Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by sodium depletion

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Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by sodium depletion. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol.: 43): R555–R560, 1998.—This study investigated the effects of bilateral injections of a serotonin (5-HT) receptor agonist into the lateral parabrachial nucleus (LPBN) on the intake of NaCl and water induced by 24-h water deprivation or by sodium depletion followed by 24 h of sodium deprivation (injection of the diuretic furosemide plus 24 h of sodium-deficient diet). Rats had stainless steel cannulas implanted bilaterally into the LPBN. Bilateral LPBN injections of the serotoninergic 5-HT12 receptor antagonist methysergide (4 µg/200 nl at each site) increased hypertonic NaCl intake when tested 24 h after sodium depletion and after 24 h of water deprivation. Water intake also increased after bilateral injections of methysergide into the LPBN. In contrast, the intake of a palatable solution (0.06 M sucrose) under body fluid-replete conditions was not changed after bilateral LPBN methysergide injections. The results show that serotonergic mechanisms in the LPBN modulate water and sodium intake. Water intake in the LPBN was not affected by LPBN serotonergic blockade. The possibility that important body fluid balance-related information from the AP reaches the LPBN through this projection.

Recent studies suggest that the lateral parabrachial nucleus (LPBN), a structure in the rat that lies dorsolateral to the superior cerebellar peduncle (SCP), is an important hindbrain area involved in the control of water and NaCl intake (4, 8, 16, 20, 21, 23, 24). Rats with electrolytic or neurotoxic (ibotenic acid) lesions of the LPBN display increased water intake in response to central and peripheral administration of angiotensin II (ANG II) and to peripheral treatment with isoproterenol (8, 23, 24). A significant increase in water and NaCl intake induced by ANG II injections made intracerebroventricularly or directly into the subfornical organ (SFO) was observed after pretreatment with bilateral LPBN injections of methysergide (a serotoninergic 5-HT12 receptor antagonist) (4, 21). The injection of serotonin (5-HT) or 2,5-dimethoxy-4-iodoamphetamine hydrobromide (DOI; a serotoninergic 5-HT2AR receptor agonist) into the LPBN reduced water intake induced by intracerebroventricular ANG II in rats (20). In addition, methysergide injected into the LPBN increased, whereas DOI reduced 0.3 M NaCl intake stimulated by combined treatment with the diuretic furosemide (Furo) and a low dose of the angiotensin-converting enzyme inhibitor captopril (Cap) (i.e., Furo-Cap). Furo-Cap treatment induces sodium and water intakes that depend on both an intact renin-angiotensin system and the presence of hypotension (9, 16, 31, 32). Therefore, these data show that salt and water intakes dependent on ANG II and hypotension are inhibited by a 5-HT receptor agonist and enhanced by a 5-HT receptor antagonist injected into the LPBN.

The LPBN receives afferent projections from the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS), and it sends efferent projections to areas of the forebrain involved in fluid and electrolyte balance, such as specific nuclei within the hypothalamus (e.g., paraventricular nucleus), the SFO, and the amygdala (e.g., central nucleus) (3, 12, 15, 18, 28). Ablation of the AP and the immediately adjacent mNTS has been shown to increase ad libitum 24-h hypertonic NaCl intake and the consumption of saline solutions in tests of short duration (5, 7). A prominent serotonergic pathway from the AP to the parabrachial nucleus has been described (19), which raises the possibility that important body fluid balance-related information from the AP reaches the LPBN through this projection.

Sodium depletion induced by first administering Furo followed by 24-h of sodium-free diet (Furo-SDF) has been employed for many years (14, 34) to induce salt appetite. In comparison to the Furo-Cap model, the Furo-SDF procedure is a more conventional and widely used protocol. Interestingly, conflicting results have been obtained with the Furo-SDF model and the effects of serotonergic drugs. Intracerebroventricular injection of ketanserin, a 5-HT2 receptor antagonist, inhibits sodium depletion-induced hypertonic NaCl intake (11), whereas peripheral injection of metocoline, a nonspecific 5-HT receptor antagonist, enhances both need-induced and need-free NaCl intake in rats (25). Nevertheless, an inhibitory influence of 5-HT on hypertonic NaCl intake has been confirmed in water-deprived rats (6, 22). Considering that ANG II is important for both the rapid onset (Furo-Cap) and the prolonged onset (Furo-SDF) models of NaCl intake (2, 26, 27, 31) and to understand the generality of the role of LPBN 5-HT across experimental models, the following studies investigated the effects of bilateral LPBN injections of a serotoninergic receptor antagonist (methysergide) on the ingestion of hypertonic NaCl and water induced by other methods. Specifically, sodium depletion with Furo-SDF and 24-h water deprivation were
used as the challenges to induce sodium deficiency and water deficit.

**MATERIALS AND METHODS**

Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300–400 g were used. The animals were housed in individual stainless steel cages with free access to normal sodium diet (Purina Rat chow 5012, sodium content 0.5%), water, and 0.3 or 0.5 M NaCl solution. Temperature was maintained at a constant 23°C, with a 12:12-h light-dark cycle, with light onset at 6:00 AM. The experiments were performed between 9:00 AM and 1:00 PM.

Cerebral cannulas. Rats were anesthetized with Equithesin (0.33 ml/100 g body wt) and placed in a Kopf stereotaxic instrument. The skull was leveled between bregma and lambda. Stainless steel 23-gauge cannulas were implanted bilaterally into the LPBN using the following coordinates: 9.4 mm caudal to bregma, 1.9 mm lateral to the midline, and 4.1 mm below the dura mater. The tips of the cannulas were positioned at a point 2 mm above each LPBN. The cannulas were fixed to the cranium using dental acrylic resin and jeweler’s screws. A 30-gauge metal obturator filled the cannulas between tests. After surgery, the rats were allowed to recover for 6 days before starting water and NaCl intake tests.

Drugs. Furo (10 mg/ml; Elkins-Sinn, Cherry Hill, NJ) was administered at a dose of 20 mg/kg sc. Methysergide maleate was purchased from Sandz Pharmaceuticals (E. Hanover, NJ). Methysergide was dissolved in propylene glycol-water 2:1. The doses of methysergide (4 µg/200 nl) used in the present study were selected on the basis of a prior study in which a dose-response analysis of LPBN injections was established for effects on water intake (20).

Water and salt ingestion. The rats were tested in their home cages. Water and 0.3 or 0.5 M NaCl were provided from burettes with 0.1-ml divisions that were fitted with metal drinking spouts.

Water and 0.5 M NaCl intakes were induced by Furo-SDF treatment, which consisted of subcutaneous Furo (20 mg/kg, 0.2 ml/kg) administration followed by access to sodium-deficient diet (rat modified chow with 0.03% NaCl, ICN Biomedical, Aurora, OH) and water for 24 h. At the conclusion of the 24-h period, the sodium-deficient food was removed from the cage and 0.5 M NaCl was returned. The intake of saline and water was recorded at 15-min intervals for the next 2 h.

Water and NaCl intakes were also induced by 24 h of water deprivation. Normal chow was available to the rats during the deprivation period. At the conclusion of the deprivation, food was removed and water was returned to the animals. Both saline and water intake were then recorded at 15-min intervals for 1 h.

In each experimental session, one-half of the rats received bilateral LPBN injections of vehicle and the remaining animals received drug injections into this structure in a counterbalanced design. Rats received no more than four tests. A recovery period of at least 3 days was allowed between tests.

Injections into the LPBN were made using 10-µl Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At the time of testing, obturators were removed and the injection cannulas were introduced into the chronically implanted guide cannulas. The injection cannulas were 2 mm longer than the guide cannulas. The injection volume into each LPBN was 200 nl. After injection, the obturators were replaced and the rats were placed back into the cage. Saline and propylene glycol-water injections into the LPBN were used as control (vehicle) treatments.

As a control, a group of rats received bilateral LPBN injections of methysergide (4 µg/200 nl each site) in the absence of any other treatment and the volumes of water and 0.3 M NaCl ingested were recorded for 1 h.

To test the specificity of the role of LPBN 5-HT injections on water and NaCl intake in comparison to another type of ingestive behavior, a group of rats on food and water ad libitum had access for 2 h every day to 0.06 M sucrose for 1 wk (i.e., a dessert test). After this adaptation period, methysergide (4 µg/200 nl each site) or vehicle was injected bilaterally into the LPBN 10 min before rats had access to 0.06 M sucrose solution. Cumulative water and 0.06 M sucrose solution intakes were measured each 30 min for 2 h.

Histology. At the end of the experiments, the animals received bilateral injections of methylene blue solution (200 nl each site) into the LPBN. They were then deeply anesthetized with pentobarbital sodium (80 mg/kg) and perfused transcardially with saline followed by 10% Formalin. The brains were removed, fixed in 10% Formalin, frozen, cut in 50-µm sections, stained with cresyl violet, and analyzed by light microscopy to confirm the injection sites in the LPBN.

Statistical analysis. The results are reported as means ± SE. Analysis of variance and preplanned follow-up comparisons used Student’s t-tests. Differences were considered significant at P < 0.05.

**RESULTS**

Histological analysis. Similar to those in previous reports (4, 20, 21), the LPBN injection sites were centered in the central lateral and dorsolateral portions of the LPBN [see Fulwiler and Saper (10) for definitions of LPBN subnuclei]. Injections reaching the ventral lateral and external lateral portions, as well as the Kölliker-Fuse nucleus, were observed in some rats, and the results from these rats were included in the analysis. As estimated from the injection of methylene blue, the spread of the injection was almost completely confined above the brachium (SCP). In some rats there was a small, limited spread of the injection into the brachium, but never below this structure. It is important to note that, as described by Edwards and Johnson (8), the brachium appears to act as a barrier to limit the spread of the drugs to more ventral and more medial structures.

From a total of 47 rats used in this study, 24 had histologically confirmed bilateral LPBN injections.

Effects of injection of methysergide into the LPBN on the ingestion of 0.5 M NaCl and water induced by Furo-SDF. Bilateral injections of methysergide (4 µg/200 nl each site) into the LPBN increased the ingestion of water \(F(1,16) = 9.43; P < 0.01\) and 0.5 M NaCl \(F(1,16) = 17.22; P < 0.01\) induced by the Furo-SDF treatment (Fig. 1).

Bilateral injections of methysergide (4 µg/200 nl each site) into the LPBN, in the presence of any other treatment, produced no effect on the ingestion of either water \(1.0 ± 0.6\) ml in 1 h) or 0.3 M NaCl \(0.03 ± 0.03\) ml/h, \(n = 10\) rats).

Effects of methysergide injection into the LPBN on the ingestion of water and 0.3 M NaCl induced by 24 h of water deprivation. Bilateral injections of methysergide (4 µg/200 nl each site) into the LPBN increased water \(F(1,12) = 13.11; P < 0.01\) and 0.3 M NaCl \(F(1,12) =
produced no significant change in the ingestion of 0.5 M NaCl (P > 0.05) or water (P > 0.05) induced by Furo-SDF treatment. M-LPBN injections of methysergide produced no change in 0.3 M NaCl intake induced by 24 h of water deprivation (P > 0.05), but a small significant increase in water intake was observed (P < 0.01).

DISCUSSION

The present results demonstrate that the intake of the hypertonic NaCl of Furo-SDF-treated rats and of water-deprived animals was enhanced by bilateral injections of methysergide into the LPBN. The water intake that accompanied the hypertonic NaCl solution intake of both Furo-SDF and water-deprived rats was also enhanced after methysergide injections into the LPBN. No change in 0.06 M sucrose intake occurred

Fig. 1. Cumulative 0.5 M NaCl intake (A) and cumulative water intake (B) induced by treatment with furosemide (20 mg/kg) followed by 24 h of sodium-deficient diet after injection of vehicle or methysergide (Methy; 4 µg/200 nl each site) bilaterally into the lateral parabrachial nucleus (LPBN). *Significantly different from Na depletion + vehicle tested by t-tests (P < 0.05). n = 9 Rats.

Fig. 2. Cumulative 0.3 M NaCl intake (A) and cumulative water intake (B) induced by 24 h of water deprivation after injections of vehicle or methysergide (4 µg/200 nl each site) bilaterally into the LPBN. *Significantly different from water deprivation + vehicle tested by t-tests (P < 0.05). n = 14 Rats.
after bilateral injections of methysergide, thus suggesting that the enhancement of fluid intake after serotonergic receptor blockade in the LPBN is not due to a nonspecific facilitation of all ingestive behaviors. In previous work (21) we demonstrated that methysergide injections into the LPBN enhance the sodium intake induced by intracerebroventricular ANG II or by the Furo-Cap treatment. Therefore, the present results obtained using a more commonly employed method for inducing salt appetite (i.e., Furo-SDF protocol) are consistent with previous studies showing that the modulation of sodium intake is under an inhibitory influence of LPBN serotonergic mechanisms. This concept can be extended further by hypothesizing that the blockade of inhibitory mechanisms by methysergide facilitates salt appetite but only when there are excitatory factors (e.g., ANG II) present. Activation of only facilitatory mechanisms or deactivation of just inhibitory mechanisms (e.g., blockade of 5-HT receptors by LPBN methysergide injection) are not sufficient to produce reliable hypertonic NaCl intake. The idea of modulation of salt appetite is similar in some ways to the proposed mechanisms associated with the interaction between oxytocin and the dilution of body fluids proposed by Stricker and Verbalis (30). In the case of oxytocin, reduction of systemic osmolality by water inhibits systemic oxytocin release and the hypothesized activity in central oxytocinergic circuits subserving salt intake that are activated by ANG II in hypovolemic animals.

A concern in studies of ingestive behavior that investigate inhibitory mechanisms is whether changes in magnitude are specific for just the response under investigation. To deal with this question, we investigated the effects of the blockade of 5-HT in the LPBN on the ingestion of 0.06 M sucrose. The ingestion of 0.06 M sucrose was not enhanced by bilateral LPBN treatment with methysergide at the same dose that produced marked increases in water and NaCl intake. This failure to increase the volume of 0.06 M sucrose intake is probably not due to a ceiling effect, because at least twice the ingested volume obtained here with 0.06 M sucrose was seen when 0.3 M sucrose was used (29). Therefore, these results argue against a nonspecific influence of the LPBN 5-HT blockade affecting all ingestive responses.

Accumulating evidence indicates that the LPBN is a major site within a central neural network that is related to the control of cardiovascular and body fluid homeostasis. For example, the LPBN projects to several brain areas (e.g., SFO, paraventricular nucleus of the hypothalamus, central nucleus of the amygdala, bed nucleus of the stria terminalis, median preoptic nucleus, NTS) that have been implicated in the control of water and electrolyte balance (3, 10, 12, 13, 15, 18, 28). The LPBN also receives afferent inputs from multiple regions, and 5-HT has been identified as a neurochemical component of an ascending pathway to the parabrachial nucleus originating in the AP/mNTS (19). Therefore, the results from both the present and previous LPBN studies provide an anatomic basis for an inhibitory site of action of 5-HT on sodium intake. Many of the systemically administered 5-HT agonists and antagonists that, respectively, decrease and increase sodium intake cross the blood-brain barrier.

Table 1. Ingestion of water and NaCl induced by different treatments in rats with missed or asymmetrical LPBN injections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Water, ml</th>
<th>NaCl, ml</th>
<th>Recording Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;-depletion + vehicle</td>
<td>8</td>
<td>4.1±1.0</td>
<td>7.5±1.0</td>
<td>120</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;-depletion + Methy</td>
<td>8</td>
<td>6.0±1.3</td>
<td>7.7±0.9</td>
<td>120</td>
</tr>
<tr>
<td>Water deprivation + vehicle</td>
<td>11</td>
<td>13.9±0.7</td>
<td>2.2±0.6</td>
<td>60</td>
</tr>
<tr>
<td>Water deprivation + Methy</td>
<td>11</td>
<td>17.1±0.8*</td>
<td>3.5±0.9</td>
<td>60</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = no. of rats). Methy, methysergide; LPBN, lateral parabrachial nucleus. Doses of methysergide were 4 µg/200 nl each site. NaCl concentration was 0.5 M for Na<sup>+</sup>-depletion tests and 0.3 M for water-deprivation tests. *Significantly different from water deprivation + vehicle tested by t-tests (P < 0.05).
Therefore, when they are injected peripherally they are likely to have access to 5-HT receptors located in the LPBN (6, 22, 25).

In prior work, Gentili and colleagues (11) demonstrated that the 5-HT receptor antagonist ketanserin, injected systemically or intracerebroventricularly, inhibited salt appetite in the Furo-SDF model. There are various reasons why there may be a difference in outcome when the 5-HT_{2} serotogenic receptor antagonist ketanserin was injected peripherally or into the anteroventral third ventricle compared with bilateral injections of methysergide directly into the LPBN. For example, the two 5-HT receptor antagonists could be acting on different brain sites, act with different efficacies on different 5-HT receptor subtypes, or differentially affect other classes of neurotransmitter/neuromodulator receptors.

It has been proposed recently (31, 32) that decreases in arterial pressure facilitate sodium intake after Furo-Cap treatment. The effects of bilateral LPBN pretreatment with methysergide on intracerebroventricular ANG II-induced pressor responses were previously investigated (20). Injections of only methysergide into LPBN increased arterial pressure. When ANG II was administered intracerebroventricularly after either vehicle or bilateral LPBN methysergide pretreatment, the pressor responses were comparable. Consequently, the overingestion of NaCl associated with the administration of bilateral LPBN methysergide is probably not due to facilitation resulting from a reduction in blood pressure below normal resting levels.

The identification of a neurochemically defined projection (i.e., 5-HT containing) from the AP/mNTS to the parabrachial nucleus (19) and the results of the present as well as other functional studies involving lesions of the AP (5, 7) or of the LPBN (7, 23, 24) raise the question as to what type of signals are transmitted in this ascending pathway. Neural afferents from arterial baroreceptors and cardiopulmonary receptors in the rat have been suggested to terminate in the AP/mNTS (1) and there is substantial evidence indicating that projections from the AP/mNTS to LPBN convey information related to blood pressure and extracellular fluid volume. It is possible that lesions or methysergide injections into the LPBN impair information flow in a central pathway related to cardiopulmonary and arterial baroreceptor input that normally plays an inhibitory role in the control of water and NaCl intake (17, 33). In this respect, it is interesting to note that electrolytic lesions of the LPBN impair the inhibition of water intake observed during the inflation of a balloon at the junction of the vena cava and right atrium (24). That is, rats with LPBN lesions may not be sensitive to activation of visceral sensory afferents associated with the control of blood volume.

Perspectives

Studies showing a specific role of LPBN serotonergic inhibitory mechanisms in the control of sodium and water intake induced by different models of experimental thirst and salt appetite broaden the generality of the concept of a hindbrain inhibitory mechanism in the behavioral control of fluid balance. Future research will be necessary to establish the origin and nature of the stimuli that activate these mechanisms and signal the brain of volume expansion. Defining the precise nature ofafferent signaling from the periphery to AP/mNTS and the mechanism of activation of the hypothesized AP/mNTS to the LPBN inhibitory pathway requires further investigation.

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