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Nociceptin/Orphanin FQ Peptide Receptor-Related Ligands as Novel Analgesics

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Abstract

Despite similar distribution patterns and intracellular events observed in the nociceptin/orphanin FQ peptide (NOP) receptor and other opioid receptors, NOP receptor activation displays unique pharmacological profiles. Several researchers have identified a variety of peptide and non-peptide ligands to determine the functional roles of NOP receptor activation and observed that NOP receptor-related ligands exhibit pain modality-dependent pain processing. Importantly, NOP receptor activation results in anti-nociception and anti-hypersensitivity at the spinal and supraspinal levels regardless of the experimental settings in non-human primates (NHPs). Given that the NOP receptor agonists synergistically enhance mu-opioid peptide (MOP) receptor agonist-induced anti-nociception, it has been hypothesized that dual NOP and MOP receptor agonists may display promising functional properties as analgesics. Accumulating evidence indicates that the mixed NOP/opioid receptor agonists demonstrate favorable functional profiles. In NHP studies, bifunctional NOP/MOP partial agonists (*e.g.*, AT-121, BU08028, and BU10038) exerted potent anti-nociception via NOP and MOP receptor activation; however, dose-limiting adverse effects associated with the MOP receptor activation, including respiratory depression, itch sensation, physical dependence, and abuse liability, were not observed. Moreover, a mixed NOP/opioid receptor agonist, cebranopadol, presented promising outcomes in clinical trials as a novel analgesic. Collectively, the dual agonistic actions on NOP and MOP receptors, with appropriate binding affinities and efficacies, may be a viable strategy to develop innovative and safe analgesics.

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CONFLICT OF INTEREST

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Keywords

Anti-nociception; bifunctional ligand; non-human primate; NOP receptor; MOP receptor; peptide ligand; non-peptide ligand; spinal cord

1. INTRODUCTION

In 1994, after the cloning of classical (μ -, δ -, and κ -) opioid receptors [1–4], a G-protein coupled receptor (GPCR) with high homology to these opioid receptors was identified.

Owing to the lower affinity for the opioid ligands, the receptor was originally named opioid receptor-like 1 (ORL1) [5–9]. Subsequently, two groups of researchers independently discovered an endogenous 17-amino acid peptide (FGGFTGARKSARKLANQ), a selective ligand of ORL1. This peptide was named nociceptin and orphanin FQ because of its ability to elicit hyperalgesia following intracerebroventricular (i.c.v.) administration in rodents and the presence of the first and last amino acid residues, respectively [10–11]. Nociceptin/orphanin FQ (N/OFQ) is produced from a precursor peptide prepro-N/OFQ (ppN/OFQ), and the amino acid sequence of ppN/OFQ is well conserved across different animal species [12–13]. Both ppN/OFQ and N/OFQ mRNA and protein expression are widely distributed in the peripheral and central nervous system (CNS) in both rodents and primates, indicating their multiple effects on CNS functions [14–18].

Following the identification of N/OFQ, ORL1 was renamed as the N/OFQ peptide (NOP) receptor [19]. The primary structure of the NOP receptor is highly conserved in mammals. Several biochemical studies and crystal structure analyses have revealed that the hydrophobic and hydrophilic parts of the binding pockets of the NOP receptor and other opioid receptors differ because of the amino acid sequences constituting the binding pocket of the NOP receptor [20–23]. These findings underlie the marked differences observed in the binding selectivity of the corresponding ligands despite high homology between the NOP receptor and other opioid receptors. Like classical opioid receptors, the NOP receptor couples with the pertussis toxin-sensitive Gi/o proteins [24–26]. Intracellular events via NOP receptor activation can reduce synaptic transmission through presynaptically or postsynaptically located NOP receptors [27–29]. The NOP receptor mRNA and protein are also abundant in multiple areas of the brain and the spinal cord [13, 30–31]. N/OFQ and NOP receptor activation inhibits the release of various neurotransmitters (*e.g.*, dopamine, noradrenaline, 5-HT, and glutamate) in the CNS [32–34], suggesting that the N/OFQ-NOP receptor system largely contributes to the regulation of several CNS functions, including memory, reward, mood disorders, motor functions, and sensory processing.

Even though NOP receptor activation drives common intracellular events associated with other opioid receptors, the NOP receptor-mediated effects on pain modulation are more complicated than the MOP receptor activation. In this review, we highlighted the functional profile of the N/OFQ-NOP receptor system in terms of pain processing and discussed the therapeutic potentials of the newly developed NOP receptor-related ligands as novel analgesics based on *in vivo* pharmacological evidence.

2. N/OFQ-NOP RECEPTOR SYSTEM IN PAIN REGULATION

2.1. N/OFQ-NOP Receptor System in Rodent Models of Acute Pain

Because NOP receptors are located in the pain processing pathways in the dorsal root ganglia (DRG), spinal dorsal horn (SDH), and brain [15, 35–36], several researchers have investigated the roles of the central N/OFQ-NOP receptor system in pain modulation. The intrathecal (i.t.) administration of N/OFQ at nanomoles has produced anti-nociceptive effects in the tail-flick test and formalin-induced pain behaviors, and N/OFQ potentiated morphine-induced anti-nociception in rodents [37–40]. In contrast, ultra-low doses (*i.e.*, femtomoles) of i.t. N/OFQ elicited pronociceptive effects in mice, suggesting the biphasic actions of N/OFQ [41–42]. Nevertheless, higher doses of i.t. N/OFQ could produce analgesia through presynaptic or postsynaptic NOP receptor activation, thereby inhibiting the excitatory glutamatergic transmission [43–46].

Conversely, supraspinal NOP receptor activation produces complicated effects on pain processing, depending on the pain modalities. Several lines of evidence determined that the NOP receptor is located in multiple areas of the brain, including the thalamus, hypothalamus, locus coeruleus, periaqueductal gray (PAG), and rostral ventromedial medulla (RVM), which participate in the ascending and descending pain pathways [15, 36, 47–48]. In rodents, i.c.v. administration of N/OFQ produced pronociceptive effects in the hot plate and tail-flick tests, and i.c.v. N/OFQ blocked morphine-induced anti-nociception [10–11, 39]. These findings are supported by the fact that i.c.v. administration of UFP-101 ([N¹Phe¹Arg¹⁴Lys¹⁵]N/OFQ-NH₂), a selective NOP receptor antagonist peptide, showed anti-nociceptive effects in the formalin test [49]. Although the underlying mechanisms of pronociceptive effects mediated by supraspinal NOP receptor activation remain unclear, it was hypothesized that the two types of neurons (ON cells and OFF cells) in the RVM might be differentially modulated by the NOP receptor [13, 31].

2.2. N/OFQ-NOP Receptor System in Rodents Models of Chronic Pain

In rodents, NOP receptor activation in the SDH effectively suppressed chronic pain. Indeed, i.t. N/OFQ inhibited carrageenan- or complete Freund's adjuvant (CFA)-induced thermal hyperalgesia [50–52]. Such anti-hyperalgesic effects by N/OFQ might be, at least partially, explained by an upregulation of the NOP receptors in the spinal cord under an inflammatory pain condition. The protein expression of N/OFQ and NOP receptor was upregulated in the SDH in the presence of carrageenan and CFA-induced inflammation [53–54]. However, like in acute pain, the supraspinal actions of the NOP receptor activation under inflammatory pain appear complicated in rodents, as microinjection of UFP-101 into the ventrolateral PAG (vlPAG) reversed carrageenan-induced mechanical allodynia [55].

Notably, the N/OFQ-NOP receptor system also exerts potent suppressive effects on neuropathic pain. The i.t. N/OFQ attenuated chronic constriction injury (CCI)- or spinal nerve ligation (SNL)-induced thermal hyperalgesia and mechanical allodynia [56–59]. Moreover, lower doses of i.t. N/OFQ attenuated mechanical hyperalgesia following diabetic neuropathic pain as compared to the normal state [58, 60]. The protein expression pattern of the NOP receptor was differentially altered in the SDH after nerve injury, while the

inhibitory effects of N/OFQ on the spinal wide dynamic range neurons might be enhanced under neuropathic pain [31, 61]. Consistent with inflammatory pain, microinjection of UFP-101 into vIPAG attenuated CCI-induced tactile allodynia in rodents [55]. Although the mRNA expression of the NOP receptor was upregulated in the vIPAG after CCI [62], it remains unclear how NOP receptor-related ligands administered at different brain areas result in different painmodulating effects. The current evidence is insufficient to reveal the supraspinal actions of the N/OFQ-NOP receptor system for pain regulation in rodents.

2.3. N/OFQ-NOP Receptor System in the Non-Human Primates

Given the anatomical and neurochemical differences between rodents and primates, it is important to assess the functional profiles of the N/OFQ-NOP receptor system in non-human primates (NHPs). The functional NOP receptor protein is expressed on different diameter DRG neurons, and the NOP receptor activation inhibits the increase of calcium levels in the human DRG neurons [63]. Moreover, the NOP receptor protein is widely distributed in the SDH of NHPs [64–66], indicating that NOP receptor-related ligands have analgesic effects. Unlike in rodents, i.t. N/OFQ over a wide dose range (*i.e.*, from femtomoles to nanomoles) only produced anti-nociception in NHPs and i.t. N/OFQ dose-dependently produced anti-nociception and enhanced morphine-induced anti-nociception [67]. Importantly, N/OFQ is the most potent endogenous peptide among all opioid peptides, including β -endorphin and enkephalins, in terms of spinal analgesia under inflammatory pain conditions in NHPs [68]. This is because the i.t. administration of N/OFQ completely inhibits carrageenan-induced hyperalgesia in NHPs. Therefore, NOP receptor activation can be an attractive target for spinal analgesia.

Recently, the supraspinal actions of N/OFQ were determined using improved surgical techniques in NHPs [69]. Contrary to the finding in rodents, intracisternal administration of N/OFQ produced anti-nociceptive effects that were reversed by a NOP receptor antagonist. Additionally, the supraspinal NOP receptor activation failed to attenuate morphine-induced anti-nociception [69]. These findings reveal the distinct functional profiles of the supraspinal N/OFQ-NOP receptor system between rodents and primates and propose the impact of the N/OFQ-NOP receptor system for attenuating pain processing in primates.

2.4. Benefits of Bifunctional NOP/MOP Ligands

The potent mu-opioid peptide (MOP) receptor agonists (*i.e.*, morphine, oxycodone, and fentanyl) are the gold standard analgesics for relieving severe pain, whereas the clinical use of MOP agonists is restricted owing to their several adverse effects, such as reinforcing effects (abuse liability), respiratory depression, itch sensation, and constipation [70–71]. In the past few decades, several studies on classical opioid receptors enabled an advanced understanding of how opioid drugs act and exert beneficial and non-desirable effects [72–74]. Nevertheless, there is an unmet need for discovering novel opioid analgesics without MOP receptor-associated adverse effects [75–76]. Unlike the MOP receptor agonists, the NOP receptor agonists demonstrate no such non-desirable adverse effects in NHPs, and more importantly, they synergistically enhance MOP receptor agonist-induced analgesia [13, 77]. According to the current literature, ligands with dual NOP and MOP receptor agonist activities can produce potent analgesic effects across different pain modalities and display a

much wider therapeutic window when compared with the selective MOP or NOP receptor agonists in NHPs [66, 75]. Hence, further research focusing on NOP receptor-related agonists opens a new avenue for developing efficacious and safe analgesics, inducing fewer adverse effects. Given that the MOP receptor agonists can exert potent analgesic effects on severe pain, it is hypothesized that the bifunctional NOP and MOP receptor agonists may display promising functional properties as analgesics.

3. PEPTIDIC NOP RECEPTOR LIGANDS

3.1. Structure-Activity Relationship

The primary amino acid sequence of N/OFQ is similar to those of the other endogenous opioid peptides (*i.e.*, β -endorphins, enkephalins, and dynorphins) [78]. Despite the similarities, N/OFQ does not bind to the other opioid receptors owing to its unique structure. In particular, the first four amino acid residues (FGGF) at the N-terminal of N/OFQ are responsible for NOP receptor activation, whereas the last C-terminal residues dictate receptor selectivity and binding affinity [78–79]. Unlike the other endogenous opioid peptides, which have Thy^1 , N/OFQ has Phe^1 at the N-terminus, which is essential to determine the characteristics of the NOP receptor ligands. Indeed, the chemical modification of Phe^1 benzyl moiety or replacement of Phe^1 with any other amino acid differentiates the pharmacological properties of N/OFQ [78].

Regarding the sequence of N/OFQ at the C-terminal, the introduction of the $\text{Arg}^{14}\text{-Lys}^{15}$ couple can generate a highly potent NOP agonist [80–81], whereas N/OFQ(1–13) was observed to be a minimally active form of N/OFQ [82–83]. Moreover, in these N/OFQ derivatives, amidation of the C-terminal can be resistant to digestion by carboxypeptidases [84]. Through crystal structure analysis of the NOP receptor, the $\text{Gly}^2\text{-Gly}^3$ couple provided a suitable distance between Phe^1 and Phe^4 , resulting in a highly flexible conformation [22, 85]. Importantly, such a sequence consisting of four amino acids at the N-terminal (FGGF) provided an ionic interaction with the Asp^{130} of the NOP receptor, critical for binding with the NOP receptor and its activation [86–87]. Two structures of the NOP receptor have been identified by evaluating the receptor stability and crystallogenesis using different NOP receptor ligands. Moreover, docking studies revealed the mechanisms for ligand-induced NOP receptor stabilization and may illustrate the effects of several compounds on NOP receptor activation [87]. Based on these findings, the chemical modification of N/OFQ can synthesize several NOP receptor ligands with different pharmacological properties (*i.e.*, full agonists, partial agonists, and antagonists).

3.2. N/OFQ-Related Peptides

As mentioned above, N/OFQ, a full agonist of the NOP receptor, exhibited anti-nociceptive effects at higher doses in rodents. Among the N/OFQ derivatives (*i.e.*, UFP-112 ($[(\text{pF})\text{Phe}^4\text{Aib}^7\text{Arg}^{14}\text{Lys}^{15}]\text{N/OFQ-NH}_2$), a full agonist; UFP-113 ($[\text{Phe}^1\text{Ψ}(\text{CH}_2\text{-NH})\text{Gly}^2(\text{pF})\text{Phe}^4\text{Aib}^7\text{Arg}^{14}\text{Lys}^{15}]\text{N/OFQ-NH}_2$), a partial agonist; and UFP-101, an antagonist) produced by chemical modifications to increase their potency and reduce degradation [78, 86], the anti-nociceptive effects of UFP-112 have been well investigated in rodents, as well as NHPs. Indeed, *i.t.* UFP-112 exerted potent anti-nociceptive effects with

higher potency and longer duration of action than N/OFQ in an acute pain model in mice [88–89]. Consistently, i.t. UFP-112 demonstrated potent anti-nociceptive effects when compared with N/OFQ and morphine in both acute and chronic pain models in NHPs [90].

Recently, an advanced chemical approach, peptide welding technology (PWT) has been utilized to develop several N/OFQ derivatives [91]. Although the development of multimeric peptides using convergent approaches is restricted by the selectivity of the reaction to link the core and peptide sequences, the reaction between a thiol and an α,β -unsaturated carbonyl group (*i.e.*, thiol-Michael conjugation) of three different tetra-maleimide functionalized cores with four linear peptides allowed researchers to produce high affinity, potent, and selective NOP receptor agonists [91–92]. Among the three different types of PWT derivatives (PWT1, PWT2, and PWT3), i.t. PWT2-N/OFQ (Fig. 1) was approximately 40-fold more potent than the endogenous N/OFQ and exerted long-lasting anti-nociceptive effects in mice [93]. Importantly, such findings can be translated from rodents to NHPs as i.t. PWT2-N/OFQ also produced potent anti-nociceptive effects in NHPs. In NHPs, PWT2-N/OFQ was about 30-fold more potent than N/OFQ and the duration of the anti-nociceptive effects of PWT2-N/OFQ was 10-fold longer than that of N/OFQ [94]. Given the potent and long-lasting effects of UFP-112 and PWT2-N/OFQ in NHPs without MOP-related adverse effects [90, 94], such as itching, the NOP receptor agonists possess great potential as spinal analgesics.

3.3. Bifunctional NOP/MOP Agonists

The NOP receptor agonists exert robust anti-nociceptive and anti-allodynic effects in acute and chronic pain [13, 31, 61]. The effects are synergistically enhanced by MOP agonists at the spinal level in both rodents and NHPs. As the Phe¹ or Tyr¹ residues at the N-terminal of N/OFQ determine the selectivity over the NOP receptors and other opioid receptors, introduction of a 2,6-dimethyltyrosine (Dmt) residue at the N-terminal generates bifunctional peptide ligands, [Dmt¹]N/OFQ(1–13)-NH₂ and [Dmt¹]N/OFQ-NH₂, that activate not only the NOP receptor but also the MOP receptor [95]. Although [Dmt¹]N/OFQ(1–13)-NH₂ displayed similar pharmacological properties as that of N/OFQ *in vitro*, i.t. [Dmt¹]N/OFQ(1–13)-NH₂ produced anti-nociceptive effects in mice, and the anti-nociceptive effects in NHPs were approximately 30-fold more potent than that observed with N/OFQ [13, 95].

Furthermore, PWT was used to synthesize a tetrameric ligand with mixed NOP/MOP agonist actions [96]. PWT-2[Dmt¹]N/OFQ(1–13)-NH₂ demonstrated a lower affinity to the NOP receptor than other linear peptides, with a similar selectivity among the NOP and MOP receptors. In NHPs, i.t. PWT2-[Dmt¹]N/OFQ(1–13)-NH₂ produced complete anti-nociceptive effects with increased potency and a much longer duration of action [96]. These lines of evidence indicate that the bifunctional NOP/MOP receptor ligands have strong therapeutic potential in pain treatment.

4. NON-PEPTIDIC NOP RECEPTOR LIGANDS

4.1. Structure-Activity Relationship

In the 1990s, several non-peptidic NOP receptor ligands were discovered via high-throughput screening from compound libraries, and these lead non-morphinan compounds were used for the synthesis of new NOP receptor ligands [97–98]. Using chemical modifications to enhance the binding affinity and selectivity for the NOP receptor, J-113397 (Fig. 2) was discovered as the first non-peptidic NOP receptor antagonist [99]. Next, the NOP agonist Ro64–6198 (Fig. 2), the first non-peptidic NOP agonist with a high affinity, was reported [100–101]. Recent technological advances, such as bioinformatics, for predicting the structure-activity relationship between small non-peptidic compounds and the NOP receptor can facilitate the discovery of novel functional NOP receptor ligands [85, 98].

By pharmacophore and crystal structure analysis of the early reported NOP receptor ligands [22, 97], three key features (an alicyclic core containing protonatable nitrogen, an aromatic or heterocyclic moiety distal to the protonatable nitrogen, and a lipophilic substituent on the protonatable nitrogen) determining the binding affinity and selectivity for the NOP receptors were identified. The lipophilic nitrogen substituent is important for the intrinsic activity of the NOP receptor ligands [102]. Furthermore, protonatable nitrogen is an essential feature for all the non-peptidic NOP receptor ligands in terms of dictating the interaction with the NOP receptor Asp¹³⁰, as well as the Phe¹ of N/OFQ [85]. Based on this knowledge, several NOP receptor-related non-peptide ligands have been developed. Notably, the chemical modifications of these key features can increase the binding affinity for the MOP receptor, resulting in the bifunctional NOP/MOP receptor ligands.

4.2. NOP Selective Agonists

The effects of systemically administered NOP receptor agonists demonstrated integrated peripheral, spinal, and supraspinal actions [66]. Although the roles of the N/OFQ-NOP receptor system in the pain processing pathways are complicated depending on the routes of administration in rodents, anti-nociceptive effects were produced regardless of the sites of action in NHPs [67, 103–104]. Thus, it is postulated that the systemic administration of the NOP receptor agonists exerts anti-nociceptive effects in NHPs. In the thermal tail withdrawal test, systemic (*i.e.*, subcutaneous, intramuscular, or intravenous) administration of Ro64–6198 dose-dependently produced anti-nociceptive effects [105], and attenuated capsaicin-induced allodynia and carrageenan-induced hyperalgesia in NHPs [104–105]. Similar to Ro64–6198, systemic SCH221510 (Fig. 2), a non-peptidic NOP receptor agonist [106], also produced anti-nociceptive effects in NHPs [77, 107]. Furthermore, the systemic administration of these NOP full agonists exerted anti-allodynic and anti-hyperalgesic effects in NHPs. NOP full agonists displayed anti-nociception comparable to morphine, while the adverse effects associated with MOP receptor activation were not observed, including respiratory depression, itching, abuse liability, and physical dependence [66, 75]. These lines of evidence suggest that the NOP receptor agonists possess potent and safer profiles than clinically used MOP receptor agonists, and are effective for treating pain by systemic administration in primates. Nonetheless, in NHPs, Ro64–6198 caused sedation at

higher doses than at the anti-nociceptive dose [103], and further evidence underlying this non-desirable effect needs to be considered.

4.3. Bifunctional NOP/MOP Partial Agonists

Notably, selective NOP receptor agonists exert potent anti-nociceptive effects and synergistically enhance the analgesic effects of MOP receptor agonists, while attenuating the drug reinforcing effects in NHPs [66, 77]. Given such preferable functional properties of the NOP receptor agonists, it has been hypothesized that the bifunctional NOP/MOP receptor agonists may be safer opioid analgesics, lacking the non-desirable adverse effects, including abuse liability [75]. Based on this hypothesis, a non-morphinan compound, AT-121 (Fig. 3), was identified as a bifunctional partial agonist of the NOP/MOP receptor, demonstrating a high binding affinity and partial agonist efficacy for NOP and MOP receptors [108]. In NHPs, systemic administration of AT-121 produced anti-nociceptive and anti-allodynic effects comparable with morphine, without inducing respiratory depression, itching, opioid-induced hyperalgesia, and physical dependence. Additionally, AT-121 failed to demonstrate reinforcing effects and attenuated the reinforcing effects of oxycodone, an abused prescription opioid, without disrupting food intake [108]. AT-121-induced anti-nociception was antagonized by either J-113397, a NOP receptor antagonist, or naltrexone, a MOP receptor antagonist, indicating that the coactivation of NOP and MOP receptors provides a promising pharmacological profile as a novel potent and safer analgesic [97, 108].

Conversely, several researchers' groups attempted to develop different NOP/MOP receptor ligands with a morphinan-like structure [109–110]. Buprenorphine, a semisynthetic multifunctional opioid, has been widely used for relieving several kinds of pain syndromes, including neuropathic pain [111–112]. Importantly, buprenorphine shows partial agonist activity for MOP receptors but does not completely lack abuse liability. According to previous reports indicating that an appropriate balance in the NOP/MOP receptor activation displays an ideal pharmacological profile (*i.e.*, potent analgesia without adverse effects) [13, 75, 77, 113], BU08028 was identified as a buprenorphine analog and a bifunctional NOP/MOP receptor agonist [114]. BU08028 (Fig. 3) showed receptor binding profiles similar to buprenorphine and other opioid receptors, with higher binding affinity and efficacy to the NOP receptor [114]. BU08028 produced potent and long-lasting (up to 30 h) anti-nociceptive and anti-allodynic effects in NHPs, and J-113397 and naltrexone resulted in the same degree of the rightward shift in the dose-response curve in BU08028-induced analgesia [115]. Furthermore, unlike in rodents, BU08028 did not produce reinforcing effects in NHPs and was devoid of respiratory depression, itching, and physical dependence at higher doses than at the analgesic dose [115].

Recently, a naltrexone-derived analog, BU10038 (Fig. 3), was further synthesized as a novel bifunctional NOP/MOP receptor partial agonist [116]. In NHPs, systemic or i.t. administration of BU10038 produced potent and long-lasting (up to 30 h) anti-nociceptive and anti-allodynic effects without inducing itching and analgesic tolerance when compared with morphine [116]. Importantly, following chronic administration, tolerance to BU10038 developed slower than with morphine. Collectively, these findings support the notion that the

bifunctional NOP/MOP receptor agonists demonstrate higher potency and a wider therapeutic window, and such ligands may be innovative, safer opioid analgesics in humans.

4.4. A Novel Analgesic with Mixed NOP and Opioid Receptor Agonist Activities

In 2014, cebranopadol (Fig. 3) was discovered as a non-morphinan compound and was characterized as a potent agonist with mixed NOP and opioid receptor agonist activities [117]. Cebranopadol displayed a high affinity for NOP and MOP and kappa-opioid peptide (KOP) receptors, with nearly full agonistic activity for NOP and MOP and partial agonistic activity for KOP receptors based on the [^{35}S]GTP γ S binding assay [117–119]. In rodents, cebranopadol demonstrated a half-life of 4.5 h and oral bioavailability of 13–23%, exerting dose-dependent anti-nociceptive effects via NOP and MOP receptor activation following systemic administration [117, 120–121]. Notably, cebranopadol was more than 100-fold potent and long-lasting when compared with morphine-induced analgesia, producing potent anti-hyperalgesic and anti-allodynic effects in different models of chronic pain, including SNL, CCI, and chemotherapy-induced neuropathic pain model in rodents [117, 119]. As mentioned, regarding the sites of action of the NOP receptors in pain modulation, cebranopadol exerted potent and efficacious anti-hyperalgesic and anti-allodynic effects at the peripheral, spinal, and supraspinal levels in rodents. Importantly, cebranopadol also showed potent anti-nociceptive and anti-allodynic effects after systemic (1–5.6 $\mu\text{g}/\text{kg}$) or i.t. (1 μg) administration without eliciting itch in NHPs [66, 122].

Considerable attention has been paid to cebranopadol as it is currently used in clinical studies as an analgesic. The pharmacokinetic properties of cebranopadol were demonstrated in phase I and phase II clinical trials. Cebranopadol displayed a maximum plasma concentration for 4–6 h and a half-life of 14–15 h [118, 123]. An operational half-life of 24 h was calculated after multiple once-daily dosing in patients. Oral administration of 0.6 mg of cebranopadol produced analgesia and opioid-like adverse effects, including miosis and respiratory depression, although such adverse effects demonstrated lower intensities and shorter durations than the analgesic effects [124]. These findings suggest that cebranopadol exhibits safer pharmacological profiles than the other clinically used opioid analgesics [118–119]. Subsequently, the analgesic efficacy of cebranopadol in patients with low back pain was evaluated in randomized, double-blind, placebo-controlled trials. Cebranopadol produced significant analgesic effects over the placebo control and showed less non-desirable adverse effects (*e.g.*, constipation, dizziness, and nausea). In patients with cancer pain or diabetic neuropathy, cebranopadol also revealed an effective and safe profile [118–119, 125]. Overall, these findings indicate that cebranopadol is an effective analgesic with an improved side-effect profile, and it is anticipated to advance to phase III trials in the future [119].

CONCLUSION

Collectively, the N/OFQ-NOP receptor system plays an important role in pain modulation both in rodents and primates. The *in vivo* pharmacological properties of several peptidic and non-peptidic NOP receptor-related ligands, with different binding affinities and efficacies, have been investigated. Notably, NOP receptor agonists potentially produce anti-nociceptive

and anti-allodynic effects and synergistically enhance buprenorphine- or morphine-induced analgesia in NHPs without eliciting non-desirable adverse effects [66, 75]. Despite some controversial phenomena, the bifunctional NOP/MOP receptor agonists display favorable functional profiles. The NOP/MOP partial agonists (*e.g.*, AT-121, BU08028, and BU10038) exerted morphine-like analgesic effects via NOP and MOP receptor activation, whereas no dose-limiting adverse effects were associated with MOP receptor activation in NHPs. Given the similarities of the anatomical and neurochemical features between humans and NHPs, the above-mentioned pharmacological evidence observed in NHPs provides valid translational relevance to facilitate basic research and drug development. Promising phase II clinical trial outcomes have been observed with cebranopadol, suggesting that the NOP/MOP receptor coactivation may exhibit efficacious pain-relief and allow the development of bifunctional NOP/MOP receptor agonists as innovative and safer analgesics.

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Glossary

Aib	2-Aminoisobutyric acid
CCI	Chronic constriction injury
CFA	Complete Freund's adjuvant
CNS	Central nervous system
Dmt	2,6-dimethyltyrosine
DRG	Dorsal root ganglia
GPCR	G-protein coupled receptor
i.c.v.	Intracerebroventricular
i.t.	Intrathecal
KOP	Kappa-opioid peptide
MOP	Mu-opioid peptide
NHP	Non-human primates
NOP	Nociceptin/orphanin FQ peptide
ORL1	Opioid receptor-like 1
PAG	Periaqueductal gray

PWT	Peptide welding technology
RVM	Rostral ventromedial medulla
SDH	Spinal dorsal horn
SNL	Spinal nerve ligation
vIPAG	Ventrolateral PAG

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Fig. (1).
Chemical structure of PWT2-N/OFQ.

Ro64-6198

SCH221510

J-113397

Fig. (2).
Chemical structures of non-peptidic NOP receptor ligands.

Fig. (3).
Chemical structures of mixed NOP/opioid receptor agonists.

Table 1.

Binding affinities and functional activities of NOP receptor-related agonists for human opioid receptors. DAMGO, [DAla², N-MePhe⁴, Gly-ol]-enkephalin, is a MOP receptor agonist.

-	Receptor binding <i>K_i</i> (nM)				NOP [³⁵ S]GTPγS		MOP [³⁵ S]GTPγS		References
	NOP	MOP	KOP	DOP	EC ₅₀ (nM)	%Stimulation	EC ₅₀ (nM)	%Stimulation	
N/OFQ	-	-	-	-	-	100	-	-	-
DAMGO	-	-	-	-	-	-	-	100	-
Morphine	-	-	-	-	-	-	-	93	[108]
AT-121	3.7	16.5	301.3	145.6	34.7	41.1	19.6	14.2	[108]
BU08028	8.5	2.1	5.6	1.6	78.6	48	6.0	21.1	[114]
BU10038	14.8	0.9	1.2	10.5	44	34		18	[116]
Cebranopadol	0.9	0.7	2.6	18.0	13.0	88.9	1.2	103.5	[120]