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Central kappa opioid receptors modulate salt appetite in rats ☆,☆☆

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ABSTRACT

The role of the central opioid system in the control of water and salt intake is complex, with both stimulatory and inhibitory effects having been observed. The aim of the present study was to investigate the participation of the central κ-opioid receptors in the control of salt appetite. Male Wistar rats were submitted to two different experimental protocols: sodium deficit produced by the diuretic, furosemide, and brain angiotensinergic stimulation in rats under normal sodium balance. Lateral ventricle (LV) injections of Nor-binaltorphimine (Nor-BNI) at different doses (5, 10 and 20 nmol) inhibited hypertonic saline solution (1.5%) intake in sodium-depleted rats. The salt appetite induced by an LV injection of angiotensin II (AngII) (10 ng) was also blocked by Nor-BNI injections into the LV, while no significant change was observed in water intake. Furthermore, the decrease in salt intake seems not to have been due to a general inhibition of locomotor activity or to any change in palatability, since central administration of Nor-BNI failed to modify the intake of a 0.1% saccharin solution when the animals were submitted to a "dessert test" or to induce any significant locomotor deficit in the open-field test. Also the central administration of Nor-BNI was unable to modify blood pressure in sodium-depleted animals. The present results suggest that activation of endogenous κ-opioid receptors modulates salt appetite induced by sodium depletion and by central angiotensinergic stimulation in rats.

1. Introduction

The different families of opioid peptides include endorphins, enkephalins, dynorphins, and nociceptin/orphanin that act through four major types of receptor: mu (μ), kappa (κ), delta (δ) and nociceptin (N/OFQ). There is evidence of distinctive but overlapping distributions of μ, κ, δ and N/OFQ receptors in different areas of the brain [1–7]. The endogenous opioid system is involved in a variety of functions and is found in multiple networks throughout the brain, especially in areas related to feeding and drinking behavior, cardiovascular control and endocrine regulation [1,8]. The role of the brain opioid system in regulating feeding behavior and modulating reward-related responses and the palatability of different substances has been well-documented [8–11]. The participation of the central opioid system in the control of water and salt intake is rather complex, with both stimulatory and inhibitory effects having been observed depending on the anatomical location, the type of opioid receptor involved and the doses of the opioidergic agents used.

The physiological regulation of body sodium depends on a multifaceted mechanism involving homeostatic responses and palatability and reward aspects of salt intake. Sodium deficit motivates behavior to specifically seek out and ingest the sodium ion [12–15]. Some studies have also shown the participation of endogenous opioid peptides in salt preference. The antagonist opioid, naloxone, administered both systemically and in the central nervous system, reduces the intake of hypertonic, hypotonic and isotonic saline solutions which are preferred over water in water-deprived rats [16–19]. Furthermore, in water-deprived rats central administration of μ- and κ-antagonists, but not δ-antagonists, decreases water intake in a two-bottle choice test between water and saline solution (0.6% and 1.7%). However, any of the antagonists used in this study were able to alter the intake of saline solution [20]. Conversely, in non-deprived rats, saline intake is increased by injecting selective δ-, μ- and κ-opioid agonists into the cerebroventricular system and into the parabrachial nuclei [18,19,21]. In addition, systemic injections of morphine increase hypertonic saline intake in sodium-depleted rats [22]. However, in this study, the opioid agents were administered subcutaneously after repeated sodium depletion. It has been shown that repeated sodium depletion may alter the intake of saline solution [23,24], as well as the palatability of salty solutions. Further studies are necessary to clarify the role of the different central opioid peptides receptors in the control of salt appetite.

Because of the contradictions in the literature with respect to the participation of opioid receptors, particularly the k-opioid receptors, in salt appetite, the objective of the present study was to investigate

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the participation of the central κ-opioid receptors in the control of salt appetite in two different models: 1) sodium deficiency produced by single administration of the diuretic, furosemide; 2) cerebral administration of a natriorexigenic drug, angiotensin II, in rats with normal sodium balance; two approaches that have largely been used in the literature to study salt preference. In addition, the effect of central k-opioid receptor blockade on locomotor activity, on palatable solution intake (0.1% saccharin) and on blood pressure was investigated. The hypothesis evaluated in this study is that opioid peptides are released during homeostatic challenges and that interaction with κ-opioid receptors may modulate different aspects of salt intake (homeostatic, palatability and reward) in order to correct sodium imbalance.

2. Methods

2.1. Animals

Adult male Wistar rats weighing 220–250 g were used in the present study and kept under controlled light (lights on from 5 AM to 7 PM) and temperature (22 ± 2 °C) conditions. They had 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 according to the regulations established by the National Council for the Control of Animal Experiments (*Conselho Nacional de Controle de Experimentação Animal* — CONCEA, Brazil).

2.2. Surgical procedures

Five days before the experimental sessions the animals were anesthetized with ketamine/xylazine (80/7 mg/kg i.p.) to enable a guide cannula (22-gauge) to be implanted into the lateral ventricle (LV) according to 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. To avoid obstruction of the guide cannula, an obturator was provided. After surgery, the animals were housed in individual cages and had free access to two different bottles, one containing distilled water and the other containing hypertonic saline solution (1.5%). The animals were handled every day in order to minimize the stress of the experimental procedure. The location of the guide cannula in the LV and the intracerebroventricular injection site was 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 Nor-binaltorphimine (Nor-BNI), an opioid antagonist selective to κ-opioid receptors [25,26], $ICI_{199,441}$ an opioid agonist preferentially binding to κ-opioid receptors [27], both acquired from Tocris Bioscience, Ellisville, MO, USA, and angiotensin II (AngII), which was purchased from Sigma Chemical, Co., St. Louis, MO, USA. The doses of the drugs used in this study were compatible with those used by other research groups: the doses of Nor-BNI were 5, 10 and 20 nmol/rat [20], while the dose of $ICI_{199,441}$ was 9.4 nmol/rat [28] and the dose of AngII was 10 ng/rat [29]. pH was neutral in all solutions and no acid or basic solutions were injected. 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 and 2)

Animals received a subcutaneous injection of furosemide (10 mg/kg) to induce renal sodium loss 24 h prior to the experimental sessions. After the injections, the rats had free access to distilled water and the standard rat chow was replaced by a low sodium diet (0.001% Na + and 0.33% K+). Control animals not submitted to sodium depletion received subcutaneous injections of isotonic saline solution instead of furosemide. In *experiment 1*, the participation of central kappa opioid receptors in the salt appetite of sodium-depleted rats was tested in different groups of sodium-depleted animals receiving LV injections of Nor-BNI at different doses (5, 10 and 20 nmol). In *experiment 2*, another group of sodium-depleted animals received an LV injection of 20 nmol of Nor-BNI plus $ICI_{199,441}$, a κ-opioid receptor agonist, at a dose of 9.4 nmol, to confirm the specificity of Nor-BNI. Sodium-depleted control animals received LV injections of isotonic saline solution. Bottles containing hypertonic saline solution (1.5%) and distilled water were reintroduced into the cages 15 min after the injections. The first measurement of fluid intake was recorded 5 min after this and measurements continued for the next 120 min. In an additional control group, the animals received subcutaneous injections of isotonic saline solution instead of furosemide and LV injections of isotonic saline solution.

2.5. Central angiotensinergic stimulation (experiment 3)

To study the participation of κ-opioid receptors in water and salt intake under conditions of normal sodium balance, pharmacological stimulation of central angiotensinergic pathways was performed. Different groups of rats received LV injections of Nor-BNI at different doses (5, 10 and 20 nmol) 15 min before receiving AngII (10 ng/rat). Bottles containing 1.5% saline solution and distilled water were made available immediately after the LV injections of AngII. As in the previous experimental sets, the first measurement of fluid intake was recorded 5 min afterwards and measurement continued for the next 120 min. In an additional control group, the animals received central administration of saline instead of AngII.

2.6. Open field test (experiment 4A)

Different groups of rats receiving LV injections of Nor-BNI (20 nmol) or saline solution were submitted to an open field test to exclude the possibility that this agent could have induced a locomotor alteration that would explain the inhibition of salt intake observed here. In this test, the animals were placed in a circular acrylic box (60 cm in diameter and 60 cm high) with an open top 30 min after an injection of Nor-BNI or saline solution into the LV. The floor was divided into eight areas of equal size with a circle at the center (42.43 cm). Hand operated counters and stopwatches were used to score locomotion over a 10 min period by measuring the number of areas into which the rats entered with all four paws. The behavioral experiments took place in a sound-attenuated, temperature-controlled (24 ± 1 °C) room between 7 AM and 11 AM. A white-noise generator provided constant background noise and the apparatus was cleaned with 70% ethanol and dried before each session to minimize olfactory cues.

2.7. Dessert test (experiment 4B)

The effect of an LV injection of Nor-BNI on the intake of a 0.1% saccharin solution, a well-established model of hedonic behavior in rats [30], was used to exclude the possibility of a non-specific, general inhibition of the central nervous system induced by this agent. In this experiment, after LV cannulation the animals were kept in the usual individual cages during the training period with free access only to