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Research report

Neuropeptide FF attenuates the acquisition and the expression of conditioned place aversion to endomorphin-2 in mice

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HIGHLIGHTS

- NPFF inhibited both the acquisition and the expression of conditioned place aversion of endomorphin-2 via NPFF receptors.
- NPFF did not modify the locomotor activity of endomorphin-2.
- These data further support an anti-opioid character of NPFF system.

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ABSTRACT

It has been demonstrated that the endogenous mu opioid (MOP) agonist endomorphin-2 (EM-2) produces conditioned place aversion (CPA) and in contrast, morphine exerts opposite action. Neuropeptide FF (NPFF) was reported to act as a functional antagonist of mu opioid receptor and to exert opioid-modulating activities. The present study examined the influence of NPFF on the rewarding action of EM-2, using the unbiased conditioned place preference (CPP) paradigm. For testing the effect of NPFF on the acquisition of EM-2-induced CPA, NPFF and EM-2 were co-injected on the conditioning days without drug treatment on the followed test day. To explore the effect of NPFF on the expression of EM-2-induced CPA, EM-2 was administered alone on the conditioning days, and NPFF was given 5 min before placement in the CPP apparatus on the test day. The results showed that NPFF (2.5, 5 and 10 nmol, i.c.v.) alone caused little place preference change. However, NPFF dose-dependently reversed the acquisition of CPA induced by 30 nmol EM-2 (i.c.v.). Similarly, the expression of EM-2-induced CPA was also reduced by NPFF. Moreover, the effects of NPFF on the acquisition and the expression of EM-2-induced CPA were completely blocked by the NPFF receptors antagonist RF9 (10 nmol, i.c.v.). However, central injection of NPFF neither changed the locomotor activity nor modified the locomotor action of EM-2. These data provide the first evidence for a functional interaction of the endogenous ligands for NPFF and MOP receptors, and further support an anti-opioid character of NPFF system.

1. Introduction

Endomorphin-1 (EM-1, YPWFamide) and endomorphin-2 (EM-2, YPFFamide), have been found as the endogenous ligands for MOP receptor [1], and activate MOP receptor with high affinities and selectivities [2,3]. Interestingly, the pharmacological studies strongly implied that different mechanisms were involved in the antinociceptive and rewarding actions produced by EM-1 and EM-2. EM-1 given i.c.v. in mice produced CPP in a manner similar to morphine or DAMGO, whereas EM-2 induced CPA in mice [4–6]. The possible mechanisms for endomorphins to exert opposite rewarding effects might be the activation of different subtypes of MOP receptors in the brain [7–9].

Neuropeptide FF (NPFF, FLFQPQRFamide) belongs to an opioid-modulating peptide family [10]. Recent reports have shown that NPFF belongs to a neuropeptide family including two G-protein coupled receptors ($NPFF_1$ and $NPFF_2$) [11,12]. It has been indicated that NPFF plays important roles in modulation of the biological activities induced by morphine. The previous studies suggested that NPFF reversed morphine analgesia, or potentiated morphine-induced antinociception when injected intracerebroventricularly or intrathecally in rats and mice, respectively [13–15]. The

Abbreviations: CNS, central nervous system; CPA, conditioned place aversion; CPP, conditioned place preference; Dyn, dynorphin A (1–17); EM-1, endomorphin-1; EM-2, endomorphin-2; NAc, nucleus accumbens; NPFF, neuropeptide FF; MOP, mu-opioid; KOP, kappa-opioid; VTA, ventral tegmental area.

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opposite effects of supraspinal and spinal injection of NPFF may be due to the multiplicity of receptors, or a functional blockade of delta opioid autoreceptors in the spinal cord [10,16]. In addition, NPFF was reported to be involved in morphine tolerance and physical dependence. The increase of NPFF level in the central nervous system might contribute to the development of tolerance and dependence for opioid [17–19], whereas NPFF antagonist RF9 significantly blocks opioid tolerance and physical dependence [20,21].

In agreement with these in vivo studies, the biochemical studies also demonstrated that NPFF receptors exert a cellular opioid-modulating action. Recently, $NPFF_2$ and MOP receptors were shown to physically interact in transfected SH-SY5Y cells, which naturally expresses opioid receptors [22,23]. Fluorescence resonance energy transfer and co-immunoprecipitation were used to monitor the interaction between $NPFF_2$ and MOP receptors tagged with variants of the green fluorescent protein, which suggested that NPFF agonists induced functional blockade of MOP receptor [22,23]. It is notable that activation of MOP receptor could exert complex pharmacological activities via different subtype receptors [24,25]. Though the link between the functions of NPFF and morphine has been studied, very few studies investigated the effects of NPFF on the biological activities induced by other MOP receptor agonists, especially the endogenous agonists toward MOP receptor.

In recent studies, NPFF and its stable agonist 1DMe were found to inhibit the acquisition and the expression of the conditioned place preference of morphine [26,27]. To further evaluate the interaction between NPFF and EM-2, in the present study, an unbiased conditioned place preference paradigm was used to investigate the effects of NPFF (i.c.v.) on CPA induced by EM-2 in mice. Furthermore, receptor specificity of the modulating action induced by NPFF was evaluated by using selective NPFF receptors antagonist RF9, which behaves as a truly pure NPFF receptors antagonist in vivo and in vitro [21,28].

2. Materials and methods

2.1. Animals

Kunming mice were obtained from the Experimental Animal Center of Lanzhou University. All animals were cared for and experiments were carried out in accordance with the European Community guidelines for the use of experimental animals (86/609/EEC). Animals were housed in an animal room that was maintained at $22 \pm 2\,^{\circ}C$ with a 12-h light:12-h dark cycle. Food and water were available ad libitum. All the protocols in this study were approved by the Ethics Committee of Lanzhou University, China.

2.2. Chemicals

EM-2, NPFF and RF9 were synthesized on a solid support following the previous report [13]. Peptides were prepared by manual solid-phase synthesis using standard N-fluorenylmethoxycarbonyl (Fmoc) chemistry. Fmoc-protected amino acids (GL Biochem (Shanghai) Ltd.) were coupled to a Rink Amide MBHA resin (Tianjin Nankai Hecheng Science & Technology Co., Ltd., China). Gel filtration (Sephadex G-10) was performed to desalt the crude peptides. The desalted peptide was purified by preparative reversed-phase HPLC using a Waters Delta 600 system coupled to a UV detector. Fractions containing the purified peptides were pooled and lyophilized. The purity of the peptide was established by analytical HPLC. The molecular weight of the peptide was confirmed by an electrospray ionization mass spectrometer (Mariner ESI-TOF MS, Applied Biosystems, CA).

In addition, the opioid receptors antagonist naloxone was obtained from Sigma Chemical Company (USA). All drugs were dissolved in sterilized saline, and the solutions were divided into aliquots and stored in 2 ml tubes at $-20\,^{\circ}C$. The aliquots were thawed and used on the day of the experiment.

2.3. Administration of drugs

Surgical implantation of cannula was conducted in an aseptic environment [29]. Mice (18–20 g) were anesthetized with pentobarbital sodium (80 mg/kg, intraperitoneally), and placed in a stereotaxic apparatus. The incision area of the scalp was shaved, and a sagittal incision was made in the midline exposing the surface of the skull. A single hole was drilled through the skull targeted above the left or right lateral ventricle, 3.0 mm posterior and 1.0 mm lateral to the bregma. A stainless steel guide cannula was implanted 3.0 mm ventrally from the surface of skull. To prevent occlusion, a dummy cannula was inserted into the guide cannula. The dummy cannula protruded 0.5 mm from the guide cannula. Dental cement was used to fix the guide cannula to the skull. After surgery, the animals were allowed to recover for at least 5 days, and during this time, mice were gently handled daily to minimize the stress associated with manipulation of the animals throughout the experiments.

Saline, EM-2, NPFF, RF9, and NPFF plus RF9 were injected intracerebroventricularly (i.c.v.) in a volume of 4 μl at a rate of 6 μl/min per mouse by using a 25-μl microsyringe. Following, the catheter was flushed with 1 μl of saline. After completion of behavioral testing, the proper injection site was verified in pilot experiments by administration and localization of methylene blue dye. Only the data from those animals with dispersion of the dye throughout the ventricles were used in the study.

2.4. Place conditioning experiment

2.4.1. CPP apparatus

The conditioned place conditioning experiment was performed according to our previous study [29]. CPP apparatus was divided into three compartments. Two identical-sized compartments (20 cm × 20 cm × 20 cm) were connected by a narrower one (5 cm × 20 cm × 20 cm). The large compartments are visually and tactually distinct (black-and-white striped walls with rough floor versus black-dotted white walls with smooth floor). These boxes could be isolated by guillotine doors.

2.4.2. Acquisition of EM-2-induced CPA

On the pre-conditioning day (day 1), mice were given free access to the entire apparatus for 15 min, and the time spent in each compartment was measured. Mice that spent more than 60% of the time in the same compartment were excluded from the tests. On the conditioning days, mice were i.c.v. injected with saline and confined to one of the compartments for 15 min. Approximately 6 h later, animals were administered intracerebroventricularly with saline, EM-2, saline plus NPFF, NPFF plus EM-2 (saline or NPFF was injected i.c.v. 10 min prior to the second drug), and confined to the opposite compartment. This conditioning procedure was carried out for a total of three identical conditioning sessions (days 2–4). On the test day (post-conditioning day, day 5), mice were also given free access to the entire apparatus for 15 min, and the time spent in each compartment was measured.

2.4.3. Expression of EM-2-induced CPA

On the pre-conditioning day (day 1) and the conditioning days (days 2–4), the same general procedure describe above was made, but mice only received saline or EM-2 (30 nmol). On the test day (day 5), mice were i.c.v. administered with NPFF (2.5, 5 and 10 nmol) or saline, and then, 5 min later were also given free access to the entire apparatus for 15 min, and the time spent in each compartment was measured.

2.5. Locomotor activity test

Locomotor activity of mice was measured using the Morris Water Maze Tracking System (Taimeng Technology Corporation of Chengdu, China). The animals were placed individually in a plexiglas box (50 cm × 50 cm × 30 cm) after injection of saline or drugs. Drugs were i.c.v. administered uniformly in a volume of 4 μl, and the catheters were also flushed with 1 μl of saline. Horizontal activity (distance traveled) was recorded for 15 min.

2.6. Statistical analysis

CPP score was expressed as time spent in the drug associated compartment on post-conditioning day minus time spent in the drug-associated compartment on preconditioning day. Results were presented as means ± S.E.M. Data obtained from CPP test and locomotor activity assay were statistically compared by means of one-way ANOVA followed by the Tukey HSD test. Probabilities of less than 5% ($P < 0.05$) were considered statistically significant.

3. Results

3.1. Endomorphin-2-induced conditioned place aversion

Data were expressed as CPP score, i.e., time spent in drug associated chamber on post-conditioning day minus that on the pre-conditioning day. As shown in Fig. 1, saline given i.c.v. did not significantly induce the place preference change, indicating that central injections were not aversive or rewarding in the unbiased balanced paradigm of conditioned place preference. Compared with saline vehicle treated, i.c.v. injection of EM-2 (7.5, 15 and 30 nmol) induced a significant place aversion in a dose-dependent manner ($F_{3,39} = 67.37$; $P < 0.001$) in mice.