Nociceptin/Orphanin FQ Receptor Activation Attenuates Antinociception Induced by Mixed Nociceptin/Orphanin FQ/μ-Opioid Receptor Agonists

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ABSTRACT

Activation of brain nociceptin/orphanin FQ (NOP) receptors leads to attenuation of μ-opioid receptor (MOP receptor)-mediated antinociception. Buprenorphine, a high-affinity partial MOP receptor agonist also binds to NOP receptors with 80 nM affinity. The buprenorphine-induced inverted U-shaped dose-response curve for antinociception may be due to NOP receptor activation, given that, in the presence of the NOP receptor antagonist, 1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one (J113397), or in NOP receptor knockout mice, buprenorphine has a steeper dose-response curve and acts as a full agonist. To further explore the involvement of the direct activation of NOP receptors by buprenorphine and other compounds that activate both NOP and MOP receptors, the antinociceptive effects of 1-[(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)piperidin-4-yl]-indolin-2-one (SR16507), 3-ethyl-1-[(4-isopropylcyclohexyl)piperidin-4-yl]-indolin-2-one (SR16435), buprenorphine, pentazocine, and morphine, compounds with varying levels of MOP and NOP receptor affinity and efficacy, were assessed in mice using the tail-flick assay. The ability of the selective NOP receptor antagonist (-)-(2R,3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one (SB-612111) to potentiate antinociception induced by the above compounds was examined to investigate whether activation of NOP receptors leads to attenuation of MOP receptor-mediated antinociception. SB-612111 potentiated antinociception induced by buprenorphine and the other mixed NOP/MOP receptor agonists SR16435 and SR16507. However, SB-612111 had no effect on pentazocine or morphine antinociception, two compounds with no NOP receptor-binding affinity. These results further support the hypothesis that activation of NOP receptors can lead to attenuation of MOP receptor-mediated antinociception elicited by mixed NOP/MOP receptor compounds such as buprenorphine, SR16435, and SR16507 and that, although buprenorphine has low efficacy in vitro, it has significant NOP receptor agonist activity in vivo.

Buprenorphine is a potent analgesic that is widely used both in the clinic and in the laboratory because of its high therapeutic index. In analgesia experiments in rodents, buprenorphine has been demonstrated to have a very flat, or even an inverted U-shaped dose-response curve, under appropriate experimental conditions (Cowan et al., 1977; Lutfy et al., 2003). Buprenorphine is also approved as a medication for heroin abuse. Buprenorphine’s approval as an addiction medication was based on many years of preclinical experiments and clinical trials demonstrating that a daily dose of buprenorphine attenuates heroin abuse while maintaining the patient in a condition suitable for daily life (Jasinski et al., 1978). Because of its safety, buprenorphine has replaced

ABBREVIATIONS: MOP receptor, μ-opioid receptor; CHO cells, Chinese hamster ovary cells; N/OFQ, nociceptin/orphanin FQ; MPE, maximum possible effect, NOP receptor, nociceptin/orphanin FQ receptor; J113397, 1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one; SB-612111, (-)-(2R,3S,4R,5R,6S)-5-amino-6-[(1R,2S,3S,4R,6S)-4,6-diamino-3-[(2R,3R,4R,5R)-3,5-dihydroxy-5-methyl-4-methylinnoxa-2-yl]hydroxy-2-hydroxy-2-cyclohexyl][oxy-2-[1-hydroxyethyl]oxo](3,4-diol; Ro 64-6198, (1S,3aS)-8-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4,5]decane-4-one; UFP-101, [Nphe1,Arg14,Lys15]nociceptin-NH2; DAMGO, (2S)-2-[[2R]-2-[[2S]-2-amino-3-(4-hydroxyphenyl)propionyl][amino][propanoyl][amino][acetyl][methyl]laminino]-N-[(2-hydroxyethyl)-3-phenylpropanamide; GTP-γS, guanosine 5’-3-O-thiotriphosphate; ANOVA, analysis of variance; CPP, conditioned place preference; SR16835, 1-[(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)piperidin-4-yl]-indolin-2-one.
methadone maintenance in a large number of methadone-maintained addicts. Although it is not approved for the treatment of cocaine or alcohol abuse, buprenorphine has been shown to decrease the self-administration of both of those drugs as well in rodents, non-human primates, and people (Martin et al., 1983; Mello et al., 1989; June et al., 1998). However, the exact mechanism of this action is not known.

Buprenorphine is a long-lasting, high-affinity opiate with partial agonist activity at MOP receptors (Lewis, 1985). It also has high affinity to the other opioid receptors, and is a partial agonist at δ-opioid receptors and an antagonist at κ-opioid receptors (Spagnolo et al., 2008). More recently, it was reported that buprenorphine also binds to the NOP receptors (Wnendt et al., 1999; Huang et al., 2001; Spagnolo et al., 2008). The affinity of buprenorphine for NOP receptors (Kᵢ = 80–100 nM) is not as high as its affinity for the other opioid receptors, at which the Kᵢ is in the low nanomolar range (Toll et al., 1998). It is thought that the activity of buprenorphine at the NOP receptor contributes to its shallow dose-response curve for antinociception.

NOP receptors, like the opioid receptors, are involved in a large number of central nervous system and peripheral actions. However, nociceptin/orphanin FQ (N/OFQ), the endogenous ligand for NOP receptors, in general does not have actions similar to the opioid peptides. In fact, when delivered intracerebroventricularly, N/OFQ is not analgesic, it blocks opioid analgesic activity (Meunier et al., 1995; Reinscheid et al., 1995), and it is not rewarding (Devine et al., 1996). Furthermore, N/OFQ blocks the reward induced by opiates and many other drugs of abuse (Murphy et al., 1999; Kotlińska et al., 2003; Ciccocoppo et al., 2004; Sakoori and Murphy, 2004).

Despite its moderate to low affinity at NOP receptors, there are reports of agonist or partial agonist activity of buprenorphine at NOP receptors, with EC₅₀ values ranging from 8 to 100 nM, in a variety of in vitro activity assays (Wnendt et al., 1999; Hashimoto et al., 2000; Huang et al., 2001; Lutfy et al., 2003). However, other reports have shown a lack of any intrinsic activity for buprenorphine at NOP receptor (Lester and Traynor, 2006; Spagnolo et al., 2008). Based on the agonist activity of buprenorphine at NOP receptors, Lutfy et al. (2003) hypothesized that the NOP receptor agonist activity of buprenorphine attenuated its own antinociceptive activity, and this was the reason for the partial agonist activity of buprenorphine and the inverted U-shaped dose-response curve in the tail-flick test. Experiments using NOP receptor knockout mice, and a selective NOP receptor antagonist, J113397, demonstrated full agonist activity for buprenorphine, in the tail-flick test, under conditions when NOP receptor agonist activity would be absent, supporting their hypothesis (Lutfy et al., 2003).

Certainly, the most straightforward explanation for the high-dose activity of buprenorphine is that it is activating NOP receptors to attenuate its MOP receptor-mediated antinociceptive activity. However, there are reasons to question this hypothesis. First, the affinity of buprenorphine for NOP receptors is far less than its affinity for any of the opioid receptors. Nevertheless, in the tail-flick experiments, its activity was potentiated by the NOP receptor antagonist J113397, even at the lowest doses of buprenorphine (Lutfy et al., 2003). Second, it is a weak partial agonist, at best, at NOP receptor in most in vitro assays. It is conceivable that other properties could explain the behavior discussed above.

To further test the hypothesis that buprenorphine acts at NOP receptors to attenuate its own antinociceptive activity, we conducted experiments by use of agonists with varying affinities and efficacies at NOP and MOP receptors, and determined whether their antinociceptive activity could be potentiated by the selective high-affinity NOP receptor antagonist SB-612111 (Zaratin et al., 2004).

**Materials and Methods**

**Cell Culture**

All receptors were in CHO cells transfected with human receptor cDNA. The cells were grown in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, in the presence of 0.4 mg/ml G418 and 0.1% penicillin/streptomycin, in 100-mm plastic culture dishes. For binding assays, the cells were scraped off the plate at confluence.

**Receptor Binding**

Binding to cell membranes was conducted in a 96-well format, as described previously (Dooley et al., 1997). Cells were removed from the plates by scraping with a rubber policeman, homogenized in 50 mM, Tris pH 7.5, with use of a Polytron homogenizer (Kinematica, Littau-Lucerne, Switzerland), then centrifuged once, and washed by an additional centrifugation at 27,000g for 15 min. The pellet was resuspended in Tris, and the suspension incubated with [³H]DAMGO (51 Ci/mmol, 1.6 nM) or [³H]N/OFQ (120 Ci/mmol, 0.2 nM) for binding to MOP receptor and NOP receptor, respectively. Nonspecific binding was determined with 1 µM unlabeled DAMGO and N/OFQ, respectively. Total volume of incubation was 1.0 ml, and samples were incubated for 60 min at 25°C. The amount of protein in the binding assay was 15 µg. The reaction was terminated by filtration by use of a Tomtec 96 harvester (Tomtec, Orange, CT) through glass fiber filters. Bound radioactivity was counted on a Pharmacia Biotech β-plate liquid scintillation counter (PerkinElmer Life and Analytical Sciences, Waltham, MA) and expressed in counts per minute. IC₅₀ values were determined by use of at least six concentrations of each compound and calculated with Prism (GraphPad Software, Inc., La Jolla, CA).

**[³¹S]GTPγS Binding**

[³¹S]GTPγS binding was conducted basically as described previously (Toll et al., 1998). Cells were scraped from tissue culture dishes into 20 mM HEPES, 1 mM EDTA, then centrifuged at 500g for 10 min. Cells were resuspended in this buffer and homogenized by use of a Polytron homogenizer (Kinematica). The homogenate was centrifuged at 27,000g for 15 min, and the pellet was resuspended in buffer A, containing: 20 mM HEPES, 10 mM MgCl₂, 100 mM NaCl, pH 7.4. The suspension was re-centrifuged at 27,000g and suspended once more in buffer A. For the binding assay, membranes (8–15 µg of protein) were incubated with [³¹S]GTPγS (50 µM), GDP (10 µM), and the appropriate compound, in a total volume of 1.0 ml, for 60 min at 25°C. Samples were filtered over glass fiber filters and counted as described for the binding assays. Percentage of stimulation was determined, in each experiment, with respect to the full agonist N/OFQ or DAMGO. Statistical analysis was conducted by use of the program Prism.

**Assessing Acute Thermal Nociception**

**Animals.** Male ICR mice weighing 25 to 30g 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 3 to 4 days before the experiments were conducted. On the test day, animals were transported to the testing room and acclimated to the environment for 1 h. Mice were maintained in accordance with the guidelines of

**Tail-Flick Assay.** Acute nociception was assessed by use of the tail-flick assay with an analgesia instrument (Stoelting, Wood Dale, IL) that uses radiant heat. This instrument is equipped with an automatic quantification of tail-flick latency, and a 15-s cutoff to prevent damage to the animal’s tail. During testing, the focused beam of light was applied to the lower half of the animal’s tail, and tail-flick latency was recorded. Baseline values for tail-flick latency were determined before drug administration in each animal. The mean basal tail-flick latency was 5.05 ± 0.3 S.E.M.

After baseline measures, animals received a subcutaneous injection of their assigned dose of drug and were tested for tail-flick latencies at 10-, 30-, and 60-min after injection. Controls received an injection of vehicle before testing.

**Drug Regimen.** Animals (n = 8–14/group) received subcutaneous injections of buprenorphine (0.3–3.0 mg/kg), morphine (1.0–10.0 mg/kg), pentazocine (0.3–3.0 mg/kg), SR16435 (3.0–30.0 mg/kg), SR16507 (0.3–1.0 mg/kg), or SB-612111 (3 or 10 mg/kg). After assessment of baseline values for tail-flick latency, animals received an injection of SB-612111 (3 or 10 mg/kg) or vehicle, and 10 min later they received an injection of buprenorphine, morphine, pentazocine, SR16435, or SR16507. Testing occurred at 10, 30, and 60 min after the final drug injection. A group of animals also served as vehicle controls.

**Statistical Analyses.** Antinociception (percentage of maximum possible effect, %MPE) was quantified by the following formula: %MPE = 100 × [(test latency – baseline latency)/(15 – baseline latency)]. If the animal did not respond before the 15-s cutoff, the animal was assigned a score of 100%. Behavioral results were analyzed by use of repeated-measures ANOVAs with drug treatment as between-group variables and post–drug-injection time (10, 30, and 60 min) as the repeated measure followed by Student Newman-Keuls post hoc tests where appropriate. The level of significance was set at P < 0.05.

**Results**

**In Vitro Findings.** Binding affinity and in vitro functional activity were determined for the prototypical MOP receptor agonist morphine (which does not bind to NOP receptors), the nonsel ective NOP receptor partial agonists buprenorphine and pentazocine, and the mixed NOP/MOP receptor compounds SR16435 and SR16507. As seen in Table 1, each of the compounds tested has high affinity for the MOP receptor, whereas only SR16435 and SR16507 have high affinity for the NOP receptor. Buprenorphine has moderate affinity for the NOP receptor (80 nM), whereas neither morphine nor pentazocine has any affinity for NOP receptors, even when tested up to 10 μM.

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<th>NOPr</th>
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<td><strong>Receptor Binding Ki</strong></td>
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<td>SR 16435</td>
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<td>7.5</td>
<td>2.7 ± 0.5</td>
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<td>SR16507</td>
<td>5.22 ± 0.65</td>
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<td>8.5 ± 0.8</td>
<td>95 ± 12</td>
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<td>Buprenorphine</td>
<td>77.4 ± 16</td>
<td>1.5 ± 0.8</td>
<td>251.2 ± 94.0</td>
<td>15.5 ± 5.8</td>
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<td>Morphine</td>
<td>&gt;10,000</td>
<td>1.1 ± 0.1</td>
<td>15.6 ± 0.5</td>
<td>90 ± 2.6</td>
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<tr>
<td>Pentazocine</td>
<td>&gt;10,000</td>
<td>3.9 ± 0.7</td>
<td>0</td>
<td>39.5 ± 0.25</td>
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<td>SB-612111</td>
<td>1.42 ± 0.12</td>
<td>674 ± 43.9</td>
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<td>SR 16435</td>
<td>28.7 ± 0.6</td>
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<td>Buprenorphine</td>
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NOPr, NOP receptor; MOPr, MOP receptor; Stim, stimulation.
antinociceptive activity ($F(3,46) = 15.1, P < 0.05$). The 10 and 30 mg/kg doses of SR16435 produced an increase in tail-flick latency 10, 30, and 60 min after injection (Fig. 2), whereas the 3 mg/kg produced a significant increase in %MPE at the earlier time points of 10 and 30 min after injection ($P < 0.05$). Unlike buprenorphine, SR16435 had a steep dose-response curve and approached maximal inhibition of tail-flick (15-s cutoff) at 30 mg/kg SR16435. In the presence of 10 mg/kg SB-612111, the overall ANOVA indicated a significant interaction effect [$F(3,90) = 9.3, P < 0.05$]. SB-612111 significantly potentiated the tail-flick latency of 3 to 10 mg/kg SR16435 at all of the time points tested, whereas with the 30 mg/kg dose %MPE was potentiated at 10 and 60 min after injection, because at 30 min, SR16435%MPE was close to the ceiling effect of 100% in the absence of the NOP receptor antagonist and could not be greatly potentiated in its presence.

The mixed NOP receptor full agonist/MOP receptor partial agonist SR16507 also produced an increase in tail-flick latency relative to controls [$F(2,22) = 15.8, P < 0.05$], where the 1 mg/kg dose produced an increase in tail-flick latency at 10, 30, and 60 min after injection (Fig. 3) and the 0.3 mg/kg dose alone was ineffective. SR16507 did not produce maximal effects possibly because of the increase in efficacy at NOP receptor. When SB-612111 was given before SR16507, the ANOVA indicated a significant interaction [$F(2,43) = 7.0, P < 0.05$], such that SB-612111 potentiated the antinociceptive activity of SR16507. SB-612111 not only potentiated the effects of 1.0 mg/kg SR16507, which alone produced an increase in antinociception, but also produced an increase in tail-flick latency when coadministered with the 0.3 mg/kg dose that alone was ineffective. Because the antinociceptive activity of both SR16435 and SR16507 is blocked by naloxone (Khroyan et al., 2007; Toll et al., submitted), these studies indicate that the analgesic activity of these mixed NOP/MOP receptor agonists is mediated by the MOP receptor, and that NOP receptor agonist activity attenuates the analgesic activity.

Antinociceptive Effects of MOP Receptor Agonists

Pentazocine and Morphine Alone or Coadministered with SB-612111. Morphine, has a steep dose-response curve [$F(3,39) = 62.5, P < 0.05$] with nearly 100% MPE at 10 mg/kg (Fig. 4, A and B), whereas the MOP receptor partial agonist pentazocine demonstrates a very shallow dose-response curve [$F(3,31) = 6.7, P < 0.05$], never approaching 100% MPE even up to 30 mg/kg (Fig. 4, C and D). Unlike buprenorphine, SR16435, and SR16507, which bind to both MOP receptor and NOP receptor, morphine and pentazocine are selective for MOP receptor. Probably because of the lack of NOP receptor agonist activity of morphine and pentazocine, SB-612111 was unable to potentiate the antinociceptive activity of morphine and only potentiated pentazocine at a single dose (30 mg/kg) and at only the 60 min time point.

Discussion

There is considerable evidence that N/OFQ and the NOP receptor are involved in a multitude of opioid actions. N/OFQ, given intracerebroventricularly, has been shown to block morphine analgesia (Mogil et al., 1996); however, it has antinociceptive activities when administered intrathecally (Tian et al., 1997). Although selective peptide antagonists...
Fig. 2. Acute thermal antinociceptive effect of mixed NOP receptor/MOP receptor partial agonist SR16435 alone or coadministered with SB-612111 by use of the tail-flick assay at 10 min (A), 30 min (B), and 60 min (C) after injection. Data are mean %MPE (± S.E.M.). An asterisk (*) represents a significant difference from their respective vehicle controls, whereas a plus sign (+) represents a significant difference from SR16435 alone (P < 0.05).

Fig. 3. Acute thermal antinociceptive effect of mixed NOP receptor/MOP receptor full agonist SR16507 alone or coadministered with SB-612111 by use of the tail-flick assay at 10 min (A), 30 min (B), and 60 min (C) after injection. Data are mean %MPE (± S.E.M.). An asterisk (*) represents a significant difference from their respective vehicle controls, whereas a plus sign (+) represents a significant difference from SR16507 alone (P < 0.05).
have antinociceptive activity when given intracerebroventricularly (Calo et al., 2000, 2005), selective small-molecule antagonists are not analgesic (Ozaki et al., 2000; Zaratin et al., 2004). The analgesic action of small-molecule agonists, when given systemically, is also unclear. Initial publications suggested that the selective agonist Ro 64-6198 is not analgesic, and attenuates morphine analgesia (Jenck et al., 2000). However, recent studies have shown that Ro 64-6198 has antinociceptive activity in mice using the hotplate but not the tail-flick test (Reiss et al., 2008), and in monkeys when measuring tail withdrawal (Ko et al., 2009). In both of these latter experiments, the antinociceptive activity of Ro 64-6198 was not naloxone reversible. Clearly, the actions of the NOP receptor system on acute pain are still not well understood.

Recently, it was discovered that the opioid partial agonist buprenorphine has affinity for the NOP receptor. In vitro, the affinity of buprenorphine for NOP receptors is considerably lower than for the opioid receptors (see Table 1). In addition, in functional assays, buprenorphine ranges from having no agonist efficacy for stimulation of \[^{35}\text{S}\]GTP\textsubscript{S} binding to dog brain membranes or transfected cells (Lester and Traynor, 2006; Spagnolo et al., 2008), partial agonist activity for stimulation of \[^{35}\text{S}\]GTP\textsubscript{S} binding in transfected cells (Huang et al., 2001), to being a full agonist for stimulation of mitogen-activated protein kinase (Lutfy et al., 2003) or when using the NOP reporter gene assay in transfected cells (Wnendt et al., 1999). Because the efficacy of a partial agonist depends on the receptor reserve and the particular assay being performed, it is possible that buprenorphine could display NOP receptor agonist activity in vivo.

In this regard, Lutfy et al. (2003) have demonstrated that the antinociceptive activity of buprenorphine in the tail-flick assay was potentiated by the NOP receptor antagonist J113397, and at higher heat intensity, the inverted U-shaped dose-response curve for buprenorphine straightened out in the presence of the antagonist or in NOP receptor KO mice (Lutfy et al., 2003). These results suggest that the shallowness of the dose-response curve is, at least to some extent, due to NOP receptor agonist activity attenuating the MOP receptor-mediated antinociceptive activity of buprenorphine.

In addition to modulating opiate analgesic activity, N/OFQ modulates the reward induced by a variety of drugs of abuse (Murphy et al., 1999; Kotlinska et al., 2003; Sakoori and Murphy, 2004), and the small-molecule NOP receptor agonists Ro 64-6198 (Shoblock et al., 2005) and SR16835 (Toll et
al., 2009) both attenuate morphine place preference. Cicco-
cioppo and colleagues demonstrated that buprenorphine in-
creased alcohol consumption at low doses, but attenuated
alcohol consumption at higher nonselecting doses in Sardin-
ian alcohol-preferring rats (Ciccocioppo et al., 2007). The
reduction in alcohol consumption at high buprenorphine
doses was blocked by the NOP antagonist UFP-101. It was
concluded that buprenorphine acts through MOP receptors
at low doses, but at higher doses NOP receptor activity con-
tacts the opioid-mediated effects, resulting in the overall
attenuation of alcohol consumption.

Although it is possible that the NOP receptor component
of buprenorphine results in a shallow dose-response curve (for
antinociception) or a decrease in reward (decrease in alcohol
intake), there were several reasons why this seemed un-
likely. Buprenorphine is a potent opiate, having antinocicep-
tive activity at less than 0.3 mg/kg. Its affinity for NOP
receptors is only approximately 80 to 100 nM. Furthermore,
in most paradigms, buprenorphine is only a partial NOP
receptor agonist, at best, even in CHO cells that overexpress
NOP receptors. In addition, other opioid partial agonists,
such as pentazocine, that do not bind NOP receptor also have
very shallow or inverted U-shaped dose-response curves
(Cowan et al., 1977). Finally, other MOP/NOP receptor ago-
nists, such as SR14635, that have a considerably greater
NOP receptor component have potent analgesic activity
(Khroyan et al., 2007). Thus, although it is plausible that the
NOP receptor system plays a role in attenuating opioid-
mediated actions of buprenorphine, this attenuation could be
caused by some other phenomenon, such as inhibition of
endogenous N/OFQ, rather than direct interaction of bu-
premorphine with the NOP receptor.

To resolve this issue, we determined the in vitro and in vivo
actions of five compounds: morphine, a high-efficacy MOP
receptor agonist with no affinity for NOP receptors; pentazo-
cine, a MOP receptor partial agonist with no affinity for NOP
receptors; buprenorphine, a MOP receptor partial agonist
with low affinity for NOP receptors; SR14635, a high-affinity
partial agonist at MOP and NOP receptors; and SR16507, a
full NOP receptor agonist and partial MOP receptor agonist
with high affinity for both receptors. The results indicated
that compounds with any measurable NOP receptor activity
and affinity, including buprenorphine, SR14635, and
SR16507, produced acute antinociception by use of the tail-
flick assay that was potentiated by the NOP receptor antag-
ionist SB-612111. Potentiation was greatest for SR16507,
which is a potent full agonist at the NOP receptor followed by
SR14635, which also has a high affinity for NOP receptors.

This is consistent with these compounds having a greater
NOP receptor component. However, pentazocine and mor-
phine, which do not bind to NOP receptors, produced antino-
ciception that was not potentiated by SB-612111. These re-
results are consistent with NOP receptor agonist activity
within a molecule being required for analgesic activity to be
potentiated by a NOP receptor antagonist, and greatly
strengthen the argument that NOP receptor agonist activity
of buprenorphine mediates a decrease in antinociceptive ac-
tivity and alcohol consumption.

The fact that SB-612111 and J113397 do not have antino-
ciceptive activity on their own, or even potentiate morphine,
is important and suggests that there is no significant NOP
receptor tone with respect to acute radiant heat in normal
mice. This finding is in contrast to what has been reported
with the NOP receptor antagonist JTC-801. JTC-801 is a less
selective NOP receptor antagonist, but has antinociceptive
activity in both acute and chronic pain models that is not
naloxone reversible (Yamada et al., 2002; Suyama et al.,
2003). The fact that the other, more selective antagonists do
not act as analgesics suggests that JTC-801 may be acting
through some other mechanism.

Buprenorphine is a unique compound. In humans it is a
very potent long-lasting analgesic that is useful after sur-
gery. Nevertheless, buprenorphine is safer than most opiate
analgesics because it has low opioid efficacy resulting in
reduced respiratory depression, and has reduced addiction
liability (Jasinski et al., 1978; Lewis, 1985). These properties
also make it useful for opioid maintenance therapy (Mello
and Mendelson, 1980). In addition to these beneficial actions,
buprenorphine has been known for many years to attenuate
cocaine use (Schottenfeld et al., 1993), and to attenuate al-
cohol consumption (Mannelli et al., 1993). The mechanism by
which it attenuates consumption of abused drugs may very
well involve activation of NOP receptors. It is not clear,
however, how a compound with such weak NOP receptor
agonist activity can be acting through this receptor, in par-
ticular, because full agonist activity seems to be required
to attenuate morphine conditioned place preference (CPP)
by SR16507 and SR16835, two compounds with much higher
affinity for the NOP receptor (Toll et al., 2009). The binding
affinity of buprenorphine and the potency for stimulation of
[35S]GTP\gammaS binding through the MOP receptors are also not
particularly impressive, but none-the-less it is a very potent
analgesic. It is possible that buprenorphine maintains un-
usually high in vivo activity at whichever receptor it is acti-
vating, because of its lipophilicity and slow dissociation rate
(Lewis, 1985). In addition, receptors involved in analgesia
might have a very high receptor reserve allowing weak par-
tial agonists, such as buprenorphine, to possess significant
agonist activity, whether it be antinociception through MOP
receptors or inhibition of antinociception through NOP recep-
tors. Another possibility to explain buprenorphine’s NOP
receptor-mediated activity is the presence of MOP/NOP het-
erodimers. Such dimers have been demonstrated to attenu-
ate MOP receptor-mediated activity in transfected HEK cells
(Wang et al., 2005). Such a mechanism may also exist in vivo
to attenuate buprenorphine’s antinociceptive activity.

These experiments also suggest that modulation of the
balance of affinity and efficacy of mixed MOP/NOP receptor
compounds, such as buprenorphine, SR14635, and SR16507
could lead to the development of novel compounds that may
have clinical use as both analgesics and drug abuse medica-
tions. Apparently, NOP receptor agonist activity can attenu-
ate the MOP receptor-mediated analgesic activity, but not
completely block it. In addition, the NOP receptor agonist
activity can attenuate the reward induced by the MOP re-
ceper component. SR14635 is an analgesic, but it induces a
conditioned place preference (Khroyan et al., 2007). SR16507
has higher efficacy at NOP receptor, is a potent analgesic,
and induces a CPP (Toll et al., 2009). In fact, another novel
NOP/MOP receptor compound, SR14150, has lower efficacy
at MOP receptor but still has naloxone-reversible antinocio-
ceptive activity, and no apparent CPP (Toll et al., 2009).
Compounds such as these, or additional NOP/MOP receptor
compounds based on a buprenorphine scaffold, as we are now
synthesizing, may prove useful as analogues with reduced addiction liability or potentially as drug abuse medications. In conclusion, these results are consistent with the hypothesis that the NOP receptor agonist activity of buprenorphine, and other mixed NOP/MOP receptor compounds, is able to attenuate the MOP receptor-mediated antinociceptive activity of this compound. These results further suggest that the development of additional compounds with mixed MOP/NOP receptor agonist activity could be beneficial for discovery of novel analogues with reduced addiction liability and drug abuse medications.

References

abuse medications. Novel analgesics with reduced addiction liability and drug development of additional compounds with mixed MOP/NOP receptor agonist activity could be beneficial for discovery of novel analogues with reduced addiction liability and drug abuse medications.

References


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