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Effect of nociceptin/orphanin FQ on the rewarding properties of morphine

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Abstract

The present study investigated the effect of nociceptin/orphanin FQ, the endogenous ligand of the opioid receptor-like 1 (ORL1) receptor, on the rewarding properties of morphine in the place conditioning paradigm. Intracerebroventricular (i.c.v.) injections of nociceptin/orphanin FQ, 500 or 1000 (but not 250) ng/rat, abolished conditioned place preference induced by subcutaneous (s.c.) injections of morphine (3 mg/kg). These doses of nociceptin/orphanin FQ induced neither place aversion nor preference per se. The same doses did not modify the rat performance in the Morris water test, suggesting that they do not disrupt spatial learning and memory. Moreover, these doses of nociceptin/orphanin FQ did not modify the development of morphine-induced locomotor sensitization, suggesting that they do not interfere with sensitization processes to morphine. The present results confirm and extend previous reports that nociceptin/orphanin FQ is able to abolish morphine-induced conditioned place preference, and raise interest for the possible role of nociceptin/orphanin FQ and ORL1 receptors in the control of opiate abuse.

Keywords: Nociceptin; Orphanin FQ; ORL1 receptor; Morphine; Conditioned place preference; Spatial learning; Locomotor sensitization

1. Introduction

Nociceptin, also referred to as orphanin FQ is the endogenous ligand of the opioid receptor-like 1 (ORL1) receptor (Meunier et al., 1995; Reinscheid et al., 1995). It is a 17-amino acid neuropeptide, structurally related to the opioid peptide dynorphin A (Nothacker et al., 1996; Reinscheid et al., 1998).

Activation of ORL1 receptors by nociceptin/orphanin FQ results in a sequence of intracellular events similar to those observed after stimulation of μ, κ, and δ opioid receptors. The ORL1 receptor is negatively coupled with adenylyl cyclase; moreover, its stimulation inhibits Ca^{2+} current in a pertussis toxin sensitive manner and activates inwardly rectifying K^+ channels (Henderson and McKnight, 1997; Meunier, 1997). However, nociceptin/orphanin FQ does not bind to opioid receptors, nor do opioid peptides activate the ORL1 receptor (Reinscheid et al., 1996).

Nociceptin/orphanin FQ has been proposed to be a functional "antiopioid peptide", since it can block opioid-induced supraspinal analgesia (Mogil et al., 1996a,b; Morgan et al., 1997). This finding has raised interest also for the effect of this "antiopioid peptide" on the motivational properties of morphine.

In this regard, a preliminary study of our group (Angeletti et al., 1999) and the study of other authors (Murphy et al., 1999a,b) has reported that nociceptin/orphanin FQ is able to inhibit morphine-induced conditioned place preference. The conditioned place preference paradigm is widely used to assess the rewarding properties of drugs, and to study the biological mechanisms subserving them (Swerdlow et al., 1989). In this paradigm, the unconditioned rewarding stimulus is paired with environmental (visual, tactile) cues, which, by association, acquire incentive properties. Morphine evokes a marked conditioned place preference in rats, which reflects its abuse potential in humans.

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The present study was aimed at further investigating the effect of nociceptin/orphanin FQ on morphine-induced conditioned place preference. First, low intracerebroventricular (i.c.v.) doses of nociceptin/orphanin FQ, 250–1000 ng/rat, were tested in order to assess the selectivity of the effect of nociceptin/orphanin FQ on morphine-induced conditioned place preference. In our previous study, these doses abolished conditioned place preference induced by alcohol in alcohol-preferring rats (Ciccocioppo et al., 1999); on the other hand, they are lower than those necessary for the effects of the peptide on morphine-induced analgesia (Meunier, 1997), on learning and memory processes (Sandin et al., 1997) and on feeding behavior (Pomonis et al., 1996; Polidori et al., 2000). Second, since nociceptin/orphanin FQ has been reported to impair spatial learning (Sandin et al., 1997; Manabe et al., 1998; Mamiya et al., 1999), the present study investigated whether its effects on place conditioning might be secondary to interference with spatial learning. Lastly, since it has been proposed that sensitization to morphine may influence its rewarding properties, as measured by place conditioning paradigm (Lett, 1989; Carlezon et al., 1999; Robinson and Berridge, 1993), the present study evaluated whether nociceptin/orphanin FQ blocks the development of locomotor sensitization to morphine.

2. Materials and methods

2.1. Animals

Male adult Wistar rats (Charles River), weighing 300–350 g, were employed. Except for the sensitization study, in which animals were housed in groups of three, rats were individually housed in a room at the temperature of 20–22°C, humidity of 45–55%, under a 12:12 h light/dark cycle (lights off at 10:00 a.m.). They were offered free access to food pellets and tap water ad libitum throughout the study. Animal testing was carried out according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals, and to the European Community Council Directive for Care and Use of Laboratory Animals. Before the experiments, rats received two sham subcutaneous (s.c.) and (i.c.v.) injections to make them familiar with drug administration procedures.

2.2. Surgery

For intracranial surgery, with the exception of the sensitization study in which rats were prepared under halothane anaesthesia (1.5–2.0%), rats were anaesthetised by intramuscular injection of 100–150 μl/100 g body weight of a solution containing ketamine (86.2 mg/ml) and acepromazine (1.3 mg/ml). A guide cannula for injections into the lateral cerebroventricle was stereotaxically implanted and cemented to the skull. The following coordinates were used for the guide cannula: AP = 1 mm behind the bregma, $L = 1.8$ mm from the sagittal suture, $V = 2$ mm from the surface of the skull. Drug injections were made by means of a stainless-steel injector 2.5 mm longer than the guide cannula, so that its tip protruded into the lateral ventricle (Paxinos and Watson, 1986). Validation of cannula placement was done by histological analysis after completion of the experiments.

2.3. Drugs

Nociceptin/orphanin FQ (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asp-Glu) was synthesised at the Department of Pharmacological Sciences and Biotechnology Centre, University of Ferrara, Italy. It was dissolved in sterile isotonic saline and given by i.c.v. injection in a volume of 1 μl/rat. Morphine sulphate, provided by the National Institute of Drug Abuse, was dissolved in sterile water and injected subcutaneously.

2.4. Conditioned place preference

The apparatus used for place conditioning consisted of a wooden box divided by a guillotine door into two square-base compartments ($40 \times 30 \times 30$ cm), one with white striped grey walls and a white floor, the other with black striped grey walls and black striped white floor. The box was placed in a dimly illuminated room with white noise. Place conditioning sessions were run from 10:00 to 12:00 a.m. (after the beginning of the dark phase).

The number of white and black strips in the walls and in the floor of the cage has been adjusted in preliminary experiments so that the animal had no preference for either compartment. Then, a 15-min trial (i.e. 900 s) was carried out with a group of 18 rats to investigate whether they exhibited side preference. The time spent under basal conditions in the two compartments was 436 ± 22 s for the white striped compartment and 464 ± 24 s for the black striped compartment; the two values were not significantly different. Thus, an unbiased procedure (Swerdlow et al., 1989) for place conditioning was used, in which rats were randomly assigned to one of the two box compartments. For each experimental group, half of the rats was conditioned with morphine in the white striped compartment, while the other half was conditioned in the black striped side of the box.

The conditioning phase consisted of 6 days. On alternate days, rats had three conditioning sessions with s.c. injections of 3 mg/kg of morphine and three conditioning sessions with morphine vehicle. Nociceptin/orphanin FQ or its vehicle was given by i.c.v. injection 5 min before the s.c. treatment. One group of eight rats received both i.c.v. and s.c. vehicles and served as a control. Other four groups of animals were conditioned with s.c. morphine. One

group of 10 rats was treated with i.c.v. vehicle on the six conditioning sessions; the other three groups of rats (n = 9–11/group) received 250, 500 or 1000 ng/rat of nociceptin/orphanin FQ in the morphine conditioning sessions and i.c.v. vehicle in the vehicle conditioning sessions. On the first day of training, half of the animals received s.c. morphine and the other half s.c. vehicle. During conditioning, the guillotine door was closed and rats were confined for 1 h in one compartment of the box.

To test the place conditioning effect of nociceptin/orphanin FQ alone, other four groups of rats were employed. As described above, every other day for 6 days, three groups of animals (n = 7–8/group) received three conditioning sessions with i.c.v. nociceptin/orphanin FQ, 250, 500 or 1000 ng/rat and three conditioning sessions with i.c.v. vehicle. The fourth group of rats (n = 7) was conditioned with i.c.v. vehicle and served as a control.

The day following the last conditioning session, rats were allowed to explore the entire box for 15 min and the time spent in each compartment was measured. Place preference score (referred to as Δ time) was obtained by subtracting from the time spent in the drug-paired compartment the time spent in the other compartment. Positive Δ values indicate development of preference, while negative values indicate development of aversion for the paired compartment. To measure Δ time in controls, for each animal, an arbitrary paired compartment was defined. The Δ time values were submitted to statistical analysis.

2.5. Morris water test

A blue circular pool (diameter 150 cm) was used, equipped with a hidden escape circular platform (17 cm of diameter) made of transparent glass. The pool was filled with tap water (t = 22–23°C) and the escape platform, that was in off-centre position, was located 1 cm below the water surface. Rats were given four trials per day for 6 consecutive days. In each trial, the rat was placed in water facing the pool wall at one of the four selected starting positions (north, south, east or west). Everyday, the order of the starting position was changed. After reaching the platform, the rat was allowed to remain on it for 30 s. For rats that did not find the platform within 60 s, the trial was terminated and a maximum score of 60 s was assigned; they were led by hand to the platform and were permitted to remain on it for 30 s. Animal performances were monitored, the escape latency and the swimming length were recorded. Five minutes before the beginning of the four consecutive swimming trials, two groups of eight rats were i.c.v. injected with nociceptin/orphanin FQ, 500 or 1000 ng/rat. Another group of eight animals received i.c.v. injections of vehicle and served as a control. From day 1 to day 6, rats were tested after drug or vehicle treatment. On day 7, animals were tested in drug-free conditions. The mean values of the four daily trials were used for statistical analysis.

2.6. Locomotor sensitization

Locomotor activity was measured in 16 identical metal wire hanging cages, with a base of 36 × 25 cm, and 20 cm high. Each cage contained two sets of infrared emitter-detector photocells positioned along the long axis 1 cm above the grid floor and 8 cm from the front and back of the cage. Movements within the cages produced photocell interruptions, which were automatically recorded at 10-min intervals (number of beam breaks) by an IBM-compatible computer. Daily sessions consisted of placing each animal in one of the locomotor cages and monitoring its locomotor activity.

A week after intracranial surgery, for 2 days, rats were placed for 1 h/day in the locomotor activity cage to make them familiar with the testing environment. The locomotor activity score of day 2 was used to divide the animals into four different groups with similar baseline. On day 3, the experiment started. Every other day, for four times, rats were placed in the locomotor activity cages for a 3-h session. For the first hour, rats were allowed to explore the cage, to reduce the locomotor reactions resulting from the impact with a new environment. Immediately after, the animals were returned to their home cages and were injected with drugs. One group of rats (n = 7) was treated with i.c.v. nociceptin/orphanin FQ vehicle followed by s.c. morphine (3 mg/kg). The second (n = 7) and the third groups (n = 6) were injected with i.c.v. nociceptin/orphanin FQ (1000 ng/rat) followed by s.c. morphine (3 mg/kg) or its vehicle, respectively. The last group (n = 6) received only vehicles and served as a control. Following drug treatments, rats were placed again in the cages and their locomotor activity was registered for 2 h. A week after the last treatment day, rats were placed again in the locomotor activity cages and the effect of a challenging drug treatment was evaluated. The first group received i.c.v. nociceptin/orphanin FQ vehicle plus s.c. morphine, the second and third groups received i.c.v. nociceptin/orphanin FQ plus s.c. morphine or s.c. morphine vehicle, respectively; the fourth group received i.c.v. and s.c. vehicles.

2.7. Statistical analysis

Data are reported as means ± S.E.M. The place conditioning data were analysed by one-way analysis of variance with between-groups comparisons. Post-hoc comparisons were carried out by means of the Newman–Keuls test. Data obtained in the Morris water task and the locomotor sensitization experiments were analysed by means of two-way analysis of variance with between-groups comparisons for drug treatment and within-groups

comparisons for time (treatment day). Post-hoc comparisons were carried out by means of the Dunnett's test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Effect of nociceptin / orphanin FQ on morphine-induced conditioned place preference

The analysis of variance revealed a statistically significant treatment effect [$F(4,41) = 25.95$; $P < 0.01$]. Post-hoc comparisons revealed a significant increase in the time spent in the drug-paired compartment following morphine administration ($P < 0.01$). Pretreatment with nociceptin/orphanin FQ, 500 or 1000 ng/rat, completely abolished this effect of morphine (Fig. 1A). No effect was observed after injection of 250 ng/rat.

On the other hand, no effect on place conditioning was observed when nociceptin/orphanin FQ was given in the conditioning paradigm in the absence of morphine treatment [$F(3,26) = 0.58$; $P > 0.05$] (Fig. 1B).

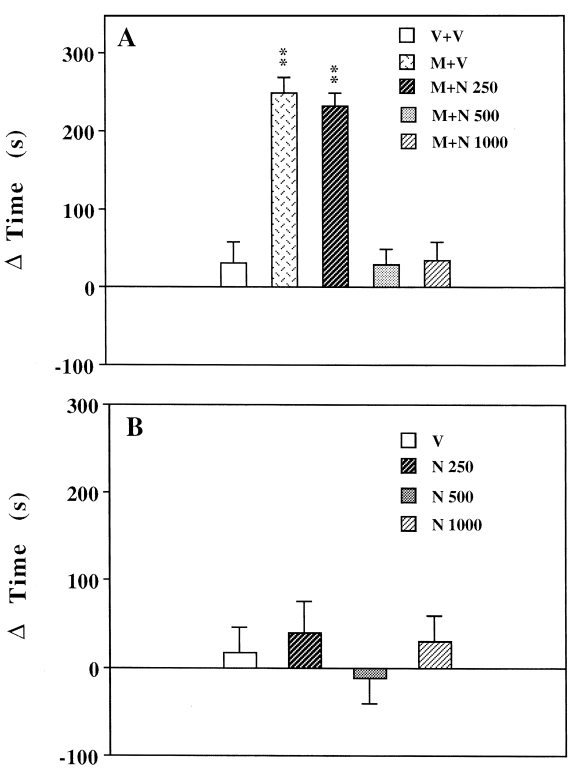

Fig. 1. Panel A shows the effects on place conditioning of s.c. injections of 3 mg/kg of morphine after i.c.v. pretreatment with either vehicle (M+V) or nociceptin/orphanin FQ, 250 (M+N 250), 500 (M+N 500) or 1000 ng/rat (M+N 1000). Panel B shows the effect on place conditioning of i.c.v. injections of either vehicle (V) or nociceptin/orphanin FQ, 250 (N 250), 500 (N 500) or 1000 ng/rat (N 1000). Δ Time is obtained by subtracting to the time spent in the drug-paired compartment that spent in the other compartment. Values are means ± S.E.M. of 8–11 subjects/group. Difference from controls: $^{**}P < 0.01$; where not indicated, difference was not statistically significant.

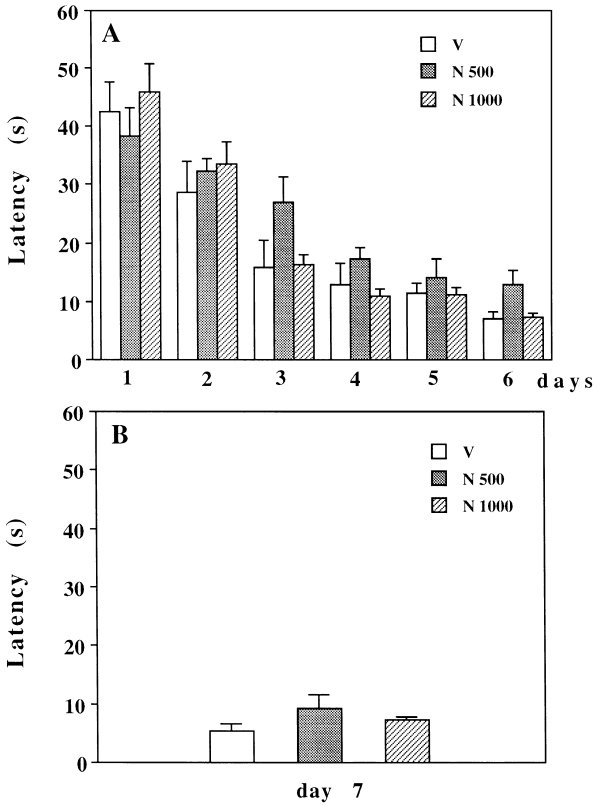

Fig. 2. Panel A shows the effect on spatial navigation in the Morris water test in 6 consecutive days of i.c.v. injections of either vehicle (V) or nociceptin/orphanin FQ, 500 (N 500) or 1000 ng/rat (N 1000). Panel B shows the swimming performance of the same groups of rats in drug-free conditions on day 7. Data are expressed as mean latency ± S.E.M. (time average of the four trials per session) of eight subjects per group. Difference from controls was not statistically significant.

3.2. Effect of nociceptin / orphanin FQ in the Morris water test

The analysis of variance revealed no statistically significant treatment effect [$F(2,21) = 0.95$; $P > 0.05$] (Fig. 2A). In addition, the swimming length measured in the two groups of rats injected with nociceptin/orphanin FQ did not differ from that of controls (data not shown).

In analogy with the experimental conditions used in the place conditioning experiments, the rats' performance in the Morris water task was also evaluated under drug-free conditions on day 7. The analysis of variance revealed no effect of treatment [$F(2,21) = 1.32$; $P > 0.05$] (Fig. 2B).

3.3. Effect of nociceptin / orphanin FQ on morphine-induced locomotor sensitization

A progressive increase in locomotor activity was observed as a result of repeated s.c. morphine injections. The statistical analysis revealed a significant treatment effect [$F(3,23) = 8.46$; $P < 0.01$] and a significant time effect [$F(3,9) = 8.63$; $P < 0.001$]. The locomotor activity of rats

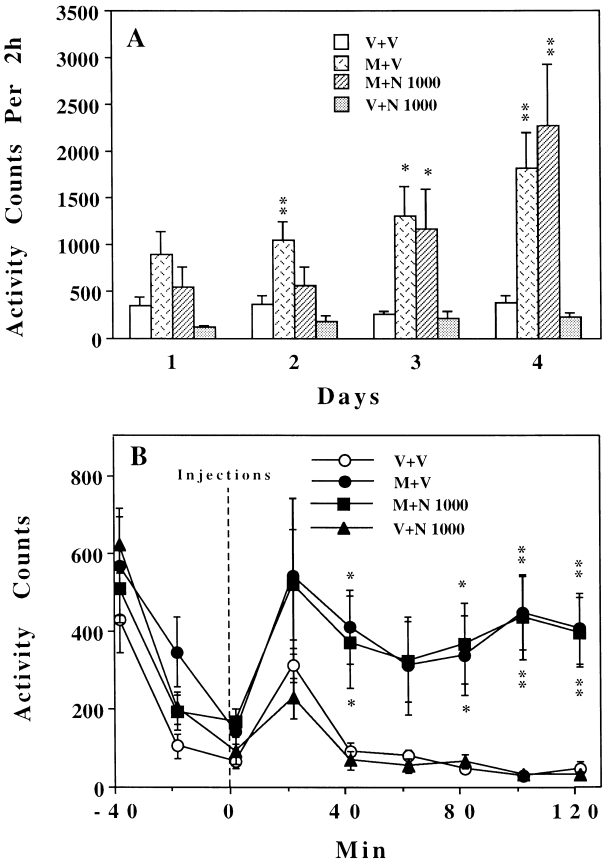

Fig. 3. Panel A shows the effects of i.c.v. nociceptin/orphanin FQ on the development of locomotor sensitization induced by repeated (four times on alternate days) s.c. injections of morphine, 3 mg/kg. Data are expressed as activity counts (number of beam brakes) in the 2-h test. Panel B shows the effect of i.c.v. nociceptin/orphanin FQ on locomotor response to morphine at 7 days after the last sensitization treatment with morphine 3 mg/kg. Data are expressed as activity counts per 20-min intervals. Values are means ± S.E.M. of 6–7 subjects per group. Difference from controls: $^{**}P < 0.01$ or $^{*}P < 0.05$; where not indicated, difference was not statistically significant.

treated with nociceptin/orphanin FQ and morphine vehicle did not differ from that of controls. Pretreatment with nociceptin/orphanin FQ did not block the effect of morphine (Fig. 3A).

Seven days after the last treatment, the effect of a challenge dose of morphine was studied. The analysis of variance yielded a significant effect of morphine treatment [$F(3,23) = 6.37$; $P < 0.01$]. A statistically significant increase in locomotor activity was observed in morphine-treated rats. On the other hand, as shown in Fig. 3B, the increased locomotor activity of morphine-treated rats was not modified following i.c.v. nociceptin/orphanin FQ pretreatment.

4. Discussion

The results of the present study show that nociceptin/orphanin FQ blocks the rewarding effects of morphine in the place conditioning paradigm at i.c.v. doses of 500 and 1000 ng/rat, but not 250 ng/rat. These data confirm the findings of a preliminary study of our group (Angeletti et al., 1999), in which a biased design (Swerdlow et al., 1989) was used for place conditioning. The present findings are also in accordance with the results of Murphy et al. (1999a); however, in their study, higher doses of nociceptin/orphanin FQ, 5000–20,000 ng/rat, were tested. A recent study of our group has shown that nociceptin/orphanin FQ inhibits ethanol-induced conditioned place preference in genetically selected alcohol-preferring rats at the same i.c.v. doses that in the present study abolished morphine-induced conditioned place preference (Ciccocioppo et al., 1999). Interestingly, these doses are lower than those necessary to observe in rats the effects of nociceptin/orphanin FQ on morphine-induced analgesia (Meunier, 1997), on learning and memory processes (Sandin et al., 1997), on feeding behavior (Pomonis et al., 1996; Polidori et al., 2000).

Moreover, the present results confirm that nociceptin/orphanin FQ does not induce conditioned place preference or aversion on its own, supporting the idea that this peptide is devoid of intrinsic motivational properties (Ciccocioppo et al., 1999; Devine et al., 1996). This may represent an interesting difference between nociceptin/orphanin FQ and opioid receptor antagonists, such as naloxone or naltrexone. These antagonists abolish, like nociceptin/orphanin FQ, morphine-induced conditioned place preference, but they produce aversion in the place conditioning paradigm (Acquas et al., 1989; Dymshitz and Lieblich, 1987; Parker and Rennie, 1992; Williams and Woods, 1999), while nociceptin/orphanin FQ does not.

Administration of nociceptin/orphanin FQ into the hippocampus has been reported to impair spatial learning in rats (Sandin et al., 1997). Moreover, knockout mice for ORL1 receptors perform better in a Morris water task (Mamiya et al., 1999; Manabe et al., 1998). Since impairment of spatial learning and memory may interfere with place conditioning, it was evaluated whether nociceptin/orphanin FQ influences spatial learning in the Morris water test at the i.c.v. doses of 500 or 1000 ng/rat that blocked conditioned place preference. However, the results obtained suggest that these doses do not influence spatial learning or memory processes.

Induction of locomotor sensitization is a phenomenon common to major classes of drugs of abuse (Robinson and Berridge, 1993). Evidence has been provided that pre-exposure to drugs which induce locomotor sensitization can also increase their rewarding impact (Horger et al., 1990; Lett, 1989); accordingly, repeated administrations of morphine have been shown to induce locomotor sensitization and to increase its rewarding properties (Carlezon et al., 1999; Lett, 1989). However, the results of the present study suggest that nociceptin/orphanin FQ does not block the development of morphine-induced locomotor sensitization, suggesting that its effect on morphine-induced condi-

tioned place preference is not related to interference with sensitization processes.

A large body of evidence suggests that drugs of abuse, including morphine, potently increase dopamine release in the nucleus accumbens (Di Chiara, 1995; Spanagel and Weiss, 1999). Facilitation of dopamine neural activity in this nucleus is important for the establishment of conditioned place preference (Di Chiara, 1995; Spanagel and Weiss, 1999), and drugs that block dopamine release or dopamine receptors can inhibit the place conditioning effects of opioids (Maldonado et al., 1997; Shippenberg et al., 1993). Interestingly, a microdialysis study in freely moving rats has shown that i.c.v. nociceptin/orphanin FQ abolishes morphine-induced dopamine release in these brain areas, without modifying extracellular dopamine levels under basal conditions (Di Giannuario et al., 1999). On the other hand, in anaesthetised rats, perfusion of nociceptin/orphanin FQ in the ventral tegmental area reduces dopamine levels in the nucleus accumbens (Murphy et al., 1999b). The dopaminergic neuronal pathway from the ventral tegmental area to the nucleus accumbens is a key component of the brain reward system and may mediate, at least in part, the rewarding properties of opioids (Herz, 1998; Koob et al., 1998; Spanagel et al., 1990). Nociceptin/orphanin FQ immunoreactive cells are mostly intermediate-size interneurons (Ikeda et al., 1998), and the ventral tegmental area shows relatively high levels of nociceptin/orphanin FQ (Houtani et al., 1996; Neal et al., 1999) and of ORL1 receptors (Anton et al., 1996; Florin et al., 1997; Ikeda et al., 1998; Sim and Childers, 1997). In the ventral tegmental area, nociceptin/orphanin FQ cells could modulate the activity of dopaminergic neurons projecting to the nucleus accumbens, and may reduce the ability of morphine to stimulate these neurons, thus, reducing its rewarding properties.

In conclusion, the present study shows that nociceptin/orphanin FQ acts as an "antiopioid" peptide to abolish the rewarding properties of morphine in the place conditioning paradigm at i.c.v. doses lower than those required for other central effects. Moreover, compared to direct opioid antagonists, such as naloxone or naltrexone, which are known to induce place and taste aversion (Parker and Rennie, 1992; Dymshitz and Lieblich, 1987), nociceptin/orphanin FQ does not possess motivational effects per se. Lastly, while opioid receptor antagonists have anxiogenic properties (Agmo and Belzung, 1998; De Cabo de la Vega et al., 1995), recent studies demonstrated that nociceptin/orphanin FQ exerts anxiolytic effects and can inhibit stress-induced drug-seeking behavior (Martin-Fardon et al., 2000).

Altogether, these findings support the view that drugs directed at central ORL1 receptors may represent a novel and promising approach to the treatment of drug abuse that could offer interesting advantages over direct opioid antagonists.

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