REVIEW

CNS Neuroscience & Therapeutics

UFP-112 a Potent and Long-Lasting Agonist Selective for the Nociceptin/Orphanin FQ Receptor

Girolamo Calo',[1] Anna Rizzi,[1] Carlo Cifani,[2] Maria Vittoria Micioni Di Bonaventura,[2] Domenico Regoli,[1] Maurizio Massi,[2] Severo Salvadori,[3] David G. Lambert[4] & Remo Guerrini[3]

1 Department Experimental and Clinical Medicine, Section of Pharmacology and Neuroscience Center, University of Ferrara, and National Institute of Neuroscience, Ferrara, Italy
2 Department of Experimental Medicine and Public Health, University of Camerino, Camerino (MC), Italy
3 Department of Pharmaceutical Sciences, and Biotechnology Center, University of Ferrara, Ferrara, Italy
4 Department of Cardiovascular Sciences (Pharmacology and Therapeutics Group), Division of Anaesthesia, Critical Care and Pain Management, University of Leicester, Leicester Royal Infirmary, Leicester, UK

Keywords
In vitro and *in vivo* studies; Nociceptin/orphanin FQ; NOP receptor; NOP(−/−) mice; Pharmacological profile; UFP-112.

Correspondence
Girolamo Calo', M.D., Ph.D.,
Via Fossato di Mortara 19, 44100 Ferrara, Italy.
Tel.: (39)-0532-455 221;
Fax: (39)-0532-455 205;
E-mail: g.calo@unife.it

Nociceptin/orphanin FQ (N/OFQ) controls several biological functions via selective activation of the N/OFQ peptide receptor (NOP). [(pF)Phe^{4}Aib^{7}Arg^{14}Lys^{15}]N/OFQ-NH$_2$ (UFP-112) is an NOP receptor ligand designed using a combination of several chemical modifications in the same peptide sequence that increase NOP receptor affinity/potency and/or reduce susceptibility to enzymatic degradation. In the present review article, we summarize data from the literature and present original findings on the *in vitro* and *in vivo* pharmacological features of UFP-112. Moreover, important biological actions and possible therapeutic indications of NOP receptor agonists are discussed based on the results obtained with UFP-112 and compared with other peptide and nonpeptide NOP receptor ligands.

doi: 10.1111/j.1755-5949.2009.00107.x

Introduction

G-protein-coupled receptors (GPCRs) historically represent the most important target for drug discovery [1]. This is particularly true as reverse pharmacology strategies over the last 20 years have led to the deorphanization of more than 300 GPCRs [2]. Thus, novel molecules modulating these previously orphan GPCRs may open new avenues for treating human diseases in the near future. Identification of nociceptin/orphanin FQ (N/OFQ) and the N/OFQ peptide receptor (NOP) represent the first successful example of reverse pharmacology [3]. Soon after the cloning of opioid receptors that started with the delta opioid peptide (DOP) receptor [4,5] followed by the kappa opioid peptide (KOP) [6] and mu opioid peptide (MOP) [7] receptors, different research groups started programs aimed at the identification of opioid receptor subtypes. This led, in 1994, to the simultaneous identification of a GPCR showing an overall 60% homology with classical opioid receptors which, unexpectedly, did not bind opioid ligands [8–11]. This receptor, named ORL-1 (opioid receptor like receptor 1) [12], was then used for "fishing" for its endogenous ligand, assuming for the receptor the same type of coupling (i.e., G_i-mediated inhibition of cAMP levels) as classical opioid receptors and for its ligand the same chemical nature (i.e., a peptide) as endogenous opioids. This strategy and these assumptions were indeed correct as demonstrated, 1 year later, by the successful identification from brain extracts of a heptadecapeptide able to inhibit forskolin stimulated cAMP in cells expressing the ORL-1 receptor but not in wild type cells [13,14]. This peptide whose primary sequence (H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH) resembles that of opioid peptides, was named nociceptin [14] or orphanin FQ [13]. Interestingly enough, nociceptin/orphanin FQ (N/OFQ) did not bind to the classical opioid receptors [13]. The N/OFQ peptide precursor (ppN/OFQ) was then cloned; it displayed organizational features that were strikingly similar to those of the genes of

preproenkephalin, preprodynorphin, and preproopiomelanocortin, the precursors to endogenous opioid peptides. This suggests that the four genes have a common evolutionary origin and belong to the same family [15,16]. Thus, the novel peptide-receptor system is similar to classical opioid systems in terms of structure of both receptors and peptides and of receptor cellular actions ($G_{i/o}$ coupling with subsequent inhibition of cAMP and calcium currents and stimulation of potassium currents [17]) while being completely different from classical opioids in terms of pharmacology; in fact, N/OFQ does not bind opioid receptors and opioid ligands (including naloxone) do not bind to the N/OFQ peptide (NOP) receptor. On this basis the NC-IUPHAR subcommittee suggested that the N/OFQ–NOP receptor system is considered as "a nonopioid branch of the opioid family" [18].

Understanding of the biological roles played by the N/OFQ–NOP receptor system was dependent upon the generation of useful research tools, particularly transgenic animals and NOP selective ligands. Mice knockout for the N/OFQ precursor (ppN/OFQ(−/−), [19]) as well as for the NOP receptor (NOP(−/−), [20]) gene have been available since 1999 and 1997, respectively. Recently, NOP(−/−) rats were generated [21]. These transgenic animals represent very important research tools both for investigating the role played by endogenous N/OFQ in regulating various biological functions and for performing *ex vivo* and *in vivo* studies on ligand selectivity.

A wide range of NOP ligands are now available (see for recent reviews on this topic [3,22]) and these can be divided into three groups based on their chemical nature: (1) N/OFQ-related peptides, (2) N/OFQ unrelated short peptides, (3) small nonpeptide molecules. A series of N/OFQ-related peptides were identified by our group using classical peptide structure–activity relationship studies and using the electrically stimulated mouse vas deferens as an N/OFQ sensitive pharmacological preparation [23,24]. The peptide N/OFQ(1–13)-NH_2 was soon identified as the shortest N/OFQ sequence maintaining the same affinity [25] and functional potency and efficacy as the natural ligand [24,26]. This peptide was then used as template for performing further structure–activity relationship studies [27–29] that led to the identification of interesting pharmacological tools such as the partial agonist [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]N/OFQ(1–13)-$NH_2$ ([F/G]N/OFQ(1–13)-NH_2, [30]), the low potency pure antagonist [$Nphe^1$]N/OFQ(1–13)-NH_2 [31], and the highly potent agonist [(pF)Phe^4]N/OFQ(1–13)-NH_2 [32,33]. It has been shown that the introduction in position 14 and 15 of N/OFQ of an extra pair of basic residues Arg, Lys generates a highly potent agonist, [$Arg^{14}Lys^{15}$]N/OFQ [34]. This chemical modification was combined with that able to eliminate efficacy ($Nphe^1$) to generate the highly potent and selective antagonist [$Nphe^1Arg^{14}Lys^{15}$]N/OFQ-NH_2; UFP-101 [35]. This peptide, whose *in vitro* and *in vivo* pharmacological properties are reviewed in [36], is among the most frequently used NOP receptor selective antagonists.

Small N/OFQ unrelated peptides were identified by screening of synthetic peptide combinatorial libraries. In this way compounds such as the nonselective NOP antagonist III-BTD [37] and the NOP selective partial agonist hexapeptides Ac-RYYRWK-NH_2 and Ac-RYYRIK-NH_2 [38] were identified. The hexapeptides were used as templates for a series of structure–activity studies [39–45], for generating useful radiolabeled probes [46], MOP/NOP bivalent ligands [47], and for the development of novel NOP ligands such as the highly potent partial agonist ZP120 [48–52]. This latter peptide is now under clinical development as a novel diuretic.

Small molecules acting as NOP selective ligands have been discovered by pharmaceutical industries via high throughput screening. The first molecule of this class reported in literature was the NOP selective antagonist J-113397 (1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-benzimidazol-2-one, [53]). This compound, also known as compB, represents the most frequently used nonpeptide NOP receptor ligand with more than 80 papers in the literature reporting its *in vitro* and *in vivo* activity. Structure–activity studies on J-113397 led to the identification of an interesting achiral analog named Trap-101 (1-[1-(cyclooctylmethyl)-1,2,3,6-tetrahydro-5-(hydroxymethyl)-4-pyridinyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one, [54]) whose *in vivo* NOP antagonist properties and antiparkinsonian actions have been recently demonstrated [55]. In 2004, GlaxoSmithKline researchers reported the identification and pharmacological characterization of a potent and selective NOP antagonist named SB-612111 ((−)-cis-1-Methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol, [56]). The excellent pharmacological profile of SB-612111 has been confirmed and extended in follow-up studies performed by our group both *in vitro* [57,58] and *in vivo* [59]. This molecule represents the most potent and selective nonpeptide antagonist available to date. More recently Banyu researchers identified a novel potent and selective NOP receptor antagonist, 1-benzyl-N-{3-[spiroisobenzofuran-1(3H),4'-piperidin-1-yl]propyl} pyrrolidine-2-carboxamide [60], Compound 24. The NOP antagonist properties of this molecule have been recently confirmed in different laboratories [61–63].

With respect to nonpeptide NOP agonists, the first series of such compounds was reported in the literature in 1999 by Roche [64]. However, these compounds

Table 1 Research milestones in the N/OFQ–NOP receptor field: pharmacology and research tools

Year	Milestone	Comment	References
1994	NOP receptor	Cloning of the NOP receptor	[8–11]
1995	N/OFQ	Identification of N/OFQ as the endogenous ligand of the NOP receptor	[13,14]
1996	ppN/OFQ	Cloning of the gene coding for ppN/OFQ	[15,16]
	mouse vas deferens	The electrically stimulated mouse vas deferens is a N/OFQ sensitive preparation	[23,24]
	N/OFQ(1–13)-NH_2	The minimum N/OFQ sequence maintaining the same potency as the natural peptide	[24,26]
1997	NOP(−/−) mice	Generation of mice knockout for the NOP receptor gene	[20]
	Ac-RYYRWK-NH_2	Identification of hexapeptides acting as selective partial agonists at NOP receptors	[38]
1998	[F/G]N/OFQ(1–13)-NH_2	The first N/OFQ-related peptide with reduced efficacy at NOP receptors	[30]
1999	ppN/OFQ(−/−) mice	Generation of mice knockout for the ppN/OFQ gene	[19]
2000	[Nphe^1]N/OFQ(1–13)-NH_2	The first selective peptide antagonist	[31]
	J-113397	The first selective nonpeptide antagonist	[53]
	Ro 64–6198	The first selective nonpeptide agonist	[67]
2002	UFP-101	A potent and selective peptide antagonist	[35]
2004	SB-612111	A potent and selective nonpeptide antagonist	[56]
2009	NOP(−/−) rats	Generation of rats knockout for the NOP receptor gene	[21]

displayed only moderate NOP potency and particularly selectivity over classical opioid receptors. These pharmacological features were substantially improved with the identification of 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], [65]). Ro 64–6198 behaved as a potent and selective NOP full agonist in *in vitro* assays and after peripheral administration mimicked the anxiolytic-like effects of supraspinally injected N/OFQ [66,67]. Interestingly, no signs of tolerance to Ro 64–6198 anxiolytic-like effects were detected following 15 days of daily drug exposure in rats [68]. The pharmacokinetic and pharmacodynamic features of Ro 64–6198 as well as its biological actions have been recently reviewed [69]. Ro 64–6198 is certainly a valuable research tool that has been extensively used and characterized in several laboratories thus becoming the standard nonpeptide NOP agonist. Several nonpeptide NOP agonists have been recently reported by Schering–Plough [70–73] and Pfizer [74–76] investigators. The best compounds among these series appear to be SCH 221510 (8-[bis(2-methylphenyl)methyl]-3-phenyl-8-azabicyclo[3.2.1]octan-3-ol, [72]) and MCOPPB (1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3R)-3-piperidinyl]-1H-benzimidazole, [74]). Similar to N/OFQ and Ro 64–6198, both compounds are able to evoke dose dependent anxiolytic like effects in rodents. The most important research tools and selective NOP receptor ligands identified to date are summarized, in chronological order, in Table 1. Moreover, the chemical structure of the above-mentioned nonpeptide NOP ligands is shown in Figure 1.

Anatomical studies have revealed high levels of expression of the ppN/OFQ and NOP receptor mRNA in various areas of the central nervous system [12,77]. NOP mRNA and binding sites exhibit approximately the same distribution pattern, suggesting that the NOP receptor is mainly located on local neuronal circuits. The NOP receptor is also expressed in the peripheral nervous system [12]. The diffuse distribution of N/OFQ and its receptor in the brain, spinal cord and peripheral nervous system indicates that this peptidergic system may control several biological actions. This has been confirmed by a series of *in vitro* and *in vivo* studies demonstrating that N/OFQ, via selective NOP receptor activation, modulates several biological functions in the central nervous system including pain transmission, stress, anxiety and emotional states, learning and memory, locomotor activity, food intake, and the motivational properties of drugs of abuse (see for reviews on these topics [3,22,78–81]). N/OFQ may also behave as an important regulatory signal of the functions of peripheral systems such as the cardiovascular, gastrointestinal, renal, genitourinary, respiratory, and immune systems (see for reviews [3,22,82–85]). The most important findings in terms of biological functions regulated by the N/OFQ–NOP receptor system and possible indications for NOP selective ligands are summarized, in chronological order, in Table 2.

It should, however, be emphasized that evidence for the importance of the N/OFQ–NOP receptor system in regulating these biological functions is, in most cases, restricted to rodent studies. Only for N/OFQ-evoked spinal analgesia [86,87] and for the beneficial effects of NOP receptor antagonists in models of Parkinson's disease [88,89], this evidence has been confirmed in nonhuman primates. Finally, clinical studies with N/OFQ were only performed in the urological field. These studies demonstrated that both acute [90,91] and subchronic

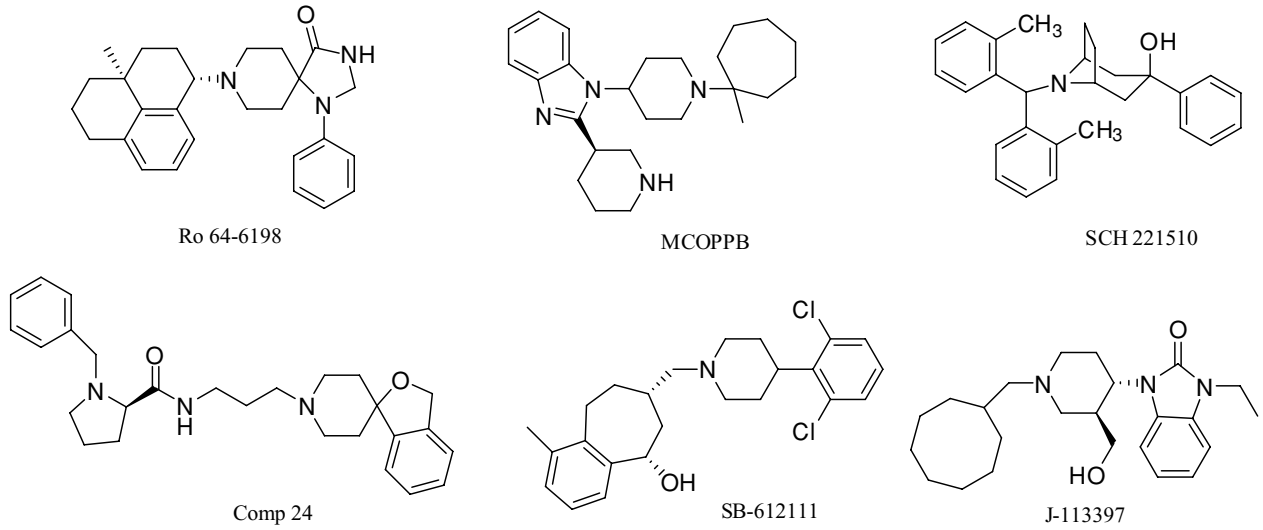

Figure 1 Chemical structure of nonpeptide NOP receptor agonists (top row) and antagonists (bottom row).

Table 2 Research milestones in the N/OFQ–NOP receptor field: biological actions and putative therapeutic indications of NOP ligands

Year	Milestone	Comment	Selected references
1995	Pain	Supraspinal N/OFQ produces hyperalgesia while spinal N/OFQ naloxone insensitive analgesia. This latter effect has been confirmed in nonhuman primates. Spinal NOP receptors may be a potential target for novel analgesics.	[13,14,86,148,149]
1997	Diuresis	N/OFQ evokes aquaresis; NOP partial agonists may be indicated for patients with water-retaining diseases.	[150,151]
	Anxiety	N/OFQ evokes robust anxiolytic-like effects. These results, later confirmed with Ro 64–6198, suggest NOP agonists as a novel class of anxiolytics.	[66,67]
	Memory	N/OFQ severely impairs spatial learning after intrahippocampal administration. NOP(−/−) have greater learning ability then NOP(+/+) mice. NOP antagonists may have memory enhancing properties.	[152,153]
1998	Micturition reflex	N/OFQ inhibits the micturition reflex in rats. Intravesical N/OFQ produces beneficial effects in patients suffering from overactive bladder. NOP agonists may represent innovative drugs for treating urinary incontinence.	[90,154]
1999	Drug addiction	N/OFQ inhibits the rewarding properties of alcohol and morphine. NOP agonists can be developed as a novel treatment for drug addiction.	[129,155]
2001	Cough	N/OFQ inhibits cough after both central and peripheral administration. NOP agonists as novel antitussive therapy.	[156]
2002	Depression	[Nphe^1]N/OFQ(1–13)-NH_2 and J-113397 reduced immobility time in the forced swimming test. NOP(−/−) mice display an antidepressant phenotype. NOP antagonists may act as innovative antidepressant drugs.	[157,158]
2004	Parkinson disease	J-113397 and UFP-101 facilitate motor activity in normal animals and relieve akinesia in hemiparkinsonian rats. NOP(−/−) outperformed NOP(+/+) mice on the rotarod and are less sensitive to haloperidol-induced motor depression. NOP receptor antagonists may represent a novel approach for Parkinson's disease.	[121,122]
2008	Sepsis	UFP-101 reduces animal mortality in a rat model of sepsis. Plasma N/OFQ levels are higher in human subjects who died as a result of sepsis. NOP antagonists as innovative drugs for the treatment of sepsis.	[159,160]

(once a day for 10 days, [92]) intravesical instillation of N/OFQ produces beneficial effects in patients suffering from urinary incontinence due to neurogenic detrusor overactivity.

The pleiotropic actions evoked by N/OFQ via selective activation of the NOP receptor imply that different therapeutic indications can be addressed with NOP selective ligands. However, this also implies that a rather large

number of possible side effects can be encountered by developing molecules acting at the NOP receptor. This certainly represents a challenging issue for the drug development process in this field.

Design of UFP-112

[(pF)Phe^{4}Aib^{7}Arg^{14}Lys^{15}]N/OFQ-NH_2 (UFP-112, [93,94]) is an NOP receptor ligand designed by combining several chemical modifications in the same peptide sequence (see Fig. 2) that were previously reported to increase NOP receptor affinity/potency and/or reduce susceptibility to enzymatic degradation. These chemical modifications and their effects on peptide pharmacological features are summarized below.

As far as C-terminal amidation of N/OFQ is concerned, this modification was reported in the first generation of structure–activity studies [25,26]. N/OFQ-NH_2 binds to NOP receptors expressed in the rat [25], mouse [95] and guinea pig [96] brain membranes as well as to recombinant human NOP receptors expressed in CHO cells [97] with pK_i values slightly higher than N/OFQ. These receptor binding data were confirmed in *in vitro* functional studies performed in different preparations measuring inhibition of cAMP accumulation in CHO_{hNOP} cells, glutamate release from rat cerebrocortical slices [97], and electrically induced contractions in several isolated tissues [98–100]. In all these studies N/OFQ-NH_2 mimicked N/OFQ actions with similar maximal effects but potency values 2–8-fold higher. Interestingly, when rat vas deferens experiments were repeated in the presence of a cocktail of peptidase inhibitors, the potency of N/OFQ was increased by 6-fold while that of N/OFQ-NH_2 by only 2-fold [101] thus suggesting that amidation of the C-terminus of N/OFQ confers higher metabolic stability to the peptide. This may explain why in some *in vivo* assays, that is, the mouse locomotor activity assay [102] and the rat plantar test [103], N/OFQ-NH_2 was found to be significantly more potent than the natural peptide. Similar considerations can be drawn analyzing the pharmacological profile of N/OFQ(1–13)-NH_2. A large series of *in vitro* and *in vivo* studies reviewed in [101] demonstrated that N/OFQ actions are mimicked by N/OFQ(1–13)-NH_2. C-terminal amidation of this peptide is, however, crucial for biological activity because N/OFQ(1–13)-OH displayed strongly reduced affinity in binding studies [95,104], very low potency in the mouse vas deferens assay [26] and inactivity in the rat *in vivo* [105]. Interestingly, *in vivo* in mice N/OFQ(1–13)-OH is inactive under control conditions whereas it mimics the bradycardic and hypotensive action of N/OFQ and N/OFQ(1–13)-NH_2 when injected in the presence of the peptidase inhibitor thiorphan [106]. Collectively, this evidence corroborates the findings obtained with N/OFQ-NH_2 and demonstrates that amidation of the C-terminus of N/OFQ counteracts the action of peptidases making the peptide more resistant to enzymatic degradation.

The (pF)Phe^4 modification has been identified as part of a detailed study performed on the Phe^4 residue of N/OFQ(1–13)-NH_2 that demonstrated that the biological activity of a series of Phe^4 substituted peptides positively correlates with the electron withdrawal properties of groups in the para position and inversely with their size [29]. The most potent compound of this series, [(pF)Phe^4]N/OFQ(1–13)-NH_2, was then selected for further *in vitro* and *in vivo* characterization. [(pF)Phe^4]N/OFQ(1–13)-NH_2 displayed higher affinity than N/OFQ(1–13)-NH_2 in receptor binding studies performed using CHO cells expressing the recombinant human NOP (CHO_{hNOP}) and rat cerebrocortical membranes.

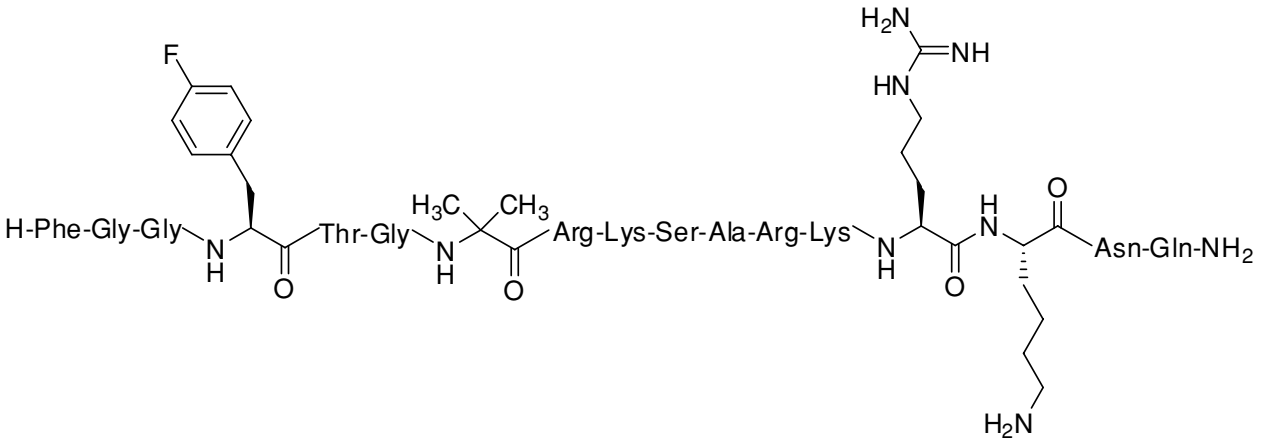

Figure 2 Primary structure of UFP-112.

In a series of functional assays (stimulation of [^{35}S]GTPγS binding in CHO_{hNOP} and rat cerebrocortical membranes, inhibition of cAMP accumulation in CHO_{hNOP} cells, bioassay in the mouse colon and in the electrically stimulated guinea pig ileum and mouse and rat vas deferens) [(pF)Phe^4]N/OFQ(1–13)-NH_2 behaved as full agonist showing potency values 3–10-fold higher than N/OFQ(1–13)-NH_2 or the natural peptide. In addition, the effects of [(pF)Phe^4]N/OFQ(1–13)-NH_2 were not modified by naloxone while being antagonized by [Nphe^1]N/OFQ(1–13)-NH_2. This NOP antagonist displayed similar pA_2 values against [(pF)Phe^4]N/OFQ(1–13)-NH_2 and N/OFQ [32]. *In vivo* in the locomotor activity assay in mice, 1 nmol N/OFQ(1–13)-NH_2 given intracerebroventricularly (i.c.v.) caused a significant decrease (about 70% inhibition) in activity for the first 15 min following injection; [(pF)Phe^4]N/OFQ(1–13)-NH_2 at the same dose exerted a similar inhibitory effect that lasted until the end of the observation period (30 min). This effect was prevented by 10 nmol [Nphe^1]N/OFQ(1–13)-NH_2. In the mouse tail-withdrawal assay, after i.c.v. administration [(pF)Phe^4]N/OFQ(1–13)-NH_2 mimicked the actions of N/OFQ(1–13)-NH_2 producing pronociceptive effects when given alone and blocking morphine-induced analgesia when administered with the alkaloid. In both experimental paradigms, the actions of [(pF)Phe^4]N/OFQ(1–13)-NH_2 were longer lasting (>60 min) compared to those of N/OFQ(1–13)-NH_2 (≈30 min). In unanaesthetized normotensive mice, bolus intravenous (i.v.) injection of 100 nmol/kg of [(pF)Phe^4]N/OFQ(1–13)-NH_2 decreased mean blood pressure and heart rate; these effects were longer lasting than those elicited by the same dose of N/OFQ(1–13)-NH_2. Finally, the i.c.v. administration of [(pF)Phe^4]N/OFQ(1–13)-NH_2 dose-dependently stimulated feeding in rats, and the peptide was about 10-fold more potent than N/OFQ(1–13)-NH_2 [33].

In a follow-up study, the (pF)Phe^4 modification was applied in the N/OFQ-NH_2 template generating *in vitro* results superimposable to those obtained with [(pF)Phe^4]N/OFQ(1–13)-NH_2 [107]. Collectively, these findings demonstrated that the (pF)Phe^4 modification produces an increase in peptide potency and duration of action without affecting its selectivity and pharmacological activity.

The Aib^7 modification was first reported by Zhang et al. [108]. These authors performed a structure–activity study in which alpha-aminoisobutyric acid (Aib) or N-methylalanine (MeAla) were inserted as replacement(s) for Ala^7, Ala^{11}, or Ala^{15} in the N/OFQ sequence. They found that [Aib^{7,11}]N/OFQ-NH_2 behaved as a highly potent NOP receptor full agonist in the [^{35}S]GTPγS binding assay. Based on the known alpha helix structure induction properties of Aib [109], the authors suggested that N/OFQ might adopt an amphipathic helix conformation in its C-terminal sequence [108]. This suggestion was later confirmed by NMR conformational analysis studies [110]. Based on these findings, we synthesized and pharmacologically evaluated in the mouse vas deferens assay a series of N/OFQ-NH_2 analogs substituted in position 7 and 11 with Calpha,alpha-disubstituted cyclic, linear and branched amino acids. None of the novel N/OFQ analogs produced better results than [Aib^7]N/OFQ-NH_2 that displayed 3-fold higher potency (pEC_{50} 8.35) than the parent peptide N/OFQ-NH_2 (pEC_{50} 7.80) [93]. Importantly, the inhibitory effects of both [Aib^7]N/OFQ-NH_2 and N/OFQ-NH_2 in the mouse vas deferens are similarly sensitive to the antagonist action of UFP-101 while not being modified by naloxone [110].

The Arg^{14}Lys^{15} modification has been reported by Okada et al. [34]. The very first structure–activity studies on N/OFQ demonstrated that the pair of dipeptides Arg-Lys at positions 8–9 and 12–13 plays a very important role in NOP receptor binding [25,111]. This is probably due to the formation of high energy ionic bonds between the peptide basic residues Arg-Lys and the acidic amino acid cluster in the second extracellular loop of the NOP receptor [112]. With the aim of obtaining a N/OFQ analog that binds more strongly to the NOP receptor, Okada et al. [34] synthesized a series of N/OFQ analogs in which the Arg-Lys dipeptide unit was placed at positions 6–7, 10–11, or 14–15 adjacent to the parent Arg-Lys. This design strategy was indeed successful since, among these N/OFQ analogs, [Arg^{14}Lys^{15}]N/OFQ was found to be more potent than the natural peptide by 3-fold in receptor binding and by 17-fold in [^{35}S]GTPγS binding experiments [34]. These results were later confirmed and extended by us *in vitro* and *in vivo* [113]. Indeed, in isolated tissues, [Arg^{14}Lys^{15}]N/OFQ mimicked the effects of N/OFQ, with similar maximal effects but higher potencies (17-fold in the mouse vas deferens, 10-fold in the rat vas deferens, and about 5-fold in the guinea pig ileum and mouse colon). In these preparations, the effects of [Arg^{14}Lys^{15}]N/OFQ were not modified by 1 μM naloxone, but were blocked by the NOP antagonists [Nphe^1]N/OFQ(1–13)-NH_2 and J-113397. Interestingly, in the rat vas deferens, a cocktail of peptidase inhibitors increased the potency of N/OFQ (by 4-fold) but not that of [Arg^{14}Lys^{15}]N/OFQ, thus suggesting that the [Arg^{14}Lys^{15}] modification confers to the peptide some resistance to enzymatic degradation. This was recently corroborated by demonstrating that the half-life of N/OFQ and [Arg^{14}Lys^{15}]N/OFQ in the presence of trypsins are 13 and 30 min, respectively [114]. In *in vivo* experiments in mice, [Arg^{14}Lys^{15}]N/OFQ mimicked the effects of N/OFQ administered i.c.v, producing pronociceptive effects in the tail-withdrawal assay and inhibiting locomotor activity.

Table 3 Comparison of N/OFQ and N/OFQ-related peptide potencies in the electrically stimulated mouse vas deferens

	N/OFQ pEC_{50}	Peptide pEC_{50}	CR	References
N/OFQ-NH_2	7.84	7.96	1.3	[99]
[(pF)Phe^4]N/OFQ-NH_2	8.27	8.59	2	[107]
[Aib^7]N/OFQ-NH_2	7.82	8.35	3	[110]
[Arg^{14}Lys^{15}]N/OFQ-NH_2	8.27	9.12	7	[107]
[(pF)Phe^{4}Aib^{7}Arg^{14}Lys^{15}]N/OFQ-NH_2 (UFP-112)	7.45	9.24	62	[93]

The maximal effects evoked by N/OFQ-related peptides were similar to those evoked by N/OFQ. CR, concentration ratio.

In both assays, [Arg^{14}Lys^{15}]N/OFQ was about 30-fold more potent than N/OFQ and produced longer lasting effects [113]. Collectively, these results demonstrate that the [Arg^{14}Lys^{15}] modification has a 2-fold effect: first, it increases peptide binding to the NOP receptor and, second, it makes the peptide less susceptible to enzymatic degradation thus increasing its *in vivo* duration of action.

As described above and summarized in Table 3 all of these chemical modifications produced an increase in N/OFQ potency in the electrically stimulated mouse vas deferens. This increase ranged from 1.3-fold for the C-terminal amidation to 7-fold for the [Arg^{14}Lys^{15}] substitution. When all modifications are applied in the same molecule, that is, UFP-112, their effects on peptide potency were more than additive. In fact, the addition of the concentration ratios obtained with the single modifications yielded a number (13.3) that is far from the concentration ratio measured with UFP-112 (62). In contrast, multiplication of the concentration ratios of the single modifications yields a number (54.6) close to the experimentally obtained concentration ratio (62) for UFP-112. This clearly suggests that the combination of the single modifications into the same molecule produces synergistic effects on peptide potency.

In vitro Pharmacological Profile of UFP-112

In receptor binding experiments performed on CHO_{hNOP} cell membranes, UFP-112 produced a concentration-dependent inhibition of [^{3}H]N/OFQ binding with a pK_i value (10.55) 10-fold higher than that of the natural peptide N/OFQ (9.50). UFP-112 displayed high (>100-fold) selectivity over classical opioid receptors. In CHO_{hNOP} cell membranes, N/OFQ stimulated [^{35}S]GTPγS binding in a concentration-dependent manner with a pEC_{50} of 9.04 and maximal effect corresponding to approximately 8-fold over basal values. UFP-112 mimicked the stimulatory effect of N/OFQ producing similar maximal effects but being 30-fold more potent [93]. Original data generated as described in [97], indicate that forskolin-stimulated cAMP accumulation in CHO_{hNOP} cells is inhibited in a concentration-dependent manner by N/OFQ with very high potency. UFP-112 mimicked the inhibitory effects of N/OFQ displaying a superimposable concentration-response curve (Table 4).

The effects of N/OFQ and UFP-112 were also compared measuring calcium mobilization in CHO_{hNOP} cells stably expressing the $G\alpha_{qi5}$ chimeric protein that forces the NOP receptor to couple to calcium signaling [58]. In this kind of experiment, UFP-112 mimicked N/OFQ effects both in terms of shape and kinetics of the calcium transients and of maximal effects. However, UFP-112 potency was unexpectedly lower than that of the natural peptide although only by 3-fold. UFP-112 effects were also evaluated in isolated tissues where N/OFQ inhibits electrically induced contractions. In the mouse and rat vas deferens and guinea pig ileum N/OFQ and UFP-112, concentration-dependently inhibited electrically induced twitches eliciting similar maximal effects. However, UFP-112 was found to be 60-, 30-, and 10-fold more potent than the natural peptide [93]. It is worthy of mention that the kinetics of the inhibitory effects evoked by N/OFQ and UFP-112 were very different in these tissues. The inhibitory effects induced by N/OFQ occurred rapidly after adding the peptide (≈3 min) to the bath and were immediately reversible after washing. In contrast, UFP-112 displayed a slower onset of action and its inhibitory effects reached a plateau only after ≈10 min; moreover, tissues treated with UFP-112 did not fully recover to the control twitch even after washing the tissue for more than 2 h (data not shown). As an example of these different kinetics, an original tracing obtained in the electrically stimulated mouse vas deferens with equieffective concentrations of N/OFQ and UFP-112 is reported in Figure 3.

In order to establish the receptor mechanism involved in their action, the effects of N/OFQ and UFP-112 in the electrically stimulated mouse vas deferens were challenged with receptor antagonists and reassessed in tissues taken from NOP(+/+) and NOP(−/−) mice. The

Table 4 *In vitro* pharmacological profile of UFP-112

Preparation	N/OFQ action	pEC_{50}	UFP-112 action	pEC_{50}	References
CHO_{hNOP}	↑ [^{35}S]GTPγS	9.04	↑ [^{35}S]GTPγS	10.55	[93]
CHO_{hNOP}	↓ cAMP	10.29	↓ cAMP	10.34	present article
$CHO_{hNOP-G\alpha qi5}$	↑ intracellular calcium	9.54	↑ intracellular calcium	9.05	[58]
Mouse vas deferens (Swiss)	↓ contractions	7.45	↓ contractions	9.24	[93]
Rat vas deferens	↓ contractions	6.83	↓ contractions	8.34	[93]
Guinea pig ileum	↓ contractions	8.05	↓ contractions	9.17	[93]
Mouse vas deferens NOP(+/+)	↓ contractions	7.62	↓ contractions	9.40	[94]
Mouse vas deferens NOP(−/−)	Inactive		Inactive		[94]
Mouse lung NOP(+/+)	↓ capsaicin-induced bronchoconstriction	≈7.5	↓ capsaicin-induced bronchoconstriction	≈8.5	[115]
Mouse lung NOP(−/−)	Inactive		Inactive		[115]

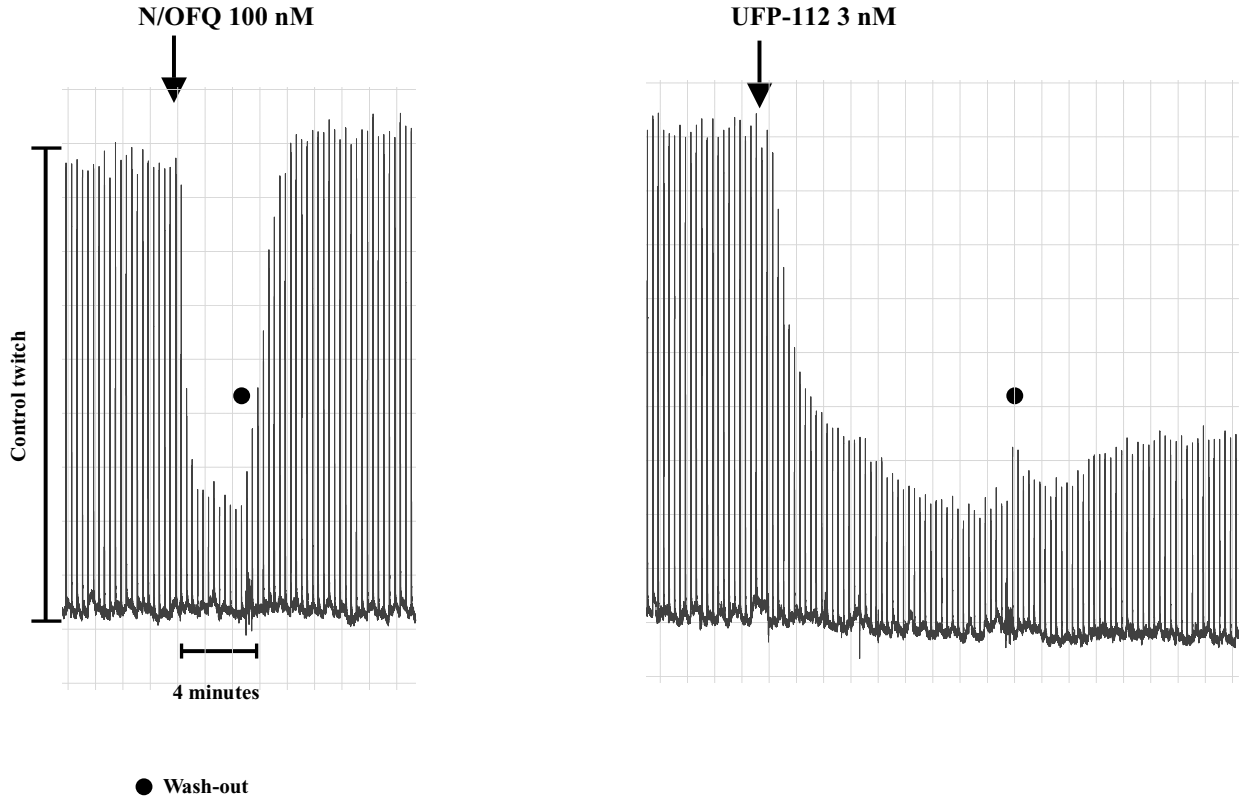

Figure 3 Typical tracing showing the kinetics of the inhibitory effects of equieffective concentrations of N/OFQ and UFP-112 in the electrically stimulated mouse vas deferens.

nonselective opioid receptor antagonist naloxone at 1 μM did not affect the concentration-response curve to either N/OFQ or UFP-112. In contrast UFP-101 displaced to the right the concentration-response curve to N/OFQ and UFP-112 to a similar extent without modifying their maximal effects and with superimposable pK_B values (6.81 and 6.91, respectively). The effects of the two peptides in tissues taken from NOP(+/+) mice were very similar to those previously described in tissues from Swiss mice that is, no difference in maximal effects and UFP-112 more potent than N/OFQ by approximately 70-fold. In contrast, in parallel experiments performed on tissues from NOP(−/−) mice both N/OFQ and UFP-112 were found inactive up to 1 μM. The selective DOP agonist [D-Pen^2,D-Pen^5]-enkephalin (DPDPE) produced identical inhibitory effects in tissues taken from both NOP(+/+) and

NOP(−/−) mice [94]. Finally, in the isolated and perfused mouse lung, capsaicin-induced bronchoconstriction was reversed by N/OFQ in a concentration-dependent manner. UFP-112 mimicked the effects of the natural peptide being, however, at least 10-fold more potent. The inhibitory effects of both N/OFQ and UFP-112 were not present in lungs taken from NOP(−/−) mice [115]. The *in vitro* actions of UFP-112 in comparison with those evoked by the natural NOP agonist N/OFQ are summarized in Table 4.

Thus, these *in vitro* findings clearly demonstrated that UFP-112 behaves (1) as NOP receptor full agonist, since its maximal effects were always superimposable with those of N/OFQ, (2) as a highly selective NOP ligand, as demonstrated by receptor binding studies and receptor antagonist and knockout studies in the mouse vas deferens and lung, (3) as an NOP ligand characterized by slow onset and long duration of action, (4) as a highly potent NOP ligand with 10-fold higher affinity in receptor binding and 10–70-(depending on the preparation) fold higher potency in functional studies. The only exceptions to the latter statement are data obtained in the cAMP and calcium assays and these findings deserve a comment. Both assays are characterized by a high efficiency of the stimulus/response coupling due to the presence of amplification phenomena. Indeed values for N/OFQ potency in these particular assays are higher than in the other assays (Table 4). Thus ceiling effects may prevent identification of ligands with potency higher than N/OFQ in these particular tests. However, this consideration does not explain why in the $G\alpha_{qi5}$ NOP receptor calcium assay UFP-112 displayed slightly lower potency than that of N/OFQ. In this assay all the NOP receptor antagonists tested displayed potency values perfectly in line with the literature [58]. This also applies to most full and partial agonists tested. Only a subset of NOP ligands including the full agonist Ro 64–6198 and the partial agonist ZP120 displayed in this assay, similar to UFP-112, values of potency significantly lower than expected [58]. The chemical nature of the small molecule Ro 64–6198, that of the N/OFQ analog UFP-112, and that of the Dooley hexapeptide derivative ZP120 are very different suggesting that chemical features are not relevant. It is worthy of mention that isolated tissue experiments demonstrated an important characteristic common to the three ligands. As depicted in Figure 3 for UFP-112 and described in detail in previous publications for Ro 64–6198 [116] and ZP120 [48,52], the kinetics of the inhibitory effect elicited by N/OFQ on the electrically induced twitch response is rapid and immediately and completely reversible after washing while that of UFP-112, Ro 64–6198, and ZP120 is characterized by slow onset, and slow and partial reversibility after washing. The slow kinetics of action of these ligands may be relevant for the estimation of their potency in the $G\alpha_{qi5}$ NOP receptor calcium assay. Indeed, the long time required to obtain activation of NOP receptors with these agonists may be incompatible with the rapid kinetics that characterizes the calcium transient response. This might be the reason for the underestimation of the UFP-112, Ro 64–6198, and ZP120 potencies in the $G\alpha_{qi5}$ NOP receptor calcium assay. For details and discussion of this issue see [58].

The conclusion that UFP-112 behaves *in vitro* as a potent and selective full agonist for the NOP receptor is corroborated by the findings by the group of Wang [117] who synthesized a series of N/OFQ analogs, including the peptide [(pF)Phe^{4}Aib^{7}Aib^{11}Arg^{14}Lys^{15}]N/OFQ-NH_2, which is very similar to UFP-112. [(pF)Phe^{4}Aib^{7}Aib^{11}Arg^{14}Lys^{15}]N/OFQ-NH_2 evaluated in receptor binding (rat brain membranes) and bioassay (mouse vas deferens) experiments displayed an affinity of 10.78 and an agonist potency of 9.37. These values are virtually superimposable to those obtained in our laboratories with UFP-112.

Finally, the degradation half-life ($T_{1/2}$) of N/OFQ and UFP-112 in mouse plasma and brain homogenates was evaluated in HPLC studies by least-square linear regression analysis of peptide peak area versus time [94]. Results of this analysis indicate that N/OFQ showed a relatively long half-life in plasma (about 1 h) compared to that obtained in brain homogenate (approximately 3 min). UFP-112 exhibited significantly longer half-lives compared to the natural peptide. In particular, the plasma $T_{1/2}$ of UFP-112 is about 3-fold longer than that of N/OFQ and this difference was even more pronounced in the mouse brain homogenate [94]. Thus, UFP-112 seems to associate to full agonist activity, slow onset and long duration of action, high NOP potency and selectivity another desirable characteristic: low susceptibility to enzymatic degradation. This latter feature might be relevant for the interpretation of the *in vivo* pharmacological actions of UFP-112 discussed below.

In vivo Actions of UFP-112

Pain transmission—N/OFQ modulates pain transmission in a complex manner. Although some conflicting results are reported in the literature most of the available evidence indicates that N/OFQ produces inhibitory effects on nociception (antinociceptive action) at peripheral and spinal levels while facilitates pain transmission (pronociceptive action) in the brain [3,78]. In line with this view, experiments performed in our laboratories with the mouse tail-withdrawal assay demonstrated pronociceptive effects of i.c.v N/OFQ [118] and

antinociceptive effects when the peptide is given intrathecally (i.t.) [119]. Both of these actions are resistant to naloxone, antagonized by UFP-101 and SB-612111 and not observed in NOP(−/−) mice [35,52,59,118,119]. Under the same experimental conditions, the effects of i.c.v. and i.t. UFP-112 were assessed in the mouse tail-withdrawal assay [94]. UFP-112 (1–100 pmol) produced dose-dependent pronociceptive effects after i.c.v. administration and antinociceptive effects when given i.t. over the same dose range. Thus, UFP-112 mimicked the actions of N/OFQ. However, UFP-112 was approximately 100-fold more potent than the natural peptide, and clearly produced longer lasting effects. In fact, the effects induced by UFP-112 in the tail-withdrawal assay were still statistically significant 120 min after i.c.v. or i.t. injection of the peptide, while those elicited by N/OFQ lasted for only 15–30 min [118,119]. Interestingly, the antinociceptive properties of spinally administered N/OFQ were confirmed in nonhuman primates [86,87]. These studies demonstrated that i.t. N/OFQ produces naltrexone resistant and J-113397 sensitive antinociceptive effects that lasted for about 120 min. Moreover, N/OFQ is able to potentiate morphine antinociceptive action. Studies on the effects of UFP-112 on pain transmission in monkeys are currently underway in the laboratories of Dr Ko at the Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan, USA. Preliminary results (Ko MC, personal communication) demonstrated that i.t. UFP-112 is able to produce dose-dependent antinociceptive effects that are similar to those evoked by N/OFQ and blocked by J-113397. However, in this assay UFP-112 is about 10-fold more potent than N/OFQ and, more importantly, produces longer lasting effects (statistically significant effects are recorded 4.5 h after i.t. injection). Collectively, these findings demonstrate that the spinal antinociceptive actions of N/OFQ and UFP-112 are similar in rodents and nonhuman primates. Moreover, these results suggest that spinal NOP receptors represent a promising target for innovative analgesic drugs and indicate that UFP-112 is worthy of further development for this particular indication.

Locomotor activity—one of the first biological actions described in response to the i.c.v. administration of N/OFQ in mice was a reduction of locomotor activity [13]. This effect was later confirmed in rats [120] and the involvement of the NOP receptor in this N/OFQ action was demonstrated by receptor antagonist [35,102] and knockout [20] studies. UFP-112 was compared with N/OFQ for its ability to inhibit locomotor activity in mice. A 10 nmol N/OFQ given i.c.v. produced a clear inhibitory effect on spontaneous locomotor activity that lasted for only 60 min. In contrast, UFP-112 at 100-fold lower doses (i.e., 0.1 nmol) elicited long lasting effects, inhibiting locomotor activity for approximately 6 h [94].

Endogenous N/OFQ—NOP receptor signaling also seems to play an important inhibitory role on motor behavior in pathological conditions. Indeed, NOP receptor antagonists (UFP-101, J-113397, and Trap-101) produce beneficial effects in rodent models of Parkinson's disease [55,88,121–124]. These results were recently confirmed in nonhuman primates [88,89]. Moreover, recent studies [125] implicate the N/OFQ–NOP receptor system in L-DOPA-induced dyskinesia, a long-term side effect of the therapy of Parkinson's disease. In fact, in dyskinetic rats i.c.v. treatment with N/OFQ, as well as systemic treatment with the NOP agonist Ro 65–6570, significantly attenuated abnormal involuntary movements and produced a full recovery of the animals motor performance on the rotarod. These same effects were observed in response to i.c.v. UFP-112, which was at least 10-fold more potent than the natural peptide. These antidyskinetic properties of NOP agonists were no longer observed in rats treated with the antagonist J-113397 demonstrating the exclusive involvement of the NOP receptor in their actions [125]. Collectively, these findings demonstrated that NOP receptor antagonists are worth evaluating as novel treatments for Parkinson's disease while NOP receptor agonists may represent an innovative strategy for controlling L-DOPA-induced dyskinesia.

Food intake—several studies demonstrated that i.c.v. N/OFQ is able to stimulate food intake in rats (for recent reviews see [80,126]). A similar hyperphagic effect could be measured after i.c.v. administration of N/OFQ in mice [59,94]. N/OFQ was inactive at 0.1 nmol, while at 1 nmol produced a statistically significant orexigenic effect. Increasing the dose of peptide to 10 nmol resulted in a loss of effect, thus making the dose-response curve to N/OFQ bell shaped. The involvement of NOP receptors in this action of N/OFQ was demonstrated by its sensitivity to the antagonist action of SB-612111 [59]. UFP-112 mimicked the hyperphagic effect of N/OFQ producing a bell-shaped dose–response curve with the maximum reached at 10 pmol. Thus UFP-112 was found to be approximately 100-fold more potent than N/OFQ in this assay [94]. In addition, it is worthy of mention that the amount of the orexigenic effect elicited by UFP-112 was approximately double that evoked by N/OFQ. Since food intake was measured cumulatively over the 60 min time course of the experiment, the larger orexigenic effect of UFP-112 can be interpreted assuming a more prolonged stimulation of the NOP receptor by the synthetic peptide than by N/OFQ. The involvement of the NOP receptor in the orexigenic action of N/OFQ and UFP-112 was demonstrated in knockout studies. Indeed, 1 nmol N/OFQ and 10 pmol UFP-112 elicited a robust hyperphagic effect in

NOP(+/+) mice while the two peptides were found completely inactive in NOP(−/−) animals [94].

Recently, experiments were carried out to investigate the effects of i.c.v. injections of UFP-112 on food intake in rats. These studies were performed using the protocols and experimental conditions described in details in [127].

The results obtained indicate that the peptide exerts a potent and very pronounced hyperphagic effect following i.c.v. administration in rats (Fig. 4). A statistically significant hyperphagic effect was even observed at the dose of 0.03 nmol, while 2.1 nmol of N/OFQ were required. Therefore, the present findings are in keeping with previous reports in mice showing that UFP-112 is about 100 times more potent than N/OFQ [94]. Both the effect of UFP-112 and that of N/OFQ appears to be behaviorally selective since they are not accompanied by evident modifications of the gross behavior of the animals. Moreover, other aspects of the ingestive behavior such as water drinking are not significantly modified following single i.c.v. injection.

In addition, the effect of UFP-112 appears to be much longer lasting than that of N/OFQ. In fact, the hyperphagic effect was still detected when food was presented even 6 h after the i.c.v. injection of UFP-112 (Fig. 5), while the hyperphagic effect of N/OFQ was completely over 1 h after administration (data not shown). These data perfectly matched those obtained in similar experiments comparing the time course of N/OFQ and UFP-112 effects on locomotor activity in mice [94], and may likely explain why the intensity of the hyperphagic effect of UFP-112 was markedly more pronounced than that of N/OFQ.

The experiment in which the NOP receptor antagonist UFP-101 was given before UFP-112 administration provides evidence that the hyperphagic effect of this molecule is completely dependent upon activation of NOP receptors. In fact, when UFP-101 was given in two injections of 20 nmol the 15 and 30 min food intake of UFP-112-treated rats was not statistically different from that of controls (Fig. 6). On the other hand, even in rats treated with UFP-101 (2 × 20 nmol) UFP-112 was able to produce a delayed stimulation of food intake. This finding can be interpreted considering the lower susceptibility to enzymatic degradation of the agonist than the antagonist.

Interestingly, the same pretreatment with UFP-101 did not significantly modify the hyperphagic effect of i.c.v. injection of NPY, 6 μg/rat (data not shown), thus providing further evidence on the high selectivity of action of this NOP receptor antagonist [36].

Alcohol intake—in contrast to opioids N/OFQ does not produce conditioned place preference *per se* [128] while it is able to counteract the rewarding properties of drugs of abuse including alcohol [3]. In fact N/OFQ

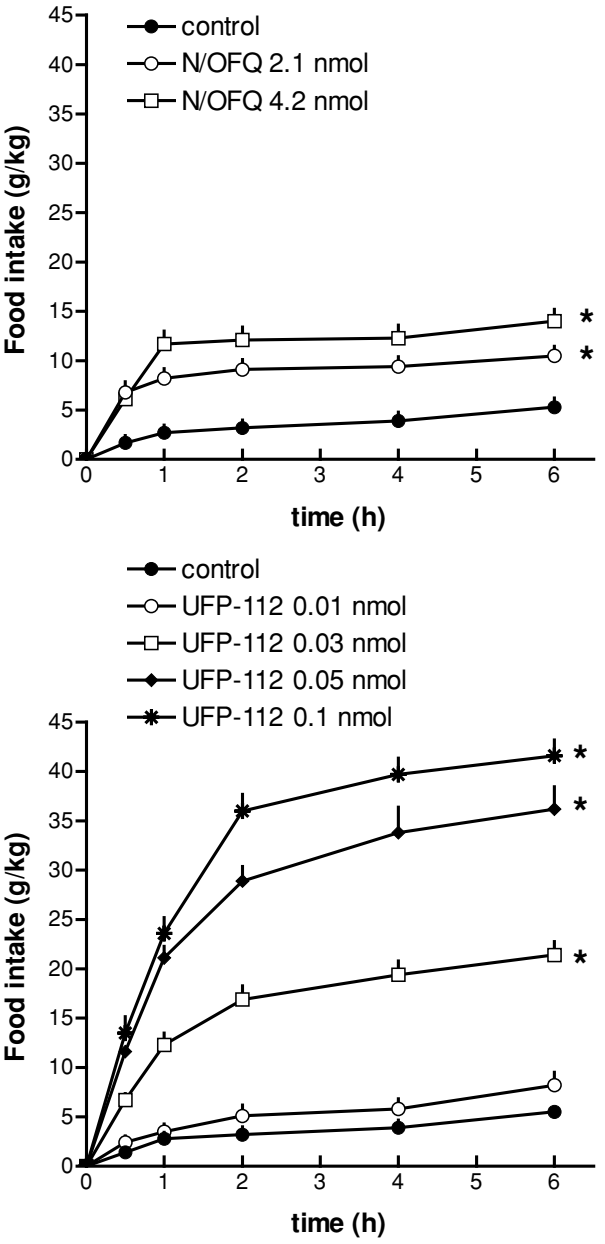

Figure 4 Cumulative food intake following i.c.v. injection of different doses of N/OFQ (top panel) and UFP-112 (bottom panel). Values are means ± sem of four separate experiments. *$P<$ 0.05 versus control, according to ANOVA followed by Dunnett test for multiple comparisons. *indicates that all the data points of the relative treatment are statistically different from control.

reduced ethanol intake in genetically selected alcohol-preferring rats [129] and this effect was prevented by the NOP antagonist [Nphe^1]N/OFQ(1–13)NH_2 [130]. Moreover, N/OFQ abolished conditioned place preference induced by ethanol [129] and inhibited reinstatement of alcohol-seeking behavior induced by electric footshock

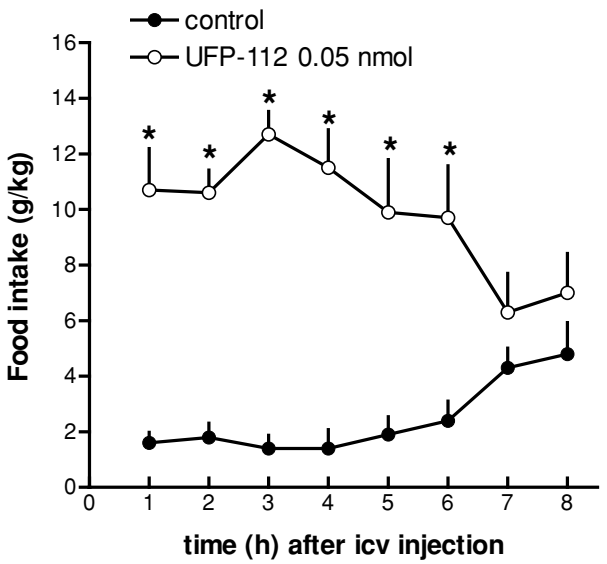

Figure 5 Thirty-minute food intake in rats treated with 0.05 nmol UFP-112 i.c.v. at different times (1–8 h) before access to food. Values are means ± sem of four separate experiments. *$P < 0.05$ versus control, according to the Student's t-test for unpaired data.

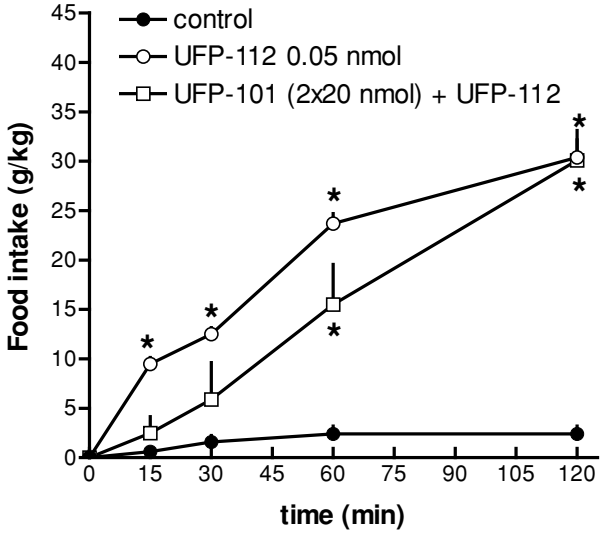

Figure 6 Cumulative food intake following i.c.v. injection of 0.05 nmol UFP-112 alone or with 2×20 nmol UFP-101 (the first injection 15 min before and the second just before injection of UFP-112). Values are means ± sem of four separate experiments. *$P < 0.05$ versus control, according to ANOVA followed by Dunnett test for multiple comparisons.

stress, as well as that induced by ethanol-paired cues [131]. Converging evidence indicates that the central amygdala may likely represent the key area for the action of N/OFQ on alcohol dependence [132,133]. Interestingly, the inhibitory effect of N/OFQ on ethanol intake is mimicked by different NOP receptor agonists including N/OFQ-NH_2, N/OFQ(1–13)-NH_2, OS-426 and UFP-102 [130,134] and, but only at high doses, by the nonselective agonist of buprenorphine [135]. The involvement of the NOP receptor in the effect of high doses buprenorphine was demonstrated with the use of the NOP selective antagonist UFP-101 [135]. Finally, conflicting results were reported on this topic on the effects of the nonpeptide NOP agonist Ro 64–6198 [134,136,137].

UFP-112 was reported to mimic the reduction in ethanol intake of genetically selected alcohol-preferring rats evoked by N/OFQ [134]. In fact subchronic i.c.v. treatment with UFP-112 produced statistically significant inhibitory effects on ethanol intake already at the low dose of 10 ng/rat after 4 days while higher doses (i.e., 50 ng/rat) evoked significant effects after 2 days of treatment [134]. Neither food nor water intake were modified by UFP-112 in this range of doses. Thus UFP-112 behaved as a potent and selective agonist at NOP receptors controlling ethanol intake mimicking the inhibitory effects elicited by N/OFQ at 500 ng/rat [129] at doses 50-fold lower (i.e., 10 ng/rat). These data obtained with UFP-112 substantiate the proposal that NOP receptor selective agonists are worthy of development for the treatment of alcohol abuse.

Cardiovascular and renal function—N/OFQ controls cardiovascular and renal function both acting in the brain and in the periphery [85]. The cardiovascular and renal effects of N/OFQ and UFP-112 were compared after i.v. bolus administration [94]. At 10 nmol/kg N/OFQ produced a slight but significant reduction in arterial pressure and a slight non significant reduction in heart rate. Similarly, immediately following drug injection, a 100-fold lower dose of UFP-112 (0.1 nmol/kg, i.v.) produced a comparably small but significant hypotension and bradycardia. However, when administered at the same dose as N/OFQ (i.e., 10 nmol/kg), UFP-112 profoundly reduced arterial pressure and heart rate. In addition to increased potency, UFP-112 also produced markedly longer cardiovascular responses than N/OFQ. In the same animals i.v. bolus injection of UFP-112 (0.1 and 10 nmol/kg) also produced a concurrent diuretic response. At the higher dose tested, the UFP-112-induced increase in urine flow rate was delayed in onset (approximately, 30 min), of relatively long duration (50–60 min), and associated with a reduction in urinary sodium excretion (not statistically significant). Of note, similar diuretic responses could be obtained with N/OFQ only after i.v. infusion but not bolus injection [85]. Thus UFP-112 given i.v. bolus not only mimicked the effects of N/OFQ on cardiovascular function with higher potency and longer lasting effects but it was also able to evoke renal responses (i.e., diuresis associated with antinatriuresis) which can be obtained only after i.v. infusion of N/OFQ. This different action

may likely derive from the ability of UFP-112, but not N/OFQ, to reach peripheral sites (e.g., the kidney [50]) at pharmacologically relevant concentrations when administered as an i.v. bolus. This can likely be attributed to the lower susceptibility to enzymatic degradation of UFP-112 compared to N/OFQ.

No data are yet available on the receptor mechanism mediating the cardiovascular and renal actions of UFP-112. Future studies performed with NOP knockout animals and selective NOP antagonists will demonstrate if these effects of UFP-112 could be solely attributed to NOP receptor activation.

Gastrointestinal function—both ppN/OFQ and the NOP receptor are widely expressed in the intestinal tract and N/OFQ has been reported to modulate stomach, small intestine and colon contractility in several species [138]. *In vitro* studies demonstrated that N/OFQ-inhibited neurogenic contractions of the stomach and small intestine while in the colon the peptide evokes direct contraction probably via inhibition of tonic NO release from enteric neurons [138,139]. These effects of N/OFQ are very robust and some intestinal preparations such as the mouse colon and particularly the electrically stimulated guinea pig ileum are widely used N/OFQ sensitive pharmacological assays. Data obtained in electrically stimulated guinea pig ileum are available for all the NOP ligands listed in Table 1. In addition, the effects of UFP-112 in this preparation are displayed in Table 4. As far as the *in vivo* actions of N/OFQ are concerned, several studies suggest an important role of this peptide in the central and peripheral control of different gastrointestinal functions including gastric empting, secretion, and damage (induced by ethanol or stress), gastrointestinal transit and colonic propulsor motility. In some recent studies, the gastrointestinal actions of N/OFQ were compared to those evoked by UFP-112. Following i.c.v. injection, N/OFQ dose-dependently delayed gastric emptying of a phenol red meal, decreased gastric secretion in water-loaded rats and reduced ethanol-induced gastric lesions. All these actions were mimicked by UFP-112 that was 20- to 50-fold more potent than the natural peptide and produced longer lasting effects. The action of N/OFQ at 2.5 nmol on gastric emptying was short lasting (10 min) while the same effect elicited by UFP-112 at 0.1 nmol was still statistically significant 2 h after injection. In addition, the effects of UFP-112 were sensitive to the NOP receptor antagonist, UFP-101 [140]. When the peptides were given intraperitoneally (i.p.) they evoked a different pattern of action: no effect on gastric emptying, a gastric hypersecretory response and antiulcer effects. This suggests that central and peripheral NOP receptors have distinct roles in controlling gastric function [140]. The protective role of peripheral NOP receptor activation was confirmed on gastric damage induced by cold-restraint stress. Again this effect of N/OFQ was sensitive to UFP-101 and mimicked by lower doses of UFP-112 [141]. As far as colonic functions are concerned, these appear to be regulated by NOP receptor signaling both under normal and pathological conditions [142]. Both i.c.v. or i.p., N/OFQ and UFP-112 increased bead expulsion time in a statistically significant and dose-related manner and reduced the percentage of rats with castor oil-induced diarrhea. UFP-112 showed greater efficacy, higher potency and longer-lasting inhibitory effects than N/OFQ. These effects of UFP-112 were sensitive to the antagonist action of UFP-101. When injected i.c.v., N/OFQ and UFP-112 inhibited corticotrophin releasing factor- and restrain stress-stimulated fecal pellet excretion in a dose-related manner. Conversely, when injected peripherally both peptides inhibited colonic propulsive motility only partially and this was not dose-related [142].

The *in vivo* actions of UFP-112 in comparison to those evoked by the natural NOP agonist N/OFQ are summarized in Table 5. Analysis of this series of data suggests the following. UFP-112 always mimicked N/OFQ actions showing, with few exceptions, 10–100-fold higher potencies than the natural peptide. UFP-112 consistently evoked longer lasting effects and, in some assays (indicated in Table 5 by an asterisk), it elicited larger effects than N/OFQ. The 10-fold higher affinity reported for UFP-112 compared to N/OFQ in receptor binding studies [93] could, at least in part, explain its high potency but not the high duration of action and size of effect. Thus, other factors should be considered. One of these is likely represented by the lower susceptibility of UFP-112 to enzymatic degradation as demonstrated in experiments using mouse plasma and brain homogenate where UFP-112 displayed 3–4 fold longer half-lives than N/OFQ [94]. Another factor possibly relevant to this issue is the different kinetics of interaction with the NOP receptor suggested by isolated tissue experiments (see Fig. 3). Therefore, the combination of higher NOP affinity, lower susceptibility to protease action, and a slow onset and long-lasting kinetics of interaction with the NOP receptor may likely be relevant to explain the *in vivo* features of UFP-112. These features that is, high potency associated with the ability to induce long-lasting effects, appear to be very consistent among species (mouse, rat, monkeys), route of administration (i.c.v., i.t., i.v., i.p.), and target organ (brain, spinal cord, airways, gut, kidney, cardiovascular system). Data obtained *in vivo* with the peptide [(pF)Phe^{4}Aib^{7}Aib^{11}Arg^{14}Lys^{15}]N/OFQ-NH_2 (which is very similar to UFP-112) measuring its ability to evoke pronociceptive effects in mice after i.c.v. administration and hypotensive effects in rats after i.v. injection [143] are virtually superimposable to those

Table 5 *In vivo* pharmacological profile of UFP-112

Test/Assay	N/OFQ action	Effective dose	UFP-112 action	Effective dose	References
Tail-withdrawal (m, icv)	↓ TW latencies	1 nmol	↓ TW latencies	0.01 nmol	[94]
Tail-withdrawal (m, it)	↑ TW latencies	1 nmol	↑ TW latencies	0.01 nmol	[94]
Locomotion (m, icv)	↓ spontaneous LA	10 nmol	↓ spontaneous LA	0.1 nmol	[94]
Rotarod (dyskinetic r, icv)	↑ motor performance	0.1 nmol	↑ motor performance	0.01 nmol	[125]
AIM (dyskinetic r, icv)	attenuation of AIM	0.1 nmol	attenuation of AIM	0.01 nmol	[125]
Food intake (Swiss m, icv)	↑ food intake	1 nmol	↑ food intake*	0.01 nmol	[94]
Food intake (NOP(+/+)m, icv)	↑ food intake	1 nmol	↑ food intake*	0.01 nmol	[94]
Food intake (NOP(−/−)m, icv)	inactive	1 nmol	Inactive	0.01 nmol	[94]
Food intake (r, icv)	↑ food intake	2.1 nmol	↑ food intake*	0.03 nmol	present article
Ethanol intake (msP r; icv)	↓ ethanol consumption	500 ng	↓ ethanol consumption	10 ng	[129,134]
HR and BP (r, iv bolus)	↓ HR and BP	10 nmol/kg	↓ HR and BP*	0.1 nmol/kg	[94]
Diuresis (r, iv bolus)	inactive	10 nmol/kg	↑ dieresis	0.1 nmol/kg	[94]
Gastric emptying (r, icv)	↓ gastric emptying	100 pmol	↓ gastric emptying	2 pmol	[140]
Gastric secretion (r, icv)	↓ acid secretion	500 pmol	↓ acid secretion	30 pmol	[140]
Gastric secretion (r, ip)	↑ acid secretion	1000 pmol	↑ acid secretion	30 pmol	[140]
Gastric damage (r, icv)	↓ alcohol- induced lesions	1000 pmol	↓ alcohol-induced lesions	100 pmol	[140]
Gastric damage (r, ip)	↓ alcohol- induced lesions	2000 pmol	↓ alcohol-induced lesions	20 pmol	[140]
Gastric damage (r, ip)	↓ stress-induced lesions	1 μg/kg/h	↓ stress-induced lesions	0.3 μg/kg/h	[141]
Colon propulsion (r, icv)	↑ mean expulsion bead time	10 pmol	↑ mean expulsion bead time*	10 pmol	[142]
Colon propulsion (r, ip)	↑ mean expulsion bead time	10 nmol	↑ mean expulsion bead time*	1 pmol	[142]
Castor oil-induced diarrhea (r, icv)	↓% rats with diarrhea	3000 pmol	↓% rats with diarrhea	300 pmol	[142]
Castor oil-induced diarrhea (r, ip)	↓% rats with diarrhea	100 pmol	↓% rats with diarrhea	10 pmol	[142]
CRF-induced fecal output (r, icv)	↓ n fecal pellet	500 pmol	↓ n fecal pellet	50 pmol	[142]
CRF-induced fecal output (r, ip)	↓ n fecal pellet	500 pmol	↓ n fecal pellet	250 pmol	[142]
RS-induced fecal output (r, icv)	↓ n fecal pellet	100 pmol	↓ n fecal pellet	2 pmol	[142]

m, mouse; r, rat; gp, guinea-pig; msP r, Marchigian Sardinian alcohol-preferring rats; icv, intracerebrentricular; it, intrathecal; iv, intravenous; TW, tail-withdrawal; LA, locomotor activity; AIMs, abnormal involuntary movements in hemiparkinsonian rats that become dyskinetic after chronic treatment with L-DOPA; HR, heart rate; BP, blood pressure; CRF, corticotropin releasing factor; RS, restrein stress; *indicates that the maximal effects elicited by UFP-112 in these assays are significantly higher than those produced by N/OFQ.

measured in response to UFP-112: very high potency and longer lasting effects.

Finally, it should be mentioned that the available data on the pharmacological features of UFP-112 were mainly obtained after acute administration. The only exception is represented by alcohol intake studies where UFP-112 has been given once a day for 6 days [134]. UFP-112 behaves as a full agonist at NOP receptors and tolerance to long-term exposure to agonists is a rather general phenomenon in the GPCR field; thus chronic studies with UFP-112 are mandatory in order to establish the therapeutic potential of NOP agonists in those conditions in which a chronic treatment is required.

Conclusions

In conclusion, the present review suggests that UFP-112 is a highly potent and selective full agonist for the NOP receptor, partially resistant to enzymatic degradation and able to produce long lasting effects *in vivo*. UFP-112 can represent a useful research tool to be used together with Ro 64–6198 [69] and the recently identified nonpeptide NOP agonists SCH 221510 [72] and MCOPPB [74] in future studies aimed at identification of the potential for NOP agonists as an innovative drug class. These studies should particularly investigate those conditions and states in which a selective and prolonged stimulation of the NOP receptor is beneficial, including anxiety [79], drug addiction [81,144], stress-induced anorexia [145], cough and possibly other respiratory diseases [84], and visceral hypersensitivity triggered by inflammation or stress [146]. Last but not least, UFP-112 may represent more than a simple research tool for some selected therapeutic indications. In fact, the peptide nature of this molecule does not limit its usefulness and possible drug development for those conditions in which systemic administration of drugs is not required; these include urinary incontinence due to overactive bladder where UFP-112 can be administered intravesically [92] or chronic pain states in patients intolerant or refractory to systemic opioids where the peptide can be administered via implantable intrathecal pumps. This latter strategy was recently demonstrated to be clinically successful with the omega conotoxin analog peptide Ziconotide [147].

Acknowledgments

This work was supported financially by the University of Ferrara (FAR grant to GC and SS), by the Italian Ministry of University (PRIN grant to RG and MM), and by UF-Peptides s.r.l. We would like to thank Stefano Molinari for preparing Figure 3.

Disclosures

The following authors Girolamo Calo', Domenico Regoli, Severo Salvadori, and Remo Guerrini are inventors of the patent application (WO2006087340) that includes UFP-112 and are founders of the University of Ferrara spin off company UFPeptides s.r.l. the assignee of such patent application.

Conflict of Interest

The authors have no conflict of interest.

References

1. Jacoby E, Bouhelal R, Gerspacher M, Seuwen K. The 7 TM G-protein-coupled receptor target family. *Chem Med Chem* 2006;**1**:761–782.
2. Chung S, Funakoshi T, Civelli O. Orphan GPCR research. *Br J Pharmacol* 2008;**153**:S339–S346.
3. Lambert DG. The nociceptin/orphanin FQ receptor: A target with broad therapeutic potential. *Nat Rev Drug Discov* 2008;**7**:694–710.
4. Evans CJ, Keith DE, Jr., Morrison H, Magendzo K, Edwards RH. Cloning of a delta opioid receptor by functional expression. *Science* 1992;**258**:1952–1955.
5. 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 U S A* 1992;**89**:12048–12052.
6. 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 U S A* 1993;**90**:6736–6740.
7. 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.
8. Mollereau C, Parmentier M, Mailleux P, et al. ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. *FEBS Lett* 1994;**341**:33–38.
9. Bunzow JR, Saez C, Mortrud M, Bouvier C, Williams JT, Low M, Grandy DK. Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a mu, delta or kappa opioid receptor type. *FEBS Lett* 1994;**347**:284–288.
10. Chen Y, Fan Y, Liu J, Mestek A, Tian M, Kozak CA, Yu L. Molecular cloning, tissue distribution and chromosomal localization of a novel member of the opioid receptor gene family. *FEBS Lett* 1994;**347**: 279–283.
11. Wang JB, Johnson PS, Imai Y, Persico AM, Ozenberger BA, Eppler CM, Uhl GR. cDNA cloning of an orphan opiate receptor gene family member and its splice variant. *FEBS Lett* 1994;**348**:75–79.
12. Mollereau C, Mouledous L. Tissue distribution of the opioid receptor-like (ORL1) receptor. *Peptides* 2000;**21**: 907–917.
13. 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.
14. 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.
15. Mollereau C, Simons MJ, Soularue P, Liners F, Vassart G, Meunier JC, Parmentier M. Structure, tissue distribution, and chromosomal localization of the prepronociceptin gene. *Proc Natl Acad Sci U S A* 1996;**93**:8666–8670.
16. Nothacker HP, Reinscheid RK, Mansour A, et al. Primary structure and tissue distribution of the orphanin FQ precursor. *Proc Natl Acad Sci U S A* 1996;**93**:8677–8682.
17. Hawes BE, Graziano MP, Lambert DG. Cellular actions of nociceptin: Transduction mechanisms. *Peptides* 2000;**21**:961–967.
18. Cox BM, Chavkin C, Christie MJ, et al. Opioid receptors. In: Girdlestone D, editor. *The IUPHAR compendium of receptor characterization and classification*. second edition ed. London: IUPHAR Media, 2000;321–333.
19. 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 U S A* 1999;**96**:10444–10449.
20. Nishi M, Houtani T, Noda Y, et al. Unrestrained nociceptive response and disregulation of hearing ability in mice lacking the nociceptin/orphaninFQ receptor. *Embo J* 1997;**16**:1858–1864.
21. Homberg JR, Mul JD, de Wit E, Cuppen E. Complete knockout of the nociceptin/orphanin FQ receptor in the rat does not induce compensatory changes in mu, delta and kappa opioid receptors. *Neuroscience* 2009;**163**:308–315.
22. Chiou LC, Liao YY, Fan PC, Kuo PH, Wang CH, Riemer C, Prinssen EP. Nociceptin/orphanin FQ peptide receptors: Pharmacology and clinical implications. *Curr Drug Targets* 2007;**8**:117–135.
23. Berzetei-Gurske IP, Schwartz RW, Toll L. Determination of activity for nociceptin in the mouse vas deferens. *Eur J Pharmacol* 1996;**302**:R1–2.

24. 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.
25. Dooley CT, Houghten RA. Orphanin FQ: Receptor binding and analog structure activity relationships in rat brain. *Life Sci* 1996;**59**:PL23–PL29.
26. 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.
27. Calo G, Guerrini R, Bigoni R, Rizzi A, Bianchi C, Regoli D, Salvadori S. Structure-activity study of the nociceptin(1–13)-NH2 N-terminal tetrapeptide and discovery of a nociceptin receptor antagonist. *J Med Chem* 1998;**41**:3360–3366.
28. 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.
29. 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.
30. Guerrini R, Calo G, Rizzi A, Bigoni R, Bianchi C, Salvadori S, Regoli D. A new selective antagonist of the nociceptin receptor. *Br J Pharmacol* 1998; **123**:163–165.
31. 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.
32. Bigoni R, Rizzi D, Rizzi A, et al. Pharmacological characterisation of [(pX)Phe4]nociceptin(1–13)amide analogues. 1. In vitro studies. *Naunyn Schmiedebergs Arch Pharmacol* 2002;**365**:442–449.
33. Rizzi A, Salis MB, Ciccocioppo R, et al. Pharmacological characterisation of [(pX)Phe4]nociceptin(1–13)NH2 analogues. 2. In vivo studies. *Naunyn Schmiedebergs Arch Pharmacol* 2002;**365**:450–456.
34. 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.
35. Calo G, Rizzi A, Rizzi D, et al. [Nphe1, Arg14,Lys15]nociceptin-NH2, a novel potent and selective antagonist of the nociceptin/orphanin FQ receptor. *Br J Pharmacol* 2002;**136**:303–311.
36. Calo G, Guerrini R, Rizzi A, et al. UFP-101, a Peptide Antagonist Selective for the Nociceptin/Orphanin FQ Receptor. *CNS Drug Rev* 2005;**11**:97–112.
37. 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.
38. 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.
39. Kawano C, Okada K, Honda T, Nose T, Sakaguchi K, Costa T, Shimohigashi Y. Structural requirements of nociceptin antagonist Ac-RYYRIK-NH2 for receptor binding. *J Pept Sci* 2002;**8**:561–569.
40. Judd AK, Kaushanskaya A, Tuttle DJ, Sanchez A, Khroyan T, Polgar W, Toll L. N-terminal modifications leading to peptide ORL1 partial agonists and antagonists. *J Pept Res* 2003;**62**:191–198.
41. Judd AK, Tuttle DJ, Jones RW, Sanchez A, Polgar W, Berzetei-Gurske I, Toll L. Structure-activity studies on high affinity NOP-active hexapeptides. *J Pept Res* 2004;**64**:87–94.
42. Carra G, Calo G, Spagnolo B, et al. Tryptophan replacement in the nociceptin/orphanin FQ receptor ligand Ac-RYYRWK-NH2. *J Peptide Res* 2005;**66**:39–47.
43. Gunduz O, Rizzi A, Baldisserotto A, et al. In vitro and in vivo pharmacological characterization of the nociceptin/orphanin FQ receptor ligand Ac-RYYRIK-ol. *Eur J Pharmacol* 2006;**539**:39–48.
44. Gunduz O, Sipos F, Spagnolo B, et al. In vitro binding and functional studies of AcRYYRIK-ol and its derivatives, novel partial agonists of the nociceptin/orphanin FQ receptor. *Neurosignals* 2006;**15**:91–101.
45. Li J, Isozaki K, Okada K, Matsushima A, Nose T, Costa T, Shimohigashi Y. Designed modification of partial agonist of ORL1 nociceptin receptor for conversion into highly potent antagonist. *Bioorg Med Chem* 2008;**16**:2635–2644.
46. Thomsen C, Valsborg JS, Platou J, et al. [3H]ac-RYYRWK-NH2, a novel specific radioligand for the nociceptin/orphanin FQ receptor. *Naunyn Schmiedebergs Arch Pharmacol* 2000;**362**:538–545.
47. Kawano S, Ambo A, Sasaki Y. Synthesis and receptor binding properties of chimeric peptides containing a mu-opioid receptor ligand and nociceptin/orphanin FQ receptor ligand Ac-RYYRIK-amide. *Bioorg Med Chem Lett* 2006;**16**:4839–4841.
48. Rizzi A, Rizzi D, Marzola G, Regoli D, Larsen BD, Petersen JS, Calo G. Pharmacological characterization of the novel nociceptin/orphanin FQ receptor ligand, ZP120: In vitro and in vivo studies in mice. *Br J Pharmacol* 2002;**137**:369–374.
49. Kapusta DR, Thorkildsen C, Kenigs VA, Meier E, Vinge MM, Quist C, Petersen JS. Pharmacodynamic characterization of ZP120 (Ac-RYYRWKKKKKKK-NH2), a novel, functionally selective nociceptin/orphanin FQ peptide receptor partial agonist with sodium-potassium-sparing aquaretic activity. *J Pharmacol Exp Ther* 2005;**314**:652–660.
50. Hadrup N, Petersen JS, Praetorius J, et al. Opioid receptor-like 1 stimulation in the collecting duct induces aquaresis through vasopressin-independent aquaporin-2 downregulation. *Am J Physiol Renal Physiol* 2004;**287**:F160–168.

51. Simonsen U, Laursen BE, Petersen JS. ZP120 causes relaxation by pre-junctional inhibition of noradrenergic neurotransmission in rat mesenteric resistance arteries. *Br J Pharmacol* 2008;**153**:1185–1194.
52. Fischetti C, Rizzi A, Gavioli EC, et al. Further studies on the pharmacological features of the nociceptin/orphanin FQ receptor ligands ZP120. *Peptides* 2009;**30**:248–255.
53. 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.
54. Trapella C, Guerrini R, Piccagli L, et al. Identification of an achiral analogue of J-113397 as potent nociceptin/orphanin FQ receptor antagonist. *Bioorg Med Chem* 2006;**14**:692–704.
55. Marti M, Trapella C, Morari M. The novel nociceptin/orphanin FQ receptor antagonist Trap-101 alleviates experimental parkinsonism through inhibition of the nigro-thalamic pathway: Positive interaction with L-DOPA. *J Neurochem* 2008;**107**:1683–1696.
56. 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.
57. Spagnolo B, Carra G, Fantin M, et al. Pharmacological characterization of the nociceptin/orphanin FQ receptor antagonist SB-612111 [(−)-cis-1-Methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol]: In vitro studies. *J Pharmacol Exp Ther* 2007;**321**:961–967.
58. Camarda V, Fischetti C, Anzellotti N, et al. Pharmacological profile of NOP receptors coupled with calcium signaling via the chimeric protein $G\alpha_{qi5}$. *Naunyn Schmiedebergs Arch Pharmacol* 2009;**379**:599–607.
59. Rizzi A, Gavioli EC, Marzola G, et al. Pharmacological characterization of the nociceptin/orphanin FQ receptor antagonist SB-612111 [(−)-cis-1-Methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol]: In vivo studies. *J Pharmacol Exp Ther* 2007;**321**:968–974.
60. Goto Y, Arai-Otsuki S, Tachibana Y, et al. Identification of a novel spiropiperidine opioid receptor-like 1 antagonist class by a focused library approach featuring 3D-pharmacophore similarity. *J Med Chem* 2006;**49**:847–849.
61. Yan-Yu L, Chiou LC. Effect of compound 24, a novel nociceptin/orphanin FQ (NOP) receptor antagonist, on NOP receptor-mediated k+ channel activation in rat periaqueductal gray slices. In: *Society for Neuroscience; 2008 November 15–19, 2008*; Washington, DC, USA; 2008.
62. Ruiz-Velasco V, Trapella C, Calo G, Margas W. Pharmacology of constitutively active NOP opioid receptors heterologously expressed in rat sympathetic neurons. In: *Society for Neuroscience; 2008 November 15–19, 2008*; Washington, DC, USA; 2008.
63. Fischetti C, Camarda V, Rizzi A, et al. Pharmacological characterization of the nociceptin/orphanin FQ receptor non peptide antagonist Compound 24. *Eur J Pharmacol* 2009;**614**:50–57.
64. Wichmann J, Adam G, Rover S, Cesura AM, Dautzenberg FM, Jenck F. 8-acenaphthen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one derivatives as orphanin FQ receptor agonists. *Bioorg Med Chem Lett* 1999;**9**:2343–2348.
65. Wichmann J, Adam G, Rover S, et al. Synthesis of (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, a potent and selective orphanin FQ (OFQ) receptor agonist with anxiolytic-like properties. *Eur J Med Chem* 2000;**35**:839–851.
66. Jenck F, Moreau JL, Martin JR, et al. Orphanin FQ acts as an anxiolytic to attenuate behavioral responses to stress. *Proc Natl Acad Sci U S A* 1997;**94**:14854–14858.
67. 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 U S A* 2000;**97**:4938–4943.
68. Dautzenberg FM, Wichmann J, Higelin J, et al. Pharmacological characterization of the novel nonpeptide orphanin FQ/nociceptin receptor agonist Ro 64–6198: Rapid and reversible desensitization of the ORL1 receptor in vitro and lack of tolerance in vivo. *J Pharmacol Exp Ther* 2001;**298**:812–819.
69. Shoblock JR. The pharmacology of Ro 64–6198, a systemically active, nonpeptide NOP receptor (opiate receptor-like 1, ORL-1) agonist with diverse preclinical therapeutic activity. *CNS Drug Rev* 2007;**13**:107–136.
70. Ho GD, Bercovici A, Tulshian D, et al. Synthesis and structure-activity relationships of 4-hydroxy-4-phenylpiperidines as nociceptin receptor ligands: Part 2. *Bioorg Med Chem Lett* 2007;**17**:3028–3033.
71. Ho GD, Bercovici A, Tulshian D, Greenlee WJ, Fawzi A, Smith Torhan A, Zhang H. Synthesis and structure-activity relationships of 4-hydroxy-4-phenylpiperidines as nociceptin receptor ligands: Part 1. *Bioorg Med Chem Lett* 2007;**17**:3023–3027.
72. Varty GB, Lu SX, Morgan CA, et al. The anxiolytic-like effects of the novel, orally active nociceptin opioid receptor agonist 8-[bis(2-methylphenyl)methyl]-3-phenyl-8-azabicyclo[3.2.1]octan-3-ol (SCH 221510). *J Pharmacol Exp Ther* 2008;**326**:672–682.
73. Yang SW, Ho G, Tulshian D, et al. Structure-activity relationships of 3-substituted N-benzhydryl-nortropane analogs as nociceptin receptor ligands for the treatment of cough. *Bioorg Med Chem Lett* 2008;**18**:6340–6343.
74. Hayashi S, Hirao A, Imai A, Nakamura H, Murata Y, Ohashi K, Nakata E. Novel non-peptide

nociceptin/orphanin FQ receptor agonist, 1-[1-(1-Methylcyclooctyl)-4-piperidinyl]-2-[(3R)-3-piperidinyl]-1H-benzimidazole: Design, synthesis, and structure-activity relationship of oral receptor occupancy in the brain for orally potent antianxiety drug (1, 2). *J Med Chem* 2009;**52**:610–625.

75. Hirao A, Imai A, Sugie Y, Tamura T, Shimokawa H, Toide K. Pharmacological properties of a novel nociceptin/orphanin FQ receptor agonist, 2-(3,5-dimethylpiperazin-1-yl)-1-[1-(1-methylcyclooctyl)piperidin-4-yl]-1H-benzim idazole, with anxiolytic potential. *Eur J Pharmacol* 2008;**579**: 189–195.
76. Hirao A, Imai A, Sugie Y, Yamada Y, Hayashi S, Toide K. Pharmacological characterization of the newly synthesized nociceptin/orphanin FQ-receptor agonist 1-[1-(1-methylcyclooctyl)-4-piperidinyl]-2-[(3R)-3-piperidinyl]-1H-benzimidazole as an anxiolytic agent. *J Pharmacol Sci* 2008;**106**:361–368.
77. 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.
78. Zeilhofer HU, Calo G. Nociceptin/orphanin FQ and its receptor–potential targets for pain therapy? *J Pharmacol Exp Ther* 2003;**306**:423–429.
79. Gavioli EC, Calo G. Antidepressant- and anxiolytic-like effects of nociceptin/orphanin FQ receptor ligands. *Naunyn Schmiedebergs Arch Pharmacol* 2006;**372**:319–330.
80. Olszewski PK, Levine AS. Minireview: Characterization of influence of central nociceptin/orphanin FQ on consummatory behavior. *Endocrinology* 2004;**145**: 2627–2632.
81. Reinscheid RK. The Orphanin FQ/Nociceptin receptor as a novel drug target in psychiatric disorders. *CNS Neurol Disord Drug Targets* 2006;**5**:219–224.
82. Malinowska B, Godlewski G, Schlicker E. Function of nociceptin and opioid OP4 receptors in the regulation of the cardiovascular system. *J Physiol Pharmacol* 2002;**53**:301–324.
83. Lecci A, Giuliani S, Meini S, Maggi CA. Nociceptin and the micturition reflex. *Peptides* 2000;**21**:1007–1021.
84. McLeod RL, Bolser DC, Jia Y, et al. Antitussive effect of nociceptin/orphanin FQ in experimental cough models. *Pulm Pharmacol Ther* 2002;**15**:213–216.
85. Kapusta DR. Neurohumoral effects of orphanin FQ/nociceptin: Relevance to cardiovascular and renal function. *Peptides* 2000;**21**:1081–1099.
86. Ko MC, Wei H, Woods JH, Kennedy RT. Effects of intrathecally administered nociceptin/orphanin FQ in monkeys: Behavioral and mass spectrometric studies. *J Pharmacol Exp Ther* 2006;**318**:1257–1264.
87. Ko MC, Naughton NN. Antinociceptive effects of nociceptin/orphanin FQ administrered intrathecally in monkeys. *J Pain* 2009;**10**:509–516.
88. Viaro R, Sanchez-Pernaute R, Marti M, Trapella C, Isacson O, Morari M. Nociceptin/orphanin FQ receptor blockade attenuates MPTP-induced parkinsonism. *Neurobiol Dis* 2008;**30**:430–438.
89. Visanji NP, de Bie RM, Johnston TH, McCreary AC, Brotchie JM, Fox SH. The nociceptin/orphanin FQ (NOP) receptor antagonist J-113397 enhances the effects of levodopa in the MPTP-lesioned nonhuman primate model of Parkinson's disease. *Mov Disord* 2008;**23**:1922–1925.
90. 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.
91. 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.
92. Lazzeri M, Calo' G, Spinelli M, et al. Daily intravescical instillation of 1 mg nociceptin/orphanin FQ for the control of neurogenic detrusor overactivity – a multicenter, placebo controlled, randomized exploratory study. *J Urol* 2006;**176**:2098–2102.
93. Arduin M, Spagnolo B, Calo G, et al. Synthesis and biological activity of nociceptin/orphanin FQ analogues substituted in position 7 or 11 with Calpha, alpha-dialkylated amino acids. *Bioorg Med Chem* 2007;**15**: 4434–4443.
94. Rizzi A, Spagnolo B, Wainford RD, et al. In vitro and in vivo studies on UFP-112, a novel potent and long lasting agonist selective for the nociceptin/orphanin FQ receptor. *Peptides* 2007;**28**:1240–1251.
95. Varani K, Calo G, Rizzi A, et al. Nociceptin receptor binding in mouse forebrain membranes: Thermodynamic characteristics and structure activity relationships. *Br J Pharmacol* 1998;**125**:1485–1490.
96. Varani K, Rizzi A, Calo G, et al. Pharmacology of [Tyr1]nociceptin analogs: Receptor binding and bioassay studies. *Naunyn Schmiedebergs Arch Pharmacol* 1999;**360**: 270–277.
97. Okawa H, Nicol B, Bigoni R, et al. Comparison of the effects of [Phe1psi(CH2-NH)Gly2]nociceptin(1–13)NH2 in rat brain, rat vas deferens and CHO cells expressing recombinant human nociceptin receptors. *Br J Pharmacol* 1999;**127**:123–130.
98. Bigoni R, Giuliani S, Calo G, et al. Characterization of nociceptin receptors in the periphery: In vitro and in vivo studies. *Naunyn Schmiedebergs Arch Pharmacol* 1999;**359**:160–167.
99. Calo G, Rizzi A, Bodin M, et al. Pharmacological characterization of nociceptin receptor: An in vitro study. *Can J Physiol Pharmacol* 1997;**75**:713–718.
100. Rizzi A, Calo G, Trevisani M, et al. Nociceptin receptor activation inhibits tachykinergic non adrenergic non

cholinergic contraction of guinea pig isolated bronchus. *Life Sci* 1999;**64**:PL157–163.
101. Calo G, Bigoni R, Rizzi A, Guerrini R, Salvadori S, Regoli D. Nociceptin/orphanin FQ receptor ligands. *Peptides* 2000;**21**:935–947.
102. Rizzi A, Bigoni R, Marzola G, Guerrini R, Salvadori S, Regoli D, Calo G. Characterization of the locomotor activity-inhibiting effect of nociceptin/orphanin FQ in mice. *Naunyn Schmiedebergs Arch Pharmacol* 2001; **363**:161–165.
103. Bertorelli R, Corradini L, Rafiq K, Tupper J, Calo G, Ongini E. Nociceptin and the ORL-1 ligand [Phe1psi (CH2-NH)Gly2]nociceptin(1–13)NH2 exert anti-opioid effects in the Freund's adjuvant-induced arthritic rat model of chronic pain. *Br J Pharmacol* 1999;**128**: 1252–1258.
104. Albrecht E, Samovilova NN, Oswald S, Baeger I, Berger H. Nociceptin (orphanin FQ): High-affinity and high-capacity binding site coupled to low-potency stimulation of guanylyl-5′-O-(gamma-thio)-triphosphate binding in rat brain membranes. *J Pharmacol Exp Ther* 1998;**286**:896–902.
105. Sandin J, Georgieva J, Silberring J, Terenius L. In vivo metabolism of nociceptin/orphanin FQ in rat hippocampus. *Neuroreport* 1999;**10**:71–76.
106. Madeddu P, Salis MB, Milia AF, Emanueli C, Guerrini R, Regoli D, Calo G. Cardiovascular effects of nociceptin in unanesthetized mice. *Hypertension* 1999;**33**:914–919.
107. 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.
108. Zhang C, Miller W, Valenzano KJ, Kyle DJ. Novel, potent ORL-1 receptor agonist peptides containing alpha-Helix-promoting conformational constraints. *J Med Chem* 2002;**45**:5280–5286.
109. Toniolo C, Crisma M, Formaggio E, et al. Structures of peptides from alpha amino acids methylated at the alpha carbon. *Biopolymers* 1993;**33**:1061–1072.
110. Tancredi T, Carra G, Guerrini R, et al. The Interaction of Highly Helical Structural Mutants with the NOP Receptor Discloses the Role of the Address Domain of Nociceptin/Orphanin FQ. *Chemistry* 2005;**11**:2061–2070.
111. Reinscheid RK, Ardati A, Monsma FJ, Jr., Civelli O. Structure-activity relationship studies on the novel neuropeptide orphanin FQ. *J Biol Chem* 1996;**271**: 14163–14168.
112. Topham CM, Mouledous L, Poda G, Maigret B, Meunier JC. Molecular modelling of the ORL1 receptor and its complex with nociceptin. *Protein Eng* 1998;**11**: 1163–1179.
113. 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.
114. Okada K, Isozaki K, Li J, Matsushima A, Nose T, Costa T, Shimohigashi Y. Synergistic effect of basic residues at positions 14–15 of nociceptin on binding affinity and receptor activation. *Bioorg Med Chem* 2008;**16**: 9261–9267.
115. D'Agostino B, Orlotti D, Calo G, et al. Nociceptin modulates bronchoconstriction induced by sensory nerve activation in mouse lung. *Am J Respir Cell Mol Biol* 2010;**42**:250–254.
116. Rizzi D, Bigoni R, Rizzi A, et al. Effects of Ro 64–6198 in nociceptin/orphanin FQ-sensitive isolated tissues. *Naunyn Schmiedebergs Arch Pharmacol* 2001;**363**: 551–555.
117. Chang M, Peng YL, Dong SL, et al. Structure-activity studies on different modifications of nociceptin/orphanin FQ: Identification of highly potent agonists and antagonists of its receptor. *Regul Pept* 2005;**130**: 116–122.
118. 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.
119. Nazzaro C, Rizzi A, Salvadori S, Guerrini R, Regoli D, Zeilhofer HU, Calo G. UFP-101 antagonizes the spinal antinociceptive effects of nociceptin/orphanin FQ: Behavioral and electrophysiological studies in mice. *Peptides* 2007;**28**:663–669.
120. 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.
121. 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.
122. Marti M, Mela F, Fantin M, et al. Blockade of nociceptin/orphanin FQ transmission attenuates symptoms and neurodegeneration associated with Parkinson's disease. *J Neurosci* 2005;**25**:9591–9601.
123. 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.
124. Marti M, Trapella C, Viaro R, Morari M. The nociceptin/orphanin FQ receptor antagonist J-113397 and L-DOPA additively attenuate experimental parkinsonism through overinhibition of the nigrothalamic pathway. *J Neurosci* 2007;**27**:1297–1307.
125. Morari M, Calo G, Marti M. Nociceptin/orphanin FQ receptor agonists attenuate L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. In: *Society for Neuroscience; 2007 November 3–7, 2007*; San Diego, CA, USA; 2007. p. 892.815/R898.

126. Przydzial MJ, Heisler LK. Nociceptin/orphanin FQ peptide receptor as a therapeutic target for obesity. *Mini Rev Med Chem* 2008;**8**:796–811.
127. Economidou D, Policani F, Angellotti T, Massi M, Terada T, Ciccocioppo R. Effect of novel NOP receptor ligands on food intake in rats. *Peptides* 2006;**27**:775–783.
128. 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.
129. Ciccocioppo R, Panocka I, Polidori C, Regoli D, Massi M. Effect of nociceptin on alcohol intake in alcohol-preferring rats. *Psychopharmacology (Berl)* 1999;**141**: 220–224.
130. Ciccocioppo R, Polidori C, Antonelli L, Salvadori S, Guerrini R, Massi M. Pharmacological characterization of the nociceptin receptor which mediates reduction of alcohol drinking in rats. *Peptides* 2002;**23**:117–125.
131. Ciccocioppo R, Economidou D, Fedeli A, Angeletti S, Weiss F, Heilig M, Massi M. Attenuation of ethanol self-administration and of conditioned reinstatement of alcohol-seeking behaviour by the antiopioid peptide nociceptin/orphanin FQ in alcohol-preferring rats. *Psychopharmacology (Berl)* 2004;**172**:170–178.
132. Economidou D, Hansson AC, Weiss F, et al. Dysregulation of nociceptin/orphanin FQ activity in the amygdala is linked to excessive alcohol drinking in the rat. *Biol Psychiatry* 2008;**64**:211–218.
133. Roberto M, Siggins GR. Nociceptin/orphanin FQ presynaptically decreases GABAergic transmission and blocks the ethanol-induced increase of GABA release in central amygdala. *Proc Natl Acad Sci U S A* 2006;**103**: 9715–9720.
134. Economidou D, Fedeli A, Fardon RM, Weiss F, Massi M, Ciccocioppo R. Effect of novel nociceptin/orphanin FQ-NOP receptor ligands on ethanol drinking in alcohol-preferring msP rats. *Peptides* 2006;**27**: 3299–3306.
135. Ciccocioppo R, Economidou D, Rimondini R, Sommer W, Massi M, Heilig M. Buprenorphine reduces alcohol drinking through activation of the nociceptin/orphanin FQ-NOP receptor system. *Biol Psychiatry* 2007;**61**:4–12.
136. 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.
137. Kuzmin A, Kreek MJ, Bakalkin G, Liljequist S. The nociceptin/orphanin FQ receptor agonist Ro 64–6198 reduces alcohol self-administration and prevents relapse-like alcohol drinking. *Neuropsychopharmacology* 2007;**32**:902–910.
138. Osinski MA, Brown DR. Orphanin FQ/nociceptin: A novel neuromodulator of gastrointestinal function? *Peptides* 2000;**21**:999–1005.
139. Menzies JR, Corbett AD. Nociceptin inhibits tonic nitric oxide release in the mouse isolated proximal colon. *Eur J Pharmacol* 2000;**388**:183–186.
140. Broccardo M, Guerrini R, Morini G, Polidori C, Agostini S, Petrella C, Improta G. The gastric effects of UFP-112, a new nociceptin/orphanin receptor agonist, in physiological and pathological conditions. *Peptides* 2007;**28**:1974–1981.
141. Grandi D, Solenghi E, Guerrini R, Polidori C, Massi M, Morini G. Nociceptin/orphanin FQ prevents gastric damage induced by cold-restraint stress in the rat by acting in the periphery. *Peptides* 2007;**28**:1572–1579.
142. Broccardo M, Agostini S, Petrella C, Guerrini R, Improta G. Central and peripheral role of the nociceptin/orphaninFQ system on normal and disturbed colonic motor function and faecal pellet output in the rat. *Neurogastroenterol Motil* 2008;**20**:939–948.
143. Peng YL, Chang M, Dong SL, et al. Novel potent agonist [(pF)Phe4,Aib7,Aib11,Arg14,Lys15]N/OFQ-NH2 and antagonist [Nphe1,(pF)Phe4,Aib7,Aib11,Arg14,Lys15] N/OFQ-NH2 of nociceptin/orphanin FQ receptor. *Regul Pept* 2006;**134**:75–81.
144. Ciccocioppo R, Angeletti S, Panocka I, Massi M. Nociceptin/orphanin FQ and drugs of abuse. *Peptides* 2000;**21**:1071–1080.
145. Ciccocioppo R, Cippitelli A, Economidou D, Fedeli A, Massi M. Nociceptin/orphanin FQ acts as a functional antagonist of corticotropin-releasing factor to inhibit its anorectic effect. *Physiol Behav* 2004;**82**:63–68.
146. Agostini S, Eutamene H, Broccardo M, et al. Peripheral anti-nociceptive effect of nociceptin/orphanin FQ in inflammation and stress-induced colonic hyperalgesia in rats. *Pain* 2009;**141**:292–299.
147. Williams JA, Day M, Heavner JE. Ziconotide: an update and review. *Expert Opin Pharmacother* 2008;**9**:1575–1583.
148. Xu XJ, Hao JX, Wiesenfeld-Hallin Z. Nociceptin or antinociceptin: Potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. *Neuroreport* 1996;**7**:2092–2094.
149. Erb K, Liebel JT, Tegeder I, Zeilhofer HU, Brune K, Geisslinger G. Spinally delivered nociceptin/orphanin FQ reduces flinching behaviour in the rat formalin test. *Neuroreport* 1997;**8**:1967–1970.
150. 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.
151. Kapusta DR, Burmeister MA, Calo G, Guerrini R, Gottlieb HB, Kenigs VA. Functional selectivity of nociceptin/orphanin FQ peptide receptor partial agonists on cardiovascular and renal function. *J Pharmacol Exp Ther* 2005;**314**:643–651.
152. Sandin J, Georgieva J, Schott PA, Ogren SO, Terenius L. Nociceptin/orphanin FQ microinjected into

hippocampus impairs spatial learning in rats. *Eur J Neurosci* 1997;**9**:194–197.
153. Manabe T, Noda Y, Mamiya T, et al. Facilitation of long-term potentiation and memory in mice lacking nociceptin receptors. *Nature* 1998;**394**:577–581.
154. Giuliani S, Lecci A, Tramontana M, Maggi CA. The inhibitory effect of nociceptin on the micturition reflex in anaesthetized rats. *Br J Pharmacol* 1998;**124**: 1566–1572.
155. Murphy NP, Lee Y, Maidment NT. Orphanin FQ/nociceptin blocks acquisition of morphine place preference. *Brain Res* 1999;**832**:168–170.
156. McLeod RL, Parra LE, Mutter JC, et al. Nociceptin inhibits cough in the guinea-pig by activation of ORL(1) receptors. *Br J Pharmacol* 2001;**132**:1175–1178.
157. Redrobe JP, Calo G, Regoli D, Quirion R. Nociceptin receptor antagonists display antidepressant-like properties in the mouse forced swimming test. *Naunyn Schmiedebergs Arch Pharmacol* 2002;**365**: 164–167.
158. 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.
159. Carvalho D, Petronilho F, Vuolo F, et al. The nociceptin/orphanin FQ-NOP receptor antagonist effects on an animal model of sepsis. *Intensive Care Med* 2008;**34**: 2284–2290.
160. Williams JP, Thompson JP, Young SP, Gold SJ, McDonald J, Rowbotham DJ, Lambert DG. Nociceptin and urotensin-II concentrations in critically ill patients with sepsis. *Br J Anaesth* 2008;**100**: 810–814.