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Research report

Activation of μ opioid receptors in the LPBN facilitates sodium intake in rats

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HIGHLIGHTS

- Inhibitory mechanisms are important key for the control of sodium appetite.
- Facilitation of sodium appetite by μ opioid mechanisms in the LPBN.
- Control of sodium appetite by central facilitatory and inhibitory mechanisms.

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ABSTRACT

Important inhibitory mechanisms for the control of water and sodium intake are present in the lateral parabrachial nucleus (LPBN). Opioid receptors are expressed by LPBN neurons and injections of β-endorphin (nonspecific opioid receptor agonist) in this area induce 0.3 M NaCl and water intake in satiated rats. In the present study, we investigated the effects of the injections of endomorphin-1 (μ opioid receptor agonist) alone or combined with the blockade of μ, κ or δ opioid receptors into the LPBN on 0.3 M NaCl and water intake induced by subcutaneous injections of the diuretic furosemide (FURO) combined with low dose of the angiotensin converting enzyme inhibitor captopril (CAP). Male Holtzman rats with stainless steel cannulas implanted bilaterally in the LPBN were used. Bilateral injections of endomorphin-1 (0.1, 0.25, 0.5, 1.0, 2.0 and 4.0 nmol/0.2 μl) into the LPBN increased 0.3 M NaCl and water intake induced by FURO + CAP. The previous blockade of μ opioid receptor with CTAP (1.0 nmol/0.2 μl) into the LPBN reduced the effect of endomorphin-1 on FURO + CAP-induced 0.3 M NaCl. GNTI (κ opioid receptor antagonist; 2.0 nmol/0.2 μl) and naltrindole (δ opioid receptor antagonist; 2.0 nmol/0.2 μl) injected into the LPBN did not change the effects of endomorphin-1 on FURO + CAP-induced 0.3 M NaCl. The results suggest that μ opioid receptors in the LPBN are involved in the control of sodium intake.

1. Introduction

Sodium and water intake is controlled by forebrain and hindbrain areas, among them the lateral parabrachial nucleus (LPBN), a pontine structure located dorsolaterally to the superior cerebellar peduncle (scp) [1–9]. Different neurotransmitters and receptors like serotonin, cholecystokinin, corticotrophin releasing factor, opioid, GABA, purinergic, glutamate and noradrenaline are involved in the modulation of sodium and water intake in the LPBN, either increasing or reducing the activity of the inhibitory mechanism involved in the control of sodium and water intake [4,5,8–17].

The opioid mechanisms in the brain are involved in the control of water and/or sodium intake induced by different stimuli like water deprivation, hyperosmolality, sodium depletion and central angiotensin II (ANG II) [18–28]. In the LPBN, the activation of μ opioid receptors inhibits neuronal activity [29–32]. Specifically into the LPBN, infusion of DAMGO (μ opioid receptor agonist) increases food intake in satiated rats [33]. A more recent study has also demonstrated that injections of β-endorphin (nonspecific opioid receptor agonist) into the LPBN induce hypertonic NaCl intake in satiated rats, an effect completely reversed by the pretreatment with the nonspecific opioid antagonist naloxone [7]. In addition, naloxone injected into the LPBN also reduced sodium

Abbreviations: ANG II, angiotensin II; b. wt., body weight; CAP, captopril; CeA, central nucleus of amygdala; FURO, furosemide; LPBN, lateral parabrachial nucleus; NTS, nucleus of the solitary tract; scp, superior cerebellar peduncle.

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depletion-induced sodium intake [7,26,34]. Therefore, the opioids in the LPBN act reducing the activity of the inhibitory mechanisms which releases sodium intake [7,26,34].

β-Endorphin and naloxone are nonspecific ligands of opioid receptors (classified as μ, κ or δ opioid receptor subtypes) [35]. Therefore, it was not demonstrated yet which is the subtype of opioid receptor involved in the control of NaCl and water intake in the LPBN. In the present study, we investigated the effects of the activation of μ opioid receptors with injections of endomorphin-1, a specific μ opioid agonist, alone or combined with the blockade of μ, κ or δ opioid receptors into the LPBN on 0.3 M NaCl and water intake induced by the combined treatment with subcutaneous (sc) injection of the diuretic furosemide (FURO) and low dose of the angiotensin-converting enzyme inhibitor captopril (CAP).

2. Experimental procedures

2.1. Animals

Holtzman male rats ($n = 54$), weighing between 290 and 310 g at the day of the surgery, were housed in individual stainless steel cages on a 12 h light-dark cycle with room temperature of $23 \pm 2\,^{\circ}C$ and humidity at $55 \pm 10\%$. Animals had free access to normal sodium diet (BioBase Rat Chow, Águas Frias, Brazil), water, and 0.3 M NaCl. All the experimental procedures were approved by Ethics Committee in Animal Use (CEUA) from School of Dentistry—UNESP. 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).

2.2. Brain surgery

Rats were anesthetized with ketamine (80 mg/kg of body weight (b. wt.); Cristália, Itapira, Brazil) combined with xylazine (7 mg/kg of b. wt.; Agener União, Embu-Guaçu, Brazil) intraperitoneally, placed in a stereotaxic instrument (Kopf, Tujunga, USA) with the skull leveled between bregma and lambda. Two stainless steel 23-gauge guide cannulas were bilaterally implanted immediately above the LPBN (coordinates: 9.6 mm caudal to bregma, 2.1 mm lateral to midline, and 3.1 mm below dura mater). The tips of the guide cannulas were positioned at a point 2 mm above the LPBN. The guide cannulas were fixed to the cranium using dental acrylic resin and jeweler screws, and a 30-gauge metal obturator filled the guide cannulas between tests. At the end of the surgery, the animals received an intramuscular injection of antibiotic (benzylpenicillin, 80,000 IUs plus streptomycin, 33 mg; Pentabiótico Veterinário - Pequeno Porte, Fort Dodge Saúde Animal Ltda., Campinas, Brazil) and an sc injection of analgesic/anti-inflammatory (ketoprofen 1% - 0.03 ml/rat; Ketoflex, Mundo Animal, São Paulo, Brazil). Water and 0.3 M NaCl intake tests started 5 days after the surgery.

2.3. Injections into the LPBN

Bilateral injections into the LPBN were made using 5 μl Hamilton syringes (Hamilton, Reno, USA) connected by polyethylene tubing (PE-10; Clay Adams, Parsippany, USA) to a 30-gauge injection cannula. At the time of testing, rats were removed from the cages, metal obturators were removed and the injection cannula (2 mm longer than the guide cannula) was inserted into the guide cannula. Injection volume into the LPBN were 0.2 μl each site. The metal obturators were replaced after injections and the rats were placed back into their cages.

2.4. Drugs centrally injected

Endomorphin-1, selective μ opioid receptor agonist (Tocris Bioscience, Bristol, UK), was dissolved in saline and administered into the LPBN at the doses of 0.1, 0.25, 0.5, 1.0, 2.0 and 4.0 nmol/0.2 μl. CTAP, selective μ opioid receptor antagonist (Sigma-Aldrich, St. Louis, USA), was dissolved in saline and administered in the LPBN at the dose of 1.0 nmol/0.2 μl. GNTI dihydrochloride, selective κ opioid receptor antagonist (Tocris), was dissolved in saline and administered in the LPBN at the dose of 2.0 nmol/0.2 μl. Naltrindole hydrochloride, selective δ opioid receptor antagonist (Tocris), was dissolved in a mix of propylene glycol and water 2:1 (vehicle) and administered in the LPBN at the dose of 2.0 nmol/0.2 μl.

2.5. Water and 0.3 M NaCl intake tests

Rats were tested in their home cages with an interval of at least 2 days between the tests. Water and 0.3 M NaCl were provided from burettes with 0.1 ml divisions that were fitted with metal drinking spouts. During the tests rats had no access to food.

Water and 0.3 M NaCl intake was induced by the treatment with sc injections of FURO (10 mg/kg of b. wt.) + CAP (5 mg/kg of b. wt.). Immediately after the treatment with FURO + CAP, rats were placed back into their home cages with no access to water or 0.3 M NaCl for 1 h. After this period, water and 0.3 M NaCl were offered to the animals and the cumulative intake was recorded at every 30 min for 2 h. The injections of endomorphin-1 or saline into the LPBN were performed 45 min after FURO + CAP treatment. When endomorphin-1 was combined with CTAP, GNTI or naltrindole, the antagonists were injected into the LPBN 15 min before the injection of endomorphin or saline.

To study the effects of the injections of different doses of endomorphin-1 into the LPBN two groups of rats were used, one for the low doses (0.1, 0.25 and 0.5 nmol/0.2 μl) and one for the high doses (1.0, 2.0 and 4.0 nmol/0.2 μl). Each group was submitted to four tests in which rats received injections of saline and the three doses of endomorphin into the LPBN. In each test, the group of rats was divided in two subgroups and each subgroup received one of the treatments into the LPBN. The sequence of the treatments into the LPBN was randomized and each rat received all the four treatments (saline and the three doses of endomorphin).

One group of rats was used to test the effects of each antagonist combined with endomorphin-1 injected into the LPBN. Each group was submitted to four tests in which rats received injections of vehicle + endomorphin-1, antagonist + endomorphin-1, antagonist + saline or vehicle + saline into the LPBN. In each test, the group of rats was divided in two subgroups and each subgroup received one of the combined treatments into the LPBN. The sequence of the treatments into the LPBN was randomized and each rat received all the four combined treatments into the LPBN. The doses tested were: endomorphin-1 (0.5 nmol/0.2 μl), CTAP (1 nmol/0.2 μl), GNTI and naltrindole (2 nmol/0.2 μl).

2.6. Histology

At the end of the last intake test, the animals received bilateral injections of 2% Evans Blue solution (0.2 μl) into the LPBN. They were then deeply anesthetized with sodium thiopental (80 mg/kg of b. wt.; Cristália) and perfused transcardially with saline followed by 10% formalin. Brains were removed, fixed in 10% formalin, frozen, cut in 50 μm sections, stained with Giemsa stain, and analyzed by light microscopy to confirm the injection sites into the LPBN.