig. 2).

Double labeling for c-Fos/AVP or c-Fos/OT was counted bilaterally in one section with a greater number of magnocellular neurons in both the PVN and SON of sham and commNTS-lesioned rats. Compared with sham rats, increased number of neurons expressed double c-Fos/AVP labeling in the PVN of commNTS-lesioned rats treated with intragastric 2 M NaCl (28 ± 1, vs. sham: 22 ± 2 c-Fos/AVP cells) [F(3,9) = 145.1; P < 0.05] and in the SON (26 ± 4, vs. sham: 11 ± 1 c-Fos/AVP cells) [F(3,9) = 47.10; P < 0.05] (Fig. 3). The intragastric 2 M NaCl similarly increased the number of neurons expressing c-Fos/OT double labeling in the PVN [F(3,9) = 5.49; P < 0.05] and SON [F(3,9) = 9.44; P < 0.05] of sham and commNTS-lesioned rats (Fig. 4).

Plasma AVP and OT Levels in commNTS-Lesioned Rats Treated with Intragastric 2 M NaCl

In sham rats, intragastric 2 M NaCl increased plasma AVP (6.6 ± 1.3 pg/ml, vs. 0.15 M NaCl: 3.7 ± 0.6 pg/ml) [F(3,29) = 3.08; P < 0.05] and OT (21 ± 7 pg/ml, vs. 0.15 M NaCl: 2.7 ± 0.8 pg/ml) [F(3,33) = 5.93, P < 0.05] (Fig. 5). Compared with sham rats, the intragastric 2 M NaCl induced a stronger increase of plasma AVP in commNTS-lesioned rats (21 ± 8.5 pg/ml), whereas the increase in plasma OT was similar (27 ± 9 pg/ml) (Fig. 5). Plasma AVP and OT levels in commNTS-lesioned rats or sham rats treated with intragastric 0.15 M NaCl were similar.

Changes in Arterial Pressure and Heart Rate in commNTS-Lesioned Rats Treated with Intragastric 2 M NaCl

Compared to sham rats, the intragastric 2 M NaCl produced stronger pressor responses in commNTS-lesioned rats that reached the maximum 60 min later (ΔMAP: 25 ± 7, vs. sham: 7 ± 2 mmHg) [F(1,198) = 45.95; P < 0.05] (Fig. 6A). In sham or commNTS-lesioned rats, the intragastric 2 M NaCl did not modify HR [F(1,198) = 0.508, P > 0.05] (Fig. 6B).

CommNTS lesions also did not change the baseline MAP and HR but reduced the pressor responses to chemoreflex activation (Table 1), as described previously (14, 34, 45, 55). In addition, commNTS lesions did not affect baroreflex, either analyzed by the peak of the response (Table 1) or by baroreflex curves (sham rats: gain = −6.1 ± 1.1 bpm/mmHg; upper plateau = 456 ± 23 bpm; lower plateau = 316 ± 17 bpm; MAP50 = 113 ± 4 mmHg; HR range = 316 ± 17 bpm, vs. commNTS rats: gain = −5.9 ± 1.6 bpm/mmHg; upper plateau = 488 ± 18 bpm; lower plateau = 332 ± 20 bpm; MAP50 = 108 ± 5; HR range = 363 ± 18 bpm; P > 0.05, Student’s t test, n = 5 and 6 rats, respectively).

Changes in Arterial Pressure and Heart Rate in commNTS-Lesioned Rats Treated with Intragastric 2 M NaCl Combined with Intravenous Vasopressin Antagonist

The blockade of vasopressin V1 receptors with intravenous Manning Compound (10 μg/kg body wt) abolished the pressor response to intragastric 2 M NaCl in commNTS-lesioned rats or in sham rats [F(3,288) = 51.93; P < 0.05] (Fig. 7A). The intragastric 2 M NaCl produced no changes in HR in sham or commNTS-lesioned rats previously treated with intravenous saline or V1-antagonist [F(3,288) = 1.193; P > 0.05] (Fig. 7B).

The treatment with intravenous Manning Compound produced no significant change in MAP in sham (−3 ± 2 mmHg)
or in commNTS-lesioned rats \( (-4 \pm 2 \text{ mmHg}) \) before intragastric 2 M NaCl but did abolish the pressor response to intravenous AVP (12.5 ng/0.1 ml/rat) for at least 70 min in sham \( (0 \pm 1, \text{ vs. control: } 28 \pm 5 \text{ mmHg}) \) or commNTS-lesioned rats \( (2 \pm 1, \text{ vs. control: } 33 \pm 5 \text{ mmHg}) \).

**Water Intake and Urinary Excretion by commNTS-Lesioned Rats Treated With Intragastric 2 M NaCl**

Compared to sham, lesions of the commNTS increased intragastric 2 M NaCl-induced water intake \( (17.5 \pm 0.8, \text{ vs. sham: } 11.2 \pm 1.8 \text{ ml/120 min}) \), \( [F(3,165) = 95.57; \ P < 0.001] \) (Fig. 8).

In sham rats, intragastric 2 M NaCl-induced natriuresis \( [F(1,38) = 11.66; \ P < 0.05] \), diuresis, \( [F(1,38) = 1.26; \ P < 0.05] \), and kaliuresis \( [F(1,38) = 1.33; \ P < 0.05] \) compared with intragastric 0.15 M NaCl (Fig. 9). Compared with sham rats, lesions of the commNTS increased intragastric 2 M NaCl-induced natriuresis \( (4.85 \pm 0.76 \text{ vs. sham: } 1.43 \pm 0.26 \text{ meq/60 min}) \), without changing the diuresis or the kaliuresis (Fig. 9). Sodium and potassium excretion and urinary volume were similar in sham and commNTS-lesioned rats treated with intragastric 0.15 M NaCl (Fig. 9).

As described previously \((5, 33)\), commNTS lesions decreased body weight \( [F(1,78) = 8.73, \ P < 0.05] \) (Table 2). The intragastric 2 M NaCl induced similar increases in serum Na\(^+\) in sham and commNTS-lesioned animals \( [F(1,22) = 0.04; \ P > 0.05] \), (Table 3). Similar to a previous study \((40)\), total serum protein concentration was also similar in sham or commNTS lesioned rats that received intragastric 2 M NaCl or 0.15 M NaCl, which suggests that an intragastric 2 M NaCl load produced no change in plasma volume (Table 3).

**DISCUSSION**

The present data demonstrate that commNTS lesions \((15 \text{ to } 20 \text{ days})\) enhance intragastric 2 M NaCl-induced vasopressin secretion, c-Fos expression in the PVN and SON vasopressinergic neurons, pressor response, water intake, and natriuresis, suggesting that inhibitory mechanisms dependent on the commNTS act to limit the responses to an acute increase in plasma osmolality. Similar to previous studies, the present results also...
showed that commNTS lesions decrease the body weight (mainly in the first week after the lesion) and chemoreflex-induced pressor response, without changing baroreflex responses (5, 14, 33, 45, 55).

One point to consider is that the electrolytic lesions used in the present study destroy cell bodies and also fibers of passage. Therefore, it is necessary to exercise some caution when interpreting the present results. Nonetheless, considering that no previous study has investigated the role of the commNTS in the responses tested, the effects of electrolytic lesions are relevant as an initial approach in the functional study of commNTS involvement with responses additional to those already demonstrated (5, 14, 34). Future studies using alternative lesioning protocols (e.g., chemicals) may confirm and expand the present results. In this direction, we have recently started studies using saporin-anti-dopamine-beta-hydroxylase lesions of the commNTS, which destroy catecholaminergic neurons in the commNTS. Preliminary results have shown that the effects of these chemical lesions on intragastric 2 M NaCl-induced water intake and arterial pressure changes are similar to those of the electrolytic lesions.

Intragastric hypertonic NaCl increased serum sodium, plasma AVP levels, and c-Fos expression in the vasopressinergic neurons of the PVN and SON in sham rats, however, induced only a small (maximum of 7 mmHg) and not statistically significant increase in MAP. In spite of the non-significant increase in MAP, the treatment with vasopressin antagonist intravenously reduced MAP in sham rats that received intragastric hypertonic NaCl, suggesting that, as expected, in this condition, vasopressin is acting peripherally to maintain arterial pressure relatively elevated.

Differently from sham rats, the intragastric 2 M NaCl in commNTS-lesioned rats induced strong pressor responses almost abolished by systemic V1 antagonist pretreatment, sug-

Fig. 3. A: photomicrographs of coronal brain sections showing the colocalization of c-Fos (green fluorescence) and vasopressin (AVP; red fluorescence) in the PVN and SON to intragastric 2 M NaCl in sham or commNTS-lesioned rats representative of the groups tested. Scale bar = 200 μm. B: number of total c-Fos/AVP double-labeled neurons in the PVN and SON to 0.15 M or intragastric 2 M NaCl in sham or commNTS-lesioned rats. The results are expressed as means ± SE; n = number of rats.
suggesting that these pressor responses are dependent on peripheral vasopressin action. This increase in arterial pressure is consistent with the 3 times higher increase in plasma vasopressin levels to intragastric 2 M NaCl in commNTS-lesioned rats compared with sham rats. An increased number of vasopressinergic neurons expressing c-Fos in the PVN and SON was detected in commNTS-lesioned rats that received intragastric 2 M NaCl compared with sham rats. Thus, there is a good correlation among the changes in arterial pressure, plasma vasopressin levels, and activity of vasopressinergic neurons in the PVN and SON comparing sham and commNTS-lesioned rats, i.e., with a similar increase in plasma osmolality, all these responses are enhanced in commNTS-lesioned rats compared with sham rats, which suggests that inhibitory mechanisms dependent on the commNTS are important to reduce or limit the excitation of vasopressinergic neurons in the PVN and SON and, consequently, vasopressin secretion and the increase in arterial pressure produced by plasma hyperosmolality. Similar to the increases in the pressor response to intragastric 2 M NaCl, it was previously shown that commNTS lesions increased pressor responses dependent on sympathetic/vasopressinergic activation produced by intracerebroventricular injection of noradrenaline or the cholinergic agonist carbachol, (55). Comparable increases in MAP were also shown in rats with AVP levels similar to those found in the present study (56). Intragastric hypertonic NaCl also increased plasma OT levels; however, this response was not modified by commNTS lesion.

A residual pressor response was still present in the commNTS-lesioned rats that received intragastric 2 M NaCl combined with AVP antagonist, which suggests that another pressor mechanism might also affect arterial pressure in these animals. Hyperosmolality increases sympathetic nerve activity (2, 12, 13, 52). Even considering that commNTS lesions affect
sympathoexcitatory responses (14, 34, 44, 45, 55), it is not possible to exclude the participation of sympathetic mechanisms in the residual pressor response in commNTS-lesioned rats treated with intragastric 2 M NaCl.

Similar to what has been previously described in intact rats (40), in the present study, in sham rats, intragastric 2 M NaCl load induced diuresis, natriuresis, and kaliuresis. Lesions of the commNTS increased intragastric 2 M NaCl-induced natriuresis, without changing diuresis or kaliuresis. The intragastric 2 M NaCl increased plasma OT levels, and the action of OT in the kidney is suggested to be a mechanism involved in hyper-

Table 1. Baseline and changes expressed as peak (maximum) in MAP and HR produced by intravenous injection of phenylephrine, sodium nitroprusside, or potassium cyanide in sham or in commNTS-lesioned rats

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<th>Sham (n = 19)</th>
<th>commNTS (n = 21)</th>
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<td>MAP, mmHg</td>
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<td>Baseline</td>
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</table>

Values are expressed as means ± SE. *Significantly different from sham (Student’s t-test; P < 0.05). n = number of rats; MAP, mean arterial pressure; HR, and heart rate; SNP, sodium nitroprusside; KCN, potassium cyanide.

Similar to what has been previously described in intact rats (40), in the present study, in sham rats, intragastric 2 M NaCl load induced diuresis, natriuresis, and kaliuresis. Lesions of the commNTS increased intragastric 2 M NaCl-induced natriuresis, without changing diuresis or kaliuresis. The intragastric 2 M NaCl increased plasma OT levels, and the action of OT in the kidney is suggested to be a mechanism involved in hyper-

Fig. 5. Plasma AVP and OT levels 20 min after 0.15 M or intragastric 2 M NaCl in sham or commNTS-lesioned rats. The results are expressed as means ± SE. n = number of rats.

Fig. 6. Changes in mean arterial pressure (MAP; A) and heart rate (HR; B) to 0.15 M or intragastric 2 M NaCl in sham or commNTS-lesioned rats. The results are expressed as means ± SE. n = number of rats.

Fig. 7. Changes in MAP (A) and HR (B) to intragastric 2 M NaCl in sham or in commNTS-lesioned rats pretreated with intravenous saline or intravenous vasopressin antagonist (AVPX; Manning Compound, 10 µg/kg body wt). The results are expressed as means ± SE; n = number of rats.
tonic saline infusion-induced natriuresis (3, 22). The increase in plasma sodium after intragastric 2 M NaCl load may also result in increased sodium filtration, which, combined with a reduced reabsorption as a consequence of OT action, produces natriuresis. Although the commNTS lesions increased intragastric 2 M NaCl-induced natriuresis, the same lesions did not modify the increase in plasma OT to hyperosmolality, which suggests that the increased natriuresis to intragastric 2 M NaCl in commNTS-lesioned rats is probably not related to OT effects. Excluding OT, another possible mechanism to explain the increased natriuresis is the pressor response to hyperosmolality in commNTS-lesioned rats that may facilitate natriuresis by increasing the filtered load. In spite of the increase in sodium excretion in commNTS-lesioned rats, the diuresis and kaliuresis to intragastric 2 M NaCl was not different among groups. Increased plasma AVP levels after intragastric 2 M NaCl in commNTS-lesioned rats probably counteract the high filtered load, absorbing more water in lesioned rats, which results in similar urinary volume after intragastric 2 M NaCl in sham or commNTS-lesioned rats. Increased renal filtration due to the pressor response might also increase potassium excretion after intragastric 2 M NaCl in the commNTS-lesioned rats. However, kaliuresis to intragastric 2 M NaCl was similar in sham and commNTS-lesioned rats. Although the mechanism is not clear, an increased reabsorption of potassium may occur in this condition. More studies are necessary to investigate mechanisms involved in renal excretion to intragastric 2 M NaCl in the commNTS-lesioned rats. Similar to AVP, intragastric 2 M NaCl in commNTS-lesioned rats might increase the levels of other hormones important to control renal function, like the ANP, which increases in response to salt load (4) and OT (3). Further studies should be addressed to evaluate this hypothesis.

Baroreflex tested using a single dose or infusions of phenylephrine or sodium nitroprusside was not affected by the commNTS lesions, suggesting that the medullary circuitry activated by the baroreflex is working properly in the commNTS-lesioned rats, as demonstrated in previous studies (14, 45). However, the commNTS lesions impaired the inhibitory mechanisms involved in the control of vasopressin secretion with a consequent increased pressor response. The first synapse of arterial baroreceptor afferents is located mainly in the intermediate NTS, and from there, baroreceptor signals may reach other medullary areas involved in cardiovascular regulation or forebrain areas involved in the control of fluid-electrolyte balance (10). Although the commNTS seems not to be part of the NTS circuitry activated by arterial baroreceptors to control the cardiovascular system, perhaps signals from arterial baroreceptors may reach the commNTS before being released to other central areas involved in the control of neuroendocrine responses to plasma hyperosmolality, such as PVN, SON, or other limbic and hypothalamic areas. On the other hand, besides arterial baroreceptors, another source of inhibitory signals is the cardiopulmonary volume receptors, that also send signals to the NTS, and may affect responses mediated by the forebrain, such as water intake (15, 27), and possible PVN and SON activation and vasopressin secretion.

Fig. 8. Cumulative water intake by sham or commNTS-lesioned rats that received intragastric 0.15 M or 2 M NaCl. The results are expressed as means ± SE. n = number of rats.

Fig. 9. Urinary Na⁺, K⁺, and volume (inset) in sham and commNTS-lesioned rats treated with 0.15 M or intragastric 2 M NaCl. The results are expressed as means ± SE. Number of rats is shown in parentheses at top of each column.
mediated facilitatory influence proposed by previous studies against hyperosmolality-induced water intake, as suggested by and not involved in other behavioral responses (33).

Mechanisms specifically related to the control of water intake in rats, suggesting that commNTS lesions disrupt inhibitory which causes reduction in body weight in commNTS lesioned intake and reduce food intake in the first 10 days after lesions, precursor induced-water intake, produce no change in daily water vasopressin secretion. The same commNTS lesions that in- and hyperosmolality, which are also the main stimuli for integrated important inhibitory mechanisms involved in the present and previous studies suggest that the commNTS terenol, a stimulus that increases peripheral ANG II (5). Thus, sions increased water intake induced by subcutaneous isopro-

Table 2. Body weight of sham and commNTS-lesioned rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Presurgery</th>
<th>4 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>14</td>
<td>296 ± 4</td>
<td>304 ± 2</td>
<td>351 ± 3*</td>
</tr>
<tr>
<td>Lesion</td>
<td>14</td>
<td>305 ± 5</td>
<td>286 ± 3*#</td>
<td>335 ± 4*#</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE *Significantly different from presurgery. #Significantly different from sham (one-way ANOVA, followed by Student-Newman-Keuls test; P < 0.05). n = number of rats.

Reduction of inhibitory signals by hypotension usually increases water intake (50); however, the increase in water intake consequent to intragastric 2 M NaCl in the commNTS-lesioned rats cannot be attributed to any reduction in MAP. Water was offered to the animals 60 min after the gavage, and at this time point, the animals were still hypertensive (~20 mmHg). Similar to what has been previously demonstrated in intact rats (40), the present results show that intragastric 2 M NaCl in sham or commNTS-lesioned rats produced similar increases in plasma sodium concentration, suggesting a similar osmotic stimulus in all animals. In addition, total serum protein was not modified 1 h after the intragastric 2 M NaCl, suggesting that blood volume was similar in all groups at this time period. Consequently, the increased water intake, c-Fos expression in the PVN and SON vasopressinergic neurons, vasopressin release and, therefore, enhanced pressor response in the commNTS-lesioned rats are due to the removal of inhibitory signals arising from the commNTS, and not secondary to any differential changes in plasma osmolality or volume in these animals. A previous study demonstrated that commNTS lesions increased water intake induced by subcutaneous isoproterenol, a stimulus that increases peripheral ANG II (5). Thus, the present and previous studies suggest that the commNTS integrated important inhibitory mechanisms involved in the control of the two basic stimuli for thirst, i.e., plasma ANG II and hyperosmolality, which are also the main stimuli for vasopressin secretion. The same commNTS lesions that increase induced-water intake, produce no change in daily water intake and reduce food intake in the first 10 days after lesions, which causes reduction in body weight in commNTS lesioned rats, suggesting that commNTS lesions disrupt inhibitory mechanisms specifically related to the control of water intake and not involved in other behavioral responses (33).

The existence of inhibitory mechanisms in the commNTS against hyperosmolality-induced water intake, as suggested by the present results, is contrary to the peripheral osmoreceptor-mediated facilitatory influence proposed by previous studies (29, 30). Peripheral osmoreceptors make their first synapse in the NTS (commissural and intermediate) and area postrema (AP) and, according to previous studies (19, 28), it was expected a reduction in water intake in commNTS-lesioned rats. Similar to the peripheral osmoreceptor-induced facilitation of water intake, it was recently demonstrated that the commNTS has also an excitatory role in the control of sympathetic nerve activity and arterial pressure during the chronic increase in plasma osmolality produced by 3 days of water deprivation (12). Therefore, other signals and not those from peripheral osmoreceptors might be those that activate the inhibitory mechanisms in the commNTS.

Neuroanatomical connections exist between the NTS, including the commNTS and forebrain areas involved in the control of water intake, vasopressin secretion, and arterial pressure control, such as the AV3V region, the PVN, and SON (20, 42, 43, 46). Plasma hyperosmolality may activate peripheral osmoreceptors and mainly central osmoreceptors located in the sFO and AV3V region (6, 32, 48). The activity of these and other forebrain areas involved in the control of fluid-electrolyte balance and cardiovascular regulation might be influenced by the inhibitory signals that arise through the commNTS (11, 20, 42, 49, 54, 54). Figure 10 presents a schematic model showing possible mechanisms by which the commNTS might modulate the responses evoked by hyperosmolality. Signals from peripheral osmoreceptors reach different portions of the NTS, including the commNTS (the only projection shown in the Fig. 10), before ascending to different central areas, probably the same that receive projections from central osmoreceptors (8). Besides signals from peripheral osmoreceptors, the NTS also receives signals from different visceral receptors like arterial baroreceptors and cardiopulmonary volume receptors among others that are an important source of inhibitory signals to control fluid-electrolyte balance especially thirst, sodium appetite, and vasopressin secretion (10, 16, 51). The first synapse of these different receptors is presented in the Fig. 10.
located mainly in the intermediate NTS (as presented in the Fig. 10) and only part in the commNTS (not shown in the Fig. 10) (49). However, as described above, even arising through other portions of the NTS, the inhibitory signals from peripheral receptors perhaps reach the commNTS before ascending to the forebrain areas involved in the control of thirst and vasopressin secretion. A possible increase in vasopressin secretion by peripheral osmoreceptor activation is not supported by previous studies that tested the effects of AP lesions or subdivisions of the sensory nuclei of the solitary tract (29, 30) in Fig. 10, we maintained peripheral osmoreceptors as a possible source of facilitatory signals, whereas inhibitory signals are suggested to be those from the cardiovascular receptors.

In summary, the present results show that commNTS lesions enhance plasma hyperosmolality-induced activation of vasopressinergic neurons in the PVN and SON, which results in increased plasma vasopressin levels and vasopressin-dependent pressor responses. Lesions of the commNTS also increase hyperosmolality-induced natriuresis, perhaps as a consequence of the increase in arterial pressure or an increase of ANP secretion that has to be investigated. The results suggest that inhibitory mechanisms dependent on commNTS act to limit or counterbalance behavioral, hormonal, cardiovascular, and renal responses to plasma hyperosmolality.

**Perspectives and Significance**

The present study demonstrates that a potent inhibitory mechanism from the commNTS acts to limit the increase in the activity of vasopressinergic neurons in the PVN and SON, vasopressin release, water intake, and arterial pressure during an acute increase in plasma osmolality. Thirst and vasopressin release are important mechanisms activated to correct hyperosmolality that must be under fine tuning to correctly recover fluid-electrolyte balance. The commNTS inhibitory mechanisms seem to adjust the release of this hormone and suppress overdrinking, which restrains an increase of the extracellular fluid volume that might result in hemodynamic changes (for review, see Ref. 24). An imbalance in this mechanism dependent on commNTS may cause alterations in body fluid homeostasis and hemodynamic with consequent cardiovascular diseases like hypertension. Important questions still remain to be addressed by future studies like the source of the signals and the phenotype of cells that convey the inhibitory signals from the commNTS to the forebrain. It is also important to investigate whether the commNTS inhibitory mechanisms that limits the response to increases in plasma hyperosmolality also affect the activation of vasopressinergic neurons in the PVN and SON, water intake, or pressor responses to other stimuli like central angiotensinergic or cholinergic activation or the responses caused by hypotension/hypovolemia. Preliminary data from our laboratory have shown that commNTS Aβ adrenergic cell might be the type of neuron that conveys the inhibitory signals. Previous studies have also shown that commNTS lesions increased the pressor response to intracerebroventricular injections of carbachol or noradrenaline and water intake to subcutaneous injection of isoproterenol; however, the importance of commNTS for the other responses produced by these stimuli were not investigated yet (5, 55).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


