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Blockade of μ-opioid receptors reveals the hyperalgesic effect of orphanin FQ/nociceptin in the rat hot plate test

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1 Orphanin FQ (OFQ, also known as nociceptin) has been proposed to oppose the antinociceptive effect of endogenous opioid peptides in the brain. We sought to determine whether, conversely, the endogenous opioid peptides counteract a pronociceptive action of OFQ.

2 In testing this hypothesis, naloxone, a non-selective opioid receptor antagonist, was used to block the action of endogenous opioid peptides. We then examined whether OFQ would produce hyperalgesia in the absence of such an endogenous opioidergic tone.

3 Neither naloxone (1 mg kg^{-1}; s.c.) nor OFQ (up to 30 nmol; i.c.v.) alone induced any significant change in mean hot plate latency. However, OFQ dose-dependently produced hyperalgesia in rats pretreated with naloxone, implying that OFQ can indeed produce hyperalgesia once an endogenous opioidergic tone is inhibited.

4 In subsequent studies, we used subtype selective opioid receptor antagonists to determine which class of opioid receptor is involved in this response. The effect of naloxone was reproduced using the selective μ-opioid receptor antagonist CTOP (D-Phe-Cyc-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2), but not by administration of the δ-opioid receptor antagonist, naltrindole (NTI) or the κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI).

5 These results suggest that endogenous opioid peptides acting at the μ-, but not κ- or δ-opioid receptor may be counteracting the hyperalgesic effect of OFQ in rats.
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Keywords: Orphanin FQ; nociceptin; naloxone; CTOP; naltrindole; nor-BNI; hyperalgesia; hot plate test

Abbreviations: aCSF, artificial cerebrospinal fluid; CTOP, (D-Phe-Cyc-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2); i.c.v., intracerebroventricular(ly); nor-BNI, nor-binaltorphimine; NTI, naltrindole; OFQ, Orphanin FQ; ORL-1, opioid receptor-like; s.c., subcutaneous

Introduction

Soon after the cloning of μ-, δ- and κ-opioid receptors, an orphan opioid receptor-like (ORL-1) receptor with a distinct pharmacological profile was characterized (Mollereau *et al*., 1994; Fukuda *et al*., 1994; Bunzow *et al*., 1994; Chen *et al*., 1994). The ORL-1 receptor, which is a G protein-coupled receptor, shows approximately 60–70% homology to the conventional opioid receptors in the transmembrane domains (Mollereau *et al*., 1994; Fukuda *et al*., 1994; Bunzow *et al*., 1994; Chen *et al*., 1994). Moreover, similarly to these receptors, activation of the ORL-1 receptor leads to inhibition of the enzyme adenylyl cyclase (Meunier *et al*., 1995; Reinscheid *et al*., 1995), reduction of calcium channel conductance (Connor *et al*., 1996b; Knoflach *et al*., 1996), and activation of potassium channels (Connor *et al*., 1996a; Matthes *et al*., 1996; Vaughan & Christie, 1996). In spite of the structural homology to the traditional opioid receptors, the classical opioid ligands do not interact with the ORL-1 receptor (Mollereau *et al*., 1994; Lachowicz *et al*., 1995).

In 1995, two independent groups identified a 17-amino acid peptide as the endogenous ligand of the ORL-1 receptor (Meunier *et al*., 1995; Reinscheid *et al*., 1995). This heptadecapeptide, which was named orphanin FQ (OFQ; Reinscheid *et al*., 1995) or nociceptin (Meunier *et al*., 1995), is somewhat structurally related to endogenous opioid peptides, particularly to dynorphin A (1-17) (Meunier *et al*., 1995; Julius, 1995). However, OFQ is lacking the N-terminal tyrosine necessary for activation of μ-, δ- and κ-opioid receptors (Reinscheid *et al*., 1998).

OFQ and its mRNA are widely distributed in the central nervous system (CNS), and in particular in areas involved in motivational and emotional behaviors and processing of nociceptive information (Neal *et al*., 1999). The effect of OFQ on nociceptive responses is somewhat complex. At the spinal level, for example, OFQ is reported to produce either no effect (Reinscheid *et al*., 1995) or antinociception (Xu *et al*., 1996; Hoa *et al*., 1997; King *et al*., 1997; Jhamandas *et al*., 1998; Wang *et al*., 1999). However, when administered supraspinally it may produce hyperalgesia (Meunier *et al*., 1995; Reinscheid *et al*., 1995; Wang *et al*., 1999) and in some instances even antinociception (Rossi *et al*., 1996, 1998). The hyperalgesic effect of OFQ has been questioned and attributed to inhibition of opioid receptor-mediated stress-induced antinociception (Mogil *et al*., 1996; Suaudeau *et al*., 1998). Previous studies have also shown that OFQ blocks antinociception induced by administration of exogenous opioid analgesics (Mogil *et al*., 1996; Tian *et al*., 1997; Morgan *et al*., 1997; Lutfy *et al*., 1999). OFQ may therefore be regarded as a physiological anti-opioid peptide (Mogil *et al*., 1996). It is possible that OFQ and traditional opioid peptides represent mutually opposing systems in the control of brain nociceptive circuitry. We sought to provide evidence for this by determining if OFQ would produce hyperalgesia under conditions in which opioid receptor-mediated effects are blocked by pretreatment with naloxone or opioid receptor sub-type selective antagonists.

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Methods

Subjects

Male and female Sprague Dawley rats (180–200 g) obtained from Harlan (Madison, WI, U.S.A.) were housed two per cage with free access to food and water. The rats were housed on a 12-hr light/12-hr dark cycle for at least 4 days prior to any experimentation. Rats were anaesthetized with halothane in a mixture of oxygen and nitrous oxide (1 : 1) and stereotaxically implanted with a 22 gauge guide cannula (3.5 mm long, Plastics One, Inc., Roanoke, VA, U.S.A.) aimed at the right lateral ventricle (AP: −0.8 mm, ML: +1.4 mm; and DV: −3.5 mm) according to the atlas of Paxinos & Watson (1986). The guide cannula was fixed to two metallic screws in the skull with dental cement. At the end of the surgical procedure, a dummy cannula was inserted into the guide cannula in order to prevent blockage of the guide cannula. Rats were then allowed at least 3 days to recover from surgery. All intracerebroventricular (i.c.v.) injections were made in a volume of 5 μl through a 30-gauge injection cannula that extended 0.5 mm beyond the tip of the guide cannula. The rats were gently restrained at the time of drug microinjection.

Effects of OFQ on the mean hot plate latency in the presence of naloxone

We used the hot plate method of Woolfe & MacDonald (1944), that is believed to measure nociceptive responses in the brain as compared to the tail flick assay which is mainly a spinally mediated pain assay in rodents (Jensen & Yaksh, 1986). The hot plate test is therefore more relevant for studying the effect of drugs administered i.c.v. We first studied the effect of either naloxone alone or OFQ alone on nociceptive responses in the hot plate test in male and female rats. A basal nociceptive response was first measured for each rat in which the latency to lick/flutter one of the hind paws or jump from the hot plate was measured. Approximately 20 min later, rats were injected with either OFQ (30 nmol, i.c.v.) or naloxone (1.0 mg kg^{-1}, s.c.) and tested on the hot plate apparatus after a further 15 or 25 min delay, respectively. The choice of the dose of OFQ was based on previous studies (Meunier *et al*., 1995; Mogil *et al*., 1996; Tian *et al*., 1997; Lufty *et al*., 1999). We then examined the action of OFQ in the presence of naloxone in male and female rats. Approximately 20 min after baseline measurement, rats were injected with naloxone (1 mg kg^{-1}, s.c.) and 10 min later received an injection of OFQ (15 nmol, i.c.v.). Rats were then tested on the hot plate apparatus 15 and 60 min later (25 and 70 min after naloxone). In a subsequent study female rats were used to generate a dose-response relationship for OFQ in the presence of a fixed dose of naloxone. Rats were pretreated with naloxone (1.0 mg kg^{-1}, s.c.), 10 min later received artificial cerebrospinal fluid (aCSF) or OFQ (7.5, 15 and 30 nmol, i.c.v.) and tested on the hot plate apparatus 15 min later.

Effects of OFQ on the mean hot plate latency in the presence of a selective μ-, δ-, or κ-opioid receptor antagonist

We used CTOP (D-Phe-Cyc-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2), naltrindole (NTI) and nor-binaltorphimine (nor-BNI) which are μ- (Toll, 1992; Devine *et al*., 1993), δ- (Portoghese *et al*., 1988a, b), and κ- (Portoghese *et al*., 1987; Takemori *et al*., 1988) opioid receptor selective antagonists, respectively. Initially, the effect of each antagonist alone on nociceptive responses was examined in female rats. A baseline hot plate latency was first measured for each rat. Approximately 20 min later, rats were injected i.c.v. with either aCSF, CTOP (5, 10 or 50 μg), NTI (0.5, 1 or 5 μg) or nor-BNI (1, 10 or 50 μg) and tested on the hot plate apparatus 25 min later. In subsequent studies, female rats were treated with aCSF or an antagonist (CTOP: 5 μg; NTI: 0.5, 1 or 5 μg; nor-BNI: 50 μg) and were injected, 10 min later, with either aCSF or OFQ (15 nmol) or left untreated. Rats were tested on the hot plate apparatus 15 min later.

Data analysis

One-way analysis of variance (ANOVA) was employed throughout, except for the time-course data where repeated measure ANOVA was used. The Newman–Keuls *post-hoc* test was used to determine the significant difference among various groups. A value of $P<0.05$ was considered statistically significant.

Drugs

Naloxone hydrochloride (Sigma; St. Louis, MO, U.S.A.) was dissolved in saline prior to s.c. administration. NTI (Sigma), nor-BNI (Research Biochemicals International, Natick, MA, U.S.A.), CTOP (Bachem, Torrance, CA, U.S.A.) and OFQ (Phoenix Pharmaceuticals, Inc., Mountain View, CA, U.S.A.) were dissolved in aCSF for i.c.v. injection. The composition of aCSF was (mM): NaCl (125); KCl (2.5); NaH_2PO_4 (0.9); Na_2HPO_4 (5); $MgCl_2$ (1); d-glucose (2.5); $CaCl_2$ (1.2); bovine serum albumin (0.025%).

Results

Effects of OFQ on the mean hot plate latency in the presence of naloxone

Neither OFQ (30 nmol, i.c.v.) nor naloxone (1 mg kg^{-1}, s.c.) produced a significant change from baseline hot plate latency in male or female rats when administered separately (data not shown). However, OFQ time-dependently decreased hot plate latencies in male (Figure 1a) and female (Figure 1b) rats pretreated with naloxone. The hot plate latency was significantly different from baseline at 15, but not at 60 min, in both groups ($F_{2,5}=6.72$; $F_{2,5}=15.53$, in male and female rats, respectively, $P<0.05$). In subsequent studies we examined the dose-dependency of this response in female rats at the time of peak effect. OFQ produced hyperalgesia in the presence of naloxone in a dose-dependent manner (Figure 2). One-way ANOVA followed by the Newman–Keuls *post-hoc* test revealed that OFQ did not produce any change at the lowest dose (7.5 nmol), however OFQ produced hyperalgesia at 15 and 30 nmol ($F_{3,31}=9.91$; $P<0.05$). Furthermore, the effect of OFQ at the highest dose was significantly greater than the other two lower doses of the drug ($P<0.05$). Analysis of the baseline latencies revealed no significant difference among the groups ($F_{3,31}=0.28$, $P>0.05$).

Effects of OFQ on the mean hot plate latency in the presence of CTOP

The selective μ-opioid receptor antagonist, CTOP, produced no hyperalgesic response when administered alone i.c.v. at

any of the tested doses ($F_{3,22}=0.88$, $p>0.05$). At the two higher doses (10 and 50 μg), however, CTOP was observed to increase motor behaviors. The effect of OFQ was therefore examined in the presence of the lowest dose (5 μg) of CTOP. Despite the fact that OFQ and CTOP did not significantly change the mean hot plate latency when each drug was administered alone (see above) or in conjunction with aCSF (Figure 3), there was a significant decrease in the mean hot plate latency in rats treated with a combination of the two drugs ($F_{1,14}=10.83$; $P<0.05$). The baseline values were not significantly different between the two groups ($F_{1,14}=0.32$, $P>0.05$).

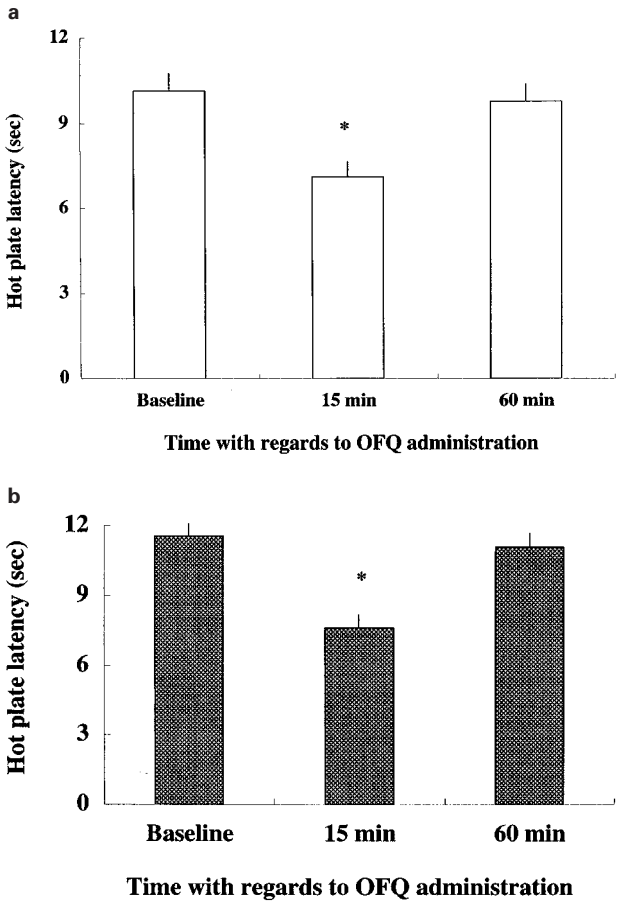

Figure 1 Pretreatment with naloxone revealed a hyperalgesic effect of OFQ in male (a) and female (b) rats. Rats were tested on the hot plate test prior to any drug administration (baseline). Twenty min later, rats were pretreated with naloxone (1 mg kg^{-1}, s.c.) and 10 min later injected with OFQ (15 nmol, i.c.v.; $n=6$ rats dose^{-1}). Rats were tested after a 15-min delay. *Significantly different from baseline and 60 min time point ($P<0.05$).

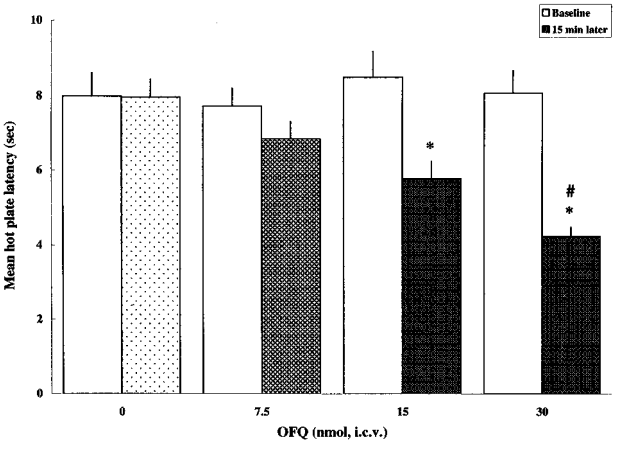

Figure 2 OFQ dose-dependently produced hyperalgesia in the presence of naloxone. Rats were initially tested for baseline and 20 min later injected with naloxone (1 mg kg^{-1}, s.c.). After a 10-min delay, rats received OFQ (7.5–30 nmol, i.c.v.; $n=6$–12 rats dose^{-1}). Rats were then tested on the hot plate machine after a further 15-min delay. *Significantly different from rats treated with aCSF or the lowest dose of OFQ ($P<0.05$). #Significantly different from all other groups ($P<0.05$).

Effects of OFQ on the mean hot plate latency in the presence of naltrindole (NTI)

I.c.v. administration of the δ-opioid receptor selective antagonist, NTI, alone decreased the baseline hot plate latency at a dose of 1 and 5 μg ($F_{5,29}=2.84$, $P<0.05$; Figure 4). The lowest dose of NTI (0.5 μg) had no effect alone and also failed to reveal a hyperalgesic effect of OFQ ($P>0.05$).

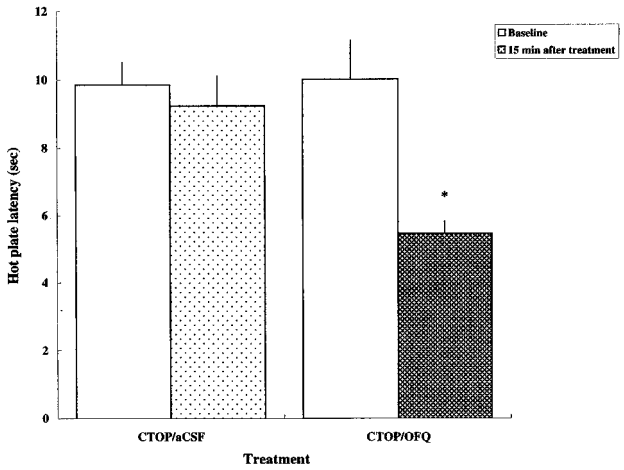

Figure 3 OFQ produces hyperalgesia in the presence of CTOP (D-Phe-Cyc-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2). Rats were tested for baseline and 20 min later injected with either aCSF or CTOP (5 μg, i.c.v.). After a 10-min delay, rats received OFQ (15 nmol, i.c.v.; $n=6$–12 rats group^{-1}) and tested on the hot plate apparatus after a 15-min delay. *Significantly different from rats treated with CTOP followed by aCSF ($P<0.05$).

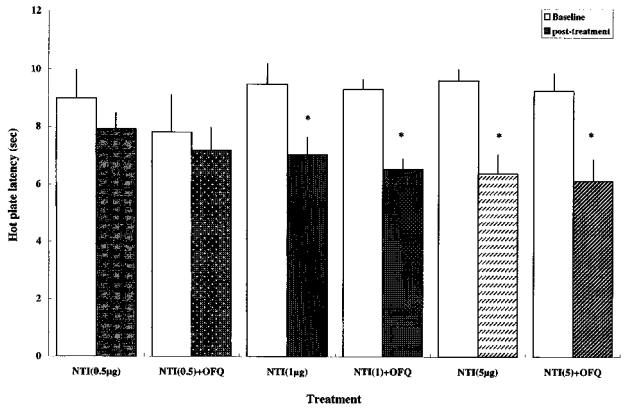

Figure 4 OFQ did not produce hyperalgesia in the presence of naltrindole (NTI). Rats were initially tested for baseline hot plate latency prior to any drug administration. Twenty min later, rats were injected with NTI (0.5, 1 and 5 μg, i.c.v.). After a 10-min delay, some rats received OFQ (15 nmol, i.c.v.; $n=6$–9 rats group^{-1}). All rats were then tested on the hot plate apparatus after a further 15-min delay. *Significantly different from baseline ($P<0.05$).

Furthermore, the higher doses of NTI (1 and 5 μg) that significantly ($P<0.05$) decreased hot plate latencies did not reveal a further hyperalgesic effect of OFQ (Figure 4).

Effects of OFQ on the mean hot plate latency in the presence of nor-binaltorphimine (nor-BNI)

The κ-opioid receptor antagonist, nor-BNI, did not produce any hyperalgesic effect when administered i.c.v. alone at any dose tested ($F_{2,17}=0.19$, $P>0.05$). Furthermore, the combination of the highest dose of nor-BNI (50 μg) with OFQ (15 nmol, i.c.v.) failed to produce a hyperalgesic effect ($F_{3,18}=0.24$, $P>0.05$; Figure 5).

Discussion

The main finding of the present study is that OFQ produced hyperalgesia in the presence of naloxone in the rat hot plate test. Moreover, OFQ elicited hyperalgesia in the presence of CTOP, a selective μ-opioid receptor antagonist, but not the δ- or κ-opioid receptor antagonists, NTI and nor-BNI, respectively, indicating that blockade of the μ-opioid receptor is a determinant factor in revealing the hyperalgesic effect of OFQ.

Initial studies showed that i.c.v. administration of OFQ produces hyperalgesia in the mouse hot plate test (Meunier *et al*., 1995) and tail flick assay (Reinscheid *et al*., 1995). The results of subsequent studies, however, suggested that OFQ, rather than producing hyperalgesia *per se*, opposes an opioid receptor-mediated stress-induced antinociception (Mogil *et al*., 1996; Suaudeau *et al*., 1998). This was further supported by the observation that OFQ blocked the antinociceptive effect of exogenous opioid receptor agonists (Mogil *et al*., 1996; Tian *et al*., 1997; Morgan *et al*., 1997; Lutfy *et al*., 1999). OFQ was therefore proposed to act simply as an endogenous counter-opioid peptide in brain nociceptive circuitry (Mogil *et al*., 1996; Suaudeau *et al*., 1998). In the present study we tested the converse hypothesis, whereby endogenous opioid peptide systems may counteract an underlying pronociceptive action of OFQ, such that if opioid receptors are blocked by pretreatment with opioid receptor antagonists OFQ would produce hyperalgesia. Our results show that blockade of an endogenous opioidergic tone does, indeed, uncover the expression of a hyperalgesic effect of OFQ in both male and female rats. The data indicate that the apparent hyperalgesic effect of OFQ observed previously (Meunier *et al*., 1995; Reinscheid *et al*., 1995) may not be due simply to inhibition of opioid receptor-mediated stress-induced antinociception for the reasons given below. First, no stress-induced antinociception was apparent in our model (Lutfy *et al*., 1999). Second, accordingly, naloxone alone did not produce hyperalgesia in the current experiments, and third, OFQ should have been without any significant effect in the presence of naloxone if OFQ were simply blocking endogenous opioid-mediated stress-induced antinociception. Rather, they suggest that the opioid and OFQ systems may function as two parallel, mutually opposing pathways. We propose that exogenously administered OFQ elicits a counter-balancing activation of endogenous opioid circuits. When this is blocked by naloxone, the hyperalgesic effect of OFQ is allowed to predominate. Conversely, concomitant blockade of the ORL-1 receptor might lead to a greater antinociceptive effect of opioid receptor agonists. The development of non-peptide ORL-1 receptor antagonists will shortly allow this to be tested.

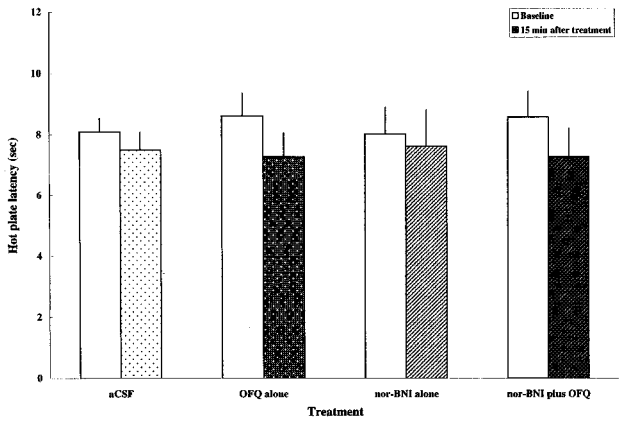

Figure 5 OFQ and nor-binaltorphimine (nor-BNI) did not produce hyperalgesia when administered alone or in combination. Rats were tested for baseline and 20 min later injected with aCSF or nor-BNI (50 μg, i.c.v.). Some rats were left untreated. After a 10-min delay, rats received an i.c.v. injection of aCSF or OFQ (15 nmol; $n=5-6$ rats treatment^{-1}) and tested after a further 15-min delay.

The apparent discrepancy between our data and those of others (Mogil *et al*., 1996; Suaudeau *et al*., 1998) could be due to the use of different experimental paradigms. In particular, the dose of OFQ may be important since OFQ produced hyperalgesia in the present study at doses that were not included in previous investigations (Mogil *et al*., 1996; Suaudeau *et al*., 1998). However, it should be noted that closer observation of the data of Mogil *et al*. (1996) reveal that a combination of naloxone and OFQ appeared to produce a greater apparent hyperalgesic response than naloxone alone, which is in concordance with our data.

At present, at least three distinct classes of opioid receptors, namely μ, δ and κ, are cloned and pharmacologically characterized (for review, see Minami & Satoh, 1995). Naloxone is considered as a non-selective opioid receptor antagonist. To determine which subtype of the opioid receptors was blocked by naloxone to reveal the hyperalgesic action of OFQ, we studied the effect of OFQ in the presence of CTOP, NTI and nor-BNI which are, respectively, μ-, δ- and κ-opioid receptor selective antagonists. Despite the fact that CTOP did not change the mean hot plate latency when administered in conjunction with aCSF, the combination of CTOP with OFQ did produce hyperalgesia, indicating that blockade of the μ-opioid receptor is sufficient to uncover the hyperalgesic effect of OFQ. Since NTI and nor-BNI were without effect in this regard, our results suggest that it is a μ-opioid receptor-mediated event that counteracts the hyperalgesic effect of exogenous OFQ, without affecting basal nociceptive responses.

In conclusion, OFQ and naloxone each were devoid of any hyperalgesic action when administered alone at doses used in the present study. However, pretreatment with naloxone revealed the hyperalgesic effect of OFQ in the rat hot plate test. Pretreatment with CTOP, a selective μ-opioid receptor antagonist, similarly allowed OFQ to produce a hyperalgesic response. In contrast, NTI and nor-BNI did not display such an effect. Our data when taken in the context of previous studies suggest that OFQ can produce hyperalgesia in the absence of opioid receptor-mediated stress-induced antinociception and support the notion that OFQ and endogenous opioid peptides, acting at the μ-opioid receptor, may act in a mutually opposing manner in brain circuitry to regulate nociceptive tone.

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References

BUNZOW, J.R., SAEZ, C., MORTRUD, M., BOUVIER, C., WILLIAMS, J.T., LOW, M. & GRANDY, D.K. (1994). Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a μ, δ or κ opioid receptor type. *FEBS Lett.,* **347,** 284 – 288.

CHEN, Y., FAN, Y., LIU, J., MESTEK, A., TIAN, M., KOZAK, C.A. & YU, L. (1994). Molecular cloning, tissue distribution and chromosomal localization of a novel member of the opioid receptor gene family. *FEBS Lett.,* **347,** 279 – 283.

CONNOR, M., VAUGHAN, C.W., CHIENG, B. & CHRISTIE, M.J. (1996a). Nociceptin receptor coupling to a potassium conductance in rat locus coeruleus neurones in vitro. *Br. J. Pharmacol.,* **119,** 1614 – 1618.

CONNOR, M., YEO, A. & HENDERSON, G. (1996b). The effect of nociceptin on Ca^{2+} channel current and intracellular Ca^{2+} in the SH-SY5Y human neuroblastoma cell line. *Br. J. Pharmacol.,* **118,** 205 – 207.

DEVINE, D.P., LEONE, P. & WISE, R.A. (1993). Mesolimbic dopamine neurotransmission is increased by administration of μ-opioid receptor antagonists. *Eur. J. Pharmacol.,* **243,** 55 – 64.

FUKUDA, K., KATO, S., MORI, K., NISHI, M., TAKESHIMA, H., IWABE, N., MIYATA, T., HOUTANI, T. & SUGIMOTO, T. (1994). cDNA cloning and regional distribution of a novel member of the opioid receptor family. *FEBS Lett.,* **343,** 42 – 46.

HAO, J.X., WIESENFELD-HALLIN, Z. & XU, X.J. (1997). Lack of cross-tolerance between the antinociceptive effect of intrathecal orphanin FQ and morphine in the rat. *Neurosci. Lett.,* **223,** 49 – 52.

JENSEN, T.S. & YAKSH, T.L. (1986). Comparison of the antinociceptive action of μ- and δ-opioid receptor ligands in the periaqueductal gray matter, medial and paramedial ventral medulla in the rat as studied by the microinjection technique. *Brain Res.,* **372,** 301 – 312.

JHAMANDAS, K.H., SUTAK, M. & HENDERSON, G. (1998). Antinociceptive and modulatory actions of spinal orphanin FQ. *Can. J. Physiol. Pharmacol.,* **76,** 314 – 324.

JULIUS, D. (1995). Home for an orphan endorphin. *Nature,* **377,** 476.

KING, M.A., ROSSI, G.C., CHANG, A.H., WILLIAMS, L. & PASTERNAK, G.W. (1997). Spinal analgesic activity of orphanin FQ/nociceptin and its fragments. *Neurosci. Lett.,* **223,** 113 – 116.

KNOFLACH, F., REINSCHEID, R.K., CIVELLI, O. & KEMP, J.A. (1996). Modulation of voltagegated calcium channels by orphanin FQ in freshly dissociated hippocampal neurons. *J. Neurosci.,* **16,** 6657 – 6664.

LACHOWICZ, J.E., SHEN, Y., MONSMA JR. F.J. & SIBLEY, D.R. (1995). Molecular cloning of a novel G protein-coupled receptor related to the opiate receptor family. *J. Neurochem.,* **64,** 34 – 40.

LUTFY, K., SHARZA, S.A. & MAIDMENT, N.T. (1999). Tolerance develops to the inhibitory effect of orphanin FQ on morphine-induced antinociception in the rat. *Neuro Report,* **10,** 103 – 106.

MATTHES, H., SEWARD, E.P., KIEFFER, B. & NORTH, A. (1996). Functional selectivity of orphanin FQ for its receptor coexpressed with potassium channel subunits in *Xenopus laevis* Oocytes. *Mol. Pharmacol.,* **50,** 447 – 450.

MEUNIER, J.-C., MOLLEREAU, C., TOLL, L., SUAUDEAU, C., BUTOUR, J.-L., GUILLEMOT, J.-C., FERREARA, P., MONSARRAT, B., MAZARGUIL, H., VASSART, G., PARMENTIER, M. & COSTENTIN, J. (1995). Isolation and structure of the endogenous agonist of the opioid receptor-like ORL_1 receptor. *Nature,* **377,** 532 – 535.

MINAMI, M. & SATOH, M. (1995). Molecular biology of the opioid receptors: structure, functions, and distributions. *Neurosci. Res.,* **23,** 121 – 145.

MOGIL, J.S., GRISEL, J.E., REINSCHEID, R.K., CIVELLI, O., BELKNAP, J.K. & GRANDY, D.K. (1996). Orphanin FQ is a functional anti-opioid peptide. *Neuroscience,* **75,** 333 – 337.

MOLLEREAU, C., PARMENTIER, M., MAILLEUX, P., BUTOUR, J.-L., MOISAND, C., CHALON, P., CAPUT, D., VASSART, G. & MEUNIER, J.-C. (1994). ORL1, a novel member of the opioid receptor family: cloning, functional expression and localization. *FEBS Lett.,* **341,** 33 – 38.

MORGAN, M.M., GRIESEL, J.E., ROBBINS, C.S. & GRANDY, D.K. (1997). Antinociception mediated by the periaqueductal gray is attenuated by orphanin FQ. *Neuro Report,* **8,** 3431 – 3434.

NEAL JR. C.R., MANSOUR, A., REINSCHEID, R., NOTHACKER, H.-P., CIVELLI, O. & WATSON JR., S.J. (1999). Localization of orphanin FQ (nociceptin) peptide and messanger RNA in the central nervous system of the rat. *J. Comp. Neurol.,* **406,** 503 – 547.

PAXINOS, G. & WATSON, C. (1986). *The rat brain in stereotaxic coordinates,* 2nd ed, New York: Academic Press.

PORTOGHESE, P.S., LIPKOWSKI, A.W. & TAKEMORI, A.E. (1987). Binaltorphimine and nor-binaltorphimine, potent and selective κ-opioid receptor antagonists. *Life Sci.,* **40,** 1287 – 1292.

PORTOGHESE, P.S., SULTANA, M., NAGASE, H. & TAKEMORI, A.E. (1988a). Application of the message-address concept in the design of highly potent and selective non-peptide δ-opioid receptor antagonists. *J. Med. Chem.,* **31,** 281 – 282.

PORTOGHESE, P.S., SULTANA, M. & TAKEMORI, A.E. (1988b). Naltrindole, a highly selective and potent non-peptide δ-opioid receptor antagonist. *Eur. J. Pharmacol.,* **146,** 185 – 186.

REINSCHEID, R.K., HIGELIN, J., HENNINGSEN, R.A., MONSMA JR. F.J. & CIVELLI, O. (1998). Structures that delineate orphanin FQ and dynorphin-A pharmacological selectivities. *J. Biol. Chem.,* **273,** 1490 – 1495.

REINSCHEID, R.K., NOTHACKER, H.-P., BOURSON, A., ARDATI, A., HENNINGSEN, R.A., BUNZOW, J.R., GRANDY, D.K., LANGEN, H., MONSMA JR. F.J. & CIVELLI, O. (1995). Orphanin FQ: A neuropeptide that activates an opioid-like G protein-coupled receptor. *Science,* **270,** 792 – 794.

ROSSI, G.C., LEVENTHAL, L. & PASTERNAK, G.W. (1996). Naloxone sensitive orphanin FQ/nociceptin analgesia in mice. *Eur. J. Pharmacol.,* **311,** R7 – R8.

ROSSI, G.C., PERLMUTTER, M., LEVENTHAL, L., TALATTI, A. & PASTERNAK, G.W. (1998). Orphanin FQ/nociceptin and its fragments. *Brain Res.,* **792,** 327 – 330.

SUAUDEAU, C., FLORIN, S., MEUNIER, J.-C. & COSTENTIN, J. (1998). Nociceptin-induced apparent hyperalgesia in mice as a result of the prevention of opioid autoanalgesic mechanisms triggered by the stress of an intracerebroventricular injection. *Fundam. & Clin. Pharmacol.,* **12,** 420 – 425.

TAKEMORI, A.E., HO, B.Y., NAESETH, J.S. & PORTOGHESE, P.S. (1988). Nor-binaltorphimine, a highly selective κ-opioid antagonist in analgesic and receptor binding assays. *J. Pharmacol. Exp. Ther.,* **246,** 255 – 258.

TIAN, J.-H., XU, W., FANG, Y., MOGIL, J.S., GRISEL, J.E., GRANDY, D.K. & HAN, J.-S. (1997). Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat. *Br. J. Pharmaco.,* **120,** 676 – 680.

TOLL, L. (1992). Comparison of μ opioid receptor binding on intact neuroblastoma cells with guinea pig brain and neuroblastoma cell membranes. *J. Pharmacol. Exp. Ther.,* **260,** 9 – 15.

VAUGHAN, C.W. & CHRISTIE, M.J. (1996). Increase by the ORL1 receptor (opioid receptor-like 1) ligand, nociceptin, of inwardly rectifying K conductance in dorsal raphe nucleus neurones. *Br. J. Pharmacol.,* **117,** 1609 – 1611.

WANG, J.-L., ZHU, C-B., CAO, X.-D. & WU, G.-C. (1999). Distinct effect of intracerebroventricular and intrathecal injections of nociceptin/orphanin FQ in the rat formalin test. *Reg. Pept.,* **79,** 159 – 163.

WOOLFE, G. & MACDONALD, A.D. (1944). The evaluation of analgesic action of pethidine hydrochloride (demerol). *J. Pharmacol. Exp. Ther.,* **80,** 300 – 307.

XU, X.-J., HAO, J.-X. & WIESENFELD-HALLEN, Z. (1996). Nociceptin or antinociceptin: potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. *Neuro Report,* **7,** 2092 – 2094.

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