Serotonergic mechanisms of the lateral parabrachial nucleus in renal and hormonal responses to isotonic blood volume expansion

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Am J Physiol Regul Integr Comp Physiol 292: R1190 –R1197, 2007. First published November 30, 2006; doi:10.1152/ajpregu.00351.2006.—This study investigated the involvement of serotonergic mechanisms of the lateral parabrachial nucleus (LPBN) in the control of sodium (Na⁺) excretion, potassium (K⁺) excretion, and atrial volume in unanesthetized rats subjected to acute isotonic blood volume expansion (0.15 M NaCl, 2 ml/100 g of body wt over 1 min) or control rats. Plasma oxytocin (OT), vasopressin (VP), and atrial natriuretic peptide (ANP) levels were also determined in the same protocol. Male Wistar rats with stainless steel cannulas implanted bilaterally into the LPBN were used. In rats treated with vehicle in the LPBN, blood volume expansion increased urinary Na⁺ and K⁺ excretion, and also plasma ANP and OT. Bilateral injections of serotonergic receptor antagonist methysergide (1 or 4 µg/200 nl) into the LPBN reduced the effects of blood volume expansion on increased Na⁺ and K⁺ excretion and urinary volume, while LPBN injections of serotonergic 5-HT₂/5-HT₃ receptor agonist, 2.5-dimethoxy-4-iodoamphetamine hydrobromide (DOI; 1 or 5 µg/200 nl) enhanced the effects of blood volume expansion on Na⁺ and K⁺ excretion and urinary volume. Methysergide (4 µg) into the LPBN decreased the effects of blood volume expansion on plasma ANP and OT, while DOI (5 µg) increased them. The present results suggest the involvement of LPBN serotonergic mechanisms in the regulation of urinary sodium, potassium and water excretion, and hormonal responses to acute isotonic blood volume expansion.

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ISOTONIC BLOOD VOLUME expansion, by reflex mechanisms, induces a series of regulatory responses, including the inhibition of the sympathetic outflow to the kidney, heart, and blood vessels, reduction of renin and vasopressin (VP) secretion, and increase in the levels of circulating atrial natriuretic peptide (ANP) and oxytocin (OT) that leads to diuresis and natriuresis (1, 2, 12, 14, 20, 24, 45, 53).

There are several reports demonstrating the correlation between natriuresis and diuresis and plasma ANP levels in response to systemic volume expansion, e.g., atrial balloon inflation, blood volume expansion, salt load, and water immersion (4, 7, 17, 32, 50, 59). These responses depend on the activation of sinoatrioarterial baroreceptors and/or cardiopulmonary receptor afferents located in the atria, lungs, great veins, and ventricles that project to the nucleus of the solitary tract (NTS) (1, 12, 22). The NTS neurons convey blood volume expansion signals and project to and excite different areas within the forebrain and hindbrain to produce neuroendocrine responses. Studies using expression of the immediate early gene c-Fos as a marker of neuronal activation have shown that, in addition to the NTS, volume-loading activates the paraventricular (PVN) and supraoptic (SON) nuclei, dorsal raphe nucleus (DRN), caudal ventrolateral medulla, locus coeruleus, and also the lateral parabrachial nucleus (LPBN) (6, 20, 46).

The LPBN, a structure lying dorsolateral to the superior cerebellar peduncle, has been shown to play an important role in the control of body fluid balance and cardiovascular regulation (10, 13, 15, 40). The LPBN is connected with hindbrain and forebrain areas that are activated by changes in body fluid volume, such as the NTS, DRN, PVN, amygdala, and the median preoptic nucleus (11, 19, 29, 51). Electrical or chemical stimulation of the LPBN modulates arterial pressure, heart rate, and VP release (25, 41, 56, 60).

Renal and hormonal responses to blood volume expansion are also strongly influenced by central serotonergic mechanisms (47, 48). Intracerebroventricular administration of 2.5-dimethoxy-4-iodoamphetamine hydrobromide (DOI) or serotonin (5-HT) increases urinary sodium excretion in water-loaded rats (47), while electrolytic lesions of DRN or intracerebroventricular injections of p-chlorophenylalanine, a tryptophan hydroxylase inhibitor that causes depletion of 5-HT from serotonergic neurons, reduce sodium and potassium excretion in water-loaded rats and ANP release under resting conditions and after blood volume expansion (48). Besides the volume load-induced increase in c-Fos immunoreactivity within the LPBN and DRN, in a recent study, we showed an enhanced number of cells double labeled for c-Fos and 5-HT in the DRN of animals that received blood volume expansion (20).

5-HT has been identified as an important neurochemical component of pathways from the raphe system and area postrema (AP) to the LPBN (33, 44). The LPBN, by means of serotonergic mechanisms, may receive signals from peripheral volume receptors that are important for neuroendocrine responses to blood volume expansion. Thus, the aim of the present study was to investigate the participation of serotonergic mechanisms of the LPBN in the control of urinary volume.
sodium and potassium excretion, and plasma ANP, OT, and VP levels in response to isotonic blood volume expansion, in conscious rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (280–350 g) from the Central Animal Facility of the Campus of Ribeirão Preto-University of São Paulo were housed in individual stainless steel cages in a room with controlled temperature (23 ± 2°C) and a 12:12-h light-dark cycle (light on at 7:00 AM), provided with normal food pellets and tap water ad libitum. The experiments were performed between 9:00 AM and 01:00 PM. All experimental protocols were approved by the Committee for Animal Use of the School of Medicine of Ribeirão Preto, University of São Paulo (052/2005).

LPBN Cannulation

Rats were anesthetized with 2,2,2-tribromoethanol (200 mg/kg of body wt; Sigma-Aldrich, St. Louis, MO) and placed in a Kopf stereotactic apparatus (model 900). The skull was leveled between bregma and lambda. Stainless steel guide cannulas (0.4 mm ID, 0.6 mm OD) were implanted bilaterally in the LPBN using the coordinates: 9.4 mm caudal to the bregma, 2.2 mm lateral to the midline, and 4.1 mm below the dura mater. The tips of the guide cannulas were targeted to terminate 2 mm above each LPBN. Cannulas were fixed to the cranium using dental acrylic resin and two jeweler’s screws. A 30-gauge metal wire filled the cannulas, except during injections. After surgery, the rats received a prophylactic injection of penicillin (20,000 units im) and were allowed to recover for 7 days, during which they were handled daily and habituated to the removal of the obturator of the guide cannula and to the gavage procedures.

Histology of LPBN

After the experiments, animals received bilateral injections of 2% Evans blue solution (200 μl/side) into the LPBN, were deeply anesthetized with sodium thiopental (80 mg/kg of body wt), and perfused transcardially with isotonic NaCl followed by 10% formalin. The brain was removed, fixed in 10% formalin, frozen, cut in 50-μm coronal sections, stained with cresyl violet, and analyzed by light microscopy (Axioskope 35M; Zeiss), to locate the injection site in the LPBN.

Blood Volume Expansion

Twenty-four hours before the experiment, in rats anesthetized with 2,2,2-tribromoethanol (200 mg/kg of body wt), a catheter was inserted into the right external jugular vein and positioned in the right atrium, as previously described (26). On the day of the experiment, blood volume expansion was performed in conscious, freely moving rats by an intravenous injection of isotonic 0.15 M NaCl solution (2 ml/100 g body wt) over 1 min.

Determination of Plasma ANP, OT, and VP Concentrations

Plasma levels of ANP, OT, and VP were measured by radioimmunoassay as previously described (16, 23, 24). VP and OT were extracted from 1 ml of plasma with acetone and petroleum ether, and ANP was extracted from 1 ml of plasma using Sep-Pak C18 cartridges (Waters, Milford, MA). The percentages of recovery after extraction were 83%, 85%, and 90% for VP, OT, and ANP, respectively. The assay sensitivity and intra- and interassay coefficients of variation were 0.9 pg/ml, 7.7% and 11.9% for VP; 0.9 pg/ml, 7.0% and 12.6% for OT; and 7.0 pg/ml, 6.0% and 10% for ANP.

Determination of Sodium and Potassium Excretion and Urinary Volume

Urinary sodium and potassium concentrations were determined by flame photometry (Micronal model b262). The rates of urinary sodium and potassium excretion were calculated by multiplying the urinary concentration times the urine volume (UNa+ /V; UK+ /V) and expressed as μeq/100 g body wt. Cumulative urinary volume was expressed as ml·100 g body wt⁻¹·60 min⁻¹.

Drugs

All drugs were purchased from Research Biomedicals. Methysergide maleate (1 and 4 μg) was dissolved in a solution of propylene glycol/water 2:1. DOI (1 and 5 μg) was dissolved in isotonic saline (0.15 M NaCl). The doses of methysergide and DOI used in the present study were selected based on a previous study (40).

The LPBN injections were performed using a 10-μl Hamilton syringe connected by polyethylene tubing (PE-10) to an injector needle (0.3 mm OD). The injector needle was 2 mm longer than the guide cannula. Each rat received only one LPBN treatment.

Statistical Analysis

The results are reported as means ± SE. Renal responses were analyzed by two-way ANOVA followed by a post hoc Newman-Keuls test, using treatment (drugs) and time as factors. Experiment 2 was analyzed using two-way ANOVA with groups (nonexpanded or volume expanded) and treatments (drugs) as factors followed by the Newman-Keuls test. Results from rats with bilateral injections outside the LPBN were analyzed by one-way ANOVA. Statistical analysis was performed using the Sigma Stat computer program with differences considered significant at P < 0.05.

Experimental Protocols

Experiment 1. Effects of serotonergic agonist and antagonist injections into the LPBN on urinary sodium, potassium, and water excretion induced by isotonic blood volume expansion. Rats had access to water, but not food, for 12 h before starting the experiments. After this period, the animals were weighed and received two intragastric water loads (5% of body wt each) with a 60-min interval between, with the purpose of increasing urine flow. Ten minutes before the second water load, the animals received bilateral LPBN injections of methysergide (1 or 4 μg), DOI (1 or 5 μg), vehicle (propylene glycol/water 2:1), or isotonic saline in a volume of 200 μl at each site. For these injections, the rats were removed from their home cages and the injection cannulas were introduced into the guide cannulas. The injections took 60 s. The animals were then placed in individual metabolic cages without access to food and water. Thirty minutes after the LPBN injections, rats were subjected to isotonic blood volume expansion. Three urine samples were collected at 20-min intervals over 60 min, starting immediately after blood volume expansion. Complete voiding of urine was manually induced by gently pressing the suprapubic region of the animal at the end of each interval. The same LPBN treatments were performed in control animals that received the two water loads without blood volume expansion.

Experiment 2. Effects of serotonergic agonist and antagonist injections into the LPBN on ANP, OT, and VP plasma levels induced by isotonic blood volume expansion. In this experiment, isotonic blood volume expansion was performed in animals subjected to two water loads and injected with vehicle, methysergide (4 μg), or DOI (5 μg) into the LPBN, as described in experiment 1. The doses of methysergide and DOI were chosen based on the most effective dose on renal responses to blood volume expansion observed in experiment 1. Five minutes after blood volume expansion, unanesthetized rats were decapitated and trunk blood was collected into chilled plastic tubes containing heparin for VP and OT determination, or EDTA (10
sergide or vehicle into the LPBN, two-way ANOVA showed significant main effects between treatments for Na⁺ excretion [F(5,36) = 40.9; P < 0.001], K⁺ excretion [F(5,36) = 57.6; P < 0.001] and urinary volume [F(5,36) = 3.5; P < 0.01].

Bilateral injections of methysergide (1 or 4 μg) into the LPBN did not change Na⁺ and K⁺ excretion and urinary volume in control rats not subjected to blood volume expansion (Fig. 2).

In contrast to methysergide, rats pretreated with bilateral injections of DOI (1 or 5 μg) into the LPBN enhanced blood volume expansion-induced increase in Na⁺ excretion (209 ± 17 and 306 ± 24 μeq·100 g⁻¹·60 min⁻¹, respectively vs. vehicle: 113 ± 12 μeq·100 g⁻¹·60 min⁻¹), K⁺ excretion (101 ± 19 and 157 ± 21 μeq·100 g⁻¹·60 min⁻¹, respectively vs. vehicle: 44 ± 4 μeq·100 g⁻¹·60 min⁻¹), and urinary volume (7.0 ± 0.5 and 9.0 ± 0.6 ml·100 g⁻¹·60 min⁻¹, respectively vs. vehicle: 4.0 ± 0.5 ml·100 g⁻¹·60 min⁻¹), (Fig. 3). In rats subjected to blood volume expansion or not that received DOI or vehicle into the LPBN, two-way ANOVA showed significant main effects between treatments for Na⁺ excretion [F(5,36) = 87.1; P < 0.001], K⁺ excretion [F(5,36) = 25.9; P < 0.001], and urinary volume [F(5,36) = 30.0; P < 0.001].

Bilateral injections of DOI (1 or 5 μg) into the LPBN did not change Na⁺ and K⁺ excretion and urinary volume in control rats not subjected to blood volume expansion (Fig. 3).

**Results**

**Histological Analysis**

The LPBN injection sites (Fig. 1) were similar to those described in previous reports in which bilateral LPBN injections of DOI or methysergide produced effects on water and sodium intake (13, 38, 40). In the present study, the injections sites were usually centered in the central lateral and external lateral portions of the LPBN. Injections reaching the ventral lateral and dorsal lateral portions, as well as the Kölliker-Fuse nucleus were observed in some rats, and these results were also included in the analysis. The spread of the injections was limited almost completely to above the brachium (superior cerebellar peduncle).

**Effects of Serotonergic Agonist and Antagonist LPBN Injections on Urinary Sodium, Potassium, and Water Excretion Induced by Isotonic Blood Volume Expansion**

In rats pretreated with vehicle in the LPBN, blood volume expansion increased Na⁺ excretion at 60 min (112 ± 12 vs. control: 9 ± 1 μeq·100 g⁻¹·60 min⁻¹), K⁺ excretion (43 ± 3 vs. control: 4 ± 1 μeq·100 g⁻¹·60 min⁻¹) and urinary volume (4.0 ± 0.5 vs. control: 2.6 ± 0.3 ml·100 g⁻¹·60 min⁻¹). (Fig. 2).

Bilateral injections of methysergide (1 or 4 μg) into the LPBN reduced the increase in Na⁺ excretion (79 ± 9 and 54 ± 6 μeq·100 g⁻¹·60 min⁻¹, respectively), K⁺ excretion (29 ± 3 and 20 ± 2 μeq·100 g⁻¹·60 min⁻¹, respectively), and urinary volume (2.8 ± 0.3 and 2.1 ± 0.3 ml·100 g⁻¹·60 min⁻¹, respectively) in response to blood volume expansion (Fig. 2). In rats subjected or not to blood volume expansion that received methysergide or vehicle into the LPBN, two-way ANOVA showed significant main effects between treatments for Na⁺ excretion [F(5,36) = 40.9; P < 0.001], K⁺ excretion [F(5,36) = 57.6; P < 0.001] and urinary volume [F(5,36) = 3.5; P < 0.01].

In rats pretreated with vehicle in the LPBN, blood volume expansion increased plasma levels of ANP (258 ± 28 vs. control: 65 ± 9 pg/ml) and OT (55 ± 4 vs. control: 9 ± 1 pg/ml), without changing VP plasma levels (1.1 ± 0.1 vs. control: 1.0 ± 0.1 pg/ml) (Fig. 4).

**Effects of Serotonergic Agonist and Antagonist Injections into the LPBN on ANP, OT, and VP Plasma Levels Induced by Isotonic Blood Volume Expansion**

In rats pretreated with vehicle into the LPBN, blood volume expansion increased plasma levels of ANP (258 ± 28 vs. control: 65 ± 9 pg/ml) and OT (55 ± 4 vs. control: 9 ± 1 pg/ml), without changing VP plasma levels (1.1 ± 0.1 vs. control: 1.0 ± 0.1 pg/ml) (Fig. 4).

Bilateral LPBN injections of methysergide (4 μg) reduced the increase on plasma ANP (80 ± 5 pg/ml) and OT (29 ± 2 pg/ml) induced by blood volume expansion (Fig. 4, A and B). Methysergide into the LPBN increased plasma VP (2.2 ± 0.2 pg/ml) in rats subjected to blood volume expansion (Fig. 4C). In contrast to methysergide, bilateral LPBN injections of DOI (5 μg) increased the effects of blood volume expansion on plasma ANP (348 ± 55 pg/ml) and OT (79 ± 7 pg/ml), without changing plasma VP levels (Fig. 4).

In rats subjected to blood volume expansion or not that received methysergide, DOI, or vehicle into the LPBN, two-way ANOVA showed significant main effects between treatments for plasma ANP [F(2,36) = 11.9; P < 0.001], OT [F(2,36) = 24.4; P < 0.001], and VP [F(2,36) = 9.2; P < 0.001].

Bilateral injections of methysergide (4 μg) or DOI (5 μg) into the LPBN did not change plasma ANP, OT, and VP in control rats that had not been subjected to blood volume expansion (Fig. 4).

**Effects of Serotonergic Agonist and Antagonist Injections Outside the LPBN on Renal and Hormonal Responses to Isotonic Blood Volume Expansion**

The specificity of the LPBN as the site in which DOI and methysergide injections produced changes in renal and hormonal responses to isotonic blood volume expansion was confirmed by results from rats in which injections did not reach...
the LPBN bilaterally (misplaced injections). These rats had injections into the cerebellum or locus coeruleus or below the brachium, including portions of the medial parabrachial nucleus. Bilateral injections of methysergide (1 or 4 μg) or DOI (1 or 5 μg) outside the LPBN produced no significant changes in urinary volume, Na and K excretion or plasma ANP, OT, and VP levels in rats subjected to blood volume expansion (Table 1).

Sodium and K" excretion, ANP and OT plasma levels, in response to blood volume expansion, in rats pretreated with methysergide or DOI into the LPBN, were significantly different compared with rats with injections outside the LPBN. Urinary volume was different in the DOI but not methysergide pretreatment, between inside and outside the LPBN-injected rats. No differences were observed comparing VP levels in response to blood volume expansion between rats with injections inside (2.2 ± 0.2 pg/ml) and outside (2.0 ± 0.3 pg/ml) the LPBN.

**DISCUSSION**

The present results show that serotonergic receptor blockade with bilateral LPBN injections of methysergide reduced the increase in urinary volume, sodium and potassium excretion, and also plasma levels of ANP and OT induced by isotonic blood volume expansion. Therefore, these results suggest that serotonergic mechanisms in the LPBN modulate water and electrolyte excretion, as well as OT and ANP secretion in response to isotonic blood volume expansion.

Previous studies have shown that intracerebroventricular administration of DOI or 5-HT increases urinary sodium excretion, while electrolytic lesions of the DRN or intracerebroventricular injections of p-chlorophenylalanine, a tryptophan hydroxylase inhibitor, which causes 5-HT depletion from serotonergic neurons, reduce sodium and potassium excretion in water-loaded rats (47, 48). In addition, the increase in synaptic concentrations of 5-HT by central administration of p-chloroamphetamine also enhances sodium and potassium excretion (55). It has also been reported that treatments with 5-HT, 5-HT precursors, 5-HT reuptake inhibitors, or serotoninergic receptor agonists stimulate the release of VP and OT into the systemic circulation (27, 34, 52, 58). Although the mechanisms involved in the maintenance of fluid homeostasis are different in water load and isotonic blood volume expansion, both conditions are likely to involve serotonergic mechanisms.

In the present study, the effects of DOI and methysergide injected into the LPBN on renal sodium, potassium, and volume excretion, and plasma ANP, OT, and VP were studied in rats subjected to intragastric water load. Although a reduction in VP plasma levels was expected in response to isotonic...
blood volume expansion (24), this was not observed in the present study, probably because a decrease in plasma osmolality, produced by water loading, inhibited VP release. In rats subjected to blood volume expansion, methysergide into the LPBN produced a small increase of plasma VP compared with vehicle-treated rats. However, plasma VP levels after methysergide into the LPBN or outside the LPBN were not different. In addition, DOI into the LPBN also did not affect plasma VP levels. Therefore, the present results do not give support to the involvement of serotonergic mechanisms of the LPBN in the modulation of VP secretion in the condition of the present study. Compared with vehicle-treated rats, methysergide into the LPBN reduced the increase in urinary volume produced by blood volume expansion, but urinary volume after methysergide into or outside the LPBN was similar. However, urinary volume increased in blood-volume-expanded rats after DOI into the LPBN but not after DOI outside the LPBN, which suggests that 5-HT in the LPBN may affect urinary volume in the condition of the present study.

In rats that had a combination of water load plus acute blood volume expansion, renal excretion and ANP and OT plasma levels were modified by injecting DOI or methysergide into the LPBN, suggesting that these responses to blood volume expansion depend on serotonergic mechanisms of the LPBN. Thus, it can be hypothesized that 5-HT in the LPBN facilitates the release of ANP and OT that act in the kidney, increasing electrolyte and water excretion. Natriuresis induced by blood volume expansion is mediated by ANP and OT release (1–3, 24, 48), and both circulate to the kidney and act on renal-specific receptors to reduce the reabsorption of sodium by a nitric oxide-cGMP mechanism (18, 24, 54). However, the blockade or the activation of the serotonergic mechanisms of the LPBN, in the absence of signals produced by blood volume expansion does not affect renal excretion or plasma levels of OT and ANP.

The present results are the first evidence suggesting that LPBN and particularly its serotonergic mechanisms are part of the central circuitry that facilitates ANP and OT release, thereby affecting renal electrolyte and water excretion during blood volume expansion to restore body fluid balance. Although changes in plasma ANP and OT mediate the renal responses described in the present study, the involvement of other mediators is possible, since tubular reabsorption also responds to subtle changes in renal sympathetic nerve activity and plasma angiotensin II (ANG II) levels. Although these two parameters were not evaluated in the present study, one would expect a decrease in renal sympathetic nerve activity and plasma ANG II during blood volume expansion.

The present results suggest that signals from pressure/volume receptors may arise from the AP, DRN, or some other not-yet-identified serotonergic projection and reach the LPBN, which then conveys these signals to the forebrain areas involved in the control of fluid and electrolyte balance. However, it is still not completely established which central pathways connect the LPBN with the oxytocinergic neurons in the PVN and SON. The LPBN projects primarily to the medial region of
and AVP levels (pg/ml) in rats previously water loaded and treated with bilateral injections of vehicle, methysergide (1 or 4 µg), or DOI (5 µg). Results are expressed as means ± SE. *P < 0.05 vs. control + vehicle; #P < 0.05 vs. BVE + vehicle; n = 6 for control groups and n = 8 for BVE groups.

The SON arising from the LPBN innervate the perinuclear zone of the supraoptic nucleus (PNZ) and influence both oxytocinergic and vasopressinergic neurons (28). This latter pathway, activated by volume load, relays arterial baroreceptor information to the VP neurons in the SON and includes the diagonal band of Broca (DBB) that projects to and activates GABAergic neurons in the SON. In turn, PNZ neurons release GABA into the SON, inhibiting VPergic neurons, and thereby inhibit VP release after blood volume expansion (21, 22, 31, 49). Recent studies have shown that volume expansion increases c-Fos positive cells in the PNZ and DBB (20–22, 30). Moreover, the LPBN is connected through the amygdala and the lateral hypothalamus with the DBB (8, 29, 51).

We may also consider another possibility of serotoninergic circuit influence on OT release from the SON and PVN, such as a coordinated activation of the LPBN and the PVN/SON mediated via separate projections arising from a common source. In this regard, Petrov et al. (44) showed collateral projections from the DRN to both the LPBN and the parvicellular subdivision of the PVN. In addition, it was shown that the SON receives direct serotonergic innervations from the DRN that project to the dorsal SON cells that are mainly oxytocinergic (5). Based on the data obtained, we propose that blood volume activation of pressure/volume receptors facilitates 5-HT release in the LPBN, activating both direct and indirect neural pathways that influence the SON and PVN oxytocinergic neurons and the release of OT. Blood volume expansion increases the release of OT, and the blockade of 5-HT in the LPBN reduces this response, which suggests that the activation of LPBN serotonergic mechanisms may also activate oxytocinergic neurons in the PVN and SON. An electrophysiological study has already shown that LPBN sends excitatory inputs to the SON (28).

Similarly to the participation on the regulation of neurohypophysial hormone release, LPBN serotoninergic mechanisms have been shown to participate in the control of water and salt intake. Electrolytic or neurotoxic (ibotenic acid) lesions of the LPBN increase water intake induced by subcutaneous ANG II or isoproterenol or intracerebroventricular ANG II in rats (15, 42, 43). Bilateral LPBN injections of methysergide strongly increase water and 0.3 M NaCl intake induced by icv ANG II or by treatment with subcutaneous injections of furosemide combined with a low dose of the angiotensin-converting enzyme inhibitor captopril (40), but have no effect on drinking or sodium intake when animals are satiated and normovolemic (39, 40). In contrast, bilateral LPBN injections of DOI reduced furosemide plus captopril-induced 0.3 M NaCl intake, while

Table 1. Urinary sodium, potassium (µEq · 100 g⁻¹ · 60 min⁻¹), and volume (ml · 100 g⁻¹ · 60 min⁻¹) and plasma ANP, OT, and AVP levels (pg/ml) in rats previously water loaded and treated with bilateral injections of vehicle, methysergide (1 or 4 µg) or DOI (1 or 5 µg) outside the LPBN, followed by isotonic blood volume expansion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Urinary Na⁺</th>
<th>Urinary K⁺</th>
<th>Urinary Volume</th>
<th>n</th>
<th>ANP</th>
<th>OT</th>
<th>AVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>16</td>
<td>96 ± 5</td>
<td>63 ± 4</td>
<td>2.8 ± 0.2</td>
<td>6</td>
<td>261 ± 18</td>
<td>23.5 ± 1.8</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Methysergide (1 µg)</td>
<td>9</td>
<td>99 ± 7</td>
<td>58 ± 4</td>
<td>2.6 ± 0.2</td>
<td>7</td>
<td>260 ± 18</td>
<td>22.4 ± 1.8</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Methysergide (4 µg)</td>
<td>15</td>
<td>98 ± 6</td>
<td>62 ± 5</td>
<td>2.6 ± 0.2</td>
<td>6</td>
<td>252 ± 30</td>
<td>22.5 ± 2.0</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>DOI (1 µg)</td>
<td>8</td>
<td>98 ± 10</td>
<td>62 ± 7</td>
<td>2.4 ± 0.1</td>
<td>2</td>
<td>252 ± 30</td>
<td>22.5 ± 2.0</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>DOI (5 µg)</td>
<td>12</td>
<td>88 ± 4</td>
<td>60 ± 6</td>
<td>2.8 ± 0.2</td>
<td>6</td>
<td>252 ± 30</td>
<td>22.5 ± 2.0</td>
<td>1.7 ± 0.3</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE. ANP, atrial natriuretic peptide; OT, oxytocin; AVP, vasopressin; DOI, 2,5-dimetoxy-4-iodoamphetamine hydrobromide; PBN, lateral parabrachial nucleus; n = number of rats.
water intake was not consistently reduced (35, 40). Thus, previous studies (36, 37, 39, 40) and the present results show that 5-HT acts in the LPBN to inhibit water and sodium intake and to increase ANP and OT release facilitating renal excretion of sodium, potassium, and water. Taken together, these observations suggest that LPBN 5-HT mechanisms may differentially modulate specific responses to body fluid volume expansion.

In conclusion, the present data demonstrate the existence of serotoninergic mechanisms in the LPBN involved in the control of ANP and OT secretion, as well as in the excretion of sodium, potassium, and water in response to isotonic blood volume expansion.

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