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Ultra-low-dose naltrexone suppresses rewarding effects of opiates and aversive effects of opiate withdrawal in rats

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Abstract *Rationale:* Ultra-low-dose opioid antagonists enhance opiate analgesia and attenuate tolerance and withdrawal. *Objectives:* To determine whether ultra-low-dose naltrexone (NTX) coadministration alters the rewarding effects of opiates or the aversive effects of opiate withdrawal. *Methods:* We used the conditioned place preference (CPP) and conditioned place aversion (CPA) paradigms to assess whether ultra-low-dose NTX alters the acute rewarding effects of oxycodone or morphine, or the aversive aspect of withdrawal from either drug. To assess the dose response for ultra-low-dose NTX, a range of NTX doses (0.03–30 ng/kg) was tested in the oxycodone CPP experiment. In order to avoid tolerance or sensitization effects, we used single conditioning sessions and female rats, as females are more sensitive to the conditioning effects of these drugs. *Results:* Ultra-low-dose NTX (5 ng/kg) blocked the CPP to morphine (5 mg/kg) and the CPA to withdrawal from chronic morphine (5 mg/kg, for 7 days). Coadministration of ultra-low-dose NTX (30 pg/kg) also blocked the CPA to withdrawal from chronic oxycodone administration (3 mg/kg, for 7 days). The effects of NTX on the CPP to oxycodone (3 mg/kg) revealed a biphasic dose response. The two lowest doses (0.03 and 0.3 ng/kg) blocked the CPP, the middle dose (3 ng/kg) was ineffective, and oxycodone combined with the highest dose (30 ng/kg) produced a trend toward a CPP. *Conclusions:* Ultra-low-dose NTX coadministration blocks the acute rewarding effects of analgesic doses of oxycodone or morphine as well as the anhedonia of withdrawal from chronic administration.

Keywords Withdrawal · Reward · Oxycodone · Morphine · Naltrexone · Place preference · Place aversion

Introduction

Opiates are powerful analgesics, but their use is hampered by side effects, analgesic tolerance, and concerns surrounding the possibility of dependence and addiction. Dependence is characterized by physical or psychological withdrawal upon discontinuation and may be independent of addiction, which itself is defined by repeated, often self-destructive behaviors focused on obtaining the drug. Still, physical dependence, or the desire to avoid withdrawal, as well as the acute rewarding or euphoric effects of opiates are both thought to contribute to opiate addiction (Koob et al. 1989). Ultra-low-dose opioid antagonists enhance analgesia and attenuate tolerance as well as somatic withdrawal signs when combined with an opiate (Crain and Shen 1995; Powell et al. 2002). The mechanism of these effects has been hypothesized to be due to prevention of excitatory signaling of opioid receptors (Crain and Shen 1995; Shen and Crain 1994). Recent molecular evidence has confirmed this hypothesis, demonstrating that ultra-low-dose naloxone combined with morphine attenuates a switch in G-protein coupling and other signaling alterations by the mu opioid receptor that occur during chronic administration of the opiate alone (Wang et al. 2005).

The current work examined whether an ultra-low dose of the opioid antagonist naltrexone (NTX) alters the acute rewarding effects of opiates as well as the aversive, affective component of withdrawal when administered in combination with the opiate. These studies used the conditioned place preference (CPP) paradigm to assess the effects of ultra-low-dose NTX on the acute rewarding effects of oxycodone or morphine, and the conditioned place aversion (CPA) paradigm to assess the effects of ultra-low-dose NTX on affective withdrawal when combined with chronic opiate (oxycodone or morphine) administration. The CPP is a well-established measure of the conditioned rewarding properties of a stimulus (i.e., drug); the aversive

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effects of drug withdrawal may be assessed as in the CPA paradigm as it provides a reliable and sensitive measure of naloxone-precipitated withdrawal from opiates (Azar et al. 2003). The dose of each opiate in this study was chosen based on previously determined analgesic effects in rodents, and the dose or dose ratio of NTX was chosen based on previous data showing optimal dose ratios for enhancing the analgesia of each opiate in mice (Shen et al. 2002a,b). Specifically, a NTX to morphine ratio of 1:10⁶ was used because this ratio greatly enhances morphine analgesia in mice, whereas a NTX to oxycodone ratio of 1:10⁸ was selected since this dose ratio more potently enhanced oxycodone analgesia.

Although chronic coadministration of an ultra-low-dose opioid antagonist with an opiate alleviates somatic withdrawal signs after precipitation by naloxone (Crain and Shen 1995), the effects of an ultra-low-dose opioid antagonist on the affective aspect of withdrawal has not been measured. The effects of ultra-low-dose NTX on the acute rewarding effects of morphine have been examined previously in the CPP paradigm, but using subanalgesic doses of morphine and a delay before conditioning (Powell et al. 2002). In that study, the combination of ultra-low-dose NTX with morphine (1 mg/kg) elicited a significant CPP when the conditioning session was delayed 2 h after injection, a delay that did not enable a significant CPP to this low dose of morphine alone. The present study investigates the effects of ultra-low-dose NTX in the standard CPP procedure (with no delay before conditioning) and with higher, analgesic doses that produce a more robust CPP. Since ultra-low-dose NTX has been shown to prevent opioid tolerance, we used single conditioning sessions to assess acute CPP effects in order to avoid any potential confound of tolerance or sensitization.

To produce strong conditioning effects with single conditioning sessions, we used female rats since female rodents are more sensitive to cocaine or methamphetamine in the CPP paradigm (Chen et al. 2003; Nazarian et al. 2004), a sex difference not explained by the more subtle sex differences in metabolism of these drugs (Bowman et al. 1999; Festa et al. 2004). Our preliminary experiments also showed females to be more sensitive to both morphine and oxycodone in the CPP paradigm, and to the aversive effects of withdrawal in the CPA paradigm. Interestingly, this finding contrasts with the known sex difference in analgesic response to opiates: female rodents are *less* sensitive than males (Kest et al. 2000).

Materials and methods

Animals

Female Sprague–Dawley rats (200–225 g at the start of the experiment) were used. Animals were housed in pairs in clear Plexiglas cages on a reversed 12-h light–dark cycle. Behavioral testing was conducted during the dark cycle. All animals had free access to food (Lab Diet, PMI Nutrition International, Inc.) and water in the home cage. All

procedures were approved by the University Animal Care Committee at Queen's University. Group sizes were ten rats for CPA experiments and eight rats for CPP experiments. All drugs were administered s.c. The effect of different doses of NTX on an oxycodone CPP was assessed in three separate experiments with three separate oxycodone-alone control groups. These control groups ($n=8$ each) were combined for statistical analysis after confirming no significant difference in the size of the CPP between groups.

Apparatus

The CPA/ CPP apparatus consists of two large compartments of equal size (45×45×30 cm) adjoined by a gray tunnel (18×18×30 cm). The two large compartments are distinguished by visual (black and white stripes or unpainted wood) and tactile cues (sawdust or wire-grid floor). The combination of visual and tactile cues is varied across the four boxes such that each large compartment is different. Beam breaks are recorded when animals enter and leave each compartment. The information is sent to an IBM-compatible, 486 computer with software written in-house.

Conditioned place preference and aversion

Before drug administration, all animals received one habituation session: they were placed in the tunnel and had free access to the entire apparatus for 20 min. None of the groups displayed a significant preference for one of the large compartments (data not shown), confirming that this procedure is unbiased. For CPA experiments, rats were then randomly assigned chronic treatment with morphine (5 mg/kg), morphine plus NTX (5 ng/kg, a ratio of 1:1,000,000), oxycodone (3 mg/kg), or oxycodone plus NTX (30 pg/kg, a ratio of 1:100,000,000). These rats were injected s.c. twice daily for 6 days and once on day 7. On day 8, 24 h after the last drug injection, half the rats in each group received an injection of naloxone (1 mg/kg s.c.) and were immediately confined to one of the large compartments for 60 min. A saline injection was paired with the other compartment in a separate session 48 h later. The other half of each group underwent the saline conditioning session first, followed by conditioning to naloxone-precipitated withdrawal 48 h later. The assignment of naloxone-paired compartment was randomized within groups.

For CPP experiments, rats were confined to one of the large compartments immediately after an s.c. injection. For the morphine CPP, rats received morphine (5 mg/kg) or morphine plus NTX (5 ng/kg, a ratio of 1:1,000,000). For the oxycodone experiment, rats received oxycodone (3 mg/kg) with NTX at the following doses: 0, 0.03, 0.3, 3, and 30 ng/kg, corresponding to NTX to oxycodone ratios of 0, 1:10⁸, 1:10⁷, 1:10⁶, and 1:10⁵. Assignment to drug group was randomized. Rats received a saline injection before confinement in the other large compartment in a separate session separated by 48 h. Half the animals received the opiate injection first, and the other half received saline first.

CPA to Morphine Withdrawal

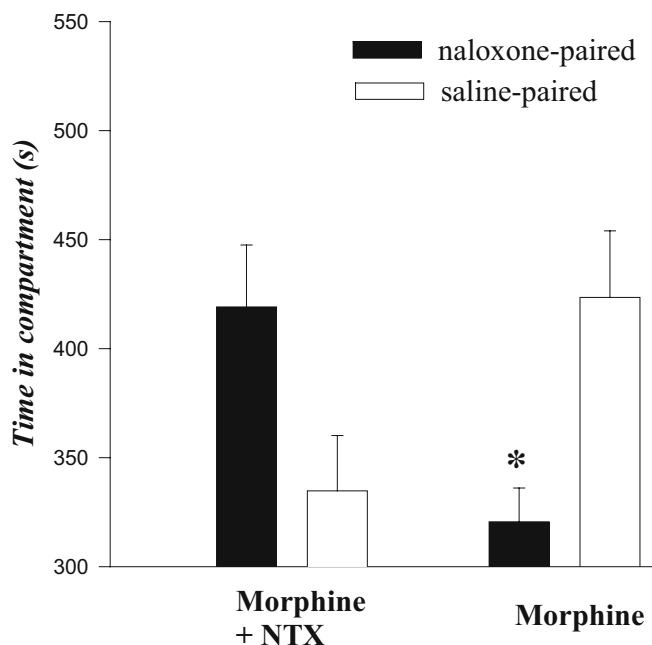


Fig. 1 Effect of NTX cotreatment (5 ng/kg s.c., a 1:10⁶ ratio) on a CPA to naloxone-precipitated withdrawal in rats treated chronically with morphine (5 mg/kg s.c. twice daily for 7 days). Bars represent the mean (\pm SEM) amount of time (s) spent in saline- and naloxone-paired compartments on test day. Rats treated with morphine alone showed a significant CPA to the naloxone-paired compartment; cotreatment with NTX blocked this effect

Again, the assignment of drug-paired compartment was randomized within groups.

Tests were conducted 48 h after the last conditioning session for both CPA and CPP experiments. Each rat was placed in the tunnel with free access to the entire apparatus for 20 min. The amount of time spent in each large compartment as well as the tunnel was recorded. A CPA is manifested as decreased time spent in the naloxone-paired vs saline-paired compartment; a CPP is manifested as increased time spent in the opiate- vs saline-paired compartment.

Statistics

Data were analyzed using *t* tests to compare the time spent in the drug- and saline-paired compartments on test day for each treatment group. In addition, *t* tests were used to compare the size of the CPA or CPP (difference scores) between treatment groups.

Results

Withdrawal from chronic administration of oxycodone or morphine induced a CPA that was absent in animals co-

treated with either opiate and ultra-low-dose NTX. Similarly, whereas morphine and oxycodone both produced a CPP, either drug combined with NTX did not. A dose-response of NTX on the CPP to oxycodone revealed a biphasic dose response, with no evidence of a CPP at the two lowest NTX doses, a modest CPP at highest NTX dose, and a strong CPP in the remaining middle dose.

CPA to opiate withdrawal

Rats chronically treated with 5 mg/kg morphine alone showed a CPA to the naloxone-paired compartment [$t(9)=-2.61$, $P<0.05$], whereas rats treated with morphine + NTX (5 ng/kg, or 1:10⁶ of the morphine dose) showed no significant CPA and in fact a trend towards a preference for the withdrawal-paired compartment [$t(9)=1.98$, $P=0.08$] (Fig. 1). After repeated treatments with 3 mg/kg oxycodone, rats showed a CPA to the compartment paired with naloxone-precipitated withdrawal [$t(9)=-2.3$, $P<0.05$]. In contrast, rats treated with 3 mg/kg oxycodone + 30 pg/kg NTX (a 1:10⁸ ratio) showed no significant CPA [$t(9)=0.01$, $P=0.99$] (Fig. 2). In addition, there was no significant difference in the size of the CPA for animals that received naloxone injections on the first vs the second conditioning day (data not shown).

CPA to Oxycodone Withdrawal

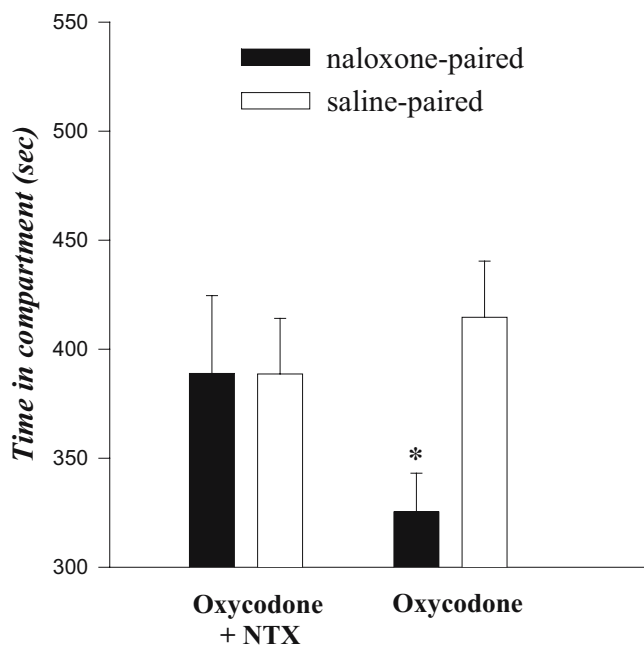


Fig. 2 Effect of NTX cotreatment (30 pg/kg s.c., a 1:10⁸ ratio) on a CPA to naloxone-precipitated withdrawal from chronic oxycodone treatment (3 mg/kg s.c. twice daily for 7 days). Bars represent the mean (\pm SEM) amount of time (s) spent in saline- and naloxone-paired compartments on test day. Rats treated with oxycodone alone showed a significant CPA to the naloxone-paired compartment and cotreatment with NTX blocked this effect

CPP to Morphine

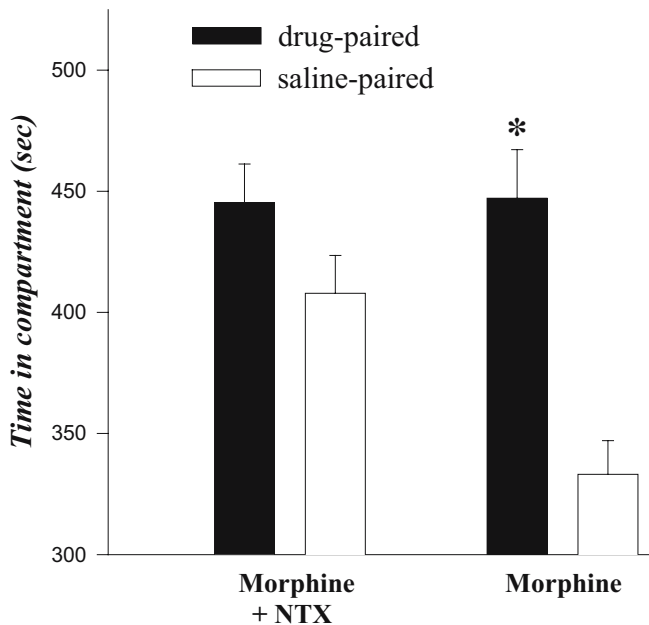


Fig. 3 Effect of NTX cotreatment (5 ng/kg s.c., a 1:10⁶ ratio) on a CPP to morphine (5 mg/kg s.c.). Bars represent the mean (\pm SEM) amount of time (s) spent in saline- and drug-paired compartments on test day. Morphine alone elicited a strong CPP that was blocked by the addition of NTX

CPP to acute opiate administration

Figure 3 shows that morphine at 5 mg/kg elicited a strong CPP [$t(7)=3.91$, $P<0.01$] that was absent in the morphine plus NTX group (5 ng/kg, or 1:1M) [$t(7)=1.43$, $P=0.2$] (Fig. 3). The effect of various doses of NTX on a CPP to 3 mg/kg oxycodone is shown in Fig. 4. The combined oxycodone control group ($n=24$) showed a strong CPP to the oxycodone-paired compartment [$t(23)=5.23$, $P<0.01$]. In contrast, there was no evidence of a CPP in animals treated with oxycodone and the lowest dose of NTX (0.03 ng/kg or a 1:10⁸ ratio) [$t(7)=.34$, $P=0.55$], the same dose chronically coadministered with oxycodone that did not elicit a CPA to oxycodone withdrawal (see Fig. 2). A tenfold higher dose of NTX (0.3 ng/kg) did not produce a CPP to oxycodone [$t(7)=1.28$, $P=0.24$], whereas a 100-fold higher dose (3 ng/kg) did [$t(7)=5.14$, $P<0.01$]. The highest NTX dose (30 ng/kg) combined with oxycodone produced a weak CPP [$t(7)=2.13$, $P=0.07$], as the upper end of this range is encroaching on doses that would classically compete with oxycodone for opioid receptor binding.

Discussion

These experiments demonstrate that ultra-low-dose NTX attenuates two processes that contribute to opiate addiction: opiate dependence, as measured by conditioning to the aversive effects of drug withdrawal, and opiate reward, as

measured by conditioning to the positive rewarding effects of acute drug administration. The lack of aversion to naloxone-precipitated withdrawal after chronic administration of ultra-low-dose NTX combined with either morphine or oxycodone suggests that no affective withdrawal was experienced. This blockade of affective withdrawal complements previous work showing that ultra-low-dose opioid antagonists block somatic withdrawal signs and the hyperalgesia associated with naloxone-precipitated withdrawal in animals chronically treated with opiates (Crain and Shen 1995; Shen et al. 2002a,b). The lack of affective withdrawal could reflect the absence of a physical withdrawal syndrome, although the anhedonia of opiate withdrawal is more likely independent of somatic withdrawal signs (Bechara et al. 1995). The lack of physical and affective withdrawal suggests that ultra-low-dose opioid antagonists, combined with opiates, prevent opioid dependence.

The suppression of the CPP to morphine or oxycodone by ultra-low-dose NTX was surprising since ultra-low-dose opioid antagonists *enhance* opiate-induced analgesia, and the rewarding and analgesic effects of opiates may involve similar substrates (Franklin 1998). By this perspective, rewarding drugs may produce analgesia by transforming the aversive state of pain into a more positive affective state. However, in light of the enhancement of analgesia by ultra-

CPP to Oxycodone

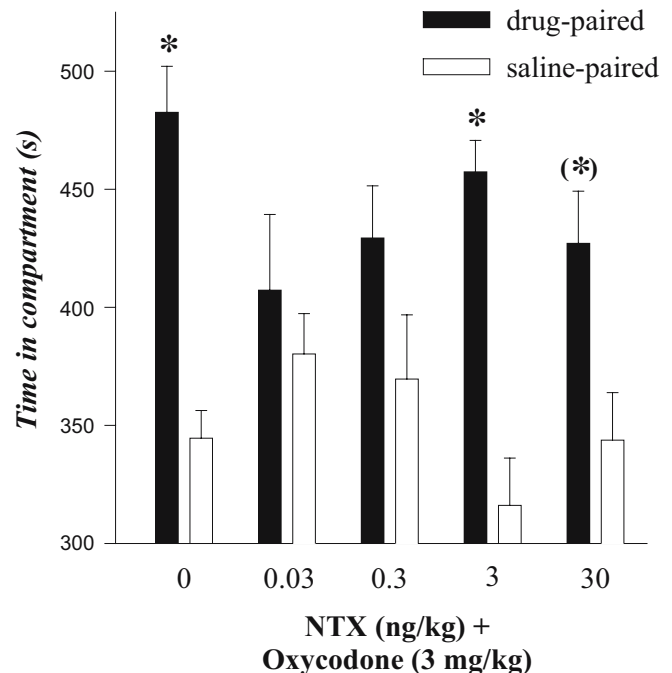


Fig. 4 Dose response of NTX on a CPP to oxycodone. Bars represent the mean (\pm SEM) amount of time (s) spent in saline- and drug-paired compartments on test day. Oxycodone (3 mg/kg s.c.) produced a significant CPP that was blocked by the addition of NTX at 0.03 ng/kg s.c. (a 1:10⁸ ratio) or 0.3 /kg (a 1:10⁷ ratio). In contrast, NTX at 3 ng/kg s.c. (a 1:10⁵ ratio) did not block the CPP to oxycodone. Oxycodone combined with the highest dose of NTX, 30 ng/kg s.c. (a 1:10⁵ ratio), produced only a trend toward a CPP

low-dose opioid antagonists, the present blockade of CPP to opiates by ultra-low-dose NTX dissociates the rewarding effects from the analgesic effects of opiates. This finding suggests that the rewarding or euphoric effect commonly experienced with opiates might be dampened by the addition of an ultra-low-dose opioid antagonist.

The suppression of CPP effects by ultra-low-dose NTX in the current study contrasts with the “paradoxical” effect on CPP previously reported by Powell et al. (2002). In that study, administration of morphine + NTX, but not morphine alone, produced a CPP. This effect occurred, however, only with subanalgesic doses of morphine and conditioning sessions 2 h after drug administration, presumably when the effects of morphine had subsided. Thus, the Powell et al. data suggest a prolongation, but not an enhancement, of a CPP. At the same time, unpublished observations from that work showed that without a delay between injection and conditioning, neither this dose of morphine nor the drug in combination with NTX produced a significant CPP, making it difficult to infer a prolongation of an effect that did not occur with no delay. In addition, the Powell et al. study used multiple conditioning sessions, which may have produced tolerance to the drug effects. An alleviation of such tolerance by ultra-low-dose NTX, known to prevent opioid analgesic tolerance, could explain the detection of rewarding effects only in the morphine + NTX group. For this reason, the present work relied on single conditioning sessions.

The experiment assessing various doses of NTX on the CPP to oxycodone in this study demonstrated a biphasic dose–response of NTX. The CPP to oxycodone was most effectively blocked by the lowest dose of NTX (0.03 ng/kg). This 0.03-ng/kg dose was also the dose coadministered with oxycodone that effectively blocked the CPA to naloxone-precipitated withdrawal. The CPP to oxycodone was also suppressed by the next lowest dose of NTX (i.e., 0.3 ng/kg) but not by the 3-ng/kg NTX dose and only marginally by the highest NTX dose of 30 ng/kg. Since oxycodone + 30 ng/kg NTX dose produced a marginal CPP, whereas oxycodone + 3 ng/kg NTX dose produced a strong CPP, it appears that this highest NTX dose may be interfering with the CPP via classical receptor antagonism, as opposed to the doses below 3 ng/kg. Presumably, much higher doses of NTX would also block the CPP to oxycodone by classical receptor antagonism.

The 0.03- and 0.3-ng/kg doses that blocked the oxycodone CPP are in the range of NTX doses shown to most effectively enhance oxycodone analgesia and alleviate tolerance and physical withdrawal (Shen et al. 2002b). Because similar NTX doses enhance analgesia while blocking tolerance, somatic and affective withdrawal as well as the conditioned rewarding effects of oxycodone, it appears that the mechanisms underlying each are similar. The lack of effect of NTX doses just outside the “ultra-low-dose” range in either blocking CPP or enhancing analgesia suggests that both phenomena are not due to a classical antagonism of opioid receptors. Indeed, receptor occupancy from these doses would be extremely low based on opioid receptor expression in rat brain (Mansour et al. 1995) and the bind-

ing affinities of NTX (Raynor et al. 1994), even assuming 100% CNS availability.

The dependence of these CPP and CPA studies on both affective experiences and associative learning begins to address the neural adaptations in response to drug exposure that contribute to addiction. Clearly, neural adaptations also underlie analgesic tolerance and dependence, and ultra-low-dose opioid antagonists attenuate both these adaptive responses. Although both learning and the adaptive responses to drug exposure contribute to addictive processes, the nature of these neural adaptations is undoubtedly complex. Nevertheless, it is possible that ultra-low-dose NTX may interfere with such processes to block the CPP and CPA effects of analgesic doses of opiates.

The effects of an ultra-low-dose opioid antagonist on the addictive properties of oxycodone were also recently investigated in a self-administration and relapse model of addiction (Leri and Burns 2005). In that study, ultra-low-dose NTX increased self-administration of oxycodone, suggesting a reduced rewarding potency similar to a reduction in dose. Furthermore, the co-self-administration of ultra-low-dose NTX attenuated reinstatement of responding triggered by oxycodone itself, by footshock stress, or by a drug-paired stimulus. The attenuation of relapse responding, however, suggests that ultra-low-dose opioid antagonists may reduce the addictive potential of opiates by reducing craving during abstinence and the vulnerability to relapse. Craving in particular is thought to result from neuroadaptations over time, even in the absence of continued drug exposure, and the attenuation of relapse measures by ultra-low-dose opioid antagonists again suggests that this treatment interferes with neural adaptations caused by opiates and associative learning around opiate effects.

In summary, these results show that ultra-low-dose NTX blocks both the rewarding effects and aversive withdrawal effects of analgesic doses of morphine or oxycodone in female rats, as expressed by environmental conditioning. Although the lack of aversive withdrawal may be explained by a lack of opioid tolerance and dependence, the attenuation of the rewarding effects of opiates contrasts with the previously established enhancement of opiate analgesia by ultra-low-dose opioid antagonists. The precise mechanism of the blockade of both these rewarding and aversive opiate effects by ultra-low-dose NTX is unclear but may be related to the suppression of neural adaptations underlying opioid tolerance and dependence. Recent data have demonstrated that ultra-low-dose opioid antagonist coadministration attenuates alterations in G protein signaling by the mu opioid receptor that occur with chronic administration of an opiate (Wang et al. 2005).

Moreover, the suppression of both the acute rewarding effects and the anhedonic aspect of withdrawal suggests that ultra-low-dose opioid antagonists may reduce the addictive potential of opiates. The enhanced analgesia of such opioid agonist/antagonist combinations was demonstrated in a phase II clinical trial of Oxytrex (oxycodone + ultra-low-dose NTX) vs oxycodone in moderate-to-severe

pain due to osteoarthritis (Chindalore et al. 2005). In a recent phase III clinical trial in low-back pain, Oxytrex produced equivalent analgesia to oxycodone with a significantly lower dose and, importantly, markedly reduced dependence as assessed by patients' withdrawal scores (L.R. Webster, manuscript in preparation).

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