CNS Drug Reviews
Vol. 11, No. 2, pp. 97–112

UFP-101, a Peptide Antagonist Selective for the Nociceptin/Orphanin FQ Receptor

Girolamo Calo[1], Remo Guerrini[2], Anna Rizzi[1], Severo Salvadori[2], Melissa Burmeister[3], Daniel R. Kapusta[3], David G. Lambert[4], and Domenico Regoli[1]

[1]Dept. Experimental and Clinical Medicine, Section of Pharmacology and Neuroscience Centre, [2]Dept. of Pharmacological Sciences and Biotechnology Centre, University of Ferrara, Ferrara, Italy; [3]Department of Pharmacology, Louisiana State University Health Sciences Center, New Orleans, LA, USA; [4]Dept. Cardiovascular Sciences, Pharmacology and Therapeutics Group, Division of Anesthesia, Critical Care and Pain Management, University of Leicester, UK

Keywords: Knockout mice — Nociceptin/orphanin FQ — NOP antagonists — NOP receptors — Opioids — UFP-101.

ABSTRACT

Nociceptin/orphanin FQ modulates various biological functions at central and peripheral levels by selectively activating a G-protein coupled receptor named N/OFQ peptide (NOP) receptor. For extending our knowledge on the biological roles of the N/OFQ – NOP receptor system the identification of selective NOP ligands, especially antagonists, is mandatory. [Nphe^1, Arg^{14}, Lys^{15}] N/OFQ-NH_2 (UFP-101) is a novel NOP ligand that was designed by combining, in the same molecule, the [Nphe^1] chemical modification which eliminates efficacy and the [Arg^{14}, Lys^{15}] substitution which increases ligand potency and duration of action *in vivo*. In the present article, we summarize the pharmacological features of UFP-101 as determined in a series of *in vitro* and *in vivo* assays. Moreover, some biological actions and possible therapeutic indications of NOP ligands are discussed on the basis of results obtained with UFP-101. Data obtained with this compound were compared with those generated using other NOP antagonists, especially J-113397 and [Nphe^1]N/OFQ(1–13)-NH_2, receptor or peptide knockout mice and other pharmacological tools useful for blocking N/OFQ – NOP receptor signaling.

Address correspondence and reprint requests to: Girolamo Calo, MD, PhD. Departrment Experimental and Clinical Medicine, Section of Pharmacology, University of Ferrara, via Fossato di Mortara, 19, 44100 Ferrara, Italy. Tel.: +39 (0532) 291-221; Fax: +39 (0532) 291-205; E-mail: g.calo@unife.it

The analysis of the available data demonstrates that UFP-101 is a useful pharmacological tool for the investigation of the central and peripheral biological functions regulated by the N/OFQ – NOP receptor system and for defining the therapeutic potential of NOP receptor ligands

INTRODUCTION

Drugs interacting with G-protein coupled receptors (GPCRs) have a variety of therapeutic indications from pain to hypertension, respiratory and gastrointestinal diseases, neurological and psychiatric pathologies, and control of food intake, making this class of membrane receptors the most important biological target for drug discovery (94). This may remain true in the future as at least 360 different genes coding for putative GPCRs (excluding sensory GPCRs) have been identified in the human genome yet the natural ligand is known only for approximately 210 receptors, the others being still orphan (94). Novel drugs acting at orphan GPCRs are likely to provide innovative treatments for a variety of pathological conditions and diseases (57,93,94). The first step in understanding the function and the potential of an orphan GPCR as drug target is the identification of its endogenous ligand (58). This process, defined as reverse pharmacology (20), has already led to the pairing of several previously orphan GPCRs with their endogenous ligands: in this way, several novel ligand-receptor systems have been recognized. These appear to be important in the regulation of a range of various biological functions including sleep, pain transmission, cardiovascular homeostasis, airways physiology, and inflammatory processes (21,44,57).

Nociceptin/orphanin FQ (N/OFQ) and its N/OFQ peptide receptor (NOP) represent the first successful example of the reverse pharmacology approach (20). The cloning of classical opioid receptors in the early 90s (14,29,46,97) led several groups to the simultaneous identification of an opioid-like receptor in 1994 which did not bind opioid ligands (66). A heptadecapeptide was identified as the natural ligand for the opioid-like receptor at the end of 1995 and was named nociceptin (67) or orphanin FQ (80). This novel peptide/receptor system is now considered "a non-opioid branch of the opioid family" of peptides and receptors (22); this definition is based from one side on close structural and transductional similarities and from the other on the pharmacological and functional differences between the N/OFQ – NOP receptor and the classical opioid systems (9,68).

The genes coding for the N/OFQ precursor (ppN/OFQ) and the NOP receptor proteins are now cloned in various species. The structural organization of ppN/OFQ is similar to opioid peptide precursors, supporting the view of a common origin for the opioid systems and the N/OFQ-NOP receptor system (69,75). In addition to N/OFQ, the precursor may encode two other biologically active peptides, named nocistatin (77) and N/OFQ2 (87), which do not bind the NOP receptor. Anatomic studies have revealed high levels of expression of the N/OFQ messenger RNA in brain structures involved in sensory, emotional and cognitive processing (81). NOP receptors are located both pre- and post-synaptically in various areas of the central nervous system, in particular in the forebrain (cortical areas, olfactory regions, limbic structures, thalamus), throughout the brainstem (central periaqueductal gray, substantia nigra, several sensory and motor nuclei), and in both the dorsal and ventral horns of the spinal cord (70). NOP mRNA and binding sites exhibit approxi-

mately the same distribution pattern, indicating that the NOP receptor is mainly located on local neuronal circuits. The NOP receptor is also expressed in the peripheral nervous system (70). The diffuse distribution of NOP mRNA and binding in the central nervous system supports an extensive role for N/OFQ in a multitude of functions. Indeed, animal studies demonstrated that N/OFQ, via NOP receptor activation, modulates several biological functions including pain transmission, stress and anxiety, learning and memory, locomotor activity, food intake, and the motivational properties of drugs of abuse. N/OFQ may also intervene in the regulation of the functions of peripheral systems such as the cardiovascular, gastrointestinal, renal, genitourinary and respiratory (9,68). It is worthy of mention that the inhibitory effect of N/OFQ on the micturition reflex, well documented in rodents (56), has been recently confirmed in patients with neurogenic bladder (54,55).

Understanding of the biological roles played by the N/OFQ-NOP receptor system is, to a major extent, dependent upon the identification of highly potent and NOP selective ligands, especially antagonists. Currently available ligands for the NOP receptor have been recently reviewed (99). They can be divided into three groups based on their chemical nature: i) small molecules discovered via high throughput screening within pharmaceutical industry, e.g., the NOP selective antagonists J-113397 (1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-benzimidazol-2-one, (78)) and SB-612111 {(–)-cis-1-methyl-7-[[4-(2,6-dichlorophenyl) piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol, (98)}, or the selective agonist Ro 64-6198 {[(1S,3aS)-8-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one], (40)}; ii) short peptides identified by screening of synthetic peptide combinatorial libraries, e.g., the NOP selective partial agonists Ac-RYYRWK-NH_2 and Ac-RYYRIK-NH_2 (28) or the non-selective NOP antagonist peptide III-BTD (2); and iii) N/OFQ related peptides identified by classical structure-activity relationship studies. Our research group contributed in this latter area with the identification and pharmacological characterization of the following NOP ligands: the full agonists N/OFQ(1–13)-NH_2 and N/OFQ-NH_2 (5,33), the partial agonist [$Phe^1\Psi(CH_2\text{-}NH)Gly^2$]N/OFQ(1–13)-$NH_2$ (6,34), and the pure antagonist [$Nphe^1$]N/OFQ(1–13)-NH_2 (8,35). More recently, two highly potent NOP agonists were identified by addition of a fluorine atom at the para position of Phe^4, generating [(pF)Phe^4]N/OFQ(1–13)-NH_2 (4,36,83), or through introduction in position 14 and 15 an extra couple of basic residues Arg, Lys generating [Arg^{14}, Lys^{15}]N/OFQ (76,86). We combined in the N/OFQ-NH_2 sequence the chemical modifications which reduce ([$Phe^1\Psi(CH_2\text{-}NH)Gly^2$]) or eliminate ([$Nphe^1$]) agonist efficacy with those which increase agonist potency ([(pF)Phe^4] and [$Arg^{14}Lys^{15}$]) thus generating a novel series of NOP ligands (37). Among these, the most interesting molecules were the pure antagonist [$Nphe^1$, Arg^{14}, Lys^{15}]N/OFQ-NH_2 (UFP-101), the full agonist [(pF)Phe^4, Arg^{14}, Lys^{15}]N/OFQ-NH_2 [UFP-102, (12)] and the partial agonist [$Phe^1\Psi(CH_2\text{-}NH)Gly^2$, (pF)$Phe^4$, Arg^{14}, Lys^{15}]N/OFQ-NH_2.

In the present article, we summarized the pharmacological features of UFP-101 as determined in a series of *in vitro* and *in vivo* assays. Moreover, some biological actions and possible therapeutic indications of NOP ligands are discussed on the basis of results obtained with UFP-101 and i) other NOP antagonists, especially J-113397 and [$Nphe^1$]N/OFQ(1–13)-NH_2; ii) results obtained in knockout animals (NOP receptor ($NOP^{-/-}$, (73)) and ppN/OFQ ($ppN/OFQ^{-/-}$ (48) mice), iii) other pharmacological tools

useful for blocking N/OFQ – NOP receptor signaling such as oligo antisense or antibodies targeting the peptide or the receptor.

PHARMACOLOGY

Basic Pharmacological Profile of UFP-101

The cellular actions induced by N/OFQ via NOP receptor activation are similar (if not superimposable) to those elicited by classical opioids. In fact, in different cell types, NOP receptor activation inhibited adenylyl cyclase and Ca^{2+} channels while activating K^+ channels via pertussis toxin-sensitive G-proteins (39). This indicates that NOP receptors couple to the $G_{i/o}$ class of G-proteins. Via these cellular actions, NOP receptors located on neurons produced robust inhibitory effects either by reducing neurotransmitter release (presynaptic localization) or cellular excitability (postsynaptic localization). Thus, the ability of N/OFQ to promote GTPγ[^{35}S] binding and to inhibit forskolin stimulated cAMP accumulation in cell cultures, to reduce neurogenic contractions in isolated tissues, and to inhibit neurotransmitter release and stimulate K^+ conductance in various brain preparations, was used for developing a rather large series of *in vitro* pharmacological preparations suitable for investigating novel NOP ligands with different biochemical, bioassay, neurochemical and electrophysiological approaches. UFP-101 data obtained by different laboratories with such approaches are summarized in Table 1.

TABLE 1. In vitro *pharmacological profile of UFP-101*

Preparation	N/OFQ action	pEC_{50}	UFP-101 action	pA_2	References
CHOhNOP	↑ GTPγ[^{35}S]	8.7	comp antagonism	9.1	(11)
CHOhNOP	↓ cAMP	11.0	comp antagonism	7.1	(11)
CHOhNOP	↑ NOP internalization	ND	antagonism	—	(27)
Vas deferens (m)	↓ contractions	7.7	comp antagonism	7.3	(11)
Vas deferens (r)	↓ contractions	7.2	comp antagonism	7.3	(11)
Ileum (gp)	↓ contractions	8.1	comp antagonism	7.2	(11)
Bronchus (h)	↓ contractions	~7	antagonism	ND	(1)
Monocytes (h)	↑ chemotaxis	~12	comp antagonism	7.0	(92)
CC synaptosomes (r)	↓ 5-HT release	7.5	comp antagonism	7.7	(11)
CC synaptosomes (r)	↓ NE release	7.7	antagonism	ND	(61)
CC synaptosomes (m)	↓ 5-HT release	8.6	antagonism	ND	(65)
Locus coeruleus slices (r)	↑ K^+ current	ND	antagonism	ND	(31)
Dorsal raphe slices (r)	↑ K^+ current	ND	antagonism	ND	(31)
Periaquedutal gray slices (r)	↑ K^+ current	7.3	antagonism	ND	(16)
Substantia nigra slices (r)	↓ DA neuron firing	ND	antagonism	ND	(63)

Abbreviations: h, human; m, mouse; r, rat; gp, guinea pig; CC, cerebrocortical; 5-HT, serotonin; NE, norepinephrine; DA, dopamine; comp, competitive.

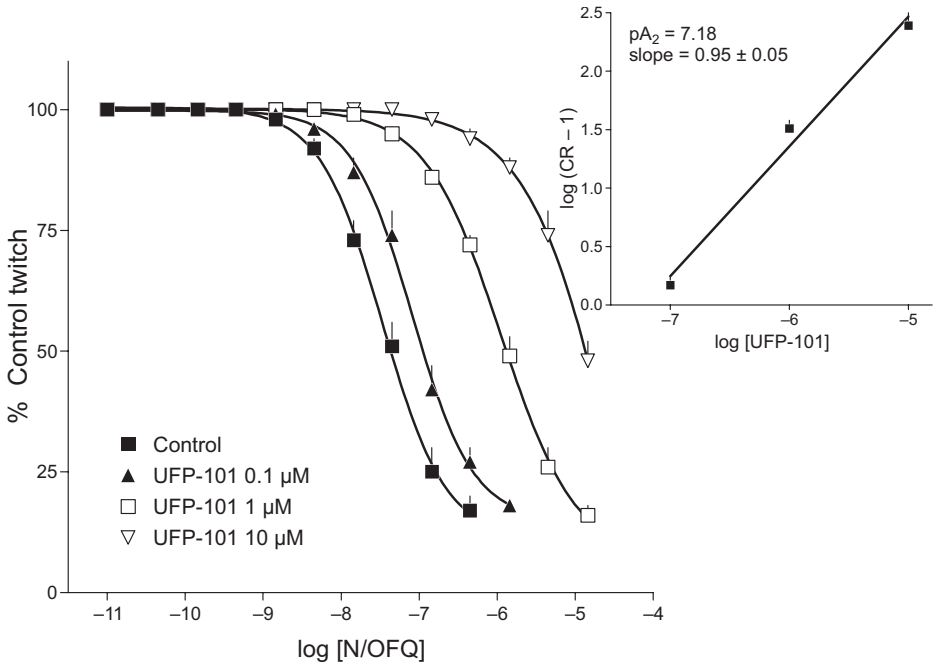

Fig. 1. Effects of UFP-101 on the inhibitory effects of nociceptin/orphanin FQ (N/OFQ) in the electrically stimulated mouse vas deferens. The relative Schild plot is shown in the insert. The data are means ± S.E.M. of at least 5 separate experiments.

UFP-101 binds with high affinity (pK_i 10.2) and selectivity (~3000 fold over classical opioid receptors) to the human recombinant NOP receptor expressed in CHO cells (11). In functional studies, such as the stimulation of GTPγ[^{35}S] binding and the inhibition of forskolin stimulated cAMP accumulation in CHOhNOP cells, UFP-101 competitively antagonized N/OFQ effects being inactive *per se* (Table 1). In the GTPγ[^{35}S] binding assay, UFP-101 antagonistic properties were also confirmed against a panel of NOP agonists including the peptides N/OFQ(1–13)NH_2, [(pF)Phe^4]N/OFQ(1–13)-NH_2 and [Arg^{14}, Lys^{15}]N/OFQ, and the non-peptide Ro 64-6198 (64). In the same CHOhNOP preparation, UFP-101 also prevented the internalization of NOP binding sites induced by N/OFQ, being inactive when tested alone (27).

The pure and competitive antagonistic profile of UFP-101 was confirmed using preparations expressing native NOP receptors and bioassay, neurochemical and electrophysiological techniques (see Table 1 and, as an example of Schild analysis, Fig. 1). Similar results were also obtained using tissues of human origin such as the isolated bronchus and monocytes where UFP-101 antagonized N/OFQ inhibition of electrically induced contractions (1) and chemotaxis (92), respectively.

With respect to antagonist potency, in the various preparations UFP-101 displayed pA_2 values in the range 7.0–7.7, with the only exception of the GTPγ[^{35}S] binding assay where a pA_2 value of 9.1 was obtained. Similar pA_2 values in the various preparations have been obtained with other NOP selective antagonists such as the peptide

[Nphe^1]N/OFQ(1–13)-NH_2 (range 6.0–6.7) (8,10) and the non peptide J-113397 (range 7.4–8.2) (3,10). Collectively, these data demonstrated that the functional sites, which mediated the effects of N/OFQ in the various preparations belong to the same receptor type, i.e., the NOP receptor.

The antagonist potency of UFP-101 is approximately ten fold higher than that of the NOP antagonist [Nphe^1]N/OFQ(1–13)-NH_2 (8,9); this difference in potency is similar to that of the agonists N/OFQ and [Arg^{14}, Lys^{15}]N/OFQ (76,86). Therefore, the insertion of Arg-Lys in position 14 and 15 produces the same effects (increase of potency, no changes in efficacy) when applied to either the agonist (N/OFQ) or antagonist ([Nphe^1]N/OFQ) chemical templates (37).

Collectively data obtained *in vitro* in a variety of preparations with different approaches demonstrated that UFP-101 behaves as a potent, competitive and selective antagonist at NOP receptors.

In Vivo Actions of UFP-101

In vivo, UFP-101 has been challenged with N/OFQ in a series of experiments aimed at the investigation of the role of the N/OFQ-NOP receptor system in regulating various biological functions including pain transmission, locomotor activity, mood-related behaviors, drug abuse, food intake and cardiovascular, kidney and gastrointestinal functions (Table 2). In the following sections the actions of UFP-101 will be briefly summarized and discussed relative to data obtained with other NOP antagonists (particularly J-113397 and [Nphe^1]N/OFQ(1–13)-NH_2), with $NOP^{-/-}$ and $ppN/OFQ^{-/-}$ mice, and oligo antisense or antibodies targeting the peptide or the receptor.

Pain transmission

The relationships between the N/OFQ – NOP receptor system and pain transmission are complex (100). Most of the available data suggest that N/OFQ signaling has opposite actions at supraspinal and spinal levels being pronociceptive in the brain and antinociceptive in the spinal cord. The data we obtained using the tail withdrawal assay in mice are consistent with this view. In fact, N/OFQ given intracerebroventricularly (i.c.v.) in the 0.1–10 nmol dose range produces clear pronociceptive effects (7) while it evokes antinociceptive actions when administered intrathecally (i.t.) (84). Both these actions of N/OFQ are no longer evident in animals treated with 10 nmol of UFP-101 (11) (Fig. 2). This indicates that NOP receptors are involved in both actions. This conclusion is corroborated by knockout studies which demonstrated that both the supraspinal pronociceptive and spinal antinociceptive effects of N/OFQ are no longer evident in $NOP^{-/-}$ mice (73, 84). Worthy of mention are the effects produced by UFP-101 alone: the peptide evoked a clear antinociceptive effect when given i.c.v. (11) whilst being inactive following spinal administration (Fig. 2). These results may suggest a tonic activation of the endogenous N/OFQ – NOP receptor supraspinal (but not spinal) system and this view is strengthened by the fact that i.c.v. administration of other NOP receptor peptide antagonists such as [Nphe^1]N/OFQ(1–13)-NH_2 (8) and retroN/OFQ-methyl-ester (41) produced similar effects. However, it should be noted that conflicting results have been reported with non-peptide NOP antagonists given systemically with no effect reported for J-113397 (78) and

TABLE 2. In vivo *pharmacological profile of UFP-101*

Test/Assay	N/OFQ action	Effective dose	UFP-101 action	Effective dose	References
Tail withdrawal (m, i.c.v.)	↓ TW latencies	1 nmol	antagonism*	10 nmol	(11)
Tail withdrawal (m, i.t.)	↑ TW latencies	1 nmol	antagonism	10 nmol	(84)
Locomotion (m, i.c.v.)	↓ spontaneous LA	1 nmol	antagonism	10 nmol	(11)
Locomotion (m, i.c.v.)	↑/↓ spontaneous LA	0.1–5 nmol	antagonism	10 nmol	(53)
Rotarod (r, SNc)	↓ performance	0.1 nmol	antagonism*	1 nmol	(63)
Electromyography (r, SNc)	↓ muscle tone	10 nmol	antagonism*	30 nmol	(63)
Bar test (r, SNc)	ND	—	↓ catalepsia	30 nmol	(62)
Forced swimming (m, i.c.v.)	no effect	—	↓ immobility time	3 nmol	(30)
Forced swimming (r, i.c.v.)	ND	—	↓ immobility time	10 nmol	(31)
Tail suspension (m, i.c.v.)	no effect	—	↓ immobility time	10 nmol	(31)
Microdialysis (r, SNc)	↑ nigral Glu	10 μM	antagonism*	10 μM	Marti et al., PC
Microdialysis (r, SNc)	↓ striatal DA	10 μM	antagonism*	10 μM	(63)
Microdialysis (m, i.c.v.)	↓ accumbal DA	7 nmol	antagonism	5 nmol	(47)
Food intake (r, i.c.v.)	↑ food intake	4 nmol	antagonism	12 nmol	Polidori et al., PC
Cardiovascular (gp, i.v.)	↓ heart rate & blood pressure	6 nmol	antagonism	60 nmol	(38)
Cardiovascular (gp, i.v.)	↓ blood NE	6 nmol	antagonism	60 nmol	(38)
Kidney function (m, i.c.v.)	↑ diuresis	3 nmol	antagonism	30 nmol	Kapusta et al., PC
Kidney function (r, i.c.v.)	↑ diuresis	3 nmol	antagonism	30 nmol	Kapusta et al., PC
Gastric function (r, i.c.v.)	↓ alcohol induced lesions	0.5 nmol	antagonism	2 nmol	(71)
Gastric function (r, i.p.)	↓ alcohol induced lesions	2 nmol	antagonism	20 nmol	(71)

Abbreviations: m, mouse; r, rat; gp, guinea pig; SNc, substantia nigra parts compacta; i.c.v., intracerebroventricular; i.t., intrathecal; i.v., intravenous; TW, tail withdrawal; LA, locomotor activity; ND, not determined; Glu, glutamate; DA, dopamine; NE, norepinephrine; PC, personal communication.

*In these assays UFP-101 per se induces changes opposite to that evoke by N/OFQ: This suggests a tonic control of these biological functions by N/OFQergic pathways.

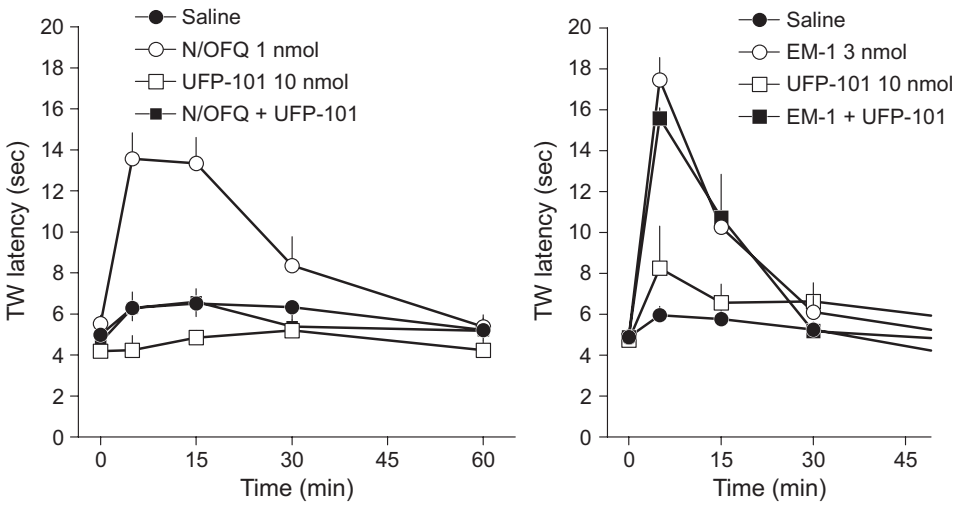

Fig. 2. Effects of UFP-101 against nociceptin/orphanin FQ (N/OFQ)- and endomorphin-1 (EM-1)-mediated antinociceptive effects in the mouse tail withdrawal assay. All compounds were given intrathecally. Data are means ± S.E.M. of at least 12 mice per group.

antinociceptive effects reported for JTC-801 (91) (which displays low NOP selectivity). Moreover, the lack of effect of UFP-101 after spinal administration in the tail withdrawal assay does not exclude the possibility that the spinal N/OFQ system can be activated under different experimental conditions. The results obtained by the group of Zeilhofer are worthy of mention: knockout mice for either the NOP receptor or the ppN/OFQ gene displayed a similar hyperalgesic phenotype in the formalin test (24) and, in this assay, pronociceptive actions have been reported in response to systemic administration of J-113397 to rats (96) and, more recently, to mice (85). Experiments are under way in our laboratories to investigate the action of UFP-101 after i.c.v. and i.t. administration in the mouse formalin test.

In summary, although much remains to be done before reaching a firm conclusion on this topic, the available information indicates that the net effect on pain threshold of blocking N/OFQergic signaling strongly depends on both the level of transmission (spinal vs. supraspinal) and on the type of nociceptive stimulus adopted (acute vs. tonic).

Locomotor activity

In the first paper reporting the identification of N/OFQ as the endogenous ligand of the NOP receptor it was shown that, after i.c.v. injection, the peptide reduces spontaneous locomotor activity in mice (80). This effect was later confirmed both in mice (74,82) and in rats (26) and was demonstrated to be exclusively due to NOP receptor activation by both receptor antagonist {e.g., [Nphe^1]NOFQ(1–13)-NH_2 (82)} and in knockout (73) studies. In agreement with these findings, UFP-101 prevented the inhibitory effect of i.c.v. N/OFQ on spontaneous locomotor activity in mice (11). Similar results were also obtained by Kuzmin et al. who demonstrated that UFP-101 antagonized the inhibitory effects of the NOP selective non-peptide agonist Ro 64-6198 (53). At doses effective against N/OFQ, UFP-101 did not modify locomotor activity *per se* (11) suggesting that this

function is not under the tonic control of the N/OFQ endogenous system. Different and interesting results were recently obtained by the group of Morari on the effects of N/OFQ on rat rotarod performance. N/OFQ microinjected in the substantia nigra pars reticulata impaired animal performance on the rotarod apparatus. This effect was sensitive to UFP-101, which, when tested alone, produced an effect opposite to that of the natural ligand enhancing animal performance (63). The involvement of the NOP receptor in this action of UFP-101 is suggested by the fact that similar results were obtained by both intranigral and systemic administration of J-113397 and that $NOP^{-/-}$ mice outperformed $NOP^{+/+}$ animals in the rotarod test (63). Collectively, these results indicate that endogenous N/OFQergic pathways are activated during exercise-driven locomotion and inhibit motor performance. On the basis of these experimental data NOP selective antagonists are worthy of evaluation in the treatment of conditions characterized by hypolocomotion, such as Parkinson's disease. The very recent observation (62) that UFP-101 reverses haloperidol induced akinesia (a model of functional parkinsonism) further strengthens this view.

Mood-related behaviors

Using the mouse forced swimming test, an animal model widely used for the screening of potential antidepressants (23), it has been shown that two chemically unrelated NOP receptor antagonists, [$Nphe^1$]N/OFQ(1–13)-NH_2 and J-113397 given i.c.v. and i.p., respectively, produce a dose-dependent reduction of immobility time. Under the same experimental conditions, N/OFQ and the non-selective opioid receptor antagonist naloxone are inactive (79). This initial observation was later confirmed and extended using UFP-101. This NOP receptor antagonist dose-dependently reduced the immobility time in the mouse forced swimming assay and its effects were reversed by the co-administration of N/OFQ, which was inactive when tested alone (30). In addition, $NOP^{-/-}$ mice showed a reduced immobility time compared to that observed in wild-type animals (30). The antidepressant-like properties of the NOP antagonist UFP-101 were further explored across species (mice and rats) and assays (forced swimming and tail suspension tests). UFP-101 reduced immobility time of mice subjected to the tail suspension test and $NOP^{-/-}$ animals also displayed an antidepressant-like phenotype in this assay (31). UFP-101 decreased immobility time and increased climbing time in rats submitted to the forced swimming test (31). Thus, results obtained using combined pharmacological and genetic approaches, indicate that blockade of the N/OFQ-NOP receptor signaling in the brain produces antidepressant-like effects in distinct species and animal models and support the NOP receptor as a candidate target for the development of innovative antidepressant drugs.

Little is known about the mechanism by which UFP-101 (and the other NOP selective antagonists) elicits its antidepressant-like effects. It should be noted at this point that UFP-101 antagonizes N/OFQ-induced presynaptic inhibition of norepinephrine (61) and serotonin (11) release in cortical preparations, and also prevents K^+-channel-mediated hyperpolarization triggered by N/OFQ in both locus coeruleus and dorsal raphe neurons (31). The antidepressant-like effects of UFP-101 in the mouse forced swimming test were clearly reduced in animals pretreated with the serotonin synthesis inhibitor PCPA (31). Although the involvement of other mechanisms and neurochemical systems can not be ruled out, these series of experiments suggest that the serotoninergic system is implicated in the antidepressant-like effects induced by NOP antagonists, which might be brought about by

their ability to prevent the inhibitory actions of N/OFQ on dorsal raphe neurons and/or on their terminals in the cerebral cortex.

Drug abuse

In contrast to opioids, N/OFQ does not produce either preference or aversion in the conditioned place preference test (25). On the other hand, the peptide inhibits conditioned place preference to various drugs of abuse including morphine (17,72,88), cocaine (49,88), alcohol (18,52), amphetamine (50), and methamphetamine (101). In some of these studies, the involvement of NOP receptors in the action of N/OFQ was demonstrated with the use of the peptide antagonist [Nphe^1]N/OFQ(1–13)-NH_2 (18,101). More recently, the ability of buprenorphine to modulate ethanol intake in alcohol-preferring rats has been investigated (19). Buprenorphine is a partial agonist at classical opioid receptors but is also able to activate the NOP receptor although with low potency (95). At low doses, buprenorphine increased ethanol consumption whereas at high doses the drug reduced it. The effects of low doses of buprenorphine were antagonized by naltrexone while those evoked by high doses were inhibited by UFP-101. These results suggest that buprenorphine possesses dualistic effects on ethanol consumption; low doses stimulate alcohol intake via activation of classic opioid receptors while higher doses reduce alcohol intake via activation of NOP receptors (19). Similar results were obtained investigating by Lutfy et al. (59) who demonstrated, using receptor antagonist (J-113397) and knockout ($NOP^{-/-}$ mice), that the reduced analgesic effect of high doses of buprenophine is due to NOP receptor activation.

Cardiovascular and kidney function

N/OFQ produces significant changes in cardiovascular and renal function following administration into the periphery or central nervous system in different animal species. With respect to peripheral effects, i.v. bolus N/OFQ produces a rapid and dose-dependent hypotension and bradycardia in conscious or anesthetized animals. These responses are insensitive to treatment with the non-selective opioid antagonist, naloxone (13,32,60). Instead, as demonstrated in rats, mice and guinea pigs, the reduction in heart rate, mean arterial pressure and corresponding decrease in plasma norepinephrine concentration produced by i.v. bolus N/OFQ are prevented by i.v. pretreatment with the selective NOP receptor antagonists [Nphe^1]N/OFQ(1–13)-NH_2 (15,89) or UFP-101 (38). At a time when the cardiovascular responses to i.v. bolus N/OFQ (100 nmol/kg) are blocked by high dose UFP-101 pre-treatment (900 nmol/kg, i.v.) the cardiovascular depressor, water diuretic and renal sympathoinhibitory responses to injection of N/OFQ (or NOP receptor partial agonists) into the brain (5.5. nmol, i.c.v.) are not altered (45). Collectively, these findings suggest that UFP-101 has limited (if any) ability to cross the blood-brain barrier and that this selective NOP receptor antagonist may be useful to differentiate cardiovascular responses evoked by activation of endogenous peripheral versus central NOP receptor systems.

N/OFQ also produces hypotension, bradycardia and water diuresis following administration into the brain (42). In urethane anesthetized rats, pretreatment with [Nphe^1]N/OFQ(1–13)-NH_2 prevented the hypotensive and bradycardic responses produced by microinjection of N/OFQ into the lateral ventricle (15) and commissural subnucleus of the nucleus tractus solitarius (90). Similarly, pretreatment of conscious rats with UFP-101 into the paraventricular nucleus of the hypothalamus antagonized the cardiovas-

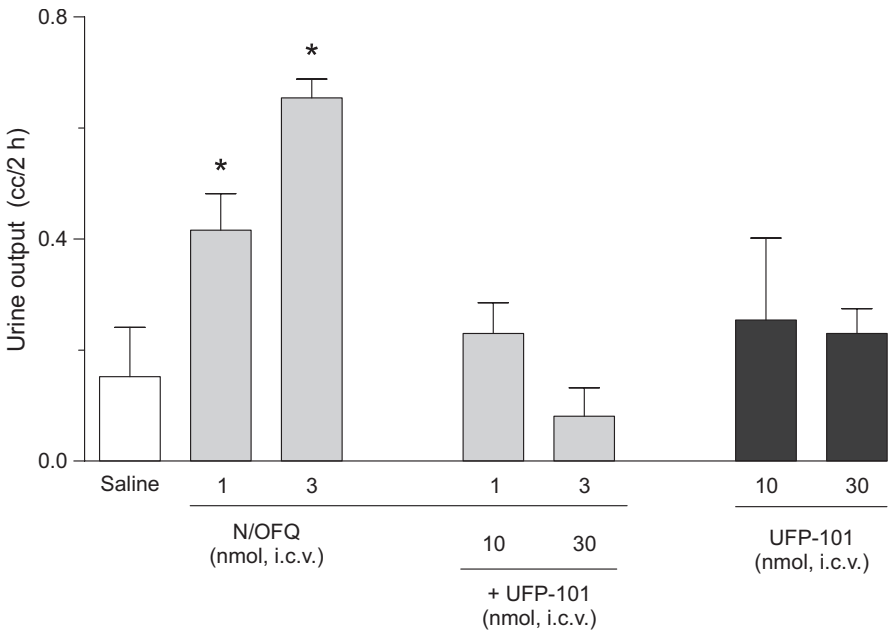

Fig. 3. Effects of UFP-101 on changes in cumulative (2 h) urine output produced by the i.c.v. injection of nociceptin/orphanin FQ (N/OFQ) in conscious CD-1 mice. i.c.v. UFP-101 (10 or 30 nmol) was administered as a pretreatment 2 h prior to N/OFQ (1 or 3 nmol) injection. Data are mean ± S.E.M. of at least 10 mice per group. *$p < 0.05$, significantly different from the effect of saline.

cular depressor and diuretic responses to microinjection of N/OFQ into this brain site (51). In recent studies and as demonstrated in Fig. 3, we have shown that central pretreatment (2 h) of CD-1 mice with UFP-101 (10 or 30 nmol, i.c.v.), which itself did not alter urine output, prevented the diuretic response produced by i.c.v. N/OFQ (1 or 3 nmol). In related studies, we have also observed that renal excretory function is not altered in CD-1 mice following i.c.v. co-injection of N/OFQ (3 nmol) and UFP-101 (30 nmol) (Rizzi and Kapusta, personal communication). In contrast to these findings, it should be noted that in rats (mice not tested), i.c.v. injection of NOP receptor partial agonists [e.g., $Phe^1y(CH_2\text{-}H)Gly^2]N/OFQ(1–13)\text{-}NH_2$] produce cardiovascular (bradycardia and hypotension) and renal excretory responses (water diuresis) similar to those elicited by central N/OFQ (43). These findings indicate that the cardiovascular and renal responses produced by i.c.v. N/OFQ involve central NOP receptor activation and that this system can be affected differently by selective NOP receptor antagonists and partial agonists.

CONCLUSIONS

Current *in vitro* and *in vivo* data obtained with different approaches, techniques and models converge in demonstrating that UFP-101 is a useful pharmacological tool for the investigation of the central and peripheral biological functions regulated by the

N/OFQ – NOP receptor system and for defining the therapeutic potential of NOP receptor ligands.

Acknowledgments. This work was supported by funds from the University of Ferrara (60% grant to GC), the Italian Ministry of University (FIRB 2001 and Cofin 2004 grant to DR and SS), and the National Institute of Health (RO1HL71212 collaborative grant to DR and DRK).

REFERENCES

1. Basso M, Naline E, Calo G, Guerrini R, Regoli D, Advenier C. Effects of NOP ligands in the electrically stimulated human bronchus. La nocicettina, Orfanina FQ ed il suo Recettore. Camerino, Italy: 2003.
2. Becker JA, Wallace A, Garzon A, et al. Ligands for kappa-opioid and ORL1 receptors identified from a conformationally constrained peptide combinatorial library. *J Biol Chem* 1999;274:27513–27522.
3. Bigoni R, Calo G, Rizzi A, et al. In vitro characterization of J-113397, a non-peptide nociceptin/orphanin FQ receptor antagonist. *Naunyn Schmiedeberg's Arch Pharmacol* 2000;361:565–568.
4. Bigoni R, Rizzi D, Rizzi A, et al. Pharmacological characterisation of [(pX)Phe(4)]nociceptin(1–13)amide analogues. 1. *In vitro* studies. *Naunyn Schmiedeberg's Arch Pharmacol* 2002;365:442–449.
5. Calo G, Rizzi A, Bogoni G, et al. The mouse vas deferens: A pharmacological preparation sensitive to nociceptin. *Eur J Pharmacol* 1996;311:R3–5.
6. Calo G, Guerrini R, Bigoni R, et al. Structure-activity study of the nociceptin(1–13)-NH_2 N-terminal tetrapeptide and discovery of a nociceptin receptor antagonist. *J Med Chem* 1998;41:3360–3366.
7. Calo G, Rizzi A, Marzola G, et al. Pharmacological characterization of the nociceptin receptor mediating hyperalgesia in the mouse tail withdrawal assay. *Br J Pharmacol* 1998;125:373–378.
8. Calo G, Guerrini R, Bigoni R, et al. Characterization of [Nphe(1)]nociceptin(1–13)NH(2), a new selective nociceptin receptor antagonist. *Br J Pharmacol* 2000;129:1183–1193.
9. Calo G, Guerrini R, Rizzi A, Salvadori S, Regoli D. Pharmacology of nociceptin and its receptor — A novel therapeutic target. *Br J Pharmacol* 2000;129:1261–1283.
10. Calo G, Rizzi A, Bigoni R, Guerrini R, Salvadori S, Regoli D. Pharmacological profile of nociceptin/orphanin FQ receptors. *Clin Exp Pharmacol Physiol* 2002;29:223–228.
11. Calo G, Rizzi A, Rizzi D, et al. [Nphe(1), Arg(14), Lys(15)]nociceptin-NH(2), a novel potent and selective antagonist of the nociceptin/orphanin FQ receptor. *Br J Pharmacol* 2002;136:303–311.
12. Carra G, Rizzi A, Guerrini R, et al. [(pF)Phe^4, Arg^{14}, Lys^{15}]N/OFQ-NH_2 (UFP-102), a highly potent and selective agonist of the nociceptin/orphanin FQ receptor. *J Pharmacol Exp Ther* 2005;312:1114–1123.
13. Champion HC, Kadowitz PJ. Nociceptin, an endogenous ligand for the ORL1 receptor, has novel hypotensive activity in the rat. *Life Sci* 1997;60:PL241–245.
14. Chen Y, Mestek A, Liu J, Hurley JA, Yu L. Molecular cloning and functional expression of a mu-opioid receptor from rat brain. *Mol Pharmacol* 1993;44:8–12.
15. Chen Y, Chang M, Wang ZZ, et al. [$Nphe^1$]nociceptin(1–13)-NH_2 antagonizes nociceptin-induced hypotension, bradycardia, and hindquarters vasodilation in the anesthetized rat. *Can J Physiol Pharmacol* 2002;80:31–35.
16. Chiou LC, Liao YY, Calo' G, Guerrini R. UFP-101 is a competitive antagonist of N/OFQ receptors mediating inwardly rectifying K^+ channel activation in rat periaquedutal gray slices. FENS 2004. Lisbon, Portugal: 2004.
17. Ciccocioppo R, Angeletti S, Sanna PP, Weiss F, Massi M. Effect of nociceptin/orphanin FQ on the rewarding properties of morphine. *Eur J Pharmacol* 2000;404:153–159.
18. Ciccocioppo R, Economidou D, Fedeli A, Massi M. The nociceptin/orphanin FQ/NOP receptor system as a target for treatment of alcohol abuse: A review of recent work in alcohol-preferring rats. *Physiol Behav* 2003;79:121–128.
19. Ciccocioppo R, Economidou D, Fedeli A, Heilig M, Massi M. Inhibition of ethanol drinking by buprenorphine: Evidence for the involvment of the nociceptin/orphanin FQ receptor system. Washington, DC: Society for Neuroscience, 2004;Abs. # 691.10.
20. Civelli O, Nothacker HP, Reinscheid R. Reverse physiology: Discovery of the novel neuropeptide, orphanin FQ/nociceptin. *Crit Rev Neurobiol* 1998;12:163–176.
21. Civelli O. GPCR deorphanizations: The novel, the known and the unexpected transmitters. *Trends Pharmacol Sci* 2005;26:15–19.

22. Cox BM, Chavkin C, Christie MJ, et al. Opioid receptors. In: The IUPHAR Compendium of Receptor Characterization and Classification, Girdlestone D. London: IUPHAR Media Ltd., 2000;321–333.
23. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends Pharmacol Sci* 2002;23:238–245.
24. Depner UB, Reinscheid RK, Takeshima H, Brune K, Zeilhofer HU. Normal sensitivity to acute pain, but increased inflammatory hyperalgesia in mice lacking the nociceptin precursor polypeptide or the nociceptin receptor. *Eur J Neurosci* 2003;17:2381–2387.
25. Devine DP, Reinscheid RK, Monsma FJ, Jr., Civelli O, Akil H. The novel neuropeptide orphanin FQ fails to produce conditioned place preference or aversion. *Brain Res* 1996;727:225–229
26. Devine DP, Taylor L, Reinscheid RK, Monsma FJ, Jr., Civelli O, Akil H. Rats rapidly develop tolerance to the locomotor-inhibiting effects of the novel neuropeptide orphanin FQ. *Neurochem Res* 1996;21: 1387–1396.
27. Di Toro R, Calienni M, Leggio GM, Spampinato S. Internalization and recycling of the human NOP receptor exposed to nociceptin-related peptides in CHO cells. In: Nocicettina/orfanina FQ ed il suo recettore — VIII Covegno Monotematico SIF. Camerino, 15 Sept 2003.
28. Dooley CT, Spaeth CG, Berzetei-Gurske IP, et al. Binding and *in vitro* activities of peptides with high affinity for the nociceptin/orphanin FQ receptor, ORL1. *J Pharmacol Exp Ther* 1997;283:735–741.
29. Evans CJ, Keith DE, Jr., Morrison H, Magendzo K, Edwards RH. Cloning of a delta opioid receptor by functional expression. *Science* 1992;258:1952195–5.
30. Gavioli EC, Marzola G, Guerrini R, et al. Blockade of nociceptin/orphanin FQ-NOP receptor signalling produces antidepressant-like effects: Pharmacological and genetic evidences from the mouse forced swimming test. *Eur J Neurosci* 2003;17:1987–1990.
31. Gavioli EC, Vaughan CW, Marzola G, et al. Antidepressant-like effects of the nociceptin/orphanin FQ receptor antagonist UFP-101: New evidence from rats and mice. *Naunyn Schmiedeberg's Arch Pharmacol* 2004;369:547–553.
32. Giuliani S, Tramontana M, Lecci A, Maggi CA. Effect of nociceptin on heart rate and blood pressure in anaesthetized rats. *Eur J Pharmacol* 1997;333:177–179.
33. Guerrini R, Calo G, Rizzi A, et al. Address and message sequences for the nociceptin receptor: A structure-activity study of nociceptin-(1–13)-peptide amide. *J Med Chem* 1997;40:1789–1793.
34. Guerrini R, Calo G, Rizzi A, et al. A new selective antagonist of the nociceptin receptor. *Br J Pharmacol* 1998;123:163–165.
35. Guerrini R, Calo G, Bigoni R, et al. Further studies on nociceptin-related peptides: Discovery of a new chemical template with antagonist activity on the nociceptin receptor. *J Med Chem* 2000;43:2805–2813.
36. Guerrini R, Calo G, Bigoni R, et al. Structure-activity studies of the Phe(4) residue of nociceptin(1–13)-NH(2): Identification of highly potent agonists of the nociceptin/orphanin FQ receptor. *J Med Chem* 2001;44:3956–3964.
37. Guerrini R, Calo G, Lambert DG, et al. N- and C-terminal modifications of nociceptin/orphanin FQ generate highly potent NOP receptor ligands. *J Med Chem* 2005;48:1421–1427.
38. Hashiba E, Hirota K, Kudo T, Calo G, Guerrini R, Matsuki A. Effects of nociceptin/orphanin FQ receptor ligands on blood pressure, heart rate, and plasma catecholamine concentrations in guinea-pigs. *Naunyn Schmiedeberg's Arch Pharmacol* 2003;367:342–347.
39. Hawes BE, Graziano MP, Lambert DG. Cellular actions of nociceptin: Transduction mechanisms. *Peptides* 2000;21:961–967.
40. Jenck F, Wichmann J, Dautzenberg FM, et al. A synthetic agonist at the orphanin FQ/nociceptin receptor ORL1: Anxiolytic profile in the rat. *Proc Natl Acad Sci USA* 2000;97:4938–4943.
41. Jinsmaa Y, Takahashi M, Fukunaga H, Yoshikawa M. Retro-nociceptin methylester, a peptide with analgesic and memory-enhancing activity. *Life Sci* 2000;67:3095–3101.
42. Kapusta DR, Sezen SF, Chang JK, Lippton H, Kenigs VA. Diuretic and antinatriuretic responses produced by the endogenous opioid-like peptide, nociceptin (orphanin FQ). *Life Sci* 1997;60:PL15–21.
43. Kapusta DR, Chang JK, Kenigs VA. Central administration of [Phe^{1}psi(CH_2-NH)Gly^2]nociceptin(1–13)-NH_2 and orphanin FQ/nociceptin (OFQ/N) produce similar cardiovascular and renal responses in conscious rats. *J Pharmacol Exp Ther* 1999;289:173–180.
44. Katugampola S, Davenport A. Emerging roles for orphan G-protein-coupled receptors in the cardiovascular system. *Trends Pharmacol Sci* 2003;24:30–35.
45. Kenigs VA, Kapusta DR. Partial agonists of the nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor mediate diuresis via separate central versus peripheral pathways. *FASEB J* 2004;18:A1259.
46. Kieffer BL, Befort K, Gaveriaux-Ruff C, Hirth CG. The delta-opioid receptor: Isolation of a cDNA by expression cloning and pharmacological characterization. *Proc Natl Acad Sci USA* 1992;89:12048–12052.

47. Koizumi M, Midorikawa N, Takeshima H, Murphy NP. Exogenous, but not endogenous nociceptin modulates mesolimbic dopamine release in mice. *J Neurochem* 2004;89:257–263.
48. Koster A, Montkowski A, Schulz S, et al. Targeted disruption of the orphanin FQ/nociceptin gene increases stress susceptibility and impairs stress adaptation in mice. *Proc Natl Acad Sci USA* 1999;96:10444–10449.
49. Kotlinska J, Wichmann J, Legowska A, Rolka K, Silberring J. Orphanin FQ/nociceptin but not Ro 65-6570 inhibits the expression of cocaine-induced conditioned place preference. *Behav Pharmacol* 2002;13: 229–235.
50. Kotlinska J, Rafalski P, Biala G, Dylag T, Rolka K, Silberring J. Nociceptin inhibits acquisition of amphetamine-induced place preference and sensitization to stereotypy in rats. *Eur J Pharmacol* 2003;474:233–239.
51. Krowicki ZK, Kapusta DR. Microinjection of nociceptin/orphanin FQ (N/OFQ) into the hypothalamic paraventricular nucleus (PVN) produces water diuresis in conscious rats. *FASEB J* 2004;18:A1259.
52. Kuzmin A, Sandin J, Terenius L, Ogren SO. Acquisition, expression, and reinstatement of ethanol-induced conditioned place preference in mice: Effects of opioid receptor-like 1 receptor agonists and naloxone. *J Pharmacol Exp Ther* 2003;304:310–318.
53. Kuzmin A, Sandin J, Terenius L, Ogren SO. Evidence in locomotion test for the functional heterogeneity of ORL-1 receptors. *Br J Pharmacol* 2004;141:132–140.
54. Lazzeri M, Calo G, Spinelli M, et al. Urodynamic and clinical evidence of acute inhibitory effects of intravesical nociceptin/orphanin FQ on detrusor overactivity in humans: A pilot study. *J Urol* 2001;166: 2237–2240.
55. Lazzeri M, Calo G, Spinelli M, et al. Urodynamic effects of intravesical nociceptin/orphanin FQ in neurogenic detrusor overactivity: A randomized, placebo-controlled, double-blind study. *Urology* 2003;61:946–950.
56. Lecci A, Giuliani S, Meini S, Maggi CA. Nociceptin and the micturition reflex. *Peptides* 2000;21: 1007–1021.
57. Lee DK, George SR, O'Dowd BF. Novel G-protein-coupled receptor genes expressed in the brain: Continued discovery of important therapeutic targets. *Exp Opin Ther Targets* 2002;6:185–202.
58. Lin SH, Civelli O. Orphan G protein-coupled receptors: Targets for new therapeutic interventions. *Ann Med* 2004;36:204–214.
59. Lutfy K, Eitan S, Bryant CD, et al. Buprenorphine-induced antinociception is mediated by mu-opioid receptors and compromised by concomitant activation of opioid receptor-like receptors. *J Neurosci* 2003;23: 10331–10337.
60. Madeddu P, Salis MB, Milia AF, et al. Cardiovascular effects of nociceptin in unanesthetized mice. *Hypertension* 1999;33:914–919.
61. Marti M, Stocchi S, Paganini F, et al. Pharmacological profiles of presynaptic nociceptin/orphanin FQ receptors modulating 5-hydroxytryptamine and noradrenaline release in the rat neocortex. *Br J Pharmacol* 2003;138:91–98.
62. Marti M, Mela F, Guerrini R, Calo G, Bianchi C, Morari M. Blockade of nociceptin/orphanin FQ transmission in rat substantia nigra reverses haloperidol induced akinesia and normalizes nigral glutamate release. *J Neurochem* 2004;91:1501–1504.
63. Marti M, Mela F, Veronesi C, et al. Blockade of nociceptin/orphanin FQ receptor signaling in rat substantia nigra pars reticulata stimulates nigrostriatal dopaminergic transmission and motor behavior. *J Neurosci* 2004;24:6659–6666.
64. McDonald J, Calo G, Guerrini R, Lambert DG. UFP-101, a high affinity antagonist for the nociceptin/orphanin FQ receptor: Radioligand and GTPgamma(35)S binding studies. *Naunyn Schmiedeberg's Arch Pharmacol* 2003;367:183–187.
65. Mela F, Marti M, Ulazzi L, et al. Pharmacological profile of nociceptin/orphanin FQ receptors regulating 5-hydroxytryptamine release in the mouse neocortex. *Eur J Neurosci* 2004;19:1317–1324.
66. Meunier J, Mouledous L, Topham CM. The nociceptin (ORL1) receptor: Molecular cloning and functional architecture. *Peptides* 2000;21:893–900.
67. Meunier JC, Mollereau C, Toll L, et al. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature* 1995;377:532–535.
68. Mogil JS, Pasternak GW. The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol Rev* 2001;53:381–415.
69. Mollereau C, Simons MJ, Soularue P, et al. Structure, tissue distribution, and chromosomal localization of the prepronociceptin gene. *Proc Natl Acad Sci USA* 1996;93:8666–8670.
70. Mollereau C, Mouledous L. Tissue distribution of the opioid receptor-like (ORL1) receptor. *Peptides* 2000;21:907–917.
71. Morini G, De Caro G, Guerrini R, Massi M, Polidori C. Nociceptin/orphanin FQ prevents ethanol-induced gastric lesions in the rat. *Regul Pept* 2005;124:203–207.

72. Murphy NP, Lee Y, Maidment NT. Orphanin FQ/nociceptin blocks acquisition of morphine place preference. *Brain Res* 1999;832:168–170.
73. Nishi M, Houtani T, Noda Y, et al. Unrestrained nociceptive response and disregulation of hearing ability in mice lacking the nociceptin/orphanin FQ receptor. *EMBO J* 1997;16:1858–1864.
74. Noble F, Roques BP. Association of aminopeptidase N and endopeptidase 24.15 inhibitors potentiate behavioral effects mediated by nociceptin/orphanin FQ in mice. *FEBS Lett* 1997;401:227–229.
75. Nothacker HP, Reinscheid RK, Mansour A, et al. Primary structure and tissue distribution of the orphanin FQ precursor. *Proc Natl Acad Sci USA* 1996;93:8677–8682.
76. Okada K, Sujaku T, Chuman Y, et al. Highly potent nociceptin analog containing the Arg-Lys triple repeat. *Biochem Biophys Res Commun* 2000;278:493–498.
77. Okuda-Ashitaka E, Minami T, Tachibana S, et al. Nocistatin, a peptide that blocks nociceptin action in pain transmission. *Nature* 1998;392:286–289.
78. Ozaki S, Kawamoto H, Itoh Y, et al. *In vitro* and *in vivo* pharmacological characterization of J-113397, a potent and selective non-peptidyl ORL1 receptor antagonist. *Eur J Pharmacol* 2000;402:45–53.
79. Redrobe JP, Calo G, Regoli D, Quirion R. Nociceptin receptor antagonists display antidepressant-like properties in the mouse forced swimming test. *Naunyn Schmiedeberg's Arch Pharmacol* 2002;365:164–167.
80. Reinscheid RK, Nothacker HP, Bourson A, et al. Orphanin FQ: A neuropeptide that activates an opioidlike G protein-coupled receptor. *Science* 1995;270:792–794.
81. Reinscheid RK, Nothacker H, Civelli O. The orphanin FQ/nociceptin gene: Structure, tissue distribution of expression and functional implications obtained from knockout mice. *Peptides* 2000;21:901–906.
82. Rizzi A, Bigoni R, Marzola G, et al. Characterization of the locomotor activity-inhibiting effect of nociceptin/orphanin FQ in mice. *Naunyn Schmiedeberg's Arch Pharmacol* 2001;363:161–165.
83. Rizzi A, Salis B, Ciccocioppo R, et al. Pharmacological characterisation of [(pX)Phe(4)]nociceptin(1–13)-NH(2) analogues. 2. *In vivo* studies. *Naunyn Schmiedeberg's Arch Pharmacol* 2002;365:450–456.
84. Rizzi A, Marzola G, Gavioli EC, et al. Antinociceptive effects induced by spinal nociceptin/orphanin FQ administration are due to NOP receptor activation: Phamacological and genetic evidences in mice. In: EPHAR meeting 2004. Porto, Portugal: 2004.
85. Rizzi A, Marzola G, Zucchini S, et al. Endogenous nociceptin/orphanin FQ (N/OFQ) signaling produces antinociceptive effects in the mouse formalin test: Pharmacological and genetic evidences. EPHAR meeting 2004. Porto, Portugal: 2004.
86. Rizzi D, Rizzi A, Bigoni R, et al. [Arg^{14}, Lys^{15}]nociceptin, a highly potent agonist of the nociceptin/orphanin FQ receptor: *In vitro* and *in vivo* studies. *J Pharmacol Exp Ther* 2002;300:57–63.
87. Rossi GC, Mathis JP, Pasternak GW. Analgesic activity of orphanin FQ2, murine prepro-orphanin FQ141-157 in mice. *Neuroreport* 1998;9:1165–1168.
88. Sakoori K, Murphy NP. Central administration of nociceptin/orphanin FQ blocks the acquisition of conditioned place preference to morphine and cocaine, but not conditioned place aversion to naloxone in mice. *Psychopharmacology (Berl)* 2004;172:129–136.
89. Salis MB, Emanueli C, Milia AF, Guerrini R, Madeddu P. Studies of the cardiovascular effects of nociceptin and related peptides. *Peptides* 2000;21:985–993.
90. Shah N, Chitravanshi VC, Sapru HN. Cardiovascular responses to microinjections of nociceptin into a midline area in the commissural subnucleus of the nucleus tractus solitarius of the rat. *Brain Res* 2003;984:93–103.
91. Shinkai H, Ito T, Iida T, Kitao Y, Yamada H, Uchida I. 4-Aminoquinolines: Novel nociceptin antagonists with analgesic activity. *J Med Chem* 2000;43:4667–4677.
92. Trombella S, Vergura R, Guerrini R, Calo G, Spisani S. Chemotactic effects of nociceptin/orphanin FQ on human monocytes are mediated by NOP receptor activation. In: Nociceptin/orphanin FQ and its receptor — Convegno momotematico SIF. Camerino, 15 Sept 2003.
93. Wilson S, Bergsma DJ, Chambers JK, et al. Orphan G-protein-coupled receptors: The next generation of drug targets? *Br J Pharmacol* 1998;125:1387–1392.
94. Wise A, Jupe SC, Rees S. The identification of ligands at orphan G-protein coupled receptors. *Annu Rev Pharmacol Toxicol* 2004;44:43–66.
95. Wnendt S, Kruger T, Janocha E, Hildebrandt D, Englberger W. Agonistic effect of buprenorphine in a nociceptin/OFQ receptor- triggered reporter gene assay. *Mol Pharmacol* 1999;56:334–338.
96. Yamamoto T, Sakashita Y, Nozaki-Taguchi N. Antagonism of ORLI receptor produces an algesic effect in the rat formalin test. *Neuroreport* 2001;12:1323–1327.
97. Yasuda K, Raynor K, Kong H, et al. Cloning and functional comparison of kappa and delta opioid receptors from mouse brain. *Proc Natl Acad Sci USA* 1993;90:6736–6740.

98. Zaratin PF, Petrone G, Sbacchi M, et al. Modification of nociception and morphine tolerance by the selective opiate receptor-like orphan receptor antagonist (–)-cis-1-methyl-7-[[4–(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol (SB-612111). *J Pharmacol Exp Ther* 2004; 308:454–461.
99. Zaveri N. Peptide and nonpeptide ligands for the nociceptin/orphanin FQ receptor ORL1: Research tools and potential therapeutic agents. *Life Sci* 2003;73:663–678.
100. Zeilhofer HU, Calo G. Nociceptin/Orphanin FQ and its receptor — Potential targets for pain therapy? *J Pharmacol Exp Ther* 2003;306:423–429.
101. Zhao RJ, Woo RS, Jeong MS, Shin BS, Kim DG, Kim KW. Orphanin FQ/nociceptin blocks methamphetamine place preference in rats. *Neuroreport* 2003;14:2383–2385.