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A neuropeptide FF agonist blocks the acquisition of conditioned place preference to morphine in C57Bl/6J mice

Stéphane Marchand [a], *Alexandre Betourne* [a], *Virginie Marty* [a], *Stéphanie Daumas* [a], *Hélène Halley* [a], *Jean-Michel Lassalle* [a], *Jean-Marie Zajac* [b], *Bernard Frances* [a,*]

[a] *Centre de Recherches sur la Cognition Animale, CNRS UMR 5169, Bât 4R3 b3, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex 4, France*

[b] *Institut de Pharmacologie et Biologie Structurale, CNRS-UMR 5089, 205 route de Narbonne, 31077 Toulouse, France*

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ABSTRACT

Neuropeptide FF behaves as an opioid-modulating peptide that seems to be involved in morphine tolerance and physical dependence. Nevertheless, the effects of neuropeptide FF agonists on the rewarding properties of morphine remain unknown. C57BL6 mice were conditioned in an unbiased balanced paradigm of conditioned place preference to study the effect of i.c.v. injections of 1DMe (D-Tyr[1](NMe)Phe[3]NPFF), a stable agonist of the neuropeptide FF system, on the acquisition of place conditioning by morphine or alcohol (ethanol). Morphine (10 mg/kg, i.p.) or ethanol (2 g/kg, i.p.) induced a significant place preference. Injection of 1DMe (1–20 nmol), given 10 min before the i.p. injection of the reinforcing drug during conditioning, inhibited the rewarding effect of morphine but had no effect on the rewarding effect of ethanol. However, a single injection of 1DMe given just before place preference testing was unable to inhibit the rewarding effects of morphine. By itself, 1DMe was inactive but an aversive effect of this agonist could be evidenced if the experimental procedure was biased. These results suggest that neuropeptide FF, injected during conditioning, should influence the development of rewarding effects of morphine and reinforce the hypothesis of strong inhibitory interactions between neuropeptide FF and opioids.

1. Introduction

Neuropeptide FF (NPFF, FLFQPQRF-NH_2) represents a neurotransmitter system acting as a modulator of endogenous opioid functions [48]. Two precursors containing several NPFF-related peptides have been cloned in mammals [22,40,46]. These peptides interact with two G-protein-coupled receptors, $NPFF_1$ and $NPFF_2$ [5,25,35] widely distributed in the central nervous systems of rodents, lagomorphs and monkeys [19]; showing that the NPFF system is phylogenetically conserved. The localization of the receptors shows noticeable species differences, previously evidenced by the measurement of $NPFF_1$- and $NPFF_2$-mRNA by quantitative RT-PCR in human and rat nervous tissues [5]. Although the functional roles of NPFF and related endogenous peptides remains to be determined, the localization of NPFF receptors and the pharmacological studies suggest that NPFF plays an important role in the modulation of pain and could act as an endogenous anti-opioid peptide in rodents [43]. High densities of $NPFF_2$ receptor, the subtype predominantly expressed in many species, are detected in the diencephalon and in the superficial layers of the spinal cord consistent with a putative role for NPFF in the modulation of sensory inputs and opioid analgesia. Pharmacological data showed that analgesia

* *Corresponding author*. Tel.: +33 5 61 55 63 36; fax: +33 5 61 55 61 54.
E-mail address: frances@cict.fr (B. Frances).

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induced by stress or morphine injection is attenuated by intracerebroventricular (i.c.v.) administration of NPFF or NPFF-analogues in rat and mouse [16,17,47]. Conversely, after intrathecal administration, NPFF induces a strong antinociception in rat and markedly enhances the antinociception produced by administration of opioid receptor agonists [18]. This effect, reversed by naloxone, could result from both an increase of met-enkephalin release in the spinal cord, which is antinociceptive, and a decrease of the release of pronociceptive dynorphins [2,3]. In mouse, intrathecal injection of NPFF agonists potentiates morphine-induced analgesia [41].

Results of several experiments suggest that NPFF could participate in morphine tolerance [28] and dependence. This peptide is able to produce some signs of a withdrawal syndrome in morphine-dependent rats [31]. Central immunoneutralization of NPFF or injection of antisense oligonucleotides to the precursor proNPFF$_A$ lowered the intensity of the withdrawal syndrome and partially restored the analgesic activity of morphine in morphine-tolerant animals [15,27]. These experiments have largely focused on the physical components of dependence but opiate addiction has also a ''psychological'' component based on their psychoactive reinforcing properties. Some neuropeptides which are also considered as modulators of the opioid systems (i.e. CCK, dynorphin or nociceptin) have been tested for their rewarding (or aversive) properties when injected alone or in combination with morphine [9,13,14,21,37,44] but the motivational properties of NPFF have not been investigated. Based on the dopamine model of reward, mesolimbic dopaminergic neurons, originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens, are thought to mediate the reinforcement process induced by many drugs including opiates and alcohol. Morphine has its most fully documented rewarding actions in the VTA. Mu opioids disinhibit the firing of dopaminergic neurons in this region by inhibiting GABAergic interneurons, leading to an increase of dopamine release in the nucleus accumbens. NPFF could modulate this opioid activity since intra-VTA injections of NPFF dose-dependently inhibit the analgesic effect and other behavioral actions of morphine in rats [1,33]. Furthermore, the NPFF binding sites in the VTA have been localized to both intrinsic non-dopaminergic neurons and afferent fibers to the VTA [19]. Intra-VTA injections of NPFF reduce the locomotor activity induced by exposure to novelty and dose-dependently reverse the potentiation of novelty-induced locomotor response produced by VTA-injection of thiorphan, an inhibitor of the degradation of enkephalins [6]. In C57Bl/6J mouse, NPFF$_2$ receptors are present in high quantities in the nucleus accumbens [19] where they could counterbalance the morphine-induced dopamine release.

Because the endogenous opioid systems seem to be essential for initiation and maintenance of excessive alcohol consumption in C57Bl/6J mice [39], we have also included place preference to ethanol in the present study.

To investigate the psychopharmacological profile of NPFF, 1DMe ([D-Tyr^1, (NMe)Phe^3]NPFF), a selective NPFF$_2$-receptors agonist resistant to proteolytic degradation, was injected intracerebroventricularly (i.c.v.) in mice submitted to the conditioned place preference (CPP) paradigm, a test which is widely employed as a valid measurement to evaluate the reinforcing effect of drugs [4,45]. In this study, we demonstrate that i.c.v. injections of 1DMe inhibit the rewarding property of morphine but not of ethanol. Additionally, we show that 1DMe is inactive on its own but can elicit place-aversion under biased conditions.

2. Materials and methods

2.1. Subjects

A total of 248 C57BL/6JIco female mice, obtained from Iffa Credo (France), aged about 3 months at the time of testing (average weight 27 g), were used in these experiments. They were housed in groups of three to five per cage in a temperature-controlled room (21 ± 1 °C) subjected to a 12-h light–dark cycle, with lights on at 8 a.m. Food and water were available ad libitum throughout the experiments.

2.2. Surgery

A stainless guide cannula was implanted unilaterally into the right or the left ventricle of all animals for i.c.v. drug injections. The mice were anaesthetized with a mixture of xylazine 2% (15 mg/kg, i.p.) and ketamine (100 mg/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments). A small hole was drilled on one side of the skull and the guide cannula (8 mm long, 0.3 mm in diameter) was positioned at the following coordinates measured from bregma, according to the atlas of Franklin and Paxinos (1997): AP 0 mm, Lat ± 1 mm, and DV−2 mm from skull. Dental cement was used to fix the guide cannula to the skull (polycarboxylate, Sigma, France) and, to prevent occlusions; a stainless steel stylet was inserted into the guide cannula and fixed in the cement. After surgery, the animals were allowed to recover for at least 1 week during which time they were gently handled daily by the experimenter to minimize the stress associated with manipulation of the animals throughout the experiments.

2.3. Drugs

1DMe ([D-Tyr^1(NMe)Phe^3]NPFF) was prepared by manual solid-phase synthesis according to [34]. The purity of the final product was assessed by analytical high-pressure liquid chromatography (HPLC) and its integrity was checked by electro-spray mass spectrometry on a TSQ 700 (Finnigan-Mat, San José, CA).

Dilutions of 1DMe were performed in 0.9% NaCl which was used as a control for possible side effects of injections. Morphine hydrochloride (10 mg/kg, i.p.) was obtained from Francopia (Gentilly, France).

2.4. Intracerebral infusion

About 1 week after surgery, the mice were manually restrained and the stylet was removed. The injector tip was inserted into the guide cannula and protruded 1 mm beyond the tip of the guide cannula. It was connected to a 10 μl Hamilton microsyringe by flexible polyethylene tubing. The syringe, mounted on a Bioblock infusion pump, delivered a volume of 2 μl at a