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Ultra-low-dose naltrexone reduces the rewarding potency of oxycodone and relapse vulnerability in rats

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Abstract

Ultra-low-dose opioid antagonists have been shown to enhance opioid analgesia and alleviate opioid tolerance and dependence. Our present studies in male Sprague–Dawley rats assessed the abuse potential of oxycodone+ultra-low-dose naltrexone (NTX) versus oxycodone alone. The lowest NTX dose (1 pg/kg/infusion), but not slightly higher doses (10 and 100 pg/kg/infusion), enhanced oxycodone (0.1 mg/kg/infusion) intravenous self-administration, suggesting a reduced rewarding potency per infusion. During tests of reinstatement performed in extinction conditions, co-self-administration of any of these three NTX doses significantly reduced drug-seeking precipitated by priming injections of oxycodone (0.25 mg/kg, SC), a drug-conditioned cue, or foot-shock stress. During self-administration on a progressive-ratio schedule, animals self-administering oxycodone (0.1 mg/kg/infusion)+NTX (1 pg/kg/infusion) reached a "break-point" sooner and showed a trend toward less responding compared to rats self-administering oxycodone alone (0.1 mg/kg/infusion). In the final experiment, the addition of ultra-low-dose NTX (10 pg/kg, SC) enhanced the acute stimulatory effect of oxycodone (1 mg/kg, SC), as well as locomotor sensitization produced by repeated oxycodone administration (7 × 1 mg/kg, SC). In summary, this work shows that ultra-low-dose NTX co-treatment augments the locomotor effects of oxycodone as it enhances opioid analgesia, but reduces oxycodone's rewarding potency and subsequent vulnerability to relapse.

Keywords: Addiction; Relapse; Self-administration; Reinstatement; Progressive-ratio; Locomotor sensitization

1. Introduction

Abuse of prescription opioids has been a longstanding concern in North America (Brands et al., 2004), and recent epidemiological studies in the United States indicate that the prevalence of abuse of prescription opioids is similar to that of heroin or cocaine abuse (for review see Zacny et al., 2003). Currently, one of the two most abused prescription opioids is oxycodone, the active ingredient in OXYCONTIN®, a controlled-release formulation that can be crushed to yield an immediate, large dose and a powerful high for the abuser. The United States Drug Abuse Warning Network estimated that in 2002, there were over 20,000 emergency room visits and hundreds of deaths involving abuse of oxycodone (U.S. Department of Health and Human Services, 2001).

The current study used rat models to estimate the abuse potential of a novel opioid analgesic that combines an "ultra-low" dose of the opioid antagonist naltrexone (NTX) with oxycodone (OXYTREX™, in clinical development by Pain Therapeutics, Inc., South San Francisco, CA). Ultra-low-dose opioid antagonists have been investigated for their paradoxical enhancement of opioid analgesia. Clinically, a variety of combinations of opioid agonists with ultra-low-dose opioid antagonists have been reported to enhance analgesia (Joshi et al., 1999; Chindalore et al., 2005), to spare opioid use by patient-controlled analgesia (Gan et al., 1997), and to provide analgesia in opioid tolerant patients (Cruciani et al., 2003). A randomized, controlled clinical trial in osteoarthritis patients showed enhanced analgesia of OXYTREX compared to oxycodone alone at an NTX oral dose of 2 μg/day (Chindalore et al., 2005).

Preclinical data have also shown ultra-low-dose opioid antagonists to enhance the antinociceptive effects of opioids, to prevent or reverse opioid antinociceptive tolerance, and to

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prevent opioid physical dependence (Crain and Shen, 1995; Shen and Crain, 1997; Powell et al., 2002; Shen et al., 2002; Oxbro et al., 2003). In rodents, these effects of opioid antagonists can be modulated by age and sex (Hamann et al., 2004), and are most potent in a dose range far below the pharmacological dose range for classical receptor antagonism, typically ng/kg or pg/kg doses by systemic delivery (Crain and Shen, 2000). Electrophysiological and behavioral studies in rodents have shown that the enhanced opioid analgesic effect and attenuated opioid tolerance/dependence by ultra-low-dose opioid antagonists may be mediated by selectively blocking excitatory signaling of opioid receptors (Crain and Shen, 1995, 1998, 2000). This excitatory signaling increases after chronic opioid administration and also appears to be regulated by GM1 ganglioside levels (Crain and Shen, 1992). Recent molecular pharmacology data has confirmed that the excitatory signaling of opioid receptors following chronic opioid administration is mediated by a switch in G protein coupling from Gi/o to Gs proteins and that ultra-low-dose antagonist co-administration prevents this switch (Wang et al., 2005).

It was recently shown that ultra-low-dose NTX in combination with analgesic doses of morphine or oxycodone blocks the development of a conditioned place preference in rats (Olmstead and Burns, 2005), suggesting a reduction in rewarding properties of the opiate. This study also showed that ultra-low-dose NTX blocks the aversive effects of withdrawal from chronic treatment with these opioids, a result consistent with earlier data showing that this co-treatment also blocks somatic signs of opiate withdrawal, e.g. withdrawal jumping (Crain and Shen, 1995; Oxbro et al., 2003), and naloxone-precipitated withdrawal hyperalgesia (Shen and Crain, 2001). The suppression of both the acute rewarding effect of opioids and the aversive effect of their withdrawal may indicate a decreased abuse potential since opioid addiction is maintained by both positive and negative reinforcement (Koob et al., 1989; Ahmed and Koob, 2005).

The present study used rat models to assess the effects of ultra-low-dose NTX on four effects of oxycodone that may be indicative of its abuse potential. First, we measured intravenous self-administration on a fixed ratio (FR) schedule because opioids promote and maintain voluntary drug taking behavior in a variety of animal species (van Ree et al., 1978; Harrigan and Downs, 1978), and oxycodone is a potent mu opioid receptor agonist that is self-administered by rats (Beardsley et al., 2004) and primates (Woods et al., 2003). Second, oxycodone self-administration, with or without ultra-low-dose NTX, was assessed using the progressive ratio (PR) schedule of reinforcement (Richardson and Roberts, 1996) because performance on this schedule is thought to reflect motivation to self-administer a drug (Arnold and Roberts, 1997). Third, because relapse vulnerability is an important component of drug addiction (Marlatt and Gordon, 1985), we used the reinstatement procedure (Shaham et al., 2003) to determine whether the addition of ultra-low-dose NTX to oxycodone may alter vulnerability to relapse precipitated by an oxycodone priming injection, by a drug-conditioned cue, or by foot-shock stress. Finally, we assessed the effects of ultra-low-dose NTX on locomotor sensitization induced by oxycodone. Repeated administration of opioids can sensitize animals to their acute stimulatory properties (Stewart and Badiani, 1993), and because locomotor sensitization often occurs in parallel to sensitization to the reinforcing properties of drugs (Vezina et al., 2002), it has been viewed as a behavioral marker for changes in drug effects that may contribute to compulsive drug-seeking (Robinson and Berridge, 2003).

2. Methods and procedures

2.1. Subjects

Male Sprague–Dawley rats (350–375 g, Charles River, QC) were individually housed on a reverse 12-h light–dark cycle (lights on: 9:00 PM; lights off: 9:00 AM) with free access to food and water. Behavioral testing was conducted during the night cycle of the animals. All procedures performed in these experiments are in compliance with the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee, University of Guelph, Canada.

2.2. Intravenous surgery (Experiments 1–3)

Rats were surgically implanted with intravenous silastic catheters (Dow Corning, Midland, MI) in the right jugular vein, under general anesthesia induced by a combination of sodium pentobarbital (18.5 mg/kg IP, MTC Pharmaceutical, Cambridge, ON), morphine (5 mg/kg SC, Ontario Veterinary College, Guelph, ON) and diazepam (1 mg/kg SC, Sabex Inc., Boucherville, QC). Rats were given atropine sulfate (4.5 mg/kg SC, Ontario Veterinary College, Guelph, ON) just before surgery and Depocillin (300,000 IU, 0.1 ml/rat IM, Intervet Canada, Whitby, ON) immediately following surgery. The catheter was secured to the vein with silk sutures and was passed subcutaneously to the top of the skull where it exited into a connector (a modified 22 gauge cannula; Plastics One, Roanoke, VA) mounted to the skull with jeweler's screws and dental cement. A plastic blocker was placed over the opening of the connector when not in use. Catheters were flushed daily with saline and every second day with 0.1 ml of a saline-heparin solution (0.2 mg/ml Hepalean 1.000 IU, Organon, Toronto, ON). Behavioral testing began after 5–7 days of recovery from surgery.

2.3. Self-administration apparatus (Experiments 1–3)

Twenty Plexiglas operant chambers (model ENV-008CT, Med Associates, Lafayette, IN) were each enclosed in larger sound-attenuating plywood chambers (model ENV-018M, Med Associates). Each operant box had a house light (28 V) and two levers, one retractable and one stationary, located 10 cm apart and 8 cm above the floor of the box. The retractable lever (active lever) was connected to an infusion pump for the delivery of drugs (Razel Scientific Instruments, Stamford, CT), positioned outside the sound-insulating chamber. The stationary lever served to control for baseline, non-reinforced operant

behavior; pressing this lever had no consequence (inactive lever), but all presses were recorded. A white light (28 V) stimulus located 3 cm above the active lever was illuminated for 30 s at the beginning of the session, and for the duration of each drug infusion (10 s), serving as a discrete cue or conditioned stimulus for drug delivery. Each self-administration chamber was fitted to deliver constant-current, intermittent, inescapable, electric foot-shock through a scrambler (model ENV-414, Med Associates) to the grid floor.

2.4. Locomotion apparatus (Experiment 4)

Locomotor activity was monitored using 12 custom chambers ($30 \times 40 \times 26$ cm) made of dark gray PVC. The entire set of chambers was located in the center of the floor of a laboratory room and covered with black wire mesh to allow video tracking of the rats during testing. The tracking software used was EthoVision (version 3, Noldus Information Technology, The Netherlands).

2.5. Intravenous self-administration procedures (Experiments 1–3)

Rats were placed in the chambers and their connectors were attached to the infusion lines. Each session started with the illumination of the house light, the entry of the retractable lever and a 30-s illumination of the light cue. If the rat pressed the active lever during this first 30-s presentation of the light cue, it received an infusion followed by termination of the light cue. Subsequently, lever presses led to drug infusions according to the schedules used. Drug was infused in a volume of 64 μl over a 10-s period. During this period, the light cue was illuminated serving as a drug-conditioned cue paired with the drug infusion. Responses on the active lever made during the infusion were recorded and used for data analyses, but did not lead to further infusions. Drug concentration was adjusted for differences in body weight.

2.5.1. Oxycodone self-administration: dose–response

To determine the optimal oxycodone infusion dose for subsequent experiments, a dose–response experiment was performed. Twelve rats were trained to self-administer 0.1 mg/kg/inf oxycodone for 4 consecutive daily sessions, each lasting 3 h, on a continuous schedule of reinforcement. After this period, 6 animals were switched to a lower dose (0.05 mg/kg/inf) and the other 6 animals to a higher dose (0.2 mg/kg/inf), and self-administration continued for 4 additional daily sessions. Finally, the doses were reversed (low to high and high to low) and self-administration continued for 4 final daily sessions. This dose range was selected based on potency ratios between oxycodone and morphine (Poyhia and Kalso, 1992; Davis et al., 2003).

2.5.2. Experiment 1—dose–response NTX/oxycodone I

Self-administration training consisted of 9 daily 3-h sessions with the first 5 sessions using a FR 1 schedule and the remaining 4 sessions using an FR3. Three groups of animals ($n = 16$ each) self-administered oxycodone alone (0.1 mg/kg/infusion), or oxycodone combined with NTX at 100 pg/kg/inf, or 10 pg/kg/inf. These NTX/oxycodone ratios of $1{:}10^6$ and $1{:}10^7$ were chosen because they enhance oxycodone analgesia in mice (Shen et al., 2002).

2.5.3. Experiment 2—dose–response NTX/oxycodone II

Self-administration training consisted of 10 daily 3-h sessions with the first 4 sessions using an FR1 schedule, the next 3 sessions an FR3, and the remaining 3 sessions an FR10. Two groups of animals self-administered oxycodone alone (0.1 mg/kg/inf; $n = 14$) or oxycodone combined with 1 pg/kg/inf NTX ($n = 16$). This NTX/oxycodone dose ratio ($1{:}10^8$) was chosen because it has been shown to be the most effective dose ratio in enhancing oxycodone analgesia in mice (Shen et al., 2002).

2.5.4. Experiment 3—reinstatement

Only animals tested in Experiments 1 and 2 underwent extinction and reinstatement testing. After self-administration training and 2 days of withdrawal during which animals were not tested, lever pressing was extinguished in three 3-h sessions on consecutive days, during which rats were placed in the operant chambers but were not connected to infusion lines. Each session started with illumination of the house light and entry of the retractable lever, but the light cue was not illuminated. Lever presses on the active lever had no consequence.

Following this extinction period, animals received 3 different tests of reinstatement on consecutive days. All animals received the Drug Reinstatement first, followed by Cue Reinstatement and finally Stress Reinstatement. This within subject design (see Shaham et al., 2003 for review of alternative methods) was chosen to reduce variability associated with levels of baseline responding in extinction conditions that is typically observed between different groups of rats trained to self-administer drugs under identical conditions. In order to eliminate any possible carry-over effect from previous reinstatement sessions, rats were placed in the operant chambers and lever pressing was extinguished for 2 h before receiving the reinstatement manipulation. Rats responding more than 15 times in the second hour received additional 1-h extinction sessions until the criterion of <15 responses/h was met. This criterion was chosen because it generally corresponds to an 80% decrease in responding from the first extinction session (Shaham et al., 2003). When the extinction criterion was met, the active lever was retracted, the house light was turned off, and a test for reinstatement was given.

All three reinstatement tests lasted 1 h and began with illumination of the house light and the entry of the active lever. The Drug Reinstatement test was preceded by a priming injection of oxycodone (0.25 mg/kg, SC) 10 min before the session started. This dose of oxycodone was chosen on the basis of estimated equipotency between oxycodone and heroin (Beardsley et al., 2004). For the Cue Reinstatement test, the light cue was illuminated for 30 s at the beginning of the session, and was also activated for 10 s after each response on

the active lever. The Stress Reinstatement test began 10 min following exposure to a 15-min period of intermittent foot-shock stress: 0.5 mA, 0.5 s ON, a mean OFF period of 40.

2.5.5. Experiment 4—progressive ratio

Two groups of rats ($n=15$ each) self-administered oxycodone (0.1 mg/kg/inf) alone or combined with NTX (1 pg/kg/inf, the most effective dose in the previous experiments). Self-administration training consisted of 6 daily 3-h sessions on an FR1 schedule. Following this acquisition period, a PR schedule adapted from Roberts and Bennett (1993) was implemented. Increasingly high ratios of responses within and between self-administration sessions were required to obtain drug infusions. The response requirements escalated through steps calculated by the equation: Response ratio $=(5\times e^{(0.2\times \text{infusion number})})-5$, rounded to the nearest integer. The same formula was used to calculate the escalation of response requirements across sessions. For example, on Session 1, rats needed to respond once for the 1st infusion, twice for the 2nd, 4 times for the 3rd, 6 times for the 4th, 9 times for the 5th, and so on. On Session 2, rats needed to respond 4 times for the 1st infusion, 6 times for the 2nd infusion, 9 times for the 3rd infusion, and so on. Table 1 indicates the response requirements for each PR self-administration session. The "break-point" was defined as the PR step that caused self-administration to cease completely (i.e., no infusions). This was indexed by the total number of responses required for the infusions taken within the session proceeding the "break-point" session. For example, if a rat obtained 3 infusions on Session 3, and no infusions on Session 4, its break-point was: responses for 1st infusion on Session 3+responses for 2nd infusion on Session 3+responses for 3rd infusion on Session 3=9+12+15=36. Self-administration on this schedule lasted a total of 8 days/sessions.

2.5.6. Experiment 5—locomotor sensitization

Before this test, rats were acclimated to the facility for 5 days and were handled on two occasions for 10 min. For the Pre-treatment Test, rats were injected with oxycodone (0.25 mg/kg, SC) immediately before the 2-h test in the locomotion chambers. This dose was chosen because it was used in the reinstatement experiment described above. Rats were subsequently assigned to the three treatment groups with baseline locomotion balanced between groups ($n=8$). Rats received 7 daily SC injections of saline, oxycodone (1 mg/kg), or oxycodone (1 mg/kg)+ultra-low-dose NTX (10 pg/kg). This NTX/oxycodone ratio ($1:10^8$) is the same as that for the groups receiving 1 pg/kg/inf NTX in Experiments 2 and 4. The dose of oxycodone was chosen on the basis of estimated equipotency between oxycodone and heroin (Beardsley et al., 2004). On the first day of sensitization treatment, all animals received a 2-h test of locomotion to assess the acute effect of the drug injections. All subsequent injections were given in the home cage. Following the last injection, animals underwent a 10-day withdrawal period during which they were left undisturbed in their home cages. The next day, they received a final locomotion test (Post-treatment Test) following an injection of oxycodone (0.25 mg/kg, SC).

2.6. Drugs

Oxycodone hydrochloride was obtained from Noramco Inc. (Wilmington, DE). Naltrexone hydrochloride was purchased from PCCA (Houston, TX). All substances were dissolved in sterile 0.9% physiological saline.

Table 1
Number of responses required for each intravenous infusions of oxycodone during the eight sessions of self-administration on a progressive ratio schedule

Infusion	Responses required							
	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8
1	1	4	9	15	25	40	145	492
2	2	6	12	20	32	50	178	603
3	4	9	15	25	40	62	219	737
4	6	12	20	32	50	77	268	901
5	9	15	25	40	62	95	328	1102
6	12	20	32	50	77	118	402	1347
7	15	25	40	62	95	145	492	1646
8	20	32	50	77	118	178	603	2012
9	25	40	62	95	145	219	737	2459
10	32	50	77	118	178	268	901	3004
11	40	62	95	145	219	328	1102	3670
12	50	77	118	178	268	402	1347	4484
13	62	95	145	219	328	492	1646	5478
14	77	118	178	268	402	603	2012	6692
15	95	145	219	328	492	737	2459	8175
16	118	178	268	402	603	901	3004	9986
17	145	219	328	492	737	1102	3670	12198
18	178	268	402	603	901	1347	4484	14900
19	219	328	492	737	1102	1646	5478	18200
20	268	402	603	901	1347	2012	6692	22230

Table 2
Mean (±S.E.M.) infusions and response in a 3-h self-administration session of oxycodone on a fixed ratio schedule of reinforcement

Oxycodone dose (mg/kg/inf)	Infusions	Responses
0.05	18.0 (2.3)	44.7 (10.4)
0.1	14.4 (4.6)	28.3 (11.6)
0.2	8.5 (0.6)	14.5 (1.3)

2.7. Statistical analyses

Data from the dose–response experiments were analyzed using a one-way ANOVA with repeated measures. Self-administration, extinction and reinstatement data were analyzed using separate mixed design, two-way ANOVAs (group as independent factor and session as repeated factor).

For the PR experiment, self-administration during acquisition was analyzed using a mixed design, two-way ANOVA (group as independent factor and session as repeated factor). Self-administration during each PR session was analyzed using separate *t*-tests because animals that reached a break-point were not tested further, precluding the use of a two-way ANOVA. Data was included for any rat that obtained at least one infusion in a given PR session. The average group "break-point responding" was compared using a *t*-test. Finally, the proportion of rats in each group that had reached a break-point by the end of each PR session was compared using a χ^2 test.

Sensitization was assessed by comparing activity on Pre-treatment Test and Post-treatment Test using a mixed design two-way ANOVA. A one-way ANOVA was used to assess the acute effect of vehicle, oxycodone or oxycodone+NTX on locomotion.

In cases of significant interactions or main effects, differences between individual group means were analyzed using the Fisher LSD test for multiple comparisons with $\alpha = 0.05$. GB-Stat School Pak statistical software (Dynamic Microsystems Inc., 1997) was used for all analyses.

3. Results

3.1. Dose–response

Table 2 represents the mean (S.E.M.) number of infusions and responses emitted on the last day of self-administration with each dose of oxycodone. In both cases, the ANOVA revealed a significant effect of dose (infusions: [$F(2,22) = 7.72$, $p < 0.01$; responses: [$F(2,22) = 8.36$, $p < 0.01$]) and multiple comparisons indicated that infusions and responses decreased significantly with increases in oxycodone dose. For the subsequent studies, the middle oxycodone dose (i.e., 0.1 mg/kg/inf) was selected because it was deemed more likely to be sensitive to possible NTX-induced shifts in self-administration.

3.2. Experiments 1 and 2—self-administration

In Experiment 1, seven animals were removed from the analysis because of catheter failure during self-administration. Final group numbers were 13 rats in the oxycodone group, 13 for oxycodone/100 NTX, and 15 for oxycodone/10 NTX. In Experiment 2, six animals were removed from the experiment for the same reason, leaving 10 rats in the oxycodone group and 14 in the oxycodone/1 NTX group. Responses on the inactive lever did not differ between groups in any of the experimental phases and therefore are not reported.

In Experiment 1, the addition of 10 or 100 pg/kg/inf NTX did not significantly alter the number of infusions taken or the number of responses (Fig. 1). There was a significant main effect of session [$F(8,304) = 43.28$, $p < 0.01$], and multiple comparisons revealed that responding significantly increased in all groups when the ratio was increased from 1 to 3.

In Experiment 2, the addition of 1 pg/kg/inf NTX to oxycodone increased the number of infusions taken beginning from the third self-administration session. Furthermore, while there were no significant changes in infusions taken over the entire self-administration period in the oxycodone group, the oxycodone+NTX group significantly increased drug intake

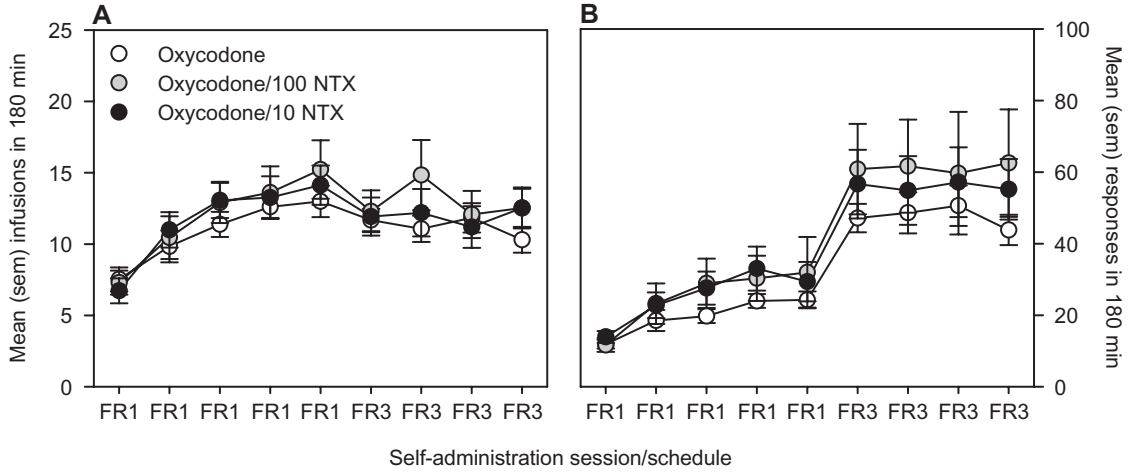

Fig. 1. There were no group differences in number of infusions (A) or responses on the active lever (B) during the 9 sessions of self-administration in rats trained with oxycodone alone (0.1 mg/kg/inf, $n = 13$) or combined with NTX (100 or 10 ng/kg/inf; $n = 13$ and $n = 15$, respectively). Data are means±S.E.M.

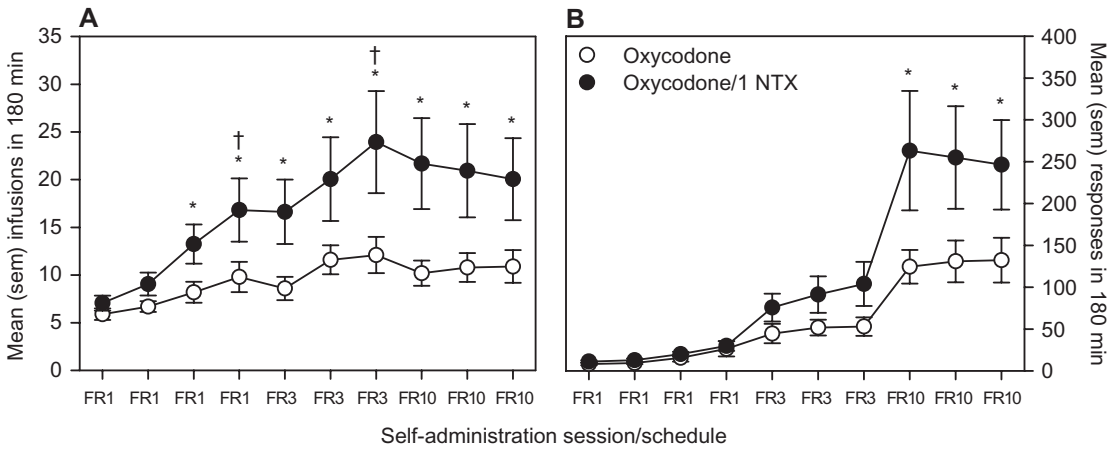

Fig. 2. Animals self-administering oxycodone (0.1 mg/kg/inf) combined with NTX (1 pg/kg/inf; $n=14$) took a significantly greater number of drug infusions (A) during the 10 sessions of self-administration compared to rats self-administering oxycodone alone (0.1 mg/kg/inf; $n=10$). The addition of NTX also increased responses on the active lever (B) in each FR10 session. Data are means ± S.E.M. *$p<0.05$ for group comparisons; †$p<0.05$ for within group escalation from the first to last session of each FR schedule.

from the first to the last day of FR1 and from the first to the last day of FR3 (Fig. 2A). The oxycodone+NTX group also responded significantly more on each FR10 session (Fig. 2B). As expected, responding increased in both groups following the shifts from FR1 to FR3, and from FR3 to FR10. Main effects of session occurred for both infusions [$F(9,216)=8.42$, $p<0.0001$] and responses [$F(9,216)=18.38$, $p<0.0001$], and session by group interactions were also seen for both infusions [$F(9,216)=1.92$, $p<0.05$] and responses [$F(9,216)=2.25$, $p<0.05$].

3.3. Experiment 3—extinction

No group differences emerged in responding during extinction in rats trained in Experiment 1. The ANOVA indicated a significant main effect of session [$F(2,76)=69.6$, $p<0.0001$], and multiple comparisons revealed that responding significantly decreased in all groups from the first to the second extinction session. Similar results were obtained in rats trained in Experiment 2: only a main effect of session was found [$F(2,44)=40.04$, $p<0.0001$]. In all groups, we observed more than an 80% decrease of responding from the first hour of the first extinction session to the third hour of the last extinction session. That is, all groups reached the extinction criterion of less than 15 responses in an hour.

3.3.1. Experiment 3—reinstatement by oxycodone (0.25 mg/kg, SC)

Because the oxycodone alone groups in Experiments 1 and 2 did not differ in each test of reinstatement, data from these two groups were pooled for analysis. The ANOVA revealed significant main effects of priming [$F(1,61)=40.03$, $p<0.0001$] and group [$F(3,61)=3.09$, $p<0.05$]. The priming injection of oxycodone significantly reinstated responding in the oxycodone and the oxycodone/100 NTX groups only. In fact, responding in these groups significantly increased in the hour following the oxycodone priming injection compared to the baseline hour preceding it (Fig. 3A). Furthermore, responding reinstated by priming in the oxycodone/10 NTX and oxycodone/1 NTX was significantly lower than that of the oxycodone alone group ($p<0.05$).

3.3.2. Experiment 3—reinstatement by the oxycodone-conditioned cue

Responding reinstated by the drug-conditioned light cue in the oxycodone/10 NTX and oxycodone/1 NTX was significantly lower than responding reinstated in the oxycodone alone group, although this cue significantly reinstated responding in all groups (Fig. 3B; main effect of reinstatement [$F(1, 61)=54.81$, $p<0.0001$]).

3.3.3. Experiment 3—reinstatement by foot-shock stress

Intermittent foot-shock stress significantly reinstated responding only in the oxycodone alone group (Fig. 3C; main effect of reinstatement [$F(1, 61)=25.75$, $p<0.0001$]). In addition, the size of the reinstatement was significantly reduced in all oxycodone+NTX groups compared to oxycodone alone (oxycodone/100 NTX: $p<0.05$, oxycodone/10 and/1 NTX: $p<0.01$).

3.4. Experiment 4—progressive-ratio experiment

One rat from the oxycodone group and three from oxycodone+NTX group were removed because of compromised health status which precluded further behavioral testing ($n=2$), or because of catheter failure ($n=2$). Thus, 14 rats comprised the oxycodone (0.1 mg/kg/inf) group and 12 rats comprised the oxycodone+NTX group. Both groups of rats acquired self-administration, as indicated by significant main effects of session for infusions [$F(5,120)=20.7$, $p<0.0001$] and responses [$F(5,120)=15.3$, $p<0.0001$].

Fig. 4 illustrates the mean number of infusions (A) and responses made on the active lever (B) during self-administration on the PR schedule. Out of 14 rats in the oxycodone group and 12 in the oxycodone+NTX group, 8 and 6 completed the entire PR phase, respectively. For infusions, the ANOVA

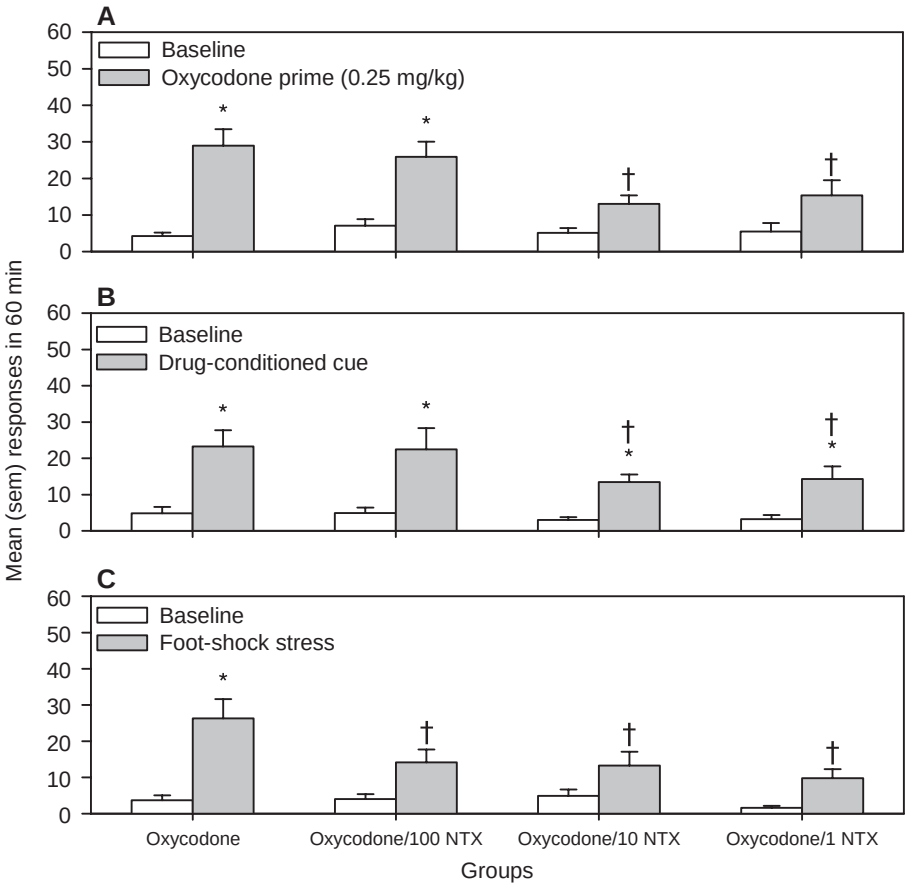

Fig. 3. Reinstatement of responding over baseline (extinction) levels precipitated by an oxycodone (0.25 mg/kg SC) priming injection (A), the drug-conditioned cue (B), or foot-shock stress (C), was significantly reduced in rats previously self-administering oxycodone combined with NTX at 10 ($n=15$) or 1 ($n=14$) pg/kg/inf compared to rats that had self-administered oxycodone alone (Experiment 1+Experiment 2; $n=23$). Co-self-administration of oxycodone+NTX at 100 pg/kg/inf ($n=13$) also reduced stress-induced reinstatement. Data are means±S.E.M. *$p<0.05$ compared to baseline responding within the same group; $^{\dagger}p<0.05$ compared to oxycodone alone.

indicated a significant main effect of session [$F(8,125)=26.9$, $p<0.0001$] but no group difference. Similarly, for responses, the ANOVA indicated a significant main effect of session [$F(8,125)=2.87$, $p<0.05$] and no group difference. Thus, as the response requirement increased across sessions, there was a significant decrease in infusions taken, occurring when animals were transferred from FR1 to the PR schedule (Session 1) and when PR requirements escalated more rapidly from Session 6 to Sessions 7 and 8 (see Table 1). In contrast to infusions, animals progressively increased their responding as the PR

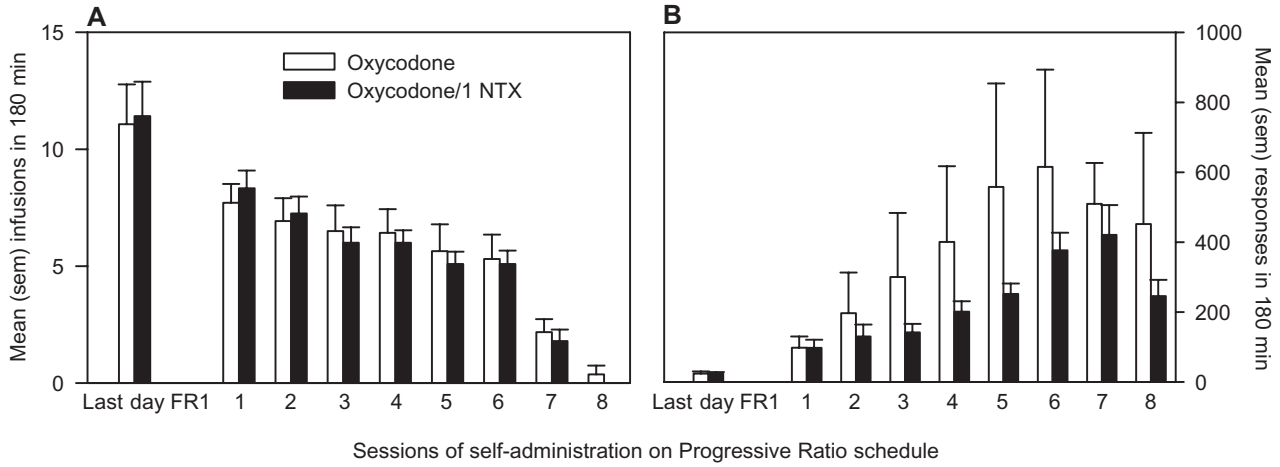

Fig. 4. The number of infusions (A) and responses on the active lever (B) during the last FR1 session and the 8 subsequent PR sessions were not significantly different in rats responding for oxycodone alone (0.1 mg/kg/inf; $n=14$) versus rats responding for oxycodone+NTX (1 pg/kg/inf; $n=12$). Data are means±S.E.M.

requirement was increased across sessions. In fact, there was a significant increase in responding when the PR1 schedule was introduced, and between Session 1 and Session 6. Responding decreased significantly over the last two PR sessions (i.e., 7 and 8), indicating that the requirements during these two sessions were too high to maintain self-administration.

In spite of an apparent trend toward reduced responding in the oxycodone+NTX group, both the ANOVA and *t*-tests performed on responses at each individual PR Session showed no significant group differences. Comparing the average break-point responding between the groups, the oxycodone+NTX group responded less than the oxycodone group, but this difference was not significant (Fig. 5A). When the percentage of rats that had reached the break-point by the end of each PR Session was compared using a χ^2 test, a significant difference was found [$\chi^2(4)=18.9$, $p<0.0001$] (Fig. 5B). This difference primarily resulted from an increased proportion of animals quitting lever pressing on Sessions 7 and 8 (Session 7: 40% of the rats in the oxycodone+NTX group had reached a break-point compared to 27% in the oxycodone group; Session 8: 100% of the rats in the oxycodone+NTX group reached a break-point compared to 88.8% in the oxycodone group).

3.5. Experiment 5—locomotor stimulation and sensitization

Repeated vehicle injections did not alter the locomotor response to the oxycodone challenge (0.25 mg/kg), but 7 injections of oxycodone (1 mg/kg each), alone or in combination with 10 pg/kg NTX did (Fig. 6A). The ANOVA revealed a significant group by test interaction [$F(2,47)=7.27$, $p<0.005$]. In the Post-treatment Test, the oxycodone+NTX group was significantly more active than both oxycodone and vehicle groups, and the oxycodone group was also significantly more active than the vehicle group. Furthermore, the oxycodone+NTX group showed a significant elevation in activity

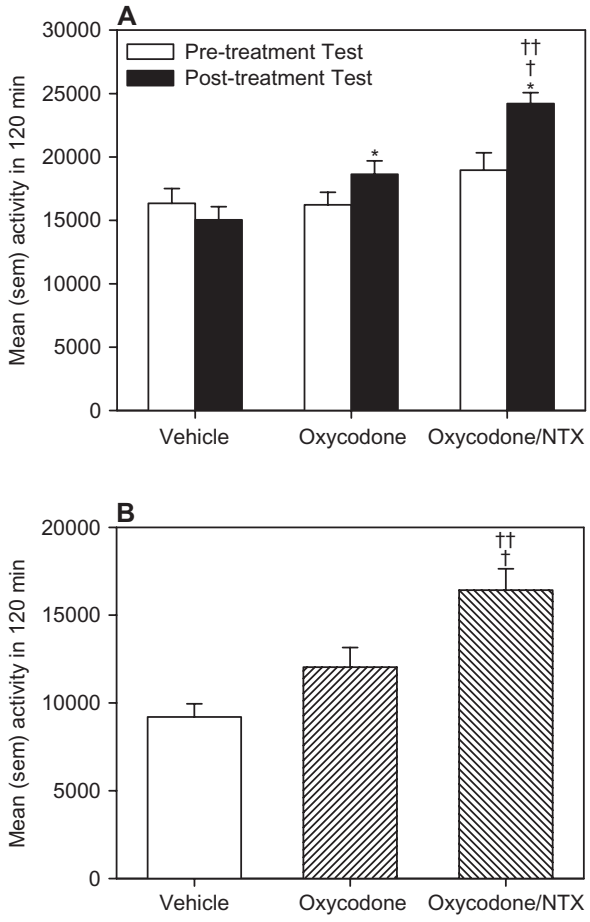

Fig. 6. Sensitization of locomotor activity (A), indicated by an enhancement of locomotor activity from the Pre-Treatment to the Post-Treatment tests, was augmented in rats administered oxycodone (1 mg/kg SC)+NTX (10 pg/kg SC, $n=8$) compared to rats treated with this dose of oxycodone alone ($n=8$). Acute locomotor stimulation (B) was also enhanced in rats treated with the oxycodone/NTX combination. Data are means±S.E.M. *$p<0.05$ compared to pre-treatment test; $^{\dagger}p<0.05$ compared to vehicle; $^{\dagger\dagger}p<0.05$ compared to oxycodone alone.

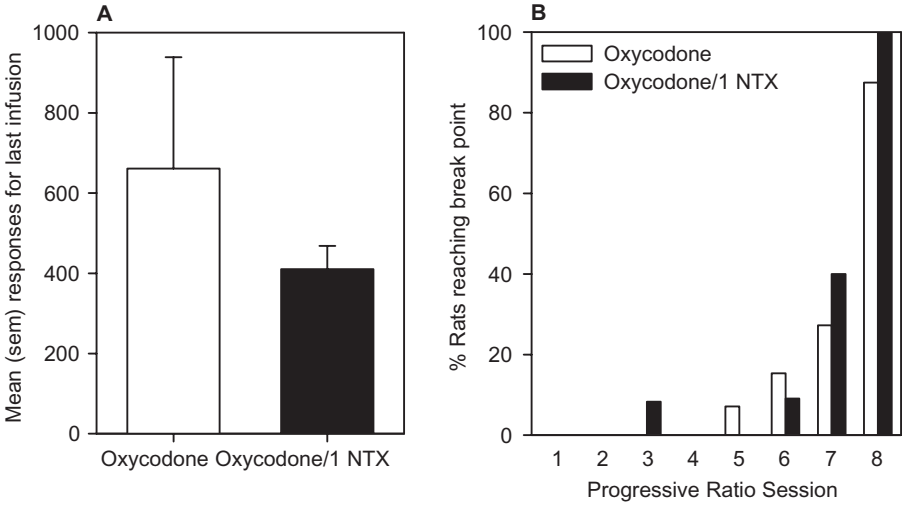

Fig. 5. The "break-point" or number of responses emitted for the last infusion was not significantly different between groups (A). Percentage of rats in oxycodone alone (0.1 mg/kg/inf) and oxycodone+NTX (1 pg/kg/inf) that reached break-point by the end of each PR Session (B). Data are means±S.E.M.

across the two tests, but the oxycodone group did not, suggesting that locomotor sensitization occurred only when ultra-low-dose NTX was added to oxycodone.

This experiment also demonstrated a significant group difference in the acute locomotor stimulatory effect [$F(2,23)=12.2$, $p<0.001$] (Fig. 6B). Oxycodone+NTX increased locomotor activity compared to either vehicle or oxycodone alone. Oxycodone alone elevated locomotion compared to vehicle injections, but this difference was not significant.

4. Discussion

The results of these experiments in rats suggest that ultra-low-dose NTX may lessen the abuse potential of oxycodone by attenuating its rewarding potency and by reducing the intensity of precipitated oxycodone-seeking.

In the self-administration experiments with FR schedules, ultra-low-dose NTX increased oxycodone intake and responding at 1 pg/kg/infusion, but not at the two higher NTX doses, suggesting that the lowest dose of NTX decreased the rewarding potency of each infusion, an interpretation consistent with the literature (Yokel and Wise, 1975, 1976) and with our dose–response experiment of oxycodone alone. This 1 pg/kg/infusion NTX dose combined with 0.1 mg/kg/infusion of oxycodone is a NTX/oxycodone ratio of $1:10^8$, the dose ratio most effective in enhancing oxycodone analgesia in mice (Shen et al., 2002). These results therefore suggest that the dose–response of ultra-low-dose NTX for attenuating oxycodone reward may coincide with its dose–response for enhancing oxycodone analgesia.

In the PR experiment, although there was no significant difference in actual break-point responding, the non-significant decrease in responding noted in each session, as well as the larger percentage reaching break-point on Sessions 7 and 8, suggests that rats administering oxycodone+NTX may have been slightly less motivated than rats administering oxycodone alone to obtain drug infusions. This subtle effect of 1 pg/kg/infusion NTX on the self-administration of oxycodone using the PR schedule may appear inconsistent with the more marked effect of this NTX dose on oxycodone self-administration on the FR schedules. However, reports of shallow dose–effect curves with self-administration of opioids indicate that PR schedules may be less sensitive in detecting increases or decreases in opioid-induced reward, in comparison to shifts in stimulant-induced reward (Ward et al., 2005). Thus, the addition of ultra-low-dose NTX, by lowering the rewarding potency of oxycodone, produced significant increases in drug intake on FR schedules, but only minor shifts on the PR schedule possibly due to the relative insensitivity of this schedule to changes in opioid-induced reward.

In the test of reinstatement, which is considered a valid animal model of human relapse (Shaham et al., 2003), responding was significantly reinstated by oxycodone priming, by the oxycodone-paired cue and by foot-shock stress in rats that had self-administered oxycodone alone. These observations are consistent with the results obtained in other studies indicating that priming injections of opioids, the re-introduction of a cue associated with opioid self-administration, or the experience of stress, can precipitate opioid-seeking behavior (Gracy et al., 2000; Shalev et al., 2000, 2002; Leri and Stewart, 2001). In contrast, rats that had self-administered oxycodone in combination with either 1 or 10 pg/kg/infusion showed no significant reinstatement of responding by priming injections of oxycodone, and all three NTX doses prevented stress-induced reinstatement of responding. Responding was significantly reinstated and maintained by the light cue previously paired with oxycodone infusions in all groups, although the level of responding was significantly reduced in rats that had self-administered oxycodone+NTX at the lowest two doses compared to rats that had self-administered oxycodone alone. The stronger effect of the drug-conditioned cue in reinstating responding compared to the drug priming or the foot-shock stress may reflect the fact that the stimulus, while initiating responding, also served as a secondary reinforcer since it was delivered after every lever press in this reinstatement test. Alternatively, the weaker effect of ultra-low-dose NTX on cue-induced reinstatement might be due to different neurobiological mechanisms suggested to mediate drug-, cue-, and stress-precipitated relapse (Stewart, 2000; Kalivas and McFarland, 2003). We previously demonstrated a similar suppression of reinstatement in rats self-administering morphine combined with ultra-low-dose naloxone, suggesting that these findings may generalize to other combinations of opioids and ultra-low-dose opioid antagonists (Burns et al., 2003).

The locomotion experiment in this study showed that the addition of ultra-low-dose NTX (10 pg/kg) to oxycodone (1 mg/kg) enhanced both its acute stimulatory effect on locomotion and its ability to induce locomotor sensitization. These results were unexpected as our reinstatement experiments suggested that ultra-low-dose opioid antagonists may prevent neuroadaptations induced by opioid self-administration that may contribute to subsequent drug-seeking behavior during periods of drug unavailability. However, the neurochemical consequences of self-administered versus experimenter-administered opioids are known to be profoundly different (Kiyatkin et al., 1993; Kiyatkin and Stein, 1995; Lee et al., 1999; Jacobs et al., 2004), possibly because the former involves a learning component. Furthermore, although locomotor sensitization often occurs in conjunction with sensitization to the reinforcing properties of drugs (Vezina et al., 2002), dissociations between these effects have also been reported, potentially questioning the reliability of locomotor sensitization as a marker of sensitization to drug reward (Bauco et al., 1993; Ciccocioppo et al., 2000).

In summary, these experiments suggest that ultra-low-dose NTX decreases the rewarding potency of oxycodone, may reduce motivation to obtain the oxycodone/NTX combination, and reduces subsequent vulnerability to relapse. These effects are not likely to be due to altered CNS concentrations of oxycodone induced by ultra-low-dose NTX since ultra-low-dose NTX co-treatment does not alter brain or plasma concentrations of systemically administered morphine (Hammarlund-Udenaes, unpublished observations). Further, such a mechanism could not explain the reduction in rewarding

potency and reinstatement coincident with the enhancement of both the locomotor stimulatory and sensitization effects of oxycodone, effects reminiscent of enhanced opioid analgesia and lack of opioid analgesic tolerance by ultra-low-dose opioid antagonists (Crain and Shen, 1995). While the mechanism of action is not fully understood (Crain and Shen, 1998, 2000), recent molecular pharmacology data has demonstrated that ultra-low-dose opioid antagonists prevent a switch in G protein coupling by the mu opioid receptor and subsequent signaling alterations that are induced by chronic opioid administration (Wang et al., 2005). Wang et al. (2005) also showed that Gi/o coupling was enhanced in spinal cord by ultra-low-dose opioid antagonist co-treatment, suggesting a mechanism for the acute enhancement of opioid analgesia. The assays in the present study provide the first direct evidence suggesting that the prevention of excitatory signaling by opioid receptors by ultra-low-dose NTX co-treatment may attenuate opioid abuse liability while enhancing the analgesic potency of oxycodone or other opioid analgesics. These experiments in rats, therefore, indicate that compared to oxycodone, OXYTREX, the novel opioid analgesic formulating ultra-low-dose NTX with oxycodone, may be a safer therapeutic agent with reduced abuse potential.

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