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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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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