Neuroscience 155 (2008) 350–358

OPIOID ACTIVATION IN THE LATERAL PARABRACHIAL NUCLEUS INDUCES HYPERTONIC SODIUM INTAKE

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Abstract—Opioid mechanisms are involved in the control of water and NaCl intake and opioid receptors are present in the lateral parabrachial nucleus (LPBN), a site of important inhibitory mechanisms related to the control of sodium appetite. Therefore, in the present study we investigated the effects of opioid receptor activation in the LPBN on 0.3 M NaCl and water intake in rats. Male Holtzman rats with stainless steel cannulas implanted bilaterally in the LPBN were used. In normohydrated and satiated rats, bilateral injections of the opioid receptor agonist β-endorphin (2 nmol/0.2 μl) into the LPBN induced 0.3 M NaCl (17.8±5.9 vs. saline: 0.9±0.5 ml/240 min) and water intake (11.4±3.0 vs. saline: 1.0±0.4 ml/240 min) in a two-bottle test. Bilateral injections of the opioid antagonist naloxone (100 nmol/0.2 μl) into the LPBN abolished sodium and water intake induced by β-endorphin into the LPBN and also reduced 0.3 M NaCl intake (12.8±1.5 vs. vehicle: 22.4±3.1 ml/180 min) induced by 24 h of sodium depletion (produced by the treatment with the diuretic furosemide s.c.+sodium deficient food for 24 h). Bilateral injections of β-endorphin into the LPBN in satiated rats produced no effect on water or 2% sucrose intake when water alone or simultaneously with 2% sucrose was offered to the animals. The results show that opioid receptor activation in the LPBN induces hypertonic sodium intake in satiated and normohydrated rats, an effect not due to general ingestive behavior facilitation. In addition, sodium depletion induced 0.3 M NaCl intake also partially depends on opioid receptor activation in the LPBN. The results suggest that deactivation of inhibitory mechanisms by opioid receptor activation in the LPBN releases sodium intake if excitatory signals were activated (sodium depletion) or not.

Key words: β-endorphin, sodium appetite, water intake, naloxone, satiety.

The lateral parabrachial nucleus (LPBN) is a pontine structure located dorsolaterally to the superior cerebellar peduncle (SCP). The LPBN is reciprocally connected with different central areas involved in the control of fluid-electrolyte balance and cardiovascular regulation like the bed nucleus of the stria terminalis, zona incerta, ventromedial and lateral hypothalamic area, preoptic area, central nucleus of the amygdala, supraoptic nucleus, nucleus of the solitary tract (NTS) and raphe nuclei (Ciriello et al., 1984; Shapiro and Miselis, 1985; Herbert et al., 1990; Krukoff et al., 1993; Jhamandas et al., 1996; Gu and Ju, 1995; Bianchi et al., 1998). Functional studies have recently shown the existence of important inhibitory mechanisms in the LPBN for the control of water and NaCl intake (Edwards and Johnson, 1991; Colombari et al., 1996; Menani et al., 1996, 1998a,b, 2000; De Gobbi et al., 2000).

Bilateral injections of methysergide (5-HT antagonist), DNQX (glutamate antagonist) or α-helical corticotropin-releasing factor$_{9-41}$ (CRF) antagonist into the LPBN increase hypertonic NaCl intake and eventually water intake induced by the treatment with the diuretic furosemide (FURO) combined with low dose of the angiotensin converting enzyme inhibitor captopril (CAP), while injections of the respective agonists (DOI, AMPA and CRF) produce opposite effects (Menani et al., 1996; Xu et al., 1997; De Castro e Silva et al., 2006). Blockade of cholecystokinin (CCK) receptors or the activation of α_2 adrenergic receptors into the LPBN also increases FURO+CAP-induced sodium intake (Menani and Johnson, 1998; Andrade et al., 2004). Sodium intake produced by other stimuli, like angiotensin II (ANG II) injected intracerebroventricular or into the subfornical organ, 24 h of sodium depletion or the mineralocorticoid deoxycorticosterone, also increased after injections of methysergide into the LPBN (Colombari et al., 1996; Menani et al., 1996, 1998a,b, 2000; De Gobbi et al., 2000). Recent results have also shown that the activation of $GABA_A$ receptors with bilateral injections of muscimol into the LPBN induces strong ingestion of hypertonic NaCl (32.5±7.3 ml of 0.3 M NaCl in 180 min) in satiated and normohydrated rats (Callera et al., 2005).

Different studies have shown the importance of opioid mechanisms in the control of ingestive behavior and specifically on the ingestion of sodium and water (Cooper, 1980; Jalowiec et al., 1981; Brown and Holtzman, 1981; Summy-Long et al., 1981; Rowland, 1982; Cooper and Gilbert, 1984; Beczkowska et al., 1992; Hubbell and McCutcheon, 1993; Eidi et al., 2003; Lucas et al., 2007). Opioid receptors and immunoreactivity for the endogenous opioid agonist enkephalin are present in the LPBN and the activation of μ-opioid receptors in the rat LPBN inhibits neuronal activity (Milner et al., 1984; Christie and North, 1988; Xia and Haddad, 1991). Although previous studies had reported the involvement of different neurotransmitters and receptors in the LPBN in the control of sodium intake, no study investigated the participation of opioid mechanisms in the LPBN in the control of sodium and water

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Abbreviations: ANG II, angiotensin II; CAP, captopril; CCK, cholecystokinin; CRF, corticotropin-releasing factor; FURO, furosemide; LPBN, lateral parabrachial nucleus; MPBN, medial parabrachial nucleus; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus of hypothalamus; SCP, superior cerebellar peduncle.

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doi:10.1016/j.neuroscience.2008.06.011

intake. In spite of some exceptions, activation of opioid mechanisms is an important step for water and sodium intake induced by different stimuli, including water intake induced by central or systemic ANG II (Cooper, 1980; Jalowiec et al., 1981; Brown and Holtzman, 1981; Summy-Long et al., 1981; Rowland, 1982; Cooper and Gilbert, 1984; Mucha and Iversen, 1986; Gosnell and Majchrzak, 1990; Beczkowska et al., 1992; Hubbell and McCutcheon, 1993; Eidi et al., 2003; Franchini et al., 2003; Lucas et al., 2007). Specifically into the LPBN, infusion of DAMGO (μ-opioid receptor agonist) induces hyperphagic effect in satiated rats (Wilson et al., 2003).

Therefore, considering the presence of opioid receptors and neurotransmitters in the LPBN and the already reported role of the LPBN and central opioid mechanisms in the control of water and sodium intake, in the present study we investigated the effects of bilateral injections of β-endorphin (opioid receptor agonist) and naloxone (opioid receptor antagonist) alone or combined into the LPBN in the control of 0.3 M NaCl and water intake in satiated and normohydrated rats and the effects of naloxone into the LPBN on sodium depletion induced 0.3 M NaCl intake.

EXPERIMENTAL PROCEDURES

Animals

A total of 55 male Holtzman rats weighing 290–310 g were used. The animals were housed in individual stainless steel cages with free access to normal sodium diet (Guabi Rat Chow, Paulinia, SP, Brazil), water and 0.3 M NaCl or 2% sucrose solution depending on the experiment to be performed. Room temperature was maintained at 23±2 °C, and humidity at 55±10% on a 12-h light/dark cycle with light onset at 7:30 AM. The Ethical Committee for Animal Care and Use from Dentistry School of Araraquara-UNESP approved the experimental protocols used in the present study. The experimental protocols followed the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23, 1996). All efforts were made to minimize animal discomfort and the number of animals used.

Cerebral cannulas

Rats were anesthetized with s.c. ketamine (80 mg/kg of body weight, Agener Uniao, Embu-Guacu, SP, Brazil) combined with xylazine (7 mg/kg of body weight, Agener Uniao) and placed in a stereotaxic instrument (Kopf, Tujunga, CA, USA). The skull was leveled between bregma and lambda. Stainless steel 23-gauge cannulas were implanted bilaterally into the LPBN using the following coordinates: 9.3 mm caudal to bregma, 2.2 mm lateral to the midline, and 4.3 mm below the dura mater (Paxinos and Watson, 1997). 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 watch screws. Metal obturators (30-gauge) filled the cannulas between tests. After the surgery, the rats received i.m. injections of the analgesic cetoprophen 1% (0.03 ml) and a prophylactic dose of the antibiotic penicillin (30,000 IU). Rats were allowed to recover for 5 days before starting ingestion tests.

Injections into the LPBN

Bilateral injections into the LPBN were made using 5-μl Hamilton syringes (Reno, NV, USA) connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At the time of testing, obturators were removed and the injection cannula (2 mm longer than the guide cannula) was carefully inserted into the guide cannula, and bolus injection was initiated 15 s later. For bilateral injections, the first injection was initially performed in one side, the needle was withdrawn and repositioned in the contralateral side, and then the second injection was made. Therefore injections were made ~1 min apart. The injection volume into the LPBN was 0.2 μl in each site. The obturators were replaced after the injections, and the rats were placed back into their cage.

Drugs

The drugs injected into the LPBN were rat β-endorphin and naloxone hydrochloride purchased from Sigma Chemicals (St. Louis, MO, USA). β-Endorphin (2 nmol/0.2 μl) was dissolved in saline and naloxone (50, 100 or 150 nmol/0.2 μl) was dissolved in a mix of propylene glycol/water 2:1 (vehicle). FURO (Sigma Chem.) was administered s.c. at 20 mg/kg of body weight.

The dose of β-endorphin (μ and δ receptor agonist, 2 nmol/0.2 μl) was based on the study of Wilson et al. (2003) that showed that infusion of DAMGO (μ receptor agonist, 2 nmol/0.5 μl) into the LPBN increased food intake in satiated rats. The dose of naloxone was also based on previous study that showed the effects of central naloxone on water and sodium intake (Gosnell and Majchrzak, 1990).

Water and 0.3 M NaCl intake in satiated and normohydrated rats

The 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. Food was not available for the rats during the tests. Cumulative intake of 0.3 M NaCl and water was measured at every 30 min during 240 min, starting immediately after bilateral injections of β-endorphin (2 nmol/0.2 μl) or saline (0.2 μl) into the LPBN.

In one group of normohydrated and satiated rats, the effects of β-endorphin injected into the LPBN on water and 0.3 M NaCl intakes (two-bottle test) were tested. The rats were submitted to two tests. In each test, the group of rats was divided in two. In the first test half of the group received saline and the other half received β-endorphin into the LPBN. In the next test the rats received the same treatments in a counterbalanced design.

The effects of β-endorphin into the LPBN were also tested in one group of normohydrated and satiated rats that had only water available (one-bottle test), following the same protocol described above for rats that had water and 0.3 M NaCl simultaneously available.

Another group of normohydrated and satiated rats was tested for the effects of the combination of naloxone and β-endorphin into the LPBN on water and 0.3 M NaCl intake (two-bottle test). Naloxone (100 nmol/0.2 μl) was injected into the LPBN 20 min before β-endorphin (2 nmol/0.2 μl) into the same area. These rats were submitted to four tests and received the following combinations of treatments into the LPBN: vehicle+saline, vehicle+β-endorphin, naloxone+β-endorphin and naloxone+saline. In each test, the group of rats was divided in two and half of the group received one of the combination of treatments cited above into the LPBN and the remaining animals received another combination of treatments into the LPBN. The sequence of the treatments in each rat in different tests was randomized and at the end of four tests each rat received all the four treatments.

A recovery period of at least 3 days was allowed between tests.

Water and 0.3 M NaCl intake by 24 h sodium-depleted rats

Sodium depletion was produced by s.c. injection of the diuretic FURO (20 mg/kg of body weight) followed by sodium deficient

food (powdered corn meal, 0.001% sodium, 0.33% potassium) and water available for 24 h. After 24 h of sodium depletion the rats had access to water and 0.3 M NaCl (two-bottle test), without food available.

Cumulative water and 0.3 M NaCl intakes were measured at 15, 30, 60, 120, 150 and 180 min starting 15 min after bilateral injections of naloxone or vehicle into the LPBN. These rats were submitted to four tests and received injections of naloxone (50, 100 or 150 nmol/0.2 μl) or vehicle into the LPBN. In each test, the group of rats was divided in two and half of the group received one treatment into the LPBN and the remaining animals received another treatment into the LPBN. The sequence of the treatments in the LPBN in each rat in different tests was randomized and at the end of the experiments each rat received all four treatments.

A recovery period of at least 3 days was allowed between tests.

Sucrose intake by satiated and normohydrated rats

To show that the effects of opioid activation in the LPBN were not due to nonspecific facilitation of all ingestive behaviors we tested the effects of β-endorphin into the LPBN on 2% sucrose intake. The rats had free access to water, food and 2% sucrose.

In the day of experiment, food, water and 2% sucrose were removed and the rats received bilateral injections of β-endorphin (2 nmol/0.2 μl) or saline into the LPBN. Immediately after bilateral injections into the LPBN, water and 2% sucrose, but not food, were available to the animals. Cumulative intake of 2% sucrose and water was measured at every 30 min during 240 min.

The rats were submitted to two tests. In each test, the group of rats was divided in two. In the first test half of the group received saline and the other half received β-endorphin into the LPBN. In the next test the rats received the same treatments in a counterbalanced design.

Histology

At the end of the experiments, the animals received bilateral injections of 2% Evans Blue solution (0.2 μl) into the LPBN. They were then deeply anesthetized with thiopental 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 serial coronal sections, stained with Giemsa, and analyzed by light microscopy to confirm the injection sites into the LPBN.

Statistical analysis

The results are reported as means±S.E.M. Repeated-measures ANOVA (RMANOVA) and Fisher's LSD tests were used for comparison. Differences were considered significant at $P<0.05$.

RESULTS

Histological analysis

The LPBN injection sites were centered in the central lateral and dorsolateral portions of the LPBN [see Fulwiler and Saper (1984) for definitions of LPBN subnuclei]. Fig. 1 shows the typical LPBN injection sites. Injections reaching the ventral lateral and external lateral portions, as well as the Kolliker-Fuse nucleus were observed in some rats and the results from these rats were included in the analysis. In some rats, injections also spread to the brachium (SCP), or slightly ventral to this structure, reaching the dorsal portions of the medial parabrachial nucleus (MPBN) uni- or bilaterally. There was no difference in the effects whether injections were restricted to the LPBN or also spread to brachium and dorsal portions of MPBN.

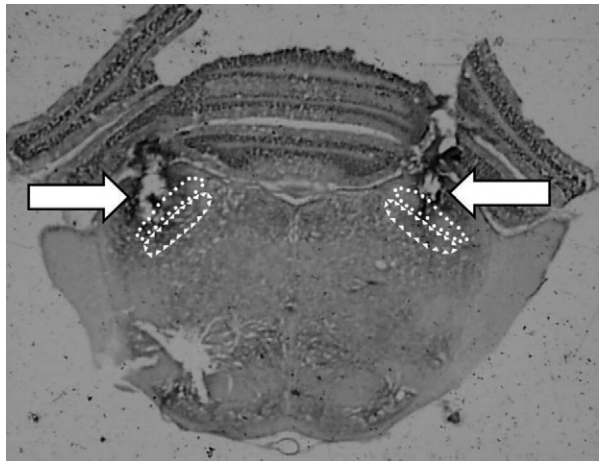

Fig. 1. Photomicrograph showing the sites of injections into the LPBN (arrows). The circle of dots is showing the SCP and the circle of triangles represents the MPBN.

Effects of bilateral injections of β-endorphin into the LPBN on water and 0.3 M NaCl intake in satiated and normohydrated rats

Bilateral injections of β-endorphin (2 nmol/0.2 μl at each site) into the LPBN induced 0.3 M NaCl and water intake when both fluids were simultaneously available (Fig. 2). ANOVA showed significant interaction between treatments and time for 0.3 M NaCl [$F(7, 42)=3.5$; $P<0.05$] (Fig. 2A) and significant difference between treatments for water intake [$F(1, 6)=8.6$; $P<0.05$] (Fig. 2B).

When only water was available, bilateral injections of β-endorphin (2 nmol/0.2 μl) into the LPBN in satiated and normohydrated rats did not affect water intake [$F(1, 7)=2.0$; $P>0.05$] (Fig. 3).

The 0.3 M NaCl intake in satiated rats treated with β-endorphin into the LPBN started to be statistically significant from 60 to 120 min of test (Fig. 2). At this moment 50% of the rats injected with β-endorphin had already ingested around 26 ml of 0.3 M NaCl, while the rest of the rats ingested less than 1 ml of NaCl. From the group of seven rats treated with β-endorphin (Fig. 2), three of them (43%) ingested 0.3 M NaCl in the first hour of test, and the other four rats (57%) started the ingestion between 120 and 150 min of test. At the end of 240 min of test all the rats treated with β-endorphin into the LPBN ingested at least 1.1 ml of 0.3 M NaCl (the 0.3 M NaCl intake ranged from 1.1–48.7 ml at end of 240 min of test) and five rats (72% of the group) ingested significant amount of 0.3 M NaCl (from 6.4–48.7 ml) in this same period. When saline was injected in the LPBN, four rats (57% of the group) ingested from 0.5–3.5 ml in the whole test and three rats ingested less than 0.5 ml of 0.3 M NaCl (Fig. 2).

Effects of the combination of naloxone and β-endorphin into the LPBN on water and 0.3 M NaCl intake in satiated and normohydrated rats

Bilateral injections of the opioid receptor antagonist naloxone (100 nmol/0.2 μl each site) into the LPBN abol-

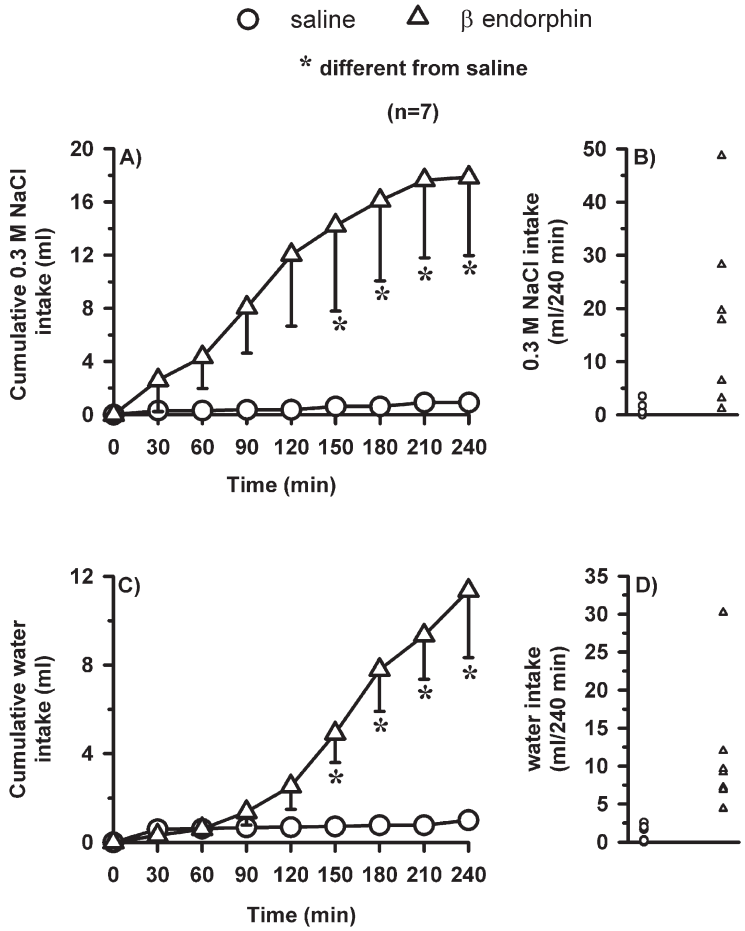

Fig. 2. (A) Cumulative 0.3 M NaCl intake; (B) individual 0.3 M NaCl intakes; (C) cumulative water intake; (D) individual water intakes in normohydrated and satiated rats that received bilateral injections of β-endorphin (2 nmol/0.2 μl) or saline into the LPBN. (A, C) Results are expressed as means±S.E.M.; *n*, number of rats.

ished the effects of β-endorphin (2 nmol/0.2 μl) injected in the same area on water and 0.3 M NaCl intake (Fig. 4). ANOVA showed significant differences among treatments for 0.3 M NaCl intake [$F(3, 18)=7.4$; $P<0.05$]

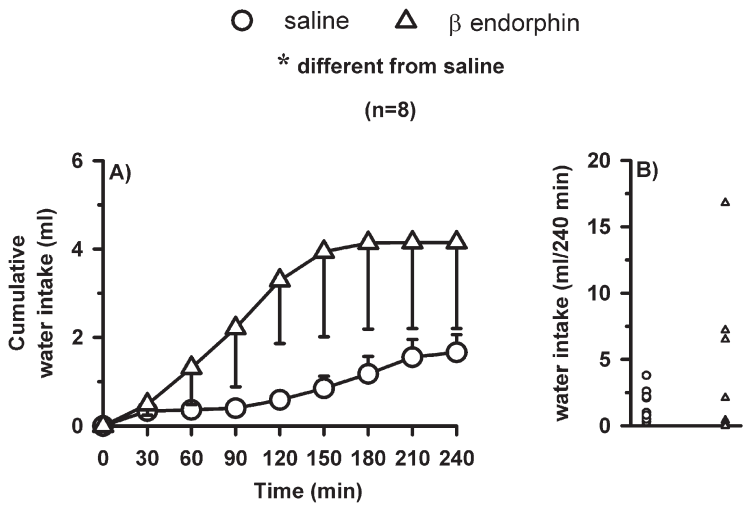

Fig. 3. (A) Cumulative water intake; (B) individual water intakes in normohydrated and satiated rats that received bilateral injections of β-endorphin (2 nmol/0.2 μl) or saline into the LPBN when only water was available. (A) Results are expressed as means±S.E.M.; *n*, number of rats.

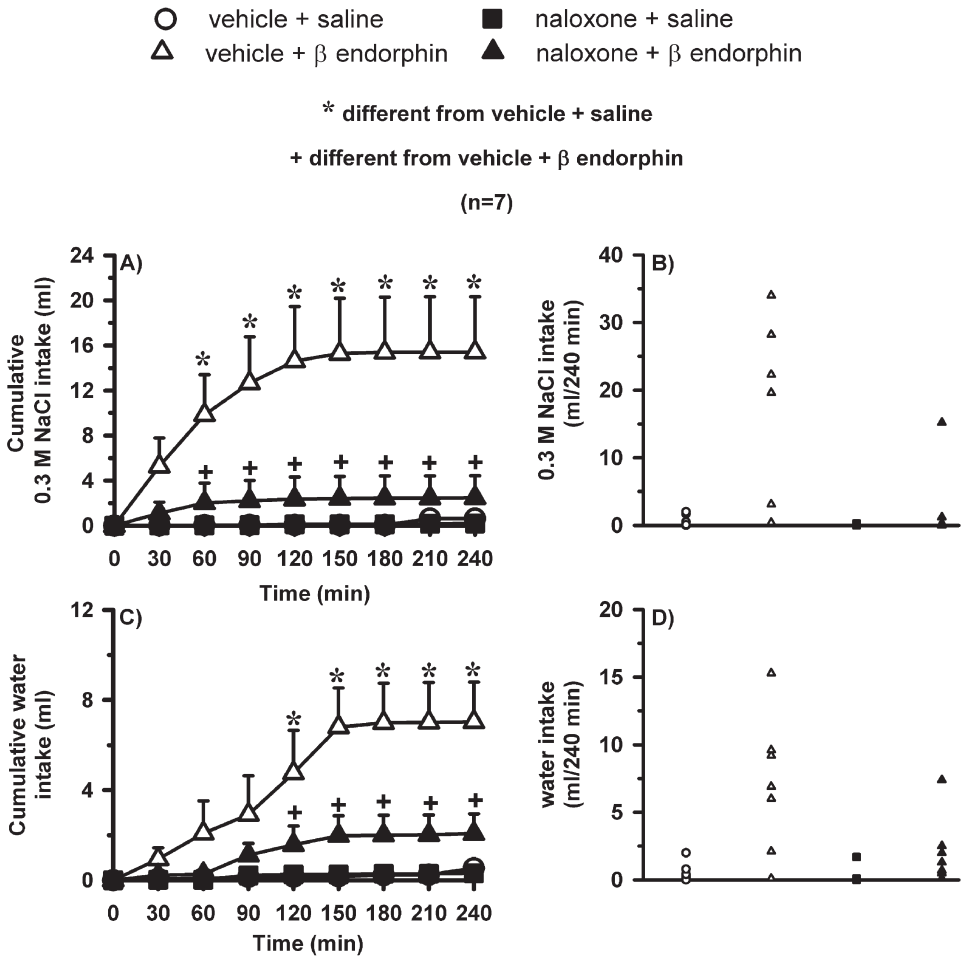

Fig. 4. (A) Cumulative 0.3 M NaCl intake; (B) individual 0.3 M NaCl intakes; (C) cumulative water intake; (D) individual water intakes in satiated and normohydrated rats that received bilateral injections of naloxone (100 nmol/0.2 μl) or vehicle combined with β-endorphin (2 nmol/0.2 μl) or saline into the LPBN. (A, C) Results are expressed as means±S.E.M., *n*, number of rats.

and water intake [$F(3, 18)=5.9$; $P<0.05$] in this group of rats (Fig. 4).

Effects of naloxone into the LPBN on water and 0.3 M NaCl intake by 24 h sodium-depleted rats

Bilateral injections of naloxone (50, 100 or 150 nmol/0.2 μl) into the LPBN reduced 24 h sodium depletion induced 0.3 M NaCl intake as shown by the significant differences among treatments [$F(3, 15)=4.8$; $P<0.05$] (Fig. 5A). Naloxone at the same doses did not affect water intake that followed sodium intake [$F(3, 15)=2.6$; $P>0.05$] (Fig. 5B).

Effects of bilateral injections of β-endorphin into the LPBN on 2% sucrose intake by satiated and normohydrated rats

To show that the effects of opioid activation in the LPBN were not due to nonspecific facilitation of ingestive behaviors we tested the effects of β-endorphin into the LPBN on 2% sucrose intake.

Bilateral injections of β-endorphin (2 nmol/0.2 μl) into the LPBN in satiated and normohydrated rats did not affect either 2% sucrose [$F(1, 7)=2.8$; $P>0.05$] or water intake [$F(1, 7)=0.3$; $P>0.05$], when both fluids were simultaneously available (Fig. 6).

Specificity of the LPBN as the site of action of β-endorphin and naloxone to produce effects on 0.3 M NaCl intake

Results from rats that received bilateral injections of β-endorphin or naloxone outside the LPBN (misplaced injections) were also analyzed to show that the effects on water and 0.3 M NaCl intake were due to a specific activation of opioid receptors in the LPBN. Bilateral injections of β-endorphin (2 nmol/0.2 μl) or naloxone (50, 100 or 150 nmol/0.2 μl) in sites outside the LPBN produced no effect on water or 0.3 M NaCl intake in satiated or sodium-depleted rats (Table 1). Misplaced injections were ventral (MPBN) or dorsal to LPBN, and part of them were rostral to LPBN. Some rats had a partial unilateral injection into the LPBN.

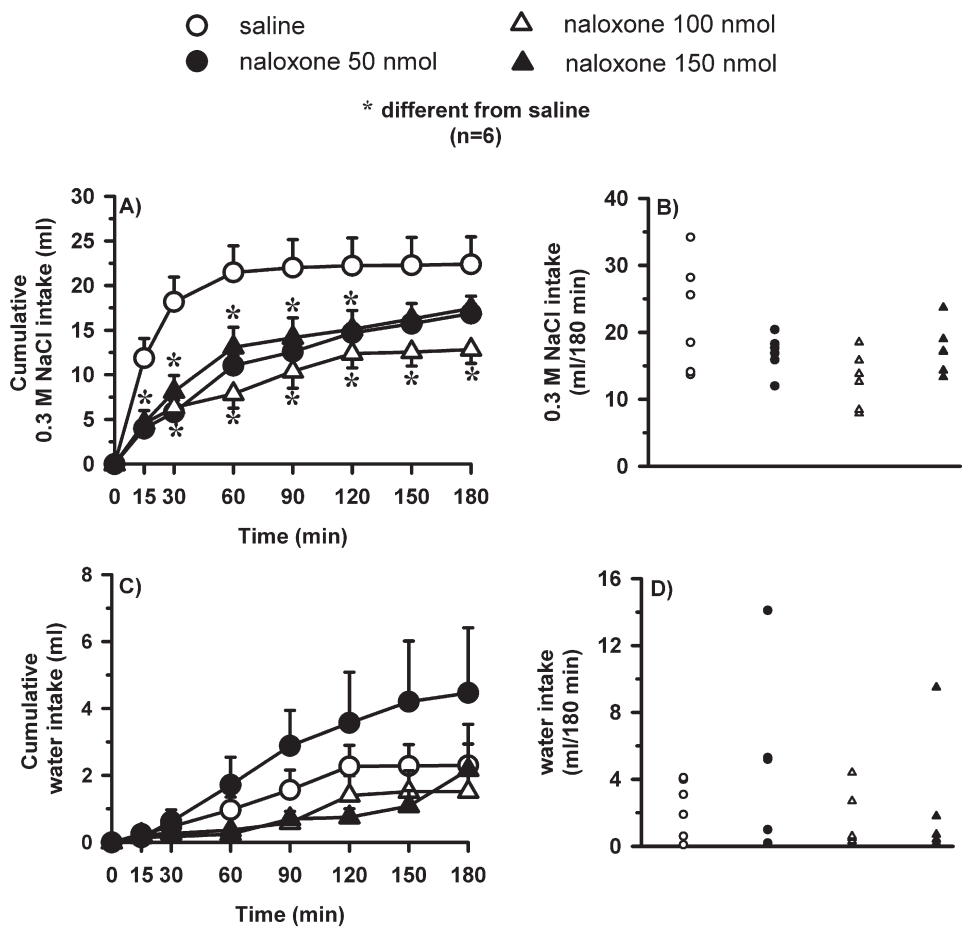

Fig. 5. (A) Cumulative 0.3 M NaCl intake; (B) individual 0.3 M NaCl intakes; (C) cumulative water intake; (D) individual water intakes in 24 h sodium depleted rats that received bilateral injections of naloxone (50, 100 or 150 nmol/0.2 μl) or vehicle into the LPBN. (A, C) Results are expressed as means±S.E.M.; *n*, number of rats.

DISCUSSION

The present results show that activation of opioid receptors with bilateral injections of β-endorphin into the LPBN rapidly (in the next 2–3 h) induces strong ingestion of hypertonic NaCl in satiated and normohydrated rats. The blockade of opioid receptors with naloxone into the LPBN not only abolished the effects of β-endorphin, but also reduced sodium depletion-induced sodium intake. Rats treated with β-endorphin into the LPBN ingested also water if 0.3 M NaCl was simultaneously available, however, no water was ingested if rats had access only to water, suggesting that the ingestion of water was a consequence of the increased plasma osmolarity due to the excessive ingestion of hypertonic NaCl. Injections of β-endorphin into the LPBN did not affect 2% sucrose intake. Naloxone or β-endorphin injected in sites outside the LPBN (misplaced injections) produced no effect on 0.3 M NaCl intake. Therefore the activation of opioid receptors specifically in the LPBN induces sodium intake by satiated and normohydrated rats and this effect is not due to a nonspecific activation of any ingestive behavior. In addition the results suggest that the release of opioid peptides in the LPBN is important for sodium depletion–induced sodium intake.

Similar to the present study, previous studies also showed that systemic injections of naloxone reduce hypo- and hypertonic NaCl intake (Brown and Holtzman, 1981; Summy-Long et al., 1981; Rowland, 1982; Cooper and Gilbert, 1984). Sodium depletion–induced salt intake is also attenuated by the blockade of delta opioid receptors with naltrindole into the shell of the nucleus accumbens or ventral tegmental area or in knockout mice lacking β-endorphin (Franchini et al., 2003; Lucas et al., 2007). All these previous studies showed that the activation of central opioid mechanisms is an important step to facilitate hypertonic sodium intake-induced by a stimulus like sodium depletion which is similar to the effects of naloxone injected into the LPBN reported in the present study. However, the present study shows that opioid activation in the LPBN in the absence of any other stimuli induces strong ingestion of hypertonic NaCl in normohydrated and satiated rats.

The LPBN is connected with forebrain areas that control fluid and electrolyte balance, such as the paraventricular nucleus of hypothalamus (PVN), central nucleus of the amygdala and median preoptic nucleus, and to medullary regions, like NTS that receives visceral and taste informa-

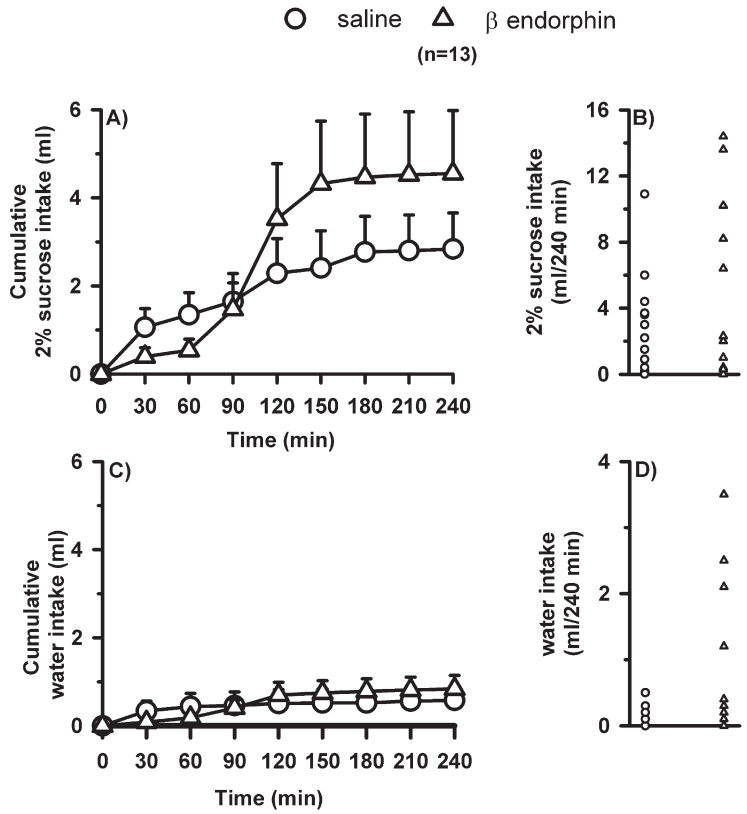

Fig. 6. (A) Cumulative 2% sucrose intake; (B) individual 2% sucrose intakes; (C) cumulative water intake; (D) individual water intakes in normohydrated and satiated rats that received bilateral injections of β-endorphin (2 nmol/0.2 μl) or saline into the LPBN. (A, C) Results are expressed as means±S.E.M.; *n*, number of rats.

tion (Norgren, 1981; Ciriello et al., 1984; Fulwiler and Saper, 1984; Shapiro and Miselis, 1985; Herbert et al., 1990; Krukoff et al., 1993; Jhamandas et al., 1996). Cells in the LPBN are activated by ingestion of NaCl solution by dehydrated rats or by rats that received intragastric load of hypertonic NaCl (Kobashi et al., 1993; Yamamoto et al., 1993; Franchini and Vivas, 1999). The LPBN also receives signals from arterial baroreceptors and volume receptors that arise through NTS. Therefore, osmotic, volume or gustatory signals may modulate the activity of LPBN inhibitory mechanisms that project to inhibit the neural circuits subserving salt appetite. Anatomical studies have shown enkephalin- and dynorphin-immunoreactive cells projecting from the NTS and PVN to parabrachial nucleus (Milner et al., 1984; Riche et al., 1990; Moga et al., 1990). Activated by changes in volume or osmotic receptor activity during hypovolemia or hyponatremia, these projections may signal for opioid release in the LPBN. Opioid receptors are usually located presynaptically and their activation reduces the release of excitatory neurotransmitters. Activation of μ or δ opioid receptors produces membrane hyperpolarization due to potassium channel activation (North et al., 1987). β-Endorphin binds to μ and δ opioid receptors that are present in the LPBN (Hewlett and Barchas, 1983; Christie and North, 1988; Xia and Haddad, 1991). Therefore β-endorphin similar to GABAergic activation in the LPBN may inhibit neuronal activity in this area reducing or blocking the inhibitory mechanisms and releasing sodium intake.

Table 1. Water and 0.3 M NaCl intake by normohydrated and satiated or sodium-depleted rats that received bilateral injections of β-endorphin or naloxone in sites outside the LPBN (misplaced injections)

Treatment	Water intake (ml/240 min)	0.3 M NaCl intake (ml/240 min)
Satiated rats (*n*=11)		
Saline	0.2±0.1	0.3±0.2
β-Endorphin (2 nmol)	1.7±0.6	0.8±0.4
Sodium-depleted rats (*n*=3)		
Vehicle	2.2±1.5	16.7±2.5
Naloxone (50 nmol)	2.6±1.6	15.1±1.7
Naloxone (100 nmol)	1.7±1.1	12.9±0.6
Naloxone (150 nmol)	0.4±0.1	13.7±1.6

Results are expressed as means±S.E.M.
n, number of rats.

Different neurotransmitters and receptors in the LPBN may affect the activity of the LPBN inhibitory mechanisms. 5-HT, CCK, glutamate and corticotropin-releasing factor acting in the LPBN activate the inhibitory mechanisms and reduce NaCl intake and eventually water intake (Menani et al., 1996; Xu et al., 1997; Menani and Johnson, 1998; De Castro e Silva et al., 2006). In the opposite side, GABAergic and

opioid neurotransmitters or the α_2 adrenergic receptors seems to act in the LPBN reducing the activity of the LPBN inhibitory mechanisms which increases induced sodium intake or even releases nonstimulated sodium intake (Andrade et al., 2004; Callera et al., 2005; De Oliveira et al., 2007 and the present results). Blockade of serotoninergic, cholecystokinergic, glutamatergic or corticotropin-releasing factor receptors or activation of α_2 adrenergic receptors in the LPBN releases the ingestion of NaCl only if the animals received a prior dipsogenic or natriorexigenic treatment, not in normohydrated and satiated animals, which suggested that the blockade of these LPBN inhibitory mechanisms alone is not enough to stimulate sodium intake (Colombari et al., 1996; Menani et al., 1996; Xu et al., 1997; Menani and Johnson, 1998; Andrade et al., 2004; De Castro e Silva et al., 2006). These results have suggested that the ingestion of a significant amount of sodium easily occurs if facilitatory mechanisms are activated and the inhibitory mechanisms simultaneously deactivated. However, the results showing strong ingestion of hypertonic NaCl in satiated and normohydrated rats after the activation of opioid or $GABA_A$ receptors in the LPBN suggest that sodium intake may occur even in the absence of facilitatory stimuli if the LPBN inhibitory mechanisms were deactivated (Callera et al., 2005 and the present results). However, it is not clear why opioid or $GABA_A$ receptor activation in the LPBN induces sodium ingestion in normohydrated and satiated rats, while other receptor blockade (5-HT, CCK, glutamate, CRF) or activation (α_2 adrenergic) in the LPBN only increases stimulated sodium intake, i.e. if rats were simultaneously stimulated to ingest sodium and/or water by treatments like central ANG II, s.c. mineralocorticoid, sodium depletion, but not in normohydrated and satiated rats (Menani et al., 1996; Xu et al., 1997; Menani and Johnson, 1998a,b; Andrade et al., 2004; Callera et al., 2005; De Castro e Silva et al., 2006). Perhaps each specific neurotransmitter, except GABA and opioid, is related only to part of the inhibitory mechanisms, i.e. blocking 5-HT, CCK, glutamate or CRF in the LPBN we can only partially reduce the activity of the inhibitory mechanisms, which combined with facilitatory mechanisms increases sodium intake. Although using different cellular actions, activation of opioid or $GABA_A$ receptors inhibits neuronal activity which might completely and simultaneously block all the inhibitory mechanisms present in the LPBN releasing NaCl intake.

Acknowledgments—We thank Reginaldo C. Queiróz and Sílvia Fóglia for expert technical assistance, Silvana A. D. Malavolta for secretarial assistance and Ana L. V. de Oliveira for animal care. This research was supported by public funding from FAPESP and CNPq. Lisandra B. Oliveira was a student at the Joint UFSCar/UNESP Graduate Program in Physiological Sciences.

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(Accepted 3 June 2008)
(Available online 11 June 2008)