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Blockade of central delta-opioid receptors inhibits salt appetite in sodium-depleted rats

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ABSTRACT

Various studies have investigated the role of central opioid peptides in feeding behavior; however, only a few have addressed the participation of opioids in the control of salt appetite. The present study investigated the effect of intracerebroventricular injections of the δ-opioid antagonist, naltrindole (5, 10 and 20 nmol/rat) and the agonist, deltorphin II (2.5, 5, 10 and 20 nmol/rat) on salt intake. Two protocols for inducing salt intake were used: sodium-depletion and the central injection of angiotensin II. In addition, the effect of a central δ-opioid receptor blockade on locomotor activity, on palatable solution intake (0.1% saccharin) and on blood pressure was also studied. The blockade of central δ-opioid receptors inhibits salt intake in sodium-depleted rats, while the pharmacological stimulation of these receptors increases salt intake in sodium-replete animals. Furthermore, the blockade of central δ-opioid receptors inhibits salt intake induced by central angiotensinergic stimulation. These data suggest that during sodium-depletion activation of the δ-opioid receptors regulates salt appetite to correct the sodium imbalance and it is possible that an interaction between opioidergic and angiotensinergic brain system participates in this control. Under normonatremic conditions, δ-opioid receptors may be necessary to modulate sodium intake, a response that could be mediated by angiotensin II. The decrease in salt intake following central δ-opioid receptors blockade does not appear to be due to a general inhibition of locomotor activity, changes in palatability or in blood pressure.

1. Introduction

The endogenous opioid system is widely distributed throughout the brain and has been shown to be involved in multiple functions including antinociception, mood, feeding and drinking behavior, cardiovascular control and endocrine regulation [8]. Opioid peptides are classified into four well-defined pharmacological families: endorphins, enkephalins, dynorphins and nociceptin/orphanin FQ that act through G-protein-coupled receptors referred to as mu, delta, kappa and nociceptin/orphanin FQ receptor [8,68,69]. Of these opioid receptors, δ-opioid receptors are particularly interesting as an alternative to μ-opioid drugs for the clinical treatment of chronic pain [5,10,73]. In the near future, drugs targeted at δ-opioid receptors may also be used as therapeutic tools in addictive and impulsive disorders, as well as in anxiety and depression [58].

Different studies have demonstrated the participation of the brain opioid system in the regulation of feeding behavior through a mechanism that may involve modulation of the reward-related responses and the palatability of different substances [9,42,44,66]. Indeed, morphine and opioid agonists induce a preference for fat and sweet food/solutions [9,26]. Few studies have addressed the role of opioid peptides in salt intake. The antagonist opioid, naloxone, administered both systemically and into the central nervous system, reduces the intake of hypertonic, hypotonic and isotonic saline solutions, which are preferred over water by fluid-deprived rats [15,25,27,28]. On the contrary, intracerebroventricular injections of selective δ-, μ- and κ-opioid agonists increase saline intake in non-deprived rats [27,28]. Previous data from our laboratory have shown that κ-opioid receptors may regulate salt appetite, since intracerebroventricular injections of nor-binaltorphimine (Nor-BNI), a κ-opioid receptor antagonist, inhibit salt intake in sodium-depleted rats [55]. In addition, δ-opioid receptors also appear to be involved in the control of ingestive behavior related

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to palatable substances. Deltorphin II, a δ-opioid receptor agonist, injected intracebroventricularly, has been shown to increase sucrose solution intake [60], while the antagonist, naltrindole, had no effect on sucrose solution intake [3]. In a study using the two-bottle choice test approach (water and saline solution 0.6% and 1.7%) in fluid-deprived rats, central administration of μ- and κ-antagonists, but not δ-antagonists, decreases water intake. In addition, all the antagonists used in that study were able to alter saline solution intake [7]. On the other hand, salt intake in sodium-depleted rats is decreased by subcutaneous injection of naltrindole, a δ-opioid antagonist [45]. However, in that study, a single dose of naltrindole was used and this was the only approach used to study salt appetite. In order to clarify the role of δ-opioid receptors in the control of salt appetite, the present study was designed to investigate the effect of intracerebroventricular injections of different doses of a δ-opioid agonist and antagonist using two different approaches. These approaches have been largely used in the literature to study salt intake: (1) sodium deficiency produced by a single administration of the diuretic furosemide; and (2) intracerebroventricular injection of angiotensin II in rats with normal sodium balance. In addition, the effect of central δ-opioid receptor blockade on locomotor activity, on palatable solution intake (0.1% saccharin) and on blood pressure was also investigated. The hypothesis is that central δ-opioid receptors participate in modulating salt intake during homeostatic challenges and may be important for the correction of sodium imbalance.

2. Methods

2.1. Animals

The present study used adult male Wistar rats weighing 250–280 g. The animals were kept under controlled light (lights on from 5 AM to 7 PM) and temperature (22 ± 2 °C) conditions with free access to tap water and laboratory chow (Nuvital Nutrientes Ltda., Curitiba, Brazil). Groups of rats used in one experimental set were not reused in any other part of the study. All experiments were conducted between 7 AM and 11 AM. The experimental protocols were performed in accordance with the regulations for the care of laboratory animals and were approved by the Institution's Animal Ethics Committee (CEUA-ICS-UFBA # 024/2012).

2.2. Surgical procedures

The animals were anesthetized with ketamine/xylazine (80/7 mg/kg i.p.) to allow the guide cannula (22-gauge) to be inserted into the lateral ventricle (LV) in accordance with the following coordinates: anteroposterior = 0.9 mm behind the bregma; lateral = 1.5 mm; vertical = 4.0 mm below the skull. The guide cannula was fixed to the skull with metal screws and dental cement and an obturator was provided to avoid obstruction. After surgery, the animals were housed in individual cages and had free access to laboratory chow, distilled water and 1.5% saline solution. The animals were handled every day to minimize the stress of the experimental procedure. The position of the guide cannula in the LV and the intracerebroventricular injection site were confirmed at the end of the experiment with the use of Evans Blue dye injected through the cannula. The brains were removed, placed in formalin, and later frozen and cut into 40 μm sections. The slices were stained with cresyl violet and analyzed using light microscopy. Only data from the animals in which the tip of the cannula was restricted to the cerebroventricular space and the Evans Blue dye could not be seen in the brain tissue surrounding the ventricle were included in the study.

2.3. Drugs and microinjections

The drugs used were naltrindole (NTI), an opioid antagonist selective to δ-opioid receptors [57]; deltorphin II (Delt-II), an opioid agonist with high selectivity and affinity to δ-opioid receptors [20,38]; and angiotensin II (AII). Both NTI and Delt-II were acquired from Tocris Bioscience, Ellisville, MO, USA, while AII was purchased from Sigma Chemical, Co., St. Louis, MO, USA. The doses of the drugs used in this study were compatible with previous reports: 5, 10 and 20 nmol/rat of naltrindole [7], while the doses of deltorphin II were 2.5, 5, 10 and 20 nmol/rat [72] and 10 ng/rat of AII [21]. Central injections were given using a Hamilton microsyringe connected to a 30-gauge injector through polyethylene tubing (PE10). A total volume of 2 μl was slowly injected (60 s). Furosemide, a loop diuretic, was purchased from Sanofi-Aventis Ltd., São Paulo, Brazil.

2.4. Sodium depletion (Experiments 1, 2 and 3)

Sodium depletion was achieved by means of a subcutaneous injection of furosemide (20 mg/kg). After the injections, the rats had free access to distilled water, while the standard rat chow was replaced by a low sodium diet (0.001% Na^+ and 0.33% K^+). In *Experiment 1*, the participation of central δ-opioid receptors in the regulation of salt appetite was tested in different groups of sodium-depleted animals receiving LV injections of NTI at different doses (5, 10 and 20 nmol) or saline. In *Experiment 2*, sodium-depleted animals received an LV injection of 20 nmol of NTI plus Delt-II at the doses of 2.5, 5, 10 and 20 nmol, or saline, to confirm the specificity of NTI. In both experimental sets, sodium-depleted control animals received LV injections of isotonic saline solution. In *Experiment 3*, the aim was to test the effect of pharmacological stimulation of the central δ-opioid receptors on salt appetite. Sodium depleted rats and normonatremic animals (not submitted to sodium-depletion) received an LV injection of 20 nmol of Delt-II or saline. In all three experimental sets, bottles containing hypertonic saline solution (1.5%) and distilled water were reintroduced into the cages 15 min after the central injections. Fluid intake measurement began 5 min later and continued for the next 120 min. To confirm the efficacy of sodium depletion, an additional control group was included. In this group, the animals received subcutaneous injections of isotonic saline solution instead of furosemide and LV injections of isotonic saline solution. As expected, salt intake was significantly higher in the sodium depleted (furosemide treated) rats compared to the normonatremic (saline treated) rats, as shown in Table 1.

2.5. Central angiotensinergic stimulation (Experiment 4)

In this experimental group, the objective was to study the role of δ-opioid receptors in the salt appetite induced by the pharmacological stimulation of central angiotensinergic pathways. Different groups of normonatremic rats received LV injections of NTI at different doses (5, 10 and 20 nmol) 15 min before receiving AII (10 ng/rat). Bottles containing 1.5% saline solution and distilled water were replenished and made available immediately after the LV injections of AII. As in the previous experimental sets, the measurement of fluid intake began 5 min after reintroduction of the bottles into the cages and continued for the next 120 min.

2.6. Open field test (Experiment 5A)

The aim of this experimental set was to exclude the possibility that the δ-opioid receptor antagonist could have induced a locomotor alteration that would explain the inhibition of salt intake observed in experiments 1 and 3. Different groups of sodium-depleted rats receiving LV injections of NTI (20 nmol) or saline solution were submitted to an open field test. In this test, the