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Pharmacological studies of NOP receptor agonists as novel analgesics

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14. ABSTRACT The studies proposed in this project will test the hypotheses that in the non-human primate (1) the functions and behavioral effects of the NOP receptor are independent of classical opioid receptors, (2) activation of the NOP receptor produces strong antinociception without abuse liability, and (3) NOP receptor agonists possess a promising therapeutic profile as analgesics compared to mu opioids following repeated administration in primates. Several key findings have been obtained and some have been published. First, intrathecal administration of N/OFQ only produced antinociception in primates. The functional profiles of spinal NOP receptors are different between primates and rodents. Second, intrathecal administration of N/OFQ and other NOP receptor agonists produced antinociception without eliciting itch/scratching responses, indicating that NOP receptor agonists represent a therapeutic target as spinal analgesics. Third, NOP receptor agonists produced antinociceptive effects comparable to clinically used mu opioids such as morphine and alfentanil in three different primate pain models, indicating that the analgesic effectiveness of NOP receptor agonists may be similar to that of mu opioid analgesics in humans. Finally, unlike mu opioids, NOP receptor agonists did not produce reinforcing effects, respiratory depressant, sedation, or itch/pruritic side effects, indicating that NOP receptor agonists may be a new generation of novel analgesics without abuse liability.					
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INTRODUCTION

Proposed studies intend to investigate the potential antinociceptive effects of NOP receptor agonists in monkeys. Both intrathecal and systemic administration are common routes for delivery of analgesics in the clinic. Future studies characterizing and comparing the behavioral effects of intrathecal and systemic administration of OFQ/N and Ro 64-6198 in monkeys would provide a great deal of information for potential pain management in humans. In particular, the pharmacological profile and behavioral effects of NOP receptor agonists can be systematically compared with those of mu opioid receptor agonists in monkeys following acute and repeated administration, and they will make a notable advance in our understanding of pain and analgesia in relation to the fourth member of the opioid receptor family in primates. The studies proposed in this project will test the hypotheses that in the non-human primate (1) the functions and behavioral effects of the NOP receptor are independent of classical opioid receptors, (2) activation of the NOP receptor produces strong antinociception without abuse liability, and (3) NOP receptor agonists possess a promising therapeutic profile as analgesics compared to mu opioids following repeated administration in primates.

BODY

TASK 1.

Extensive evaluation of the behavioral effects of intrathecally administered N/OFQ in non-human primates.

(a) Study behavioral effects of ultra-low doses of intrathecal N/OFQ over a wide dose range using a warm water tail withdrawal assay and behavioral observations.

This experiment has been conducted. We have found that intrathecal administration of N/OFQ over a wide dose range from 1 fmol to 1 nmol did not produce hyperalgesia, scratching, or any pain-like behavioral responses in monkeys. Ultra-low doses of intrathecal N/OFQ (i.e., fmol) produced pain-like behavior manifested by scratching, biting, and licking behaviors in mice (Sakurada et al., 1999). The pharmacological profile of intrathecal N/OFQ is clearly different between rodents and primates (Ko et al., 2006b).

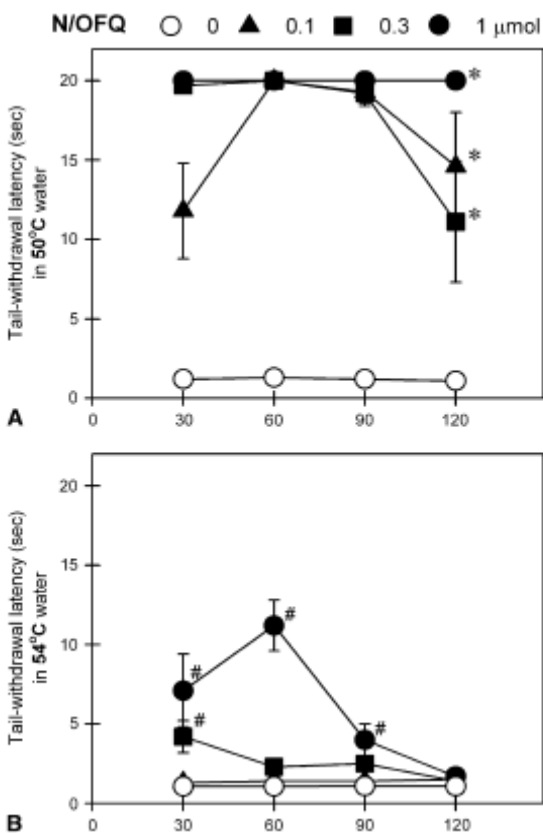


Figure 1. Antinociceptive effects of intrathecally administered N/OFQ at doses between 0.1 and 1 μmol. Panels A and B represent changed tail-withdrawal latencies in 50°C

and 54°C water, respectively.

In addition, intrathecal N/OFQ at doses between 10 nmol and 1 µmol dose-dependently produced antinociceptive effects against a noxious stimulus at different intensities. Combined administration of intrathecal N/OFQ and morphine significantly potentiated morphine-induced antinociception without inhibiting morphine-induced itch/scratching responses. Although these experiments were time-consuming and labor-intensive, these results provide a unique functional profile of intrathecal N/OFQ over a wide dose range in primates. Overall, intrathecal N/OFQ produced thermal antinociception without anti-morphine actions or eliciting itch/scratching responses, indicating that N/OFQ or NOP receptor agonists may represent a promising target as spinal analgesics.

Findings relevant to Task 1 have been published in the *Journal of Pain*, the official journal of *American Pain Society* ([Ko & Naughton \(2009\) Antinociceptive effects of nociception/orphanin FQ administered intrathecally in monkeys. Journal of Pain 10\(5\):509-516](#), see Appendices for other details).

TASK 2.

Comparison of effectiveness of systemically administered Ro 64-6198 in different experimental pain models in non-human primates.

(a) Determine the doses of systemic Ro 64-6198, a non-peptidic NOP receptor-selective agonist, that produce antinociception in monkeys using a warm water 50°C tail withdrawal assay.

This experiment has been conducted. Systemic administration of Ro 64-6198 (0.001-0.03 mg/kg), a NOP receptor-selective agonist, dose-dependently produced antinociceptive effects against a noxious stimulus, 50°C water. Systemic Ro 64-6198 0.03 mg/kg produced full antinociception under this context. The warm water tail-withdrawal assay has been widely used to determine the antinociceptive effects of the test compound in monkeys (Butelman et al., 1993; Ko et al., 1998a). Previous studies have shown that systemic morphine 3 mg/kg produced full antinociception measured by this procedure (Butelman et al., 1996; Lee et al., 2007).

(b) Compare the antinociceptive effects of systemic Ro 64-6198 with those of systemic morphine in capsaicin-induced allodynia and carrageenan-induced hyperalgesia in the same monkeys.

This experiment has been conducted. **Figure 2** shows the antinociceptive effectiveness and potency of morphine and Ro 64-6198 against two different nociceptive assays. Both Ro 64-6198 and morphine are effective in producing antinociception against two different noxious stimuli. More importantly, Ro 64-6198 is more potent (~50-100 fold) than morphine to produce anti-allodynic/anti-hyperalgesic effects under this context.

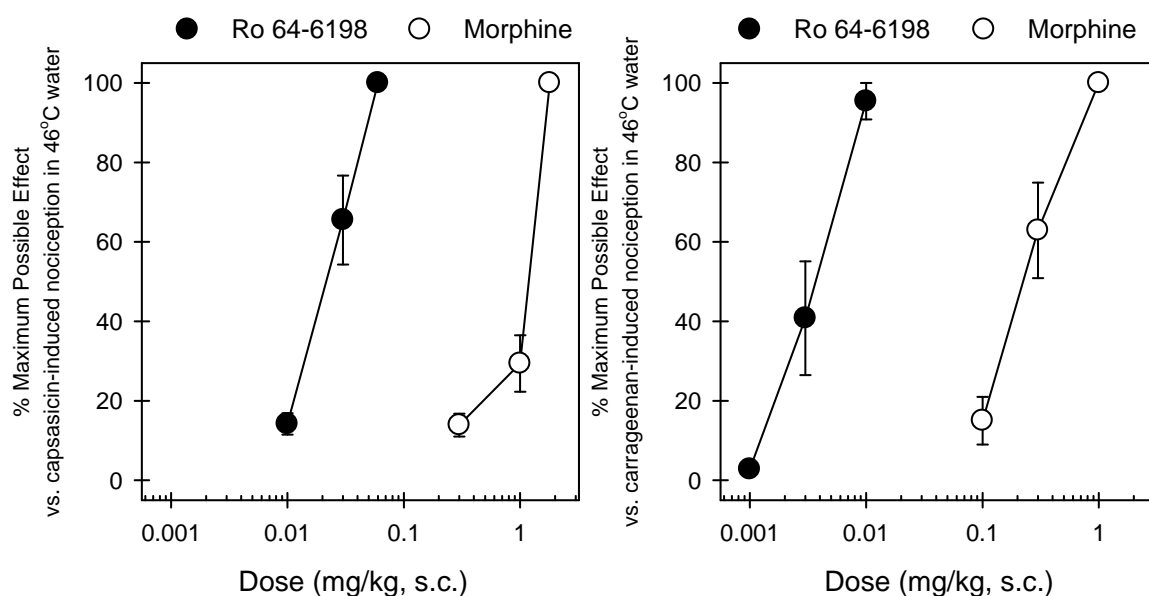


Figure 2. Antinociceptive effects of Ro 64-6198 and morphine against capsaicin- and carrageenan-induced allodynia/hyperalgesia in 46 °C water.

Both capsaicin- and carrageenan-induced pain models have been established in monkeys to determine and compare the effectiveness of clinically used analgesics and experimental compounds (Ko et al., 1998b; Ko and Lee, 2002; Butelman et al., 2004). In particular, a capsaicin-based pain model is practical and valuable on many levels. Capsaicin is a natural irritant found in hot-chili peppers that evokes pain sensation by activating at the TRPV1. TRPV1 and the up-regulation of its expression have been

strongly implicated in the integration and transduction of a variety of pain signaling including tissue-injury induced thermal hyperalgesia, diabetic neuropathy, and neurogenic inflammatory response associated with many disease states (Szallasi et al., 2007; Knotkova et al., 2008). Furthermore, capsaicin-induced allodynia has been previously utilized as a pain model in both monkeys (Ko et al., 1998b; Butelman et al., 2004) and humans (Park et al., 1995; Eisenach et al., 1997) to study experimental compounds as analgesics. Considering the variety of pain modalities capsaicin-sensitive fibers are linked to, the ability to attenuate capsaicin-induced allodynia would suggest a prominent clinical value of NOP receptor agonists.

Part of findings relevant to Task 2 has been published in *Neuropsychopharmacology*, the official journal of the *American College of Neuropsychopharmacology* (Ko et al. (2009) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys. *Neuropsychopharmacology* 34:2088-2096, see Appendices for other details).

TASK 3.

Clarification of the receptor selectivity and site of actions of NOP receptor agonists by conducting receptor antagonist studies *in vivo*.

(a) Determine the *in vivo* apparent pA_2 value of J-113397, a non-peptidic NOP receptor-selective antagonist, against systemic Ro 64-6198-induced antinociception in monkeys.

This experiment has been conducted. Pretreatment with J-113397 dose-dependently produced rightward shifts of the dose response curve of Ro 64-6198-induced antinociception. These dose-dependent antagonist effects of J-113397 were graphed in a Schild plot with values derived from individual dose ratios for each subject. The mean pA_2 value of J-113397 was 7.98 (7.85-8.11) with a slope of -1. The doses of J-113397 alone did not change the thermal threshold of monkeys (i.e., no changes in the tail withdrawal latencies in 42, 46, or 50°C water).

(b) Cross-examine the antagonist potency of naltrexone, an opioid receptor antagonist, on Ro 64-6198-induced antinociception and the antagonist potency of J-113397 on morphine-induced antinociception.

This experiment has been conducted. **Figure 3** compares the antagonist effects of naltrexone and J-113397 on the antinociceptive effects produced by s.c. Ro 64-6198 and alfentanil.

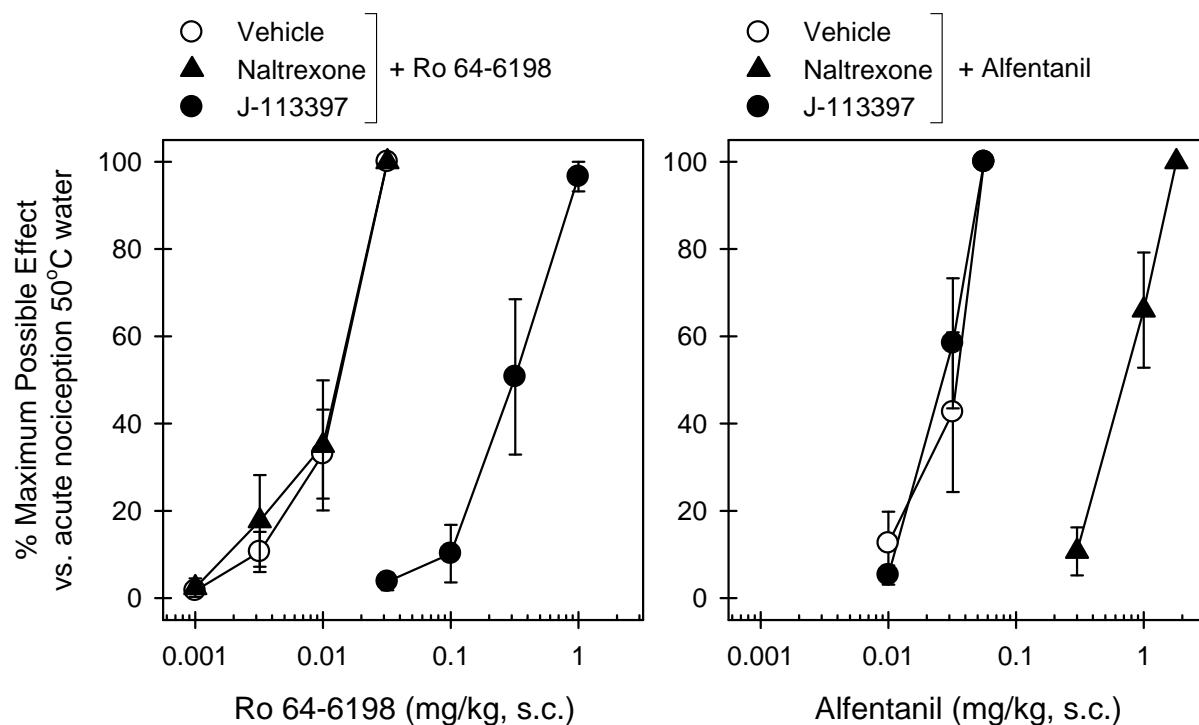


Figure 3. Effects of mu opioid receptor and NOP receptor antagonists on alfentanil- and Ro 64-6198-induced antinociceptive effects in monkeys.

The left panel shows that a single dose (0.1 mg/kg) of J-113397 produced a large rightward shift of the dose response curve of Ro 64-6198-induced antinociception. The mean J-113397 pK_B value was 8.02 (7.78-8.26) under this condition. Naltrexone 0.03 mg/kg failed to block Ro 64-6198-induced antinociception; the ED₅₀ value of Ro 64-6198 dose response for vehicle pretreatment (0.012 mg/kg) was similar to that for naltrexone pretreatment (0.013 mg/kg). In contrast, the right panel shows that a single dose of naltrexone 0.03 mg/kg produced a large rightward shift of the dose response curve of alfentanil-induced antinociception. The mean naltrexone pK_B value was 8.44 (8.18-8.70) under this condition. J-113397 0.1 mg/kg failed to block alfentanil-induced

antinociception; the ED50 value of alfentanil dose response for vehicle pretreatment (0.031 mg/kg) was similar to that for J-113397 pretreatment (0.026 mg/kg).

(c) Compare the antagonist potency of intrathecal versus subcutaneous J-113397 on systemic Ro 64-6198-induced antinociception.

This experiment has been conducted. Pretreatment with a single dose 0.01 mg/kg of subcutaneous J-113397 produced approximately a 30-fold rightward shift of the dose-response curve of Ro 64-6198-induced antinociception. In contrast, pretreatment with a single dose 0.001 mg (i.e., 100-fold less than total amount 0.1 mg afforded by subcutaneous J-113397 0.01 mg/kg in monkeys with averaged body weight of 10 kg) of intrathecal J-113397 produced approximately a 25-fold rightward shift of the dose response curve of Ro 64-6198-induced antinociception.

Taken together, these findings showed that systemic Ro 64-6198 alone produced antinociceptive effects which could be blocked dose-dependently by J-113397, a selective NOP receptor antagonist. In vivo apparent pA_2 analysis was used because this quantitative procedure offers a powerful approach to establish receptor-mediated drug effects (Arunlakshana and Schild, 1959; Tallarida et al., 1979). J-113397 dose-dependently produced parallel rightward shifts of the dose response curve of Ro 64-6198-induced antinociception, indicating that the agonist and antagonist compete for the same NOP receptors in a reversible manner. More importantly, cross-examination of both antagonists against different agonists demonstrated that both alfentanil- and Ro 64-6198-induced antinociceptive effects were mediated by mu opioid receptors and NOP receptors, respectively. These experiments provide a pharmacological basis for the role of spinal NOP receptors in Ro 64-6198-induced antinociception and indicate that antinociceptive effects of opioid analgesics can be produced by two independent opioid receptor mechanisms in monkeys.

Part of findings relevant to Task 3 has also been published in Neuropsychopharmacology (Ko et al. (2009) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys. Neuropsychopharmacology 34:2088-2096, see Appendices for other details).

TASK 4.

Evaluation of potential abuse liability of NOP receptor agonists using the self-administration assay.

(a) Determine and compare reinforcing effects of Ro 64-6198 with those of the mu opioid agonist fentanyl, the psychomotor stimulant cocaine, and the barbiturate anesthetic methohexital in the monkey intravenous self-administration assay to assess whether NOP receptor agonists possess abuse liability.

This experiment has been conducted. Response rates (responses/sec) for saline, alfentanil, and Ro 64-6198 across a dose range of 0.03 – 30 µg/kg/inj were assessed. To aggregate data across all subjects, mean response rates engendered by each dose of each drug were averaged. Under this multiple component schedule, contingent saline infusions engendered very low response rates (less than 0.3 responses/sec). All animals self-administered alfentanil within the dose range tested, generating a biphasic dose-effect curve characteristic of intravenous drug self-administration. In contrast, Ro 64-6198 did not maintain high rates of responding at any of the doses tested, resulting in a flat dose-effect curve indicative of a compound without reinforcing effects under the present conditions. Likewise, Ro 64-6198 did not maintain high rates of responding at doses tested, but all subjects self-administered cocaine, under the same schedule.

(b) Assess the effects of Ro 64-6198 pretreatment on remifentanil- and cocaine-maintained self-administration behavior.

This experiment has been conducted. Pretreatment with an antinociceptive dose 0.03 mg/kg of Ro 64-6198 did not significantly attenuate the monkey's self-administration responses maintained by either cocaine (0.01 mg/kg/injection) or remifentanil (0.1 µg/kg/injection) under a single test session.

Taken together, these findings showed lack of reinforcing effects of Ro 64-6198 in alfentanil-, cocaine-, and methohexital-maintained monkeys. The presence of a behavioral effect (i.e., antinociception at 10-30 µg/kg) in the absence of any indication of a reinforcing effect indicates that we have tested sufficiently large doses for potential reinforcing effects. For example, the antinociceptive doses of intravenous alfentanil were 10-30 µg/kg (Ko et al., 2002), but the doses of alfentanil producing reinforcing

effects were 0.1-1 $\mu\text{g/kg}$ (i.e., a 30-100 fold difference) (Winger et al., 1992; Ko et al., 2002). Lack of reinforcing effects by Ro 64-6198 might be expected because several studies have shown that activation of NOP receptors inhibited dopamine release in the striatum and supported the notion that NOP receptor agonists do not have reinforcing or aversive properties of their own (Murphy and Maidment, 1999; Flau et al., 2002). The relation between NOP receptors and dopamine release was also supported by the findings that pretreatment with Ro 64-6198 did not attenuate opioids such as remifentanyl- or cocaine-mediated reinforcing effects in monkeys.

Part of findings relevant to Task 4 has also been published in *Neuropsychopharmacology* (Ko et al. (2009) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys. *Neuropsychopharmacology* 34:2088-2096, see Appendices for other details).

TASK 5.

Determination of the receptor selectivity and functional efficacy of NOP receptors at the cellular level.

(a) Characterize the density of NOP receptors in membranes of cortex, thalamus, and spinal cord of monkeys by using a receptor binding assay.

This experiment has been conducted. Table 1 illustrates the apparent affinity and Bmax data in cortical and thalamic membranes of rhesus monkeys. Compared to previous studies (Ko et al., 2003), the Bmax value of NOP receptor is higher than that of mu opioid receptors in the cortex, but Bmax values for both receptors in the thalamus remain similar. We are currently characterizing the Bmax value of NOP receptors in the membranes of spinal cord.

Table 1.

Mean values in both cortical and thalamic membranes of monkeys determined by the receptor binding assay

	Cortex		Thalamus	
	Bmax	Kd	Bmax	Kd
	fmol/mg	nM	fmol/mg	nM
NOP receptor	282 \pm 41	0.1 \pm 0.01	149 \pm 20	0.1 \pm 0.01

Note: The density of NOP receptor binding sites was measured with [^3H]N/OFQ. Shown are mean values and S.E.M. of individual data of four monkeys.

(b) Compare the potency and magnitude of concentration-responses curves of N/OFQ- and Ro 64-6198-stimulated $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding in membranes of cortex, thalamus, and spinal cord of monkeys.

This experiment has also been conducted. We have found that both N/OFQ and Ro 64-6198 produced similar magnitudes in $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding in the cortical membranes but with different potencies.

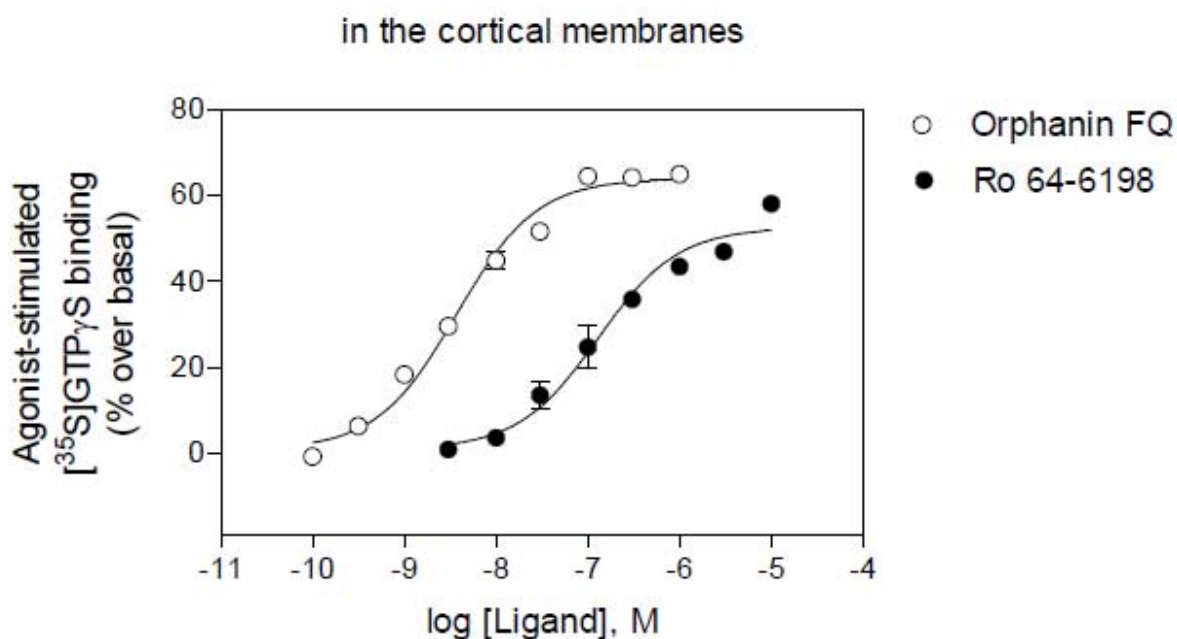


Figure 4. Comparison of stimulation of $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding by N/OFQ and Ro 64-6198 in cortical membranes of one monkey. Shown are mean values \pm SEM from two independent experiments, each conducted in duplicate.

(c) Determine the antagonist selectivity of J-113397 and opioid receptor antagonists on N/OFQ- and MOR, KOR, and DOR agonist-stimulated $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding in the cortical membranes of monkeys.

This experiment has been conducted. In the cortical membranes, we have found that N/OFQ-stimulated $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding could not be antagonized by selective MOR, KOR, and DOR antagonists, i.e., naloxone, nor-BNI, and naltrindole, respectively. In

addition, the NOP receptor antagonist J-113397 did not antagonize MOR, KOR, and DOR agonist-stimulated $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding (i.e., DAMGO, U69593, and SNC80). These data clearly demonstrate that the functions of the NOP receptor are independent of classical opioid receptors at the cellular level.

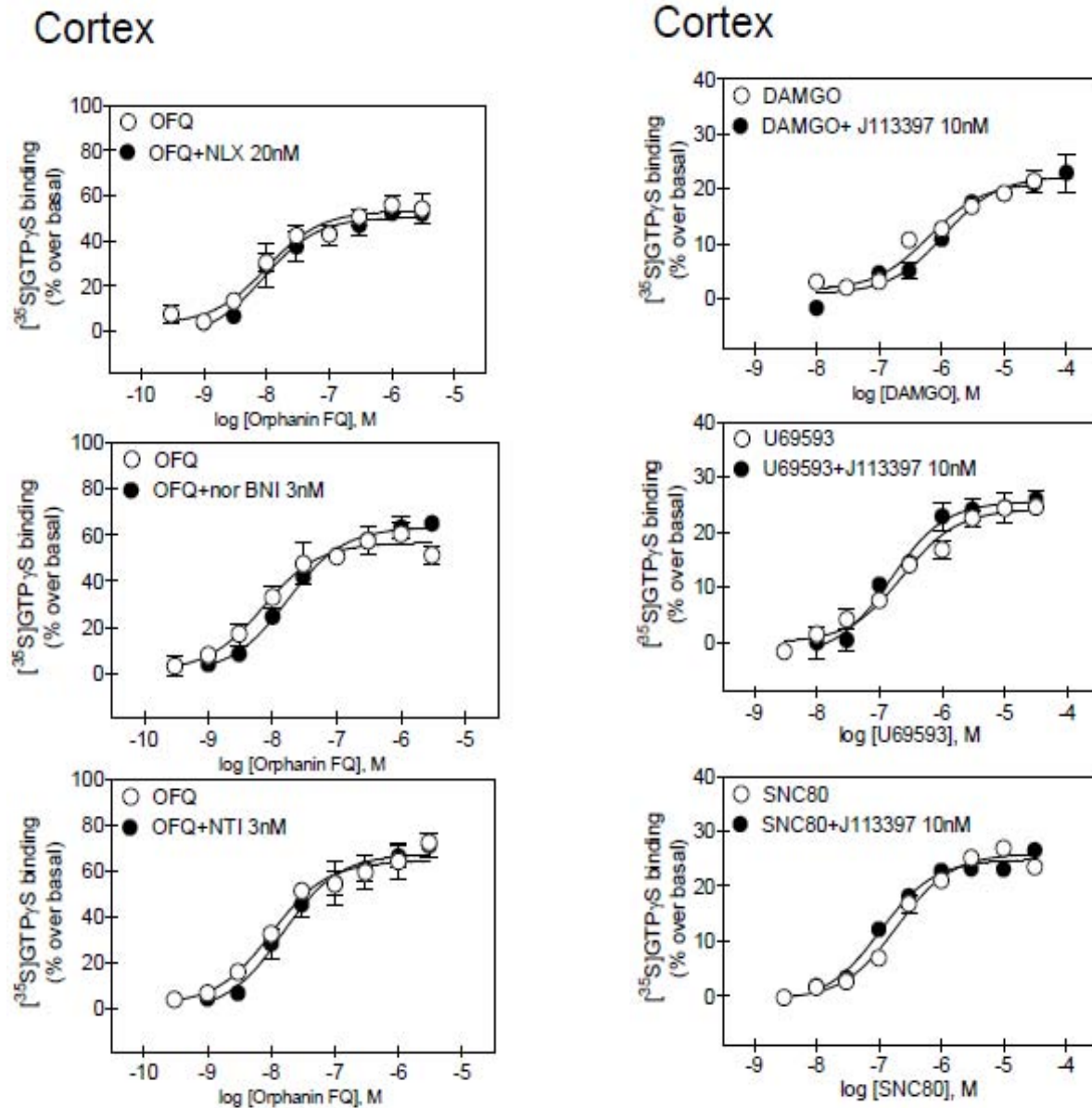


Figure 5. Cross-examination of stimulation of $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding by N/OFQ, and MOR agonist DAMGO, KOR agonist U69593, and DOR agonist SNC80 in the monkey's cortical membranes.

TASK 6.

Evaluation of the behavioral profile and safety margin of the NOP receptor agonist in non-human primates.

(a) Determine whether the dose equal to or larger than antinociceptive doses of systemic Ro 64-6198 produce side effects such as respiratory depression, sedation, and convulsions and whether J-113397 can reverse the side effects of Ro 64-6198 in monkeys.

This experiment has been conducted. We have found that the mu opioid receptor agonist, alfentanil, dose-dependently decreased f and VE responses, but Ro 64-6198 did not significantly decrease the respiratory function, compared with the vehicle condition in monkeys under both breathing cycles. More importantly, a dose (0.06 mg/kg) larger than the antinociceptive dose (0.01-0.03 mg/kg) of Ro 64-6198 did not significantly decrease the respiratory parameters under this context. In addition, intravenous Ro 64-6198 at 0.01 mg/kg produced full antinociceptive effects, but it is up to 0.3 mg/kg (i.e., a 30-fold window between the antinociceptive dose and the dose producing side effects) Ro 64-6198 started to produce sedative effects and suppressed operant responding for remifentanil self-administration (see Appendix #4, a manuscript under revision). Furthermore, a novel NOP receptor agonist, UFP-112, also produced a promising pharmacological profile as a potential analgesic compared to mu opioids following spinal administration in primates (Hu et al., 2010).

(b) Compare behavioral withdrawal signs in monkeys abruptly withdrawn from acute administration of either morphine or Ro 64-6198.

This experiment has been conducted. Following the dosing regimen for detecting acute dependence in monkeys (Ko et al., 2006a), morphine-dependent monkeys showed a variety of behavioral withdrawal signs, especially with increased lethargic responses, vocalization, and self-injury behaviors. In contrast, Ro 64-6198-dependent monkeys did not show dramatic behavioral changes. These data indicate that Ro 64-6198 may have less liability to produce physical dependence following short-term repeated administration.

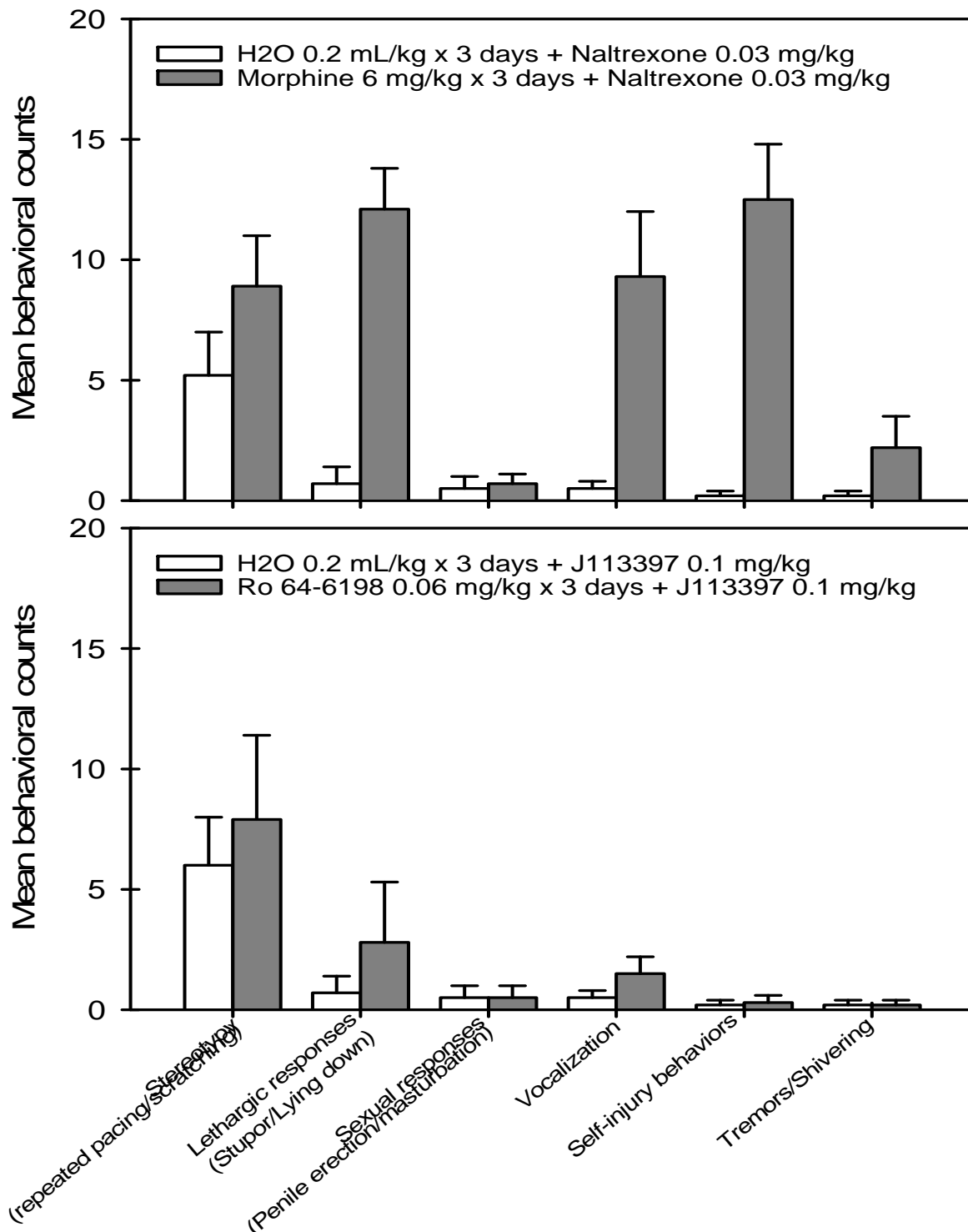


Figure 6. Behavioral withdrawal signs in monkeys following acute repeated administration of either morphine or Ro 64-6198. The antagonist, either naltrexone or J-113397, was used to participate withdrawal under this context (see Ko et al., 2006a).

Part of findings relevant to Task 6 has also been published in *Neuropsychopharmacology* (Ko et al. (2009) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys. *Neuropsychopharmacology* 34: 2088-2096) and *Pain* (Hu et al. (2010) Long-lasting antinociceptive spinal effects in primates of the novel nociception/orphanin FQ receptor agonist UFP-112. *Pain* 148: 107-113).

TASK 7.

Comparison of the potential development of tolerance and physical dependence following chronic administration of either the MOR agonist or NOP receptor agonist.

(a) Determine whether chronic administration of either morphine or Ro 64-6198 leads to tolerance to the antinociceptive potency of either compound and determine whether such chronic administration changes the nociceptive threshold of monkeys.

This experiment has just been started. More data will be collected to complete this task. Therefore, we request a no-cost time extension for the project in order to complete all proposed studies.

KEY RESEARCH ACCOMPLISHMENTS

These findings indicate that -

- Intrathecal administration of N/OFQ and other NOP receptor agonists only produced antinociception in primates. The functional profiles of spinal NOP receptors are different between primates and rodents.
- Intrathecal administration of N/OFQ and other NOP receptor agonists produced antinociception without eliciting itch/scratching responses and sedation, indicating that N/OFQ or other NOP receptor agonists represent a therapeutic target as spinal analgesics.

- NOP receptor agonists produced antinociceptive effects comparable to clinically used mu opioids such as morphine and alfentanil in three different primate pain models, indicating that the analgesic effectiveness of NOP receptor agonists may be similar to that of mu opioid analgesics in humans.
- Actions produced by NOP receptor agonists produced are independent from classical opioid receptors at both behavioral responses and the cellular level.
- Unlike mu opioids, NOP receptor agonists did not produce reinforcing effects, respiratory depressant, or itch/pruritic side effects, indicating that NOP receptor agonists may be a new generation of novel analgesics without abuse liability.

REPORTABLE OUTCOMES

1. Ko MC and Naughton NN (2009)

Antinociceptive effects of nociception/orphanin FQ administered intrathecally in monkeys.

Journal of Pain, 10: 509-516. (see Appendix #1).

2. Ko MC, Woods JH, Fantegrossi WE, Galuska CM, Wichmann J, Prinssen EP (2009)

Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys.

Neuropsychopharmacology 34: 2088-2096 (see Appendix #2).

3. Hu E, Calo G, Guerrini R, Ko MC (2010)

Long-lasting antinociceptive spinal effects in primates of the novel nociception/orphanin FQ receptor agonist UFP-112.

Pain 148: 107-113 (see Appendix #3).

4. Podlesnik CA, Ko MC, Winger G, Wichmann J, Prinssen EP, Woods JH (2010)

The effects of nociception/orphanin FQ agonist Ro 64-6198 and diazepam on antinociception, remifentanil self-administration, and anxiolytic-like responding in rhesus monkeys.

Psychopharmacology (provisionally accepted, under revision, see Appendix #4).

CONCLUSION

These experiments conducted so far demonstrated two important points. The first point is in the field of using spinal opioid analgesics. Spinal administration of mu opioid analgesics is an important method for pain management in the past few decades. However, itch/pruritus is the most common side effects derived from spinal opioids. Intrathecal administration of morphine dose-dependently produces antinociception with simultaneous scratching responses in monkeys, and this observation parallels closely with the functional profile of spinal morphine in humans. Using the monkey model, NOP receptor agonists only produced antinociceptive effects without eliciting itch/scratching responses. Such findings strongly indicate that NOP receptor agonists represent a therapeutic target as spinal analgesics (Ko and Naughton, 2009; Hu et al., 2010).

The second point is in the research and development of novel opioid analgesics. As a recent review (Corbett et al., 2006) pointed out, much effort aimed at developing powerful analgesics without the side effects associated with mu opioids. Using the monkey model, NOP receptor agonists display a very different pharmacological profile compared to rodents. Like mu opioids, Ro 64-6198 produced full antinociceptive effects in three primate pain models. Unlike mu opioids, Ro 64-6198 did not producing reinforcing effects, respiratory depression, or itch/pruritic effects, indicating that NOP receptor agonists may be a new generation of novel analgesics without abuse liability (Ko et al., 2009). Such a promising pharmacological profile warrants additional monkey studies to investigate effects of other NOP receptor agonists and initiation of clinical trials of NOP receptor agonists in humans.

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APPENDICES (see attachment)

Antinociceptive Effects of Nociceptin/Orphanin FQ Administered Intrathecally in Monkeys

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Abstract: Nociceptin/orphanin FQ (N/OFQ) is the endogenous peptide for the NOP receptors. Depending on the doses, intrathecal administration of N/OFQ has dual actions (ie, hyperalgesia and antinociception) in rodents. However, the pharmacological profile of intrathecal N/OFQ is not fully known in primates. The aim of this study was to investigate behavioral effects of intrathecal N/OFQ over a wide dose range and to compare its effects with ligands known to produce hyperalgesia or antinociception in monkeys. Intrathecal N/OFQ from 1 fmol to 1 nmol did not produce any hyperalgesic or scratching responses. In contrast, intrathecal substance P 100 nmol produced hyperalgesia, and intrathecal DAMGO 10 nmol produced antinociception. At the dose range between 10 nmol and 1 μ mol, intrathecal N/OFQ dose-dependently produced thermal antinociception against a noxious stimulus in 2 intensities. More importantly, N/OFQ in combined with intrathecal morphine dose-dependently potentiated morphine-induced antinociception without inhibiting morphine-induced itch/scratching. Taken together, this study is the first to provide a unique functional profile of intrathecal N/OFQ over a wide dose range in primates. Intrathecal N/OFQ produces thermal antinociception without anti-morphine actions or scratching responses, indicating that N/OFQ or NOP receptor agonists represent a promising target as spinal analgesics.

Perspective: Intrathecal administration of N/OFQ only produced thermal antinociception, not hyperalgesia, in monkeys. In addition, intrathecal N/OFQ does not have anti-morphine actions or itch/scratching responses. This study strongly supports the therapeutic potential of N/OFQ or NOP receptor agonists as spinal analgesics for clinical trials.

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Key words: Spinal cord, analgesia, NOP receptors, substance P, thermal hyperalgesia.

Spinal administration of μ -opioid receptor agonists is an important method for pain management, and it is widely used for obstetric analgesia.^{8,10} However, itch/pruritus is the most common side effect derived from spinal opioids, and it reduces the value of pain relief afforded by spinal opioids.^{8,14} Previously, we have established an experimental model of spinal opioid-induced itch/scratching in monkeys.^{18,21} Intrathecal administration of morphine dose-dependently produces

antinociception with simultaneous scratching responses in monkeys,¹⁸ and this observation parallels closely with the behavioral effects of spinal morphine in humans.^{1,34} This experimental model using the intrathecal route for drug delivery in primates provides a valuable tool for identifying a novel, viable target as spinal analgesics.

Interestingly, a recent study found that intrathecal administration of an endogenous peptide, nociceptin/orphanin FQ (N/OFQ),^{28,36} in the dose range of nanomoles produced antinociceptive effects without itch/scratching responses in monkeys.²² Such naltrexone-insensitive effects could be blocked by the selective N/OFQ peptide receptor (NOP) antagonist J-113397 indicating that activation of spinal NOP receptors may be a promising target for spinal analgesia.^{22,24} However, ultra low doses of N/OFQ administered intrathecally at the dose range of femtomoles produced spontaneous agitation and pain manifested by biting, scratching, and licking behavioral

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responses in mice, suggesting that spinal N/OFQ has biphasic actions in rodents.^{15,40} Anatomical studies indicated that species differences may exist in the distribution of N/OFQ and NOP receptors.^{2,4} Nevertheless, most studies report that there is a high expression of N/OFQ and NOP receptors in the spinal cord of both rodents and humans.^{31,44} It is worth investigating whether spinal N/OFQ has both antinociceptive and pronociceptive/hyperalgesic actions and further characterizing the physiological functions of spinal N/OFQ in primates.

Therefore, the aim of this study was to extensively investigate and directly compare the behavioral effects of intrathecally administered N/OFQ over a wide dose range in monkeys. As noted, rodent studies have shown that intrathecal DAMGO and substance P produced antinociceptive and pronociceptive effects, respectively.^{29,30,41} By using both behavioral end points (ie, antinociception/hyperalgesia and scratching responses), effects of intrathecal DAMGO and substance P were compared with those of intrathecal N/OFQ. Antinociceptive effects of intrathecal N/OFQ were further studied against a noxious stimulus in 2 intensities. In addition, the potential interaction between intrathecal N/OFQ and morphine was determined to explore whether N/OFQ modulated intrathecal morphine-induced antinociception and scratching responses.

Materials and Methods

Subjects

Eighteen adult intact male and female rhesus monkeys (*Macaca mulatta*) with body weights ranging between 6.7 and 12.2 kg were used. The monkeys were housed individually with free access to water and were fed approximately 25 to 30 biscuits (Purina Monkey Chow; Ralston Purina, St. Louis, MO) and fresh fruit daily. No monkey had exposure to any opioid 1 month before the present study. The monkeys were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. The studies were conducted in accordance with the University Committee on the Use and Care of Animals in the University of Michigan (Ann Arbor, MI) and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health (Bethesda, MD).

Procedures

Nociceptive Responses

The warm water tail-withdrawal assay was used to evaluate thermal antinociceptive or hyperalgesic effects of the test compound.^{19,22} Briefly, monkeys were seated in primate restraint chairs, and the lower part of their shaved tails (approximately 15 cm) were immersed in a thermal flask containing water maintained at either 42°, 46°, 50°, or 54°C. Tail-withdrawal latencies were measured using a computerized timer by an experimenter who did not know dosing conditions. In each test session, monkeys were evaluated once with 4 temperatures given in a random order. If the monkeys

did not remove their tails within 20 seconds (cutoff), the flask was removed and a maximum time of 20 seconds was recorded. Test sessions began with determining a control value at each temperature. Subsequent tail-withdrawal latencies were determined at multiple time points after intrathecal administration.

Itch/Scratching Responses

Scratching behavior, inferred to be a response to itch sensation,^{18,21} was recorded on videotape while the monkeys were in their home cages. Each recording session was conducted for 15 minutes per test session. A scratch was defined as 1 short-duration (<1 second) episode of scraping contact of the forepaw or hind paw on the skin surface of other body parts. Scratches occurred repetitively at the same location. Scratching responses were scored by trained individuals who were blinded to experimental conditions. In addition, monkeys were rated for sedation and muscle relaxation according to 2 behavioral rating scales⁶ while in their home cages. The monitoring of potential side effects was conducted by an observer at the last minute of each test session.

Experimental Designs

The first part of the study was to determine behavioral responses of intrathecally administered N/OFQ over a wide range of ultra-low doses (ie, from 1 fmol to 1 nmol). In addition, effects of DAMGO and substance P were used as control conditions to compare with those of intrathecal N/OFQ. The doses of intrathecal DAMGO and substance P were selected based on a previous monkey study and our pilot study.²¹ The tail-withdrawal latency in the temperatures 46°C (non-noxious) and 50°C (noxious) of warm water was used to detect potential hyperalgesic/pronociceptive and antinociceptive effects, respectively, in monkeys.^{19,22} The second part of the study was to determine the degree of antinociception produced by intrathecal N/OFQ. The temperature 54°C of warm water represents a higher intensity of the nociceptive stimulus. The tail-withdrawal latency in both 50 and 54°C of warm water were used to characterize the antinociceptive effectiveness of intrathecal N/OFQ with increasing doses from 10 nmol to 1 μ mol. The third part of the study was to investigate how behaviorally active doses of N/OFQ modulated intrathecal morphine-induced antinociception and scratching responses. The dose of intrathecal morphine 50 nmol was selected based on previous studies,^{20,26} showing that it produced maximal scratching responses and antinociception, and it could be used to detect whether intrathecal N/OFQ could interfere with morphine-mediated actions.

Statistical Analysis

Mean values (mean \pm SEM) were calculated from individual values for all behavioral end points. Comparisons were made for the same monkeys across all test sessions in the same experiment. Data were analyzed by a 2-way

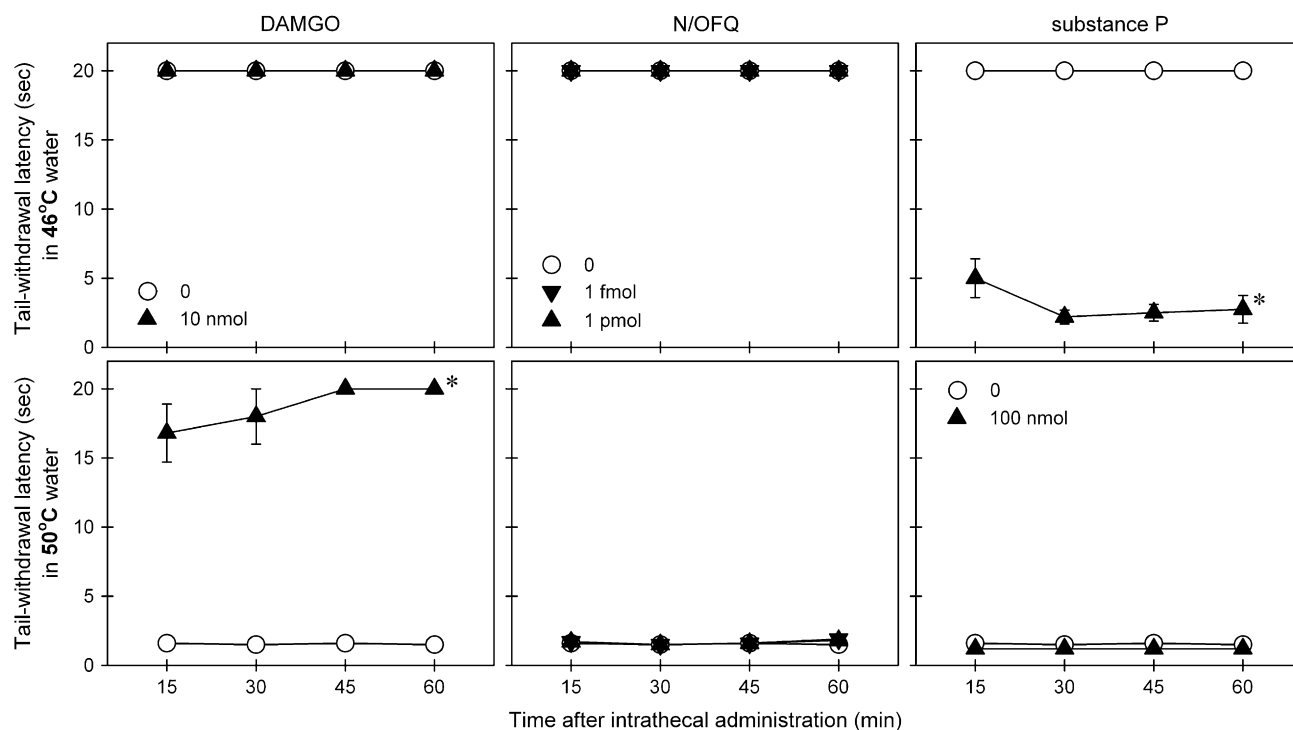


Figure 1. Comparison of warm water tail-withdrawal responses of intrathecally administered N/OFQ, DAMGO, and substance P. Top panels: Tail-withdrawal latency in 46°C water. Bottom panels: Tail-withdrawal latency in 50°C water. Behavioral responses were measured at 15-, 30-, 45-, and 60-minute time points after intrathecal administration of test compound, using a single dosing procedure. Each value represents mean \pm SEM ($n = 6$). Symbols represent different dosing conditions for the same monkeys. Asterisk represents a significant difference from the vehicle condition for all time points ($*P < .05$).

analysis of variance (ANOVA) followed by the Newman-Keuls test for multiple (post hoc) comparisons. For comparison of data at a single time point, data were analyzed by 1-way ANOVA followed by the Dunnett test for multiple comparisons. The criterion for significance was set at $P < .05$.

Drugs

N/OFQ, morphine sulfate (National Institute on Drug Abuse, Bethesda, MD), DAMGO, and substance P (Sigma-Aldrich, St. Louis, MO) were dissolved in sterile water. Doses are presented in the compound forms listed above. For intrathecal administration, N/OFQ, morphine, or the mixture of N/OFQ and morphine was administered at a total volume of 1 mL. The detailed description for intrathecal drug delivery can be referred to previous studies.^{20,21} All experiments using intrathecal administration were conducted with a 10-day inter-injection interval.

Results

Fig 1 illustrates distinct responses to nociceptive stimuli of monkeys receiving intrathecal administration of N/OFQ, DAMGO, and substance P. Intrathecal N/OFQ over a wide range of ultra-low doses (ie, from 1 fmol to 1 nmol) did not produce either hyperalgesic or antinociceptive responses (**Table 1**). In contrast, intrathecal substance P 100 nmol produced hyperalgesic responses in 46°C water [$F(1,5) = 1025.2$; $P < .05$] and intrathecal

DAMGO 10 nmol produced antinociceptive responses in 50°C water [$F(1,5) = 335.9$; $P < .05$].

Fig 2 compares distinct behavioral responses of monkeys after intrathecal administration of N/OFQ, DAMGO, and substance P. Intrathecal N/OFQ over a wide dose range of ultra-low doses did not elicit scratching responses (**Table 1**). Although intrathecal substance P 100 nmol significantly produced hyperalgesic effects, this dose of substance P did not elicit scratching responses. In contrast, intrathecal DAMGO 10 nmol significantly evoked scratching responses [$F(1,5) = 124.3$; $P < .05$] in addition to its antinociceptive effects. Scratching evoked by intrathecal DAMGO peaked at the first observation period (ie, 15 minutes after intrathecal administration) and continued throughout the 1 hour observation period (**Fig 2** and **Table 1**). It is worth noting that intrathecal administration of N/OFQ, DAMGO, and substance P at these doses did not cause any observable side effects including sedation and muscle relaxation.

Fig 3 shows behavioral responses of intrathecal N/OFQ at doses between 10 and 100 nmol. Intrathecal N/OFQ dose-dependently produced antinociceptive effects against a nociceptive stimulus, 50°C water [$F(3,15) = 28.1$; $P < .05$]. However, N/OFQ at these doses did not produce significant antinociception against a higher intensity of nociceptive stimulus, 54°C water and it did not elicit scratching responses under these conditions. For comparison, **Fig 4** shows behavioral responses of intrathecal N/OFQ at higher doses from 0.1 to 1 μ mol.

Table 1. Behavioral Responses of Intrathecal Administration of N/OFQ Over a Wide Range of Ultra-Low Doses as Compared to a Single Dose of DAMGO and Substance P.

Compound/Dose	WARM WATER TAIL-WITHDRAWAL LATENCY (sec)*		ITCH/SCRATCHING† NUMBER/15 MIN
	46°C	50°C	
N/OFQ			
0 (vehicle)	20 ± 0‡	1.6 ± 0.1	50.0 ± 12.9
1 fmol	20 ± 0	1.7 ± 0.1	33.8 ± 9.9
10 fmol	20 ± 0	1.6 ± 0.2	49.3 ± 15.7
100 fmol	20 ± 0	1.4 ± 0.2	44.3 ± 14.0
1 pmol	20 ± 0	1.7 ± 0.1	57.2 ± 15.3
10 pmol	20 ± 0	1.8 ± 0.1	57.5 ± 10.2
100 pmol	20 ± 0	1.9 ± 0.2	41.0 ± 15.1
1 nmol	20 ± 0	1.6 ± 0.2	35.2 ± 7.1
Substance P			
100 nmol	4.9 ± 1.4§	1.2 ± 0.1	48.5 ± 8.6
DAMGO			
10 nmol	20 ± 0	16.8 ± 2.1§	910.5 ± 103.9§

*The latency was measured at 15 min after intrathecal administration of test compound.

†The scratching number was scored between 15th and 30th min after intrathecal administration of test compound.

‡Each value represents mean ± S.E.M. (n = 6).

§The asterisk represents a significant difference from the vehicle condition ($P < 0.05$).

All 3 doses of intrathecal N/OFQ produced significant antinociception against 50°C water [$F(3,15) = 198.4$; $P < .05$]. In addition, N/OFQ dose-dependently produced antinociceptive effects against 54°C water [$F(3,15) = 15.1$, $P < .05$] without evoking scratching responses. It is worth noting that intrathecal administration of N/OFQ at these doses did not cause any observable side effects including sedation and motor impairment.

Fig 5 illustrates behavioral responses of intrathecal N/OFQ in combination with morphine. A single dose of intrathecal morphine 50 nmol produced antinociceptive effects against 50°C, not 54°C water (top 2 panels). This antinociceptive effect of intrathecal morphine was

accompanied by profound scratching responses (bottom panel). When N/OFQ was combined with intrathecal morphine, N/OFQ dose-dependently increased the mixture's antinociceptive effects against 54°C water [$F(3,15) = 14.2$; $P < .05$]. Under these conditions, increasing doses of N/OFQ did not attenuate intrathecal morphine-induced scratching responses.

Discussion

The present study showed that intrathecal administration of N/OFQ over a wide dose range (ie, from 1 fmol to 1 μ mol) produced thermal antinociception in the absence of hyperalgesia, scratching, sedation, and muscle relaxation. There were no sequelae to intrathecal N/OFQ, administered over several occasions consecutively in the same primates. For comparison, intrathecal administration of substance P 100 nmol significantly produced pronociceptive/hyperalgesic effects, manifested as reduced tail-withdrawal latencies in 46°C water. These results agree with rodent studies, indicating that intrathecal substance P causes hyperalgesic effects.^{27,30} Intrathecal administration of substance P and N/OFQ both produced a similar degree of hyperalgesic effects, as shown by decreased response latency approximately for 2 to 3 seconds in rodents.^{30,39} It has been suggested that intrathecal N/OFQ-induced hyperalgesia may be mediated by tachykinin NK1 receptors in the mouse spinal cord.^{39,40} Although intrathecal N/OFQ did not produce hyperalgesic effects like intrathecal substance P in monkeys, more studies are warranted to elucidate the relationship of intrathecal substance P with other neurotransmitter systems in the modulation of nociceptive processing of the primate spinal cord.

In contrast, intrathecal administration of DAMGO 10 nmol significantly produced antinociceptive effects, manifested as elevated tail-withdrawal latencies in 50°C water. These effects are consistent with rodent studies, indicating that intrathecal DAMGO is a potent μ -opioid antinociceptive agent.^{29,41} By testing intrathecal N/OFQ, substance P, and DAMGO in the same animals, they displayed distinct effects on modulating the nociceptive threshold. Such findings may suggest that intrathecal N/

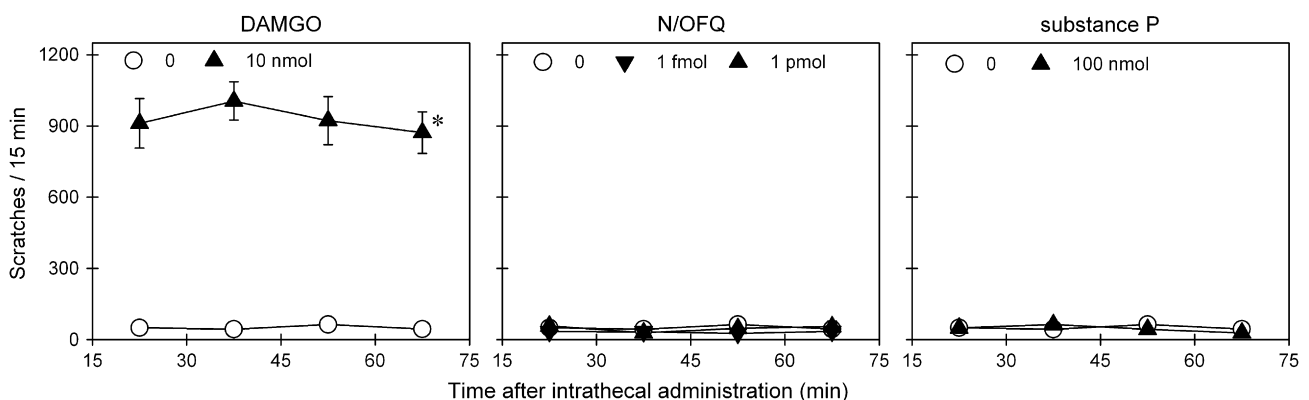


Figure 2. Comparison of itch/scratching responses of intrathecally administered N/OFQ, DAMGO, and substance P. Behavioral responses were scored for each 15-minute session after intrathecal administration of test compound, using a single dosing procedure. Each value represents mean ± SEM (n = 6). Symbols represent different dosing conditions for the same monkeys. Asterisk represents a significant difference from the vehicle condition ($*P < .05$).

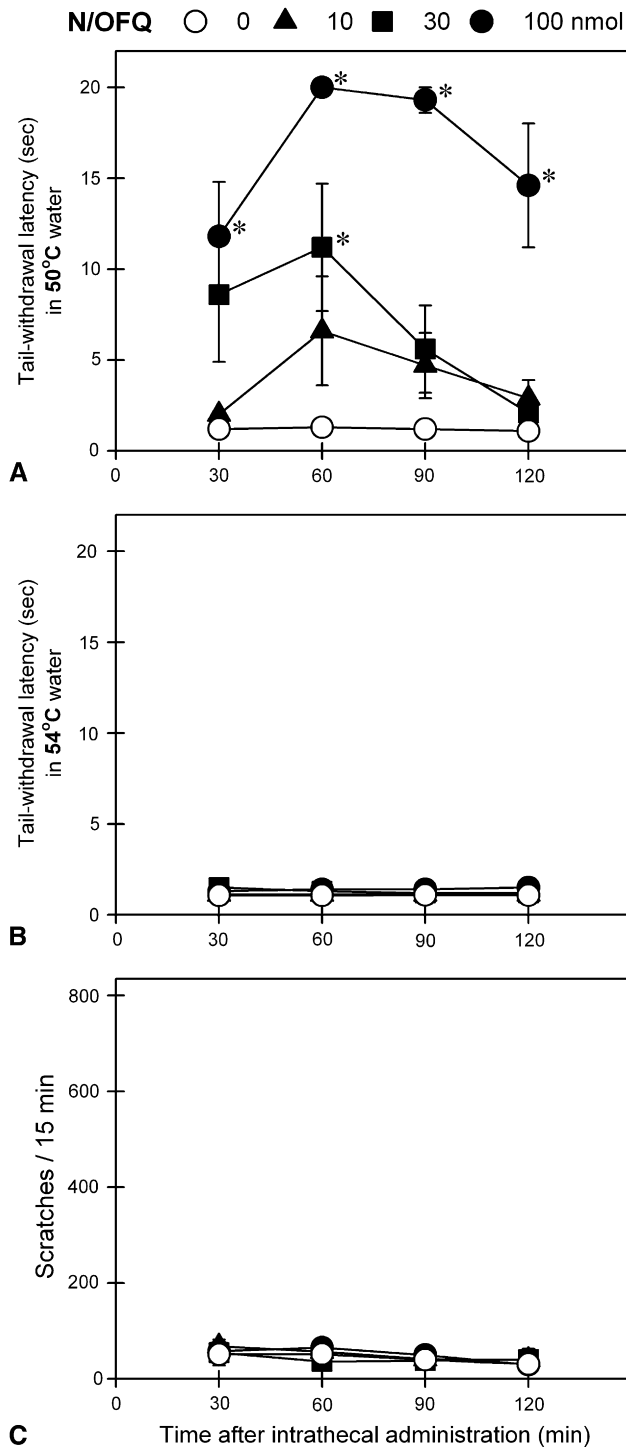


Figure 3. Behavioral responses of intrathecally administered N/OFQ at doses between 10 and 100 nmol. **A** and **B**, tail-withdrawal latency in 50° and 54°C water, respectively. **C**, itch/scratching responses for each 15-minute session crossing the time points, 30, 60, 90, or 120 minutes after intrathecal N/OFQ (ie, scratching number between 23rd and 38th minutes for the time point, 30 minutes). Each value represents mean \pm SEM ($n = 6$). Symbols represent different experimental conditions for the same monkeys. Asterisk represents a significant difference from the vehicle condition at corresponding time point ($*P < .05$).

OFQ over a wide dose range does not produce pronociceptive/hyperalgesic responses in monkeys under this context.

Intrathecal administration of either N/OFQ or substance P did not significantly elicit scratching responses, but only intrathecal DAMGO elicited profound scratching responses (Fig 2 and Table 1). Behavioral responses of intrathecal DAMGO are expected because previous studies have demonstrated that antinociceptive doses of μ -opioid receptor agonists elicited scratching responses in monkeys.^{18,20,26} It is well known that intrathecal morphine produces pain relief accompanied by simultaneous itch sensation in humans.^{1,34} These findings strongly support the notion that increased scratching responses in monkeys may represent a behavioral end point selective for itch sensation^{18,21} and may suggest that intrathecal N/OFQ and substance P do not elicit itch sensation in primates.

It is interesting to know that intrathecal administration of substance P and N/OFQ both elicited scratching responses in rodents.^{3,13,15,40} Nevertheless, rodents' scratching behavior may be neither necessary nor sufficient to be indicative of pain or itch sensation. For example, early studies showed that intrathecal substance P-induced scratching was not attenuated by pretreatment with analgesics, indicating that scratching is not pain-related.^{3,13} In contrast, increased scratching is considered as a sign of chronic pain in arthritic rats.¹¹ Perhaps a series of behavioral responses including scratching, biting, and licking^{15,40} after intrathecal substance P or N/OFQ represents a general behavioral spectrum in rodents under the state of pain or/and agitation, especially when additional measurements such as decreased response latency to a noxious stimulus were provided.^{30,39} On the other hand, increased scratching is also considered as a behavioral response to itch sensation in rodents receiving pruritogenic agents.^{16,23,25} Whether scratching behavior is pain-related or itch-related depends on the context. Several factors such as administration routes and species differences may also contribute to different results or interpretations in the behavioral pharmacology of itch. Therefore, it is very important to conduct more psychophysical studies in humans and functional studies in animals^{9,17,42} to further integrate and elucidate the physiological role of each neurotransmitter in the modulation of itch and pain sensation.

Intrathecal administration of N/OFQ at the dose range from 10 nmol to 1 μ mol dose-dependently produced antinociception against a noxious stimulus in 2 intensities (Figs 3 and 4). The magnitude of N/OFQ's antinociceptive effects in this assay is potentially similar to that of clinically available μ -opioid analgesics, such as nalbuphine, morphine, and fentanyl.^{5,18,43} Importantly, these antinociceptive doses of intrathecal N/OFQ did not elicit scratching responses. As previously demonstrated, intrathecal N/OFQ-induced antinociception was blocked by pretreatment with a selective NOP receptor antagonist, J-113397³³ but not by a classic opioid receptor antagonist, naltrexone.²² These findings together suggest that intrathecal N/OFQ or other NOP receptor agonists may have the therapeutic potential as spinal analgesics without side effects derived from μ -opioid receptor agonists. The degree of antinociception produced by an

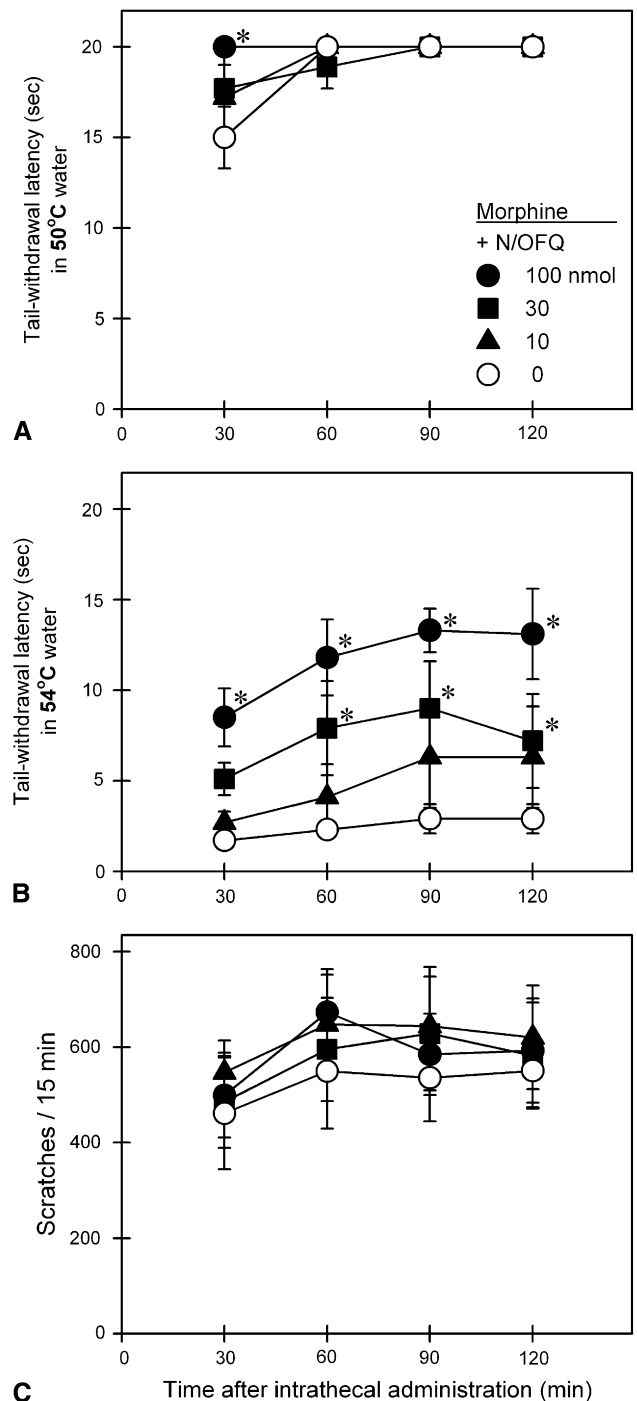
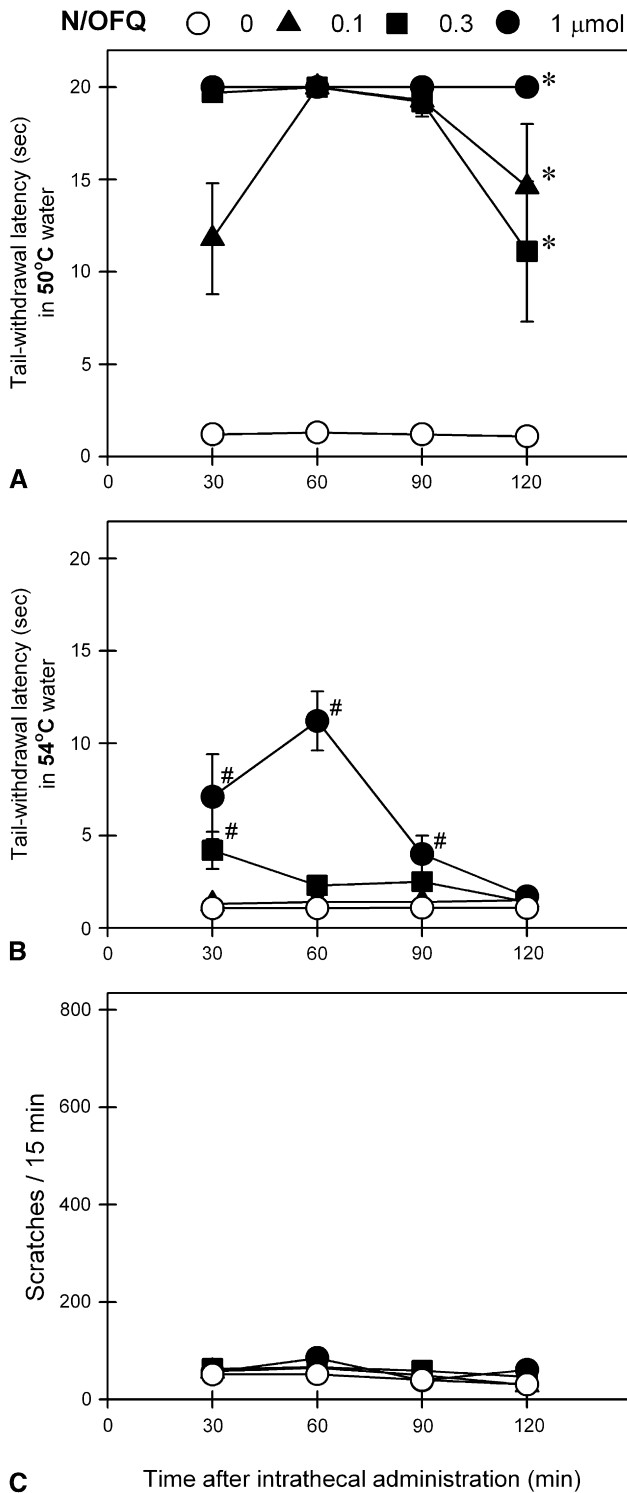


Figure 5. Behavioral responses of intrathecally administered N/OFQ in combination with morphine. Open circles represent the effects of intrathecal morphine 50 nmol alone. Other symbols represent effects of the same dose of morphine in combination with different doses of N/OFQ in the same monkeys. Each value represents mean \pm SEM ($n = 6$). Asterisk represents a significant difference from the control condition (ie, intrathecal morphine alone) at corresponding time point ($*P < .05$). See Fig 3 for other details.

experimental compound depends on its intrinsic efficacy and the nociceptive stimulus intensity.^{12,35,38} Future studies are needed to further investigate whether intrathecal N/OFQ or other NOP receptor agonists produce the same degree of antinociception as μ -opioid receptor agonists in

monkeys under different pain modalities. In particular, long-lasting NOP receptor agonists^{32,37} such as UFP-112 have been identified, and it would be important to study such agonists in the context of spinal delivery in primates.

When N/OFQ was combined with a single dose of intrathecal morphine, this addition potentiated intrathecal morphine-induced antinociception, manifested as elevated tail-withdrawal latencies in 54°C water, by increasing the dose of N/OFQ (Fig 5). Interestingly, addition of intrathecal N/OFQ did not attenuate intrathecal morphine-elicited scratching responses. These results may indicate that intrathecal N/OFQ potentiates morphine-induced antinociception without producing motor-related side effects because monkeys still display profound scratching responses. Furthermore, in contrast to anti-morphine actions of supraspinal N/OFQ,^{7,45} intrathecal N/OFQ did not produce anti-morphine actions, indicating that N/OFQ has different actions on spinal versus supraspinal sites.⁴⁵ It would be reasonable to expect that intrathecal administration of a mixture of morphine with NOP receptor agonists produces antinociceptive effectiveness with fewer side effects. It also would be interesting to investigate the development of tolerance

to antinociceptive effects of spinally administered morphine or/and NOP receptor agonists in future studies.

In summary, this study reveals a unique functional profile of intrathecal N/OFQ in primates. Unlike dual actions (ie, both pronociceptive and antinociceptive effects) of intrathecal N/OFQ observed in rodents, intrathecal N/OFQ over a wide dose range only produced antinociception. More importantly, intrathecal N/OFQ did not produce anti-morphine actions when it was combined with intrathecal morphine. The therapeutic potential of N/OFQ and the NOP receptors has been emphasized for its broad medical applications.^{24,45} Given that intrathecal N/OFQ produces antinociception without eliciting itch/scratching responses in monkeys, N/OFQ or other NOP receptor agonists represent a viable target as spinal analgesics for future clinical trials.

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Behavioral Effects of a Synthetic Agonist Selective for Nociceptin/Orphanin FQ Peptide Receptors in Monkeys

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Behavioral effects of a nonpeptidic NOP (nociceptin/orphanin FQ Peptide) receptor agonist, Ro 64-6198, have not been studied in primate species. The aim of the study was to verify the receptor mechanism underlying the behavioral effects of Ro 64-6198 and to systematically compare behavioral effects of Ro 64-6198 with those of a μ -opioid receptor agonist, alfentanil, in monkeys. Both Ro 64-6198 (0.001–0.06 mg/kg, s.c.) and alfentanil (0.001–0.06 mg/kg, s.c.) produced antinociception against an acute noxious stimulus (50°C water) and capsaicin-induced allodynia. An NOP receptor antagonist, J-113397 (0.01–0.1 mg/kg, s.c.), dose-dependently produced rightward shifts of the dose–response curve of Ro 64-6198-induced antinociception. The apparent pA_2 value of J-113397 was 8.0. Antagonist studies using J-113397 and naltrexone revealed that Ro 64-6198 produced NOP receptor-mediated antinociception independent of μ -opioid receptors. In addition, alfentanil dose-dependently produced respiratory depression and itch/scratching responses, but antinociceptive doses of Ro 64-6198 did not produce such effects. More important, Ro 64-6198 did not produce reinforcing effects comparable with those of alfentanil, cocaine, or methohexital under self-administration procedures in monkeys. These results provide the first functional evidence that the activation of NOP receptors produces antinociception without reinforcing effects in primates. Non-peptidic NOP receptor agonists may have therapeutic value as novel analgesics without abuse liability in humans. *Neuropsychopharmacology* (2009) **34**, 2088–2096; doi:10.1038/npp.2009.33; published online 11 March 2009

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INTRODUCTION

Opioid analgesics are the most effective and widely used drugs for pain management; the most clinically used opioids are μ -opioid receptor agonists (Zollner and Stein, 2007). However, there are several side effects associated with the use of μ -opioid agonists. These include constipation, respiratory depression, and itch/pruritus (Zollner and Stein, 2007). Importantly, the abuse liability derived from μ -opioid agonists has been and remains a serious public health concern and limits the opioid analgesics' value for pain management (Cicero *et al*, 2007; Katz *et al*, 2007). Research to identify potential analgesics with fewer side effects and reduced abuse liability is pivotal to advances in health care of all individuals.

Given that the neuroanatomical and physiological aspects of opioid receptors are similar between humans and monkeys

(Kuhar *et al*, 1973; Mansour *et al*, 1988; Peckys and Landwehrmeyer, 1999), the functions of opioid receptor subtypes can be investigated in nonhuman primates using a variety of behavioral assays and experimental compounds that are likely to be relevant to humans. In particular, the self-administration assay in monkeys has been used extensively, and it provides useful information for the abuse liability of drugs in humans (Weerts *et al*, 2007). Depending on the experimental schedules, most abused drugs in humans have been shown to have reinforcing effects in monkey self-administration procedures (Winger *et al*, 1975; Ator and Griffiths, 1987; Weerts *et al*, 2007). Although neither κ - nor δ -opioid agonists produce reinforcing effects, drugs in these categories do not have promising pharmacological profiles as strong analgesics because of their undesirable side effects. Centrally penetrating κ -opioid agonists' antinociceptive effects are compromised by sedation, and δ -opioid agonists are weak analgesics limited by potential convulsant effects (Dykstra *et al*, 1987; Negus *et al*, 1998).

The NOP receptor, previously called the ORL1 receptor, is defined as the fourth member within the opioid receptor family by the International Union of Pharmacology (Mollereau *et al*, 1994; Foord *et al*, 2005). An endogenous peptide selective for the NOP receptor, nociceptin/orphanin

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FQ (N/OFQ), has been identified and shown to have similar actions as other opioid peptides at the cellular level (Meunier *et al*, 1995; Reinscheid *et al*, 1995). Although activation of supraspinal NOP receptors may produce hyperalgesic effects (Meunier *et al*, 1995; Rizzi *et al*, 2007), most studies have shown that activation of peripheral and spinal NOP receptors produces antinociceptive effects in a variety of pain models in rodents (Erb *et al*, 1997; Zeilhofer and Calo, 2003; Obara *et al*, 2005). Interestingly, both peripheral and spinal administration of N/OFQ produce antinociceptive effects in monkeys, indicating a potential therapeutic value of NOP receptor agonists as analgesics (Ko *et al*, 2002b, 2006).

The development of a selective nonpeptidic NOP receptor agonist, Ro 64-6198 (Jenck *et al*, 2000; Wichmann *et al*, 2000), and antagonist, J-113397 (Kawamoto *et al*, 1999), provides an opportunity to study integrated behavioral effects of a NOP receptor agonist in animals following systemic administration (Chiou *et al*, 2007; Shoblock, 2007). However, to date, there is no study investigating the behavioral pharmacological actions of Ro 64-6198 in primates. In particular, it is important to investigate whether Ro 64-6198 produces any reinforcing effect/abuse liability in monkey self-administration procedures. Therefore, the aim of the study was to clarify the receptor mechanism underlying Ro 64-6198-induced behavioral responses. Antinociceptive effects of Ro 64-6198 were further examined using different pain modalities and various behavioral assays were applied to systematically compare effects between Ro 64-6198 and alfentanil, a μ -opioid receptor agonist, in monkeys.

MATERIALS AND METHODS

Subjects

Twenty seven adult gonadally intact male and female rhesus monkeys (*Macaca mulatta*) with body weights ranging between 6.6 and 11.7 kg were used. Twelve monkeys participated in the antinociception and itch/scratching studies, and another six monkeys participated in the respiration study. The remaining nine monkeys were used in the self-administration study. The monkeys were housed individually with free access to water and were fed approximately 25–30 biscuits (Purina Monkey Chow, product No. 5045; Ralston Purina, St Louis, MO) and fresh fruit daily. No monkey had exposure to any opioid receptor agonist or antagonist for 1 month before this study. The monkeys were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. The studies were conducted in accordance with the University Committee on the Use and Care of Animals at the University of Michigan and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health (National Academy Press, Washington DC, revised 1996).

EXPERIMENTAL PROCEDURES

Antinociception

The warm water (50°C) tail-withdrawal assay was used to evaluate thermal antinociceptive effects of the test

compound (Ko *et al*, 1998a). Briefly, monkeys were seated in primate restraint chairs, and the lower part of their shaved tails (approximately 15 cm) were immersed in a thermal flask containing water maintained at either 42, 46, or 50°C. Tail-withdrawal latencies were measured using a computerized timer by an experimenter who was blinded to experimental conditions. In each test session, monkeys were tested once with three temperatures given in a random order, and only the 50°C water was tested twice to confirm the full antinociceptive effect. If the monkeys did not remove their tails within 20 s, the flask was removed and a maximum time of 20 s was recorded. Test sessions began with control determinations at each temperature. Then, the test compound was administered subcutaneously by a cumulative dosing procedure with a 30-min interinjection interval. Subsequent tail-withdrawal latencies were determined starting 20 min after each injection.

The NOP receptor antagonist potency of J-113397 against Ro 64-6198-induced antinociception was determined by giving subjects different doses of s.c. J-113397 (0.01, 0.03, and 0.1 mg/kg) for *in vivo* apparent pA_2 analysis. In particular, the dose-response curve of s.c. Ro 64-6198 for antinociception was redetermined 15 min after pretreatment with a single dose of J-113397. A single dose of naltrexone (0.03 mg/kg) and J-113397 (0.1 mg/kg) was used to compare their antagonist effects against both alfentanil- and Ro 64-6198-induced antinociception. The dose and pretreatment time (ie, 15 min) for both naltrexone and J-113397 were chosen based on an earlier study (Ko *et al*, 1998a).

The tail-withdrawal latency in 46°C water following 0.1 mg of capsaicin administration was measured to evaluate the potential antiallodynic effects of analgesics (Ko *et al*, 1998b, 2002b). The procedure for studying thermal allodynia was slightly different from the general procedure for measuring thermal antinociception. The dose-response studies were measured by using a single-dosing procedure. The 46°C water was the thermal threshold for these subjects for expressing allodynic responses following the local injection of the capsaicin (Ko *et al*, 1998b, 2002b). After the chemical was administered s.c. in the tail, it dose-dependently produced thermal allodynia that peaked 15 min following the injection. This allodynic response was manifested as a reduced tail-withdrawal latency from a maximum value of 20 s to approximately 2–3 s in 46°C water. The test compounds, Ro 64-6198 and alfentanil, were administered s.c. 15 min before the capsaicin administration.

Scratching Responses

Scratching responses, inferred as an itch sensation (Ko *et al*, 2004), were recorded on videotapes when monkeys were in their home cages. The test compound was administered i.m. by a cumulative dosing procedure with a 30-min interinjection interval. Each recording session was conducted for 15 min/test session (ie, from 15 to 30 min for each drug injection cycle). A scratch was defined as one short-duration (<1 s) episode of scraping contact of the forepaw or hindpaw on the skin surface of other body parts. Scratching responses were scored by trained individuals who were blinded to experimental conditions. In addition, sedation was monitored by cumulative time for eye closure or lying down at the bottom of the cage. Both scratching

and sedation end points were summed into one score per session.

Respiratory Function

The apparatus is similar to that described previously (Butelman *et al*, 1993). The monkey was seated in a primate restraint chair, enclosed within a sound-attenuating chamber. A rectangular helmet ($13.5 \times 17.0 \times 13.5$ cm) was placed over the head of the monkey and sealed around its neck by two closely fitting latex shields. Gas (either air or a mixture of 5% CO₂ in air) flowed into the helmet and was pumped out at a rate of 8 l/min. The monkeys' breathing produced changes in pressure inside the helmet that were measured with a pressure transducer connected to a polygraph (Grass Model 7). The data were recorded on a polygraph trace and in a microprocessor (IBM PC) through an analog-to-digital converter. The polygraph integrator was connected to a computer, which analyzes the data collected over a 3-min period. The rate of breathing (f , respiratory frequency) is determined directly. The minute volume (V_E), the number of liters of air inspired per min, is determined from the integration of the plethysmograph system. The test compound was given i.m. in a cumulative dosing procedure, the test session contained 5–6 consecutive cycles of exposure to air. Each cycle was 30 min, which included a 23-min exposure to air alone and a 7-min exposure to 5% CO₂ mixed in air. The test compound was administered in the beginning of each test cycle and the doses were increased by a 0.25 or 0.5 log unit throughout the test sessions.

Self-Administration

Three groups of monkeys ($n = 3$ per group), with baselines of either alfentanil, cocaine, or methohexital self-administration were used to evaluate the reinforcing effects of Ro 64-6198. The common elements of the groups were that drug availability was signaled by a red stimulus light in the monkeys' home cages, and a fixed number of responses on a lever located beneath the stimulus light resulted in an infusion of drug or saline. The red light was extinguished and a green light was paired with the infusion. The red light remained off for a brief period after the infusion (timeout), during which time responding on the lever had no programmed consequence. Ro 64-6198 or saline was substituted for the baseline drug no more often than once every fourth session; two 2-h sessions were scheduled each day. In the two groups with alfentanil and cocaine baselines, each infusion followed 30 responses, which in turn, was followed by a 45-s timeout. In addition, each session comprised four components, each 25 min or 20 infusions in duration. The duration of the infusion pump, and therefore, the dose of the drug, was varied across components, so that dose-response observations could be made in each session (Winger *et al*, 1992).

A more rigorous evaluation of the reinforcing effects of Ro 64-6198 was made in the monkeys that had sodium methohexital as a baseline drug. In this case, a single dose of drug (0.1 mg/kg methohexital as baseline) was available throughout each twice-daily session on an FR 10–60 s schedule. The simpler schedule with a smaller response requirement as well as a comparison with a drug that is less

reinforcing than cocaine or alfentanil was used in these animals to increase the possibility of observing a reinforcing effect of Ro 64-6198.

Data Analysis

Mean values (mean \pm SEM) were calculated from all behavioral endpoint. Comparisons were made for the same monkeys across all test sessions in the same experiment. For the dose-response curves for antinociception, individual tail-withdrawal latencies were converted to percentage of maximum possible effect. The formula of the percentage of maximum possible effect is defined as ((test latency—control latency)/(cutoff latency, 20 s—control latency)) \times 100. ED₅₀ values were calculated by least-squares regression with the portion of the dose-response curves spanning the 50% maximum possible effect. The 95% confidence limits were also determined. Mean ED₅₀ values were considered to be significantly different when their 95% confidence limits did not overlap. For *in vivo* apparent pA₂ analysis (ie, multiple doses of antagonist), dose ratios between dose and response curves were analyzed in a Schild plot, and the mean J-113397 pA₂ value was averaged from the individual values following linear regression lines in the Schild plot. In addition, apparent pK_B values were determined for a single dose of antagonist by using a modified equation, $pK_B = -\log (B/(\text{dose ratio} - 1))$, where B equals the antagonist dose in moles/kg. Mean pK_B values \pm 95% confidence limits were averaged from individual pK_B values for J-113397 and naltrexone.

Mean number of injections earned or response rates for each dose of self-administered drug were calculated by averaging the results of each substitution trial for a given dose across all experimental subjects. The one-way ANOVA was conducted for data obtained from scratching, respiration, and self-administration experiments. Where appropriate, *post hoc* comparisons using the Tukey's test were made between the drug effect and the vehicle effect. The criterion for significance was set at $P < 0.05$.

Drugs

Alfentanil HCl, naltrexone HCl, (–)cocaine HCl, and (+)J-113397, provided by the National Institute on Drug Abuse (Bethesda, MD), were dissolved in sterile water. Ro 64-6198, provided by F. Hoffmann-La Roche AG (Basel, Switzerland), was dissolved in a solution of DMSO/Tween 80/sterile water in a ratio of 1:1:8. Capsaicin (Sigma, St Louis, MO) was dissolved in a solution of ethanol/Tween 80/saline in a ratio of 1:1:8, and it was administered s.c. in the terminal 3–6 cm of the tail with constant 0.1 ml volume. Methohexital, purchased from Ace Surgical Supplies (Brockton, MA), was diluted with sterile water. Doses are presented in the compound forms listed above. For systemic administration in antinociception, scratching, and respiration experiments, all test compounds were administered at a volume of 0.1 ml/kg.

RESULTS

Figure 1 illustrates the antagonist effect of J-113397 against Ro 64-6198-induced antinociception in 50°C water.

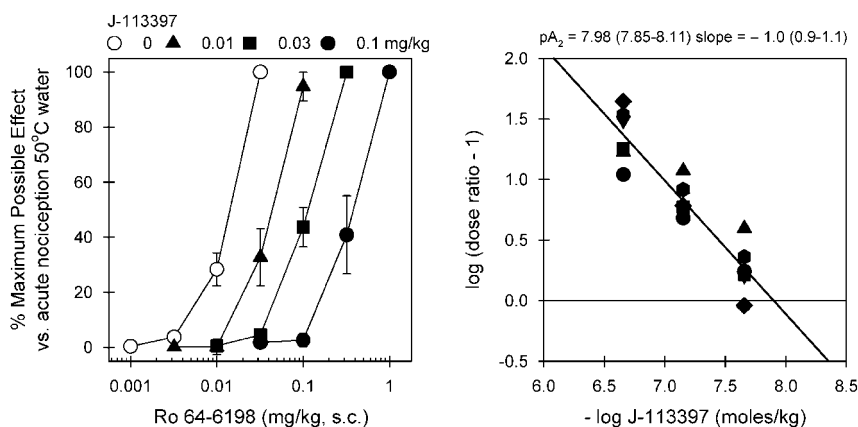


Figure 1 *In vivo* antagonist potency of J-113397 against Ro 64-6198-induced antinociception in monkeys. Left panel, antagonist effects of s.c. J-113397 on the dose-response curve of Ro 64-6198-induced antinociception in 50°C water. Each data point represents a mean \pm SEM ($n = 6$). Right panel, a Schild plot for J-113397. Abscissa, negative log unit for J-113397 in moles/kg. Ordinate, log of (dose ratio: 1). Each point was converted from individual dose ratio for each dosing condition presented in the left panel. Closed symbols represent different subjects. The mean pA_2 value and slope of J-113397 are shown with 95% confidence limits in parentheses.

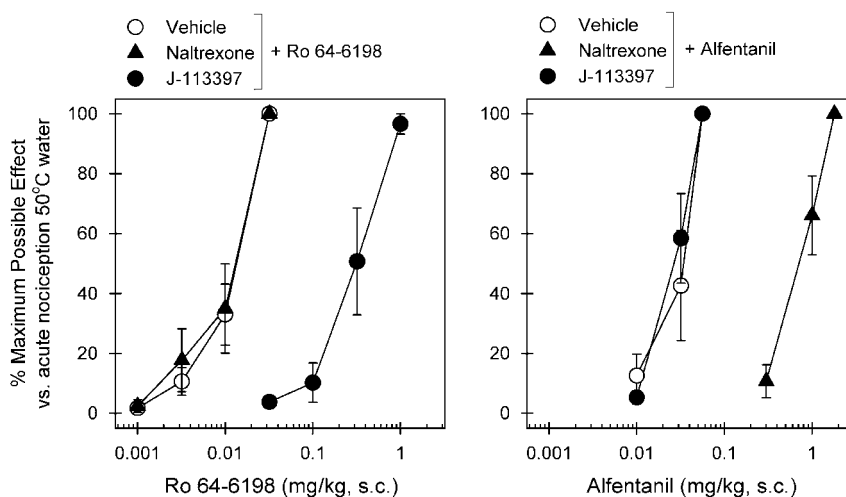


Figure 2 Effects of μ -opioid receptor and NOP receptor antagonists on alfentanil- and Ro 64-6198-induced antinociceptive effects in monkeys. A μ -opioid receptor antagonist (naltrexone, 0.03 mg/kg, s.c.) or an NOP receptor antagonist (J-113397, 0.1 mg/kg, s.c.) was administered s.c. 15 min before redetermination of the dose-response curve of alfentanil and Ro 64-6198. Left panel, antagonist effects of s.c. naltrexone and J-113397 on the dose-response curve of Ro 64-6198-induced antinociception in 50°C water. Right panel, antagonist effects of s.c. naltrexone and J-113397 on the dose-response curve of alfentanil-induced antinociception in 50°C water. Each data point represents a mean \pm SEM ($n = 6$).

Mean ED_{50} (95% confidence limit) value of s.c. Ro 64-6198-induced antinociception with vehicle pretreatment was 0.014 mg/kg (0.011–0.016). Pretreatment with J-113397 dose-dependently produced rightward shifts of the dose-response curve of Ro 64-6198-induced antinociception. These dose-dependent antagonist effects of J-113397 were graphed in a Schild plot with values derived from individual dose ratios for each subject. The mean pA_2 value of J-113397 was 7.98 (7.85–8.11) with a slope of -1 . The doses of J-113397 alone did not change the thermal threshold of monkeys (ie, no changes in the tail-withdrawal latencies in 42, 46, or 50°C water).

Figure 2 compares the antagonist effects of naltrexone and J-113397 on the antinociceptive effects produced by s.c. Ro 64-6198 and alfentanil. The left panel shows that a single dose (0.1 mg/kg) of J-113397 produced a large rightward shift of the dose-response curve of Ro 64-6198-induced antinociception. The mean J-113397 pK_B value was 8.02

(7.78–8.26) under this condition. Naltrexone 0.03 mg/kg failed to block Ro 64-6198-induced antinociception; the ED_{50} value of Ro 64-6198 dose-response for vehicle pretreatment (0.012 mg/kg) was similar to that for naltrexone pretreatment (0.013 mg/kg). In contrast, the right panel shows that a single dose of naltrexone 0.03 mg/kg produced a large rightward shift of the dose-response curve of alfentanil-induced antinociception. The mean naltrexone pK_B value was 8.44 (8.18–8.70) under this condition. J-113397 0.1 mg/kg failed to block alfentanil-induced antinociception; the ED_{50} value of alfentanil dose-response for vehicle pretreatment (0.031 mg/kg) was similar to that for J-113397 pretreatment (0.026 mg/kg).

Figure 3 illustrates the antinociceptive effects of Ro 64-6198 and alfentanil against capsaicin-induced allodynia. Normally, monkeys kept their tails in 46°C water for 20 s, but withdrew their tails within 1–3 s after capsaicin injection (mean \pm SEM, 1.7 ± 0.2 s). Pretreatment with Ro 64-6198

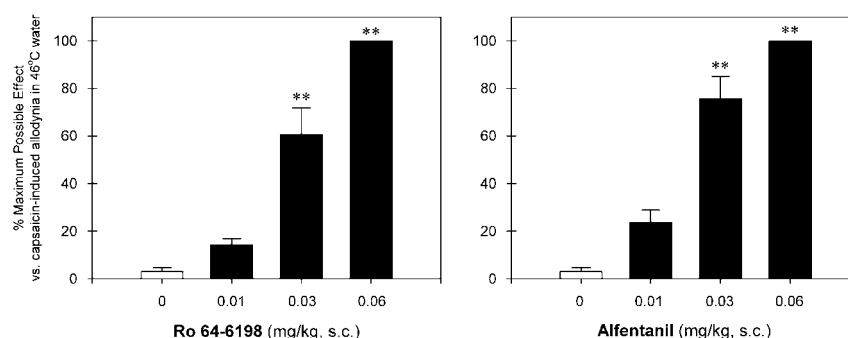


Figure 3 Antinociceptive effects of Ro 64-6198 and alfentanil against capsaicin-induced allodynia in 46°C water. Each data point represents a mean \pm SEM ($n = 6$). The asterisks represent a significant difference from the vehicle condition (** $p < 0.01$). Each data point was measured at 15 min after administration of capsaicin.

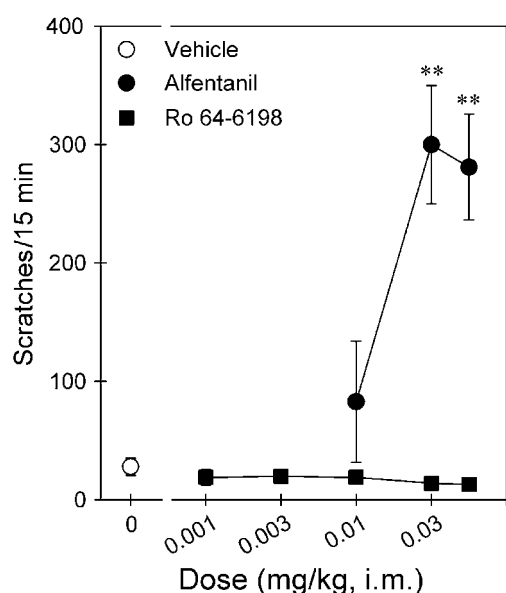


Figure 4 Comparison of the dose-response curves for itch/scratching effects produced by i.m. administration of alfentanil and Ro 64-6198. Each data point represents a mean \pm SEM ($n = 6$). The asterisks represent a significant difference from the vehicle condition (** $p < 0.01$).

($F(3,20) = 60.6$; $p < 0.01$) and alfentanil ($F(3,20) = 68.3$; $p < 0.01$) both dose-dependently attenuated allodynia in 46°C water. The ED_{50} value for Ro 64-6198 dose-response (0.024 mg/kg) was similar to that for alfentanil (0.019 mg/kg) under this condition.

Figure 4 compares the itch/scratching responses of alfentanil and Ro 64-6198 after i.m. administration. Alfentanil produced a dose-dependent increase in scratching ($F(3,20) = 11.0$; $p < 0.05$). *Post hoc* comparisons indicated that both doses of alfentanil 0.03 and 0.06 mg/kg significantly increased scratching responses ($p < 0.01$). The peak effect was 300 ± 49.9 (mean \pm SEM) scratches evoked by 0.03 mg/kg of alfentanil. In contrast, Ro 64-6198 did not increase scratching responses ($F(5,30) = 0.7$; $p > 0.05$), compared with the vehicle condition in the same monkeys. These doses of Ro 64-6198 (ie, 0.001–0.06 mg/kg) did not produce any observable sedation in monkeys.

Figure 5 compares the respiratory depressant effects of alfentanil and Ro 64-6198 after i.m. administration. The top panels show the dose-response curves of alfentanil and Ro 64-6198 for the changes of respiratory parameters f and V_E during air breathing. Alfentanil produced dose-dependent changes for both f ($F(4,25) = 3.3$; $p < 0.05$) and V_E ($F(4,25) = 9.3$; $p < 0.05$). *Post hoc* comparisons indicated that alfentanil 0.06 mg/kg significantly decreased f responses ($p < 0.05$). In addition, both doses of alfentanil, 0.03 and 0.06 mg/kg, significantly decreased V_E responses ($p < 0.05$). The maximum depressant effect of V_E responses produced by alfentanil 0.06 mg/kg was $55 \pm 5\%$ of control response (ie, before drug administration). In contrast, Ro 64-6198 did not decrease the respiratory function manifested by f ($F(5,30) = 0.2$; $p > 0.05$) and V_E ($F(5,30) = 1.4$; $p > 0.05$) responses, compared with the vehicle condition in the same monkeys.

The bottom panels show the dose-response curves of alfentanil and Ro 64-6198 for the changes of respiratory parameters f and V_E during breathing of a mixture of 5% CO_2 in air. This increase in CO_2 enhances the sensitivity of the assay to the potential respiratory depressant effects of test compounds. Alfentanil produced dose-dependent changes of both f ($F(4,25) = 14.1$; $p < 0.05$) and V_E ($F(4,25) = 19.4$; $p < 0.05$) under these conditions. *Post hoc* comparisons indicated that both alfentanil 0.03 and 0.06 mg/kg significantly decreased f and V_E responses ($p < 0.05$). The maximum respiratory depressant effect produced by alfentanil 0.06 mg/kg was 67 ± 3 and $46 \pm 4\%$ of control f and V_E responses, respectively. In contrast, Ro 64-6198 did not significantly decrease the respiratory parameters f ($F(5,30) = 1.3$; $p > 0.05$) and V_E ($F(5,30) = 2.4$; $p > 0.05$), compared with the vehicle condition in the same monkeys.

Figure 6 top panel shows the reinforcing effects of Ro 64-6198 in alfentanil-maintained monkeys. Response rates (responses/s) for saline, alfentanil, and Ro 64-6198 across a dose range of 0.03–30 μ g/kg per injection were assessed. To aggregate data across all three subjects, mean response rates engendered by each dose of each drug were averaged. Under the multiple component schedules, contingent saline infusions engendered very low response rates (< 0.3 responses/s). The top panel of Figure 6 presents the aggregate dose-response curves for alfentanil and Ro 64-6198. All animals self-administered alfentanil within the

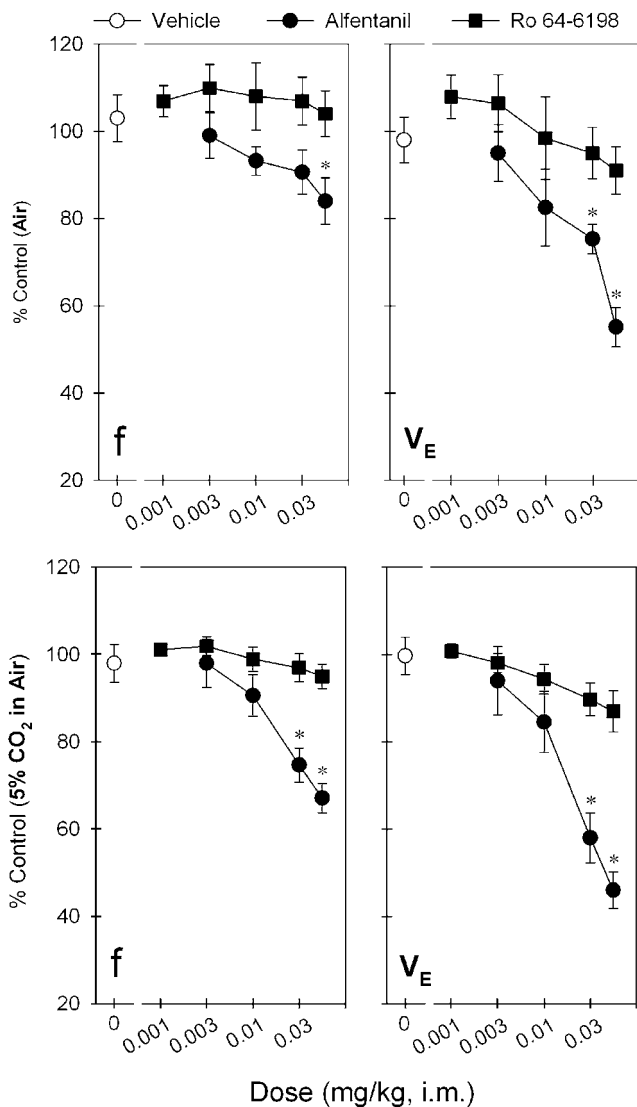


Figure 5 Comparison of the dose-response curves for respiratory depressant effects produced by i.m. administration of alfentanil and Ro 64-6198. Top and bottom panels show the changes of both f and V_E parameters when monkeys breathing air and air mixed with 5% CO₂, respectively. Each data point represents a mean \pm SEM ($n = 6$). The asterisk represent a significant difference from the vehicle condition (* $p < 0.05$).

dose range tested, generating a biphasic dose-effect curve characteristic of i.v. drug self-administration. In contrast, Ro 64-6198 did not maintain high rates of responding at any of the doses tested, resulting in a flat dose-effect curve indicative of a compound without reinforcing effects under the present conditions. Likewise, the middle panel indicates that Ro 64-6198 did not maintain high rates of responding at the doses tested, although all subjects self-administered cocaine under the same schedule.

Figure 6 bottom panel presents the aggregate dose-response curves for Ro 64-6198 compared with responding maintained by a reference dose of methohexital or saline. The number of injections earned of Ro 64-6198 across a dose range of 1–30 μ g/kg per injection were compared to the number of self-injections earned of 0.1 mg per kg/injection methohexital or saline. To aggregate data across all three

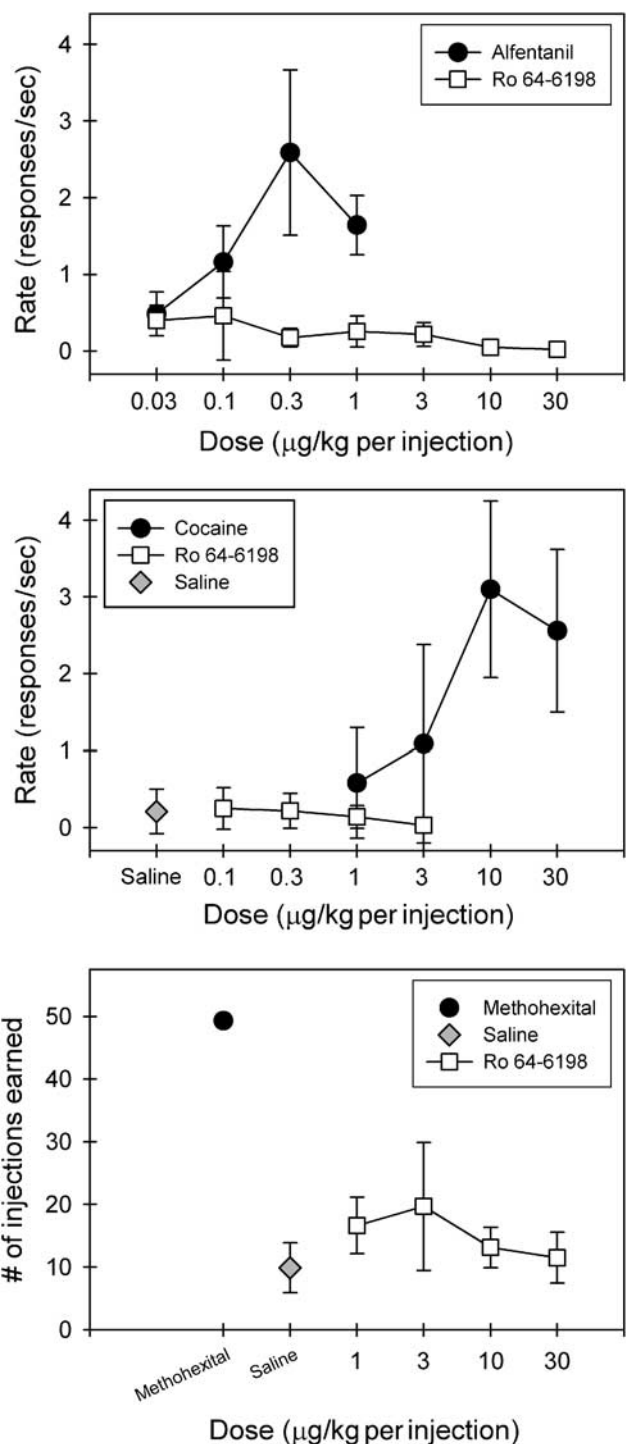


Figure 6 Lack of reinforcing effects of Ro 64-6198 in alfentanil-, cocaine-, and methohexital-maintained monkeys. Top and middle panels: Symbols represent aggregated dose-response curves for alfentanil, cocaine, and Ro 64-6198 under a fixed ratio of 30 timeout 45 s multiple component schedule. Data are the means \pm SEM ($n = 3$) for the response rates. Bottom panel: symbols represent aggregated dose-response curves for Ro 64-6198 compared with responding maintained by a single dose of methohexital 0.1 mg/kg/injection or saline. Data are the means \pm SEM ($n = 3$) for the numbers of injection earned.

experimental animals, mean number of injections earned by each monkey at each dose were averaged. Methohexital-maintained responding occurred at a high, regular rate

across the entire session. When contingent saline was available, animals tended to 'sample' early in the session, but behavior generally abated entirely within 15 min. No dose of Ro 64-6198 reliably maintained responding above levels observed when saline was available, indicating that Ro 64-6198 had no reinforcing effects under the present conditions.

DISCUSSION

Systemic Ro 64-6198 alone produced antinociceptive effects that were blocked dose-dependently by J-113397, a selective NOP receptor antagonist. *In vivo* apparent pA_2 analysis was used because this quantitative procedure offers a powerful approach to establish receptor-mediated drug effects (Arunlakshana and Schild, 1959; Tallarida *et al*, 1979). In this study, J-113397 dose-dependently produced parallel rightward shifts of the dose-response curve of Ro 64-6198-induced antinociception (Figure 1), indicating that the agonist and antagonist compete for the same NOP receptors in a reversible manner. The pA_2 value of J-113397, 8.0, was approximately threefold less than the naltrexone pA_2 value of 8.5 under the same behavioral context using an antinociceptive assay (Ko *et al*, 1998a), indicating that both naltrexone and J-113397 are potent antagonists *in vivo* for μ -opioid and NOP receptors, respectively, in monkeys. More important, examination of both antagonists against different agonists showed that alfentanil- and Ro 64-6198-induced antinociceptive effects were mediated by μ -opioid receptors and NOP receptors, respectively (Figure 2). J-113397 0.1 mg/kg failed to block alfentanil-induced antinociception and naltrexone 0.03 mg/kg failed to block Ro 64-6198-induced antinociception. These results indicate that antinociceptive effects of opioid analgesics are produced by two independent opioid receptor mechanisms in monkeys.

Systemic administration of Ro 64-6198-produced antinociception against capsaicin-induced allodynia in monkeys (Figure 3). Capsaicin evokes pain sensation by activating at the vanilloid receptor and stimulating the release of pronociceptive neuropeptides, such as substance P from primary afferents (Szallasi *et al*, 2007). Studies have shown that the vanilloid receptor is required for inflammatory sensitization to noxious stimuli and is essential for tissue injury-induced allodynia and hyperalgesia (Caterina *et al*, 2000; Davis *et al*, 2000). Capsaicin-induced allodynia has been used in both monkeys (Ko *et al*, 1998b; Butelman *et al*, 2004) and humans (Park *et al*, 1995; Eisenach *et al*, 1997) to show its prominent value for studying pain mechanisms *in vivo* and pharmacological interventions. Given that capsaicin-sensitive nerve fibers are involved in a variety of nociceptive conditions (Szallasi *et al*, 2007), the effectiveness of Ro 64-6198 in inhibiting capsaicin-induced allodynia indicates that NOP receptor agonists may be effective for treating pain derived from different nociceptive origins.

It is worth noting that systemic Ro 64-6198 did not produce antinociceptive effects in rodents (Jenck *et al*, 2000). Perhaps supraspinal NOP receptor-mediated hyperalgesia in rodents (Meunier *et al*, 1995; Rizzi *et al*, 2007) counteract antinociceptive effects mediated by spinal and

peripheral NOP receptors when rodents receive systemic administration of non-peptidic NOP receptor agonists. Given that both systemic and spinal administration routes are commonly used for delivery of analgesics in humans, it may not be practical to study the effects of intracerebroventricular administration of NOP receptor agonists in monkeys. Nevertheless, the degree of integrated physiological outcome from activating supraspinal, spinal, and peripheral NOP receptors together following systemic administration of NOP receptor agonists may vary across species. Anatomical studies have indicated that differences between rodents and primates may exist in the distribution of N/OFQ and NOP receptors (Berthele *et al*, 2003; Bridge *et al*, 2003). In addition, functional studies have also revealed that species differences exist in the pharmacological profiles of spinal N/OFQ between rodents and primates (Inoue *et al*, 1999; Sakurada *et al*, 1999). Unlike dual actions (ie, both pronociceptive and antinociceptive effects) of intrathecal N/OFQ observed in rodents, intrathecal N/OFQ only produced antinociceptive effects in monkeys (Ko and Naughton, 2009). More research should be conducted to elucidate whether the signal transduction pathways of NOP receptors or/and functions of sensory neurons expressing NOP receptors are different between rodents and primates.

The antinociceptive doses of systemic Ro 64-6198 (ie, 0.01–0.06 mg/kg) did not produce undesirable side effects compared with the μ -opioid agonist alfentanil (Figures 3 and 4). Both respiratory depression and itch/scratching have been documented as physiological responses to μ -opioid receptor activation in monkeys (Butelman *et al*, 1993; Ko *et al*, 2004). Given that these doses of Ro 64-6198 did not produce any sedation or motor dysfunction in monkeys, systemic Ro 64-6198 provides a promising pharmacological profile of NOP receptors as a novel analgesic in primates. On the other hand, rodent studies have found that higher doses of systemic Ro 64-6198 (10 mg/kg) interfered with behavioral performance (Jenck *et al*, 2000; Shoblock, 2007). These results suggest that Ro 64-6198 may have a wide therapeutic window between the antinociceptive doses and doses eliciting undesirable side effects. Whereas this study suggests that Ro 64-6198 may have a wide therapeutic index relative to the μ -opioid agonist alfentanil, it does not establish what the dose-limiting effects of this compound might be. Administration of larger doses of Ro 64-6198 and other systemically active NOP receptor agonists are needed to establish dose-limiting effects.

No reinforcing effects of Ro 64-6198 in alfentanil-, cocaine-, and methohexital-maintained monkeys (Figure 6) were observed. The presence of a behavioral effect (ie, antinociception at 10–30 μ g/kg) in the absence of any indication of a reinforcing effect indicates that we have tested sufficiently large doses for potential reinforcing effects. For example, the antinociceptive doses of i.v. alfentanil were 10–30 μ g/kg (Ko *et al*, 2002a), but the doses of alfentanil-producing reinforcing effects were 0.1–1 μ g/kg (ie, a 30–100-fold difference; Winger *et al*, 1992; Ko *et al*, 2002a). Lack of reinforcing effects by Ro 64-6198 might be expected because several studies have shown that the activation of NOP receptors inhibited dopamine release in the striatum, and supported the notion that NOP receptor

agonists do not have reinforcing or aversive properties of their own (Murphy and Maidment, 1999; Flau *et al*, 2002). Given that increased dopamine neuronal activity is closely associated with reinforcing effects of several drugs of abuse, it will be valuable to study further whether NOP receptor agonists can suppress the reinforcing effects of other drugs that have abuse potential in primates.

Taken together, this study showed that antinociceptive effects of systemic Ro 64-6198 were independent of μ -opioid receptors and activation of NOP receptors produced antinociception without reinforcing effects in monkeys. Ro 64-6198 has previously been studied in only rodent species (Chiou *et al*, 2007; Shoblock, 2007). This is the first study to investigate the behavioral effects of Ro 64-6198 in primates. Like alfentanil, Ro 64-6198 produced antinociception in two primate nociceptive models. Unlike alfentanil, Ro 64-6198 did not produce reinforcing effects, respiratory depressant, or itch/pruritic side effects, indicating that NOP receptor agonists may be a new generation of novel analgesics without abuse liability. Such a promising pharmacological profile warrants additional studies to document potential therapeutic value of NOP receptor agonists in humans.

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DISCLOSURE/CONFLICT OF INTEREST

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Long-lasting antinociceptive spinal effects in primates of the novel nociceptin/orphanin FQ receptor agonist UFP-112

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ABSTRACT

Chemical modifications of nociceptin/orphanin FQ (N/OFQ) peptide that result in increased potency and resistance to degradation have recently lead to the discovery of [(pF)Phe⁴Aib⁷Arg¹⁴Lys¹⁵]N/OFQ-NH₂ (UFP-112), a novel N/OFQ peptide (NOP) receptor agonist. The aim of this study was to investigate the pharmacological profile of intrathecally administered UFP-112 in monkeys under different behavioral assays. Intrathecal UFP-112 (1–10 nmol) dose-dependently produced antinociception against an acute noxious stimulus (50 °C water) and capsaicin-induced thermal hyperalgesia. Intrathecal UFP-112-induced antinociception could be reversed by a NOP receptor antagonist, J-113397 (0.1 mg/kg), but not by a classic opioid receptor antagonist, naltrexone (0.03 mg/kg). Like intrathecal morphine, UFP-112 produced antinociception in two primate pain models with a similar magnitude of effectiveness and a similar duration of action that last for 4–5 h. Unlike intrathecal morphine, UFP-112 did not produce itch/scratching responses. In addition, intrathecal inactive doses of UFP-112 and morphine produced significant antinociceptive effects when given in combination without increasing scratching responses. These results demonstrated that intrathecal UFP-112 produced long-lasting morphine-comparable antinociceptive effects without potential itch side effect. This study is the first to provide functional evidence that selective NOP receptor agonists such as UFP-112 alone or in conjunction with morphine may improve the quality of spinal analgesia.

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1. Introduction

Spinal administration of morphine is one of the most common clinical procedures for pain relief because of its long-lasting analgesic effects [7,8]. However, itch/pruritus is characteristic of intrathecal morphine treatment, with reported incidence rates ranging from 30% to 100% in humans [36]. Such unique physiological functions of intrathecal morphine can also be observed in non-human primates. For example, a single antinociceptive dose of intrathecal morphine elicited profound long-lasting itch/scratching responses in monkeys [17]. Importantly, pharmacological studies using monkeys have demonstrated that opioid analgesic-induced scratching response is selectively mediated by mu opioid receptors (MOP) [18] and that kappa opioid receptor (KOP) agonists have the therapeutic potential as antipruritics under this context [20,25]. These findings support that clinically used drugs with low or moderate

intrinsic efficacy on MOP or/and KOP are effective in alleviating opioid-induced itch [9,12,38]. Nevertheless, identification of novel targets as spinal analgesics devoid of MOP-induced pruritic effects still remains a challenge to the drug development.

Since the identification of the nociceptin/orphanin FQ (N/OFQ) peptide as the endogenous ligand of the N/OFQ peptide (NOP) receptor [26,33], this novel peptide receptor system has been implicated in the modulation of pain [23,39]. A variety of pain assays in rodents have shown the effect of intrathecally administered N/OFQ to be antinociceptive [11,29]. Peculiarly, unlike dual actions of intrathecal N/OFQ in rodents [13,35], intrathecal N/OFQ over a wide dose range only produced antinociceptive effects in monkeys [21]. More interestingly, using an established itch behavioral assay in monkeys [17], intrathecal N/OFQ produced dose-dependent antinociception without eliciting scratching responses [19,21]. These findings indicate that NOP receptor agonists may represent a promising target as spinal analgesics.

[(pF)Phe⁴Aib⁷Arg¹⁴Lys¹⁵]N/OFQ-NH₂ (UFP-112) is a recently designed NOP receptor agonist that results from chemical modifications to the N/OFQ peptide by increasing its agonist potency and decreasing its susceptibility to peptidase actions [1,34]. In rodent

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assays investigating a variety of physiological functions, UFP-112 consistently mimicked the effects of N/OFQ with markedly higher potency and longer duration of action [6]. Given that intrathecal morphine-induced antinociception only last for 1–2 h in rodents [4,27] and the duration of antinociception can be distinguished between intrathecal morphine (4–5 h) and N/OFQ (2–3 h) in monkeys [19], it is important to examine whether the high potency and long duration of action of UFP-112 can translate to non-human primates in the absence of an itch side effect.

Therefore, the aim of this study was to investigate the pharmacological profile of intrathecally administered UFP-112 in monkeys. Of chief concern were the two behavioral endpoints of antinociception and scratching. The potency and duration of intrathecal UFP-112- and morphine-induced antinociceptive effects were compared using both monkey models of acute nociception and capsaicin-induced hyperalgesia. Antagonist studies were conducted to determine the receptor mechanism underlying UFP-112-induced effects. In addition, the drug combination study was conducted to explore whether intrathecal UFP-112 in conjunction with morphine produced antinociception with less scratching responses.

2. Materials and methods

2.1. Subjects

Eighteen adult female and male rhesus monkeys (*Macaca mulatta*) ranging in body weight (7.1–12.1 kg) were used. The monkeys were individually housed and their daily diet consisted of approximately 25–30 biscuits (Purina Monkey Chow; Ralston Purina Co., St. Louis, MO), fresh fruit, and free access to water. All monkeys used were previously trained in the warm water tail-withdrawal assay and acclimated to being video-recorded in-cage. For 1 month prior to the study, the monkeys did not have exposure to any opioid compound. Six monkeys (three males and three females) participated in the first two parts of the study (see details in Section 2.3). Another six monkeys (two males and four females) participated in the third part of the study. The remaining six monkeys (three males and three females) were used in the last part of the study. The monkeys were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. The studies were conducted in accordance with the University Committee on the Use and Care of Animals in the University of Michigan (Ann Arbor, MI) and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health (Bethesda, MD).

2.2. Procedures

2.2.1. Nociceptive responses

2.2.1.1. Acute thermal nociception. The warm water tail-withdrawal assay was used to measure nociceptive responses to thermal stimuli and antinociceptive effects of test compounds [16]. Monkeys were seated in primate-restraining chairs, which allowed access to their shaved backs and exposure of their shaved tails (approximately 15 cm) to thermal flasks containing water maintained at 42, 46, or 50 °C. Forty-two and 46 °C water were used as normally non-noxious stimuli whereas 50 °C water was used as an acute noxious stimulus. If monkeys did not remove their tails within 20 s, the flask was removed and a maximum time of 20 s was recorded. Test session began with control determinations at each temperature. Then, tail-withdrawal latencies were determined at multiple time points after intrathecal administration of the test compound.

2.2.1.2. Capsaicin-induced hyperalgesia. Capsaicin 0.1 mg was administered subcutaneously in the terminal 5 cm of the unanesthetized monkey's tail. The monkey's tail wagged within the first 2 min after capsaicin administration. The 15 min after administration was the time of peak hyperalgesic effects of capsaicin and it was the time point to measure the tail-withdrawal latency in 46 °C water in order to evaluate antihyperalgesic effects of test compounds [16,22]. This hyperalgesic response was manifested as a reduced tail-withdrawal latency from a maximum value of 20 s to approximately 2–3 s in 46 °C water. The test compounds were administered at different time points before capsaicin administration. As noted, all behavioral responses were measured by individuals blinded to experimental conditions.

2.2.2. Itch/scratching responses

Monkeys were recorded in-cage for scratching behavior, which has been previously associated to an itch sensation [18]. Recording was done in 15-min intervals and scored by trained individuals blinded to experimental conditions. A scratch was counted and defined as a short (≤ 1 s) episode of scraping contact of the monkey's forepaw or hind paw on the skin surface of other body parts. Scratches often occurred by a hind paw repetitively at the same location. In addition, monkeys were monitored for sedation and muscle relaxation while in their home cages as previous studies [19,21,25].

2.3. Experimental design

The first part of the study was to determine and compare the behavioral responses of intrathecally administered UFP-112 (1–10 nmol) and morphine (100 nmol) along with corresponding dose-dependent effects. The dose range was selected based on previous studies and our pilot study [19,34]. Tail-withdrawal latency measurements were made at hour intervals for the duration of a 5-h time course following the intrathecal injection. The tail-withdrawal latencies at 42 and 46 °C were used to detect potential hyperalgesic/pronociceptive effects whereas the 50 °C latencies were used to measure antinociceptive effects. Recording for scratching observation was also done at 1 h intervals for the duration of a 5-h time course after each session of tail-withdrawal latency measurements. For clarification, the antinociceptive effects were measured during the 25th to 40th min of each hour. Subsequently, monkeys were returned to their home cages and scratching responses were recorded during the 45th to 60th min of each hour. Monkeys were monitored for their general motor functions such as gait and balance during each transition between their home cages and the procedure room.

The second part of the study was to determine the receptor mechanism underlying intrathecal UFP-112-induced antinociception. A single dose of 0.03 mg/kg naltrexone and 0.1 mg/kg J-113397 (1-[3R,4R/3S,4S]-1-(Cyclooctylmethyl)-3-(hydroxymethyl)-4-piperidiny]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one) was used to compare their antagonist effects against both morphine (100 nmol)- and UFP-112 (10 nmol)-induced antinociception. The doses for both naltrexone and J-113397 were chosen based on a recent study [22] showing that both antagonists produced similar degrees of rightward shifts of dose-response curves of MOP and NOP agonists, respectively. The dose of J-113397 did not produce pronociceptive effects by itself [19,22].

The third part of the study was to assess the potency and duration of antihyperalgesia of intrathecally administered UFP-112 and morphine against capsaicin. Transient receptor potential vanilloid subfamily member 1 (TRPV1) is a transduction molecule for noxious stimuli. Capsaicin elicits pain sensation by activating TRPV1 and it has been used in monkeys [5,16] and humans [10,32] to study experimental analgesics in a broader therapeutic-relevant

context. Given that TRPV1-containing nerve fibers are involved in various pain origins, it is essential to determine and compare the antinociceptive effectiveness of intrathecal UFP-112 and morphine in monkeys receiving capsaicin. The drug was administered intrathecally 1 h prior to administration of capsaicin. Then, tail-withdrawal latencies were measured 15 min following capsaicin administration. The dose–response curves of intrathecal UFP-112 (0.3–10 nmol) and morphine (3–100 nmol) were established by using a single dosing procedure. In addition, intrathecal UFP-112 (10 nmol) and morphine (100 nmol) were used to determine their durations of antinociception at different time points before capsaicin administration.

The last part of the study was to investigate whether combination of inactive doses of intrathecal UFP-112 (1 nmol) and morphine (3 nmol) produced increased antihyperalgesia with less scratching responses. UFP-112, morphine, or the combination of UFP-112 and morphine was administered intrathecally 1 h before capsaicin administration. For scratching measurement, the same dosing conditions were conducted separately in the same subjects without capsaicin administration. In addition, antagonist studies were conducted to investigate the receptor mechanism underlying antihyperalgesia by the mixture of intrathecal UFP-112 (1 nmol) and morphine (3 nmol). A single dose of 0.03 mg/kg naltrexone or/and 0.1 mg/kg J-113397 were administered 30 min after administration of the mixture to determine the degree of antagonist effects.

2.4. Data analysis

Mean values (mean \pm SEM) were calculated from individual values for all behavioral endpoints. As noted, we did not find a significant difference in nociceptive responses or effects of drugs between male and female monkeys, so mean values for all monkeys in the same dosing condition were used for data analysis.

Measurement differences were compared across all tests sessions in the same experiment. Data were analyzed by using two-way analysis of variance followed by the Newman–Keuls test for multiple comparisons. Comparisons of data at a single time point were conducted by using one-way analysis of variance followed by the Dunnett test for multiple comparisons. The criterion for significance for all tests was set at $p < 0.05$.

2.5. Drugs

UFP-112 (synthesized and purified as described by Arduin et al. [1] at the University of Ferrara, Ferrara, Italy), morphine sulfate (Mallinckrodt, Hazelwood, MO), (\pm)J-113397 (1-[3R,4R/3S,4S)-1-(Cyclooctylmethyl)-3-(hydroxymethyl)-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one) (Tocris Bioscience, Ellisville, MO), and naltrexone HCl (National Institute on Drug Abuse, Bethesda, MD) were dissolved in sterile water. Capsaicin (Sigma, St. Louis, MO) was dissolved in a solution of ethanol/Tween 80/saline in a ratio of 1:1:8. Intrathecal doses were administered in a random order at a total volume of 1 mL. A detailed description of the intrathecal drug delivery procedure has been previously described [18]. All experiments (i.e., all four parts of the study) using intrathecal administration were conducted with a 10-day inter-injection interval as previous studies did [18–21].

3. Results

Fig. 1 compares the behavioral responses of intrathecally administered UFP-112 and morphine in monkeys. Intrathecal UFP-112 produced antinociception against acute nociceptive stimulus, 50 °C water, in both dose- [$F(3, 15) = 33.7$; $p < 0.05$] and time [$F(4, 20) = 7.8$; $p < 0.05$]-dependent manners. Post hoc comparisons indicated that both 3 and 10 nmol of intrathecal UFP-112 produced significant antinociception between 0.5- and 4.5-h time points. Although intra-

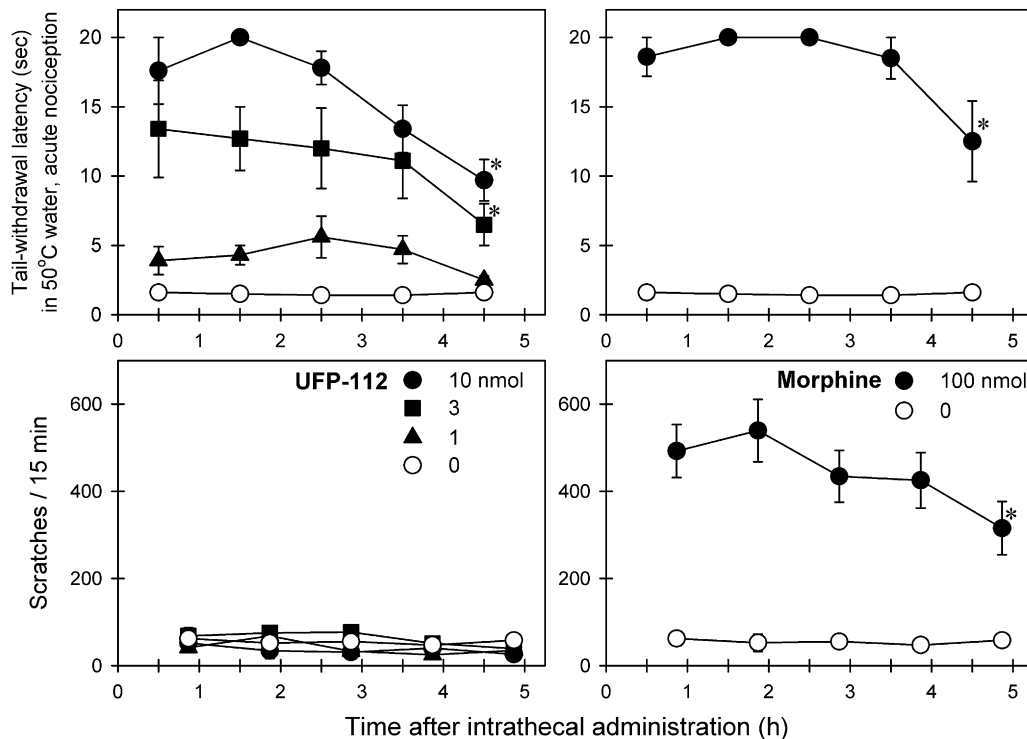


Fig. 1. Comparison of behavioral effects produced by intrathecal administration of UFP-112 and morphine. Abscissas: time in hours after intrathecal administration. Ordinates: latency to withdraw the tail in 50 °C water (top panels) and scratches per 15 min (bottom panels). Each value represents mean \pm SEM ($n = 6$). Symbols represent different dosing conditions in the same monkeys. The asterisk represents a significant difference from the vehicle condition at corresponding time points ($*p < 0.05$).

thecal UFP-112 produced long-lasting antinociception, it did not elicit significant scratching responses [$F(3, 15) = 2.3$; $p = 0.1$] at any dose or time point. In contrast, 100 nmol of intrathecal morphine produced both antinociception and scratching during the entire 5-h session ($p < 0.05$). Administration of either UFP-112 or morphine at these doses did not cause any overt side effects including sedation and muscle relaxation. In addition, there was no motor dysfunction detected during monkeys' transition between their home cages and the procedure room.

Fig. 2 illustrates the effects of antagonists on intrathecal UFP-112 (10 nmol)- and morphine (100 nmol)-induced antinociception in monkeys. For antagonist effects on UFP-112 antinociception (left panel), there were significant differences among the dosing conditions [$F(2, 10) = 458.4$; $p < 0.05$]. Post hoc comparisons indicated that a post-injection with a NOP receptor antagonist, J-113397 (0.1 mg/kg), significantly blocked intrathecal UFP-112-induced antinociception ($p < 0.05$). However, naltrexone 0.03 mg/kg failed to block intrathecal UFP-112-induced antinociception. For antagonist effects on morphine antinociception (right panel), there were significant differences among the dosing conditions [$F(2, 10) = 673.1$; $p < 0.05$]. Post hoc comparisons indicated that a post-injection with the same dose of an opioid receptor antagonist, naltrexone (0.03 mg/kg), significantly blocked intrathecal morphine-induced antinociception ($p < 0.05$). However, J-113397 (0.1 mg/kg) failed to block intrathecal morphine-induced antinociception.

Fig. 3 compares the effectiveness and potency of intrathecal UFP-112- and morphine-induced antihyperalgesia. Pretreatment with UFP-112 [$F(4, 25) = 36.5$; $p < 0.05$] or morphine [$F(4, 25) = 31.4$; $p < 0.05$] dose-dependently attenuated capsaicin-induced hyperalgesia in 46 °C water. The ED_{50} (95% confidence limit) values of intrathecal UFP-112 and morphine were 2.0 (1.2–3.4) and 11.8 (4.7–29.5) nmol, respectively. Post hoc comparisons indicated that 3–10 nmol of UFP-112 or 10–100 nmol of morphine significantly produced antihyperalgesia as compared with the vehicle condition under this context ($p < 0.05$).

Fig. 4 illustrates the duration of antihyperalgesia of intrathecal UFP-112 and morphine. A single dose 10 nmol of intrathecal UFP-112 produced antihyperalgesia in a time-dependent manner [$F(5, 30) = 59.7$; $p < 0.05$]. A single dose 100 nmol of intrathecal morphine also produced antihyperalgesia time-dependently

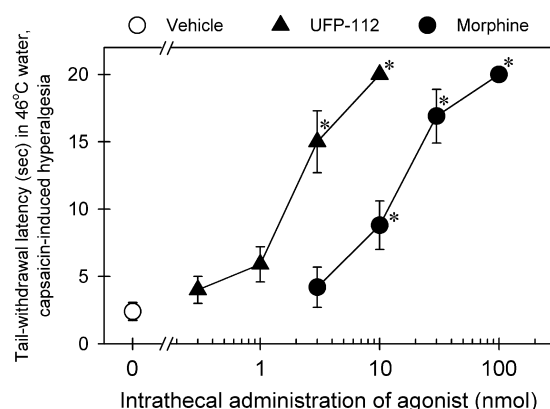


Fig. 3. Antinociceptive effects of intrathecally administered UFP-112 and morphine against capsaicin-induced hyperalgesia in 46 °C water. The agonist was given intrathecally 1 h before administration of capsaicin (0.1 mg/tail). Each data point was determined 15 min after capsaicin administration and represents mean \pm SEM ($n = 6$). Symbols represent effects with different dosing conditions in the same monkeys. The asterisk represents a significant difference from the vehicle condition (* $p < 0.05$).

[$F(5, 30) = 71.4$; $p < 0.05$]. Both intrathecal UFP-112 and morphine had similar durations of antihyperalgesia, i.e., full antihyperalgesia at the 4-h time point followed by partial antihyperalgesia at the 5-h time point ($p < 0.05$).

Fig. 5 illustrates behavioral responses of intrathecal UFP-112 in combination with morphine. The top panel shows the antihyperalgesic effects of intrathecal UFP-112 (1 nmol), morphine (3 nmol), and the combination of UFP-112 and morphine against capsaicin. There were significant differences among the dosing conditions [$F(3, 20) = 16.9$; $p < 0.05$]. Intrathecal UFP-112 or morphine alone did not produce statistically significant effects. In contrast, intrathecal UFP-112 in combination with morphine significantly produced antihyperalgesia ($p < 0.05$). The bottom panel shows the scratching-eliciting effects of intrathecal UFP-112 (1 nmol), morphine (3 nmol), and the combination of UFP-112 and morphine. There was no significant difference among the dosing conditions [$F(3, 20) = 1.6$; $p = 0.2$]. Intrathecal UFP-112 in combination with morphine did not increase scratching responses.

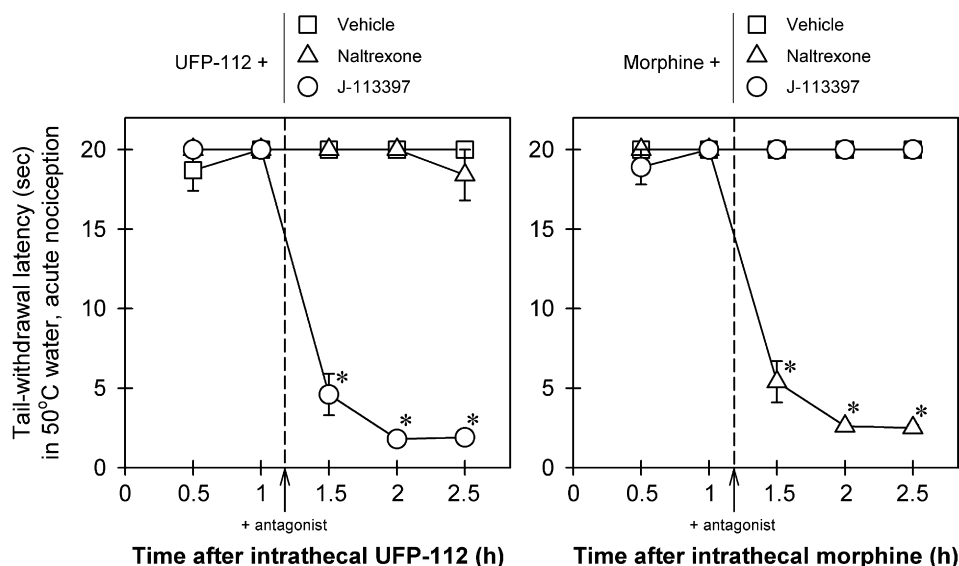


Fig. 2. Effects of antagonists on intrathecal UFP-112 and morphine-induced antinociception in monkeys. The antagonist, either naltrexone (0.03 mg/kg) or J-113397 (0.1 mg/kg), was given subcutaneously 10 min after the 1-h time point. Abscissas: time in hours after intrathecal administration. Ordinates: latency to withdraw the tail in 50 °C water. Each value represents mean \pm SEM ($n = 6$). Symbols represent effects with different post-injection conditions for the same monkeys. The asterisk represents a significant difference from the vehicle post-injection condition between time points 1.5 and 2.5 h (* $p < 0.05$).

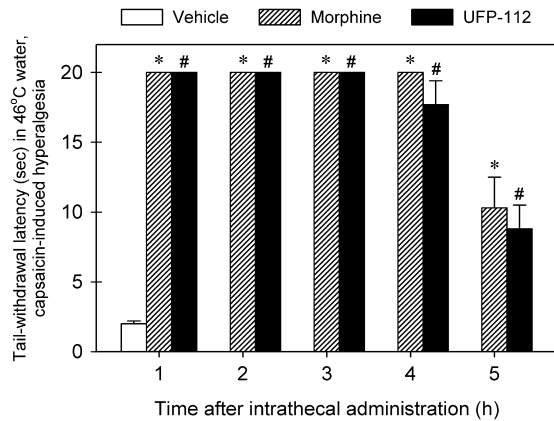


Fig. 4. Comparison of durations of intrathecal UFP-112- and morphine-induced antihyperalgesia in 46 °C water. The antihyperalgesic effect of the agonist, either morphine (100 nmol) or UFP-112 (10 nmol), was determined by using a single dosing procedure. The agonist was given intrathecally at different time points before administration of capsaicin (0.1 mg/tail). Each data point was determined 15 min after capsaicin administration and represents mean \pm SEM ($n = 6$). Symbols represent effects with different dosing conditions for the same monkeys. The asterisk represents a significant difference between morphine-treated condition and the vehicle condition ($*p < 0.05$). The symbol # represents a significant difference between UFP-112-treated condition and the vehicle condition ($*p < 0.05$).

Fig. 6 compares the effects of antagonists on antihyperalgesia produced by intrathecal combination of UFP-112 (1 nmol) and morphine (3 nmol) (i.e., a mixture). There were significant differences among the dosing conditions [$F(3, 20) = 8.8$; $p < 0.05$]. Post-injection with naltrexone (0.03 mg/kg) or J-113397 (0.1 mg/

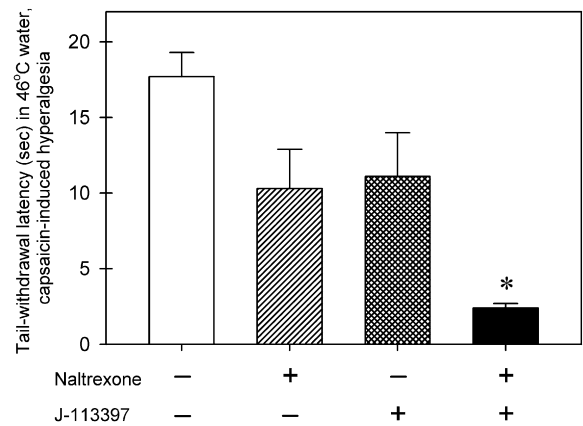


Fig. 6. Effects of antagonists on antihyperalgesia by intrathecal combination of UFP-112 (1 nmol) and morphine (3 nmol) (i.e., a mixture). The antagonist, either naltrexone (0.03 mg/kg) or J-113397 (0.1 mg/kg), was given subcutaneously 30 min after intrathecal administration of a mixture. The symbol “+” indicates the corresponding compound was given. The symbol “-” indicates the corresponding compound was not given. Each value represents mean \pm SEM ($n = 6$). The asterisk represents a significant difference from the vehicle condition ($*p < 0.05$).

kg) alone did not produce a significant blockade of intrathecal mixture-induced antihyperalgesia. However, combined systemic administration of naltrexone and J-113397 significantly blocked intrathecal mixture-induced antihyperalgesia ($p < 0.05$).

4. Discussion

This study demonstrated that intrathecal administration of UFP-112 produced long-lasting morphine-comparable antinociception against both acute pain and capsaicin-induced hyperalgesia. Intrathecal UFP-112-induced antinociception was not accompanied by itch/scratching responses and its action was exclusively mediated by NOP receptor activation. In addition, combination of inactive doses of intrathecal UFP-112 and morphine produced antihyperalgesia without scratching responses. This study is the first to provide direct functional evidence and translational value in primates that NOP receptor agonists such as UFP-112 alone or in conjunction with morphine will improve the quality of spinal analgesia.

As previously reported, intrathecal N/OFQ produced antinociception for 2–3 h in monkeys [19,21]. The duration of intrathecal N/OFQ-induced antinociception is significantly shorter than intrathecal morphine-induced antinociceptive effects that have at least 4–5 h in primates [17,19]. With improved resistance to enzymatic degradation, a novel NOP receptor agonist, UFP-112 [1,34], produced antinociception that last for 4–5 h in the same experimental context. Similar longer durations of actions were obtained in rodent studies while the same effects elicited by N/OFQ lasted only for approximately 1 h [6,34]. Furthermore, UFP-112 (10 nmol) is approximately 10-fold more potent than N/OFQ (i.e., 100 nmol) [19] in producing full antinociceptive effects in monkeys. This potency ratio between intrathecal UFP-112- versus N/OFQ-induced antinociception is consistent with rodent studies showing that UFP-112 displayed 10- to 100-fold higher potency than N/OFQ in a variety of rodent *in vivo* assays [6]. To our knowledge, UFP-112 is the first reported peptide that has such a long-lasting antinociceptive action comparable to morphine in a primate species. It will be interesting to further conduct pharmacokinetic studies comparing the CSF levels of UFP-112 and morphine following intrathecal administration.

The pharmacological profile of intrathecal morphine in producing antinociception with profound itch/pruritic effect is well

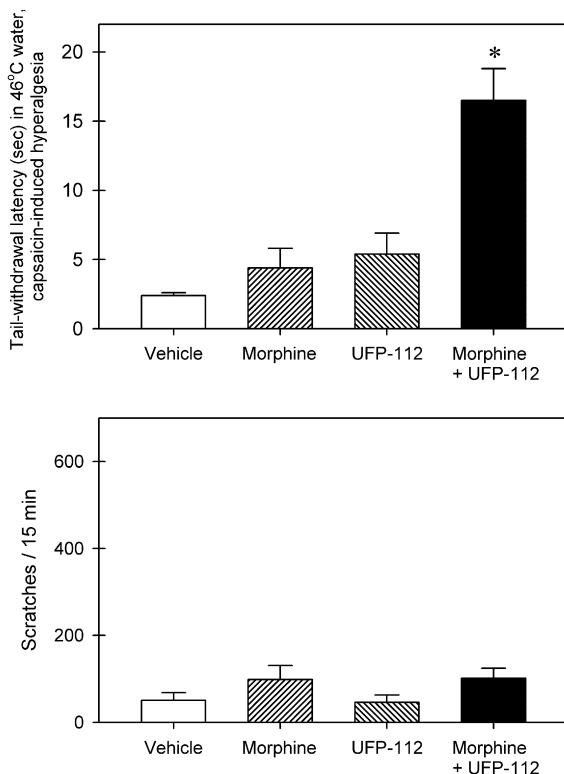


Fig. 5. Behavioral responses of intrathecally administered UFP-112 in combination with morphine. Effects of intrathecal morphine (3 nmol) or UFP-112 (1 nmol) alone were determined separately. Then effects of intrathecal administration of a mixture, i.e., 3 nmol of morphine combined with 1 nmol of UFP-112, were tested in the same monkeys. Each value represents mean \pm SEM ($n = 6$). The asterisk represents a significant difference from the vehicle condition ($*p < 0.05$).

known in the clinical setting [2,3,31]. Interestingly, such unique effects of intrathecal morphine in humans [2,31] can be modeled in monkeys, but not in rodents, as a single dose of morphine produced both antinociception and itch/scratching responses simultaneously in monkeys [17,20,24]. For studying opioid analgesics *in vivo*, the scratching response can be used as a selective behavioral endpoint corresponding to activation of MOP [18,25]. Given that pruritus is a long standing side effect associated with the use of intrathecal morphine [7,8], lack of scratching responses by intrathecal UFP-112 in monkeys strongly suggests that UFP-112 has the therapeutic potential as a spinal analgesic.

The results of the antagonist study on intrathecal UFP-112 mirrored those of antagonist studies on intrathecal N/OFQ [19]. Naltrexone, a classical opioid receptor antagonist, failed to block the antinociception produced by UFP-112, indicating that the actions of intrathecal UFP-112 are not mediated by classic opioid receptor subtypes. In contrast, J-113397, a selective NOP receptor antagonist [14,30], significantly blocked the antinociceptive effects of intrathecal UFP-112, but not morphine. The dose 0.1 mg/kg of J-113397 has been previously used to provide a large rightward shift (~10- to 30-fold) of the dose–response curve to the non-peptide NOP receptor agonist Ro 61-6198 in monkeys [22]. Intrathecal UFP-112-induced antinociception can be fully reversed by J-113397, demonstrating that the antinociceptive action of UFP-112 in monkeys is due to selective NOP receptor activation. These data confirm and extend to non-human primates the high selectivity of NOP action of UFP-112 that has been previously demonstrated in rodents. In fact, all the *in vitro* and *in vivo* actions of UFP-112 (and N/OFQ) in rats and mice are sensitive to the NOP selective antagonist UFP-101 and no longer present in the NOP receptor knockout mice [6].

While the acute noxious stimulus stands as a convenient pain model to test experimental analgesics, this study further demonstrated that intrathecal UFP-112 alleviated capsaicin-induced thermal hyperalgesia in monkeys. Capsaicin is a natural irritant found in hot-chili peppers that evokes pain sensation by activating at the TRPV1. TRPV1 and the up-regulation of its expression have been implicated in the transduction of a variety of noxious stimuli including tissue-injury induced thermal hyperalgesia, diabetic neuropathy, and neurogenic inflammatory response associated with many disease states [15,37]. Furthermore, capsaicin-induced hyperalgesia has been previously utilized as a pain model in both monkeys [5] and humans [10,32] to study experimental compounds as analgesics. Considering the variety of pain modalities capsaicin-sensitive fibers are linked to, the UFP-112's ability to attenuate capsaicin-induced hyperalgesia would suggest a prominent clinical value.

The dose–response curves established under the capsaicin pain model illustrated that intrathecal UFP-112 was more potent than morphine. Importantly, the results illustrated equally maximum antihyperalgesic effect for both UFP-112 and morphine. A key characteristic of antinociceptive effects of NOP receptor agonists previously studied in both rodents and primates was a relatively short duration of action (2–3 h) compared to morphine (4–5 h) [19,21,28,34]. Then, it is clinically promising that, not only did UFP-112 produce long-lasting antinociception against acute thermal nociception, the time course of intrathecal UFP-112-induced antihyperalgesia essentially matched that of intrathecal morphine. Like intrathecal morphine, UFP-112 produced antinociception in two primate nociceptive models with a similar magnitude of effectiveness and a similar duration of action. Unlike intrathecal morphine, UFP-112 did not produce scratching responses. These findings together suggest that similar antinociceptive effects can be produced by two independent receptor mechanisms in the spinal cord of monkeys.

It is interesting to observe that when an inactive dose of intrathecal UFP-112 was combined with that of morphine, such a mixture produced significant antihyperalgesia. Such effects could be antagonized by combined administration of naltrexone and J-113397. Importantly, given its independent receptor mechanism for antinociception, UFP-112 was able to do so without increasing or decreasing the itch side effect. This finding is another supporting evidence to the therapeutic potential of UFP-112 as a spinal analgesic in both active and inactive doses as methods to alleviate morphine-induced itch while maintaining antinociception. It would be interesting to further investigate whether UFP-112 or other NOP agonist is able to additively or synergistically potentiate morphine-induced antinociception. Nevertheless, a recent study has demonstrated that N/OFQ enhanced intrathecal morphine-induced antinociception without producing motor-related side effects [21]. These findings along with this study suggest that intrathecal administration of a mixture of morphine with UFP-112 may produce antinociception with much less pruritic side effects.

In summary, this study reveals a promising functional profile of intrathecal UFP-112 in primates. Over the dose range of 1–10 nmol, intrathecal UFP-112 potently produced antinociceptive effects that were longer lasting than other NOP receptor agonists and comparable to those of intrathecal morphine. The antinociceptive effects were active against both acute nociception and capsaicin-induced hyperalgesia, providing support for its clinical value. Importantly, in all behavioral assays conducted, intrathecal UFP-112 produced antinociceptive effects without an itch side effect. Along with the finding that an inactive dose of UFP-112 in combined with morphine produced antihyperalgesia without itch/scratching, these results strongly suggest that UFP-112 has potential as a therapeutic spinal analgesic candidate for future clinical trials.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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The effects of nociceptin/orphanin FQ receptor agonist Ro 64-6198 and diazepam on
antinociception, remifentanyl self-administration, and anxiolytic-like responding
in rhesus monkeys

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Abstract

The synthetic nonpeptide NOP (nociceptin/orphanin FQ peptide) receptor agonist Ro 64-6198 produces antinociception in rhesus monkeys. In rodents, it has much more variable effects on pain responses, but is active in tests of anxiety, and decreases drug reward. The aim of this study was to compare Ro 64-6198 with the anxiolytic diazepam in three behavioral models in rhesus monkeys: analgesia, anxiety, and drug self-administration. Ro 64-6198 (0.001 – 0.01 mg/kg, i.v.) produced antinociception against an acute noxious stimulus (50° C water) in the absence of sedation, whereas diazepam (0.32 – 3.2 mg/kg, i.v.) did not have analgesic effects without sedation. Diazepam (1.0 – 5.6 mg/kg, i.v.) and the largest dose of Ro 64-6198 (0.32 mg/kg, i.v.) decreased lever pressing maintained by intravenous self-administration of the mu-opioid agonist, remifentanyl, but neither effect could be distinguished from sedative effects. Although neither drug consistently increased low rates of non-reinforced responding, a model of anxiety, such effects were observed more frequently following diazepam administration. This suggests that the NOP receptor system might have less anxiolytic-like effects in monkeys than rats. The effects of Ro 64-6198 on lever pressing were blocked by the NOP-receptor antagonist, J-113397, but not by the benzodiazepine antagonist, flumazenil. These findings suggest the effects of Ro 64-6198 on operant lever pressing are mediated by NOP receptors and that larger doses are required to impact operant behavior when compared directly with those that produce antinociception. Therefore, the present findings support previous literature suggesting NOP receptors are a viable target for pain management.

Introduction

The endogenous nociceptin/orphanin FQ peptide (NOP) binds to receptors located throughout the central and peripheral nervous systems (see Lambert, 2008, for a review). The NOP receptor is considered a member of the opioid receptor family (Mollereau et al., 1994; Foord et al., 2005) in that it shares structural features with mu, delta, and kappa opioid receptors. In addition, NOP receptor agonists produce actions similar to other opioid receptor agonists at the cellular level (Meunier et al., 1995; Rizzi et al., 2007). However, the effects of NOP receptor agonists are not blocked by administration of naltrexone, a drug that traditionally antagonizes opioid agonist effects (Ko et al., 2009; Varty et al., 2005). NOP receptors are implicated in numerous biological and behavioral processes, including immunity, pain, stress, anxiety, and drug abuse/addiction (see Lambert, 2008, for a review). Investigation of integrated behavioral responses to NOP receptor activation in animal models has been facilitated by the development of the selective nonpeptidic NOP receptor agonist, Ro 64-6198, and antagonist, J-113397 (see Shoblock, 2007).

There has been particular focus on the role of NOP receptors in mediating pain responses. The effect of NOP receptor agonists on pain measures appears to be influenced by a number of experimental variables, including species, dose of NOP receptor agonist, route of administration, and the particular test conditions (Heinricher, 2005). In rodents, systemically administered Ro 64-6198 produced antinociceptive effects in some studies (e.g., Obara et al., 2005; Reiss et al., 2008), and pronociceptive effects in others (e.g., Jenck et al., 2000). In cases of pronociception, it is possible that any spinal and peripheral antinociceptive effects were counteracted by supraspinal NOP receptor-mediated hyperalgesia (Meunier et al., 1995; Rizzi et al., 2007) or that stress-induced analgesia during testing was blocked by Ro 64-6198 (Reiss et al., 2008).

Nonetheless, the effects of systemically administered Ro 64-6198 in rodent pain models are mixed.

Ko et al. (2009) published the only study to assess the behavioral effects of systemic Ro 64-6198 in rhesus monkeys. Given that monkeys' opioid receptor systems are similar to humans (Mansour et al., 1988), monkeys might be a more appropriate species in which to study the behavioral effects of NOP receptor agonists, including nociception. As with the mu-opioid agonist alfentanil, Ro 64-6198 produced antinociception using both warm water and capsaicin stimuli. These effects were blocked by J-113397, but not by naltrexone. Furthermore, unlike the mu-opioid receptor agonist alfentanil, Ro 64-6198 did not produce scratching, respiratory depression, or maintain intravenous self-administration. These findings in monkeys suggest that Ro 64-6198 might have therapeutic value as an analgesic at the NOP receptor without some of the undesirable effects typical of mu-opioid receptor agonists.

Ro 64-6198 has shown promise as a potential therapeutic agent in other behavioral models using rodents as experimental subjects. For instance, Ro 64-6198 has been shown to diminish the rewarding effects of drugs of abuse, including alcohol (Kuzmin et al., 2007) and morphine (Shoblock et al., 2005). In addition, Ro 64-6198 produced antianxiety-like responses in the absence of negative side effects (e.g., tolerance, sedation) both in neophobic tests (e.g., Jenck et al., 2000; Nicholas et al., 2006; Wichmann et al., 2000) and conflict tests (e.g., Jenck et al., 2000; Varty et al., 2005). The effects of Ro 64-6198 on intravenous drug self-administration and anxiolytic-like responses, however, have yet to be assessed in rhesus monkeys.

The aim of the present study was to characterize further the behavioral effects of Ro 64-6198 with rhesus monkeys as experimental subjects. Specifically, antinociceptive effects investigated by Ko et al. (2009) were explored further by examining the duration of action of

intravenous Ro 64-6198 on antinociception. In addition, the effect of Ro 64-6198 on self-administration of the short-acting mu opioid receptor agonist, remifentanyl, was assessed, and the receptor mechanisms mediating the effects of Ro 64-6198 on remifentanyl self-administration were assessed using the selective NOP receptor antagonist, J-113397. Responding during periods of signaled nonreinforcement was assessed as an index of anxiolytic-like effects (Wedeking, 1974).

The effects of Ro 64-6198 were compared on each endpoint to those produced by the benzodiazepine diazepam, a drug with demonstrated anxiolytic effects (e.g., Rowlett et al., 2006), mixed analgesic effects (Morichi & Pepeu, 1979; Zambotti et al., 1991), and some ability to suppress drug self-administration (Hedlund & Wahlstrom, 1998).

Materials and Methods

Subjects

Six adult (3 males and 3 females) rhesus monkeys (*Macaca mulatta*) with body weights ranging from 7.9 to 11.9 kg participated in the nociception experiment. Three adult (2 males and 1 female) rhesus monkeys with body weights ranging from 11.7 to 14.1 kg participated in the remifentanyl self-administration experiment. All monkeys were housed individually with free access to water in stainless steel cages (83.3 cm high x 76.2 cm wide x 91.4 cm deep). Diets consisted of 25 to 30 Purina Monkey Chow biscuits (Ralston Purina Co., St. Louis, MO) and fresh fruit daily. Housing was accredited by the American Association for the Accreditation of Laboratory Animal Care. Methods were in accordance with the University Committee on the Use and Care of Animals at the University of Michigan (Ann Arbor, MI) and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health (Bethesda, MD).

In the monkeys participating in the remifentanyl self-administration study, silicone rubber indwelling i.v. catheters were implanted in a jugular, femoral, external jugular, or brachial vein and were routed subcutaneously to the midscapular area of each monkey. Flexible tethers protecting the catheters were held in place by a Teflon mesh jacket (Lomir, Quebec, Canada) and connected behind the cages to infusion pumps. Catheters were implanted under ketamine (10 mg/kg, i.m.) and xylazine (2 mg/kg, i.m.) anesthesia.

Experimental Procedures

Acute thermal nociception

The warm water tail-withdrawal assay was used to measure nociceptive responses to thermal stimuli and antinociceptive effects of test compounds (Ko et al., 1999). Monkeys were seated in primate-restraining chairs, which allowed access to their shaved tails (approximately 15 cm). Nociception evaluation was performed by placing the tail in thermal flasks containing water maintained at 50° C. The time required for the monkey to remove its tail from the warm water was recorded. If a monkey did not remove its tail within 20 sec, the flask was removed and a maximum time of 20 sec was recorded. Test sessions began with control determinations (data not shown) with 50° C water prior to intravenous administration of the test compound. Test compounds were administered intravenously over a 30 sec period through a temporary catheter (Angiocath, 24G/0.75") placed into the saphenous vein, and removed once the infusion was given. Tail-withdrawal latencies were determined every 30 min for 3 hours after administration of the test compound. In addition, before the initial tail-withdrawal latency was determined, drug-induced sedation was scored based on a scale used previously (Ko et al., 1999).

Remifentanil self-administration

The three monkeys had prior exposure to remifentanil self-administration under similar reinforcement schedules as those used in the present experiment and therefore did not require preliminary training. A panel was mounted on one side of the cages containing three depressible levers (Model 121-07, BRS-LVE) requiring 0.10 to 0.15 N to operate. Levers were separated by 0.3-cm dividers that extended 8 cm from the panel. Stimulus lights with a diameter of 2.5 cm were located directly above each lever. Only the left lever and left and center stimulus lights were used in the present experiment. Computers located in an adjacent room operating MED-PC IV interfacing and software (Med-Associates, Georgia, VT, USA) controlled all experimental events.

Sessions were approximately 2 hrs long and conducted twice daily (6:00 AM and 12:15 PM), seven days per week. Each of three components of a multiple schedule of reinforcement was signaled by a different colored stimulus light over the left lever. Each component was 5 min long and was presented eight times per session. Sessions were divided into 3-component blocks in which the order of component presentations was randomized. Pressing the lever in two of the components, signaled by red and green stimulus lights over the left lever, resulted in an injection of remifentanil on random-ratio (RR) 30 schedules of reinforcement. Thus, each response had a 3% chance in resulting in reinforcement. Reinforcement consisted of a 5-s infusion of 1 ml of a solution containing 0.0001 mg/kg/inj remifentanil. During drug delivery, the stimulus light over the left lever was turned off and the green stimulus light over the center lever was illuminated. This was followed by a return to the component stimulus on the left lever. Given that the reinforcement contingencies were identical and performance was similar across these two components, response rates were averaged across these components and hereafter is referred to

as the RR component. The third component was signaled by a white keylight and no remifentanyl was presented for lever pressing in that component. In addition, responses made within the last 10 s of that component delayed the onset of the following component by an additional 10 s (i.e., DRO 10-s schedule). This component hereafter is referred to as the DRO component.

Pre-session treatments with drugs occurred following three sessions of stable responding in all components, determined by visual inspection that indicated no increasing or decreasing trends and with at least three days between pretreatments. When the agonists diazepam or Ro 64-6198 were administered, they were injected i.v. through the catheter 10 min prior to the experimental session. The dose-effect curve was completed for diazepam followed by Ro 64-6198. When antagonists flumazenil or J-113397 were administered, they were injected i.m. 20 min prior to the session alone or followed by the agonists. Finally, the 0.1 mg/kg dose of Ro 64-6198 and the 3.2 mg/kg dose of diazepam were retested following evaluation of the antagonists.

Drugs

Ro 64-6198 was provided by F. Hoffmann-LaRoche AG (Basel, Switzerland). (+) J-113397 was obtained from the National Institute on Drug Abuse (Bethesda, MD, USA). Diazepam and flumazenil were purchased from Henry Schein Medical Supplies (henryschein.com). Ro 64-6198, flumazenil, and (+) J-113397 were dissolved in a solution of DMSO/Tween 80/sterile water in a ratio of 1:1:8. Diazepam was provided in solution containing 40% propylene glycol, 10% alcohol, 5% sodium benzoate and benzoic acid and 1.5% benzyl alcohol. In the nociception study, all drugs were administered in a volume of 0.1 ml/kg.

Data Analysis

In the analgesia assays, mean values (mean \pm S.E.M.) were calculated from individual values for tail-withdrawal latencies. Measurement differences were compared across all tests

sessions in the same experiment. Data were analyzed by using two-way repeated measures (dose x time) analysis of variance followed by the Newman-Keuls test for multiple comparisons. The criterion for significance for all tests was set at $p < 0.05$. In the self-administration assays, data were collected as responses made in the presence of each of the stimulus lights divided by the sec that the light was illuminated. Data from the remifentanyl self-administration study were examined on an individual-subject basis assessing responding on a session-by-session basis for visual stability and effects resulting from dosing conditions.

Results

Acute thermal nociception

Figure 1 shows the antinociceptive effects of Ro 64-6198 (top panel) and diazepam (bottom panel) as a function of time after intravenous administration. As shown in the top panel, Ro 64-6198 produced dose- [$F(3,15)=62.6$; $p < 0.05$] and time- [$F(5,25)=82.7$; $p < 0.05$] dependent increases in tail withdrawal latency. Post hoc comparisons indicate differences from vehicle at 30 and 60 min for all doses and up to 120 min for the largest dose (0.01 mg/kg). For all doses, peak effects occurred during the first observation period of 30 min, as well at 60 min for the 0.01 mg/kg dose. At the 30- and 60-min time points, the 0.01 mg/kg dose induced the maximal effect of a 20-sec latency. Although Ro 64-6198 produced antinociception at doses between 0.001 and 0.01 mg/kg, at these doses this compound did not cause sedation, according to the sedation rating scale described by Ko et al. (1999). As shown in the bottom panel of Figure 1, diazepam also produced dose- [$F(3,15)=7.4$; $p < 0.05$] and time- [$F(5,25)=14.6$; $p < 0.05$] dependent increases in latency to withdraw the tail from 50° C water. Post hoc comparisons indicate that the largest dose of 3 mg/kg of intravenous diazepam produced slight but significant antinociception during the first hour. However, this mild antinociceptive effect was associated with sedation as monkeys

showed heavy eyelids but responded to noises in the procedure room (i.e., scores of 2-3 in the sedation rating scale of Ko et al., 2009).

Remifentanil self-administration

Figure 2 shows response rates for individual monkeys averaged across the two RR components and in the DRO component during baseline and following intravenous injections of Ro 64-6198 (0.03 – 0.3 mg/kg) in the left column and diazepam (0.3 – 5.6 mg/kg) in the right column. In all monkeys, mean baseline (BL) response rates in the RR component were between 0.7 and 1.0 responses per sec and below 0.1 responses per sec in the DRO component. The left column reveals a response rate decreasing effect on RR component responding with increasing dose of Ro 64-6198 and little to no change in response rates in the DRO component at any dose. Small response rate increases in the DRO component occurred for monkeys Ki and Pe with the 0.1 mg/kg dose, but those effects were not consistent. Informal observations indicated that Ro 64-6198 produced sedative effects at the 0.3 mg/kg dose.

The right column of Figure 2 shows that response rates in the RR component decreased with increasing doses of diazepam in all monkeys. Diazepam dose dependently increased monkey Me's responding in the DRO component across a range of doses (1.0 – 3.0 mg/kg). Monkey Ki's response rates increased at the 1.8 mg/kg dose and to a lesser extent at the 3.0 mg/kg dose, although these increases were not consistent across determinations, as indicated by no increase at the 1.8 mg/kg dose replication. Finally, there were no clear or consistent increases in responding in the DRO component for monkey Pe.

Figure 3 shows the effects of the NOP receptor antagonist, J-113397 (0.1 mg/kg, i.v.), and the benzodiazepine antagonist, flumazenil (1.0 mg/kg, i.v.) on remifentanil self-administration. When administered in the absence of Ro 64-6198 and diazepam, neither J-

113397 nor flumazenil produced systematic changes in response rates in the RR or DRO components (labeled as BL in figure). In the left column, J-113397 diminished the response rate decreasing effect of the 0.3 mg/kg dose of Ro 64-6198 in the RR component in all monkeys. Conversely, the effects of flumazenil followed by Ro 64-6198 were not different from the effects of Ro 64-6198 given alone. Neither flumazenil nor J-113397 consistently altered the ability of any dose of Ro 64-6198 to modify responding in the DRO component. The right column shows that flumazenil blocked the response rate decreasing effects of the largest diazepam doses examined in the three monkeys. Conversely, J-113397 did not block the rate-decreasing effects of diazepam in the RR component for any monkey. For monkey Me, flumazenil blocked increases in response rates produced by 3.2 mg/kg diazepam in the DRO component, but J-113397 did not. For monkeys Ki and Pe, response rates in the DRO component were not altered consistently by flumazenil or J-113397 under any dosing condition. Given the lack of systematic effects of diazepam on DRO component responding for monkeys Ki and Pe, it is difficult to interpret the interaction of flumazenil or J-113397 with diazepam on responding in the DRO component.

Discussion

Intravenous Ro 64-6198 induced antinociceptive effects in rhesus monkeys; diazepam had no antinociceptive effects at doses less than those producing sedation. Ro 64-6198 and diazepam both decreased lever pressing maintained by remifentanyl self-administration; the dose of Ro 64-6198 required to produce these decreases were substantially larger than those that produced antinociception. Importantly, NOP receptors and benzodiazepine-receptor sites mediated decreases in remifentanyl self-administration with Ro 64-6198 and diazepam, respectively: the NOP antagonist, J-113397, but not the benzodiazepine antagonist, flumazenil,

attenuated the effects Ro 64-6198; flumazenil but not J-113397 attenuated the effects of diazepam. Finally, anxiolytic-like effects, as indicated by increases in nonreinforced responding, were more apparent with diazepam than Ro 64-6198. These findings demonstrate that Ro 64-6198 and diazepam are behaviorally and pharmacologically distinct in rhesus monkeys.

The antinociceptive effects of intravenous Ro 64-6198 in the present study were consistent with the effects of NOP receptor agonists in other studies in rhesus monkeys. NOP receptor agonists appear to have clear and consistent antinociceptive effects in the monkey following systemic (Ko et al., 2009) and intrathecal (Hu et al., 2010; Ko & Naughton, 2009; Ko et al., 2006) administration. Conversely, in rodents, both anti- and pro-nociceptive effects of NOP receptor agonists have been reported across a range of experimental conditions (Heinricher, 2005). Differences between monkeys and rodents in pain responses to NOP receptor agonists might be a result of differences in NOP receptor localization (Berthele et al., 2003; Bridge et al., 2003). The extent to which species differences are responsible for these effects is unclear at this time because the effect of NOP receptor systems in responses to pain have not been examined nearly as extensively in monkeys as in rodents. Given that the analgesic effects of Ro 64-6198 in the present study were large and occurred in the absence of sedation, the present findings add to the existing literature suggesting NOP receptors as a potential target as therapeutics for pain management in humans.

In addition to pain management, NOP receptor agonists have been implicated as a target for treating drug abuse and addiction (Lambert, 2008; Shoblock, 2007). In the present study, Ro 64-6198 decreased remifentanyl self-administration, consistent with studies suggesting NOP receptor agonists attenuate the rewarding effects of some drugs of abuse. For instance, studies in rodents have found Ro 64-6198 disrupts acquisition and reinstatement of morphine conditioned

place preference (Shoblock et al., 2005). Moreover, NOP receptor agonists decreased morphine-induced dopamine release in the nucleus accumbens, part of the brain reward pathway (Di Giannuario & Pieretti, 2000). It is unclear, however, whether purported attenuation of drug reward by Ro 64-6198 is an artifact of its sedative effects (Shoblock, 2007). In the present study, whereas Ro 64-6198 consistently decreased remifentanyl self-administration in all monkeys, it did so only at the largest dose tested (0.32 mg/kg, i.v.), and only under conditions of general sedation. Decreases in motor activity are also a primary direct effect of Ro 64-6198 in rodents (Higgins et al., 2001; Jenck et al., 2000; Varty et al., 2005) and provide an index of doses with limited therapeutic potential. Because Ro 64-6198-induced decreases in rates of remifentanyl self-administration were not distinguished from a general disruption in operant behavior in the present study, it is difficult to suggest that this drug might have a selective effect in the treatment of drug abuse. However, one important implication of these effects is that therapeutically relevant effects of Ro 64-6198 are limited to doses below 0.32 mg/kg (i.v.) in rhesus monkeys.

As another proposed therapeutic use of NOP receptor agonists, anxiolytic-like effects have been one of the most promising endpoints of Ro 64-6198 (Shoblock, 2007). Although anxiolytic-like effects of Ro 64-6198 have most commonly been demonstrated in rodents across a range of tests (Shoblock, 2007), such effects were not observed in the present study. Unfortunately, diazepam's anxiolytic-like effects were inconsistent both within and among monkeys (Figure 2), so a clear positive comparison for Ro 64-6198 was not established. It is not clear why diazepam failed to demonstrate anxiolytic effects in this preparation, although the nature of the behavioral assay, the use of diazepam, the use of rhesus monkeys, or a combination of these variables are the most obvious possibilities. However, nonreinforcement/DRO procedures have been sensitive to the disinhibiting effects of anxiolytics in both monkeys and

rodents in early studies (Hanson et al., 1967; Wedeking, 1974). And, in a conflict situation using rhesus monkeys, diazepam reliably disinhibited punished responding (Rowlett et al., 2006).

Nevertheless, to the extent that diazepam produced variable increases in low rates of DRO responding, and Ro 64-6198 generally failed to do so, a tentative conclusion can be made that the mixed anxiolytic-like effects of diazepam and absence of such effects with Ro 64-6198 suggest Ro 64-6198 might have less anxiolytic potential than diazepam. This finding is supported by data that showed that Ro 64-6198 did not increase punished responding in mice in a conflict situation in which diazepam did increase punished responding (Varty et al., 2005). These mixed effects with diazepam in the present study suggests additional studies using more traditional conflict procedures to compare anxiolytic-like effects of diazepam and Ro 64-6198 in rhesus monkeys would be useful.

The present findings also provide additional pharmacological evidence that the behavioral effects of Ro 64-6198 in rhesus monkeys are mediated by NOP receptors. Specifically, the suppression of remifentanyl self-administration by Ro 64-6198 was attenuated by pretreatment with the NOP receptor antagonist, J-113397, but not by the benzodiazepine-receptor-site antagonist, flumazenil. Likewise, the effects of diazepam were reduced by flumazenil, but not by J-113397. These findings join those demonstrating thermal antinociceptive effects of Ro 64-6198 that were blocked dose dependently by J-113397 but not by naltrexone (e.g., Ko et al., 2009). Previous studies in rodents have shown involvement of GABA/benzodiazepine systems in behavioral effects of NOP-receptor agonists (Gavioli et al., 2008; Uchiyama et al., 2008). Those studies have found the GABA_A-receptor antagonist, bicuculline and/or flumazenil reduced the anxiolytic-like effects of NOP receptor agonists in rodent models of anxiety. Given the lack of disinhibition produced by Ro 64-6198 in the present

study, potential involvement of GABA/benzodiazepine systems in such effects in rhesus monkeys cannot be substantiated. Nonetheless, the present findings do suggest that behavioral effects of Ro 64-6198 are mediated by NOP receptors in rhesus monkeys, even at large doses that disrupt operant performance.

Doses of Ro 64-6198 producing therapeutic-like effects and those producing negative side effects are different across rodent species (Shoblock, 2007). For instance, the therapeutic window between anxiolytic-like effects and those producing motor disturbances appears larger for rats than for mice (Varty et al., 2005). Although the present study suggests there are little or no anxiolytic-like effects of Ro 64-6198 relative to diazepam in rhesus monkeys, the present findings suggest a fairly large therapeutic window between doses producing antinociceptive effects (0.001 – 0.01 mg/kg) and those producing sedation (0.32 mg/kg). In addition, because both effects were antagonized by J-113397, the present findings suggest these behavioral effects of Ro 64-6198 are mediated by NOP receptors and that Ro 64-6198 could be a useful analgesic at doses producing few motor disturbances. Overall, these findings add to the literature suggesting NOP receptors are promising targets for pain management.

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Figure Captions

Figure 1. Antinociceptive effects of intravenously administered Ro 64-6198 (top panel) and diazepam (bottom panel) against an acute noxious stimulus, 50°C water. Each point represents mean and error bars represent S.E.M. (n=6). Symbols represent different dosing conditions in the same monkeys. Asterisks represents a significant difference from the vehicle condition from the time point 30 min to the corresponding time point for each dose (*, $p < 0.05$).

Figure 2. Response rates in the RR and DRO component during baseline (BL) and as a function of dose of intravenous Ro 64-6198 (left column) and diazepam (right column).

Figure 3. Effects of J-113397 and flumazenil on response rates in the RR and DRO component during baseline (BL) and as a function of dose of intravenous Ro 64-6198 (left column) and diazepam (right column). Note that mean effects of Ro 64-6198 and diazepam alone from Figure 2 are presented as solid and dashed lines, respectively.

Figure 1

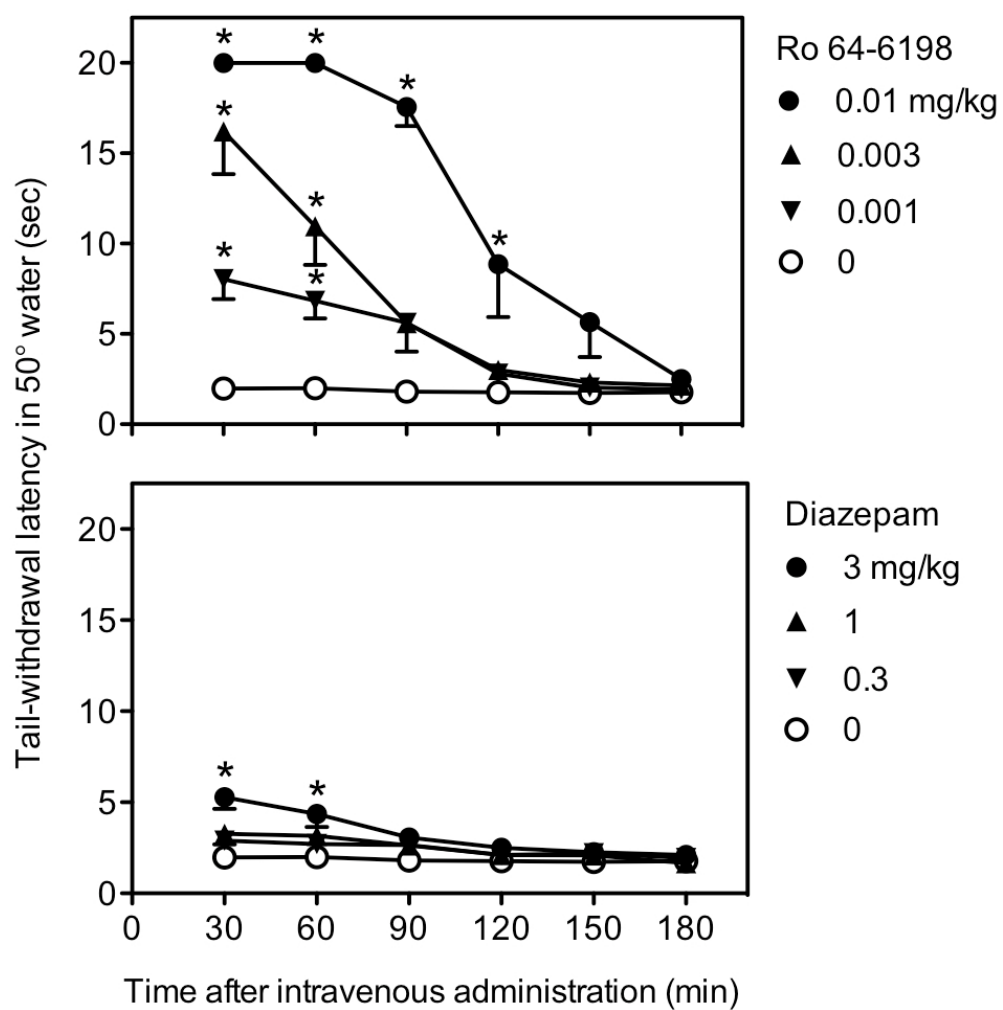


Figure 2

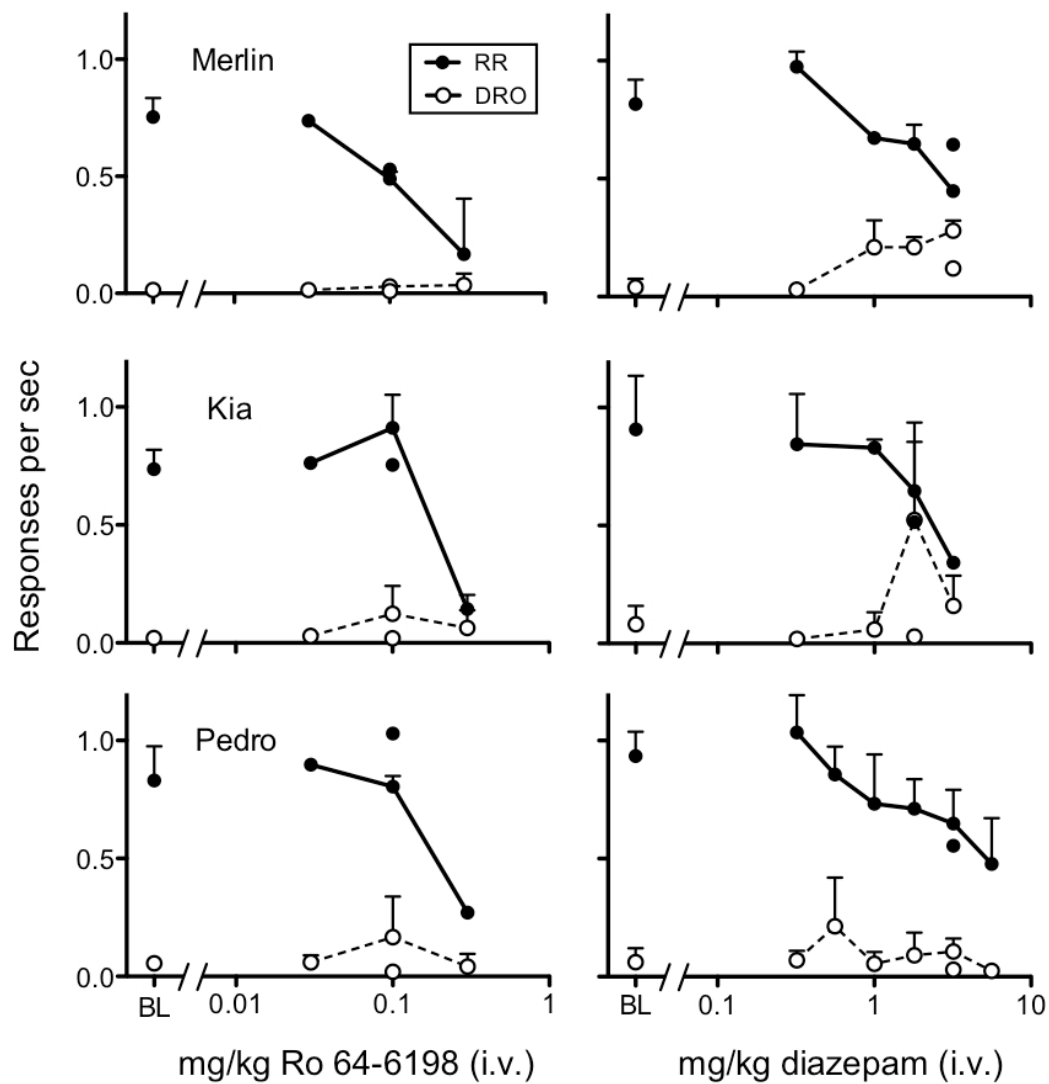


Figure 3

