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Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat

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1 The present study was designed to investigate further the effects of the newly discovered orphanin FQ (OFQ) – the endogenous ligand for the orphan opioid receptor (called, e.g., ORL_1 and LC132) – on pain modulation in the rat. We used the tail-flick assay as a nociceptive index.

2 When injected into a cerebral ventricle, OFQ (4 fmol – 10 nmol) has no effect on basal tail-flick latency by itself at any dose, but dose-dependently antagonizes systemic morphine analgesia (400 fmol – 50 nmol).

3 Injected intrathecally, OFQ (3 and 10 nmol) displayed an analgesic effect without producing motor dysfunction, and potentiated morphine analgesia (1 and 10 nmol).

4 The anti-opioid effect of OFQ in rat brain and the high level of expression of LC132/ORL_1 receptor in the locus coeruleus indicated a possible role of OFQ in the precipitation of opiate withdrawal symptoms. However, no such precipitation was observed by OFQ in morphine-dependent rats.

Keywords: Analgesia; anti-opioid; ORL_1/LC132; orphanin FQ/nociceptin; morphine withdrawal

Introduction

Unlike three other opioid receptors (μ, δ and κ) that were cloned after the discovery of their ligands, the orphan opioid receptor (Bunzow *et al*., 1994; Mollereau *et al*., 1994; Wang *et al*., 1994) was discovered before the isolation of its endogenous ligand (Meunier *et al*., 1995; Reinscheid *et al*., 1995). This receptor, named LC 132 (Bunzow *et al*., 1994) or ORL_1 (Mollerau *et al*., 1994), shows approximately 50% identity with μ-, δ-, or κ-opioid receptors, belongs to the superfamily of G-protein coupled receptors, and inhibits adenylate cyclase, all suggesting that it is an opioid receptor, despite the fact that typical opioid ligands bind it with very low affinity. Therefore, it has been considered an 'orphan receptor', until last year two laboratories purified its endogenous agonist, a 17-amino acid peptide named orphanin FQ (OFQ) (Reinscheid *et al*., 1995) or nociceptin (Meunier *et al*., 1995).

The ORL_1/LC132 receptor is distributed widely in brain, as mapped by the *in situ* hybridization (Bunzow *et al*., 1994; Mollereau *et al*., 1994) and immunohistochemistry (Anton *et al*., 1996). The high level expression in brain areas, such as amygdala, hypothalamus, periaqueductal gray(PAG), dorsal raphe nucleus and spinal cord dorsal horn suggested its involvement in nociceptive processing. Interestingly, although OFQ is structurally similar to the opioid peptides, and shows the same intracellular effects as the opioids in lowering the adenosine 3′, 5′-cyclic monophosphate (cyclic AMP) level (Meunier *et al*., 1995; Reinscheid *et al*., 1995), decreasing calcium conductance (Conner *et al*., 1996) and increasing potassium conductance (Vaughan & Christie, 1996), it was shown to induce hyperalgesia rather than analgesia when injected into the cerebral ventricle of mice (Meunier *et al*., 1995; Reinscheid *et al*., 1995). However, more recently Mogil *et al*. (1996b) have shown that the apparent hyperalgesic effect of OFQ was in fact a reversal of opioid-mediated analgesia related to the i.c.v. injection procedure (which in mice proceeds directly through the skull into the ventricle). Their suggestion that OFQ has anti-opioid properties in the mouse was further supported by findings that OFQ blocks supraspinal (but not spinal) morphine, [D-Ala^2, N-Me-Phe^4-Gly-ol]enkephalin (DAMGO), [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and U-50, 488H analgesia (Grisel *et al*., 1996; Mogil *et al*., 1996a,b).

The aim of the present study was to observe the effects of OFQ, injected either intracerebroventricularly (i.c.v.) or intrathecally (i.t.) in the rat, on systemic morphine-induced analgesia as tested by the tail-flick assay. The results indicate that OFQ antagonizes analgesia in brain and potentiates morphine analgesia in the spinal cord. The underlying mechanisms of this bidirectional modulatory effect remain to be elucidated.

Methods

Subjects

Adult male Wistar rats weighing 220 – 280 g were provided by the Animal Centre in Beijing Medical University. The implantation of i.c.v. cannulae was performed stereotaxically under 10% chlorohydrate anaesthesia (0.3 ml 100 g^{-1} body weight). Stainless steel tubing of 0.8 mm outer diameter was fixed on the skull at coordinates A 5.4, L 1.5, H 3.0 mm, according to the Pellegrino *et al*. (1979) system A. Experiments with i.c.v. injection were started 3 – 4 days after the operation. The injection volume was 10 μl, administered over 10s.

Intrathecal catheterization was performed under 10% chlorohydrate anaesthesia (0.3 ml 100 g^{-1} body weight) according to Yaksh and Rudy (1976). PE-10 tubing of 13 cm in length was introduced through the incised atlanto-occipital membrane and the dura down to the subarachnoid space for 7.5 cm to reach the upper border of the lumbar enlargement. Experiments with i.t. injection were started 1 day after the operation. The injection volume was 10 μl, administered over 10 s followed by flushing with normal saline (NS, 0.9% NaCl) at the same rate.

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Nociceptive test

Experiments were performed at room temperature ($20 \pm 1^{\circ}C$). Nociception was tested by radiant heat tail-flick test (Ren & Han, 1979). Rats were kept in a plastic restrainer with hindlimbs and tail extending. Focused light from a 12.5 W projection bulb was applied to the lower 1/3 of the tail and the latency of tail flick reaction (TFL) was recorded to the nearest 0.1 s. Values from the first 3 measurements, with an interest interval of 5 min, were averaged as the basal TFL, which was usually in the range of 4–6 s. TFLs obtained in subsequent tests were expressed as percentage changes from the basal level, with a cutoff limit of 150% in order to avoid unnecessary skin damage. In every experiment of tail flick test, we measured tail temperature, and if it was changed more than 1°C (compared with room temperature), the tail flick latency would be corrected by a coefficient of -0.25 s/°C (Ren & Han, 1979).

Evaluation of motor function

(1) Neuroscore (Long *et al*., 1989). Level 0: total paralysis of hindlimbs; level 1: severe paralysis of hindlimbs allowing a little movement, but cannot support the body or crawl; level 2; moderate paralysis of hindlimbs allowing crawling, but cannot support the body to walk; level 3: slight paralysis of hindlimbs, walks lamely, level 4: no paralysis of hindlimbs, walks freely.

(2) Sliding plate test (Rivlin & Tator, 1977). An inclined plane was used to evaluate motor function. The rat was placed on a sloping board with a changeable angle. The greatest slope of the board that the rat could maintain itself on was the motor function score. Typically, a saline-treated rat could maintain its position at a slope of 53°, values from first three measurements were averaged as the basal angle. Angles obtained in subsequent measurements were expressed as angle change from the basal level.

Morphine dependence and withdrawal precipitation

Rats were injected subcutaneously (s.c.) 3 times a day (06 h, 15 h, 22 h) for 7 days with increasing dose of morphine (5, 10, 20, 40, 50, 60, 70 mg kg^{-1}). On the 6th and 8th day (06 h), rats received an i.c.v. injection of OFQ (2, 10 or 50 nmol), or an i.p. injection of naloxone (1 mg kg^{-1}) as a positive control. Classical withdrawal symptoms, including for quantified signs (escape attempts, wet-dog shakes, penile licking, weight loss) and one binary sign (teeth chattering), were evaluated for 45 min after injection. The results obtained on day 6 and 8 of each rat were averaged.

Chemicals

Orphanin FQ (OFQ, full sequence of 17 amino acids) was a product of Phoenix Pharmaceuticals, Inc. (U.S.A.). Morphine HCl is a product of Qinghai Drug House (China). Naloxone HCl was obtained from Sigma (U.S.A.). All drugs were dissolved in sterile NS.

Statistical analysis

The data are expressed as the mean ± s.e. Group differences were assessed by two-way analyses of variance (ANOVAs) followed by Newman-Keuls post-hoc test. $P < 0.05$ was taken as the significant level of difference.

Results

The effect of i.c.v. OFQ on morphine analgesia

Naive Wistar rats ($n = 9 \sim 11$ per group) were injected s.c. with 5 mg kg^{-1} morphine. TFL was measured before and at 10 min intervals after the s.c. injection. By measurements taken at 20 min, we identified analgesic rats (those with a percentage increase over 100%) to be given i.c.v. injection of normal saline (NS) or OFQ (4, 40 or 400 fmol; 4, 40 or 400 pmol; 10 or 50 nmol), and retested every 10 min for 100 min. The results are shown in Figure 1. In the NS control group, morphine produced an increase in TFL for more than 60 min. I.c.v. injection of OFQ, ranging from 40 fmol to 50 nmol, produced a dose-dependent reversal of morphine-induced analgesia.

To assess whether OFQ itself affected TFL, OFQ alone (from 4 fmol to 10 nmol) was injected i.c.v., and TFL was tested before and at 10-min intervals after the injection for 120 min. No significant difference in TFL was found between the OFQ group (at any dose) and the NS group, as can be seen in Figure 2.

The effect of i.t. OFQ on morphine analgesia

Cumulative s.c. injection of morphine (1, 1, 2, 4, 8 mg kg^{-1} at 30-min intervals) was used to induce an increasing analgesic effect. OFQ was administered i.t. 10 min after the first injection of morphine. Results are shown in Figure 3. The cumulative dose-effect curve for morphine was shifted to the left by 1 and 10 nmol OFQ ($P < 0.05$), indicating that i.t. OFQ potentiated the morphine-induced analgesia.

The effect of i.t. OFQ on basal TFL and motor function

I.t. injection of OFQ produced a dose-dependent increase in TFL, as shown in Figure 4. TFL was increased by 3 nmol and

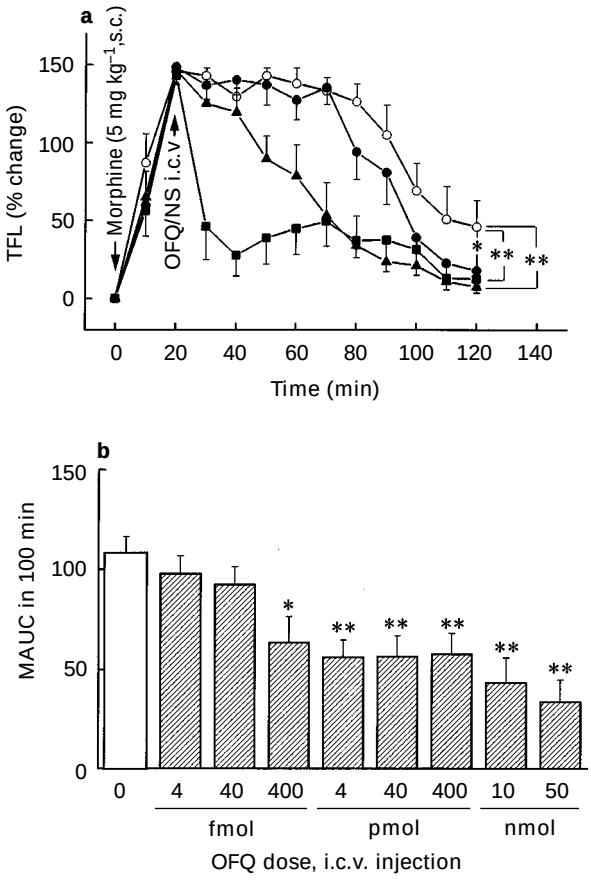

Figure 1 Effect of i.c.v. OFQ on morphine analgesia in rats. (a) Dose-dependent reversal of morphine analgesia by OFQ at (●) 40 fmol ($n = 11$), (▲) 400 fmol ($n = 10$) and (■) 50 nmol ($n = 9$); (○) normal saline (NS) ($n = 11$). (b) Morphine analgesia expressed as the mean area under the curve (MAUC) over the 20 ~ 120 min period of the testing session in the presence (hatched columns) and absence (open column) of OFQ; $n = 9-11$. Symbols (columns) represent mean and vertical lines s.e. $^{*}P < 0.05$ $^{**}P < 0.01$ compared with NS (normal saline) group, tested by ANOVA followed by Newman-Keuls post-hoc test.

10 nmol OFQ, reached a peak at 10 min after i.t. injection, declined quickly thereafter. The 1 nmol OFQ dose had no effect on basal TFL (Figure 4a). Figure 4b shows the mean area under the curve (MAUC) over total testing session. The effects of 1, 3 and 10 nmol ORQ revealed a good dose-effect relationship ($r=0.9964$).

In order to evaluate whether the effect of OFQ was secondary to motoric impairment, we tested motor function after i.t. injection of OFQ every 10 min for a total of 60 min by the neuroscore (Long *et al*., 1989) and sliding plate (Rivlin & Tator, 1977) tests. Evaluation by neuroscore showed that in all 41 rats given OFQ (1 nmol, $n=9$; 3 nmol, $n=10$; 10 nmol, $n=13$) or NS ($n=9$), only 2 rats given 10 nmol OFQ had slight paralysis of one hindlimb (neuroscore: Level 3) which was not spontaneously reversible (data not shown). In the sliding plate test, no significant angle changes were observed in any group (Figure 5). These results suggested that at or below the dose of 10 nmol, ORQ did not cause apparent motor dysfunction in rats.

OFQ did not precipitate withdrawal symptoms in morphine-dependent rats

In morphine-dependent rats, naloxone (1 mg kg^{-1}, i.p.) precipitated all withdrawal symptoms except penile licking. No such precipitation was observed with any dose of OFQ administered i.c.v. (2, 10 or 50 nmol), as shown in Figure 6. We also noted that 50 nmol OFQ tended to inhibit wet-dog shakes and escape attempts, possibly due to a decease in locomotor activity caused by this high dose of OFQ.

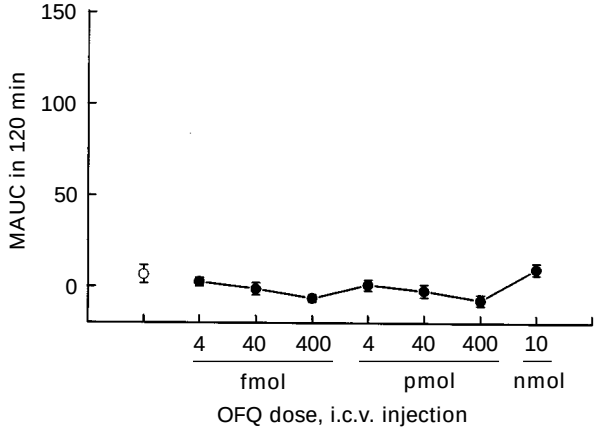

Figure 2 Effect of i.c.v. OFQ on basal tail-flick latency (TFL) in rats. Shown are mean area under the curve (MAUC) over the entire 2 h testing session in the presence (●) and absence (○) of OFQ. Symbols represent mean and vertical lines s.e., $n=8-10$.

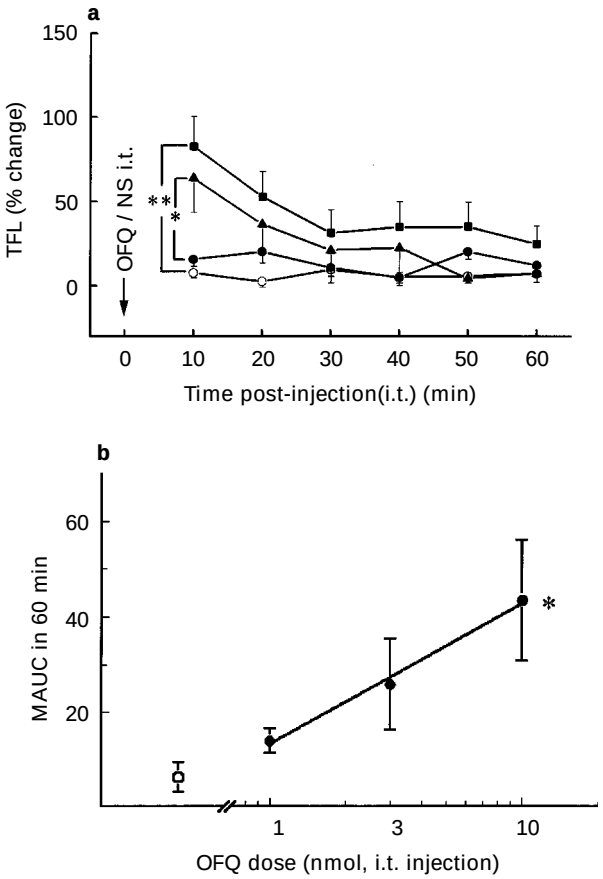

Figure 4 Effect of i.t. OFQ on basal tail-flick latency (TFL) in rats. (a) The dose-effect curves of OFQ during 60 min after i.t. injection at (●) 1 nmol ($n=9$), (▲) 3 nmol ($n=10$) and (■) 10 nmol ($n=13$); (○) normal saline ($n=10$). (b) Data from (a) expressed as mean area under the curve (MAUC) over 60 min. (○) normal saline; (●) OFQ. r (regression coefficient) = 0.9964. Symbols represent mean and vertical lines show s.e. $n=9-13$. $^*P<0.05$, $^{**}P<0.01$, compared with NS (normal saline) group, assessed by ANOVA followed by the Newman-Keuls post-hoc test.

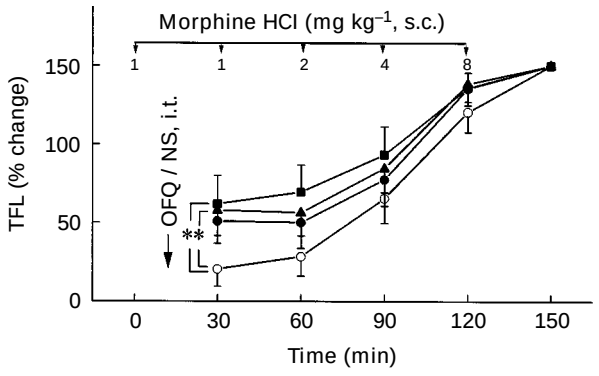

Figure 3 Effect of i.t. OFQ on morphine analgesia in rats. The dose-effect curves after cumulative s.c. injection of morphine (1, 1, 2, 4, 8 mg kg^{-1} every 30 min) are shown. (○) Normal saline (NS) ($n=14$); (●) 0.1 nmol OFQ ($n=13$); (▲) 1 nmol OFQ ($n=15$); (■) 10 nmol ($n=13$). Symbols represent mean and vertical lines s.e. $^*P<0.05$ compared with NS group, tested by ANOVA followed by Newman-Keuls post-hoc test.

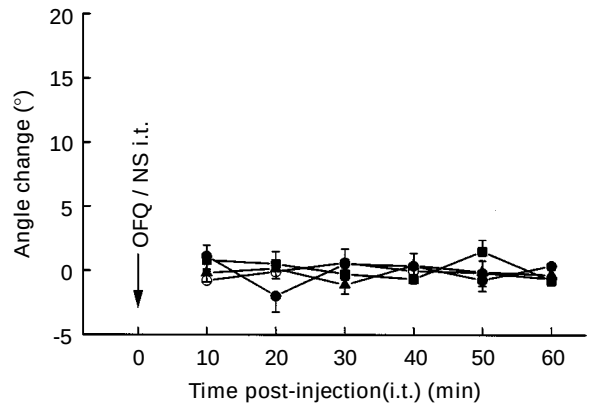

Figure 5 Effect of i.t. OFQ on motor function of rats as assessed by the angle change on the sliding plate test at (●) 1 nmol ($n=9$), (▲) 3 nmol ($n=10$) and (■) 10 nmol ($n=13$); (○) normal saline (NS) ($n=9$). Symbols represent mean and vertical lines show s.e. Tested by ANOVA followed by Newman-Keuls post-hoc test.

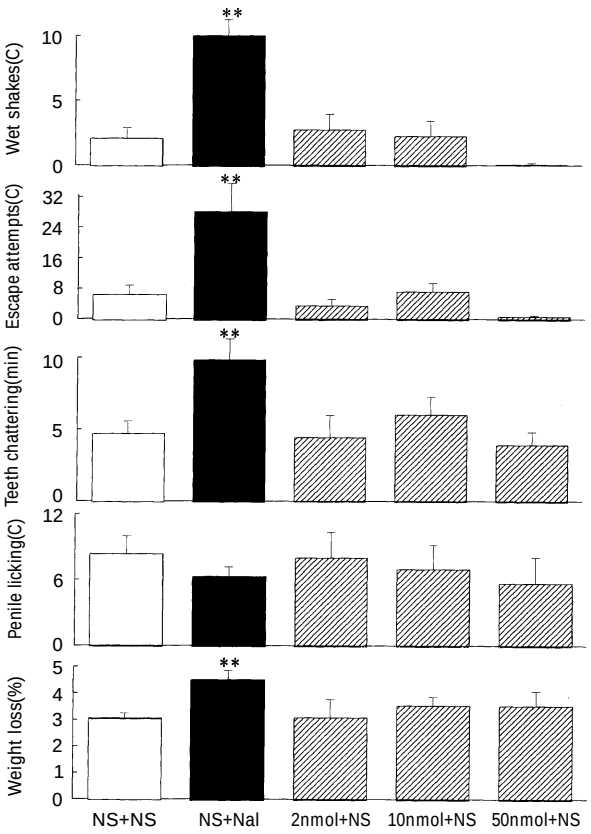

Figure 6 Effect of OFQ (2, 10 or 50 nmol, i.c.v.) or naloxone (Nal, 1 mg kg^{-1}, i.p.) in morphine-dependent rats. Shown are four classic withdrawal signs and weight loss during the whole 45-min observation period in the absence (open columns) and presence of naloxone (solid columns) or OFQ (hatched columns). Columns represent mean ± s.e., $n = 10-12$. $^{**}P < 0.01$, compared with NS (normal saline) group, assessed by ANOVA followed by Newman-Keuls post-hoc test.

Discussion

This study demonstrated that: (1) i.c.v. injection of OFQ, ranging from 40 fmol to 50 nmol, dose-dependently reverses systemic morphine-induced analgesia in rats, while itself producing neither analgesia nor hyperalgesia; (2) i.t. injection of OFQ not only produces analgesia by itself, but also potentiates cumulative morphine-induced analgesia; and, (3) i.c.v. OFQ does not precipitate a withdrawal syndrome in morphine-dependent rats.

Research on morphine-induced analgesia has had a long history, and has been studied in great detail. Systemically administered morphine produces its antinociceptive effect through two synergistic mechanisms: synergy between μ, δ, and/or κ receptors (Sutters *et al*., 1990), the other is synergy between spinal and supraspinal opioid systems (Yeung & Rudy, 1980; Roerig *et al*., 1984; Miyamoto *et al*., 1991). The descending noradrenergic system, in addition to the 5-hydroxytryptaminergic sytem, may be an important component in the spinal/supraspinal interaction (Wigdor & Wilcox, 1987). It is also important to point out that morphine inhibits pain transmission neurones directly (by reducing transmitter release from primary afferent neurones and hyperpolarizing nociceptive dorsal horn neurones) as well as indirectly through pain-modulating systems, involving the midbrain PAG and the rostral ventromedial medulla (RVM), which project to the spinal cord (see, e.g., Basbaum & Fields, 1984).

The site(s) and mechanism of the anti-opioid action of OFQ remain elusive at the present time. OFQ may remain in supraspinal loci after i.c.v. injection (Tseng & Fujimoto, 1984), but the range of its diffusion and the precise site of its action are not clear. Among the regions to which OFQ possibly diffuses, the hypothalamus, PAG, locus coeruleus and raphe complex, all show a high level of expression of the ORL_1/LC132 receptor (Anton *et al*., 1996), affording a possible effect of OFQ. The fact that OFQ has low affinity for other opiate receptors but binds ORL_1/LC132 in a saturable manner and with high affinity (Reinscheid *et al*., 1995) suggests that OFQ needs to bind a specific receptor to produce its actions. Of course, we cannot be sure of this until after the development of a selective and specific antagonist for ORL_1/LC132.

Recently, Mogil and colleagues (1996a,b) demonstrated that i.c.v. OFQ can antagonize systemic morphine analgesia, opioid-mediated stress-induced analgesia, and μ- δ- and κ-mediated analgesia in mice. Our results replicate theirs and extend them to another species, suggesting that OFQ has a functional interaction with the opioid system. These kinds of effects of OFQ are similar to those of other anti-opioid peptides, including cholecystokinin octapeptide (CCK-8) (Han, 1995). However, unlike CCK-8, OFQ shares amino acid homology with the known members of the opioid family, and when acting at the ORL_1/LC132 receptor has the same effect on cyclic AMP levels, calcium conductance and potassium conductance as the opiates. We can thus conjecture that OFQ may have a different anti-opioid mechanism compared to that of CCK-8. OFQ probably exerts its anti-opioid actions indirectly, through the pain-modulation system. Further study is needed to evaluate the effect of OFQ on opioid analgesia in the PAG, the locus coeruleus and raphe complex, all of which show high level expression of the ORL_1/LC132 receptor.

In contrast to our results in brain, it was somewhat surprising when we found the analgesic effect of OFQ in the spinal cord. We showed that OFQ can dose-dependently induce analgesia (but not hyperalgesia) after i.t. injection without producing motor dysfunction. In addition, when administered 10 min after the first s.c. injection of morphine (1 mg kg^{-1}), 1 nmol OFQ potentiated cumulative morphine analgesia. We consider this effect a potentiation because 1 nmol OFQ has no analgesic effect on its own (Figure 4). In the mouse, Grisel *et al*. (1996) have observed no anti-opioid effects but a trend towards potentiation of morphine analgesia by 10 nmol OFQ. They did not, however, observe any analgesic actions of i.t. OFQ, suggesting that some of the effects of OFQ may be species-specific.

Our data suggest that spinally, the analgesic effect of OFQ works only at certain high doses and has a modest and short-lasting action, whereas supraspinally, OFQ works over a large dose range (40 fmol to 50 nmol) and has anti-opioid effects lasting for at least 60 min (Figure 1a). There are some possible explanations for this disparity: (1) OFQ might be degraded more rapidly in the spinal cord; (2) the ORL_1/LC132 receptor might desensitize more quickly; and, (3) in brain, OFQ might produce its effects through the induction of other anti-opioid mechanisms. These possibilities and others remain to be evaluated in the future.

In addition to pain-related areas, ORL_1/LC132 receptor transcripts are highly expressed in limbic system regions such as the cerebral cortex, amygdala and hippocampus (Bunzow *et al*., 1994; Mollereau *et al*., 1994). The euphoric and addictive properties of opiate drugs are considered to be related to the actions of opioid ligands on limbic system function and plasticity. There are also high levels of the ORL_1/LC132 receptors in the locus coeruleus, which is an important site for the expression of opiate withdrawal syndromes (Aghajanian, 1978). These findings suggest to us that OFQ might be involved in morphine tolerance and dependence. Although OFQ did not precipitate a withdrawal syndrome in morphine-dependent rats in the present study, the possible role of OFQ in this field will still be worth studying.

A last point concerns the similarity between OFQ and dynorphin, in both structure and function. Dynorphin also displays a bidirectional modulatory effect on morphine-induced

analgesia (Ren *et al*., 1985). It can act as a neurotransmitter at glutamatergic mossy fibre synapses within the hippocampus, depress synaptic transmission and inhibit the induction and expression of long-term potentiation through activation of κ-opioid receptors (Weisskopf *et al*., 1993). All of these results suggest that OFQ, acting at the ORL_1/LC132 receptor, may play a similar role to dynorphin in modulating pain transmission and synaptic plasticity in the central nervous system. OFQ and dynorphin might represent a class of neuropeptides which would be better designated as 'opioid-modulating peptides' due to their complicated function.

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