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# 8 Ultra-low-dose opioid antagonists enhance opioid analgesia while reducing tolerance, dependence and addictive properties

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## Abstract

*Ultra-low-dose opioid antagonists, when combined with opiates, increase the analgesic efficacy and duration of analgesia of the opiate. This enhanced and prolonged analgesia was recently demonstrated in a 350-patient Phase II clinical trial of Oxytrex™, a novel drug candidate that combines oxycodone with an ultra-low dose of the opioid antagonist naltrexone. Extensive preclinical data also show that the addition of ultra-low-dose opioid antagonists prevents analgesic tolerance to opiates as well as opioid dependence, measured by withdrawal signs. These drug combinations can also reverse established tolerance in rodents, unlike many other approaches shown to alleviate tolerance. The mechanism of action of ultra-low-dose opioid antagonists has been shown to be the prevention of excitatory signaling of opioid receptors, a phenomenon that opposes the normal inhibitory*

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*signaling of opioid receptors and contributes to opioid tolerance. Specifically, ultra-low-dose opioid antagonists prevent the mu opioid receptor from coupling to G proteins that stimulate adenylyl cyclase, instead of coupling to the G proteins normally used by this receptor that inhibit this enzyme. Finally, while ultra-low-dose opioid antagonists enhance the analgesic efficacy of opiates, they decrease the addictive potential of opiates in rat models of drug reward, drug-taking and drug-seeking. These results suggest that the addictive properties of opiates can be partially dissociated from their analgesic effects and may be mediated in part by opioid-induced neuroadaptations interacting with learning processes. Moreover, Oxytrex and similar combinations of opiates with ultra-low-dose opioid antagonists, might significantly improve opioid therapy available today by enhancing analgesic efficacy, alleviating tolerance and physical dependence, and reducing the potential for opioid addiction.*

# Introduction

Opiates are powerful analgesics, but their use is hampered by non-trivial side effects, tolerance to the analgesic effects, physical dependence resulting in withdrawal effects, and finally, concerns surrounding the possibility of addiction. By itself, enhanced analgesic efficacy of an opiate would result in opioid sparing, and therefore a reduction in opioid-related side effects. The side effects of opiates include nausea, vomiting, pruritus, insomnia, constipation, sedation and impaired physical function (Ballantyne and Mao, 2003). In many cases, patients taking opioids are balancing side effects with analgesia, often choosing to tolerate a certain amount of pain so as to avoid side effects. The more severe side effect of respiratory depression can also limit the tolerated dose, and hence the effective analgesia in many patients.

One of the most problematic aspects of opioid therapy is analgesic tolerance with prolonged treatment. Tolerance may be defined as the need for progressively higher doses in order to maintain the same reduction in pain. While opioid rotation is currently used to minimize tolerance, this approach requires close monitoring due to variable cross-tolerance and side effect profiles among different patients (Fine, 2004). In its most severe form, opioid tolerance can manifest as opioid-induced hyperalgesia; that is, the opiate no longer reduces pain but actually increases or induces pain (Arner et al., 1988; Simonnet and Rivat, 2003; Fine, 2004). This hyperalgesia is clinically similar to the hyperalgesia of neuropathic pain, and *in vivo* models show that brainstem descending pain facilitation pathways are activated in both syndromes (Vanderah et al., 2001). Like neuropathic pain, opioid-induced hyperalgesia is extremely difficult to treat and is often a physician's greatest fear in initiating opioid therapy.

Some of the greatest fears of pain patients surrounding the use of opiates are dependence and addiction. Dependence is characterized by physical or psychological withdrawal upon discontinuation of the opiate and may be independent of addiction, which itself is defined by repeated, often self-destructive behaviors focused on obtaining the drug, according to DSM-IV criteria (American Psychiatric Association, 2000). Still, physical dependence, or the desire to avoid withdrawal, is thought to contribute to opiate addiction, particularly at later stages of addiction; whereas, a craving for the euphoric effects of opiates may dominate in earlier stages (Koob et al., 1989). The somatic withdrawal signs that can occur when opioid therapy is abruptly stopped in physically dependent individuals include agitation,

irritability, muscular jerks, abdominal pain, diarrhea, burning sensations, "gooseflesh," and itching (Miser et al., 1986; Heit, 2003). Abrupt cessation of opioid treatment can also cause a hyperalgesia, which has also been called opioid-induced hyperalgesia (Li et al., 2001). While patients receiving prolonged opioid analgesic therapy may or may not develop analgesic tolerance, they usually become physically dependent, requiring careful tapering off of the opiate in order to minimize withdrawal effects (Heit, 2003; Woolf and Hashmi, 2004).

While the incidence of actual addiction or even misuse of prescription opiates by pain patients is difficult to assess due to insufficient epidemiological data (Joranson et al., 2000), this risk continues to darken the general public's perception of opioid therapy. A further cloud over prescription opiates for patients and physicians alike, is their wide-scale diversion and abuse that has grown in recent years, particularly involving controlled-release formulations that are easily crushed to yield an immediate, large dose and a powerful high for the abuser. In 2002 in the US, this problem resulted in over 20,000 emergency room visits and hundreds of deaths involving abuse of oxycodone alone, according to the Drug Abuse Warning Network (DAWN). In reaction, the US Drug Enforcement Administration (DEA) has heightened investigations into prescription drug diversion and physicians prescribing opiates to high-risk patients. The US Food and Drug Administration (FDA) has also required "Risk Management" programs to be implemented by manufacturers of opiate medications that consist of ensuring proper use through prescriber and patient education, reducing abuse through the use of community interventions, and working closely with law enforcement to minimize diversion. This problem of abuse and diversion of prescription opiates, as well as the increased scrutiny of physician practices by health officials, has worsened the stigma around opiate analgesic therapy for both patients and physicians. While the feasibility of a non-addictive opiate analgesic may or may not be a realistic hope (Evans, 2004), any treatment that effectively reduces the abuse or addictive potential of opiates while maintaining analgesic potency would be a significant advance for opiate therapy.

The development of novel therapeutics that combine ultra-low-dose opioid antagonists with opiates may alleviate many of these undesirable aspects of opioid therapy. This approach shows promise for an improvement in analgesic efficacy, an increased duration of analgesia, an alleviation of tolerance and withdrawal, and reduced addictive potential. All of these properties together would be a vast improvement over existing opiate therapies. This review will first summarize preclinical data demonstrating the ability of ultra-low-dose opioid antagonists to enhance and prolong the analgesic efficacy of opiates as well as to prevent opioid tolerance and physical dependence. Next, enhanced and prolonged analgesia will be demonstrated in data from a 350-patient Phase II clinical trial comparing Oxytrex (oxycodone + ultra-low-dose naltrexone) to oxycodone in a three-week treatment of moderate-to-severe pain due to osteoarthritis (Chindalore et al., 2005). An overview of the mechanism of action of ultra-low-dose opioid antagonists at the level of the mu opioid receptor will next be presented. Finally, I will describe recent preclinical data suggesting that ultra-low-dose opioid antagonists may reduce the addictive potential of opiates by reducing euphoric side effects and drug craving after self-administration (Leri and Burns, 2005; Olmstead and Burns, 2005).

## Ultra-low-dose opioid antagonists enhance opiate analgesia and prevent tolerance

Extensive preclinical data have shown ultra-low-dose opioid antagonists to enhance and prolong the analgesic efficacy of opiates and prevent opioid tolerance (Crain and Shen, 1995; Powell et al., 2002; Shen et al., 2002a, b). The enhancement of analgesia is both an increase in potency and an increase in efficacy of the opiate. For example, in the mouse hot water tailflick assay, the antinociceptive effect of the $EC_{50}$ dose of oxycodone (1 mg/kg, s.c.) was enhanced by the addition of 1 pg/kg naltrexone, while the antinociceptive effect of an $EC_{100}$ dose of oxycodone (3 mg/kg, s.c.) was also enhanced by ultra-low-dose naltrexone, here at 3 pg/kg (Fig. 1). In addition, the duration of action is prolonged.

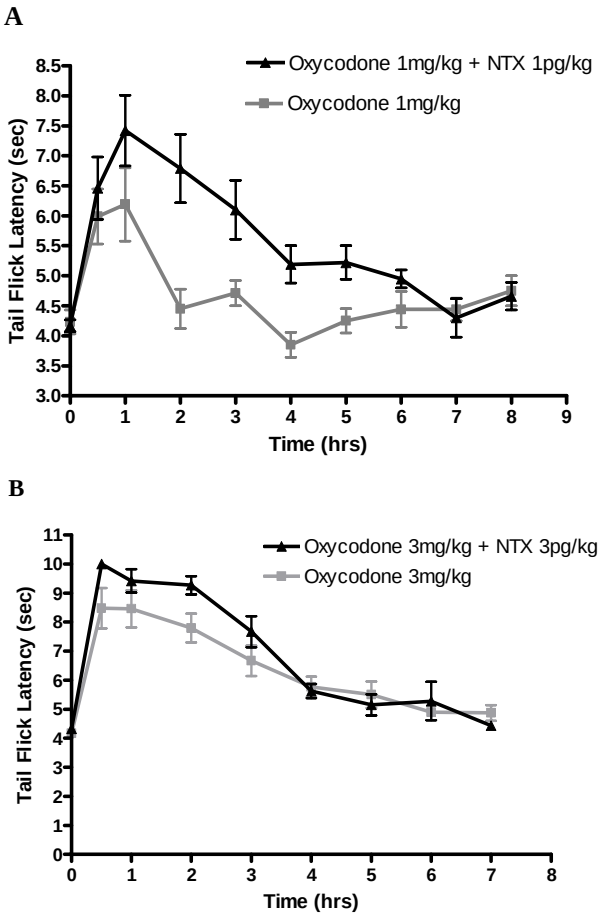


**Figure 1.** Effect of ultra-low-dose naltrexone (NTX) on potency and efficacy of oxycodone antinociception in male Swiss Webster mice using a 52ºC hot water immersion tail-flick test. **A**: Ultra-low-dose NTX (1 pg/kg, s.c.) enhanced and prolonged the antinociceptive effect of an $EC_{50}$ dose of oxycodone (1 mg/kg, s.c.) **B**: NTX (3 pg/kg, s.c.) enhanced the antinociceptive effect of an $EC_{100}$ dose of oxycodone (3 mg/kg, s.c.). Data are means ± s.e.m., $n$=9. Data courtesy of Ke-Fei Shen.

Opioid analgesic tolerance can be alleviated with the addition of ultra-low-dose opioid antagonists (Crain and Shen, 1995; Powell et al., 2002). In rodents as well as in humans, opioid tolerance can be a simple reduction or loss of analgesia, or it can progress to hyperalgesia caused by the opiate (Arner et al., 1988). Using osmotic minipumps for continuous subcutaneous delivery, oxycodone alone (8.5 mg/kg/day s.c.) produced a profound and stable hyperalgesia beginning at day 3, while with the addition of ultra-low-dose naltrexone (0.85 ng/kg/day s.c.) to this treatment produced continuous analgesia for the 28 days of testing (Fig. 2).

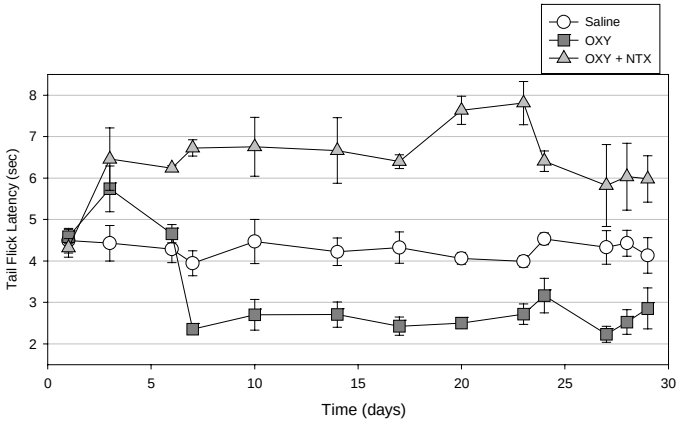


**Figure 2.** Time-effect curves of 52ºC hot water immersion tail-flick tests in male Swiss Webster mice with subcutaneous osmotic minipumps. The analgesic effect of oxycodone (OXY; 8.5 mg/kg/day) declined to baseline by day 6 and converted to hyperalgesia from day 7 through the remainder of the test period. Co-treatment with ultra-low-dose naltrexone (NTX; 0.85 ng/kg) prevented this tolerance-associated hyperalgesia through the 29 days of testing. Saline released at the same rate had no effect on baseline tail-flick latency. Data are means ± s.e.m., *n*=3. Data courtesy of Ke-Fei Shen.

In addition to preventing the development of tolerance, ultra-low-dose opioid antagonists can reverse established tolerance. Mice treated twice daily with oxycodone alone (0.3 mg/kg s.c.) show hyperalgesia by Day 3 of administration (Fig. 3). When these same mice were given this dose of oxycodone combined with administered ultra-low-dose naltrexone (0.3 ng/kg s.c.) the next day, they demonstrated a marked analgesia.

## Prevention of opioid dependence and "paradoxical" hyperalgesia

Although prolonged opioid treatment can lead to both analgesic tolerance and opioid dependence, analgesic tolerance has been attributed to desensitization of opioid receptors (Breivogel et al., 1997), while dependence is thought to be more closely related to the increased production of cAMP that occurs after chronic opioid treatment (Avidor-Reiss et al., 1995). In addition, mice deficient in β-arrestin-2 (Bohn et al., 2000), PKCγ (Zeitz et al., 2001), or the delta opioid receptor (Nitsche et al., 2002) do not develop opioid tolerance, although withdrawal effects in these knockouts are unaffected or, as in the latter two cases, actually increased. In addition to preventing analgesic tolerance, ultra-low-

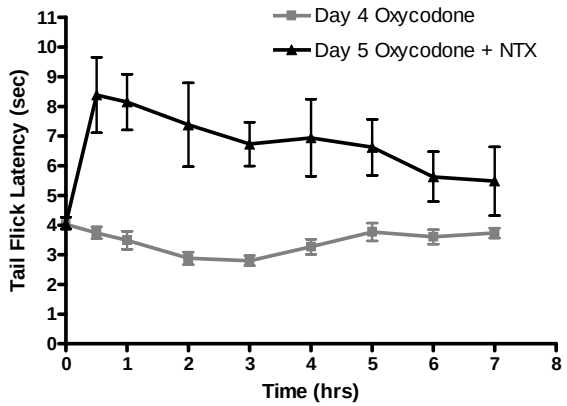


**Figure 3.** The effect of ultra-low-dose naltrexone (NTX) on established oxycodone antinociceptive tolerance in the 52ºC hot water immersion tail-flick tests in male Swiss Webster mice. Mice were treated daily with oxycodone (3 mg/kg, s.c.) for the first 4 days and showed a hyperalgesic response on day 4 (*n*=9). On day 5, a subset of mice (*n*=3) were administered the same dose of oxycodone + NTX (0.3 ng/kg, s.c.). The addition of ultra-low-dose NTX reversed the oxycodone-induced hyperalgesia from day 4 and produced an anti-nociceptive response on day 5. Data are means ± s.e.m. Data courtesy of Ke-Fei Shen.

dose opioid antagonists prevent physical dependence, demonstrated by a virtual elimination of naloxone-precipitated somatic withdrawal signs after chronic treatment (Crain and Shen, 1995; Oxbro et al., 2003).

In addition, a withdrawal-associated hyperalgesia can be precipitated by lower doses of naloxone than those used to precipitate somatic withdrawal signs, and this hyperalgesia is prevented by chronic co-treatment with ultra-low-dose opioid antagonists (Shen et al., 2002a, b). This experimental hyperalgesia models the hyperalgesia that follows prolonged opiate therapy (Miser et al., 1986). Hyperalgesia can also be elicited by low doses of opiates, a phenomenon described as "paradoxical hyperalgesia" (Kayser et al., 1987; Kiyatkin, 1989). While the mechanism for the hyperalgesia caused by low doses of opiates is unclear, ultra-low-dose opioid antagonists not only prevent this hyperalgesia but actually convert it into marked analgesia (Crain and Shen, 2001).

## Atypical dose-response curve of ultra-low-dose opioid antagonists

The enhancement of analgesia and prevention of analgesic tolerance by ultra-low-dose opioid antagonists are both counterintuitive and occur in a dose range far below the physiological dose range for opioid antagonists in blocking the effects of opiates. The effects of ultra-low-dose naloxone or naltrexone have been demonstrated in rodents most often in ng/kg or pg/kg doses, normally corresponding to antagonist:agonist ratios of $1{:}10^6$ to $1{:}10^8$. Even within the "ultra-low-dose" range, lower doses have been more effective than slightly higher doses, but with a lower limit. In an early study, 100 ng/kg naltrexone was more effective at enhancing the acute antinociceptive potency of morphine than either 1 μg/kg or 1 ng/kg naltrexone (Shen and Crain, 1997). Subsequently, Powell et al. (2002) showed that 10 ng/kg naltrexone more potently

reversed morphine tolerance than a 50 ng/kg dose. A more thorough dose-response study of naltrexone and oxycodone combinations demonstrated an even lower effective dose range for naltrexone when combined with oxycodone instead of morphine. Here, a dose response of naltrexone:oxycodone ratios (from $1:10^9$ to $1:10^5$) was superimposed on dose-response curves of oxycodone (from 0.03 to 3.0 mg/kg) (Fig. 4). This study illustrates the greater enhancement of analgesia by the lower doses of naltrexone. Specifically, 3 pg/kg and 0.3 ng/kg were more effective than 30 ng/kg of naltrexone in enhancing the analgesic effect of 3 mg/kg oxycodone. In a separate study examining naltrexone doses on preventing oxycodone tolerance, with naltrexone doses ranging from 1 ng/kg to 1 μg/kg (dose ratios of $1:10^6$ to $1:10^3$), the greatest and most persistent prevention of tolerance occurred in the group receiving the lowest dose of naltrexone (1 ng/kg), even though differences in acute enhancement of analgesia were not as dramatic in this study.

While the binding sites of ultra-low-dose naloxone and naltrexone are not currently known, it is clear they are high-affinity targets and that these "ultra-low" doses are working differently than classical receptor antagonism. Wang et al (2005) estimated receptor occupancy of a 10 ng/kg dose of naloxone to be about 1% of the MOR population, based on a saturation binding curve and assuming 100% CNS availability. Therefore, since it is unlikely that less than 1% receptor occupancy could explain the behavioral effects seen, we hypothesize that a high-affinity upstream target controls a

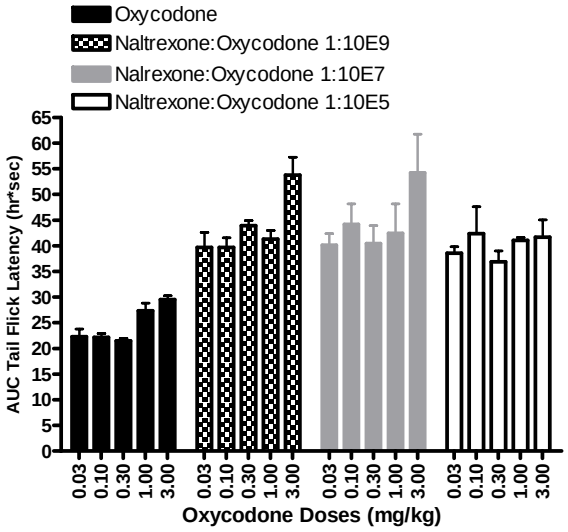


**Figure 4.** Dose response of ultra-low-dose naltrexone:oxycodone ratios in enhancing oxycodone antinociception in the 52ºC hot water immersion tail-flick tests in male Swiss Webster mice. Three different naltrexone:oxycodone ratios ($1:10^9$, $1:10^7$, and $1:10^5$) were superimposed onto a dose response of oxycodone (0.03, 0.1, 0.3, 1 and 3 mg/kg, s.c.) for antinociception. While all naltrexone:oxycodone ratios enhanced the effects of all doses of oxycodone, the $1:10^9$ and $1:10^7$ ratios more potently enhanced the antinociceptive effect of the 3 mg/kg oxycodone dose than did the $1:10^5$ dose ratio. Data are means ± s.e.m., *n*=3. Data courtesy of Ke-Fei Shen.

greater number of receptors. By this perspective, the dose-response curve of ultra-low-dose opioid antagonists at this putative high-affinity site may be complicated by interference from binding at classical receptor sites as doses increase.

## Prevention of mu opioid receptor – G protein coupling switch

Opioid receptors are inhibitory G-protein-coupled receptors. When activated by an agonist, the mu opioid receptor preferentially couples to an inhibitory, pertussis toxin-sensitive G proteins (Gi or Go), which in turn inhibits the adenylyl cyclase / cAMP pathway (Laugwitz et al., 1993; Connor and Christie, 1999). While the Gα or "signaling" subunit of these heterotrimeric G proteins that couple to mu opioid receptors inhibits the adenylyl cyclase enzyme, the Gβγ dimer portion also signals. This Gβγ inhibits cellular activities via interactions with ion channels that hyperpolarize the cell. Hence, opiates activating opioid receptors typically exert inhibitory effects. However, opiates have also been shown to exert *excitatory* effects, i.e. to induce excitatory signaling by opioid receptors, particularly after chronic opiate exposure (Shen and Crain, 1989; Crain and Shen, 1990; Wang and Gintzler, 1997; Gintzler and Chakrabarti, 2001).

The mechanism of action of ultra-low-dose opioid antagonists in combination with opiates is thought to be a prevention of such excitatory signaling of opioid receptors. Specifically, ultra-low-dose opioid antagonists prevent a switch in G protein coupling that has been shown to occur during chronic opiate administration and is thought to contribute to opioid tolerance (Crain and Shen, 1995; Wang et al., 2005). Crain and Shen first demonstrated that opiates can produce excitatory as well as inhibitory effects by measuring action potential durations in electrophysiological recordings from mouse dorsal root ganglion neurons *in vitro* (Shen and Crain, 1989; Crain and Shen, 1990). They observed that the excitatory effect (a prolongation instead of a shortening of the action potential) could be blocked by cholera toxin, an agent that blocks activation of the excitatory G protein Gs (Shen and Crain, 1990a). They therefore hypothesized that the excitatory effects of opiates were mediated by opioid receptors coupling to Gs instead of their usual inhibitory G proteins, Gi and Go. The research of Crain and Shen also suggested that the excitatory signaling of opioid receptors underlies opioid tolerance (Crain and Shen, 1992a) and is regulated by GM1 ganglioside, since GM1 ganglioside administration essentially mimicks opioid tolerance (Shen and Crain, 1990b; Shen et al., 1991; Crain and Shen, 1992b). The role of excitatory signaling of opioid receptors in tolerance was more explicitly demonstrated when Crain and Shen discovered that ultra-low-dose opioid antagonists prevented both the excitatory effects *in vitro* (Shen and Crain, 1994) and opioid tolerance and dependence *in vivo* (Shen and Crain, 1994; Crain and Shen, 1995). Based on their data, Crain and Shen (2000) theorized that ultra-low-dose opioid antagonists prevent opioid tolerance and dependence by blocking opioid receptor coupling to Gs proteins.

The hypothesis that opioid receptors couple to Gs proteins during opioid tolerance has been somewhat controversial, supported initially by the electrophysiological and pharmacological data of Crain and Shen. An alternative hypothesis has been that excitatory signaling of opioid receptors occurs by Gβγ activation of adenylyl cyclase without a switch in G protein coupling (Wang and Gintzler, 1997; Gintzler and Chakrabarti, 2001). Experiments using cholera toxin to block Gs and pertussis toxin to block Gi/o suggested that low doses of opiates may elicit excitatory effects via Gs, but

that opioid tolerance is instead mediated by Gβγ stimulation of adenylyl cyclase (Wang and Gintzler, 1997).

A more recent and extensive study examined the mechanism of opioid receptor excitatory signaling in opioid tolerance by assessing G protein coupling profiles of mu opioid receptors in CNS tissues from rats chronically treated with vehicle, morphine, morphine plus ultra-low-dose naloxone, or ultra-low-dose naloxone alone (Wang et al., 2005). Wang showed that chronic morphine induced a pronounced Gs coupling by the mu opioid receptor that was not seen in vehicle-treated rats. This research also revealed that the Gβγ dimer that is released from this Gs heterotrimeric Gs protein also interacts with adenylyl cyclase. In other words, the excitatory effect of the switch in G proteins is a result of both the α subunit and the βγ dimer stimulating adenylyl cyclase. Co-treatment with ultra-low-dose naloxone attenuated both these morphine-induced signaling changes. When administered alone, ultra-low-dose naloxone did not change the signaling profile of mu opioid receptors seen in vehicle controls.

In this work, co-immunoprecipitation of the mu opioid receptor with its associated G protein by antibodies to the various Gα proteins clearly showed that chronic morphine induced novel Gs coupling by the mu opioid receptor, while reducing the normal level of coupling to Gi and Go proteins. Ultra-low-dose naloxone co-treatment attenuated the Gs coupling and restored or even enhanced the normal Gi/o coupling. This pattern of G protein coupling alterations induced by morphine and attenuated by ultra-low-dose naloxone co-treatment was also shown by specific [$^{3}$H]DAMGO binding to mu opioid receptors after precipitation by antibodies to the various Gα proteins (Fig. 5, from Wang et al., 2005). A similar pattern of G protein coupling changes was demonstrated using an agonist-induced [$^{35}$S]GTPγS binding assay. Here, quantification of [$^{35}$S]GTPγS-bound Gα proteins that were subsequently immunoprecipitated with specific antibodies to the various Gα proteins showed the same treatment effects on G protein coupling by the mu opioid receptor (Fig 6, from Wang et al., 2005).

The chronic morphine-induced signaling to adenylyl cyclase by Gβγ was demonstrated by co-immunoprecipitation of complexes of Gβγ and adenylyl cyclase type II or IV after activation of the mu opioid receptor. Fig. 7 (from Wang et al., 2005) shows the interaction of Gβγ with adenylyl cyclase type II, and similar results were obtained for adenylyl cyclase type IV. Ultra-low-dose naloxone co-treatment also attenuated this chronic morphine-induced Gβγ – adenylyl cyclase interaction. The similar treatment effects on Gs coupling and on the Gβγ coupling to adenylyl cyclase suggested that this Gβγ was derived from the Gs heterotrimeric G protein. Isotyping of the Gβ comprising Gs versus the native Go heterotrimers that couple to the mu opioid receptor together with Gβ isotyping of the Gβγ dimers that associate with adenylyl cyclase II and IV confirmed this Gs origin.

This recent data of Wang et al. (2005) confirms the G protein coupling switch first hypothesized by Crain and Shen and links this switch to the Gβγ interaction with adenylyl cyclase first demonstrated by Gintzler and colleagues. Importantly, the prevention of both these mu opioid receptor signaling changes by ultra-low-dose naloxone co-treatment provides molecular evidence for its mechanism of action in enhancing analgesia and alleviating opioid tolerance and dependence.

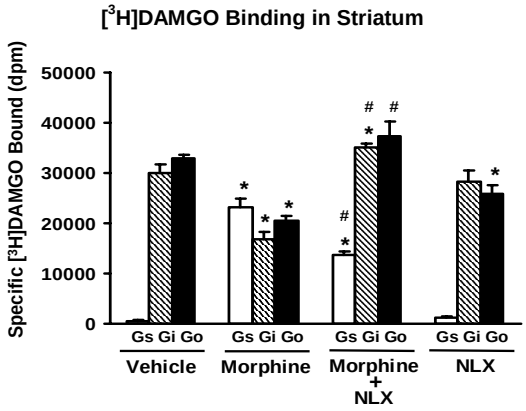


**Figure 5.** Specific [$^3$H]DAMGO binding in mu opioid receptor – G protein complexes precipitated with selective antibodies to the various Gα proteins in spinal cord membranes from rats treated twice daily for 7 days with vehicle, morphine (10 mg/kg, s.c.), naloxone (NLX; 10 ng/kg, s.c.) or these doses of morphine and NLX combined. [$^3$H]DAMGO binding was used to quantitate the amount of mu opioid receptor protein in the respective anti-Gα immunoprecipitates. A marked amount of [$^3$H]DAMGO binding was detected in Gαs immunoprecipitates in spinal cord from morphine- but not vehicle-treated rats. In membranes from rats treated with morphine + NLX, [$^3$H]DAMGO binding was decreased in Gαs immunoprecipitates compared to the morphine group, and increased in Gαi/o immunoprecipitates compared to the vehicle group. These data show that chronic morphine caused the mu opioid receptor to couple to Gs while decreasing coupling to Gi and Go and that the addition of ultra-low-dose NLX partially reversed this effect. $n = 4$. Data are means ± s.e.m. * $p < 0.05$ versus same Gα protein in vehicle group. # $p < 0.05$ versus same Gα protein in morphine group. Data courtesy of Hoau-Yan Wang.

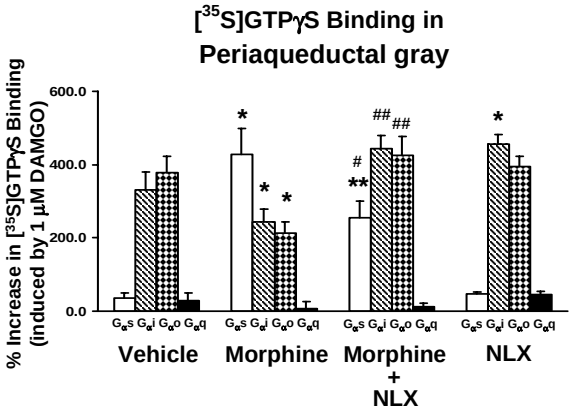


**Figure 6.** Chronic morphine treatment induced activation of Gαs in PAG that was attenuated by naloxone (NLX) co-treatment, shown here by [$^{35}$S]GTPγS binding. Membranes were incubated with 0.5 nM [$^{35}$S]GTPγS followed by incubation with vehicle or 1 μM DAMGO. Membranes were then solubilized and [$^{35}$S]GTPγS-bound Gα proteins were immunoprecipitated using anti-Gα antibodies. $n = 4$. Data are means ± s.e.m. * $p < 0.05$; ** $p < 0.01$ versus same Gα protein in vehicle group. # $p < 0.05$; ## $p < 0.01$ versus same Gα protein in morphine group. Data courtesy of Hoau-Yan Wang.

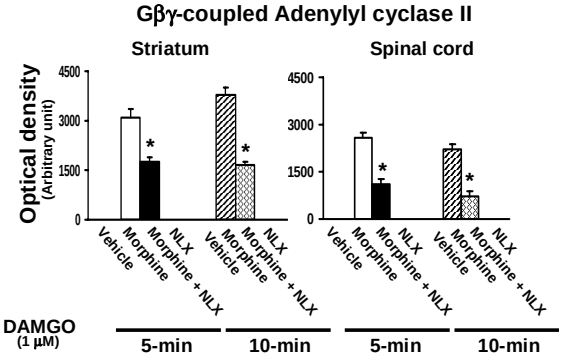


**Figure 7.** Densitometric quantitation of western blots detecting adenylyl cyclase type II in immunoprecipitates of Gβ proteins from striatal or spinal cord membranes from rats treated twice daily for 7 days with vehicle, morphine (10 mg/kg, s.c.), naloxone (NLX; 10 ng/kg, s.c.) or these doses of morphine and NLX combined. Membranes were stimulated with DAMGO for 5 or 10 min in the presence of Gpp(NH)p before co-immunoprecipitation of Gβ proteins with adenylyl cyclase II proteins. Chronic morphine treatment caused the direct association of Gβγ proteins with adenylyl cyclase type II, and this interaction was attenuated by NLX co-treatment. The blots were stripped and re-probed with an anti-Gβ antibody to demonstrating equivalent Gβ protein levels were precipitated in each lane (data not shown). $n = 4$. Data are means ± s.e.m. * $p < 0.05$ versus morphine group. Data courtesy of Hoau-Yan Wang.

## Clinical trials of Oxytrex: Improved therapeutic index compared to oxycodone

Clinical experience with opiates combined with low-dose opioid antagonists was initially limited to case reports and a few small clinical studies (Gan et al., 1997; Joshi et al., 1999; Cepeda et al., 2002; Cruciani et al., 2003; Cepeda et al., 2004). The first controlled clinical study demonstrated an opioid-sparing effect and a reduction in side effects by a continuous infusion of naloxone at 0.25 μg/kg/hr added to morphine administered by Patient Controlled Analgesia (PCA) (Gan et al., 1997). In a subsequent study, patients receiving a single 15- or 25-μg injection of nalmefene before morphine PCA reported decreased severity of pain 24 hours later and had a decreased need for antiemetics and antipruretics (Joshi et al., 1999). Cepeda and colleagues were unable to replicate these effects using a higher dose of naloxone mixed with morphine for PCA (Cepeda et al., 2002). A more recent study by Cepeda demonstrated a decrease in side effects, but no opioid-sparing effect and no enhancement of analgesia (Cepeda et al., 2004).

In a three-week 350-patient Phase II clinical trial of moderate-to-severe chronic pain due to osteoarthritis, Oxytrex was compared to placebo and oxycodone alone delivered at the same total daily dose as the oxycodone component of Oxytrex (Chindalore et al., 2005). Patients with a pain score ≥5 were stratified by sex and randomly assigned to receive placebo (51 patients), oxycodone QID (4 times daily; 102 patients), Oxytrex QID (104 patients) or Oxytrex BID (twice daily; 103 patients). To maintain blinding, the Oxytrex BID group received two active treatments and two placebos each day. All active treatment groups received the same total daily dose and dose escalation of oxycodone starting at 10 mg/day and ending at 40 mg/day. Because each dose of

Oxytrex was formulated to contain 0.001 mg naltrexone, the total daily dose of naltrexone was 0.002 mg/day for the Oxytrex BID group and 0.004 mg/day for the Oxytrex QID group. With the dose escalation of oxycodone and the naltrexone dose fixed, the naltrexone:oxycodone ratio shifted slightly over the study, i.e. from 1:5000 to 1:20,000 for the Oxytrex BID group.

Oxytrex BID produced a 39% reduction in pain intensity from baseline by study completion and this was significantly greater than the 21% reduction for placebo ($p<0.001$), the 25% reduction for oxycodone QID ($p=0.006$) and the 26% reduction for Oxytrex QID ($p=0.003$). Actual pain intensity scores for each week are shown in Table 1 (from Chindalore et al., 2005). Oxytrex BID most effectively reduced pain in both males and females and there were no significant effects of gender or gender x treatment interactions. However, treatment groups better separated in males, although only 30% of the patients were male (Fig 8, from Chindalore et al., 2005). In addition to the greatest reduction in pain intensity scores, Oxytrex BID showed the greatest separation from placebo on all secondary measures. These included quality of analgesia, duration of pain control each day (Fig. 9, from Chindalore et al., 2005), patients' global assessments and the WOMAC Osteoarthritis Index total score and subscales of pain, stiffness and physical function. Side effects were mostly typical opioid-related side effects, and their incidence was comparable between active treatment groups. While oxycodone alone did not significantly separate from placebo in this study, similar doses of oxycodone have also failed to separate from placebo in other clinical trials (Roth et al., 2000; Matsumoto et al., 2002).

The efficacy of the Oxytrex BID treatment versus placebo and oxycodone alone, in contrast to the Oxytrex QID treatment, warrants discussion. While the Oxytrex BID group received a total daily naltrexone dose of 0.002 mg, the Oxytrex QID group received naltrexone at 0.004 mg/day. It is possible that this 4 microgram total daily naltrexone dose was too high to enhance analgesia. It should also be noted that the greatest difference between Oxytrex BID and the other groups occurred at Week 3, when the opiate dose was highest, and the ratio of naltrexone to oxycodone, therefore, the lowest (1:20,000). Pharmacokinetic differences in humans versus rodents for both drugs combined with the poor oral bioavailability of naltrexone (Kogan et al., 1977) make it difficult to compare the dose ratios in this study to those of rodent studies that have used non-oral delivery methods. However, these clinical data show that 2 but not 4 μg/day of

**Table 1.** Pain Intensity Scores (Mean ± SD)

| | Placebo | Oxycodone QID | Oxytrex QID | Oxytrex BID |
|---|---|---|---|---|
| ***Baseline*** | **7.7 ± 1.3** | **7.4 ± 1.3** | **7.7 ± 1.4** | **7.6 ± 1.4** |
| ***Week 1*** | **6.5 ± 2.1** | **6.1 ± 2. 2** | **6.3 ± 2. 1** | **5.5 ± 2. 1**[1] |
| ***Week 2*** | **6.2 ± 2.5** | **5.8 ± 2. 3** | **6.0 ± 2. 2** | **5.0 ± 2. 2**[2] |
| ***Week 3*** | **6.1 ± 2.8** | **5.6 ± 2. 3** | **5.7 ± 2. 4** | **4.5 ± 2. 4**[3] |

[1] $P=0.01$ vs. placebo.
[2] $P=0.002$ vs. placebo and $P=0.05$ vs. oxycodone.
[3] $P<0.0001$ vs. placebo and $P=0.009$ vs. oxycodone.

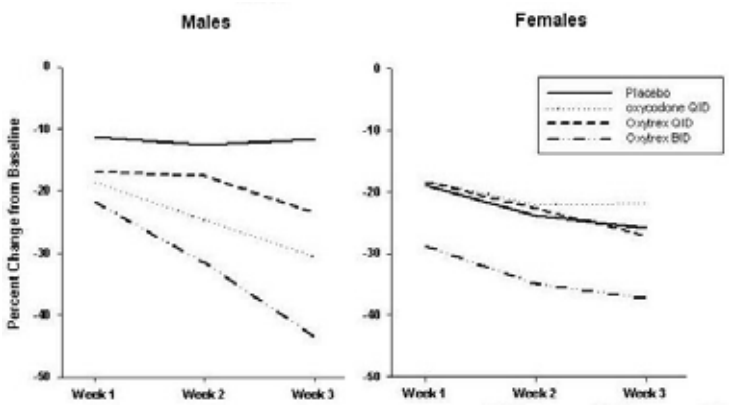


**Figure 8.** Reduction in pain intensity in males and females in a Phase II clinical trial in moderate-to-severe pain due to osteoarthritis. Oxytrex BID provided the greatest reduction in pain intensity scores in both males and females, and there were no significant effects of gender. At Week 3, Oxytrex BID was significantly better than placebo in males and significantly better than oxycodone in females. Data are mean pain intensity scores on a 0-10 numerical scale. Data courtesy of Vishala Chindalore and Pain Therapeutics, Inc.

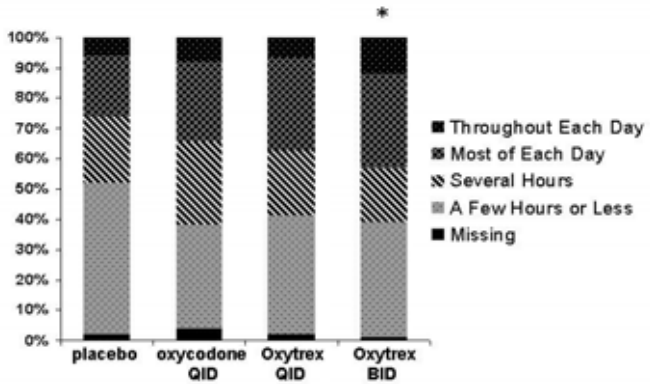


**Figure 9.** Duration of pain control each day in a Phase II clinical trial in moderate-to-severe pain due to osteoarthritis. Despite less frequent dosing, Oxytrex BID was the only active treatment that was significantly better than placebo in the assessment of duration of pain control at Week 3, indicated by percentages of patients in each level of assessment for each treatment. Data courtesy of Vishala Chindalore and Pain Therapeutics, Inc.

oral naltrexone enhanced the analgesia of oxycodone. Prior clinical studies that did not show enhanced analgesia used IV naloxone and doses of approximately 20 μg/day (Cepeda et al., 2004), 475 μg/day (Cepeda et al., 2002), and 540 μg/day (Gan et al., 1997), assuming similar patient weights between studies.

Moreover, the enhanced analgesia of the Oxytrex BID treatment compared to oxycodone alone not only demonstrates an enhanced analgesic effect but also a prolonged analgesic effect since the BID regimen of Oxytrex outperformed the QID dose

regimen of oxycodone. Immediate release oxycodone is only approved for QID dosing, and the duration of action and short half-life of immediate-release oxycodone, reported as 2.6-5.5 hrs (Leow et al., 1992; Poyhia et al., 1992; Poyhia et al., 1993), do not suggest that a BID dose regimen would produce greater analgesia than a QID regimen, even with higher doses. Furthermore, the superiority of Oxytrex BID in patients' assessments of how well their pain was controlled throughout each day also suggests a prolonged duration of analgesia.

A 719-patient, placebo- and active-controlled Phase III clinical trial in chronic low-back pain also demonstrated enhanced analgesic efficacy of Oxytrex compared to oxycodone (unpublished data of Lynn Webster and Pain Therapeutics, Inc.). In this trial, patients were dose escalated every week up to 6 weeks until they attained adequate pain relief (≤ 2) or just bearable side-effects using 10 to 80 mg of drug (or placebo) per day and then remained on that dose for an additional 12 weeks. Patients taking Oxytrex BID (with 2 micrograms/day naltrexone) achieved the same analgesia with a 12% lower average daily oxycodone dose than patients taking oxycodone QID ($p=0.03$). Importantly, physical dependence was reduced by 55% in patients taking Oxytrex BID compared to oxycodone QID, as demonstrated by responses on the Short Opioid Withdrawal Scale (SOWS) after treatment was abruptly discontinued ($p=0.01$; Fig 10). In addition, Oxytrex BID produced significant reductions in moderate to severe incidents of three opioid-related side effects. These were a 44% reduction for constipation ($p=0.01$), a 33% reduction for somnolence ($p=0.03$) and a 51% reduction for pruritis ($p<0.001$). This study confirmed the superior therapeutic index of Oxytrex BID compared to oxycodone QID and was the first clinical trial to show adequate opioid analgesia with minimal opioid withdrawal effects. Since analgesic tolerance was not observed in any treatment group, the prevention of tolerance by Oxytrex could not be assessed in this trial. An additional 12-week Phase III clinical trial is ongoing.

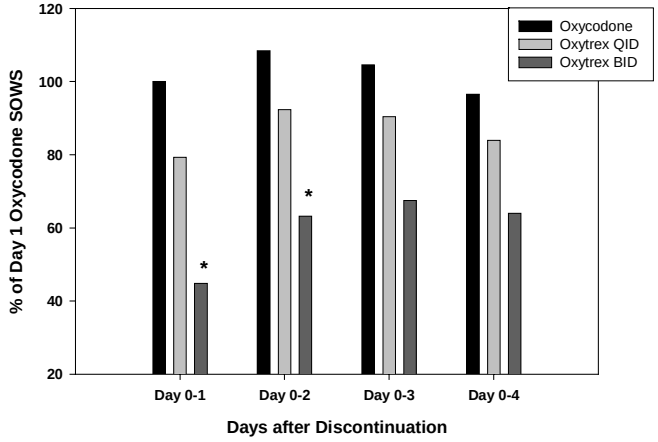


**Figure. 10.** Reduction in opioid dependence by Oxytrex demonstrated by withdrawal scores after treatment abruptly stopped. Discontinuation of Oxytrex BID resulted in a 55% reduction in the SOWS score compared to withdrawal from oxycodone ($p=0.01$). SOWS scores Days 0-2 were also significantly reduced by Oxytrex BID ($p=0.025$). Oxytrex QID produced an intermittent reduction that was not significant. Data courtesy of Lynn Webster and Pain Therapeutics, Inc.

## Aversive effects of withdrawal and acute rewarding effects are reduced

While the effects of ultra-low-dose opioid antagonists on analgesia, tolerance and dependence have been well studied, their effects on the affective component of withdrawal and on the acute rewarding effects of opiates have been examined only recently (Powell et al., 2002; Olmstead and Burns, 2005). In the conditioned place aversion (CPA) paradigm, rats chronically treated with morphine show an aversion to an environment where they experienced naloxone-induced withdrawal, an effect that may last for months (Stinus et al., 2000). This motivational effect of withdrawal is believed to reflect the anhedonia often experienced by chronic drug users during abstinence (Koob et al., 1989). Using this paradigm, Olmstead and Burns (2005) showed that withdrawal from chronic morphine or oxycodone treatment precipitated by 1 mg/kg naloxone elicited a significant CPA in rats treated with the opiate alone, but not in rats treated with the opiate in combination with ultra-low-dose naltr exone (Fig. 11, from Olmstead and Burns, 2005). This lack of an aversive response to precipitated withdrawal from chronic co-treatment with an opiate plus ultra-low-dose opioid antagonist is consistent with the absence of somatic withdrawal signs shown earlier (Crain and Shen, 1995; Oxbro et al., 2003), although the anhedonia of withdrawal is thought to be mechanistically independent of somatic signs (Bechara et al., 1995).

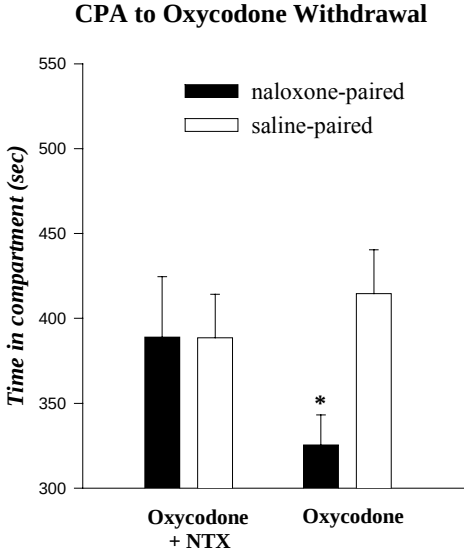


**Figure 11.** Effect of ultra-low-dose naltrexone (NTX) co-treatment (30 pg/kg s.c., a $1{:}10^8$ ratio) on a Conditioned Place Aversion (CPA) to naloxone-precipitated withdrawal from chronic oxycodone treatment (3 mg/kg, s.c. twice daily for 7 days). Rats treated with oxycodone alone showed a significant CPA to the naloxone-paired compartment, i.e. a significant decrease in time spent in the naloxone-paried compartment versus time spent in the saline-paired compartment on test day. Co-treatment with NTX blocked this CPA to oxycodone withdrawal. Data are means ± s.e.m., $n$=10. * $p$ <0.05 versus time spent in the saline-paired compartment by the same group. Data courtesy of Mary C. Olmstead.

The acute rewarding effects of opiates combined with an ultra-low-dose opioid antagonist have been tested using a similar environmental conditioning paradigm: the conditioned place preference (CPP) test (Powell et al., 2002; Olmstead and Burns, 2005). The ability of drugs to induce a CPP is one measure of their abuse potential (Bardo and Bevins, 2000). The first CPP study to assess the effects of an ultra-low-dose opioid antagonist was designed to measure an enhancement of rewarding effects and therefore used very low threshold doses of morphine. While no enhancement was found, they reported an increased duration of rewarding effects by these sub-analgesic doses of morphine (Powell et al., 2002). That is, when a 2-hr delay was implemented between drug administration and the conditioning session, animals treated with the morphine/naltrexone combination demonstrated a small but significant conditioned place preference while animals treated with morphine alone did not. In this study, rats were given four drug pairings, perhaps introducing the variable of tolerance.

A subsequent and more thorough investigation of conditioned reward, using single conditioning sessions, demonstrated that the significant place preference observed with analgesic doses of morphine or oxycodone was blocked by the addition of ultra-low-dose naltrexone (Olmstead and Burns, 2005). Further, a dose-response of naltrexone on the conditioned place preference to 3 mg/kg oxycodone in this study showed that either 0.03 or 0.3 ng/kg naltrexone blocked the place preference while a 3 ng/kg dose did not (Fig. 12, from Olmstead and Burns, 2005). Oxycodone combined with a still higher dose of 30 ng/kg naltrexone produced a marginal place preference (p=0.07), suggesting that this naltrexone dose may be interfering with the oxycodone via classical receptor antagonism, unlike the 0.03 and 0.3 ng/kg doses. The naltrexone doses most effective in blocking the rewarding effect of oxycodone are in a similar range to those shown to enhance analgesia and prevent tolerance.

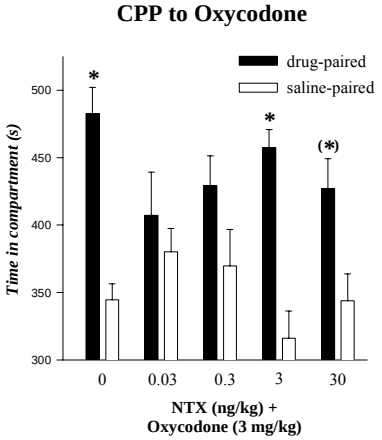


**Figure 12.** Dose response of ultra-low-dose naltrexone (NTX) on a Conditioned Place Preference (CPP) to oxycodone. Oxycodone (3 mg/kg, s.c.) produced a significant CPP, i.e. a significant increase in time spent in the drug-paired compartment versus the saline-paired compartment on test day. The addition of NTX at 0.03 ng/kg s.c., (a $1{:}10^8$ ratio) or 0.3 ng/kg (a $1{:}10^7$ ratio). In contrast, NTX at 3 ng/kg s.c. (a $1{:}10^6$ ratio) did not block the CPP to oxycodone. Oxycodone combined with the highest dose of NTX, 30 ng/kg s.c. (a $1{:}10^5$ ratio), produced only a trend toward a CPP. Data are means ± s.e.m., $n$=8. * $p$ <0.05 versus time spent in the saline-paired compartment by the same group. Data courtesy of Mary C. Olmstead.

## Attenuates potency of reward during self-administration and relapse vulnerability

Self-administration by animals is another indicator of the abuse potential of drugs (Collins et al., 1984; Koob, 1992), and opiates are well known to promote and maintain intravenous self-administration in a variety of animal species (van Ree et al., 1978; Cicero et al., 2003). In the reinstatement paradigm (Stewart and de Wit, 1987; Shaham et al., 2003), self-administration is followed by an extinction phase (drug is unavailable) and a reinstatement phase where responding is triggered by stress, drug cues or the drug itself, i.e. triggers of relapse in human addicts. In this reinstatement paradigm, the addition of ultra-low-dose naltrexone (1 pg/kg/infusion) to oxycodone (0.1 mg/kg/infusion) caused an increase in intake during the self-administration phase suggesting a decrease in the rewarding potency (Fig. 13, from Leri and Burns, 2005), an

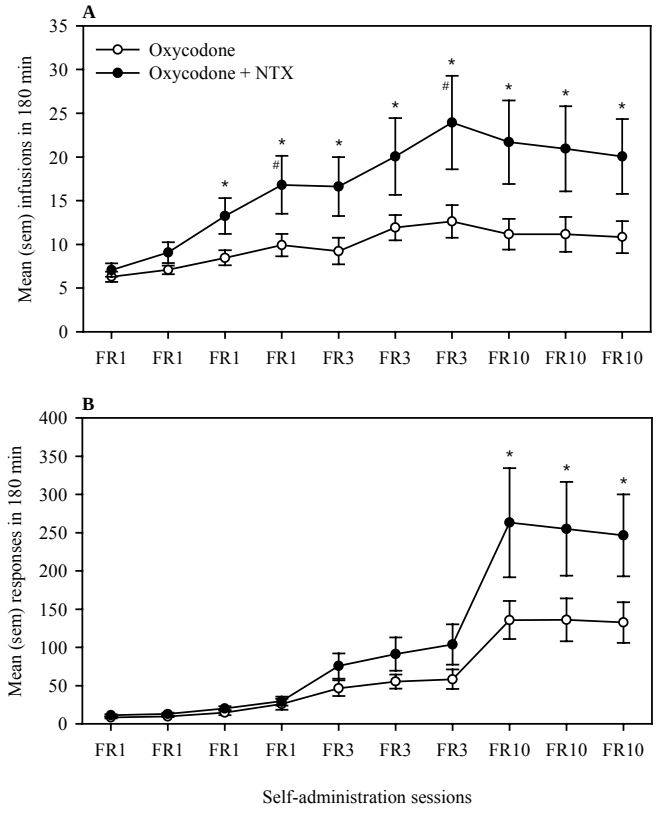


**Figure 13.** Number of infusions and lever-presses on an active lever during 10 sessions of IV self-administration of oxycodone (0.1 mg/kg/infusion) alone or in combination with ultra-low-dose naltrexone (NTX; 1 pg/kg/infusion). **A:** The oxycodone + NTX group took significantly more infusions beginning from the third self-administration session. **B:** Responses by the oxycodone + NTX group were significantly greater in each FR10 session. Data are means ± s.e.m.; $n$=12 for oxycodone, $n$=14 for oxycodone + NTX. * $p$ <0.05 versus oxycodone alone; # $p$ <0.05 versus first session in the same group. Data courtesy of Francesco Leri.

interpretation consistent with those of prior studies (Yokel and Wise, 1975, 1976). In addition, the reinstatement of responding triggered by stress or by a subcutaneous injection of oxycodone was significantly reduced in the rats that had self-administered the combination (Fig. 14, from Leri and Burns, 2005). Together, these results suggest a decreased potency of reward during self-administration that later results in decreased "drug-seeking" or drug craving during abstinence.

In a separate experiment in which rats responded on a progressive ratio schedule (lever-pressing requirements progressively increase for each drug infusion), a significantly greater percentage of rats receiving oxycodone combined with naltrexone ceased responding as the response requirements increased within each session (Leri and Burns, 2005). In addition, there was a trend toward reduced responding by the rats responding for the combination compared to those responding for oxycodone alone. Although there was no significant group difference in the mean "break-point" (the greatest number of responses emitted for a single infusion), these results may be interpreted as a slight decrease in the motivation to self-administer oxycodone when combined with ultra-low-dose naltrexone.

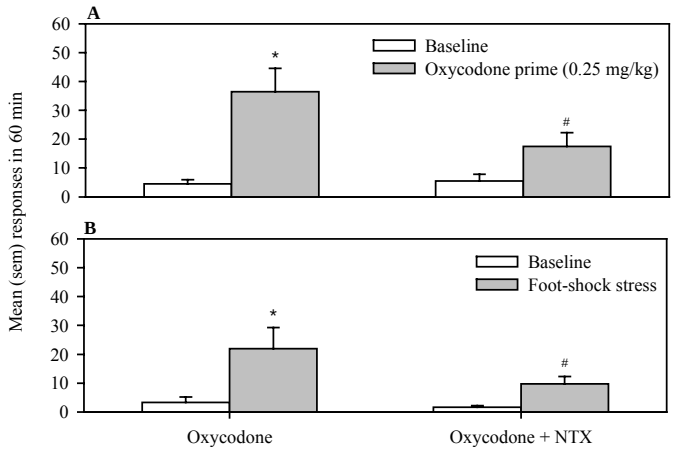


**Figure 14.** Responses during baseline and reinstatement tests in the absence of drug after extinction of lever-pressing by drug removal. **A:** Oxycodone priming elicited significantly less reinstatement of responding in the oxycodone + NTX group. **B:** Reinstatement induced by foot-shock stress was significantly reduced in the oxycodone + NTX group. Data are means ± s.e.m.; n=12 for oxycodone, *n*=14 for oxycodone + NTX. * $p < 0.05$ versus oxycodone alone. Data courtesy of Francesco Leri.

# Conclusions

Together, the work summarized in this review suggests that the addition of ultra-low-dose opioid antagonists may markedly improve several problematic aspects of opioid therapy. Although the mechanism of action of ultra-low-dose opioid antagonists appears to be the specific prevention of G protein signaling alterations of the mu opioid receptor that occur during opioid tolerance, multiple behavioral effects are seen with this co-treatment. First, the enhanced and prolonged analgesia provided by the addition of

ultra-low-dose opioid antagonists increases the therapeutic index of the opiate. Since opiate analgesic efficacy is very often limited by side effects, this augmented analgesic efficacy may help to minimize the side effects of opiates, such as the problematic gastrointestinal and respiratory depressive effects. In addition, the reductions in constipation, somnolence and pruritis observed in the Phase III clinical trial were of a greater magnitude than would be expected from an opioid sparing effect. Second, an alleviation of tolerance would preserve analgesia over time allowing more effective long-term treatment, an aspect particularly important for the chronic pain population. A lack of tolerance would also prevent the opioid-induced hyperalgesia that may occur with chronic treatment. Third, a lack of physical dependence or withdrawal would alleviate the need to carefully taper off the drug. Finally, an attenuation in the addictive potential of opiates by the addition of ultra-low-dose opioid antagonists is supported by the reduction in their rewarding or euphoric effects as well as in drug craving during abstinence in rat models of drug reward, drug taking and drug seeking. While all these properties of ultra-low-dose opioid antagonists are supported by preclinical data, so far only enhanced and prolonged analgesia, reduced physical dependence and certain reductions in side effects have been demonstrated in randomized, controlled clinical trials. Although the attenuation of addictive potential by ultra-low-dose opioid antagonists is well supported by rat data, this property will be the most difficult to confirm in humans and may emerge only after fairly extensive clinical experience with these combination therapeutics. In conclusion, the addition of an ultra-low-dose opioid antagonist has the potential to greatly improve several different aspects of current opioid therapy.

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