Differential Effects of Nociceptin/Orphanin FQ (NOP) Receptor Agonists in Acute versus Chronic Pain: Studies with Bifunctional NOP/ μ Receptor Agonists in the Sciatic Nerve Ligation Chronic Pain Model in Mice^[S]

Taline V. Khroyan, Willma E. Polgar, Juan Orduna, Jose Montenegro, Faming Jiang, Nurulain T. Zaveri, and Lawrence Toll

SRI International, Menlo Park, California (T.V.K., W.E.P., J.O., J.M., F.J., L.T.); and Astraea Therapeutics LLC, Mountain View, California (N.T.Z.)

Received May 31, 2011; accepted August 18, 2011

ABSTRACT

1-(1-Cyclooctylpiperidin-4-yl)-indolin-2-one (SR14150) and 1-(1-(2,3,3a,4,5,6-hexahydro-1*H*-phenalen-1-yl)piperidin-4-yl)-indolin-2-one (SR16835) are moderately selective nociceptin/orphanin FQ (NOP) receptor agonists. In the [³⁵S]guanosine 5'-O-(3-thiotriphosphate) assay in vitro, SR14150 is a partial agonist at both the NOP and μ -opioid receptors, whereas SR16835 is a full agonist at the NOP receptor and has low efficacy at μ receptors. These compounds were tested for antinociceptive and antiallodynic activity, using mice in chronic pain, subsequent to spinal nerve ligation (SNL) surgery. When administered subcutaneously to mice after SNL surgery, SR14150 but not SR16835 increased tail-flick latency, which was blocked by the opioid antagonist naloxone, but not by the NOP receptor antagonist (–)-*cis*-1-methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-ol (SB-612111). In contrast, both SR14150 and

SR16835 had antiallodynic activity when mechanical allodynia was measured with von Frey monofilaments. This effect was completely blocked by SB-612111 but not by naloxone. On the other hand, morphine antinociception and antiallodynia were both blocked by naloxone and potentiated by SB-612111. These results indicate that, in mice, circuitry mediating antinociceptive activity in acute and chronic pain states is different. It is possible that during a chronic pain state, an up-regulated NOP system in the spinal cord leads to NOP receptor-mediated antiallodynia, which is blocked by NOP antagonists. However, supraspinal up-regulation could lead to an attenuation of morphine antinociception and antiallodynia, which can be alleviated by an NOP receptor antagonist. Thus, although neither NOP agonists nor antagonists are effective as analgesics in acute pain, they may have efficacy as analgesics, either alone or in combination with morphine, for treatment of chronic pain.

This work was supported by the National Institutes of Health National Institute on Drug Abuse [Grants DA023281, DA014026] (to L.T. and N.T.Z., respectively).

Parts of this work were previously presented at the following conference: Toll L, Khroyan TV, Polgar WE, Orduna J, Zaveri NT, and Jiang F (2010) Mixed μ /NOP agonists, differential effects in acute and chronic pain. *International Narcotics Research Conference*; 2010 July 11–16; Malmö, Sweden. International Narcotics Research Conference Inc., Teaneck, NJ.

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.

doi:10.1124/jpet.111.184663.

^[S] The online version of this article (available at <http://jpet.aspetjournals.org>) contains supplemental material.

Introduction

Modulation of the pain response by the NOP receptor system is complex. Initial observations with its endogenous ligand nociceptin/orphanin FQ (N/OFQ) found that intracerebroventricular injection of this peptide showed pronociceptive effects in mice, decreasing hot-plate and tail-flick latency, indicating that its effects were opposite to those of morphine (Meunier et al., 1995; Reinscheid et al., 1995). Subsequently, it was determined that N/OFQ did not directly affect nociception in mice or rats but in fact decreased the intracerebroventricular injection-mediated stress-induced analgesia (Grisel et al., 1996). In ad-

ABBREVIATIONS: NOP, nociceptin/orphanin FQ; N/OFQ, nociceptin/orphanin FQ; Ro 64-6198, (1*S*,3*aS*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one; SR16835, 1-(1-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)piperidin-4-yl)-indolin-2-one; SR14150, 1-(1-cyclooctylpiperidin-4-yl)-indolin-2-one; SB-612111, (–)-*cis*-1-methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-ol; CFA, complete Freund's adjuvant; CCI, chronic constriction injury; SNL, spinal nerve ligation; UFP-101, [Nphe¹Arg¹⁴Lys¹⁵]N/OFQ-NH₂; HPCOM, [1-[4-(2-{hexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl]-1*H*-benzimidazol-1-yl)piperidin-1-yl]cyclooctyl]methanol; J-113397, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one.

dition to blocking stress-induced analgesia, intracerebroventricular N/OFQ blocks analgesia mediated by μ -, δ -, and κ -opioid ligands (Mogil et al., 1996). Intrathecal administration of N/OFQ, on the other hand, produces a distinctly different result. Rather than attenuating morphine analgesia, intrathecal N/OFQ potentiates morphine analgesia and has antinociceptive activity of its own that is not present in NOP knockout mice (Xu et al., 1996; Tian and Han, 2000; Bertorelli et al., 2002). Studies with more potent and longer-lasting peptide agonists have generally confirmed the findings that NOP receptor activation supraspinally has antiopioid activity, whereas activation in the spinal cord has antinociceptive effects, similar to opioid receptor activation (Carrà et al., 2005; Rizzi et al., 2007).

Studies with small-molecule NOP receptor agonists further underscore the complex role of the NOP system in pain. The selective NOP receptor agonist (1*S*,3*aS*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one (Ro 64-6198) was found not to produce antinociception in the tail-flick test in rats or mice after systemic administration (Jenck et al., 2000; Reiss et al., 2008), although it was effective in the mouse hot-plate test (Reiss et al., 2008). Bifunctional compounds with both NOP and μ -opioid receptor agonist activity, such as 1-(1-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)piperidin-4-yl)-indolin-2-one (SR16435) and 1-(1-cyclooctylpiperidin-4-yl)-indolin-2-one (SR14150) and even the opioid partial agonist buprenorphine, have significant μ -mediated antinociceptive activity in the tail-flick assay, which is blocked by naloxone rather than an NOP receptor antagonist. In fact, the activity of these compounds is potentiated by the NOP receptor antagonist (–)-*cis*-1-methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-ol (SB-612111), suggesting that the NOP receptor agonist activity of these compounds is attenuating its μ -agonist activity (Khroyan et al., 2007a, 2009a; Toll et al., 2009).

The effects of NOP receptor agonists have also been studied in chronic pain models. N/OFQ, given intracerebroventricularly, has no antiallodynic activity in the complete Freund's adjuvant (CFA) model of arthritic pain but can block the antiallodynic activity of morphine (Bertorelli et al., 1999). When given intrathecally, N/OFQ is antiallodynic in rats that are in chronic pain, and it does not alter morphine-induced antiallodynia (Hao et al., 1998; Corradini et al., 2001). Ro 64-6198 also has antiallodynic activity after intrathecal and intraplantar injection but not subcutaneously in rats that have undergone chronic constriction injury (CCI) (Obara et al., 2005).

As is true for many neurotransmitter systems, the NOP-N/OFQ system is plastic and can be modified under certain experimental and pathogenic conditions. In particular, there is an increase in NOP receptor mRNA in various brain regions, dorsal root ganglia, and spinal cord of CCI rats (Brisini et al., 2002; Mika et al., 2004; Ma et al., 2005). There is also an increase in NOP receptors in superficial laminae of the rat spinal cord subsequent to CFA injection, as determined by *in vitro* autoradiography (Jia et al., 1998). N/OFQ mRNA has also been shown to be up-regulated in the nucleus raphe magnus, amygdala, and periaqueductal gray after spinal nerve ligation (SNL) and CCI surgery (Sun et al., 2001; Ma et al., 2005). How this up-regulation of the NOP system in a chronic pain state affects the actions of selective small-molecule NOP agonists and antagonists is still not clear, and it appears that these compounds have differential behavioral

effects in the brain versus spinal cord. The ultimate actions of small-molecule NOP agonists and antagonists in chronic pain states probably depend on the extent to which the NOP system is modulated in the spinal cord and the brain and also on the pharmacokinetic distribution of the compound.

In this study, we describe experiments that were carried out in mice that underwent SNL to determine how chronic pain affects the ability of NOP/ μ agonists and antagonists to modulate the response to a painful stimulus. In this model, chronic pain appears to up-regulate the NOP system, resulting in NOP receptor agonist-mediated antiallodynia, potentially at the level of the spinal cord, and potentiation of morphine-induced antinociception and antiallodynia by a NOP antagonist, presumably mediated supraspinally.

Materials and Methods

Subjects. Male ICR mice weighing 20 to 25 g at the start of the experiment were used. Animals were group-housed under standard laboratory conditions and kept on a 12-h day/night cycle (lights on at 7:00 AM). Animals were handled for at least 3 to 4 days before undergoing surgery. For all behavioral experiments, animals were transported to the testing room and acclimated to the environment for 1 h before any testing. Mice were maintained in accordance with the guidelines of SRI International and the National Research Council (2003) *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*.

Drugs. SR14150 and SR16835 were synthesized in Dr. Zaveri's laboratory, as hydrochloride salts (Zaveri et al., 2004) (Fig. 1). The NOP receptor antagonist SB-612111 was also synthesized in Dr. Zaveri's laboratory using previously reported methodology (Zaratin et al., 2004; Barlocco et al., 2006). Morphine hydrochloride (Eli Lilly and Co., Indianapolis, IN) and naloxone (Sigma-Aldrich, St. Louis, MO) were dissolved in water. SR14150, SR16835, and SB-612111 were dissolved in 1 to 2% dimethyl sulfoxide and 0.5% aqueous hydroxypropylcellulose. Drugs were injected in a volume of 0.1 ml/25 g s.c. Controls received a 0.1 ml/25 g volume of the appropriate vehicle.

SNL Surgery. All surgical procedures used were performed under deep isoflurane anesthesia (5% for induction and 2% for maintenance) in 10% O₂. The SNL surgery was performed as described previously (Kim and Chung, 1992). In brief, a midline incision above the lumbar spine exposed the left L6 transverse process. The left L5 spinal nerve was isolated and tightly ligated with 6-0 silk. The animals were then given 1 to 2 weeks to recover and for the allodynia to develop.

Assessment of Thermal Nociception Using the Tail-Flick Assay. Animals received their assigned dose of drug and were tested for thermal nociception at 30 min after injection. Thermal nociception was assessed using the tail-flick assay with an analgesia instrument (Stoelting, Kiel, WI) that uses radiant heat. During testing, the focused beam of light was applied to the lower half of the animal's

Fig. 1. Chemical structures of SR14150 and SR16835.

tail, and tail-flick latency was recorded. If the animal did not respond before the 15-s cutoff, the animal was assigned a score of 15 s. Baseline values for tail-flick latency were determined before drug administration in each animal. The mean basal tail-flick latency after surgery was 4.17 ± 0.13 s (S.E.M.).

Assessment of Tactile/Mechanical Allodynia. Animals received their respective dose of drug and were tested 60 min later for mechanical allodynia with von Frey filaments using the up-down method modified for mice (Dixon, 1991; Chaplan et al., 1994). The filaments we used for these experiments had buckling weights of 0.005, 0.02, 0.03, 0.07, 0.17, 0.41, 0.7, 1.2, and 1.5 g. For each mouse, a von Frey filament that had a buckling weight of 0.07 g was applied to the right hind paw of the animal with continuous pressure for approximately 5 s. If the animal lifted its paw, the next filament with lower force was then applied. If the animal did not lift its paw, the next filament with higher force was used. Each response was recorded, and the experiment ended once the animal had made five responses after the initial positive response. The 50% paw withdrawal threshold was calculated using the formula: $(10^{Xf+\kappa\delta})/10,000$ where Xf is the final von Frey filament used (log units), κ is a value that analyzes the response pattern (taken from a published table (Dixon, 1991; Chaplan et al., 1994), and δ is the mean difference between stimuli (log units; calculated by the program used to analyze the data). If the animal made four consecutive positive responses a score of 0.005 g was assigned, whereas if the animal had five consecutive negative responses then a score of 1.5 g was assigned.

Baseline values for mechanical allodynia were determined before testing with the drugs in each animal. The mean baseline before surgery was 1.1 ± 0.09 g. The postsurgery baseline, once allodynia was induced, was 0.05 ± 0.008 g.

Experimental Design. Animals ($n = 8$ –14/group) received subcutaneous injections of morphine (1–10 mg/kg), SR14150 (3–10 mg/kg), or SR16835 alone (10–30 mg/kg). Other groups of animals served as vehicle controls. Morphine, SR14150, and SR16835 produced antinociceptive effects, so in follow-up experiments, animals received a pretreatment of 1 mg/kg opioid antagonist naloxone or a pretreatment of 10 mg/kg NOP receptor antagonist SB-612111 (Zaratin et al., 2004) to determine whether drug-mediated effects were due to opioid or NOP receptor activity. The dose of naloxone chosen has been shown to reverse the effects of morphine, and the dose of SB-612111 was chosen on the basis of our previous experiments (Khroyan et al., 2007b; Toll et al., 2009). The antagonists were given 10 min before the injection of morphine, SR14150, or SR16835. Animals then underwent testing for thermal antinociception or mechanical allodynia, as described above. The experimenters were blind as to the particular treatment and condition of each animal.

Statistical Analyses. Thermal nociception data (test latency, seconds) and mechanical allodynia (force, grams) were analyzed using an analysis of variance with drug treatment (SR14150, SR16835, morphine, SB-612111, and naloxone) as between-group variables followed by Student-Newman-Keuls post hoc tests where appropriate. The level of significance was set at $P < 0.05$.

Results

Effects of SR14150 on Tail-Flick Latency and Mechanical Allodynia in SNL Mice. In a previous study, we have shown that SR14150 produces antinociception in the tail-flick assay in animals that are not in chronic pain (Toll et al., 2009). Likewise, here we report that SR14150 produces an increase in tail-flick latency in SNL animals. The effect of SR14150 on tail-flick latency in SNL mice is shown in Fig. 2A. The overall analysis of variance indicated that there was a significant main effect [$F_{3,35} = 38.9$, $P < 0.05$]. As expected, morphine (10 mg/kg) produced a near-maximal antinociceptive effect. The 3 and 10 mg/kg doses of SR14150

Fig. 2. Effect of SR14150 administered alone (A) or coadministered with NOP receptor antagonist SB-612111 or opioid antagonist naloxone (B) on tail-flick latency in mice that have undergone SNL. Morphine is included as the positive control. Data are means $\pm$ S.E.M. *, significant difference from vehicle controls; +, significant difference from morphine control; †, significant difference from SR14150 alone ($P < 0.05$).

produced a significant increase in antinociception relative to that in vehicle controls. The antinociception produced by 3 mg/kg SR14150 was greater than that with vehicle but less than that with the 10 mg/kg morphine control, whereas 10 mg/kg SR14150 produced levels of antinociception similar to those with 10 mg/kg morphine. To determine the receptor mediating the SR14150-induced antinociception, animals received a pretreatment of SB-612111 or naloxone. Naloxone reversed SR14150-induced antinociception [$F_{1,24} = 11.6$, $P < 0.05$], whereas pretreatment with SB-612111 did not produce any significant changes (Fig. 2B), indicating that the SR14150-induced antinociceptive activity is mediated by opioid receptors and not by the NOP receptor.

The effect of SR14150 on tactile allodynia is shown in Fig. 3A. SR14150 produced an increase in antiallodynia whereby the 10 mg/kg dose produced a significant antiallodynic effect, relative to that for vehicle controls [$F_{3,35} = 11.3$, $P < 0.05$]. As expected, the positive control morphine also produced a robust antiallodynic effect when tested 60 min after injection. Levels of antiallodynia observed with SR14150 were similar to that observed with morphine. Higher doses of SR14150 were not used because they can cause sedation/loss of muscle tone as published previously

Fig. 3. Effect of SR14150 administered alone (A) or coadministered with NOP receptor antagonist SB-612111 or opioid antagonist naloxone (B) on mechanical allodynia induced by SNL. Morphine is included as the positive control. Data are means $\pm$ S.E.M. *, significant difference from vehicle controls; †, significant difference from SR14150 alone ($P < 0.05$).

(Toll et al., 2009). In contrast to its antinociceptive activity in the tail-flick assay, the antiallodynic activity of SR14150 was reversed by the NOP antagonist SB-612111 [$F_{1,24} = 11.67$, $P < 0.05$], whereas pretreatment with naloxone did not produce any significant changes in SR14150-induced antiallodynic activity (Fig. 3B), indicating that the antiallodynic activity of SR14150 is a function of its NOP agonist activity.

Effects of SR16835 on Tail-Flick Latency and Mechanical Allodynia in SNL Mice. The effect of SR16835 on tail-flick latency in SNL mice is shown in Fig. 4. Similar to previous experiments in naive mice (Toll et al., 2009), SR16835 does not produce antinociception to a thermal stimulus in SNL mice, although, as expected, morphine (10 mg/kg) produced near-maximal antinociceptive effects [$F_{3,29} = 351$, $P < 0.05$].

The effect of SR16835 on tactile allodynia is shown in Fig. 5A. In contrast to its effect on tail-flick, SR16835 produced a dose-dependent increase in antiallodynia whereby the 30 mg/kg dose produced a significant increase relative to that for vehicle controls [$F_{3,65} = 21.9$, $P < 0.05$]. Levels of antiallodynia observed with SR16835 were significantly less than levels observed after morphine administration. As with

Fig. 4. Effect of SR16835 administered alone on tail-flick latency in mice that have undergone SNL. Morphine is included as the positive control. Data are means $\pm$ S.E.M. *, significant difference from vehicle controls ($P < 0.05$).

Fig. 5. Effect of SR16835 administered alone (A) or coadministered with NOP receptor antagonist SB-612111 or opioid antagonist naloxone (B) on mechanical allodynia induced by SNL. Morphine is included as the positive control. Data are means $\pm$ S.E.M. *, significant difference from vehicle controls; +, significant difference from morphine control; †, significant difference from SR16835 alone ($P < 0.05$).

SR14150, antiallodynic activity of SR16835 was inhibited by the NOP antagonist SB-612111 [$F_{4,92} = 17.4$, $P < 0.05$], but not by naloxone, indicating that the antiallodynic activity was due to NOP receptor activation (Fig. 5B).

Fig. 6. Effect of morphine administered alone or coadministered with NOP receptor antagonist SB-612111 or opioid antagonist naloxone on tail-flick latency in mice that have undergone SNL. Data are means $\pm$ S.E.M. *, significant difference from vehicle controls; +, significant difference from morphine alone ($P < 0.05$).

Effect of SB-612111 and Naloxone on Morphine-Induced Antinociception and Antiallodynia in SNL Mice.

We also examined tail-flick latency after 3 and 10 mg/kg morphine administration in these SNL mice (Fig. 6). Both the 3 and 10 mg/kg doses of morphine produced an increase in tail-flick latency compared with that for vehicle controls [$F_{4,33} = 20.5$, $P < 0.05$]. In contrast to what we found in naive mice (Khroyan et al., 2009a), SB-612111 significantly potentiated morphine-induced antinociception that was observed at 3 mg/kg morphine in SNL mice. As expected, pretreatment with naloxone completely reversed morphine-induced antinociception, and tail-flick latencies were similar to those for vehicle controls.

As shown in Fig. 7A, morphine produced a steep dose-response curve for its antiallodynic activity, such that the 1 and 3 mg/kg doses were ineffective, whereas the 10 mg/kg dose produced significant antiallodynia compared with that in vehicle controls [$F_{3,24} = 41.1$, $P < 0.05$]. SB-612111 (10 mg/kg), which had no effect on its own, significantly potentiated morphine-induced antiallodynia [$F_{3,50} = 47.1$, $P < 0.05$] for all three doses of morphine (Fig. 7B). As expected, pretreatment with naloxone reversed morphine-induced antiallodynia.

Discussion

The possible involvement of the NOP receptor in modulating pain has been examined extensively, particularly with respect to acute pain models. Initial experiments were conducted with the 17-amino acid peptide N/OFQ injected directly in the brain or spinal cord. The natural peptide ligand N/OFQ decreases hot-plate and tail-flick latency and attenuates morphine analgesia when injected intracerebroventricularly (Meunier et al., 1995; Reinscheid et al., 1995; Mogil et al., 1996), but it is antinociceptive when injected intrathecally (Tian et al., 1997; Inoue et al., 1998). Thus, the behavioral outcome of pain modulation by the NOP system is dependent on the site of action—periphery, spinal cord, or brain. This fact has to be taken into consideration when small molecules are administered systemically because the resulting effect on pain modulation is dependent on the pharmacokinetic distribution of the compound. The selective full

Fig. 7. Effect of morphine administered alone (A) or coadministered with NOP receptor antagonist SB-612111 or opioid antagonist naloxone (B) on mechanical allodynia induced by SNL. Data are means $\pm$ S.E.M. *, significant difference from vehicle controls; +, significant difference from morphine alone ($P < 0.05$).

agonist Ro 64-6198 has been reported to block morphine-induced increases in tail-flick latency and has no effect on its own in the tail-flick paradigm but is antinociceptive in the hot-plate test in rodents (Jenck et al., 2000; Reiss et al., 2008). Similar to agonists, selective small-molecule antagonists are also generally devoid of antinociceptive activity when administered systemically (Ozaki et al., 2000; Zaratin et al., 2004), indicating that there is no endogenous tone of N/OFQ to attenuate the antinociceptive activity of endogenous opioids.

Another way to achieve antinociceptive activity is to use a compound with both NOP and μ receptor agonist activity. We have proposed that, because NOP activation attenuates μ -mediated reward, such a bifunctional compound could have μ -mediated (naloxone-reversible) antinociceptive activity with reduced rewarding properties. In fact, SR14150, which is 20-fold selective for NOP but still has μ -agonist activity, appears to possess just such a profile (Toll et al., 2009).

In the condition of chronic neuropathic pain, there are changes to the opioid and the NOP system. Studies designed to examine the effect of chronic and inflammatory pain on the NOP system have demonstrated an up-regulation of NOP receptor and prepro-N/OFQ mRNA in the spinal cord and

dorsal root ganglia of neuropathic CCI rats (Briscini et al., 2002; Mika et al., 2004). A significant increase in brain N/OFQ immunoreactivity has also been demonstrated in SNL rats (Sun et al., 2001), as well as an increase in NOP receptors in superficial laminae of the rat spinal cord subsequent to CFA injection, as determined by in vitro autoradiography (Jia et al., 1998). Furthermore, increased levels of N/OFQ have been reported in the cerebrospinal fluid of patients with chronic pain (Raffaelli et al., 2006). These studies suggest that exogenously applied NOP agonists and/or antagonists may have activity in chronic pain animals.

In studies examining the effects of NOP agonists on chronic or inflammatory pain, in general, the results are similar to what is found in acute pain studies. In rats that are in chronic pain subsequent to CCI, an intrathecal injection of N/OFQ is antiallodynic (Tian et al., 1997). In the Freund's adjuvant-induced monoarthritic rat model, intracerebroventricular injections of N/OFQ can reverse morphine-induced antinociception (Bertorelli et al., 1999). In CCI rats, the peptide antagonist, [Nphe¹]N/OFQ(1–13)/NH₂, given alone intrathecally was not antiallodynic, and, as expected, it reversed N/OFQ-induced but not morphine-induced antiallodynia (Corradini et al., 2001). However, the NOP antagonist [Nphe¹Arg¹⁴Lys¹⁵]N/OFQ-NH₂ (UFP-101) delivered directly to the ventrolateral periaqueductal gray blocked CCI-induced allodynia (Scoto et al., 2009). This last study is consistent with an up-regulation of the NOP system in the brain mediating the allodynic response and once again demonstrates the dichotomy between brain and spinal cord. In a recent study, a new, selective small-molecule NOP receptor agonist, {1-[4-(2-{hexahydropyrrolo[3,4-c]pyrrol-2(1*H*)-yl]-1*H*-benzimidazol-1-yl)piperidin-1-yl]cyclooctyl}methanol (HPCOM), was reported to have antiallodynic activity in CCI rats, which was reversed by the NOP antagonist 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397) (Hayashi et al., 2010). Of interest, HPCOM does not efficiently cross the blood-brain barrier and apparently has antiallodynic activity through a peripheral site of action. These results are consistent with Obara et al. (2005), who showed that Ro 64-6198 had antiallodynic activity after intraplantar and intrathecal but not subcutaneous administration in CCI rats. In line with findings reported by Obara et al. (2005), we have shown a lack of antiallodynic activity of SR14150 after systemic administration in CCI rats (Khroyan et al., 2009b).

In contrast to the previous studies using CCI in rats, SNL in mice appears to produce strikingly different results with respect to the pain-attenuating actions of the small-molecule NOP agonists SR14150 and SR16835. SR14150 and SR16835 have somewhat different profiles both in vitro and in vivo. In vitro, SR14150 is more selective for NOP and has higher affinity at NOP and μ receptors compared with SR16835 (Supplemental Table S1). SR14150 is 20-fold selective for the NOP versus μ receptor (K_i = 1.5 nM at NOP; K_i = 30 nM at μ) and is a partial agonist at both receptors in the [³⁵S]guanosine 5'-O-(3-thiotriphosphate) assay (Toll et al., 2009). SR16835 is 7-fold selective for the NOP receptor (K_i = 11 nM at NOP; K_i = 80 nM at μ). This compound is a full agonist at NOP and a weak partial agonist at μ (less than 20% stimulation). In vivo we have shown that SR14150 has antinociceptive activity in the acute tail-flick assay, and this is reversed by naloxone, indicating that it is μ -opioid recep-

tor-mediated (Toll et al., 2009). SR16835 has no antinociceptive activity in the tail-flick assay in naive mice, similar to the findings with Ro 64-6198 (Toll et al., 2009). In SNL mice, the effects of SR14150 and SR16835 on tail-flick latency are exactly the same (Figs. 2 and 4).

The effects of SR14150 and SR16835 on mechanical allodynia in SNL mice are very different from their effect on thermal pain. In SNL mice, both these NOP agonists display potent antiallodynic activity, with SR14150 being more potent than SR16835. In contrast to thermal nociception, the antiallodynic activity of both agonists was completely blocked by the NOP antagonist SB-612111 but was not altered by naloxone, indicating that both compounds have NOP receptor-mediated and not μ receptor-mediated antiallodynic activity. Unlike the NOP receptor agonists, the antiallodynic activity of morphine was blocked by naloxone, indicating that the μ receptor can still mediate an antiallodynic response. Unlike its effect on acute pain, SB-612111 greatly potentiated the antiallodynic activity of morphine, consistent with chronic pain-mediated changes in the NOP system.

Our hypothesis is that the NOP system is up-regulated in both the brain and spinal cord after SNL surgery, leading to changes in the activity of NOP-active compounds. Presumably, in a naive animal not in chronic pain, SR14150, SR16835, and Ro 64-6198, when administered systemically, cannot sufficiently activate spinal NOP receptors to achieve an antinociceptive response. However, in a chronic pain situation when NOP receptors are up-regulated, especially in the spinal cord, these high-efficacy NOP receptor agonists have sufficient efficacy to attenuate SNL-induced mechanical allodynia.

On the other hand, because of chronic pain-induced up-regulation of the NOP receptor system in the brain, endogenous N/OFQ now attenuates morphine antiallodynia and presumably morphine thermal antinociception. Consequently, when NOP receptors are blocked by the NOP antagonist SB-612111, there is a potentiation of morphine antiallodynia and antinociception. This result is consistent with our previous report that systemic administration of the small-molecule NOP receptor antagonists SR16430 and SB-612111 can also potentiate morphine antiallodynia in rats that are in chronic pain induced by chronic constriction injury (Khroyan et al., 2009b). Such a potentiation of morphine-induced analgesia is not evident in an acute pain model (Khroyan et al., 2009a) also suggesting that NOP brain circuitry is altered in a chronic pain state.

The present results have significant implications for the potential utility of both NOP receptor agonists and antagonists for the treatment of chronic pain. Although opiates are widely prescribed for these conditions, clinical studies have shown that neuropathic pain is not very responsive to morphine (Przewlocki and Przewlocka, 2005) and thus larger doses are generally used. NOP receptor antagonists, which we have shown to potentiate morphine analgesia and antiallodynic activity in a chronic pain state (Khroyan et al., 2009b), may provide a favorable therapeutic combination for neuropathic pain treatment that would allow for a reduction in opioid dosage. On the other hand, NOP receptor agonists can block a painful stimulus, similarly to opiate agonists, presumably at the level of the spinal cord. Thus, after SNL surgery, a NOP receptor agonist, such as SR14150 or SR16835, could have the ability to act directly through the

up-regulated NOP receptor to provide a therapeutic antiallodynic effect in a chronic pain condition. NOP receptor-mediated antiallodynic activity may be more effective than an opiate medication, whose therapeutic efficacy may be compromised by an up-regulated NOP system present in the chronic pain state and whose use (i.e., of opiates) in chronic pain may be compromised by their central rewarding activity, a liability not present in NOP agonists. Thus, paradoxically, both NOP receptor agonists and antagonists could potentially become useful treatments for neuropathic pain.

Acknowledgments

We thank Rajesh Khanna for help with the graphics and Lucita Jimenez for technical assistance with the experiments.

Authorship Contributions

Participated in research design: Khroyan, Zaveri, and Toll.

Conducted experiments: Khroyan, Polgar, Orduna, and Montenegro.

Contributed new reagents or analytic tools: Jiang and Zaveri.

Performed data analysis: Khroyan.

Wrote or contributed to the writing of the manuscript: Khroyan, Zaveri, and Toll.

References

- Barlocco D, Cignarella G, Giuseppe G, Grugni M, and Ronzoni S (2006) inventors; GlaxoSmithKline S.p.A., assignee. Benzosuberonylpiperidine compounds as analgesics. U.S. patent 7,115,633. 2006 Oct 3.
- Bertorelli R, Bastia E, Citterio F, Corradini L, Forlani A, and Ongini E (2002) Lack of the nociceptin receptor does not affect acute or chronic nociception in mice. *Peptides* **23**:1589–1596.
- Bertorelli R, Corradini L, Rafiq K, Tupper J, Calò G, and Ongini E (1999) Nociceptin and the ORL-1 ligand [Phe^ψ (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* **128**:1252–1258.
- Briscini L, Corradini L, Ongini E, and Bertorelli R (2002) Up-regulation of ORL-1 receptors in spinal tissue of allodynic rats after sciatic nerve injury. *Eur J Pharmacol* **447**:59–65.
- Carrà G, Rizzi A, Guerrini R, Barnes TA, McDonald J, Hebbes CP, Mela F, Kenigs VA, Marzola G, Rizzi D, et al. (2005) [(pF)Phe⁴, Arg¹⁴, Lys¹⁵]N/OFQ-NH₂ (UFP-102), a highly potent and selective agonist of the nociceptin/orphanin FQ receptor. *J Pharmacol Exp Ther* **312**:1114–1123.
- Chaplan SR, Bach FW, Pogrel JW, Chung JM, and Yaksh TL (1994) Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* **53**:55–63.
- Corradini L, Briscini L, Ongini E, and Bertorelli R (2001) The putative OP₄ antagonist, [Nphe¹]nociceptin(1–13)NH₂, prevents the effects of nociceptin in neuropathic rats. *Brain Res* **905**:127–133.
- Dixon WJ (1991) Staircase bioassay: the up-and-down method. *Neurosci Biobehav Rev* **15**:47–50.
- Grisel JE, Mogil JS, Belknap JK, and Grandy DK (1996) Orphanin FQ acts as a supraspinal, but not a spinal, anti-opioid peptide. *Neuroreport* **7**:2125–2129.
- Hao JX, Xu IS, Wiesenfeld-Hallin Z, and Xu XJ (1998) Anti-hyperalgesic and anti-allodynic effects of intrathecal nociceptin/orphanin FQ in rats after spinal cord injury, peripheral nerve injury and inflammation. *Pain* **76**:385–393.
- Hayashi S, Nakata E, Morita A, Mizuno K, Yamamura K, Kato A, and Ohashi K (2010) Discovery of [1-[4-(2-(hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-1H-benzimidazol-1-yl)piperidin-1-yl]cyclooctyl]methanol, systemically potent novel non-peptide agonist of nociceptin/orphanin FQ receptor as analgesic for the treatment of neuropathic pain: design, synthesis, and structure-activity relationships. *Bioorg Med Chem* **18**:7675–7699.
- Inoue M, Kobayashi M, Kozaki S, Zimmer A, and Ueda H (1998) Nociceptin/orphanin FQ-induced nociceptive responses through substance P release from peripheral nerve endings in mice. *Proc Natl Acad Sci USA* **95**:10949–10953.
- Jenck F, Wichmann J, Dautzenberg FM, Moreau JL, Ouagazzal AM, Martin JR, Lundstrom K, Cesura AM, Poli SM, Roevers S, et al. (2000) A synthetic agonist at the orphanin FQ/nociceptin receptor ORL1: anxiolytic profile in the rat. *Proc Natl Acad Sci USA* **97**:4938–4943.
- Jia Y, Linden DR, Serie JR, and Seybold VS (1998) Nociceptin/orphanin FQ binding increases in superficial laminae of the rat spinal cord during persistent peripheral inflammation. *Neurosci Lett* **250**:21–24.
- Khroyan TV, Polgar WE, Jiang F, Zaveri NT, and Toll L (2009a) Nociceptin/orphanin FQ receptor activation attenuates antinociception induced by mixed NOP/ μ -opioid receptor agonists. *J Pharmacol Exp Ther* **331**:946–953.
- Khroyan TV, Polgar WE, Orduna J, Jiang F, Olsen C, Toll L, and Zaveri NT (2009b) Activity of new NOP receptor ligands in a rat peripheral mononeuropathy model: potentiation of morphine antiallodynic activity by NOP receptor antagonists. *Eur J Pharmacol* **610**:49–54.
- Khroyan TV, Polgar WE, Orduna J, Zaveri NT, Judd AK, Tuttle DJ, Sanchez A, and Toll L (2007a) Anti-nociceptive and antiallodynic effects of a high affinity NOP hexapeptide [Ac-RY(3-Cl)YRWR-NH₂] (Syn 1020) in rodents. *Eur J Pharmacol* **560**:29–35.
- Khroyan TV, Zaveri NT, Polgar WE, Orduna J, Olsen C, Jiang F, and Toll L (2007b) SR 16435 [1-(1-(bicyclo[3.3.1]nonan-9-yl)piperidin-4-yl)indolin-2-one], a novel mixed nociceptin/orphanin FQ/ μ -opioid receptor partial agonist: analgesic and rewarding properties in mice. *J Pharmacol Exp Ther* **320**:934–943.
- Kim SH and Chung JM (1992) An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* **50**:355–363.
- Ma F, Xie H, Dong ZQ, Wang YQ, and Wu GC (2005) Expression of ORL1 mRNA in some brain nuclei in neuropathic pain rats. *Brain Res* **1043**:214–217.
- Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour JL, Guillemot JC, Ferrara P, and Monsarrat B (1995) Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature* **377**:532–535.
- Mika J, Schäfer MK, Obara I, Weihe E, and Przewlocka B (2004) Morphine and endomorphin-1 differently influence pronociceptin/orphanin FQ system in neuropathic rats. *Pharmacol Biochem Behav* **78**:171–178.
- Mogil JS, Grisel JE, Zhangs G, Belknap JK, and Grandy DK (1996) Functional antagonism of μ -, δ - and κ -opioid antinociception by orphanin FQ. *Neurosci Lett* **214**:131–134.
- National Research Council (2003) *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*. National Academies Press, Washington, DC.
- Obara I, Przewlocki R, and Przewlocka B (2005) Spinal and local peripheral antiallodynic activity of Ro64-6198 in neuropathic pain in the rat. *Pain* **116**:17–25.
- Ozaki S, Kawamoto H, Itoh Y, Miyaji M, Azuma T, Ichikawa D, Nambu H, Iguchi T, Iwasawa Y, and Ohta H (2000) In vitro and in vivo pharmacological characterization of J-113397, a potent and selective non-peptidyl ORL1 receptor antagonist. *Eur J Pharmacol* **402**:45–53.
- Przewlocki R and Przewlocka B (2005) Opioids in neuropathic pain. *Curr Pharm Des* **11**:3013–3025.
- Raffaelli W, Samolsky Dekel BG, Landuzzi D, Caminiti A, Righetti D, Balestri M, Montanari F, Romualdi P, and Candeletti S (2006) Nociceptin levels in the cerebrospinal fluid of chronic pain patients with or without intrathecal administration of morphine. *J Pain Symptom Manage* **32**:372–377.
- Reinscheid RK, Notheracker HP, Boursan A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ Jr, and Civelli O (1995) Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. *Science* **270**:792–794.
- Reiss D, Wichmann J, Tekeshima H, Kieffer BL, and Ouagazzal AM (2008) Effects of nociceptin/orphanin FQ receptor (NOP) agonist, Ro64-6198, on reactivity to acute pain in mice: comparison to morphine. *Eur J Pharmacol* **579**:141–148.
- Rizzi A, Spagnolo B, Wainford RD, Fischetti C, Guerrini R, Marzola G, Baldisserotto A, Salvadori S, Regoli D, Kapusta DR, et al. (2007) In vitro and in vivo studies on UFP-112, a novel potent and long lasting agonist selective for the nociceptin/orphanin FQ receptor. *Peptides* **28**:1240–1251.
- Scoto GM, Ariò G, Iemolo A, Ronsisvalle S, and Parenti C (2009) Involvement of the nociceptin/orphanin FQ-NOP receptor system in the ventrolateral periaqueductal gray following mechanical allodynia in chronic pain. *Life Sci* **85**:206–210.
- Sun RQ, Wang Y, Zhao CS, Chang JK, and Han JS (2001) Changes in brain content of nociceptin/orphanin FQ and endomorphin 2 in a rat model of neuropathic pain. *Neurosci Lett* **311**:13–16.
- Tian JH and Han JS (2000) Functional studies using antibodies against orphanin FQ/nociceptin. *Peptides* **21**:1047–1050.
- Tian JH, Xu W, Fang Y, Mogil JS, Grisel JE, Grandy DK, and Han JS (1997) Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat. *Br J Pharmacol* **120**:676–680.
- Toll L, Khroyan TV, Polgar WE, Jiang F, Olsen C, and Zaveri NT (2009) Comparison of the antinociceptive and antirewarding profiles of novel bifunctional nociceptin receptor/ μ -opioid receptor ligands: implications for therapeutic applications. *J Pharmacol Exp Ther* **331**:954–964.
- Xu XJ, Hao JX, and Wiesenfeld-Hallin Z (1996) Nociceptin or antinociceptin: potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. *Neuroreport* **7**:2092–2094.
- Zaratin PF, Petrone G, Sbaccchi M, Garnier M, Fossati C, Petrillo P, Ronzoni S, Giardina GA, and Scheideleer MA (2004) 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* **308**:454–461.
- Zaveri NT, Jiang F, Olsen CM, Deschamps JR, Parrish D, Polgar W, and Toll L (2004) A novel series of piperidin-4-yl-1,3-dihydroindol-2-ones as agonist and antagonist ligands at the nociceptin receptor. *J Med Chem* **47**:2973–2976.

Address correspondence to: Dr. Lawrence Toll, Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, FL 34987. E-mail: ltoll@tpims.org