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Overriding the blockade of antinociceptive actions of opioids in rats treated with extended-release naltrexone

Reginald L. Dean [a,*], Mark S. Todtenkopf [a], Daniel R. Deaver [a], Mahin F. Arastu [a], Nan Dong [a], Krystal Reitano [a], Kevin O'Driscoll [a], Kristina Kriksciukaite [a], David R. Gastfriend [b]

[a] *Department of Life Sciences and Toxicology, Alkermes, Inc., 88 Sidney St., Cambridge, MA 02139, USA*
[b] *Medical Affairs Department, Alkermes, Inc., Cambridge, MA 02139, USA*

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Abstract

A monthly extended-release formulation of the opioid antagonist naltrexone (XR-NTX) is approved for treatment of alcohol dependence. There is little research regarding overriding chronic (>21 days) competitive opioid receptor blockade with opioids for acute pain. Using the hot plate test after XR-NTX or placebo microsphere administration, rats were treated with an opioid analgesic to determine the dose required to produce the maximum response latency (MRL; 60 s). Rats were later treated with the same opioid to determine any potential effects on respiration rate or locomotor activity. In naïve rats, 15 mg/kg morphine, 0.1 mg/kg fentanyl and 8 mg/kg hydrocodone produced MRL. In XR-NTX treated rats, morphine produced 36% and 46% MRL at 90 mg/kg on days 4 and 19 and 96% MRL at 45 mg/kg on day 39. Fentanyl produced 100% MRL at 2.0 mg/kg on days 4 and 19 and at 0.5 mg/kg on day 39. Hydrocodone (80 mg/kg) produced 69%, 80% and 100% MRL on days 4, 19 and 39. Compared to placebo, these doses did not further depress respiration or alter locomotor activity. Thus, opioid receptor blockade with XR-NTX can be overcome in rats with higher doses of opioids without further affecting respiration or locomotor activity.

Keywords: XR-NTX; Analgesia; Antinociception; Morphine; Fentanyl; Hydrocodone; Respiration rate; Locomotor activity; Vivitrol®; PLG microspheres

1. Introduction

Naltrexone is a non-selective, high affinity, competitive opioid antagonist. Oral naltrexone is an approved pharmacological treatment for opioid and alcohol dependence (Johnson et al., 2004; Pettinati and Rabinowitz, 2006; Tambour and Quertemont, 2007). Unfortunately, non-adherence (i.e., failure to self-administer daily or complete a course of treatment) occurs in the majority of patients (Harris et al., 2004), undermines the efficacy of naltrexone in treating alcohol dependence and multiplies the risk of relapse by a factor of 2–4 (Baros et al., 2007; Pettinati et al., 2000). For this reason, an extended-release formulation (XR-NTX) containing naltrexone incorporated into biodegradable polymeric microspheres made of poly(D,L-lactide-co-glycolide) (PLG) was developed and recently approved by the FDA (Pettinati and Rabinowitz, 2006) for treatment of alcohol dependence. This once-a-month injectable formulation assures monthly adherence because it transfers the responsibility of daily treatment from the patient to the delivery system. XR-NTX results in clinically meaningful blockade of opiate receptors over at least 30 days (Johnson et al., 2004). In a multi-site clinical trial comparison with placebo injection, XR-NTX significantly reduced the number of heavy drinking days in alcohol dependent patients and also increased abstinence and decreased drinking days in the indicated population with initial abstinence (Garbutt et al., 2005; O'Malley et al., 2005).

The most effective and commonly used drugs to relieve acute pain are opioids, which act at the same receptors that naltrexone blocks. Despite over two decades of clinical experience

Abbreviations: (MRL), maximum response latency; (XR-NTX), extended-release naltrexone; (LLOQ), lower limit of quantitation; (HPLC), high performance liquid chromatography; (MS), mass spectrometry.

* Corresponding author. Tel.: +1 617 494 0171; fax: +1 617 494 9263.
E-mail address: reggie.dean@alkermes.com (R.L. Dean).

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managing acute pain states in the presence of oral naltrexone, a search of the clinical literature revealed limited published studies regarding the feasibility of overriding naltrexone-induced opioid receptor blockade with high doses of opioid analgesics for pain management (Comer et al., 2006; O'Brien and Cody, 2006; Vickers and Jolly, 2006). Adverse effects of opioids are multiple in number, are usually receptor mediated, difficult to separate from the desired analgesic effect and progressively increase as the opioid dose increases (Schug et al., 1992; Shook et al., 1990). One concern with the use of high doses of centrally acting opioid analgesics is their effect on respiration (Inturrisi, 2002). Even at clinically relevant doses, opioid analgesics produce some degree of respiratory depression (Borison, 1977) primarily due to their effect on μ opioid receptors (MOR) in the brain stem (Shook et al., 1990). An additional concern associated with significant dose escalation of opioids is sedation in ambulatory patients (Inturrisi, 2002). Thus, a non-clinical study examining the feasibility to overcome naltrexone-mediated opioid receptor blockade with opioid analgesics, and the relationship between this and the presence/degree of adverse effects, was conducted.

The objectives of the present studies were to determine at what doses commonly used opioid analgesics (morphine, fentanyl or hydrocodone) elicit antinociceptive effects in rats pre-treated with XR-NTX, and if such doses would produce additional adverse changes in respiration rate (as indicated with whole-body plethysmography using conscious unrestrained rats) or locomotor activity (using automated open field chambers). Three typical opioids were tested because it is possible that different opioid analgesics could elicit different results.

2. Materials and methods

2.1. Animals

These studies were approved by the Institutional Animal Care and Use Committee, Alkermes, Inc., Cambridge, MA, and were conducted in accordance with the Institute of Laboratory Animal Resources' "Guide for the Care and Use of Laboratory Animals" (1996). Male Sprague–Dawley rats (250±25 g; Charles River Laboratory, Raleigh, NC) were used in these studies. Rats were pair-housed in polypropylene cages with free access to food and water. The vivarium was maintained on a 12 hour light:dark cycle (0700:1900) with a room temperature of 22±3 °C and a relative humidity level of 35±15%.

2.2. Drug preparation

Naltrexone was incorporated into 75:25 biodegradable poly (D,L-lactide-co-gycolide) microspheres at a concentration of 337 mg of naltrexone/g microspheres (Alkermes, Wilmington, OH). XR-NTX was suspended in diluent composed of carboxymethylcellulose sodium salt, polysorbate 20, sodium chloride and water prior to injection (Dean, 2005). Morphine sulfate, fentanyl citrate and hydrocodone bitartrate were purchased from Spectrum Laboratory Supply Co. (Gardena, CA). All drugs were dissolved in physiological saline prior to use. All drugs were dosed at 1 mL/kg body weight, except for the highest dose of morphine (90 mg/kg) which was dosed at 2 mL/kg due to limited solubility at this concentration. Opioid effects were determined at the time of peak pharmacodynamic action, with the only exception being the open field test for locomotor activity assessment. This test was conducted shortly after the assessment for respiratory depression but still within an acceptable time frame based on the drugs' plasma half-life (approximately 4 h for both hydrocodone and fentanyl).

2.3. Hot plate antinociception test

Antinociception to acute thermal stimuli was assessed using a commercially available hot plate apparatus (Columbus Instruments, Columbus, OH). Rats were placed individually on the hot plate (25.4 cm×25.4 cm; surface temperature=52.5 °C) and the response latency to lick either hind paw was recorded. The MRL was set to 60 s to avoid potential thermal injury associated with longer exposure times. The latency to lick the hind paw was normalized to both the minimum time observed following saline administration and the maximum response latency (60 s) using the following equation:

$$\text{Maximum Response Latency (MRL)} = \frac{\text{Drug Latency} - \text{Saline Latency}}{\text{Maximum Latency (60 seconds)} - \text{Saline Latency}}$$

This reduces the amount of inter-test variability associated with daily testing by taking into account the non-drug associated sensitivity to thermal stimuli and the maximum range of responses.

The dose of each opioid which produced a response latency of 60 s on the hot plate was referred to as 100% MRL for that drug. To determine the minimum dose of each analgesic that results in 100% MRL, naïve (i.e., non-microsphere-treated) rats were tested with ascending doses of morphine, fentanyl or hydrocodone. The minimum doses that produced 100% MRL were found to be 15 mg/kg for morphine, 0.1 mg/kg for fentanyl and 8 mg/kg for hydrocodone (Fig. 1). These doses were used as the starting doses in the subsequent experiments.

2.4. Whole-body plethysmography

Respiration rate was determined using whole-body plethysmography performed in conscious unrestrained rats similar to methods previously reported (Raehal et al., 2005; Walker and Jennings, 1998). This is a non-invasive method which is sensitive to measures of respiratory frequency (breathing) in morphine-treated animals (Raehal et al., 2005). Our plethysmography apparatus (Buxco; Troy, NY) has 8 separate chambers that allow for the simultaneous monitoring of rats. An integrated software analysis program was used to calculate respiratory frequencies (breaths per minute; BioSystem XA software v2.9). The rejection criterion was set to record only pressure changes due to respiration. Following a 30-min acclimation period, rats were randomly dosed with saline or test drug. After injection,

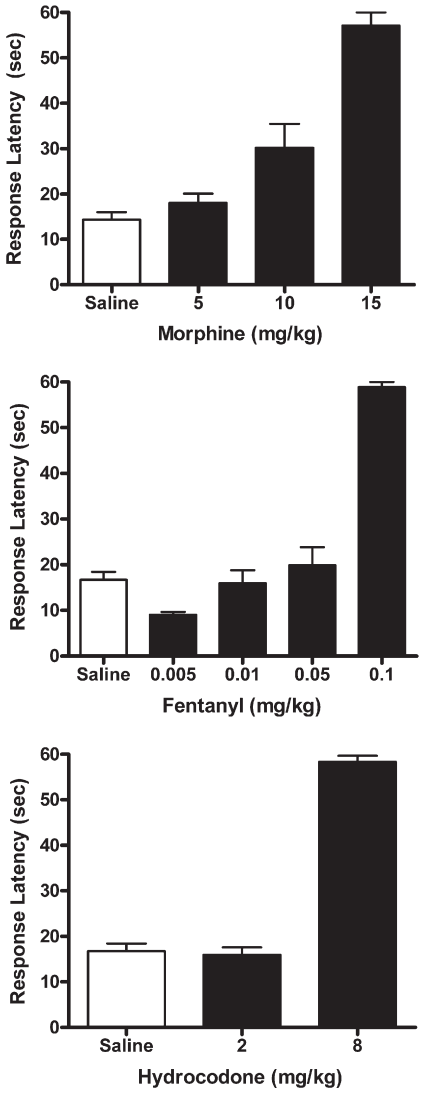

Fig. 1. The minimum dose of each opioid necessary to produce the 60 s response latency criteria (100% MRL) was determined in naïve rats prior to the beginning of the studies. Doses of each opioid were given in an ascending manner in separate groups of rats. Baseline doses that achieved MRL were 15 mg/kg morphine (top), 2 mg/kg fentanyl (middle) and 8 mg/kg hydrocodone (bottom).

rats were placed back into the chambers and data collected were for 20 min. Respiratory rate was determined by averaging the frequency per minute between 6 and 20 min of the data collection period. The first 5 min were discarded as they tended to be unreliable due to confounding effects of handling and injection. To normalize data over the test period and across days, percent change in respiratory rates from placebo microsphere pre-treated rats injected with saline were used.

2.5. Open field

Potential opioid-induced sedative effects were assessed using automated open field chambers (Columbus Instruments, Columbus, OH) to measure exploratory/locomotor activity. Each apparatus consisted of a clear, acrylic box (48.3 cm L × 48.3 cm W) with a photo beam matrix located around its perimeter. Rats were placed in the center of the box and locomotor activity was measured for 10 min using a computer-controlled program. These chambers measure several different forms of activity. For simplicity, this study focused on total distance traveled (cm) which has reliably been the most robust measure of sedation in our studies. All measurements were recorded in 1 min intervals. Locomotor activity was assessed within 10 min following completion of the respiratory frequency measures (approximately 30 min post-dosing). Locomotor activity was determined by averaging the total distance traveled for the 10 min test session for all treatment groups and the results from the placebo microsphere pre-treated rats were compared with XR-NTX pre-treated rats.

2.6. Plasma levels of naltrexone in XR-NTX pre-treated rats

Blood samples (~250 μL) were collected into tubes containing EDTA the day following each battery of tests (days 6, 21 and 41) from a lateral tail vein. Samples were centrifuged (10K ×*g*, 2 min) and plasma was separated and stored at −80 °C. Naltrexone levels were quantified using high performance liquid chromatography coupled with a PE/Sciex API 2000 mass spectrometer (LC-MS/MS; Baranczewski et al., 2006) equipped with a TurboIon Spray source.

Extraction was performed by transferring 100 μL of each standard, sample and control into microcentrifuge tubes containing 10 μL of internal standard (1 μg/mL hydrocodone in acetonitrile) and 10 μL of 10 mM sodium bicarbonate buffer. Then 250 μL of acetonitrile was used to precipitate protein, the clear supernatant was removed, concentrated to dryness, and reconstituted with 100 μL of mobile phase buffer. The high performance liquid chromatography was performed isocratically at ambient temperature using a Waters C18 3.5 μm column (XBridge, 2.1 × 50 mm i.d., Milford, MA). The mobile phase consisted of 10 mM ammonium acetate, 0.1% ammonium hydroxide buffer (pH 9.0 ± 0.5) and acetonitrile (45:55, v/v). The flow rate was 0.350 mL/min. Peak areas were measured for naltrexone (m/z 342 → 324) and the internal standard (m/z 300 → 199) in positive ion mode. Data was analyzed using Analyst software (Applied Biosystems, version 1.2). The standard curves were plotted as the peak area ratio (analyte/internal standard) vs. analyte nominal concentration with a weighting factor of 1/y. Standard curves were linear in the range from 1 ng/mL to 100 ng/mL with a coefficient of determination (r^2) >0.990 (n = 10). The lower limit of quantitation (LLOQ) was 1.0 ng/mL.

2.7. Time course and XR-NTX dosing of the study design

The time course of the experimental design is outlined in Fig. 2. On day 0, rats were lightly anesthetized with 1–2% isoflurane and injected sc using a 21 G thin walled needle with naltrexone-containing microspheres (a total of 50 mg/kg naltrexone) or a comparable mass of placebo (i.e., blank) microspheres. While the route of administration for XR-NTX in humans is intramuscular, the injection was given subcutaneously in rats due to the mass of the microspheres. Previously, it has

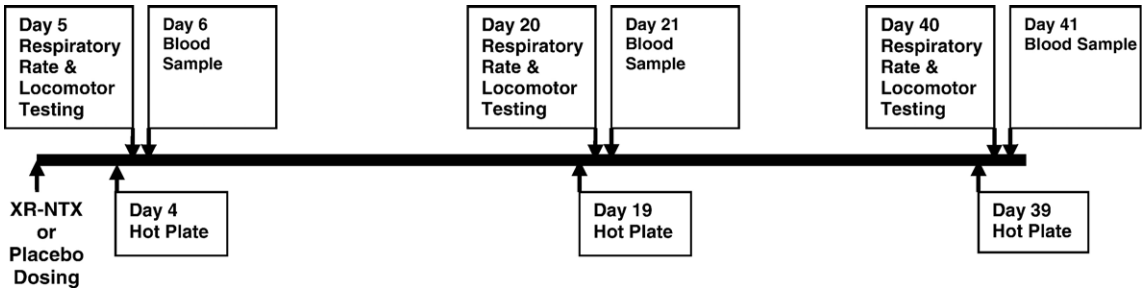

Fig. 2. Time course of the experimental design.

been shown that there is little difference in the XR-NTX PK profile between these routes of administration in rats (Bartus et al., 2003).

Because of the size and complexity of this study, a conservative approach was taken with the administration of opioids to avoid overdosing the rats. We treated one group of rats at a time: the first dose tested was that used with the placebo group. We then increased the dose in each subsequent group of rats as necessary until an antinociceptive effect was observed or the maximum dose was reached (ascending dose procedure).

2.7.1. Study 1: morphine

Morphine was the initial opioid analgesic investigated for its ability to overcome naltrexone's blockade of opioid receptors as demonstrated on the hot plate test. Separate groups of rats were treated with morphine (14–90 mg/kg; ip) on days 4, 19 and 39, in an ascending dose manner and 30 min later MRL was determined on the hot plate test. If MRL was less than 75%, the dose was titrated up in the next group of rats tested. This continued until MRL of $\geq$75% was obtained or the solubility of morphine in saline was exceeded. Because of solubility issues with morphine, a dose high enough to achieve MRL was not obtained. As a result, the studies measuring respiration frequency and locomotor activity were not conducted.

2.7.2. Study 2: fentanyl and hydrocodone

Following completion of the hot plate study with morphine, we examined the ability of fentanyl or hydrocodone to overcome XR-NTX-induced opioid receptor blockade. The dosing route for fentanyl was sc because ip administration was not well tolerated. On day 0, rats were injected sc with naltrexone-containing microspheres (a total of 50 mg/kg naltrexone) or a comparable mass of placebo microspheres (as in the morphine study). Separate groups of rats were treated in an ascending manner on days 4, 19 and 39 with fentanyl (0.1 to 2.0 mg/kg; sc) or hydrocodone (8 to 80 mg/kg; ip) and 30 min later MRL was determined on the hot plate test. MRL was determined as described above for morphine, titrating up doses of the opioid analgesics. To determine the effect of opioid analgesics (at the same doses necessary to produce MRL) on respiratory rate and locomotor activity, rats were again treated with fentanyl or hydrocodone on days 5, 20 and 40. Additionally, naltrexone concentrations in plasma were determined in these subsequent experiments.

2.8. Statistical analysis

The data are expressed as means and standard error of the mean (±SEM). For each day and drug study, statistical analysis of differences between placebo microsphere and XR-NTX pre-treated groups were assessed using Prism 4 (GraphPad Software, San Diego, CA) to perform an analysis of variance followed by Dunnett's test, if the model was significant ($p<0.05$).

3. Results

3.1. Naltrexone plasma concentration

Mean plasma concentrations of naltrexone were 6.1±0.3, 3.5±0.3 and 0.4±0.1 ng/mL (below the LLOQ; 1.0 ng/mL) on days 6, 21 and 41, respectively.

3.2. Hot plate antinociception test

The ability of three typical opioid analgesics to produce antinociceptive effects in XR-NTX treated rats and overcome naltrexone-mediated opioid receptor blockade was assessed in the hot plate test. The results are summarized in Fig. 3. In placebo microsphere-treated rats, morphine produced 100% MRL (60 s) on the hot plate test at the baseline dose of 15 mg/kg (ip). In XR-NTX-treated rats, the highest dose of morphine tested (90 mg/kg; highest dose being limited by solubility in saline) was unable to completely overcome opioid receptor blockade and produced a MRL of only 36% on day 4 and 46% on day 19. On day 39, a morphine dose of 45 mg/kg produced 100% MRL.

Fentanyl produced MRL on the hot plate test at the baseline dose of 0.1 mg/kg (sc) in placebo microsphere-treated rats. In XR-NTX-treated rats, doses of fentanyl required to produce MRL were 2.0 mg/kg on days 4 and 19, and 0.5 mg/kg on day 39 (when drug release is minimal).

In placebo microsphere-treated rats, the baseline dose of 8 mg/kg hydrocodone (ip) produced MRL on the hot plate test. In XR-NTX-treated rats, the highest dose of hydrocodone tested (80 mg/kg) produced 69% MRL on day 4, 80% on day 19 and reached 100% on day 39. A hydrocodone dose of 24 mg/kg (3 times that necessary to produce MRL in placebo microsphere-treated rats) produced 75% MRL on day 39.

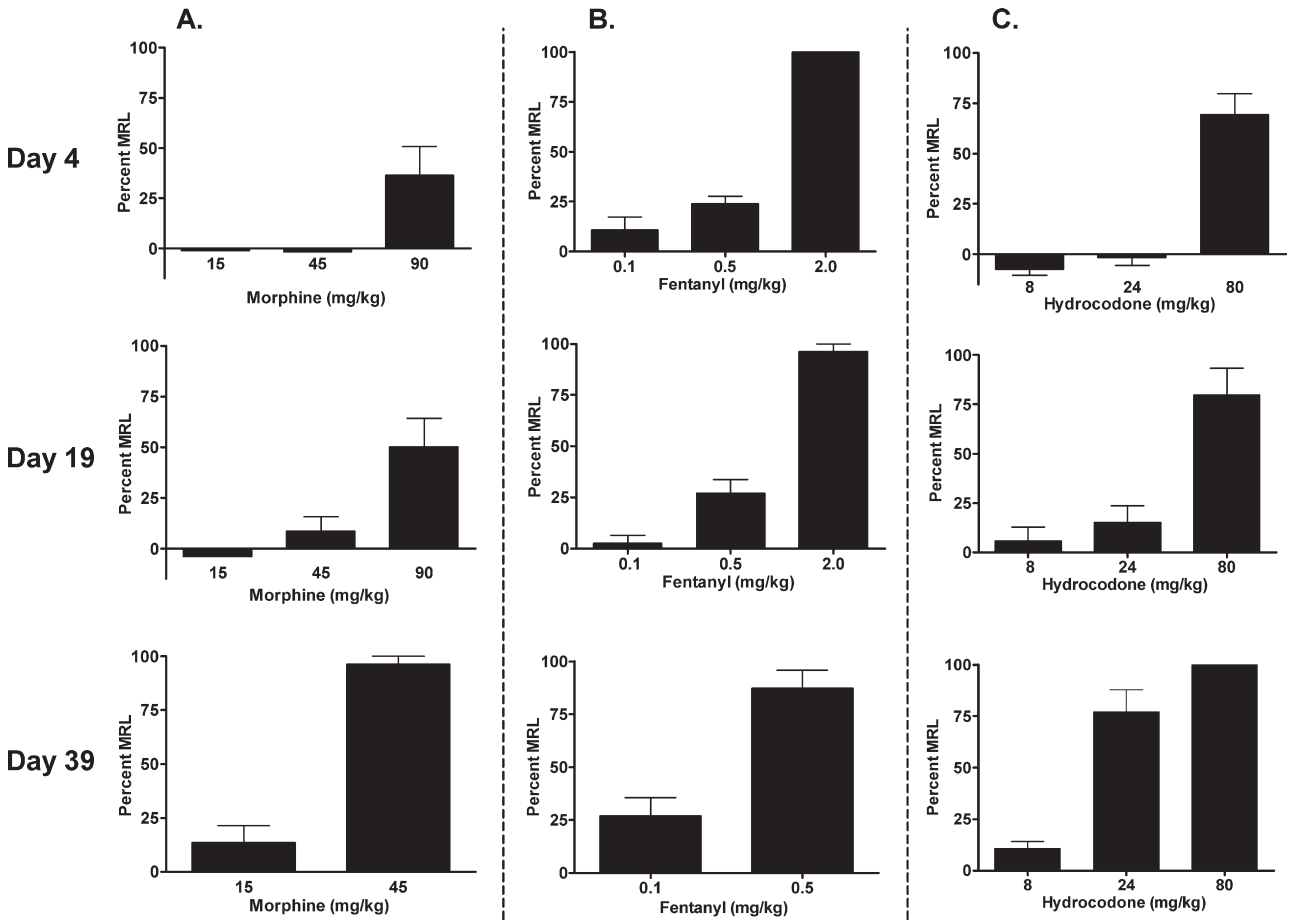

Fig. 3. Dose–response latency for morphine (A), fentanyl (B) and hydrocodone (C) on the hot plate test in XR-NTX-treated rats on days 4 (top), 19 (middle) and 39 (bottom) after subcutaneous administration of 50 mg/kg XR-NTX. The dose necessary to achieve MRL for each opioid at the time points tested (except for morphine on day 4, see text) was higher than the baseline dose. In addition, the higher dose required at each time point was related to the plasma naltrexone concentrations.

3.3. Respiration rate

The effects of fentanyl and hydrocodone on respiratory rate (a measure sensitive to respiratory depression) were assessed in placebo and XR-NTX treated rats using whole-body plethysmography in conscious, unrestrained rats. In placebo microsphere-treated rats, at doses necessary to produce antinociceptive effects on the hot plate test, only fentanyl produced decreases in respiratory rate (saline = 160.6 ± 19.6 breaths per minute compared to fentanyl at 0.1 mg/kg = 98.1 ± 3.8 breaths per minute, $p < 0.002$) Because data from day 20 and day 40 were not statistically different for the saline control placebo-treated microsphere group, these data were combined for statistical purposes. In the XR-NTX treated rats, higher doses (6 to 20 times) of the opioid analgesics necessary to overcome naltrexone-induced opioid blockade and produce MRL did not result in further respiratory depression when compared to that observed in the placebo microsphere-treated rats ($p > 0.05$; Table 1). Data collected on day 5 for fentanyl and hydrocodone were not included due to an instrument calibration error.

3.4. Locomotor activity

The effects of fentanyl and hydrocodone on locomotor activity (used here as a measure of sedation) were assessed in

Table 1
Respiration rates in placebo microsphere- and XR-NTX-treated rats

	Placebo-treated	XR-NTX-treated		
Fentanyl (mg/kg) :	0.1	0.1	0.5	2.0
Day 20	60.50 ± 1.88 (8)	*Not tested*	68.80 ± 8.00 (8)	58.24 ± 5.44 (8)
Day 40	60.16 ± 5.11 (5)	78.60 ± 13.15 (6)	55.07 ± 7.02 (8)	*Not tested*
Hydrocodone (mg/kg):	8	8	24	80
Day 20	87.13 ± 13.07 (9)	*Not tested*	92.64 ± 5.42 (9)	95.50 ± 5.23 (8)
Day 40	108.30 ± 15.63 (7)	87.59 ± 5.84 (9)	105.30 ± 5.76 (8)	*Not tested*

Data (mean ± SEM) are expressed as percent of placebo microsphere-treated saline controls. The number of rats in each group is in parenthesis.

Table 2
Locomotor activity in placebo microsphere- and XR-NTX-treated rats

	Placebo-treated		XR-NTX-treated		
Fentanyl:	Saline	0.1 (mg/kg)	0.1 (mg/kg)	0.5 (mg/kg)	2.0 (mg/kg)
Day 5	220.58±8.72 (5)	179.49±28.91 (8)	*Not tested*	106.38±28.95 (9)	164.30±28.25 (9)
Day 20	151.92±25.18 (5)	181.06±22.04 (7)	*Not tested*	145.10±31.76 (9)	123.99±38.95 (9)
Day 40	170.14±21.98 (5)	104.86±27.71 (5)	112.18±23.05 (6)	116.65±47.97 (6)	*Not tested*
Hydrocodone:	Saline	8 (mg/kg)	8 (mg/kg)	24 (mg/kg)	80 (mg/kg)
Day 5	164.34±33.13 (5)	144.88±25.63 (9)	*Not tested*	137.89±27.32 (9)	89.47±26.64 (9)
Day 20	189.55±45.95 (6)	227.10±17.15 (8)	*Not tested*	141.20±23.46 (8)	159.27±29.85 (10)
Day 40	104.63±18.24 (5)	170.58±27.81 (9)	192.17±17.42 (9)	196.74±22.16 (8)	*Not tested*

Data (total distance traveled in centimeters over 10 min) are expressed as mean±SEM. The number of rats in each group is in parenthesis.

placebo and XR-NTX treated rats using automated open field chambers. Neither fentanyl nor hydrocodone altered locomotor activity at any of the doses tested in placebo microsphere or XR-NTX pre-treated rats ($p>0.05$). While locomotor activity was not assessed in morphine-treated rats, they were observed to exhibit normal activity in their home cage up to 90 min following morphine treatment regardless of placebo or XR-NTX pretreatment (Table 2).

4. Discussion

The present study was designed to determine at what doses three typical opioid agonists elicit analgesic effects in XR-NTX pre-treated rats. These data demonstrate that higher doses of opioid analgesics were able to overcome XR-NTX induced opioid receptor blockade in rats (as demonstrated by antinociceptive effects on the hot plate test) and the dose necessary to produce antinociception corresponded with the plasma concentration of naltrexone at the time of testing. That is, as naltrexone concentration in plasma decreased, the dose necessary to produce MRL decreased. This relationship between the pharmacokinetics of XR-NTX and opioid receptor blockade confirms previous observations reported with acute morphine challenges in chronic naltrexone-treated rats (8 days via sc pellets) (Yoburn et al., 1986) and extended-release naltrexone-treated rats (Bartus et al., 2003) and humans (Comer et al., 2006).

A single dose of 50 mg/kg XR-NTX was tested because this dose reflects the human PK profile across a 1 month release period (Dunbar et al., 2006). The time points (days) selected for pharmacological evaluation of opioid effects during the 30 day XR-NTX release were based on previous studies in rats (Bartus et al., 2003) and humans (Dunbar et al., 2006), reflecting the early, mid and late phases of naltrexone release from PLG microspheres. The LC-MS/MS results of this study confirm mean plasma concentrations for the early, middle and late phases were approximately 6, 3.5 and <0.5 ng/mL on days 6, 21 and 41, respectively. These concentrations would be expected to provide varying degrees of opiate receptor blockade. In humans the relationship between plasma concentrations of naltrexone and degree of opiate receptor blockade is not completely understood. Previous studies in humans demonstrated that a plasma concentration of 1–3 ng/mL antagonized 25 mg intravenous heroin (Comer et al., 2006; Verebey et al., 1976) or 500 mg inhaled diamorphine (Brewer, 2002). However, other data suggested that naltrexone continues to inhibit brain opioid receptors in the absence of measurable plasma concentrations of naltrexone (Lee et al., 1988; Navaratnam et al., 1994; Schuh et al., 1999). The present data are in agreement with this concept given the higher doses of opioids required to induce antinociception on day 39 (compared to that in placebo microsphere-treated rats) when naltrexone plasma concentrations were near undetectable levels (day 41).

The equianalgesic dose among three typical opioid analgesics (morphine, fentanyl and hydrocodone) necessary to produce antinociceptive effects in naïve rats was determined using the hot plate test. Doses found to produce 100% MRL in this study were comparable to those reported by others using tests of acute thermal nociception in rats (Cicero et al., 1997; McLaughlin and Dewey, 1994; Meert and Vermeirsch, 2005). As expected, with extended-release naltrexone (XR-NTX) pretreatment, these “baseline” doses of opioids were not adequate to achieve MRL to the thermal stimulus (operationally defined as antinociception). Similar blockade of analgesic doses of morphine have been reported with chronic (Yoburn et al., 1986) and extended-release preparations (Bartus et al., 2003; He et al., 2001) of naltrexone.

All opioid analgesics, at clinically relevant doses, produce some degree of respiratory depression (Borison, 1977). This effect progressively increases with the dose of the drug and their affinity for μ opioid receptors (Eckenhoff and Oech, 1960). Morphine and similar opioid agonists (e.g., fentanyl and hydrocodone) produce much of their analgesic and respiratory depressant effects at μ opioid receptors (MORs). Opioids depress respiration primarily by their direct action on this receptor subtype in brain stem regions involved with respiration (Lalley, 2003). In contrast, the analgesic effects of opioids are mediated in part by MORs located in select regions of the forebrain, diencephalon and mesencephalon (Yaksh, 1999). Although effects on respiration are measurable, clinically significant respiratory depression rarely occurs with standard doses of opioid analgesics that are adequate to treat pain in patients (in the absence of underlying pulmonary disease) (Borgbjerg et al., 1996; Inturrisi, 2002). Opioid analgesics can also produce sedation and drowsiness which are not desirable effects in ambulatory patients.

We report a decrease in respiration rate (but without any noticeable signs of distress) in the placebo microsphere group

when challenged with fentanyl, at a dose that produced maximum response latency on the hot plate test. No change in respiration rate with hydrocodone was observed at the dose that produced antinociception. With XR-NTX treated rats, it was unknown how much the dose response curve for the opioid-mediated effects would shift to the right. Thus, the purpose of this study was to assess this potential shift at higher doses that are typically harmful to naïve rats (due to their adverse effects). Naltrexone blockade of opioid receptors can be overcome with high doses of opioid analgesics (6–20 times that required to produce antinociception in control rats); however, we cannot predict *a priori* adverse effects of higher doses in XR-NTX treated rats. Thus, locomotor activity and respiration rate were assessed because sedation and respiratory depression (respectively) are the greatest concern when treating with higher doses of opioids. We report that high doses of opioid analgesics necessary to overcome naltrexone-induced opioid receptor blockade (as measured on the hot plate test) do not *further* decrease respiration rate (i.e., with fentanyl and does not decrease respiration rate with hydrocodone). Neither analgesic produced a significant decrease in locomotor activity at high doses (doses necessary to produce antinociception in XR-NTX treated rats).

While morphine has been shown to be equipotent in producing antinociception and respiratory depression in rats (Meert and Vermeirsch, 2005) and humans (Green, 1959), subcutaneous hydrocodone has been reported to affect respiration (defined as >30% $PaCO_2$ increase) at doses which did not produce antinociception, while subcutaneous fentanyl in rats was reported to produce antinociception without any change in respiration (Meert and Vermeirsch, 2005). In this same study, none of these opioids at antinociceptive doses produced impairments in locomotor activity (i.e., sedation) as measured on a rotarod test. In contrast, the present study demonstrated that at doses that produced MRL on the hot plate, subcutaneous fentanyl but not intraperitoneal hydrocodone decreased respiration rates in placebo microsphere-treated rats. The contrasting data between Meert and Vermeirsch's results and this study may be attributed to both the differences in the route of drug administration as well as the acute thermal nociception test employed (tail flick versus hot plate).

In XR-NTX pre-treated rats, higher doses of opioids necessary to overcome naltrexone blockade to produce MRL did not result in further respiratory depression (compared with that observed in placebo microsphere pre-treated rats with doses of opioid analgesics necessary to produce MRL). Additionally, locomotor activity was not affected in placebo microsphere or XR-NTX-pre-treated rats with either of the opioid analgesics at these higher doses. One possibility is that the analgesic actions of opioids may be mediated by a different area of opioid receptor population than that mediating respiration or sedation. The actions of opioids at these separate receptor populations may be distinct such that the higher levels of opioids used in the present study do not affect respiration or sedation to the same extent as antinociception. Similarly, it has been suggested that in patients treated with an extended-release preparation of naltrexone, plasma levels above 1 ng/mL are sufficient to provide prophylaxis against drug overdose (i.e., severe respiratory depression) while attempting to overcome opioid receptor blockade (Comer et al., 2006; Hulse et al., 2004, 2005).

This non-clinical study addresses a concern in the clinic regarding pain management with opioids in patients being treated with extended-release naltrexone (e.g., (Vickers and Jolly, 2006). The results we report here are applicable to our proprietary formulation of naltrexone as well as others (e.g., implantable naltrexone rods (Hulse et al., 2004); a depot formulation in a clinical study (Comer et al., 2006)). While there is a large body of literature examining the effects of subchronic exposure (8 days or less) to naltrexone, the scientific literature is limited when it comes to chronic exposure (>21 days). Additionally, we did not have a washout period (i.e., drug-free state) typically used in many subchronic exposure studies. We administered opioids while the antagonist was still "on board" at different time periods along the PK curve when plasma levels of naltrexone were high, moderate or low. Thus, this study expands the current understanding of opioid antagonist pharmacology.

Patients taking naltrexone for alcohol or opioid dependence will be resistant to opioid analgesia, which poses a major therapeutic challenge when treating acute pain. At high doses, respiratory depression is one of the most serious adverse effects of opioid analgesics and is the primary cause of death from opioid overdose. The results of this animal study demonstrate that extended-release naltrexone blocks the antinociceptive effects of acute opioids at conventional doses, as expected. It has also been reported that μ opioid agonists stimulate locomotor activity in rodents (Babbini and Davis, 1972). There was no evidence of this effect in our study. Because the locomotor effects were assessed on the day following the hot plate test, it is possible that the rats developed an acute tolerance to the stimulatory effects. Given that this test was repeated after an extended drug-free period (14 and 19 days), this does not seem likely. The study, however, provides support for the concept of titrating up doses of opioids to produce analgesia by overriding XR-NTX receptor blockade without further depressing respiration or causing sedation. These results suggest that competitive blockade by naltrexone produces a generalized rightward shift in the dose–response curve while maintaining the relative relationship between antinociception, respiratory rate, and locomotor activity—rather than a marked, unpredictable alteration in the pharmacodynamic relationship between these parameters. Furthermore, these results in rats suggest that it may be feasible to manage acute pain in XR-NTX treated patients by titrating typical opioid analgesics upwards to patient comfort under medical observation without causing sedation or additional respiratory depression. For clinical purposes, it will be important to identify optimal opioid agonists for surmounting the effects of XR-NTX to produce analgesia without clinically relevant adverse effects.

References

Babbini M, Davis WM. Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. Br J Pharmacol 1972;46: 213–24.

Baranczewski P, Stanczak A, Kautiainen A, Sandin P, Edlund PO. Introduction to early in vitro identification of metabolites of new chemical entities in drug discovery and development. Pharmacol Rev 2006;58:341–52.

Baros AM, Latham PK, Moak DH, Voronin K, Anton RF. What role does measuring medication compliance play in evaluating the efficacy of naltrexone? Alcohol Clin Exp Res 2007;31:596–603.

Bartus RT, Emerich DF, Hotz J, Blaustein M, Dean RL, Perdomo B, et al. Vivitrex, an injectable, extended-release formulation of naltrexone, provides pharmacokinetic and pharmacodynamic evidence of efficacy for 1 month in rats. Neuropsychopharmacology 2003;28:1973–82.

Borgbjerg FM, Nielsen K, Franks J. Experimental pain stimulates respiration and attenuates morphine-induced respiratory depression: a controlled study in human volunteers. Pain 1996;64:123–8.

Borison HL. Central nervous respiratory depressants–narcotic analgesics. Pharmacol Ther [B] 1977;3:227–37.

Brewer C. Serum naltrexone and 6-beta-naltrexol levels from naltrexone implants can block very large amounts of heroin: a report of two cases. Addict Biol 2002;7:321–3.

Cicero TJ, Nock B, Meyer ER. Sex-related differences in morphine's antinociceptive activity: relationship to serum and brain morphine concentrations. J Pharmacol Exp Ther 1997;282:939–44.

Comer SD, Sullivan MA, Yu E, Rothenberg JL, Kleber HD, Kampman K, et al. Injectable, sustained-release naltrexone for the treatment of opioid dependence: a randomized, placebo-controlled trial. Arch Gen Psychiatry 2006;63:210–8.

Dean RL. The preclinical development of Medisorb Naltrexone, a once a month long acting injection, for the treatment of alcohol dependence. Front Biosci 2005;10:643–55.

Dunbar JL, Turncliff RZ, Dong Q, Silverman BL, Ehrich EW, Lasseter KC. Single- and multiple-dose pharmacokinetics of long-acting injectable naltrexone. Alcohol Clin Exp Res 2006;30:480–90.

Eckenhoff JE, Oech SR. The effects of narcotics and antagonists upon respiration and circulation in man. A review. Clin Pharmacol Ther 1960;1: 483–524.

Garbutt JC, Kranzler HR, O'Malley SS, Gastfriend DR, Pettinati HM, Silverman BL, et al. Efficacy and tolerability of long-acting injectable naltrexone for alcohol dependence: a randomized controlled trial. JAMA 2005;293:1617–25.

Green AF. Comparative effects of analgesics on pain threshold, respiratory frequency and gastrointestinal propulsion. Br J Pharmacol Chemother 1959;14:26–34.

Harris KM, DeVries A, Dimidjian K. Datapoints: trends in naltrexone use among members of a large private health plan. Psychiatr Serv 2004;55:221.

He GA, Hou HM, Liu XJ. Naltrexone microspheres: in vitro release and effect on morphine analgesia in mice. Acta Pharmacol Sin 2001;22:530–3.

Hulse GK, Arnold-Reed DE, O'Neil G, Chan CT, Hansson R, O'Neil P. Blood naltrexone and 6-beta-naltrexol levels following naltrexone implant: comparing two naltrexone implants. Addict Biol 2004;9:59–65.

Hulse GK, Tait RJ, Comer SD, Sullivan MA, Jacobs IG, Arnold-Reed D. Reducing hospital presentations for opioid overdose in patients treated with sustained release naltrexone implants. Drug Alcohol Depend 2005;79: 351–7.

Inturrisi CE. Clinical pharmacology of opioids for pain. Clin J Pain 2002;18: S3–S13.

Johnson BA, Ait-Daoud N, Aubin HJ, Van Den Brink W, Guzzetta R, Loewy J, et al. A pilot evaluation of the safety and tolerability of repeat dose administration of long-acting injectable naltrexone (Vivitrex) in patients with alcohol dependence. Alcohol Clin Exp Res 2004;28:1356–61.

Lalley PM. Mu-opioid receptor agonist effects on medullary respiratory neurons in the cat: evidence for involvement in certain types of ventilatory disturbances. Am J Physiol Regul Integr Comp Physiol 2003;285:R1287–304.

Lee MC, Wagner Jr HN, Tanada S, Frost JJ, Bice AN, Dannals RF. Duration of occupancy of opiate receptors by naltrexone. J Nucl Med 1988;29:1207–11.

McLaughlin CR, Dewey WL. A comparison of the antinociceptive effects of opioid agonists in neonatal and adult rats in phasic and tonic nociceptive tests. Pharmacol Biochem Behav 1994;49:1017–23.

Meert TF, Vermeirsch HA. A preclinical comparison between different opioids: antinociceptive versus adverse effects. Pharmacol Biochem Behav 2005;80: 309–26.

Navaratnam V, Jamaludin A, Raman N, Mohamed M, Mansor SM. Determination of naltrexone dosage for narcotic agonist blockade in detoxified Asian addicts. Drug Alcohol Depend 1994;34:231–6.

O'Brien B, Cody C. Analgesia and sedation in the presence of a naltrexone implant: a novel pharmacological challenge. Eur J Emerg Med 2006;13: 315–6.

O'Malley SS, Garbutt JC, Gastfriend DR, Kranzler HR, Pettinati HM, Illeperuma MS, et al. Effects of lead-in abstinence and a goal in abstinence on heavy drinking. Alcohol Clin Exp Res 2005;29:77A.

Pettinati HM, Rabinowitz AR. Choosing the right medication for the treatment of alcoholism. Curr Psychiatry Rep 2006;8:383–8.

Pettinati HM, Volpicelli JR, Pierce Jr JD, O'Brien CP. Improving naltrexone response: an intervention for medical practitioners to enhance medication compliance in alcohol dependent patients. J Addict Dis 2000;19:71–83.

Raehal KM, Walker JK, Bohn LM. Morphine side effects in beta-arrestin 2 knockout mice. J Pharmacol Exp Ther 2005;314:1195–201.

Schug SA, Zech D, Grond S. Adverse effects of systemic opioid analgesics. Drug Safety 1992;7:200–13.

Schuh KJ, Walsh SL, Stitzer ML. Onset, magnitude and duration of opioid blockade produced by buprenorphine and naltrexone in humans. Psychopharmacology (Berl) 1999;145:162–74.

Shook JE, Watkins WD, Camporesi EM. Differential roles of opioid receptors in respiration, respiratory disease, and opiate-induced respiratory depression. Am Rev Respir Dis 1990;142:895–909.

Tambour S, Quertemont E. Preclinical and clinical pharmacology of alcohol dependence. Fundam Clin Pharmacol 2007;21:9–28.

Verebey K, Volavka J, Mule SJ, Resnick RB. Naltrexone: disposition, metabolism, and effects after acute and chronic dosing. Clin Pharmacol Ther 1976;20:315–28.

Vickers AP, Jolly A. Naltrexone and problems in pain management. BMJ 2006;332:132–3.

Walker JK, Jennings DB. Respiratory effects of pressor and depressor agents in conscious rats. Can J Physiol Pharm 1998;76:707–14.

Yaksh T. Central pharmacology of nociceptive transmission. In: Wall P, Melzack R, editors. Textbook of Pain. London: Churchill Livingstone; 1999. p. 253–308.

Yoburn BC, Cohen AH, Inturrisi CE. Pharmacokinetics and pharmacodynamics of subcutaneous naltrexone pellets in the rat. J Pharmacol Exp Ther 1986;237:126–30.