Serotonergic mechanisms of the lateral parabrachial nucleus and cholinergic-induced sodium appetite

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Menani, José Vanderlei, Silas Pereira Barbosa, Laurival Antonio De Luca, Jr., Juliana Irani Fratucci De Gobbi, and Alan Kim Johnson. Serotonergic mechanisms of the lateral parabrachial nucleus and cholinergic-induced sodium appetite. Am J Physiol Regulatory Integrative Comp Physiol 282: R837–R841, 2002; 10.1152/ajpregu.00311.2001.—Central cholinergic mechanisms are suggested to participate in osmoreceptor-induced water intake. Therefore, central injections of the cholinergic agonist carbachol usually produce water intake (i.e., thirst) and are ineffective in inducing the intake of hypertonic saline solutions (i.e., the operational definition of sodium appetite). Recent studies have indicated that bilateral injections of the serotonin receptor antagonist methysergide into the lateral parabrachial nucleus (LPBN) markedly increases salt intake in models involving the activation of the renin-angiotensin system or mineralocorticoid hormones. The present studies investigated whether sodium appetite could be induced by central cholinergic activation with carbachol (an experimental condition where only water is typically ingested) after the blockade of LPBN serotoninergic mechanisms with methysergide treatment in rats. When administered intracerebroventriculatly in combination with injections of vehicle into both LPBN, carbachol (4 nmol) caused water drinking but insignificant intake of hypertonic saline. In contrast, after bilateral LPBN injections of methysergide (4 µg), intracerebroventricular carbachol induced the intake of 0.3 M NaCl. Water intake stimulated by intracerebroventricular carbachol was not changed by LPBN methysergide injections. The results indicate that central cholinergic activation can induce marked intake of hypertonic NaCl if the inhibitory serotonergic mechanisms of the LPBN are attenuated.

carbachol; water intake; thirst; salt intake; salt appetite; 5-hydroxytryptamine

The lateral parabrachial nucleus (LPBN) is a hindbrain structure that has been implicated in body fluid regulation (3, 4, 6, 17–24). The LPBN receives afferent projections from the area postrema (AP) and adjacent portions of the medial nucleus of the solitary tract (mNTS) and sends efferent projections to forebrain areas involved in fluid and electrolyte balance such as the subfornical organ, median preoptic nucleus, paraventricular hypothalamic nucleus, and the amygdala (e.g., central nucleus) (2, 9, 10, 13, 15, 25). Functional studies have implicated the LPBN and its afferent connections in the neural control of body fluid and cardiovascular homeostasis (14).

A prominent serotonergic pathway from the AP/mNTS to the parabrachial nucleus has been described (16). Previous experimental results have led to the hypothesis that information arising from this pathway acts to inhibit thirst- and sodium appetite-related behaviors. Bilateral injections of the nonselective 5-hydroxytryptamine (5-HT)1/2 receptor antagonist methysergide into the LPBN significantly increases salt intake in a number of forms of experimentally induced sodium appetite. These include intracerebroventricular angiotensin (22), systemic administration of a diuretic (furosemide; Furo) plus angiotensin-converting enzyme inhibitor (22), chronic sodium depletion [24 h (18)], water deprivation (18), and chronic treatment with DOCA (4). Conversely, the administration of the 5-HT receptor agonist 2,5-dimethoxy-4-iodoamphetamine into the LPBN substantially reduces salt intake produced by dipsogenic/natriorexigenic treatments.

In addition to the increase of an already existing sodium intake, injection of methysergide into the LPBN also results in marked sodium intake by conditions where this behavior is usually not activated. Specifically, subcutaneous treatment with the β-adrenergic agonist isoproterenol or acute (1–2 h after) subcutaneous Furo (19) induces significant intakes of salt solution when methysergide is administered into the LPBN.

Central cholinergic activation, particularly with carbachol, is another special situation that stimulates water consumption but not hypertonic saline intake. Some studies have even suggested that central injections of carbachol might actually inhibit sodium intake in rats (7, 8). Considering the results from previous

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studies that have shown increases of an already existing sodium intake (3, 4, 17, 18, 22) or induction of a de novo sodium appetite (19) after the blockade of the LPBN inhibitory serotonergic mechanism, the present experiments investigated whether treatment of the LPBN with methysergide in conjunction with central (intracerebroventricular) injections of carbachol may induce hypertonic NaCl intake in addition to water, which is typically the only fluid consumed.

**MATERIALS AND METHODS**

**Animals.** Male Holtzman rats weighing 280–320 g were used. The animals were housed in individual stainless steel cages with free access to standard sodium diet (Purina Rat chow, sodium content 0.5%), water, and 0.3 M NaCl solution. Temperature was maintained at 23 ± 1°C, with a 12:12-h light-dark cycle with light onset at 7:00 AM. All experiments were performed between 9:00 AM and 1:00 PM.

**Cerebral cannulas.** Rats were anesthetized with ketamine (10 mg/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 by using the following coordinates: 9.4 mm caudal to bregma, 2.2 mm lateral to the midline, and 4.1 mm below the dura mater. The tips of the cannulas were positioned to terminate 2 mm above each LPBN. A third 23-gauge stainless steel cannula was implanted into the lateral ventricle (LV) according to the following coordinates: 0.5 caudal to bregma, 1.5 mm lateral to the midline, and 3.5 mm below the dura mater. Cannulas were fixed to the skull with the use of dental acrylic resin and jewelers screws. A 30-gauge metal obturator filled the cannulas between tests. After surgery, the rats were allowed to recover for 6 days before testing.

**Drugs.** Carbachol hydrochloride (4 nmol/1 μl) from Sigma Chemicals (St. Louis, MO) was dissolved in isotonic saline. Methysergide maleate (Sandoz Pharmaceutical, E. Hanover, NJ) was dissolved in propylene glycol-water (2:1). The dose of methysergide (4 μg/200 nl) used in the present study was based on previous studies (3, 4, 17–20).

**Water and 0.3 M NaCl ingestion tests.** Rats were tested in their home cages. Water and 0.3 M NaCl were provided from burettes with 0.1-ml divisions that were fitted with metal drinking spouts. In each experimental session, one-half of the rats received bilateral LPBN injections of vehicle (i.e., 2:1 propylene glycol-water), and the remaining animals received methysergide injections into the same nuclei. Fifteen minutes later, carbachol was injected into the LV. The volumes of water and 0.3 M NaCl ingested were recorded each 30 min during the 90 min immediately after the injection of carbachol. Each group of rats received two tests with a recovery period of at least 3 days between tests. One group of 26 rats had water and 0.3 M NaCl available during the test, and one group of 18 rats had only water available during the test. Injections were made by using 30-gauge injection cannulas connected by polyethylene tubing (PE-10) to 10-μl Hamilton syringes. At the time of testing, the animals were taken from their home cages, the obturators were removed, and injection cannulas were introduced into the implanted guide cannulas. The injection cannulas were 2 mm longer than the guide cannulas. Injection volumes of 200 nl were delivered to each LPBN and 1 μl into the LV. After injection, the obturators were replaced, and the rats were returned to their cages.

To control for the effects of LPBN methysergide injections in the absence of any other treatment, one group of 22 rats received bilateral LPBN injections of vehicle (200 nl/site) or methysergide (4 μg/200 nl/site; order randomized), and the volumes of water and 0.3 M NaCl ingested were recorded for 1 h.

**Food intake.** To assess the specificity of LPBN serotonergic mechanisms to control sodium intake in one group of 12 rats, the effect of LPBN injections of methysergide on food intake was tested.

Rats were deprived of food but not water overnight (14 h). The next day, a preweighed amount of food (pellet Purina chow) was returned to the rats 15 min after methysergide (4 μg/200 nl) or vehicle was injected bilaterally into the LPBN. Food intake was recorded each 30 min for 90 min.

**Histology.** At the end of the experiments, the animals received bilateral LPBN injections of Evans blue dye (200 nl/site). They were then deeply anesthetized with tribromoethanol (200 mg/kg body wt) and perfused transcardially with saline followed by 10% formalin. The brains were removed, fixed in 10% formalin, frozen, cut in 50-μm sections, stained by Giemsa, and analyzed by light microscopy in single-blind fashion to confirm the injection sites in the LPBN. Only data from rats with confirmed injections into the LV and bilateral placements of cannulas in the LPBN were analyzed.

**Statistical analysis.** The results are reported as means ± SE. Repeated-measures ANOVA and Newman-Keuls tests were used for comparisons. Differences were considered significant at P < 0.05.

**RESULTS**

**Histological analysis.** As seen in studies conducted previously (3, 4, 20, 22), LPBN injection sites were centered in the central lateral and dorsal lateral portions of the LPBN [see Fulwiler and Saper (9) 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 Evans blue dye, the spread of the injection was almost completely confined above the brachium. In some rats there was a small, limited spread of the injection into the brachium.

**Effects of LPBN methysergide injection on the intake of water and 0.3 M NaCl induced by intracerebroventricular injection of carbachol.** After the injection of vehicle into the LPBN, carbachol (4 nmol) injected intracerebroventricularly induced significant water intake and only a small amount of 0.3 M saline over the 90-min period after the injection (Fig. 1). Bilateral injections of methysergide (4 μg/200 nl each site) into the LPBN combined with intracerebroventricular carbachol significantly increased the intake of 0.3 M NaCl [F(1,22) = 7.24; P < 0.05] (Fig. 1). Water intake induced by intracerebroventricular carbachol was not significantly increased by LPBN methysergide treatment [F(1,22) = 0.15; P > 0.05] (Fig. 1).

When water was the only fluid available for drinking, bilateral LPBN injections of methysergide also produced no change in carbachol-induced water intake [F(1,18) = 0.883; P > 0.05] (Fig. 2).

Bilateral LPBN injections of methysergide, in the absence 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/1 h; n = 10 rats).

Effects of LPBN injections of methysergide on food intake. Bilateral LPBN injections of methysergide produced no significant change in food intake after 14 h of food deprivation [F(1,12) = 0.14; P > 0.05] (Fig. 3).

DISCUSSION

The results of these experiments are consistent with others (7, 8) indicating that intracerebroventricular injection of carbachol immediately induces water intake but little hypertonic NaCl intake. However, the present results demonstrate that when the nonselective 5-HT receptor antagonist methysergide is administered into the LPBN in conjunction with intracerebroventricular carbachol, there is substantial 0.3 M NaCl intake. Unlike the intake of hypertonic saline, water intake in rats treated with central carbachol or food intake was not modified by LPBN injections of methysergide.

Previous studies (3, 4, 18, 22) have demonstrated that injections of methysergide into the LPBN enhance hypertonic saline intake under conditions where a sodium appetite is already being expressed. That is, sodium appetite induced by several systemic treatments [Furo + captopril (22), subcutaneous Furo + 24 h of sodium-deficient diet (18), 24 h of water deprivation (18), or subcutaneous DOCA (4)] are significantly increased by bilateral LPBN methysergide treatment. In comparison to the previous studies, the present results demonstrate that it is possible not only to enhance an existing salt intake but also to induce sodium appetite by LPBN 5-HT receptor antagonist injections in combination with intracerebroventricular carbachol, a treatment that typically produces only water drinking (i.e., thirst).

Evidence available at the present time indicates that there is a reasonable degree of specificity for the enhancement of NaCl solution intake by bilateral LPBN
methysergide injections given in conjunction with na-
triorexigenic or dipsogenic treatments. Previous re-
search (18) has shown that sucrose solution intake
motivated only by the hedonic qualities of the fluid (i.e.,
sweet taste offered to nondeprived animals) was not
increased by bilateral LPBN methysergide treatment.
Similarly, the present study examining the effects of
LPBN methysergide injections on food intake after a
moderate period (overnight) of food deprivation showed
no evidence of facilitating eating.

Most experimental situations that induce a sodium
appetite take a long time (usually on the order of hours
to days) for enhanced salt ingestion (i.e., sodium appe-
tite) to clearly manifest itself. This is in contrast with
the shorter latencies for the onset of drinking (26). It
has been hypothesized that the relatively long latency
required for a sodium appetite to become apparent is
due to the presence of some form(s) of tonic inhibition,
which must be reduced before hypertonic NaCl is con-
sumed (26). Mechanisms hypothesized to be involved
in the reduction of such inhibition include 1) further
reductions in blood volume, 2) osmotic dilution of the
blood as a consequence of water intake, and 3) moder-
ate reductions in arterial blood pressure [i.e., decreases
of ~20 mmHg below normal; see Johnson and Thun-
horst (14) and Stricker and Verbalis (26) for review].
Therefore, previous studies have suggested that the
5-HT action in the LPBN might be associated with one
or more of the mechanisms hypothesized to be inhibi-
tory mechanisms. In the present study with the deac-
tivation of LPBN serotonergic inhibitory mechanisms,
sodium appetite arose in the presence of central cho-
linergic activation, an experimental condition that typ-
ically produces water consumption but not sodium in-
take. Central carbachol induces ingestion of water
simultaneously with increases in arterial pressure and
vasopressin release, which are all responses associated
with a condition of plasma hyperosmolarity (11, 12).

Previous studies have suggested that carbachol may
actually inhibit sodium intake in rats (7, 8). The present
findings suggest that central carbachol also
activates mechanisms that drive sodium intake, but
the result of such cholinergic activation can be ob-
served only after the blockade of LPBN serotonergic
inhibitory mechanisms. Part of the inhibitory effect of
carbachol on sodium intake in rats may be a conse-
quency of inhibitory input due to the marked increase
in arterial pressure produced by intracerebroventricu-
lar carbachol. LPBN methysergide treatment may
block such inhibitory influences derived from systemic
arterial baroreceptor input.

Neural afferents from the viscera, including those
from arterial and cardiac baroreceptors, terminate in
the NTS and in portions of the AP (1). As noted previ-
ously, a prominent serotonergic pathway originates in
the AP and mNTS and projects to the lateral regions of
the LPBN (16). Of course, the possibility that manip-
ulation of the LPBN 5-HT mechanisms may alter some
other type of inhibitory input (e.g., osmotic or gastric
distension) arising from other systemic receptors that
are carried in the AP/mNTS-LPBN projection cannot
be discounted. This anatomic organization suggests
that information derived from the peripheral visceral
afferents may alter activity in the anatomically defined
AP/mNTS-LPBN serotonergic pathway (16). Accumu-
lation evidence indicates that the LPBN is an impor-
tant component of a central neural network that is
related to control of cardiovascular and body fluid ho-
meostasis. The LPBN projects to several forebrain ar-
enas (e.g., paraventricular nucleus of the hypothalamus,
central nucleus of the amygdala, bed nucleus of the
stria terminalis, median preoptic nucleus) that have
been implicated in the control of water and electrolyte
balance (2–4, 6, 9, 10, 13, 15, 17, 18, 20, 22).

Perspectives

The present study extends previous results showing
that the blockade of LPBN serotonergic mechanisms
combined with a central stimulus (cholinergic activa-
tion) that usually induces only thirst (i.e., no salt in-
take) can induce sodium appetite. Thus it seems the
control of water and sodium appetite may have similar
input signals that are modified by LPBN serotonergic
mechanisms to determine whether sodium intake vs.
water intake will be initiated. The presence or absence
of 5-HT activity in the LPBN seems to act as a switch
to turn off or turn on sodium appetite. Future research
will be necessary to show whether other stimuli or
experimental manipulations that usually evoke only
thirst also activate brain circuits that control sodium
appetite. This confirmation will provide new concepts
for the understanding of the control mechanisms sub-
erving water and sodium intake.

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