Inhibition of sodium appetite by lipopolysaccharide: involvement of α2-adrenoceptors


Departments of Physiology and Pathology, School of Dentistry and Natural Products and Toxicology, School of Pharmaceutical Sciences, São Paulo State University, Universidade Estadual Paulista, Araraquara, São Paulo, Brazil

Submitted 2 September 2009; accepted in final form 6 April 2011

Almeida RL, David RB, Constancio JF, Fracasso J, Menani JV, De Luca LA Jr. Inhibition of sodium appetite by lipopolysaccharide: involvement of α2-adrenoceptors. Am J Physiol Regul Integr Comp Physiol 301: R185–R192, 2011. First published April 6, 2011; doi:10.1152/ajpregu.00555.2009.—Lipopolysaccharide (LPS), an endotoxin from the wall of Escherichia coli, produces a general behavioral inhibition and affects several aspects of fluid-electrolyte balance. LPS inhibits thirst; however, it is not clear if it also inhibits sodium appetite. The present results show that LPS (0.3–2.5 mg/kg body wt) injected intraperitoneally produces a dose-dependent reduction of sodium appetite expressed as 0.3 M NaCl intake induced by sodium depletion (furosemide plus removal of ambient sodium for 24 h). The high doses of LPS (1.2–2.5 mg/kg) also produced transient hypothermia at the beginning of the sodium appetite test; however, no dose produced hyperthermia. LPS also increased the stomach liquid content (an index of gastric emptying) after a load of 0.3 M NaCl given intragastrically to sodium-depleted rats. The α2-adrenoceptor antagonist yohimbine (5 mg/kg ip) abolished the effect of LPS on 0.3 M NaCl intake, without changing the effect of LPS on gastric emptying. Injection of RX-821002 (160 nmol), another α2-adrenoceptor antagonist, in the lateral cerebral ventricle (LV) also reversed the inhibition of sodium appetite produced by LPS. Yohimbine intraperitoneally or RX-821002 in the LV alone had no effect on sodium intake. Although yohimbine plus LPS produced a slight hypotension, RX-821002 plus LPS produced no change in arterial pressure, suggesting that the blockade of the effects of LPS on sodium intake by the α2-adrenoceptor antagonists is independent from changes in arterial pressure. The results suggest an inhibitory role for LPS in sodium appetite that is mediated by central α2-adrenoceptors.

sodium intake; endotoxin; arterial pressure; RX-821002; fluid balance; sickness behavior

LIPOPOLYSACCHARIDE (LPS), an endotoxin derived from the wall of gram-negative bacteria, triggers an array of behavioral and systemic responses, grouped under the name of “sickness behavior” (7).

Sickness behavior has been hypothesized to be an adaptive syndrome of behavioral and physiological responses by animals coping with infectious pathogens (7). Reduced activity, feeding, and drinking are some of the behavioral alterations induced by infection. These alterations may help to shift internal energy production to increase body temperature and sustain fever, an important mechanism to combat infection.

It is possible that the reduced drinking that occurs in sickness behavior is also associated with a general effect on body-fluid balance because LPS releases hormones that influence such balance (4) and reduces several aspects related to sodium metabolism: sodium transport in the gut (5), natriuresis (1, 31), and a rapidly developing sodium intake induced by the diuretic furosemide combined with a low dose of the converting enzyme inhibitor captopril or Furo/Cap (1).

In the previous study (1), LPS injected before Furo/Cap treatment inhibited sodium intake produced within 2 h, but it also reduced sodium excretion, which may affect sodium intake. Thus it is not possible to definitively conclude if the inhibition of sodium intake was a direct effect of LPS or secondary to the reduction of sodium excretion. Moreover, recall that a strong motivation also may overcome the inhibitory effect of LPS (7). Therefore, it is important to test if, independent from its antinatriuretic effects, LPS also inhibits sodium appetite, a motivated behavior fully expressed after several hours of sodium depletion (10, 24, 35–37). Such test may be performed by sodium depleting the animals, for example, through the combination of furosemide injection plus 24-h removal of ambient sodium (10, 24, 35–37) before injecting LPS.

The antidiipsogenesis induced by LPS has been known for several years and is mediated by central production of nitric oxide, prostaglandins, and, perhaps, tumor necrosis factor-α (3, 19, 20, 22, 32). These mediators could also affect sodium appetite; however, there is also a possibility for a noradrenergic mediation through α2-adrenoceptors. LPS activates central release of norepinephrine (26), the endogenous ligand for α2-adrenoceptors, and α2-adrenoceptor antagonists increase the survival rate of rats with lethal endotoxemia (17). Moreover, α2-adrenoceptors are involved with reduced mood, immunological activity, and sodium appetite (9, 13, 14, 40, 43).

In the present work, we tested if LPS at sublethal doses inhibits sodium appetite and if α2-adrenoceptor antagonists, yohimbine (YOH) or RX-821002 (RX), counteract the effect of LPS on sodium appetite.

METHODS

Animals

Male Holtzman rats weighing 280–320 g were used. The animals were housed in individual stainless steel cages with free access to a regular diet with 0.5–1.0% sodium (Guabi Rat Chow; Paulínia, São Paulo, Brazil), water, and 0.3 M NaCl, except when differently required by the protocol. Rats were maintained at room temperature of 23 ± 2°C, humidity of 55 ± 10%, and on a 12:12-h light-dark cycle with lights on at 7:30 A.M. They were handled at least once a day by the experimenter. The protocols were approved by the Institutional Ethical Committee for Animal Care and Use from the School of Dentistry, Universidade Estadual Paulista, Araraquara, and followed the recommendations from the Brazilian College of Animal Experimentation.

Address for reprint requests and other correspondence: L. A. De Luca Jr., Dept. of Physiology and Pathology, School of Dentistry, São Paulo State Univ., UNESP, Rua Humaitá, 1680, Araraquara, São Paulo, 14801-903, Brazil (e-mail: lucajr@foar.unesp.br).
Drugs

LPS, endotoxin extracted from *Escherichia coli* serotype 026:B6 (0.3, 0.6, 1.2, 2.0, or 2.5 mg/kg body wt; Sigma), and the α2-adrenoceptor antagonists YOH (2 and 5 mg/kg body wt; Sigma) or RX (80 and 160 nmol/μL; Sigma) were dissolved in sterile isotonic saline (SAL). Furosemide (10 mg/ml; Aldrich), a natriuretic and diuretic drug, was dissolved in NaOH solution (pH = 9.0).

**Sodium Depletion and Sodium Appetite Test**

We combined the diuretic and natriuretic furosemide with 24-h removal of ambient sodium to deplete the animals of sodium (10, 24, 35–37).

On the day before all the tests, food and fluids were removed from the cage, and the animals received a subcutaneous injection of furosemide (10 mg/rat). Cages were thoroughly rinsed to remove sodium, and the animals remained with water and sodium-free diet (powdered corn meal) for the next 24 h.

Twenty-four hours after the injection of furosemide, food was removed, and the animals received intraperitoneal injection of LPS or isotonic SAL. Sodium appetite test started 1 or 2 h later (see *Protocols*) when the bottle of water was substituted by two graduated burettes (0.1-ml divisions) fitted with stainless steel spouts containing water and 0.3 M NaCl. Water and 0.3 M NaCl intakes were recorded at the end of the 2-h sodium appetite test.

**Protocols**

**Sodium appetite test in rats treated with intraperitoneal LPS**. The animals (*n* = 35) were gently handled to measure rectal temperature once a day, for habituation, in the days that preceded a sodium appetite test.

On the day of the test, 24-h sodium-depleted animals randomly received an intraperitoneal injection of LPS (0.3, 0.6, 1.2, or 2.5 mg/kg body wt) or SAL 1 h before the sodium appetite test. Rectal body temperature was measured immediately before injecting LPS to establish baseline temperature. Rectal temperature was recorded again at the beginning of the sodium appetite test immediately before offering water and 0.3 M NaCl and after the 2-h sodium appetite test.

**Sodium appetite test in rats treated with intraperitoneal LPS combined with intraperitoneal YOH**. To test the effects of LPS combined with YOH, a new batch of the same brand and serotype of LPS was used. For some reason, the new batch was less effective in pilot studies, with the 0.6 mg/kg of LPS from this batch yielding 0.3 M NaCl intake that was not different from controls that received only SAL. Therefore, the dose of LPS was increased from 0.6 to 2.0 mg/kg to obtain similar inhibition of sodium appetite without side effects as those produced by LPS at the dose of 2.5 mg/kg in the first tests (see *Sodium Appetite Test and Rectal Temperature in Rats Treated With Intraperitoneal LPS*). Because LPS at the dose of 2.0 mg/kg from the new batch inhibited 0.3 M NaCl intake with no overt side effects, this dose was used in the remaining experiments.

Because YOH combined with LPS caused a transient reduction in arterial pressure in 24-h sodium-depleted rats, which could alter the effects of LPS on sodium intake, sodium appetite test was planned to start 1 h after the injection of LPS (during the period of hypotension) or 2 h after the injection of LPS when arterial pressure had returned to basal levels (see *Changes in mean arterial pressure and heart rate in sodium-depleted rats treated with intraperitoneal LPS combined with intraperitoneal YOH*).

To test the effects of YOH or SAL intraperitoneally combined with LPS or SAL intraperitoneally on water and 0.3 M NaCl when sodium appetite tests started 1 h after LPS injection, sodium-depleted animals (*n* = 36) were divided into three groups, one that received SAL and the other two that received YOH (2 or 5 mg/kg of body wt ip). Fifteen minutes later, the groups were further subdivided into animals that received SAL or LPS (2 mg/kg body wt ip); thus, six groups were formed: SAL + SAL, YOH-2 + SAL, YOH-5 + SAL, saline + LPS, YOH-2 + LPS, and YOH-5 + LPS. The sodium appetite test began 1 h after the second injection.

For the sodium appetite test starting 2 h after LPS treatment, other animals (*n* = 36) received the same intraperitoneal injections as described above, and another identical six groups were formed. The sodium appetite test began 2 h after the second injection.

**Changes in mean arterial pressure and heart rate in sodium-depleted rats treated with intraperitoneal LPS combined with intraperitoneal YOH**. While a mild hypotension, which might be induced by YOH, attenuates inhibitory signals from the cardiopulmonary receptors to facilitate fluid intake (24), a severe hypotension, such as that reported for relatively high doses of LPS (42), may impair the ability of the animal to ingest fluid. Therefore, the effects of LPS alone or combined with YOH intraperitoneally on mean arterial pressure (MAP) and heart rate (HR) were tested in sodium-depleted animals.

To record MAP and HR, rats were anesthetized with ketamine (80 mg/kg body wt combined with xylazine (7 mg/kg body wt), and a polyethylene tubing (PE-10 connected to a PE-50) was inserted in the abdominal aorta through the femoral artery. The arterial catheter was tunneled subcutaneously and exteriorized on the back of the rat to record MAP and HR in unrestrained and freely moving rats. One day after the surgery to implant the arterial catheter, the animals (*n* = 20) were submitted to sodium depletion as described in *Sodium Depletion and Sodium Appetite Test*. After 24 h of sodium depletion, water- and sodium-deficient food were removed from the cage, and the arterial catheter was connected to a Statham Gould (P23Db) pressure transducer coupled to a preamplifier (model ETH-200 Bridge Bio Amplifier; CB Sciences, Dover, NH) that was connected to a PowerLab computer data acquisition system (model Powerlab 16SP; ADInstruments, Castle Hill, Australia) to record pulsatile arterial pressure, MAP, and HR in unanesthetized and unrestrained rats. A period of 20–30 min was necessary for MAP and HR readings to stabilize. Injections were made only after 10 min of stable MAP and HR recordings. A first sampling of MAP and HR (baseline MAP and HR values) was made at the instant defined as −15 min immediately before a first injection of either SAL or YOH (5 mg/kg body wt ip). A second sampling of MAP and HR was made at the instant defined as 0 min immediately before a second injection of either SAL or LPS (2 mg/kg body wt ip). The recording was interrupted, and the catheter was disconnected from the pressure transducer to administer each injection. The catheter was immediately reconnected to the transducer after each injection to continue the recordings. Thus four groups were formed: SAL + SAL, YOH-5 + SAL, saline + LPS, and YOH-5 + LPS. The recordings proceeded without interruption for 120 min after the second injection, and the data were sampled at 20-min intervals for statistical analysis.

**Gastric emptying in sodium-depleted rats treated with intraperitoneal LPS combined with intraperitoneal YOH**. Reduction in gastric emptying may signal satiety for hypertonic NaCl intake (39). LPS reduces gastric emptying, an effect reduced by YOH in mice (21). This could explain a recovery of 0.3 M NaCl intake when LPS is combined with YOH. Therefore, we tested the effects of LPS, alone or combined with YOH, on gastric emptying, as measured by the amount of liquid retained in the stomach in response to a gavage of hypertonic NaCl.

Animals (*n* = 22) were trained to receive a gavage in the stomach using a polyethylene cannula inserted through the mouth once a day for 5 days before the test. One day before the test, rats were submitted to sodium depletion as described in *Sodium Depletion and Sodium Appetite Test*. After 24 h of sodium depletion, rats received SAL or YOH (5 mg/kg body wt ip) combined 15 min later with SAL or LPS (2 mg/kg body wt ip); thus, four groups were formed: SAL + SAL, YOH-5 + SAL, saline + LPS, and YOH-5 + LPS. Two hours after the second injection, the animals received a 3-ml gavage of 0.3 M NaCl to emulate the effect of rapid ingestion of 0.3 M NaCl. Five minutes
later, the animals were deeply anesthetized with sodium thiopental (80 mg/kg of body wt; Cristália) for analysis of the remaining stomach liquid content, an index of gastric emptying, as adapted from previous works (27). The esophagus, just proximal to the gastric fundus, and the duodenum, just distal to the pylorus, were clamped and tied with suture thread. The clamps were removed, a transverse cut distal to the stomach was made in the vicinity of each tie, and the stomach was immediately removed and weighed to determine total weight. Next, the stomach was desiccated (60°C during 48–72 h) and weighed again to determine the dry tissue weight. The amount of liquid retained in the stomach (stomach liquid content) was calculated by subtracting the dry weight from the total weight, assuming 1 g = 1 ml. Values are for total liquid weighed, including the amount (1.6 ± 0.1 g, n = 6) found in a group of sodium-depleted animals that did not receive the gavage.

Sodium appetite test and changes in MAP and HR in sodium-depleted rats treated with intraperitoneal LPS combined with intracerebroventricular RX. To test if central α2-adrenoceptors mediate the effect of LPS on sodium appetite, a group of animals was prepared to receive intracerebroventricular injections of RX, an α2-adrenoceptor antagonist that dissociates better than YOH in isotonic SAL and therefore is more suitable for central injections.

Rats to receive intracerebroventricular injections were anesthetized as described above in Changes in mean arterial pressure and heart rate in sodium-depleted rats treated with intraperitoneal LPS combined with intraperitoneal YOH and placed in a stereotaxic instrument (Kopf). The skull was leveled between bregma and lambda. Stainless steel 23-gauge guide cannulas were unilaterally implanted in the left lateral ventricle (LV, coordinates: 0.3 mm caudal to bregma, 1.6 mm lateral to the midline, and 3.8 mm below the skull surface), with the tips positioned 2 mm above the LV. The guide cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. Thirty-gauge metal obturators filled the guide cannulas. Rats were allowed to recover at least 3 days before beginning the experiments.

Injections in the LV were made using 5-μl Hamilton syringes (Hamilton) connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At the time of testing, obturators were removed, and the injection cannulas (2 mm longer than the guide cannulas) were introduced in the brain. The injection volume in the LV was 1.0 μl. The obturators were replaced after injections, and the rats were returned to their cages. One group of animals (n = 37) to receive central injections was submitted to sodium depletion as described in Sodium depletion and sodium appetite test. After 24 h of sodium depletion, food was removed, and the group was subdivided into three groups, one that received SAL and two others that received RX (80 or 160 mmol icv). Fifteen minutes later, the groups were further subdivided into animals that received SAL or LPS (2 mg/kg body wt ip), thus forming six groups: SAL + SAL, RX-80 + SAL, RX-160 + SAL, SAL + LPS, RX-80 + LPS, and RX-160 + LPS. The sodium appetite test began 2 h after the second injection.

Another group of animals (n = 20) to receive central injections was prepared according to Changes in mean arterial pressure and heart rate in sodium-depleted rats treated with intraperitoneal LPS combined with intraperitoneal YOH for cardiovascular recordings and sodium depleted as described in Sodium depletion and sodium appetite test. At the end of 24 h of sodium depletion, after removing food and stabilization of baseline MAP and HR for 10 min, the group was subdivided into two groups, one that received RX and another that received RX (160 mmol icv). Fifteen minutes later, the groups were further subdivided into animals that received SAL or LPS (2 mg/kg body wt ip), thus forming four groups: SAL + SAL, RX-160 + SAL, SAL + LPS, and RX-160 + LPS. The recordings proceeded without interruption until 120 min after the second injection.

Statistics

Data were analyzed using ANOVA. One-way ANOVA was used to compare different treatments. Two-way ANOVA was used with treatment and time as factors for the studies involving changes in MAP and HR and rectal temperature. Pairwise comparisons were made using the Student-Newman-Keuls test. Data are expressed as means ± SE, and a probability of <0.05 was required for significance.

RESULTS

Sodium Appetite Test and Rectal Temperature in Rats Treated with Intraperitoneal LPS

ANOVA showed significant differences among treatments with LPS (0.3, 0.6, 1.2, or 2.5 mg/kg body wt ip) and SAL for 0.3 M NaCl [F(4,35) = 12.3; P < 0.05] but no differences among treatments with LPS and SAL for water intake during the sodium appetite test [F(4,35) = 0.7; P > 0.05] (Fig. 1, bottom). Post hoc tests showed that LPS (0.6, 1.2, or 2.5 mg/kg body wt ip) reduced 24-h sodium depletion-induced 0.3 M NaCl intake compared with SAL (Fig. 1, top).

ANOVA showed significant differences among treatments with LPS and SAL on rectal temperature [F(4,105) = 4.3; P < 0.05] but no differences for time [F(2,105) = 1.2; P > 0.05] or any interaction between time and treatment [F(8,105) = 1.3; P > 0.05]. Post hoc tests showed that, at the beginning of the
sodium appetite test, immediately before offering sodium to the animals, LPS at the high doses of 1.2 and 2.5 mg/kg decreased rectal temperature (34.2 ± 0.4 and 33.4 ± 0.5°C, respectively) compared with SAL (35.9 ± 0.2°C), with rectal temperature returning to baseline (35.4 ± 0.2°C average) at the end of the sodium appetite test. No grooming was observed when the animals received any dose of LPS or SAL, but LPS (2.5 mg/kg) produced behavioral side effects like piloerection and body curling with the nostrils pointing ventrally.

Sodium Appetite Test in Rats Treated With Intraperitoneal LPS Combined With Intraperitoneal YOH

ANOVA showed significant differences among treatments (SAL + SAL, YOH-2 + SAL, YOH-5 + SAL, SAL + LPS, YOH-2 + LPS, and YOH-5 + LPS) for 0.3 M NaCl intake during the sodium appetite test that began 2 h after LPS injection \( F(5,27) = 4.8; P < 0.05 \) (Fig. 3, bottom). Post hoc tests showed that the combination of YOH (5 mg/kg of body wt ip) + LPS (2 mg/kg body wt ip) again reversed the inhibition of 0.3 M NaCl intake produced by the combination of SAL + LPS (Fig. 3, top).

ANOVA showed significant differences among treatments (SAL + SAL, YOH-2 + SAL, YOH-5 + SAL, SAL + LPS, YOH-2 + LPS, and YOH-5 + LPS) for water intake during the sodium appetite test that began 2 h after LPS injection \( F(5,27) = 2.7; P < 0.05 \) (Fig. 3, bottom). Post hoc tests showed that the combination of YOH (5 mg/kg of body wt ip) + SAL increased water intake compared with SAL + SAL, and the combination of YOH (2 and 5 mg/kg of body wt ip) +
LPS increased water intake compared with SAL + LPS during the sodium appetite test that began 2 h after LPS injection (Fig. 3, bottom).

Changes in MAP and HR in Sodium-Depleted Rats Treated With Intraperitoneal LPS Combined With Intraperitoneal YOH

There was no significant difference in preinjection baseline at −15 min for MAP or HR among groups (SAL + SAL, YOH-5 + SAL, SAL + LPS, and YOH-5 + LPS) (Fig. 4).

For MAP, ANOVA showed significant differences for treatment (SAL + SAL, YOH + SAL, SAL + LPS, YOH-5 + LPS) [F(3,14) = 3.8; P < 0.05] and time [F(6,84) = 3.1; P < 0.05]; however, there was no interaction between treatment and time [F(18,84) = 2.1; P > 0.05]. Post hoc tests showed that treatments with SAL + LPS (2 mg/kg body wt ip) or YOH-5 (5 mg/kg body wt ip) + SAL did not change MAP compared with SAL + SAL treatment; however, the treatment with YOH + LPS reduced MAP from 40 to 80 min after LPS injection compared with SAL + SAL treatment (Fig. 4, top).

For HR, ANOVA showed no significant differences for treatment (SAL + SAL, YOH-5 + SAL, SAL + LPS, and YOH-5 + LPS) [F(3,14) = 1.0; P > 0.05], time [F(6,84) = 1.9; P > 0.05], or the interaction between treatment and time [F(18,84) = 1.4; P > 0.05] (Fig. 4, bottom). Post hoc tests showed that changes in HR were not different among treatments.

ANOVA showed significant differences among treatments (SAL + SAL, YOH-5 + SAL, SAL + LPS, and YOH-5 + LPS) for the stomach liquid content after a gavage of 0.3 M NaCl [F(3,18) = 10.7; P < 0.05]. Post hoc tests showed that, in rats that received a gavage of 2 ml of 0.3 M NaCl, the combination of SAL + LPS (2 mg/kg body wt ip) increased the stomach liquid content compared with SAL + SAL, an effect not modified by the combination of YOH (5 mg/kg of body wt ip) + LPS (Fig. 5). The combination of YOH-5 + SAL did not affect the stomach liquid content compared with SAL + SAL.

Sodium Appetite Test and Changes in MAP and HR in Sodium-Depleted Rats Treated With Intraperitoneal LPS Combined With Intracerebroventricular RX

ANOVA showed significant differences among treatments (SAL + SAL, RX-80 + SAL, RX-160 + SAL, SAL + LPS, RX-80 + LPS, and RX-160 + LPS) for the 0.3 M NaCl intake during the sodium appetite test [F(5,31) = 5.8; P < 0.05] (Fig. 6, top) but no differences among treatments for water intake [F(5,31) = 1.2; P > 0.05] (Fig. 6, bottom). Post hoc tests showed that the combination of RX (160 nmol icv) + LPS (2 mg/kg body wt ip) reversed the inhibition of 0.3 M NaCl intake produced by the combination of SAL + LPS, whereas the combination of RX (80 nmol icv) + LPS did not modify the inhibition of 0.3 M NaCl produced by the combination of SAL + LPS (Fig. 6, top). The combination of RX (80 or 160) + SAL produced no change on 0.3 M NaCl intake compared with SAL + SAL (Fig. 6, top).

ANOVA showed no difference for treatment (SAL + SAL, RX-80 + SAL, RX-160 + SAL, SAL + LPS, and RX-160 + LPS) for MAP [F(3,18) = 0.9; P > 0.05] and no interaction between treatment and time [F(18,108) = 1.3; P > 0.05]; however, there was a significant effect of time [F(6,108) = 3.8; P < 0.05] (Fig. 7, top). ANOVA also showed no difference in HR for treatment [F(3,18) = 2.7; P > 0.05], time [F(6,108) = 1.2; P > 0.05], or the combination of treatment and time [F(18,108) = 1.6; P > 0.05] (Fig. 7, bottom). Post hoc tests showed that there was no significant difference in basal MAP

\[ \Delta \text{MAP} \text{ (mmHg)} \]

\[ \Delta \text{HR} \text{ (mmHg)} \]

\[ \text{Stomach liquid content (g)} \]

Fig. 4. Changes in mean arterial pressure (ΔMAP) and heart rate (ΔHR) in 24-h sodium-depleted rats treated with YOH (5 mg/kg) or SAL ip combined with LPS (2 mg/kg) or SAL ip. The arrows indicate the moment of sample recording performed immediately before each injection; n = 5/group. *†P < 0.05.

Fig. 5. Stomach total liquid weight 5 min after an intragastric load of 0.3 M NaCl in 24-h sodium-depleted rats treated with YOH (5 mg/kg) or SAL ip combined with LPS (2 mg/kg) or SAL ip. No. of rats/group is in parentheses. *P < 0.05.
DISCUSSION

LPS (0.6–2.5 mg/kg ip) strongly inhibited sodium depletion-induced 0.3 M NaCl, an effect reversed by the pretreatment with the \( \alpha_2 \)-adrenoceptor antagonists YOH (5 mg/kg of body wt) injected intraperitoneally or RX (160 nmol/\( \mu l \) icv). A transient hypothermia was induced by the high doses of LPS (1.2 and 2.5 mg/kg). LPS also increased the stomach liquid content (or the retention of NaCl solution in the stomach) after a gavage of 0.3 M NaCl, and YOH did not modify this effect.

Two major conclusions derive from the present work. First, the results provide unequivocal evidence for an inhibitory role for LPS on sodium appetite. Second, the results suggest that LPS inhibits sodium appetite through activation of \( \alpha_2 \)-adrenoceptors located in the brain.

A previous study also showed that LPS inhibits sodium intake induced acutely (2 h after the combination of furosemide with a low dose of captopril); however, in those rats, LPS also reduced the natriuretic effect of furosemide, which might also be a reason for the reduced sodium intake produced by LPS in furosemide- + captopril-treated rats (1). In the present study, LPS was injected in sodium-depleted animals 24 h after furosemide injection so that sodium intake could be inhibited without interfering with sodium loss and vice versa. The results suggest that LPS probably acts by increasing inhibitory signals for sodium intake and thereby inhibits sodium appetite.

The effects of the \( \alpha_2 \)-adrenoceptor antagonists showed important differences and similarities. Unlike YOH intraperitoneally, RX (160 nmol) injected intracerebroventricularly combined with systemic SAL had no effect on sodium intake, water intake, or arterial pressure. Similar to YOH intraperitoneally, RX injected intracerebroventricularly combined with LPS abolished the inhibition of sodium intake produced by LPS combined with SAL. A central action for RX was expected from the literature describing the effects of forebrain \( \alpha_2 \)-adrenoceptor activation (2, 11, 16, 29, 40, 43). In addition to the central action of RX, we believe the effects of YOH on the inhibition of sodium appetite produced by LPS were on central \( \alpha_2 \)-adrenoceptors for two reasons. First, YOH has a rapid access to the brain by crossing the blood-brain barrier (23). Second, the present findings suggest that the effects of YOH on the inhibition produced by LPS are independent from cardiovascular or gastric responses. Thus the results strongly suggest that \( \alpha_2 \)-adrenoceptors are involved with central rather than systemic inhibitory mechanisms that mediate the antinatriorexigenic effect of LPS.

In sodium-depleted rats, YOH intraperitoneally or LPS alone produced no change on arterial pressure; however, YOH combined with LPS produced a transient hypotension (for \( \leq 1 \) h) before animals had access to sodium solution. Note that this hypotension is not the result of an additive effect of the drugs on a preexisting hypotension associated with sodium depletion because MAP of sodium-depleted rats is similar to hydrated controls (10). The unloading of cardiopulmonary receptors by hypotension may facilitate water and sodium intake (24);
therefore, it is not possible to exclude some influence of the hypotension facilitating 0.3 M NaCl intake after the combination of YOH and LPS. Moreover, YOH injected systemically may induce water and sodium intake in satiated rats (18). However, we tested sodium appetite when arterial pressure had returned to preinjection baseline levels and the behavioral responses were, presumably, no longer directly influenced by hypotension.

The exact mechanism activated by YOH injected systemically to induce fluid intake is not known, although it depends at least on the integrity of the amygdala and subfornical organ (18, 45). The present results suggest that the dipsogenic effect of YOH is independent from hypotension. Although it is not possible to rule out that the dipsogenic effect of YOH somehow helped to reverse sodium intake to normal, note that sodium intake in the YOH + SAL group did not differ from SAL + SAL.

It is important to point out that the variability shown in MAP and HR responses was very likely a consequence of the stress produced by the intraperitoneal injections. However, intraperitoneal injections also were used in the sodium appetite tests, and, for comparison, it was necessary to test MAP and HR in the same conditions. Perhaps, injections of the same drugs and doses in sodium-depleted rats using a different method of injection could produce slightly different MAP and HR responses, but this was beyond the scope of the present work.

LPS also has the potential to recruit a short-latency peripheral inhibitory mechanism, such as reduction in gastric emptying, thereby increasing satiating signals through osmo- or mechanoreceptors lying in the wall of the stomach to inhibit sodium appetite (39). This idea is consistent with the increased stomach liquid content in the presence of LPS, but contradicted by the experiments with YOH: whereas YOH, an α2-adrenoceptor antagonist, failed to influence the reduction in gastric emptying, it nonetheless restored sodium appetite in LPS-treated rats. This result contrasts with the literature describing the ability of YOH to reverse the inhibition induced by LPS on gastric motility in mice (21), a difference that might relate to the degree of dehydration or species involved.

Clonidine, an α2-adrenoceptor agonist, produces a biphasic change in body temperature, hyperthermia followed by hypothermia, in guinea pigs (15). However, hypothermia was not followed by hyperthermia in our experiments. Only the high doses of LPS produced hyperthermia in sodium-depleted rats at the beginning of the sodium appetite test. It is not clear why high doses of LPS did not produce hyperthermia or fever. Although fever is considered a typical effect of LPS (7), alterations in body temperature may be polyphasic and dependent on conditions such as ambient temperature, dose, and method of LPS injection (34). It is possible that sodium appetite tests ended before the increase in body temperature expected to follow the hyperthermia (15, 34). At any rate, hypothermia was not a causal factor to inhibit sodium appetite. First, hypothermia was produced only by high doses of LPS. Second, cold has been reported to increase hypertonic NaCl intake, a behavioral response important for thermogenesis and restoration of fluid balance (12, 44). So, it seems that the effects of LPS on body temperature and sodium appetite occur independently despite sharing similar adrenergic mechanisms.

The effects of LPS and brain norepinephrine on sodium appetite might be linked by α2-adrenoceptors to two functionally distinct ascending medullary catecholaminergic projections, one to mediate LPS-induced anorexia (38), another to restrain sodium intake in dehydrated animals (6). In addition to the catecholaminergic systems, LPS may also activate the dorsal raphe nucleus serotonergic neurons through a complex transduction mechanism localized in the blood-brain barrier involving immune cells, vascular cells, and cytokines (25). The dorsal raphe nucleus is also involved with serotonergic inhibitory mechanisms of sodium intake (33), and it sends projections to the lateral parabrachial nucleus (LPBN), which apparently is part of the same circuit (8). This raises the possibility that LPS also recruits the putative raphe-LPBN inhibitory system. However, this possibility is contradicted by the failure of LV injection of idazoxan, an analog of RX, to reverse the inhibition exerted by serotonergic receptors of the LPBN on sodium intake (28). Such a failure suggests that the hindbrain serotonergic mechanism is dissociated from the forebrain α2-adrenoceptor system in the inhibition of sodium appetite. If so, there is also a possibility that LPS activates different inhibitory systems related to the ingestion of different commodities.

Norepinephrine activates forebrain α2-adrenoceptors, which interact with imidazoline receptors to inhibit sodium appetite and thirst (29, 40, 41). Activation of these receptors may counteract facilitatory mechanisms of sodium appetite, such as angiotensin II and mineralocorticoid (11, 16, 40, 43), an effect possibly reinforced by oxytocin and atrial natriuretic peptide, antinatrioregic hormones also released by LPS (2, 4, 37). Thus it is possible that, besides activation of central α2-adrenoceptors, LPS also elicits an array of neural and endocrine mechanisms to contribute to the inhibition of sodium appetite.

**Perspectives and Significance**

The present work adds the inhibition of sodium appetite to our knowledge of the effects of LPS on behavior and suggests a mechanism for such inhibition: activation of brain α2-adrenoceptors. It remains to be demonstrated if α2-adrenoceptors also mediate the inhibition LPS exerts on other behaviors, particularly food and sweet intake. Recall that reduced sweet intake is an indicator of anhedonia (30), a depression in mood also produced by LPS (7). The inhibitory effect of forebrain α2-adrenoceptors has been the subject of intense neuropharmacological research suggesting their interaction with imidazoline receptors inhibits hydromineral fluid intake, but the knowledge of their role in physiological conditions is still incipient. The present results suggest that brain α2-adrenoceptors have a role controlling sodium intake in pathological situations associated with LPS. Understanding this role may provide an additional tool for basic and clinical research to understand and counteract the effects of the endotoxin.

**ACKNOWLEDGMENTS**

We thank Silvana A. D. Malavolta for secretarial assistance, Silvia Foglia, Silas P. Barbosa, and Reginaldo C. Queiroz for technical assistance, and Ana Vitor Oliveira for animal care.

**GRANTS**

This research was supported by Brazilian public funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP-PRONEX to Dr. Ayton C. Moreira). R. L. Almeida, R. B. David, and J. Constancio were enrolled in the Joint Universidade Federal de São Carlos-Universidade Es-
tudial Paulista Graduate Program in Physiological Sciences. This work is part of requirements to obtain a Doctoral Degree by R. L. Almeida (CNPq fellowship).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


