Hindbrain serotonin and the rapid induction of sodium appetite

JOSÉ VANDERLEI MENANI, LAURIVAL ANTONIO DE LUCA JR., ROBERT L. THUNHORST, AND ALAN KIM JOHNSON. Hindbrain serotonin and the rapid induction of sodium appetite. Am J Physiol Regulatory Integrative Comp Physiol 279: R126–R131, 2000.—Both systemically administered furosemide and isoproterenol produce water intake (i.e., thirst). Curiously, however, in light of the endocrine and hemodynamic effects produced by these treatments, they are remarkably ineffective in eliciting intake of hypertonic saline solutions (i.e., operationally defined as sodium appetite). Recent work indicates that bilateral injections of the serotonin receptor antagonist methysergide into the lateral parabrachial nucleus (LPBN) markedly enhance a preexisting sodium appetite. The present studies establish that a de novo sodium appetite can be induced with LPBN-methysergide treatment under experimental conditions in which only water is typically ingested. The effects of bilateral LPBN injections of methysergide were studied on the intake of water and 0.3 M NaCl following acute (beginning 1 h after treatment) diuretic (furosemide)-induced sodium and water depletion and following subcutaneous isoproterenol treatment. With vehicle injected into the LPBN, furosemide treatment and isoproterenol injection both caused water drinking but essentially no intake of hypertonic saline. In contrast, bilateral treatment of the LPBN with methysergide induced the intake of 0.3 M NaCl after subcutaneous furosemide and isoproterenol. Water intake induced by subcutaneous furosemide or isoproterenol was not changed by LPBN-methysergide injections. The results indicate that blockade of LPBN-serotonin receptors produces a marked intake of hypertonic NaCl (i.e., a de novo sodium appetite) after furosemide treatment as well as subcutaneous isoproterenol.

Menani, José Vanderlei, Laurival Antonio De Luca Jr., Robert L. Thunhorst, and Alan Kim Johnson. Hindbrain serotonin and the rapid induction of sodium appetite. Am J Physiol Regulatory Integrative Comp Physiol 279: R126–R131, 2000.—Both systemically administered furosemide and isoproterenol produce water intake (i.e., thirst). Curiously, however, in light of the endocrine and hemodynamic effects produced by these treatments, they are remarkably ineffective in eliciting intake of hypertonic saline solutions (i.e., operationally defined as sodium appetite). Recent work indicates that bilateral injections of the serotonin receptor antagonist methysergide into the lateral parabrachial nucleus (LPBN) markedly enhance a preexisting sodium appetite. The present studies establish that a de novo sodium appetite can be induced with LPBN-methysergide treatment under experimental conditions in which only water is typically ingested. The effects of bilateral LPBN injections of methysergide were studied on the intake of water and 0.3 M NaCl following acute (beginning 1 h after treatment) diuretic (furosemide)-induced sodium and water depletion and following subcutaneous isoproterenol treatment. With vehicle injected into the LPBN, furosemide treatment and isoproterenol injection both caused water drinking but essentially no intake of hypertonic saline. In contrast, bilateral treatment of the LPBN with methysergide induced the intake of 0.3 M NaCl after subcutaneous furosemide and isoproterenol. Water intake induced by subcutaneous furosemide or isoproterenol was not changed by LPBN-methysergide injections. The results indicate that blockade of LPBN-serotonin receptors produces a marked intake of hypertonic NaCl (i.e., a de novo sodium appetite) after furosemide treatment as well as subcutaneous isoproterenol. Furosemide; sodium intake; water intake; 5-hydroxytryptamine

THE LATERAL PARABRACHIAL NUCLEUS (LPBN) is a hindbrain structure implicated in body fluid regulation (3, 6, 18–23). The LPBN receives afferent projections from the area postrema (AP) and the adjacent medial portions of the 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 hypo-thalamic nucleus, and amygdala (e.g., central nucleus) (2, 9, 10, 12, 15, 24). Functional studies have implicated the LPBN and its afferent connections in the neural control of body fluid and cardiovascular homeostasis (see Ref. 13 for review). Rats with electrolytic or neurotoxic (ibotenic acid) lesions of the LPBN have been described in rats with AP/mNTS lesions (7, 22). Furthermore, ablation of the AP and the immediately adjacent mNTS increases ad libitum (i.e., 24 h) intake of concentrated NaCl (4) and experimentally induced sodium intake in tests of short duration (5). A significant elevation in consumption of concentrated NaCl solutions that are normally avoided or consumed in very small amounts is commonly used as an operational definition of sodium appetite.

A prominent serotonergic pathway from the AP/mNTS to the parabrachial nucleus has been described (16). Previous experimental results led to the hypothesis that body fluid and/or hemodynamic information carried in this pathway acts to inhibit thirst- and sodium appetite-related behaviors. On the one hand, bilateral injections of the nonselective 5-hydroxytryptamine (5-HT)1/2-receptor antagonist methysergide into the LPBN significantly increases sodium intake in a number of forms of experimentally induced sodium appetite, including intracerebroventricular angiotensin (20), systemic administration of a diuretic plus angiotensin-converting enzyme inhibitor (20), chronic sodium depletion (24 h) (18), and water deprivation (18). On the other hand, administering a 5-HT-receptor agonist into the LPBN substantially reduces the sodium intake produced by these dipsogenic/natriorexigenic treatments.

In rats, peripheral injections of the β-adrenergic agonist isoproterenol reduce blood pressure, release renin, and induce water intake (11, 14, 17). Moderate reductions in blood pressure and increased activity of

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the renin-angiotensin system have both been implicated as participating mediators in an experimental model of sodium appetite that has a short latency of onset (29, 30, also, see Ref. 13 for review). In this light, it is puzzling that, although systemic isoproterenol treatment readily promotes water consumption (i.e., thirst), it produces virtually no hypertonic saline intake (i.e., no sodium appetite) (8). In a similar vein, it is also curious that acute hypovolemia (i.e., 1–2 h after treatment) induced by subcutaneous furosemide induces water intake but no hypertonic NaCl ingestion. Because the results from previous studies (3, 18, 20) suggest the presence of a 5-HT-LPBN inhibitory mechanism that acts to hold in check excess sodium intake, the present experiments investigated whether LPBN-methysergide treatment would induce a de novo sodium appetite after subcutaneous isoproterenol or after sodium depletion induced by furosemide treatment.

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 standard sodium diet (Purina Rat Chow 5012, sodium content 0.5%), water, and 0.3 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. All experiments were performed between 9:00 AM and 1:00 PM.

Cerebral Cannulas

Rats were anesthetized with an Equithesin-like anesthetic cocktail (composed of 0.97 g of pentobarbital sodium and 4.25 g of chloral hydrate/100 ml distilled water and prepared by The University of Iowa Hospitals and Clinics Pharmacy; 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 directed to terminate 2 mm above the LPBN were implanted bilaterally 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. Cannulas were fixed to the skull 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 testing.

Drugs

Both furosemide (Astra, Westborough, MA) and isoproterenol (Elkins-Sinn, Cherry Hill, NJ) were administered at 10 and 30 μg/kg sc, respectively. Methysergide maleate (Sandoz Pharmaceutical, East Hanover, NJ) was dissolved in propylene glycol/water (2:1). The dose of methysergide (4 μg/200 nl) used in the present studies was selected on the basis of a prior study in which a dose-response analysis of LPBN injections was established for its effects on experimentally induced water intake (19).

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 drug injections into the same nuclei. Repeated treatments were administered in a counter-balanced design with no rat receiving more than four tests, and a recovery period of at least 3 days intervened between tests.

LPBN injections were made 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. The injection volume delivered to each LPBN was 200 nl. After injection, the obturators were replaced and the rats were returned to their cages.

Furosemide treatment. Hypovolemia was induced by furosemide (10 mg/kg sc). Animals were injected and returned to their cages, from which water and 0.3 M NaCl had been removed. Fifty minutes later, methysergide (4 μg · 200 nl⁻¹ · site⁻¹) or vehicle (200 nl/site) was injected bilaterally into the LPBN. Ten minutes later, rats were given access to water and 0.3 M NaCl. Cumulative water and 0.3 M NaCl intakes were measured every 30 min for 2 h beginning immediately after the return of the fluids. Several days later, the test was repeated with reversal of the LPBN treatments.

Isoproterenol treatment. Rats were injected with isoproterenol (30 μg/kg body wt sc) and then immediately with methysergide (4 μg · 200 nl⁻¹ · site⁻¹) or vehicle (200 nl/site) bilaterally into the LPBN. They were returned to their cages, where water and 0.3 M NaCl were available. Water and 0.3 M NaCl intakes were recorded every 30 min for 2 h. A minimum of 2 days later, the LPBN treatments were reversed in a second test.

Another pair of tests conducted with only water available for drinking began several days later. In this pair of tests, the rats also received isoproterenol (30 μg/kg body wt) in conjunction with vehicle or methysergide LPBN treatment (order randomized).

To assess the effects of LPBN-methysergide injections in the absence of any other treatment, one group of rats received bilateral LPBN injections of vehicle (200 nl/site) or methysergide (4 μg · 200 nl⁻¹ · site⁻¹; order randomized with a minimum of 2 days intervening), and the volumes of water and 0.3 M NaCl ingested were recorded for 1 h.

Histology

At the end of the experiments, methylene blue solution (200 nl/site) was injected through the cannulas. Rats 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 in a single-blind fashion to confirm the injection sites. Only data from rats with confirmed, bilateral placements of cannulas in the LPBN were analyzed. Data from rats with only unilateral placements, or bilateral misses, were discarded.

Statistical Analysis

The results are reported as means ± SE. When the data (i.e., after isoproterenol treatment) met the assumptions for conducting an ANOVA, this statistical test was used. Fisher’s least significant difference tests were used to make follow-up comparisons when at least one F ratio was significant. In the furosemide study in which the data did not conform to the assumptions underlying parametric statistical analyses, a
RESULTS

Histological Analysis

As seen in studies conducted previously in our laboratory (3, 18–20), LPBN injection sites were centered in the central lateral and dorsal lateral portions of the LPBN (see Ref. 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 methylene blue, the spread of the injectate was almost completely confined to tissue above the brachium. In some rats there was a small, limited spread of the injection into the brachium, but it did not extend below this structure.

Effects of LPBN-Methysergide Injection on the Intake of Water and 0.3 M NaCl Induced by Subcutaneous Injection of Isoproterenol

Isoproterenol injection in conjunction with LPBN-vehicle treatment was associated with ~4 ml of water intake over the 2-h period after injection and essentially no intake of 0.3 M saline (Fig. 1). In contrast, bilateral injection of methysergide into the LPBN significantly increased the intake of 0.3 M NaCl (main effect, $F_{1.8} = 7.94; P < 0.05$) to isoproterenol. A significant treatment $\times$ time interaction ($F_{3,24} = 3.97; P < 0.05$) showed that LPBN-methysergide-treated rats drank significantly more hypertonic saline within the first 30–60 min of fluid access. Water intake was not significantly increased by LPBN-methysergide treatment ($F_{1.8} = 2.88; P > 0.05$), and there was no significant treatment $\times$ time interaction ($F_{3,24} = 0.49; P < 0.05$; Fig. 1). However, there was a significant main effect of time ($F_{3,24} = 14.85; P < 0.05$) on intake.

In tests in which only water was available for drinking, bilateral LPBN injections of methysergide produced no change in isoproterenol-induced water intake ($F_{1.10} = 4.76; P > 0.05$) compared with the LPBN-vehicle treatment condition (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 $\pm$ 0.6 ml/1 h) or 0.3 M NaCl (0.03 $\pm$ 0.03 ml/1 h, $n = 10$ rats).

Effects of LPBN Injection of Methysergide on the Intake of 0.3 M NaCl and Water Induced by Furosemide

Over the 1- to 3-h time period following subcutaneous furosemide treatment, LPBN-vehicle-treated rats drank ~2.5 ml of water but essentially no 0.3 M NaCl (Fig. 3). Because of zero intakes in some cells, ANOVA was inappropriate because of the violation of assumptions underlying parametric statistics. Therefore, a nonparametric statistical test, the Wilcoxon, was employed on the final, cumulative intake data. Bilateral LPBN injections of methysergide produced a significant increase in the intake of 0.3 M NaCl ($W = 21; P < 0.032; n = 6$), but no significant change in water consumption ($W = 9; P > 0.05; n = 6$) following subcutaneous furosemide (Fig. 3).

DISCUSSION

The results of these experiments are consistent with others (8, 29), indicating that rats given isoproterenol or furosemide readily drink water but consume essentially no hypertonic NaCl after treatment. The most important finding of the present experiments is that the administration of the nonselective 5-HT$_{1/2}$-receptor antagonist methysergide into the LPBN is associated with significant intakes of 0.3 M NaCl after acute furosemide-induced hypovolemia or subcutaneous isoproterenol. Unlike the intake of hypertonic saline, water intake in rats treated with either furosemide or isoproterenol was not reliably affected by LPBN injections of methysergide. It is important to note that, as previously observed (18, 20), the present work also...
found that bilateral LPBN injections of methysergide had no effect on sodium or water intake unless the rats were subjected to a treatment that, by itself, induced the ingestion of sodium and/or water.

Previous studies (3, 18, 20) have demonstrated that injections of methysergide into the LPBN increase the ingestion of hypertonic saline under conditions in which a sodium appetite is already being expressed. That is, sodium appetite induced by several systemic treatments (furosemide + captopril (20), subcutaneous furosemide + 24 h of sodium-deficient diet (18), 24 h of water deprivation (18)) is significantly increased by injection of methysergide bilaterally into the LPBN. In light of these prior investigations, the present results are unique in that they demonstrate that it is possible not only to enhance an existing sodium intake, but also to induce sodium appetite de novo by employing bilateral LPBN 5-HT-receptor antagonist injections in combination with systemic treatments that typically produce only water drinking (i.e., thirst). In this light, it is important to consider what action methysergide may be having in the LPBN to induce NaCl intake de novo.

Most experimental models used to study sodium appetite require a long time (usually on the order of hours to days) for a significant increase in sodium intake to become manifest. This delay is in marked contrast with the shorter latencies for the onset of drinking (25). For example, polyethylene glycol treatment induces water intake with a relatively brief latency (e.g., <1–2 h), but it typically takes several hours (4–7 h) before significant consumption of hypertonic NaCl is observed (see Ref. 25 for review). It has been hypothesized that the relatively long period required for a sodium appetite to become apparent is due to some form(s) of tonic inhibition that must first be reduced (25). Mechanisms proposed to be involved in the waning of such inhibition include 1) reductions in blood volume, 2) osmotic dilution as a consequence of water intake, and 3) reductions in arterial blood pressure (i.e., decreases of ~20 mmHg below normal; see Refs. 13 and 25 for review).

Neural afferents from the viscera, including arterial and cardiac baroreceptors, terminate in the NTS and in portions of the AP (1). A prominent serotonergic pathway originates in the AP and mNTS, which projects to the lateral regions of the LPBN (16). This anatomical organization suggests that information derived from peripheral visceral afferents may alter activity in the AP/mNTS-LPBN serotonergic pathway (16). The results of the present experiments are consistent with the idea that the 5-HT antagonist acts in the LPBN to alter the throughput of information derived from systemic receptors.

Treatments that lower arterial pressure given in conjunction with furosemide induce a significant sodium appetite (27, 29). However, the dose of furosemide used in the present studies does not produce hypotension (29). Therefore, the expression of a sodium appetite (25) is not produced.

1 In the context of this discussion, it should be noted that bilateral LPBN-methysergide treatment does not by itself reduce arterial blood pressure (19).
appetite in furosemide-treated rats with injections of methysergide into the LPBN is unlikely to be due to the presence of frank hypotension. On the other hand, it is possible that blockade of 5-HT in the LPBN with methysergide in conjunction with furosemide might be mimicking a fall in systemic arterial pressure and/or an additional decrease in blood volume. Alternatively, manipulation of the LPBN serotonergic pathway may alter some other type of systemic visceral input (e.g., osmolality or gastric stretch) that is normally inhibitory and that is carried in the AP/mNTS-LPBN projection.

Peripherally injected isoproterenol produces hypotension, renin release, and water intake, but only trivial intakes of hypertonic NaCl solutions (8, 11, 14, 17). Considering the findings that indicate that moderate decreases in arterial pressure facilitate NaCl intake (27, 29) and that increases in circulating renin-angiotensin (28) importantly contribute to sodium appetite, it is difficult to understand why isoproterenol does not induce robust NaCl intake (8). Similar to the case discussed for furosemide treatment, the LPBN methysergide plus isoproterenol-induced sodium appetite may also be the result of a simulated change in an afferent input from systemic arterial (i.e., high pressure) baroreceptors or from cardiopulmonary volume receptors (i.e., low pressure baroreceptors located primarily in the vena cava and atria). However, in the case of isoproterenol, it is worth considering an alternative explanation that involves afferent activity from yet another set of systemic mechano- and/or chemoreceptors. Isoproterenol may actually generate inhibitory input from a set of systemic receptors, and this inhibition is sufficient to override facilitatory signals arising from elevated circulating ANG II and hypotension. A source of such inhibitory input associated with isoproterenol treatment per se may be from mechano- and chemosensitive receptors in the cardiac ventricles through their afferent vagal C fibers. Specifically, Thorén (26) and colleagues have demonstrated in cats that systemically administered adrenaline or isoproterenol increases activity in vagal C fibers arising from cardiac ventricular receptors. These ventricular receptors are typically activated by aortic or carotid occlusion and by severe hypovolemia. Increased afferent activity in these C fibers has been shown to inhibit sympathetic nerve activity and has been proposed to be responsible for the sympatholytic responses that accompany severe hypotensive hemorrhage (26). It seems reasonable to speculate that similar afferent input might contribute to an active inhibition of sodium appetite in response to systemic isoproterenol treatments. If isoproterenol treatment generates an increased inhibitory signal from cardiopulmonary receptors, as suggested by Thorén’s findings, it may be the case that LPBN 5-HT blocks this inhibitory input so that sodium appetite is actively expressed.

In the present series of studies, LPBN-methysergide treatment significantly increased 0.3 M NaCl, but not water, intake in response to dipsogenic stimuli. In some (18, 20), but not all (3), experiments, we have found that both water intake and hypertonic saline intake are significantly increased by LPBN-methysergide treatment. In general, we have interpreted these findings to suggest that an LPBN serotonergic-related mechanism is involved in modulating not just sodium appetite, but behaviors more generally (i.e., also water intake or thirst) that contribute to the restoration or expansion of extracellular volume (13). However, there may be other factors involved in the control of these behaviors in addition to the major signals of extracellular fluid volume status (i.e., ANG II and input from receptors monitoring hypovolemia/hypotension) that are affected by LPBN-methysergide treatment. For example, the hypertonic NaCl solutions used in these and other experiments (18, 20) also have properties associated with taste and increased osmolarity. In a previous study (18), we evaluated the specificity of LPBN-methysergide treatment in nondeprived animals drinking 2% sucrose, in which the fluid intake was motivated by palatability of the solutions (i.e., a “dessert test”). We found no significant increase in sucrose intake following methysergide treatment. This observation suggests that the effects of LPBN-methysergide treatment are selective for water and NaCl solutions when intake is induced by hypovolemic/hypotensive related treatments. Nevertheless, because the parabrachial nucleus is located in key pathways related to taste and the control of feeding, further analysis of the role of 5-HT in the inhibition of other ingestive and oral-related behaviors is clearly warranted.

In summary, accumulating evidence indicates that the LPBN is an important component of a central neural network related to the control of cardiovascular and body fluid homeostasis. The LPBN projects to several forebrain areas (e.g., paraventricular nucleus of the hypothalamus, central nucleus of the amygdala, bed nucleus of the stria terminalis, median preoptic nucleus) that are implicated in the control of water and electrolyte balance (2, 3, 6, 9, 10, 12, 15, 18–24). The LPBN receives inputs from multiple regions (10, 16), and 5-HT has been identified as a neurochemical component of an ascending pathway to the parabrachial nucleus originating in the AP/mNTS (16). Therefore, both the present and previous (3, 18, 20) studies provide a functional basis for the LPBN as an inhibitory site of action for 5-HT in the control of sodium appetite.

**Perspectives**

The present investigation extends the findings of earlier studies suggesting that 5-HT acts in the LPBN to limit the amount of hypertonic saline intake. The results reported here demonstrate that blockade of 5-HT action in the LPBN will actually induce a sodium appetite in response to experimental treatments that typically elicit only water intake (i.e., thirst). The demonstration that a de novo salt appetite occurs in response to treatments that are normally only dipsogenic provides further evidence that the LPBN is an important component in the brain system that controls body...
fluid homeostasis and that 5-HT is an important neurochemical mediator within that central neural circuitry.

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REFERENCES


