Differential Effects of NOP Receptor Agonists in Acute versus Chronic Pain: Studies with Bifunctional NOP/mu Receptor Agonists in the Sciatic Nerve Ligation Chronic Pain Model in Mice

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

SRI International, Menlo Park, CA, USA (TVK, WEP, JO, JM, FJ, and LT); Astraea Therapeutics LLC, Mountain View, CA, USA (NTZ)
Corresponding Author current address:
Lawrence Toll
Torrey Pines Institute for Molecular Studies
11350 SW Village Parkway,
Port St. Lucie, FL 34987
Tel: 1-772-345-4714
E-mail: ltoll@tpims.org

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d) Abbreviations: NOP, Nociceptin/Orphanin FQ receptor; ORL1, opioid receptor-like receptor; N/OFQ, Nociceptin/Orphanin FQ; mu-opioid receptor; s.c., subcutaneous; i.t., intrathecal; i.c.v., intracerebroventricular; i.pl., intraplantar; ANOVA, analysis of variance; GTPγS, guanosine 5’-O-(3-thiotriphosphate); SR14150, 1-(1-cyclooctylpiperidin-4-yl)-indolin-2-one; SR16835, 1-(1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)piperidinl-4-yl)-indolin-2-one; SB-612111, (-)-cis-1-methyl-7-[(4-(2,6-dichlorophenyl)piperidin-1-yl)methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol.

e) Recommended section: Behavioral Pharmacology
Abstract

SR14150 and SR16835 are moderately selective NOP receptor agonists. In the [35S]GTPγS assay in vitro, SR14150 is a partial agonist at both the NOP and mu opioid receptors, while SR16835 is a full agonist at NOP and has low efficacy at mu receptors. These compounds were tested for antinociceptive and anti-allodynic activity, using mice in chronic pain, subsequent to spinal nerve ligation (SNL) surgery. When administered subcutaneously to mice following 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 SB612111. In contrast, both SR14150 and SR16835 had anti-allodynic activity when measuring mechanical allodynia with von Frey monofilaments. This effect was completely blocked by SB612111 but not by naloxone. On the other hand, morphine antinociception and anti-allodynia were both blocked by naloxone and potentiated by SB612111. 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 upregulated NOP system in the spinal cord leads to NOP receptor-mediated anti-allodynia, which is blocked by NOP antagonists. Supraspinal upregulation, however, could lead to an attenuation of morphine antinociception and anti-allodynia, which can be alleviated by an NOP receptor antagonist. Thus, while 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.
1. 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 i.c.v. injection of this peptide showed pro-nociceptive effects in mice, decreasing hot plate and tail flick latency, indicating that its effects were opposite to that 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 i.c.v. injection-mediated stress-induced analgesia (Grisel et al., 1996). In addition to blocking stress-induced analgesia, i.c.v. N/OFQ also blocks analgesia mediated by mu-, delta-, and kappa-opioid ligands (Mogil et al., 1996). Intrathecal (i.t.) administration of N/OFQ, on the other hand, produces a distinctly different result. Rather than attenuating morphine analgesia, i.t. 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 anti-opioid activity, while activation in the spinal cord has antinociceptive effects, similar to opioid receptor activation (Carra 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 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), though it was effective in mouse hot plate (Reiss et al., 2008). Bifunctional compounds with both NOP and mu opioid receptor agonist activity, such as SR16435 and SR14150, and even the opioid partial agonist buprenorphine, have significant mu-mediated antinociceptive activity in the tail flick assay, that is blocked by naloxone rather than an NOP receptor antagonist.
In fact, the activity of these compounds is potentiated by the NOP receptor antagonist SB612111, suggesting that the NOP receptor agonist activity of these compounds is attenuating its mu agonist activity (Khroyan et al., 2007a; Khroyan et al., 2009a; Toll et al., 2009).

The effect of NOP receptor agonists have also been studied in chronic pain models. N/OFQ, given i.c.v., has no anti-allodynic activity in the complete Freund’s adjuvant (CFA) model of arthritic pain, but can block the anti-allodynic activity of morphine (Bertorelli et al., 1999). When given i.t., N/OFQ is anti-allodynic in rats that are in chronic pain and it does not alter morphine-induced anti-allodynia (Hao et al., 1998; Corradini et al., 2001). Ro 64-6198 also has anti-allodynic activity after i.t. and intraplantar (i.p.) injection, but not s.c. 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, DRG, and spinal cord of CCI rats (Briscini 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 upregulated in the NRM, amygdala, and PAG after SNL and CCI surgery (Sun et al., 2001; Ma et al., 2005). How this upregulation 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.
Here we describe experiments that were carried out in mice that underwent spinal nerve ligation (SNL) to determine how chronic pain affects the ability of NOP/mu agonists and antagonists to modulate the response to a painful stimulus. In this model, chronic pain appears to upregulate the NOP system, resulting in NOP receptor agonist-mediated anti-allodynia, potentially at the level of the spinal cord, and potentiation of morphine induced anti-nociception and anti-allodynia by a NOP antagonist, presumably mediated supra-spinally.
2. Materials and Methods

2.1 Subjects.

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

2.2 Drugs.

SR14150 [1-(1-cyclooctylpiperidin-4-yl)-indolin-2-one], and SR16835 [1-(1-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)piperidinl-4-yl)-indolin-2-one] were synthesized in Dr. Zaveri’s laboratory, as hydrochloride salts (Zaveri et al., 2004). The NOP receptor antagonist SB-612111 was also synthesized in Dr. Zaveri’s laboratory using previously reported methodology (Barlocco et al., 2006; Zaratin et al., 2004). Morphine hydrochloride (Eli Lilly & 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% dimethylsulfoxide and 0.5% aqueous hydroxypropylcellulose. Drugs were injected in a volume of 0.1 ml/25g s.c. Controls received 0.1 ml/25 g of the appropriate vehicle.

2.3 Spinal Nerve Ligation (SNL) Surgery.

All surgical procedures used were performed under deep Isoflurane anesthesia (5% for induction and 2% for maintenance) in 10% O₂. The SNL was performed as described previously (Kim and Chung, 1992). Briefly, 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 1-2 weeks to recover and for the allodynia to develop.

2.4 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 post-injection. Thermal nociception was assessed using the tail flick assay with an analgesia instrument (Stoelting) that uses radiant heat. During testing, the focused beam of light was applied to the lower half of the animal's tail, and tail-flick latency was recorded. If the animal did not respond prior to 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 post-surgery was 4.17 s ± 0.13 SEM.

2.5 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 0.005g, 0.02g, 0.03g, 0.07g, 0.17g, 0.41g, 0.7g, 1.2g, and 1.5g. 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 about 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 following the initial positive response. The 50% paw withdrawal threshold was calculated using the formula: 

\[(10^{Xf / \kappa}) / 10,000\] where \(Xf\) is the final von Frey filament used (log units), \(\kappa\) is a value that
analyzes the response pattern (taken from a published table (Dixon, 1991; Chaplan et al., 1994) and $\delta$ 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 prior to testing with the drugs in each animal. The mean baseline prior to surgery was 1.1 g ± 0.09 SEM. The post surgery baseline, once allodynia was induced, was 0.05 g ± 0.008 SEM.

2.6 Experimental Design.

Animals (N=8-14/group) received s.c. 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 of the opioid antagonist naloxone or a pretreatment of 10 mg/kg of the 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 based on previous experiments in our laboratory (Khroyan et al., 2007b; Toll et al., 2009). The antagonists were given 10 minutes prior to 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.

2.7 Statistical Analyses.
Thermal nociception data (test latency, sec) and mechanical allodynia (force, g) were analyzed using an ANOVA 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.
3. Results

3.1 Effects of SR14150 on tail flick latency and mechanical allodynia in SNL mice.

Previously we have shown that SR14150 produces antinociception in the tail flick assay in animals that are not in chronic pain (Toll et al., 2009). Similarly, 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 figure 2A. The overall ANOVA 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 produced a significant increase in antinociception relative to vehicle controls. The antinociception produced by 3 mg/kg SR14150 was greater than vehicle but less than 10 mg/kg morphine control, whereas the 10 mg/kg SR14150 produced similar levels of antinociception as 10 mg/kg morphine. To determine the receptor mediating the SR14150-induced antinociception, animals received a pretreatment of SB612111 or naloxone. Naloxone reversed SR14150-induced antinociception [F(1, 24)= 11.6, P<0.05], whereas pretreatment with SB612111 did not produce any significant changes (n.s.; Figure 2B), indicating that the SR14150 induced antinociceptive activity is mediated by opioid receptors, and not the NOP receptor.

The effect of SR14150 on tactile allodynia is shown in Figure 3A, SR14150 produced an increase in anti-allodynia whereby the 10 mg/kg dose produced a significant anti-allodynic effect, relative to vehicle controls [F(3, 35)= 11.3, P<0.05]. As expected, the positive control morphine, also produced a robust anti-allodynic effect when tested 60 min post-injection. Levels of anti-allodynia observed with SR14150 were similar to that observed with morphine. Higher doses of SR14150 were not used since they can cause sedation/loss of muscle tone as previously published (Toll et al., 2009). In contrast to its antinociceptive activity in the tail flick, the anti-allodynic activity of
SR14150 was reversed by the NOP antagonist SB612111 \([F(1, 24)= 11.67, P<0.05]\), whereas pretreatment with naloxone did not produce any significant changes in SR14150-induced anti-allodynic activity (n.s.; Figure 3B), indicating that the anti-allodynic activity of SR14150 is a function of its NOP agonist activity.

3.2 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 Figure 4. Similarly to previous experiments in naïve mice (Toll et al., 2009), SR16835 does not produce antinociception to a thermal stimulus in SNL mice, though 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 Figure 5A. In contrast to its effect on tail flick, SR16835 produced a dose-dependent increase in anti-allodynia whereby the 30 mg/kg dose produced a significant increase relative to vehicle controls \([F(3, 65)= 21.9, P<0.05]\). Levels of anti-allodynia observed with SR16835 were significantly less than levels observed following morphine administration. As with SR14150, anti-allodynic activity of SR16835 was inhibited by the NOP antagonist SB612111 \([F(4, 92)= 17.4, P<0.05]\), but not by naloxone (n.s.), indicating the anti-allodynic activity was due to NOP receptor activation (Figure 5B).

3.3 Effect of SB612111 and Naloxone on morphine-induced antinociception and anti-allodynia in SNL mice.

We also examined tail flick latency following 3 and 10 mg/kg morphine administration in these SNL mice (Figure 6). Both the 3 and 10 mg/kg doses of morphine produced an increase in tail flick latency compared to vehicle controls \([F(4,33)= 20.5, P<0.5]\). In contrast to what we found in naïve mice (Khroyan et al., 2009a), SB612111 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 vehicle controls.

As shown in figure 7A, morphine produced a steep dose response curve for its anti-allodynic activity, such that the 1 and 3 mg/kg doses were ineffective, whereas the 10 mg/kg dose produced significant anti-allodynia compared to vehicle controls [F(3, 24) = 41.1, P<0.05]. SB612111 (10 mg/kg), which had no effect on its own, significantly potentiated morphine-induced anti-allodynia [F(3,50)= 47.1, P<0.05] at all three doses of morphine (Figure 7B). As expected, pretreatment with naloxone reversed morphine-induced anti-allodynia.
4. 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 i.c.v. (Meunier et al., 1995; Reinscheid et al., 1995; Mogil et al., 1996), but it is antinociceptive when injected i.t. (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 has to be taken into consideration when administering small molecules systemically since the resulting effect on pain modulation is dependent on the pharmacokinetic distribution of the compound. The selective full agonist Ro 64-6198 has been reported to block morphine-induced increases in tail flick latency, 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 would be to use a compound with both NOP and mu receptor agonist activity. We have proposed that, since NOP activation attenuates mu-mediated reward, such a bifunctional compound could have mu-mediated (naloxone-reversible) antinociceptive activity with reduced rewarding properties. In fact, SR14150, which is 20-fold selective for NOP but still has mu 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 upregulation of NOP receptor and preproN/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 spinal nerve-ligated 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 chronic pain patients (Raffaeli et al., 2006). These studies suggest that exogenously applied NOP agonists and/or antagonists may have activity in chronic pain animals.

In the 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 i.t. injection of N/OFQ is anti-allodynic (Tian et al., 1997). In the Freund’s adjuvant-induced monoarthritic rat model, i.c.v injections of N/OFQ can reverse morphine-induced antinociception (Bertorelli et al., 1999). In CCI rats, the peptide antagonist, [Nphe(1)]N/OFQ(1-13)NH2, given alone i.t. was not antiallodynic, and, as expected, it reversed N/OFQ-induced but not morphine-induced anti-allodynia (Corradini et al., 2001). However, the NOP antagonist UFP-101 delivered directly to the vIPAG blocked CCI-induced allodynia (Scoto et al., 2009). This last study is consistent with an upregulation of the NOP system in the brain mediating the allodynic response and once again demonstrates the dichotomy between brain and spinal cord.

Recently, a new, selective small molecule NOP receptor agonist, HPCOM, was reported to have anti-allodynic activity in CCI rats, which was reversed by the NOP antagonist J-113397 (Hayashi et al., 2010). Interestingly, HPCOM does not efficiently cross the blood brain barrier, and apparently has anti-allodynic activity through a peripheral site of action. These results are consistent with Obara et al., who showed that Ro 64-6198 had
anti-allodynic activity after i.pl. and i.t., but not s.c. administration in CCI rats (Obara et al., 2005). In line with findings reported by Obara et al. above (2005), we have shown a lack of anti-allodynic 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 mu receptors compared to SR16835 (Supplementary Table S1). SR14150 is 20-fold selective for NOP versus mu receptor (Ki 1.5 nM at NOP, Ki 30 nM at mu) and is a partial agonist at both receptors in the \[^{35}\text{S}]\text{GTP}\gamma\text{S} \text{assay (Toll et al., 2009). SR16835 is 7-fold selective for the NOP receptor (Ki 11 nM at NOP, Ki 80 nM at mu). This compound is a full agonist at NOP and a weak partial agonist at mu (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 mu opioid receptor-mediated (Toll et al., 2009). SR16835 has no antinociceptive activity in tail flick in naïve 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 (Figures 2 and 4).

The effects of SR14150 and SR16835 on mechanical allodynia in SNL mice are very different than their effect on thermal pain. In SNL mice both these NOP agonists display potent anti-allodynic activity, with SR14150 being more potent than SR16835. In contrast to thermal nociception, the anti-allodynic activity of both agonists was completely blocked by the NOP antagonist SB612111, but not altered by naloxone, indicating that both compounds have NOP receptor-mediated, and not mu receptor-mediated anti-allodynic activity. Unlike the NOP receptor agonists, morphine’s anti-
alldynic activity was blocked by naloxone, indicating that the mu receptor can still mediate an anti-alldynic response. Unlike its effect on acute pain, SB612111 greatly potentiated morphine’s anti-alldynic activity, consistent with chronic pain-mediated changes in the NOP system.

Our hypothesis is that the NOP system is upregulated in both the brain and spinal cord after SNL surgery leading to changes in the activity of NOP-active compounds. Presumably, in a naïve 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 upregulated especially in the spinal cord, these high efficacy NOP receptor agonists have sufficient efficacy to attenuate SNL-induced mechanical alldynia.

On the other hand, due to a chronic pain-induced upregulation of the NOP receptor system in the brain, endogenous N/OFQ now attenuates morphine anti-alldynia, and presumably morphine thermal antinociception. Consequently, when NOP receptors are blocked by the NOP antagonist SB612111, there is a potentiation of morphine anti-alldynia and antinociception. This is consistent with our previous report that systemic administration of the small molecule NOP receptor antagonists SR16430 and SB612111 can also potentiate morphine anti-alldynia 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 potentiate morphine analgesia and anti-allodynic 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 upregulated NOP receptor to provide a therapeutic anti-allodynic effect in a chronic pain condition. NOP receptor-mediated anti-allodynic activity may be more effective than an opiate medication, whose therapeutic efficacy may be compromised by an upregulated 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.
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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Toll, Khroyan, Zaveri

Conducted Experiments: Khroyan, Polgar, Orduna, Montenegro,

Contributed new reagents or analytic tools: Jiang, Zaveri

Performed data analysis: Khroyan

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


Khroyan TV, Polgar WE, Jiang F, Zaveri NT and Toll L (2009a) NOP Receptor Activation Attenuates Antinociception Induced by Mixed NOP/Mu-Opioid Receptor Agonists. J Pharmacol Exp Ther.


Footnotes

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c) Reprint requests should be addressed to: Lawrence Toll, Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, FL 34987. E-mail: ltoll@tpims.org, Tel: 1-772-345-4714

d) Inquiries about compounds should be addressed to Nurulain Zaveri, Astraea Therapeutics, 320 Logue Avenue, Suite 142, Mountain View, CA 94043. E-mail nurulain@astraeatherapeutics.com, Tel: 650-254-0786;
Legends for Figures

**Figure 1.** Chemical structures of SR14150 and SR16835.

**Figure 2.** Effect of SR14150 administered alone (A) or co-administered with NOP receptor antagonist SB612111 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 (± S.E.M.) *, significant difference from vehicle controls; †, significant difference from morphine control; †, significant difference from SR14150 alone (P<0.05).

**Figure 3.** Effect of SR14150 administered alone (A) or co-administered with NOP receptor antagonist SB612111 or opioid antagonist naloxone (B) on mechanical allodynia induced by SNL. Morphine is included as the positive control. Data are means (± S.E.M.) *, significant difference from vehicle controls; †, significant difference from SR14150 alone (P<0.05).

**Figure 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 (± S.E.M.) *, significant difference from vehicle controls (P<0.05).

**Figure 5.** Effect of SR16835 administered alone (A) or co-administered with NOP receptor antagonist SB612111 or opioid antagonist naloxone (B) on mechanical allodynia induced by SNL. Morphine is included as the positive control. Data are means (± S.E.M.) *, significant difference from vehicle controls; +, significant difference from morphine control; †, significant difference from SR16835 alone (P<0.05).

**Figure 6.** Effect of morphine administered alone or co-administered with NOP receptor antagonist SB612111 or opioid antagonist naloxone on tail flick latency in mice that have undergone SNL. Data are means (± S.E.M.) *, significant difference from vehicle controls; +, significant difference from morphine alone (P<0.05).

**Figure 7.** Effect of morphine administered alone (A) or co-administered with NOP receptor antagonist SB612111 or opioid antagonist naloxone (B) on mechanical
allodynia induced by SNL. Data are means (± S.E.M.) *, significant difference from vehicle controls; +, significant difference from morphine alone (P<0.05).
Figure 2

A

![Bar graph showing latency in seconds for different treatment conditions: Vehicle, Morphine (10 mg/kg), 3 mg/kg Dose of SR14150 (s.c.), and 10 mg/kg Dose of SR14150 (s.c.).](image)

- Baseline
- 30-min Post-Injection

B

![Bar graph showing latency in seconds for different doses of SR14150 with different treatments: alone, + 10 mg/kg SB612111, + 1 mg/kg Naloxone.](image)

- 3 mg/kg
- 10 mg/kg

Dose of SR14150 (s.c.)
Figure 4

![Graph showing latency (sec) for different treatments.](image-url)
Figure 6

Latency (s)

Vehicle 3 mg/kg 10 mg/kg
Dose of morphine (s.c.)

- Alone
- + 10 mg/kg SB612111
- + 1 mg/kg Naloxone

* Significant difference
+ Trend towards significant difference
Figure 7

A

![Graph showing force (g) vs. dose of morphine (s.c.)](image)

- Baseline
- 60-min Post-Injection

B

![Graph showing force (g) vs. dose of morphine (s.c.)](image)

- Alone
- +10 mg/kg SB612111
- +1 mg/kg Naloxone

Dose of morphine (s.c.)