

Functional antagonism of μ -, δ - and κ -opioid antinociception by orphanin FQ

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Abstract

Orphanin FQ (OFQ) is the recently isolated endogenous ligand for the orphan opioid-like receptor, LC132. Initial reports suggested that OFQ increased pain sensitivity when injected intracerebroventricularly (i.c.v.) in mice. However, we have recently demonstrated that OFQ is instead an anti-opioid peptide that reverses morphine- and opioid-mediated stress-induced antinociception. Morphine binds to multiple opioid receptor types (μ , δ , and κ). The present study was designed to examine specific interactions of OFQ with antinociception mediated by each receptor type. To this end, mice were administered i.c.v. cocktails containing either vehicle or OFQ (10 nmol) and a μ -specific ([D-Ala², N-Me-Phe⁴-Gly-ol]enkephalin; DAMGO; 0–0.1 nmol), δ -specific ([D-Pen², D-Pen⁵]enkephalin; DPDPE; 0–50 nmol), or κ -specific (U-50,488H; 0–1000 nmol) agonist. As we have shown previously, OFQ alone had no effect on nociceptive sensitivity. OFQ was, however, able to completely block supraspinal antinociception produced by all three receptor type-selective agonists. We conclude, therefore, that OFQ functionally antagonizes μ (and opioid receptors, and may play a general role in opioid modulation.

Keywords: Anti-opiate; Mu; Delta; Kappa; LC132 receptor; Orphanin FQ; Pain inhibition

Opioid peptides are known to mediate a wide variety of physiological processes via their interaction with opioid receptors, most notably the modulation of nociception. Based on pharmacological characterization, three major opioid receptor types are currently recognized: μ , δ and κ [13]. Molecular characterization of the opioid receptor gene family has only recently become possible, subsequent to the cloning of the δ receptor. By homology screening, the μ and κ receptors were quickly cloned, as was an orphan member of the gene family, LC132 [3] (also named ORL-1 and XOR; see Ref. [16]). In addition to significant sequence homology with the other opioid receptors, LC132 displays a unique but overlapping mRNA distribution with μ , δ and κ receptors [3]. LC132 does not, however, appreciably bind opioid agonists or naloxone [3].

Recently, two laboratories independently isolated the endogenous ligand of LC132, and named it orphanin FQ

(OFQ) [16] and nociceptin [11]. This 17-amino acid peptide (FGGFTGARKSARKLANQ) displays impressive sequence homology with opioid peptides (β -endorphin, enkephalins, and dynorphins); however, it shows little affinity for μ , δ , or κ receptors. Intracellular second messenger systems coupled to the OFQ receptor are apparently identical to those linked to μ , δ and κ activation (i.e. inhibition of cAMP, cellular hyperpolarization via K⁺ channel activation) [11,16] (unpublished data). Due to its similarities to classical opioid peptides, OFQ was predicted to have antinociceptive properties. However, when injected intracerebroventricularly (i.c.v.) in mice, OFQ did not appear to produce antinociception. Rather, it was reported that mice injected with OFQ became more sensitive to pain [11,16]. We have recently demonstrated that this apparent OFQ hyperalgesia was actually a reversal of opioid-mediated stress-induced antinociception (SIA) produced by the i.c.v. injection procedure [12]. Consistent with our conclusion that OFQ is actually an anti-opioid peptide, we have observed that OFQ dose-dependently reverses systemic and supraspinal (but not spinal) morphine antinociception [7,12].

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Although morphine is a μ -preferring agonist, it can bind to additional sites that include the δ - and κ -opioid receptors as well. The purpose of the present study was to determine if OFQ can specifically modulate μ -, δ - and/or κ -mediated antinociception. To this end, OFQ's effects on supraspinal antinociception produced by specific agonists of the ([D-Ala², N-Me-Phe⁴-Gly-ol]enkephalin; DAMGO), ([D-Pen², D-Pen⁵]enkephalin; DPDPE), and (*trans*-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide; U-50,488H) receptors were assessed.

Naïve, adult (8–12 weeks old) outbred Swiss-Webster mice (Simonsen Inc., Gilroy, CA) of both sexes were used. Mice were housed four to a cage in a 12:12 h light/dark cycle (lights on at 0700 h) in a temperature-controlled environment ($21 \pm 1^\circ\text{C}$), and given food (Purina chow) and tap water ad libitum.

Nociceptive sensitivity was measured using the tail-withdrawal (TW) assay [1], a test of acute, thermal nociception. During testing, mice were lightly restrained in a cloth/cardboard holder (voluntarily entered in most cases), and the distal half of the tail was immersed in 49°C water. The latency to reflex withdrawal (or vocalization) 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. A cut-off latency of 12 s was imposed to avoid possible tissue damage.

The μ -, δ -, and κ -selective agonists, DAMGO, DPDPE, and U-50,488H, respectively, were obtained from Research Biochemicals Inc. (Natick, MA). Doses were chosen based on pilot data to bracket the effective range of antinociception (DAMGO: 0, 0.02, 0.05, and 0.1 nmol; DPDPE: 0, 10, 25 and 50 nmol; U-50,488H: 0, 100, 200, and 1000 nmol). DAMGO and U-50,488H were freely soluble in artificial cerebrospinal fluid (aCSF) vehicle; DPDPE was dissolved in a 1:5 solution of dimethylsulfoxide/aCSF. OFQ was obtained from Phoenix Pharmaceuticals (Mountain View, CA). A 10 nmol OFQ dose was chosen based on its efficacious functional antagonism of antinociception produced by systemic and i.c.v. morphine, without the concomitant ataxia produced by higher doses [7,12].

I.c.v. injections were made directly into the left lateral ventricle through the coronal suture according to the method of Laursen and Belknap [9]. Drug cocktails were made containing DAMGO, DPDPE or U-50,488H combined in appropriate final concentrations (2.5 μl injection volume) with aCSF vehicle or OFQ (10 nmol). Cocktails were injected under light halothane anesthesia, using a 10 μl Hamilton microsyringe attached to a 3-mm long, 27-gauge needle.

All experiments were conducted at least 3 h after lights-on, and at least 3 h before lights-off, to minimize circadian influences on nociceptive sensitivity. Six to nine mice were randomly assigned to each experimental group,

with both sexes equally represented. All mice were tested for baseline nociceptive sensitivity, and then anesthetized and injected. Mice were 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.

Each agonist (DAMGO, DPDPE, and U-50,488H) was tested in separate sessions, so these data were analyzed individually. TW latency data were analyzed first by three-way repeated measures analysis of variance (ANOVA), with dose, OFQ and sex as the between-subjects factors. A significant main effect of sex was observed for DAMGO and U-50, 488H ($F_{1,30} = 21.3$, $P < 0.001$; $F_{1,34} = 5.3$, $P < 0.05$, respectively), and the main effect of sex approached significance for DPDPE ($F_{1,31} = 4.0$, $P = 0.06$). These findings are reflective of both higher baseline TW latencies in male relative to female mice, and higher magnitude opioid antinociception in males. Both of these phenomena are well known and have been reported in the literature (see Ref. [6] for review). In no case, however, was a significant interaction with sex observed, so data from both sexes were pooled for all further analyses. Antinociception at each time point was assessed relative to baseline latencies using Dunnett's post-hoc test. In order to construct dose-response curves, raw TW latency data were converted to antinociceptive area under the time \times TW latency curve (AUC; min s). Percent of maximal antinociception was calculated by dividing the obtained AUC by the maximal AUC that would be obtained by a subject displaying cut-off TW latencies (>12 s) at all post-injection time points. Half-maximal antinociceptive dose (AD_{50}) estimates were calculated using linear regression of percent maximal antinociception scores at each dose. The criterion for statistical significance was chosen to be $P < 0.05$.

As shown in Fig. 1, DAMGO, DPDPE and U-50,488H produced dose-dependent supraspinal antinociception when paired with vehicle. AD_{50} values were calculated to be: 0.06 (0.02 nmol (DAMGO), 34.4 (8.6 nmol (DPDPE), and 1420 (220 nmol (U-50,488H). The antinociception produced by U-50,488H was modest (significant only at the high, 1000 nmol dose), congruent with other reports (see Ref. [19]), and higher doses were not practical due to motoric impairment. In contrast, 10 nmol OFQ completely blocked all three types of antinociception at all doses. In no case was statistically significant antinociception observed in any OFQ-treated group. Again, as demonstrated in previous studies [7,12], OFQ alone had no effect on nociceptive responsivity (data not shown).

OFQ's potent antagonism of antinociception produced through activation of μ , δ , and κ receptors supports and extends our previous observation that OFQ can act as a functional anti-opioid. We have previously shown that OFQ blocks both systemic and i.c.v. morphine analgesia as well as an opioid-mediated form of SIA [7,12]. OFQ's anti-opioid actions are not limited to nociceptive modula-

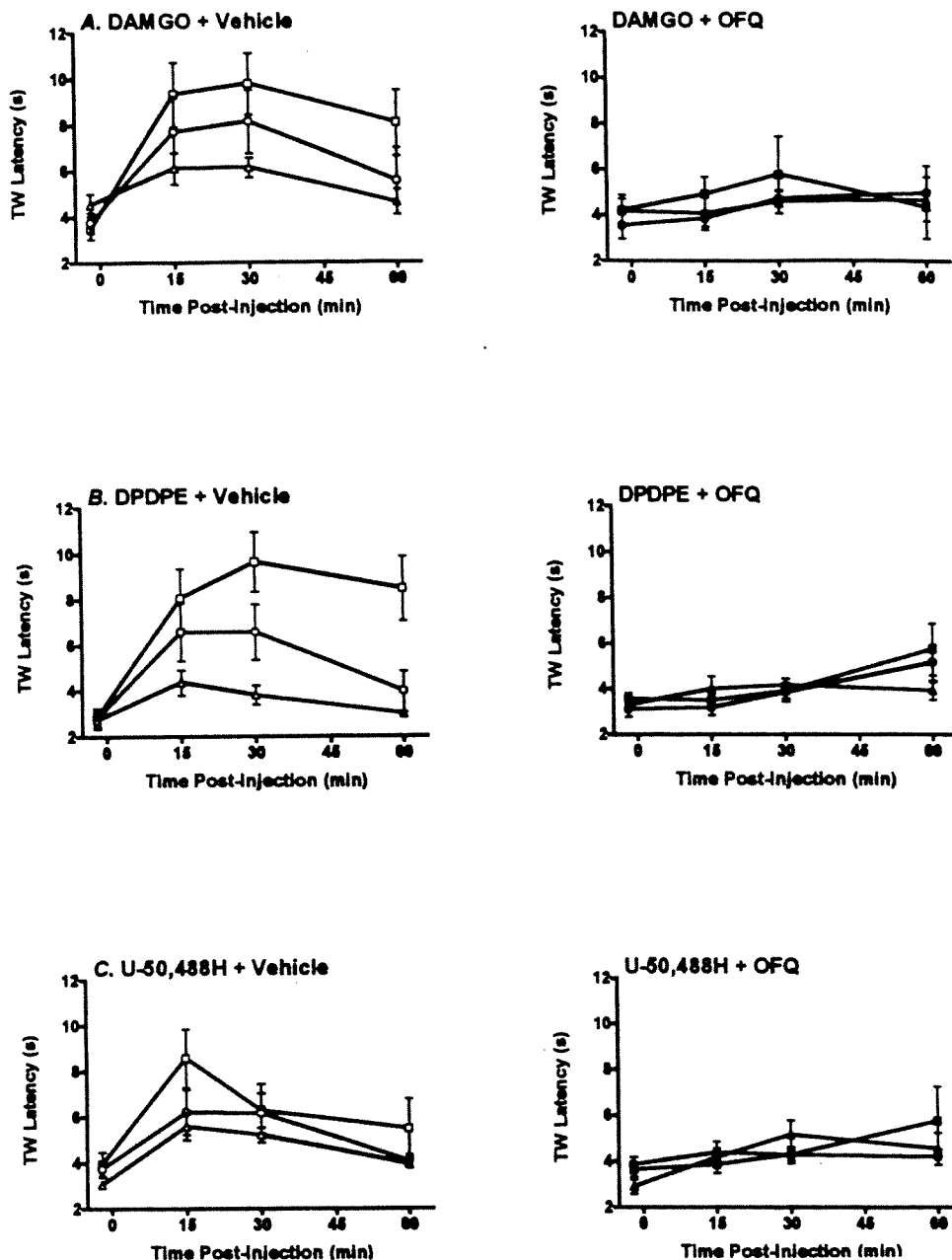


Fig. 1. Effects of OFQ on DAMGO (μ), DPDPE (δ), and U-50,488H (κ) antinociception. Mice were assessed for baseline nociceptive sensitivity on the 49°C TW test, injected i.c.v. with a combination of vehicle (2.5 μ l artificial cerebrospinal fluid; left panels) or OFQ (10 nmol; right panels) and either DAMGO (0.01, 0.02, and 0.1 nmol; A), DPDPE (10, 25, 50 nmol; B), or U-50,488H (100, 200, and 100 nmol; C), and retested 15, 30 and 60 min later. Closed and open symbols (with or without OFQ, respectively) represent mean (SEM of 6–9 mice. ct, low dose; cc, medium dose; cb, high dose. Mice treated with vehicle + vehicle or vehicle + OFQ showed no alterations in nociceptive sensitivity (not shown).

tion, as evidenced by its reversal of morphine-induced hypothermia and Straub tail (unpublished data).

The doses of DAMGO, DPDPE and U-50,488H (AD_{50} = 0.06, 34.4, and 1420 nmol, respectively) required to produce antinociception in the present study are higher than those commonly reported in the literature (e.g., Ref. [5]). This discrepancy might be explainable at least in part by genotypic factors. Swiss-Webster mice are known to display particularly low antinociceptive sensitivity to opioids [2,15]. Also, large differences in antinociceptive sensitivity in rodents of the same strain but from different

vendors are not uncommon [8]. Indeed, in a recent study we noted that spinal and supraspinal morphine AD_{50} s in these mice were similarly high [7].

Subtypes of μ , δ and κ receptors have been proposed based on pharmacological data [4,10,14]. The effect of OFQ on μ_1 and μ_2 receptors [14] cannot be easily evaluated due to the lack of subtype-specific agonists. In contrast, specific agonists at the δ receptor subtypes, δ_1 and δ_2 , are available (DPDPE and [D-Ala³, Glu⁴]deltorphin II, respectively). Although both δ agonists produce antinociception in mice, there is reason to believe that δ_1 receptors are

primarily important supraspinally, whereas δ_2 receptors are thought to mediate spinal antinociception selectively [18]. Since we have recently demonstrated that OFQ's anti-opioid actions against morphine are confined to supraspinal sites [7], only the effect of OFQ on δ_1 antinociception produced by DPDPE was evaluated. Two κ -receptor subtypes, κ_1 and κ_3 , have been shown to modulate nociception [13]. Naloxone benzoylhydrazone (NalBzoH), has been reported to produce antinociception at high doses by acting at supraspinal κ_3 receptors [13]. Our preliminary findings suggest a complex interaction between NalBzoH and OFQ (unpublished data).

An additional observation of note is that 10 nmol OFQ was sufficient to completely reverse DAMGO, DPDPE and U-50,488H analgesia of various magnitudes. In contrast, we have recently shown that this same OFQ dose is only able to partially antagonize systemic morphine analgesia (5 mg/kg, s.c.) [12] and high-dose supraspinal morphine analgesia (50 μ g, i.c.v.) [7]. This discrepancy is likely not due to higher antinociceptive magnitudes produced by morphine; indeed, 0.1 nmol DAMGO and 50 nmol DPDPE produced equipotent antinociception that was fully reversed by OFQ. Together, these data support the hypothesis that morphine, which at high doses can simultaneously activate multiple opioid receptor types, is producing some portion of its antinociceptive effect via synergy between μ , δ and/or κ receptors (e.g., Ref. [17]).

The generalizability of OFQ's modulation of opioid processes suggests that it is likely to have widespread physiological effects. Indeed, we have recently found evidence that OFQ may play an important role in reward, tolerance/dependence, and hypothermia (unpublished data). Direct confirmation of the significance of OFQ in opioid phenomena, however, awaits the development of a specific OFQ receptor antagonist.

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