

Letter to Neuroscience

ORPHANIN FQ IS A FUNCTIONAL ANTI-OPIOID PEPTIDE

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The heptadecapeptide orphanin FQ has recently been shown to be the endogenous agonist for the orphan opioid-like receptor, LC132. The molecular evidence that LC132 and orphanin FQ are evolutionarily related to other opioid receptors and their ligands suggests that these proteins may also play a role in modulating opiate actions. We now report that orphanin FQ (0.5–10 nmol), injected intracerebroventricularly in mice, does not produce hyperalgesia as suggested previously but rather reverses opioid-mediated (i.e. naloxone-sensitive) stress-induced antinociception in three different algesiometric assays. In addition to its antagonism of endogenous opioid antinociception, orphanin FQ dose-dependently (2.5–25 nmol) reverses systemic morphine antinociception (5 mg/kg, s.c.). Based on these data, we propose that orphanin FQ is a functional anti-opioid peptide. Copyright © 1996 IBRO. Published by Elsevier Science Ltd.

The drug of choice for the clinical treatment of moderate-to-severe pain is morphine. Unfortunately, there are liabilities associated with its use, including the development of tolerance, dependence and addiction. Morphine exerts its biological effects by activating endogenous opioid mechanisms, which in turn interact with a variety of other neurotransmitter systems to modulate nociception (i.e. sensitivity to pain).^{2,14} Subsequent to the cloning of the δ -opioid receptor,^{6,9} molecular techniques have been used to identify additional members of the opioid receptor gene family: μ , κ and the sequentially related orphan receptor, LC132 (e.g., Ref. 4). LC132 exhibits a pharmacological profile and a brain mRNA distri-

bution distinct from those of the classical opioid receptors (μ , δ and κ).⁴ However, expression of this receptor's mRNA is high in the periaqueductal gray, dorsal raphé nucleus, locus coeruleus and the dorsal horn of the spinal cord,⁴ brain areas importantly associated with nociceptive modulation,^{2,19} suggesting a role for the LC132 receptor in this process.

Recently, the endogenous peptidergic ligand of the LC132 receptor was purified and named both orphanin FQ (OFQ)¹⁶ and nociceptin.¹³ OFQ interacts with its receptor to potentially inhibit forskolin-stimulated cyclic AMP accumulation,^{13,16} and a radiolabeled synthetic peptide analog of OFQ displays saturable and displaceable high-affinity binding to membranes of cells transfected with the receptor.¹⁶ Despite the amino terminal Phe-Gly-Gly-Phe motif, which is very similar to the canonical Tyr-Gly-Gly-Phe sequence of the endogenous opioid peptides (β -endorphin, enkephalins and dynorphins), OFQ shows no appreciable binding to either μ , δ or κ receptors (with IC_{50} values of 2083 ± 432 , 2246 ± 339 and 754 ± 89 , respectively; unpublished observations).

In spite of its structural homology with the opioid peptides, OFQ has not been shown to produce antinociception (i.e. a decreased sensitivity to pain). Rather, OFQ has been reported to produce hyperalgesia, a supersensitivity to pain, in the tail-flick and hot-plate assays (Refs 13 and 16, respectively) when injected intracerebroventricularly (i.c.v.) in mice. To investigate whether OFQ might be producing a mild antinociception, detectable only by using a more sensitive algesiometric assay, we evaluated the effects of OFQ in the acetic acid abdominal constriction test.¹⁰ The nociceptive state produced in this assay is at least 10-fold more sensitive to morphine inhibition compared to the tail-flick and hot-plate tests.⁷ Mice were assigned to three experimental groups: one

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Abbreviations: OFQ, orphanin FQ; SIA, stress-induced antinociception; TW, tail-withdrawal.

Table 1. Effects of i.c.v. injection of vehicle or orphanin FQ on nociception in the acetic acid abdominal constriction and 50°C hot-plate assays

Assay (dependent measure)	i.c.v. injection		
	None	Vehicle	OFQ
AC (total constrictions \pm S.E.M.)	27.1 \pm 5.1	12.0 \pm 4.1*	26.4 \pm 4.4
HP [hindpaw-lick/flutter latency (s) \pm S.E.M.]	28.2 \pm 1.9	45.2 \pm 2.6*	34.0 \pm 1.8

Naïve adult Swiss-Webster mice of both sexes (20–35 g; Simonsen Inc.; $n = 7$ –12 per group) were used. For i.c.v. injections, mice were briefly anesthetized with halothane and drugs were injected into the left lateral ventricle, following the protocol described in Laursen and Belknap.¹¹ Mice received OFQ (2.5 nmol) or vehicle (2.5 μ l artificial cerebrospinal fluid) or were left undisturbed in their home cages (None). In the acetic acid abdominal constriction (AC) test, i.c.v. injected mice were allowed to recover from the anesthetic for 10 min, and then all mice received an i.p. injection of 0.6% glacial acetic acid. Immediately thereafter, mice were placed individually into cylindrical Plexiglas observation chambers (30 cm high, 30 cm diameter) to which they were previously habituated, and the number of abdominal constrictions—lengthwise stretches of the torso with a concomitant concave arching of the back—were counted for the next 30 min. In the hot-plate (HP) test, i.c.v. injected mice were returned to their home cages for 20 min, and then tested for nociceptive sensitivity to an aluminum plate maintained at 50 \pm 0.2°C. Ambulation on the plate was restricted by 15 cm high Plexiglas walls to an area of 10 \times 10 cm². Latency to respond to the heat stimulus with a behavior regarded as indicative of nociception (hindpaw-lick or -flutter; see Ref. 7) was measured to the nearest 0.1 s.

*Significantly different by Tukey's *post hoc* test, $P < 0.05$.

Both one-way ANOVAs were significant (AC: $F_{2,34} = 3.31$, $P < 0.05$; HP: $F_{2,20} = 15.87$, $P < 0.001$). In this and all subsequent experiments, no main effects or interactions of sex were found to be significant, so data from both sexes were pooled for reported analyses.

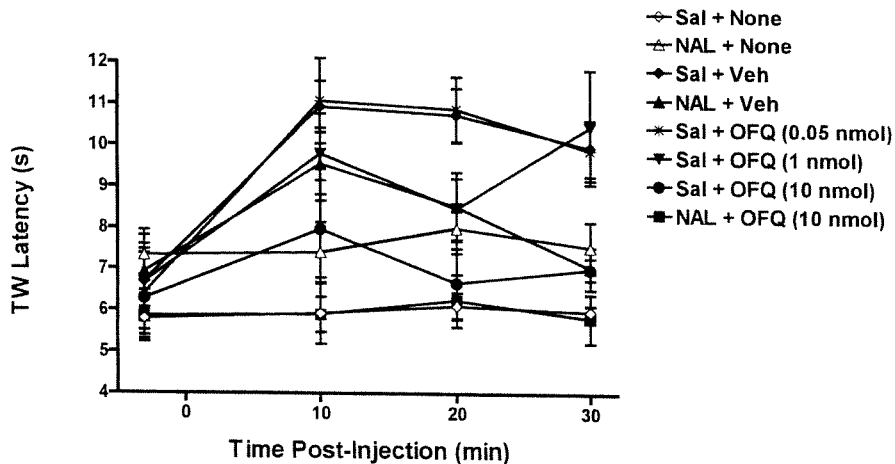
group received only an intraperitoneal (i.p.) injection of 0.6% acetic acid; in the other two groups this i.p. injection was immediately preceded by an i.c.v. injection (under halothane anesthesia) of either vehicle (2.5 μ l artificial cerebrospinal fluid) or 2.5 nmol OFQ. As shown in Table 1, OFQ-treated animals and uninjected animals displayed equivalent sensitivity to this noxious stimulus. In contrast, mice receiving vehicle injections displayed significantly reduced nociceptive sensitivity compared to either OFQ-treated or uninjected mice. In another particularly sensitive algometric assay, the 50°C hot-plate test,¹ we obtained identical results (Table 1).

We reasoned that a possible explanation for this observation is that i.c.v. vehicle-treated mice were displaying stress-induced antinociception (SIA),⁸ which was reversed by OFQ. Many forms of SIA are mediated by endogenous opioids;^{12,18} therefore, we examined the effect of the prototypic opioid antagonist, naloxone, on this phenomenon. Using an algometric assay [the 46°C tail-withdrawal (TW) test³] very similar to that used by Reinscheid *et al.*,¹⁶ we tested doses of OFQ over the same range of concentrations examined previously (0.05–10 nmol).^{13,16} In this test, i.c.v. injections of vehicle produced a \approx 4-s post-injection increase in TW latency. This TW latency increase was antagonized by naloxone (1 mg/kg, s.c.) and/or OFQ in a dose-dependent manner (Fig. 1). Again, there was no evidence of hyperalgesia relative to baseline latencies produced by OFQ at any dose. The reversal of i.c.v. vehicle injection antinociception by naloxone supports the contention that this procedure produces opioid SIA. Since OFQ also reverses this SIA, it appears to be functioning as an anti-opioid peptide.

Given that OFQ can antagonize opioid SIA, one might also expect it to reverse morphine antinociception. We tested this hypothesis by administering morphine to mice immediately following assessment of their baseline nociceptive sensitivity in the 49°C TW test. We used a higher water temperature because it has been demonstrated that SIA is less readily observed when using higher intensity noxious stimuli;⁵ indeed, there were no significant TW latency increases observed following i.c.v. injection at this water temperature (Fig. 2A, dotted line). As illustrated in Fig. 2A, 5 mg/kg morphine (s.c.) produced a profound antinociception peaking at 30 min post-injection and lasting about 2 h. OFQ reversed this antinociception in a dose-dependent manner (2.5–25 nmol; Fig. 2B). The dose of OFQ producing half-maximal inhibition of 5 mg/kg morphine antinociception was calculated to be 7.5 nmol (95% confidence interval = 2.3–12.7 nmol).

The apparently disparate conclusions reached from the present data and those of previous reports^{13,16} can be reconciled by considering the influence of test-related SIA. Many aspects of nociceptive testing are stressful to the animal, including novelty, handling, restraint and needle pricks (e.g., Ref. 15). To evaluate the influence of such stressors, baseline nociceptive sensitivity is typically assessed before any manipulation. Our current studies demonstrate that the halothane anesthesia/i.c.v. injection protocol is sufficient to cause opioid-mediated SIA. Consequently, we suggest that the apparent hyperalgesia in response to OFQ/nociceptin reported previously^{13,16} was actually a reversal of SIA. Since each animal was tested only once in the original studies, after the i.c.v. injection, these alternative possibilities could not be distinguished. Our data thus support the

A



B

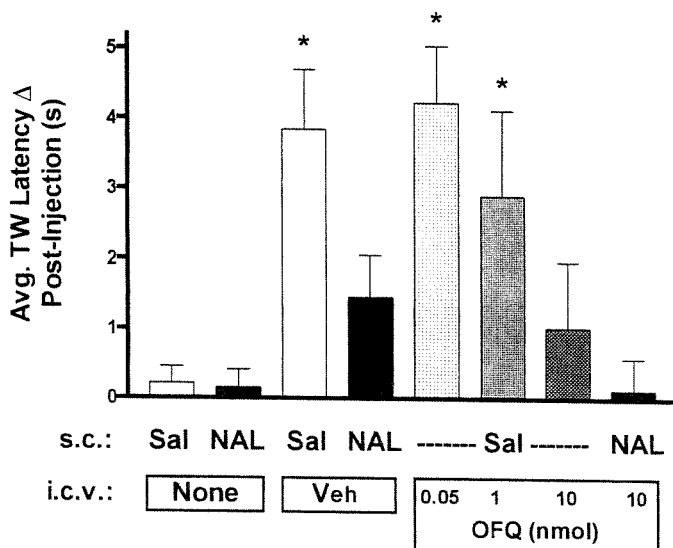


Fig. 1. Evaluation of OFQ's effects in the 46°C TW assay. Mice (Swiss-Webster, Sinonsen Inc., $n = 7$ or 8 per group) were tested for nociceptive sensitivity to immersion of the distal half of the tail in $46 \pm 0.2^\circ\text{C}$ water before, and 10, 20 and 30 min after, s.c. injection with saline (Sal; 10 ml/kg) or naloxone (NAL; 1 mg/kg) and i.c.v. injection (as described in Table 1 footnote) with vehicle (Veh) or OFQ (0.05, 1 or 10 nmol). Mice were lightly restrained in a cloth/cardboard holder (voluntarily entered by most subjects), and the latency to respond to the heat stimulus by a vigorous flexion of the tail was measured to the nearest 0.1 s by an experienced observer blind to drug condition. In the absence of a withdrawal reflex, the tail was removed from the water after a cut-off latency of 15 s. To improve accuracy, three separate TW latency determinations, separated by 20 s, were made and averaged at each time-point. (A) Symbols represent raw TW latency means (\pm S.E.M.); note that baseline TW latencies range from ≈ 6 to 8 s and that no group displays post-injection TW latencies shorter than their baseline value. (B) Summarizing the data in A, bars represent mean (\pm S.E.M.) change in TW latency relative to baseline values averaged over three tests (10, 20 and 30 min post-injection). An ANOVA performed on averaged TW change data revealed significant main effects of s.c. injection and i.c.v. injection ($F_{1,44} = 4.43$, $P < 0.05$; $F_{2,44} = 9.31$, $P < 0.001$, respectively). A significant main effect of OFQ dose was also observed ($F_{3,25} = 4.63$, $P < 0.01$). *Significantly different from Saline + None group by planned contrasts, $P < 0.05$. Pilot experiments demonstrated no significant effect of s.c. injection or repeated TW latency assessments on nociceptive sensitivity in this assay.

contention that OFQ does not facilitate nociception but rather functionally disinhibits it; therefore, referring to this peptide as "nociceptin" may be misleading.

In recent years, there have been numerous reports of endogenous neuromodulators that influence some of the effects of opioids. For example, the peptides cholecystokinin, FMRFamide and neurotensin have

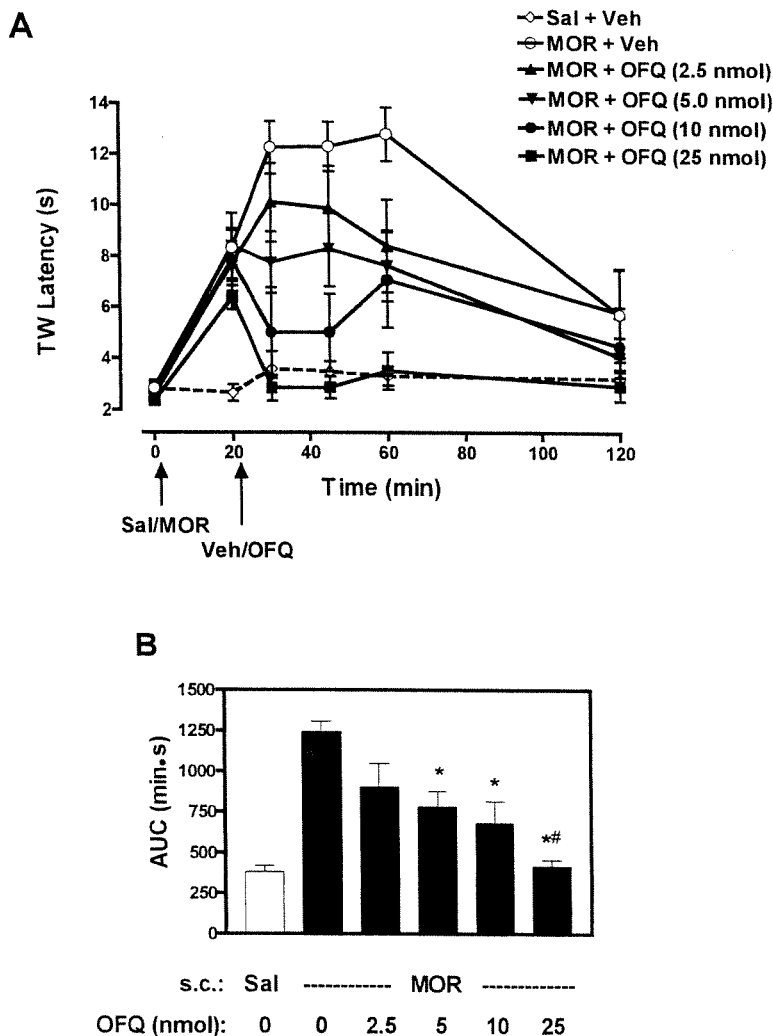


Fig. 2. Reversal of morphine antinociception by OFQ. Mice ($n = 6-11$ per group) were tested for baseline nociceptive sensitivity on the $49 \pm 0.2^\circ\text{C}$ TW test (see Fig. 1 legend), injected s.c. with morphine sulfate (MOR; 5 mg/kg) or saline (Sal; 10 ml/kg), and then retested 20 min later to establish the existence of morphine antinociception. Mice were then injected i.c.v. with vehicle (Veh) or OFQ (2.5, 5, 10 or 25 nmol), and retested 10, 25, 40 and 100 min later. (A) Time-course data showing morphine antinociception and its dose-dependent reversal by OFQ. Symbols represent mean (\pm S.E.M.) TW latencies at each time-point. The dotted line represents data from mice receiving Saline + Vehicle; no significant differences from baseline TW latency were observed in this group. (B) Data from A expressed as areas under the time \times TW latency curve (AUC; using the trapezoidal rule) over the entire 2-h testing session. Bars represent mean (\pm S.E.M.) area under the curve. ANOVA performed on area under the curve data revealed a significant main effect of OFQ dose ($F_{4,34} = 8.78$, $P < 0.001$). *Significantly different from the Morphine + Vehicle (0) group, $P < 0.05$. #Not significantly different from Saline + Vehicle (0) group, $P < 0.05$ (Tukey *post hoc* test). The 25 nmol OFQ dose produced marked atonia and flaccid paralysis in approximately 50% of mice. These motoric side-effects should not, however, be considered a confound of the present data, since mice injected with this high OFQ dose actually reacted faster to the thermal stimulus.

been shown to attenuate antinociception and promote tolerance to opioids, and antagonism of these peptides' effects can potentiate morphine potency (see Ref. 17). Unlike these peptides, OFQ shares significant amino acid homology with the known members of the opioid family.⁴ The common evolutionary lineage shared by OFQ and its receptor with the opioids and their receptors, respectively, suggests that interactions among them may be particularly relevant. The potent, efficacious and monophasic

nature of OFQ's effects also indicate a potentially important physiological role for this peptide. Due to OFQ's lack of affinity for opioid receptors, and the poor binding of endogenous and exogenous opiates to LC132 (unpublished observations, but see Ref. 20), it is likely that the OFQ neuromodulatory system affects opioid phenomena indirectly. Moreover, since OFQ functionally antagonizes opioid actions despite producing identical intracellular effects (decreased cyclic AMP production^{13,16} and cellular

hyperpolarization unpublished observations); it is likely to be influencing opioid mechanisms via a distinct inhibitory neuronal pathway. At present, it cannot be ruled out that the effects reported herein may be due to the action of an as yet uncharacterized OFQ metabolite. Moreover, antinociception can be produced by both opioid and non-opioid mechanisms,^{12,18} which are known to interact in a complex manner. Since the effects of OFQ on non-opioid antinociception have remained uncharacterized, it is possible that OFQ may be producing the present effects in part by affecting non-opioid mechanisms as well. In any case, aspects of nociceptive processing and stress responses that remain poorly understood may be clarified by the further characterization of OFQ.

Confirmation of the role of OFQ as an anti-opioid peptide will require the development of a specific OFQ receptor antagonist. In this regard, it is interesting that, using an antisense oligodeoxynucleotide

directed against a translated region of the OFQ receptor mRNA, Meunier *et al.*¹³ were able to demonstrate effects opposite to those of OFQ. Antagonism of the OFQ system may have considerable clinical utility.

The present findings suggest that, although an OFQ antagonist may not have antinociceptive actions *per se*, it may be effective as an adjunct to morphine pharmacotherapy, allowing lower doses of morphine (producing concomitantly fewer side-effects) to be used.

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