

# Orphanin FQ acts as a supraspinal, but not a spinal, anti-opioid peptide

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ORPHANIN FQ (OFQ), the endogenous ligand for the orphan opioid receptor, LC132, was recently isolated and characterized. The anti-opioid role of OFQ in supraspinal pain modulation was demonstrated by our previous observations that intracerebroventricular (i.c.v.) OFQ administration dose-dependently reverses systemic morphine antinociception and opioid-mediated stress-induced antinociception. The present study was designed to evaluate whether OFQ also modulates the antinociceptive actions of morphine in the spinal cord. Immediately after assessment of baseline nociceptive sensitivity on the 49°C tail-withdrawal assay, mice of both sexes were given i.c.v. or intrathecal (i.t.) cocktails of morphine (0, 1, 10 or 50 µg [0-135 nmol]) and OFQ (0 or 10 nmol), and re-tested 15, 30 and 60 min later. OFQ alone did not affect nociceptive sensitivity when administered by either route. Following i.c.v. administration, the antinociception produced by 10 µg morphine was completely reversed by 10 nmol OFQ; antinociception induced by 50 µg morphine was significantly antagonized. In contrast, OFQ was completely ineffective against antinociception induced by i.t. morphine. These findings indicate that the anti-opioid actions of OFQ are restricted to supraspinal central nervous system sites.

**Key Words:** Nociception; LC132 receptor; Morphine; Pain inhibition

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## Introduction

Pharmacological approaches have identified a family of opioid receptors ( $\mu$ ,  $\delta$  and  $\kappa$ ) which, when activated by endogenous opioid peptides or exogenous opiate alkaloids, play a major role in nociceptive modulation (see Ref. 1 for review). Subsequent to the cloning of the  $\delta$  opioid receptor, homology screening was used to clone  $\mu$  and  $\kappa$  receptor cDNAs, as well as the orphan opioid-like receptor LC132<sup>2</sup> (also called ORL-1 and XOR; see Ref. 3). Although LC132 shares considerable sequence homology with the classical opioid receptors (~65% in the putative transmembrane domains), and shows a high level of expression in many brain areas relevant to nociceptive processing,<sup>2</sup> it does not appreciably bind opioid agonists or naloxone<sup>2</sup> (but see Ref. 4).

Recently, the endogenous ligand of the LC132 receptor was purified from rat and porcine brain tissue.<sup>3,5</sup> This heptadecapeptide - FGGFTGARK-SARKLANQ; named orphanin FQ (OFQ)<sup>3</sup> and nociceptin<sup>5</sup> - shares significant homology with opioid peptides ( $\beta$ -endorphin, enkephalins and dynorphins) but shows little affinity for  $\mu$ ,  $\delta$  or  $\kappa$  receptors. When tissue culture cells expressing the OFQ receptor (i.e., LC132) are exposed to OFQ, forskolin-stimulated cAMP production is inhibited.<sup>3,5</sup> OFQ is active

*in vivo*, producing locomotor depression at high concentrations,<sup>3</sup> yet intracerebroventricular (i.c.v.) administration does not produce antinociception.<sup>3,5</sup> Indeed, although it was reported that mice injected with OFQ became more sensitive to pain as measured by the tail-flick<sup>3</sup> and hot-plate<sup>5</sup> assays, we have recently provided evidence that the apparent hyperalgesic effect of OFQ was actually a reversal of opioid-mediated stress-induced antinociception (SIA) associated with the i.c.v. injection procedure.<sup>6</sup> Furthermore, we demonstrated that OFQ acts as a potent and efficacious anti-opioid peptide against exogenous opiates; OFQ dose-dependently reverses antinociception following systemic (5 mg kg<sup>-1</sup>, s.c.) morphine administration<sup>6</sup> and i.c.v. injections of  $\mu$ -,  $\delta$ - and  $\kappa$ -selective agonists (Mogil *et al. Neurosci Lett.* In press (1996)). Since OFQ was administered i.c.v. in these studies it is likely that the observed effects of this peptide were mediated supraspinally. However, morphine is known to exert both spinal and supraspinal actions.<sup>7,8</sup> Furthermore, all known anti-opioid peptides, including cholecystinin (CCK), dynorphin, FMRFamide,  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), neurotensin, and thyrotropin releasing hormone (TRH), appear to have important spinal actions (see Discussion). Therefore, in an attempt to further characterize the

anti-opioid effects of OFQ, we assessed antinociceptive dose-response relationships for morphine administered by the i.c.v. and intrathecal (i.t.) routes, in the presence and absence of co-administered OFQ.

## Materials and Methods

Naive, adult (8–12 weeks old) outbred Swiss-Webster mice (Simonsen, Gilroy, CA) of both sexes were housed four to a cage in a 12:12 h light:dark cycle (lights on at 07:00 h) in a temperature-controlled environment ( $21 \pm 1^\circ\text{C}$ ), with food (Purina chow) and tap water *ad lib*.

Nociceptive sensitivity was assessed using the tail-withdrawal (TW) assay.<sup>9</sup> This test of acute, thermal nociception measures the latency to reflexive withdrawal of the distal half of the tail after its immersion in  $49 \pm 0.2^\circ\text{C}$  water. The withdrawal latency was measured to the nearest 0.1 s by an experienced observer blind to drug condition. To improve accuracy, two separate TW latency determinations, separated by 20 s, were made and averaged at each time point. During testing, mice were lightly restrained in a cloth/cardboard holder, which was voluntarily entered in most cases. A cut-off latency of 10 s was imposed to prevent possible tissue damage.

Morphine sulfate (Mallinckrodt, St. Louis, MO) and OFQ (Phoenix Pharmaceuticals, Garden View, CA) were dissolved in artificial cerebrospinal fluid (vehicle) and combined in appropriate final concentrations. I.c.v. injections were made directly into the left lateral ventricle through the coronal suture following the method of Laursen and Belknap,<sup>10</sup> and i.t. injections were made at L4–L5 following the method of Hylden and Wilcox.<sup>11</sup> In both cases, 2.5  $\mu\text{l}$  of drug cocktail was injected under light halothane anesthesia, using a 10  $\mu\text{l}$  Hamilton microsyringe attached to a 3-mm long, 27-gauge needle (for i.c.v. injections) or a 0.5"-long, 30-gauge needle (for i.t. injections).

Mice were randomly assigned to either route of administration (i.c.v. or i.t.), and one of four doses of morphine: 0 (vehicle), 1, 10 or 50  $\mu\text{g}$  (0, 2.7, 27 or 135 nmol). These doses were chosen based on pilot data to produce a range of antinociceptive responses commensurate with the construction of dose-response curves. Within each route/morphine dose group, half the mice received vehicle and half received OFQ (10 nmol). This OFQ dose was chosen because it produced a robust antagonism of systemic morphine antinociception (5 mg  $\text{kg}^{-1}$ , s.c.) in a previous study without concomitant motor side-effects.<sup>6</sup> The i.c.v. and i.t. groups were tested in separate sessions. Every testing session contained equal numbers of mice receiving morphine + vehicle and morphine + OFQ. In a follow-up i.t. morphine experiment, mice

received 50  $\mu\text{g}$  morphine plus either vehicle, 1 or 25 nmol OFQ. All groups were composed of 6–9 mice, with both sexes equally represented.

Mice were assessed for baseline nociceptive sensitivity on the TW test, briefly anesthetized, injected and then returned to their home cages, where they recovered from anesthesia within 5 min. At 15, 30 and 60 min post-injection, mice were retested for TW latencies. All experiments proceeded near mid-photophase (10:00–16:00 h), to minimize circadian influences on nociceptive sensitivity.

Since i.c.v. and i.t. data were collected in separate sessions, they were analyzed separately, initially by three-between (morphine dose  $\times$  OFQ dose  $\times$  sex), one-within (baseline, 15, 30 and 60 min post-injection) repeated measures analyses of variance (ANOVAs). Antinociception at each time point was assessed relative to baseline latencies using Dunnett's *post-hoc* test. For purposes of group comparisons and dose-response curve construction, raw TW latency data were converted to antinociceptive area under the time  $\times$  TW latency curve (AUC;  $\text{min s}^{-1}$ ). Percentage maximal antinociception was calculated by comparing the obtained AUC with the maximal AUC that would be obtained from a subject displaying cut-off TW latencies ( $> 10$  s) at all post-injection time points. Half-maximal antinociceptive dose ( $\text{AD}_{50}$ ) estimates were calculated using linear regression of percentage maximal antinociception scores at each dose. The criterion for statistical significance was chosen as  $p < 0.05$ .

## Results

In addition, males displayed higher magnitudes of morphine antinociception, administered by both routes and at all doses, than females. Both of these phenomena are well known and not uncommon (see Ref. 12 for a review). In no case, however, was a significant interaction of sex with morphine dose or OFQ observed. Therefore, we collapsed data from both sexes for all further analyses.

Data from vehicle + vehicle and vehicle + OFQ groups were analyzed separately to determine whether OFQ produced alterations in baseline nociceptive sensitivity by either route of administration. Repeated measures ANOVAs performed on vehicle + vehicle data revealed very modest ( $< 1$  s) but significant post-injection increases in TW latencies (Fig. 1, dotted lines). These TW latency increases probably reflect SIA resulting from the i.c.v. and i.t. injection procedures. OFQ produced a partial but non-significant antagonism of these latency increases. It is important to note that, as in our previous study,<sup>6</sup> in no case was hyperalgesia (i.e. decreased TW latencies relative to baseline values) noted. We believe that the

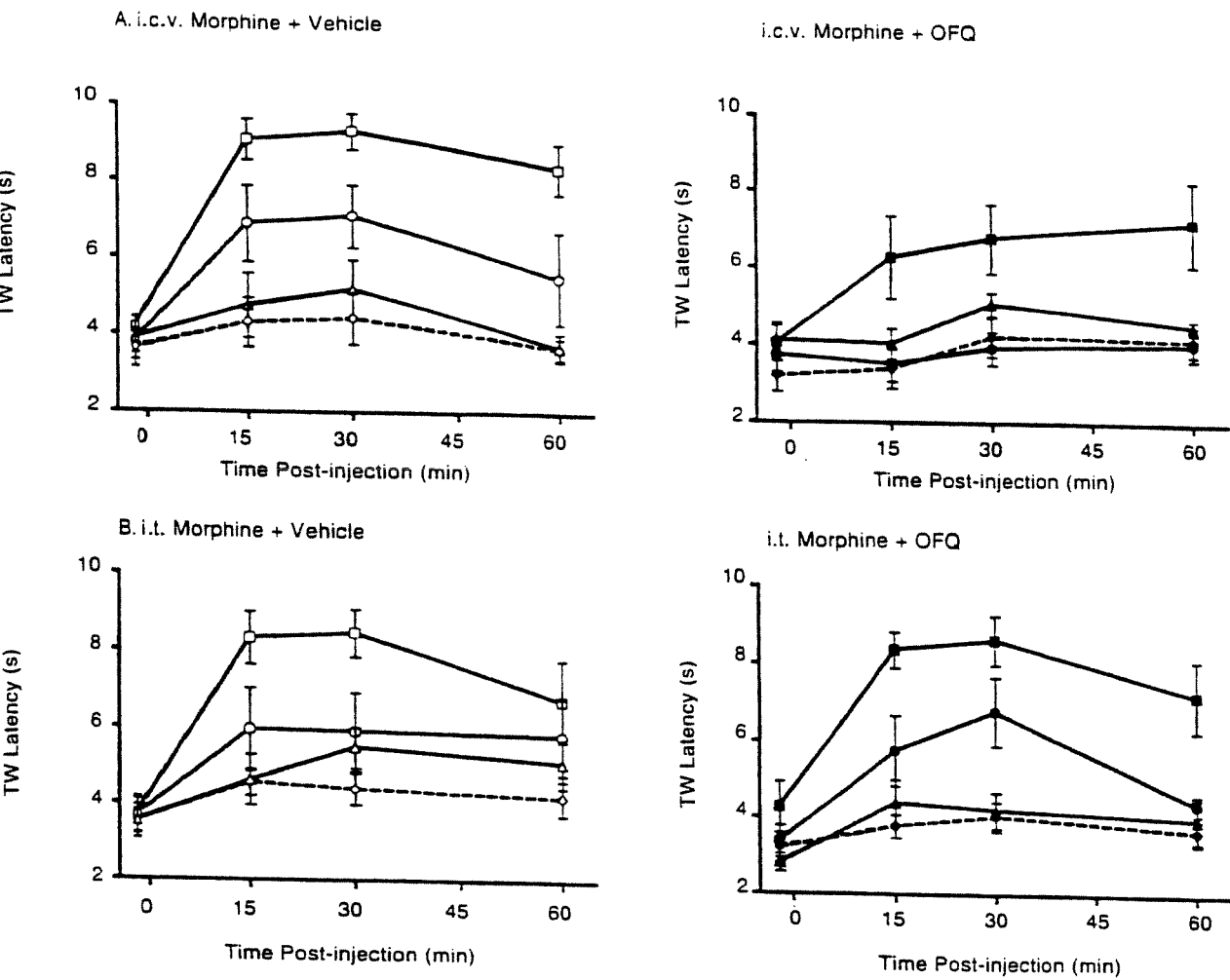


FIG. 1. Effects of OFQ on spinal and supraspinal morphine antinociception on the TW test. Mice were assessed for baseline nociceptive sensitivity to 49°C water, injected i.c.v. (A; top panels) or i.t. (B; bottom panels) with a combination of morphine (0, 1, 10 or 50  $\mu\text{g}$ ) and either vehicle (2.5  $\mu\text{l}$  artificial cerebrospinal fluid; left panels) or OFQ (10 nmol; right panels), and retested for TW latencies 15, 30 and 60 min later. Closed and open symbols (with or without OFQ, respectively) represent the mean  $\pm$  s.e.m. of 6–9 mice. (◆) 0  $\mu\text{g}$  morphine; (▲) 1  $\mu\text{g}$  morphine; (●) 10  $\mu\text{g}$  morphine; (■) 50  $\mu\text{g}$  morphine. \* $p < 0.05$  compared with corresponding morphine + vehicle group.

very modest magnitude of injection-related SIA observed here does not meaningfully affect the quantification of the much higher-magnitude morphine antinociception.

No significant differences in baseline TW latency (~4 s) were observed between any group. Repeated measures ANOVA performed on i.c.v. data revealed highly significant main effects of morphine dose ( $F_{2,39} = 11.64$ ) and OFQ ( $F_{1,39} = 8.91$ ), and a non-significant morphine  $\times$  OFQ interaction ( $F_{2,39} = 1.75$ , n.s.). As shown in Figure 1A, i.c.v. morphine + vehicle dose-dependently increased TW latencies (significantly at the 10 and 50  $\mu\text{g}$  doses), which peaked at 30 min post-injection. In contrast, mice receiving i.c.v. morphine + OFQ displayed significantly increased TW latencies at the high (50  $\mu\text{g}$ ) dose only, and here the latencies were significantly atten-

uated at both 15 min and 30 min post-injection relative to animals receiving i.c.v. morphine + vehicle. The failure of OFQ to antagonize 50  $\mu\text{g}$  morphine antinociception at 60 min post-injection may reflect degradation of OFQ by this time.

Repeated measures ANOVA performed on i.t. data revealed a highly significant main effect of morphine dose ( $F_{2,41} = 7.96$ ), but no significant main effect of OFQ ( $F_{1,41} = 0.18$ , n.s.) or morphine  $\times$  OFQ interaction ( $F_{2,41} = 0.35$ , n.s.) were evident. As shown in Figure 1B, i.t. morphine produced dose-dependent increases in TW latency (significant at the 10 and 50  $\mu\text{g}$  doses), peaking at 30 min post-injection. In contrast to data obtained with i.c.v. administration, effects of i.t. morphine were not attenuated by co-administration of 10 nmol OFQ. No significant differences between OFQ- and vehicle-treated

**Table 1.** Effects of OFQ on i.c.v. and i.t. morphine dose-response relationships

Route	CNS injection <sup>a</sup>	Morphine AD <sub>50</sub> <sup>b</sup> (µg)	Potency ratio <sup>c</sup>
i.c.v.	Vehicle	27.5 ± 10.6	2.2*
	OFQ	61.2 ± 8.3	
i.t.	Vehicle	33.6 ± 12.2	1.1
	OFQ	35.8 ± 11.8	

<sup>a</sup> Vehicle (2.5 µl artificial cerebrospinal fluid) or OFQ (10 nmol) were mixed and co-administered with morphine (0, 10 or 50 µg).

<sup>b</sup> Half-maximal antinociceptive dose (AD<sub>50</sub>) ± S.E.M. was calculated by linear regression of the dose-response curve as described in Materials and Methods.

<sup>c</sup> Calculated as OFQ AD<sub>50</sub>: Vehicle AD<sub>50</sub>.

\* Significant dose-response curve shift,  $p < .05$ .

mice were observed at any morphine dose and/or time point. Table 1 shows AD<sub>50</sub>s and potency ratios calculated from linear regression of AUC data.

In a separate group of mice, we tested two other doses of OFQ (1 and 25 nmol) against 50 µg i.t. morphine (higher OFQ doses were impractical because they produce atonia). Again, neither of the OFQ doses tested significantly altered i.t. morphine antinociception. However, we observed a trend towards potentiation of spinal morphine antinociception by OFQ in this experiment (AUC for vehicle, 1 nmol OFQ and 25 nmol OFQ, respectively: 224.3 ± 42.5, 291.4 ± 40.8, 293.0 ± 31.6;  $p = 0.053$ ). We have in fact, demonstrated this spinal potentiation of morphine antinociception in rats and further studies are currently underway to determine the reproducibility of this effect in mice.

## Discussion

We interpret our data to suggest that OFQ is a selective supraspinal anti-opioid within the central nervous system (CNS), and does not attenuate spinal opioid antinociception. We have previously shown that OFQ given i.c.v. dose-dependently reverses systemic morphine antinociception.<sup>6</sup> In the present study, a dose of OFQ previously shown to be effective against 5 mg kg<sup>-1</sup> (s.c.) morphine was injected in combination with various doses of morphine given i.c.v. or i.t. Although morphine produced antinociception by both routes of administration, OFQ was only able to antagonize this antinociception when co-administered i.c.v. We observed that the antagonistic effect of OFQ was constrained by both morphine dose and time. Although the antinociception resulting from 10 µg morphine was completely abolished by 10 nmol OFQ, this same OFQ dose was unable to antagonize fully the antinociceptive effect of 50 µg morphine. Furthermore, at 60 min post-injection,

OFQ was no longer effective against this high dose of morphine, which may reflect the time window within which OFQ is biologically active. The metabolism of OFQ is at present unknown, although likely to be fairly rapid. It remains to be determined whether higher doses of OFQ would be more effective against antinociception induced by 50 µg morphine.

It is worth noting that doses of morphine required to produce antinociception in the present study, by both routes of administration, are considerably higher than those commonly reported in the literature.<sup>11,13,14</sup> Possible reasons for differences in morphine potency between studies include genotype of the subject, algometric assay (stimulus type and intensity, testing protocol), method of potency calculation, and/or environmental factors including stress and circadian effects. Genotype is particularly likely to be relevant, having substantial effects on pain sensitivity and antinociception (see Ref. 15 for review). In fact, Swiss-Webster mice display very low sensitivity to antinociceptive drugs; antinociceptive ED<sub>50</sub>s for morphine in various algometric assays are 2- to 4-fold higher in Swiss-Webster mice than in other strains.<sup>16,17</sup> In addition, surprisingly large differences in antinociceptive potency in rodents of the same strain but from different vendors have been noted.<sup>18</sup> Despite the high doses of morphine required in this study, the antagonism of morphine antinociception by supraspinal OFQ was unambiguous, and thus supports and extends our previous assertion that OFQ acts as an anti-opioid peptide.

OFQ is not unique in its anti-opioid activity; many other peptides have been purported to have such antagonistic effects. These include CCK, dynorphin, FMRFamide (and its analogs including NPFF), MIF-1/Tyr-MIF-1, α-MSH, neurotensin and TRH.<sup>19-21</sup> Whether OFQ blocks opioid actions via mechanism(s) similar to those of other anti-opioids remains unknown. However, the present study reveals an interesting difference between OFQ and other anti-opioid peptides, in that the latter all appear to have spinal anti-opioid actions<sup>20,22-25</sup> whereas OFQ does not. The supraspinal specificity of OFQ may imply a different mechanism of interaction with opioid systems. Another notable difference between OFQ and other anti-opioids is that OFQ appears to have evolved as part of the opioid gene family. Further research should clarify whether common or divergent mechanisms of anti-opioid action exist between OFQ and other anti-opioids, in addition to possible interactions among them.

## Conclusions

The results of the present study demonstrate that OFQ, the endogenous ligand of the orphan opioid

ceptor, LC132, selectively antagonizes supraspinal  
morphine antinociception. These data support the  
hypothesis that OFQ may play an important role in  
modulation of opioid processes.

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