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Ultra-low concentrations of naloxone selectively antagonize excitatory effects of morphine on sensory neurons, thereby increasing its antinociceptive potency and attenuating tolerance/dependence during chronic cotreatment

(excitatory opioid receptor antagonist/dorsal root ganglion neuron/opioid-sensitive action potential/naltrexone/withdrawal jumping assay)

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ABSTRACT **Ultra-low picomolar concentrations of the opioid antagonists naloxone (NLX) and naltrexone (NTX) have remarkably potent antagonist actions on excitatory opioid receptor functions in mouse dorsal root ganglion (DRG) neurons, whereas higher nanomolar concentrations antagonize excitatory and inhibitory opioid functions. Pretreatment of naive nociceptive types of DRG neurons with picomolar concentrations of either antagonist blocks excitatory prolongation of the Ca^{2+}-dependent component of the action potential duration (APD) elicited by picomolar–nanomolar morphine and unmasks inhibitory APD shortening. The present study provides a cellular mechanism to account for previous reports that low doses of NLX and NTX paradoxically *enhance*, instead of attenuate, the analgesic effects of morphine and other opioid agonists. Furthermore, chronic cotreatment of DRG neurons with micromolar morphine plus picomolar NLX or NTX prevents the development of (*i*) tolerance to the inhibitory APD-shortening effects of high concentrations of morphine and (*ii*) supersensitivity to the excitatory APD-prolonging effects of nanomolar NLX as well as of ultra-low (femtomolar–picomolar) concentrations of morphine and other opioid agonists. These *in vitro* studies suggested that ultra-low doses of NLX or NTX that selectively block the excitatory effects of morphine may not only enhance the analgesic potency of morphine and other bimodally acting opioid agonists but also markedly attenuate their dependence liability. Subsequent correlative studies have now demonstrated that cotreatment of mice with morphine plus ultra-low-dose NTX does, in fact, enhance the antinociceptive potency of morphine in tail-flick assays and attenuate development of withdrawal symptoms in chronic, as well as acute, physical dependence assays.**

Many clinical reports have noted the unexpected and paradoxical observation that administration of low doses (<30 μg/kg) of the opioid antagonist naloxone (NLX) results in analgesia or enhances, rather than attenuates, the analgesic effects of morphine or other opioid agonists (refs. 1–8; see reviews in refs. 9 and 10). Similarly, low doses of NLX induce analgesia in normal and especially in arthritic rats, whereas only high doses elicit hyperalgesia (11–17). "Paradoxical" analgesia is also induced in rats by a brief series of daily injections of NLX or naltrexone (NTX) (18–21). The mechanism underlying NLX-induced analgesia and NLX enhancement of morphine-induced analgesia is unknown, although some studies have suggested that low-dose NLX may selectively block a putative opioid system that is antagonistic to analgesia (9, 10) or an endogenous dynorphin "antianalgesic system" (22). NLX may also elicit analgesia by blocking specific κ opioid-mediated hyperalgesic systems in the central nervous system (ref. 23; see also refs. 24–27).

Electrophysiologic studies of opioid effects on nociceptive types of dorsal root ganglion (DRG) neurons in culture have led us to propose that just as G_i/G_o-coupled inhibitory opioid receptor-mediated effects [e.g., shortening of the Ca^{2+}-dependent component of the action potential duration (APD)] on these neurons provide a useful cellular model of opioid analgesia (28–32), G_s-coupled excitatory opioid receptor-mediated effects (e.g., prolongation of the APD) (31–37) elicited by lower concentrations of morphine and other bimodally acting opioids in the *same* cells may provide insights into mechanisms underlying opioid hyperalgesia (31–33) and antianalgesia (22, 34, 35, 38, 39).

We recently reported that ultra-low (picomolar) concentrations of diprenorphine are remarkably effective in blocking morphine-induced excitatory effects in DRG neurons, whereas higher nanomolar levels are required to block opioid inhibitory receptor functions in these cells (35, 39). In the presence of picomolar diprenorphine, picomolar–nanomolar concentrations of morphine become effective in shortening the APD of DRG neurons, in contrast to the much higher micromolar levels generally required in tests with morphine alone. These and related results suggest that selective antagonism of higher-affinity excitatory opioid receptor functions in DRG neurons by picomolar diprenorphine markedly enhances the inhibitory potency of morphine and other μ, δ, and κ bimodally acting (excitatory/inhibitory) opioid agonists (35, 39), consonant with ultra-low-dose NLX (intrathecal) enhancement of morphine-induced analgesia in mice by antagonism of a dynorphin-mediated antianalgesic (excitatory) system (22).

The present study demonstrates that at picomolar concentrations, NLX and NTX have similar selective antagonist actions as diprenorphine on excitatory opioid receptor functions in DRG neurons, thereby unmasking potent inhibitory effects of morphine as well as other bimodally acting μ, δ, and κ opioid agonists. These results on nociceptive types of DRG neurons in culture provide a cellular mechanism to account for many previous reports of the paradoxical analgesic effects of NLX and NTX *in vivo* as well as the present evidence of enhanced antinociceptive potency of morphine in mice treated with ultra-low dose NTX. Furthermore, our findings that chronic cotreatment of DRG neurons with morphine together with picomolar NLX or NTX prevent development of the usual opioid tolerance and dependence effects (as observed after cotreatment with other excitatory opioid receptor antagonists, e.g., picomolar etorphine, which are not available for clinical

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Abbreviations: DRG, dorsal root ganglion; APD, Ca^{2+}-dependent component of the action potential duration; NLX, naloxone; NTX, naltrexone.

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use; refs. 35 and 39) have now been confirmed *in vivo* by evidence that cotreatment of mice with morphine plus low-dose NTX attenuates development of tolerance/dependence.

MATERIALS AND METHODS

Tissue Cultures. Organotypic explants of spinal cord with attached DRGs (from 13-day fetal mice) were grown on collagen-coated coverslips in Maximow chambers or in Petri dishes (31, 40). The cells were fed with standard serum/embryo extract culture medium, supplemented with 0.5 μg of nerve growth factor (NGF-7S) per ml during the first week *in vitro*, as in our previous studies (33–35, 40).

Electrophysiologic Procedures. After >3 weeks of maturation *in vitro*, intracellular current-clamp recordings of action potentials were obtained from small- and medium-size ($\approx$10–30 μm) nociceptive types of DRG perikarya (33), using similar procedures as in our previous studies (see Fig. 1 legend) (31–37). Drugs were applied by bath perfusion with a manually operated, push–pull syringe system at a rate of 2–3 ml/min. The APD was measured as the time between the peak of the action potential and the inflection point on the repolarizing phase. Opioid-induced changes in the APD (>10% of the control value) were considered significant only if they were reversed after washout with balanced salt solution (BSS).

Antinociception and Dependence Tests in Mice. Swiss–Webster male mice (25–30 g, Charles River Breeding Laboratories) were housed in groups of five, maintained on a 12-hr light/dark cycle, and provided water and food ad libitum until testing. Antinociceptive effects of opioids were measured using a warm-water tail-flick assay similar to methods previously described (e.g., ref. 41). The mouse was inserted into a plastic restraining device that permitted the tail to be dipped into a water bath maintained at 55°C. The latency to a rapid tail flick was recorded; mice with control latencies >5 s were excluded from these tests and a 10-s cutoff was used to minimize tissue damage. Six sequential control tests were made, each with a 10-min interval. The latencies of the last four tests were averaged to provide a control value. Percent antinociception was calculated according to the following formula: $100 \times [(\text{test latency} - \text{control latency})/10 - \text{control latency}]$.

Acute physical dependence was assessed by recording NLX-precipitated withdrawal jumping behavior in mice that had been injected 3–4 hr earlier with a 100 mg/kg (s.c.) dose of morphine (e.g., refs. 41–43), administered either alone or together with a low dose of NTX. Each mouse was placed individually in a tall container and the number of abrupt, stereotyped jumps was recorded during a 15-min period after administration of NLX (10 mg/kg, i.p.). Chronic physical dependence was assessed by similar NLX-precipitated withdrawal jumping behavior tests in mice that had been injected for 4 days (twice daily) with increasing doses of morphine (20–50 mg/kg, s.c.), alone or together with a low dose of NTX (41, 44, 45). On the fifth day, the animals were primed with morphine (10 mg/kg) and challenged 1 hr later with NLX (10 mg/kg, i.p.), as in previous chronic morphine-dependence assays (43, 45, 46).

Differences between treatment groups were examined for statistical significance by means of ANOVA with Neuman–Keuls tests (antinociceptive assays) or by means of χ^2 tests (dependence assays).

Materials. The following drugs were used: dynorphin A-(1–13), NLX (Endo Laboratories, New York), NTX (Sigma), and morphine (gift from Eric J. Simon, New York University). For the *in vitro* studies, opioids were generally prepared as 1 mM solutions in H_2O and then diluted with BSS to the desired concentrations, systematically discarding pipette tips after each successive 1:10 or 1:100 dilution step to ensure accuracy of extremely low (femtomolar–picomolar) concentrations (33–36, 47).

RESULTS

***In Vitro* Studies.** Intracellular recordings were made from DRG neuron perikarya that generated relatively long APDs (>3 ms in Ca/Ba BSS) and that showed characteristic responsiveness to opioid agonists and other properties of primary afferent nociceptive neurons (31–33, 35) as occur *in vivo* (48). Acute application of selective inhibitory opioid receptor agonists—e.g., etorphine—to these DRG neurons shortens the APD in 80–90% of the cells, whereas low concentrations of bimodally acting opioids—e.g., morphine, dynorphin, enkephalins—prolong the APD in the same cells (33, 35, 49).

At Ultra-Low Concentrations NLX and NTX Act as Selective Antagonists at Excitatory Opioid Receptors on DRG Neurons, Thereby Unmasking Potent Inhibitory Effects of Bimodally Acting Opioid Agonists. At nanomolar concentrations NLX blocks inhibitory APD shortening in DRG neurons by micromolar μ, δ, and κ opioid agonists as well as excitatory APD prolongation by lower picomolar–nanomolar levels (31, 32). Tests with lower concentrations of NLX have now revealed that picomolar NLX acts selectively as an antagonist at excitatory opioid receptors. In DRG neurons where femtomolar–nanomolar morphine elicited dose-dependent APD prolongation, subsequent tests on the same neurons in the presence of 1 pM NLX showed a complete block of opioid excitatory effects ($n = 6$), and in most of the cells APD *shortening* was evoked at these low (femtomolar–nanomolar) morphine concentrations ($n = 5$) (Fig. 1). Similar unmasking of potent inhibitory effects of low concentrations of morphine was obtained in another series of DRG neurons tested with femtomolar–nanomolar morphine in the presence of picomolar NTX ($n = 8$), whereas higher concentrations of NTX (nanomolar–micromolar) blocked inhibitory as well as excitatory opioid effects (Fig. 2).

Chronic Cotreatment of DRG Neurons with Morphine and Ultra-Low-Dose NLX or NTX Prevents Development of Opioid Excitatory Supersensitivity ("Dependence") and Tolerance. Coadministration of ultra-low (picomolar) concentrations of NLX or NTX during chronic treatment of DRG neurons with micromolar levels of morphine was remarkably effective in preventing development of the opioid excitatory supersensitivity and tolerance that generally occurs after sustained exposure to bimodally acting opioids. Acute application of femtomolar dynorphin A-(1–13) ($n = 17$) as well as 1 nM NLX ($n = 11$) to DRG neurons chronically exposed to 1 μM morphine together with 1 pM NLX or NTX (for 1–11 weeks) did *not* evoke the usual hyperexcitable (APD-prolonging) responses observed in chronic morphine-treated cells (34, 50, 51) when tested after washout with BSS (data not shown). Furthermore, there was no evidence of tolerance to the usual inhibitory effects of micromolar opioids ($n = 6$); even picomolar–nanomolar concentrations of dynorphin A-(1–13) still shortened the APD as observed in naive cells cotreated with picomolar NTX or NLX (Fig. 1).

***In Vivo* Studies.** If significant numbers of excitatory opioid receptors are distributed on neurons in nociceptive networks *in vivo*, then selective blockade of excitatory, but not inhibitory, opioid receptors by low concentrations of NLX or NTX might enhance the antinociceptive potency of morphine as well as prevent development of dependence, as predicted by the results of our studies on DRG neurons in culture. This hypothesis was tested on mice using NTX in view of its clinical advantages as a long-lasting and orally effective opioid antagonist (52–54).

Cotreatment of Mice with Morphine and Low-Dose NTX Enhances Opioid Antinociceptive Potency. Antinociceptive tests were carried out at various intervals up to 4 hr after drug injection, using a warm-water (55°C) tail-flick assay. Untreated mice showed tail-flick latencies of 2.15 ± 0.4 s (mean ± SD; $n = 58$). Cotreatment of mice with 10 mg of morphine per kg plus a 1000-fold lower dose of NTX (10 μg/kg; i.p.) resulted in moderate attenuation and no significant enhance-

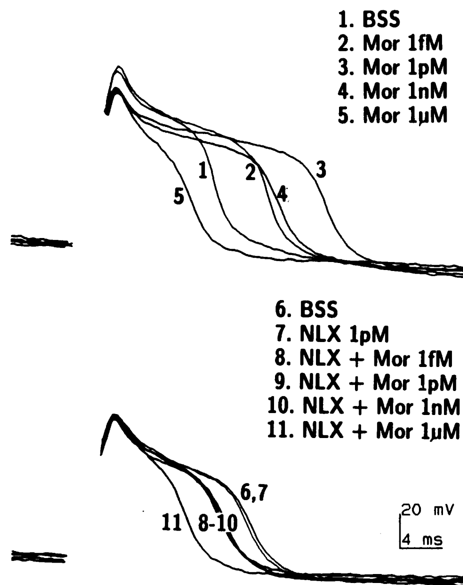

FIG. 1. Low picomolar concentrations of NLX selectively block excitatory APD-prolonging effects of morphine or other bimodally acting opioid agonists on DRG neurons, thereby unmasking potent inhibitory APD-shortening effects of low concentrations of these agonists. *Record 1*: action potential (AP) generated by a DRG neuron in BSS containing 5 mM Ca^{2+} and 5 mM Ba^{2+}. AP response in this record (and in all subsequent records) is evoked by a brief (2 ms) intracellular depolarizing current pulse. *Records 2 and 3*: APD is progressively prolonged by application of 1 fM and 1 pM concentrations of morphine (Mor) (5-min test periods, in these and all subsequent records, unless otherwise specified). *Record 4*: application of 1 nM Mor also prolongs the APD, but to a lesser degree than occurs in 1 pM Mor. *Record 5*: after a further increase in Mor concentration to 1 μM, APD shortens well below control value (cf. record 1). *Record 6*: after a 10-min washout in BSS, the APD becomes stabilized at a more prolonged value (which may be due to sustained second messenger-mediated activities following activation of excitatory opioid receptors by morphine; see text). *Record 7*: APD is not altered by subsequent application of 1 pM NLX. *Records 8–10*: in the presence of 1 pM NLX, 1 fM–1 nM Mor now *shorten* the APD, in contrast to the marked APD prolongation generally evoked by these low concentrations of Mor (cf. records 2–4 above and Fig. 2). Note: records in this and subsequent figures are from DRG neurons in organotypic DRG-spinal cord explants maintained for 3–5 weeks in culture.

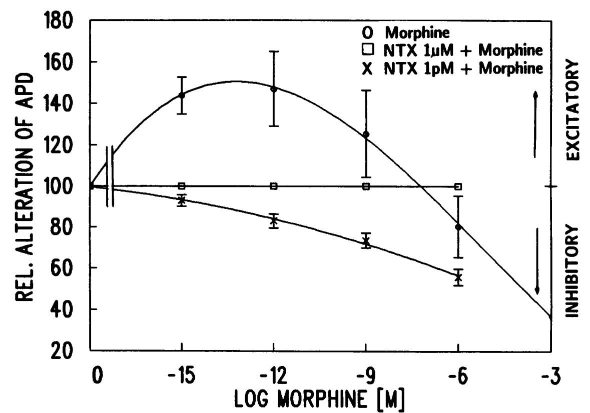

FIG. 2. Excitatory APD-prolonging effects elicited by acute application of femtomolar–nanomolar morphine to DRG neurons are selectively blocked by coadministration of a low picomolar concentration of NTX, thereby unmasking potent dose-dependent, inhibitory APD-shortening effects by low concentrations of morphine. A dose–response curve (upper) demonstrates the bimodal excitatory APD-prolonging effects of low femtomolar–nanomolar concentrations of morphine and inhibitory APD shortening at higher micromolar concentrations (○) ($n = 13$) (data obtained as in Fig. 1). In the presence of 1 μM NTX, the excitatory and inhibitory effects of morphine are completely blocked (□) ($n = 3$). By contrast, in the presence of a much lower picomolar concentration of NTX, potent dose-dependent inhibitory APD shortening by morphine is unmasked (X) in a group of DRG neurons ($n = 7$), most of which showed APD prolongation when tested with femtomolar–nanomolar morphine prior to introduction of NTX.

ment of the analgesic potency of morphine injected alone (data not shown). This result was not unexpected in view of the potent antagonist action of NTX and NLX on inhibitory (analgesic) opioid receptor functions (e.g., ref. 4). In contrast, cotreatment of mice with 1 mg of morphine per kg plus a 100,000-fold lower dose of NTX (10 ng/kg, i.p.) demonstrated that in the presence of this extremely low dose of NTX, the peak values of tail-flick latencies at 1 hr were maintained during the subsequent hour, whereas the antinociceptive effects of morphine alone rapidly decreased during this same period. Furthermore, a remarkable degree of antinociception was maintained for >1.5 hr after the effects of 1 mg of morphine per kg alone were no longer detectable ($n = 25$; Fig. 3). The marked enhancement of the analgesic potency of morphine in mice during cotreatment with 10 ng of NTX per kg is quite consonant with the unmasking of potent inhibitory effects of 1 pM–1 nM morphine in DRG neurons *in vitro* by cotreatment with 1 pM NTX (Fig. 2).

Cotreatment of Mice with Morphine Plus Low-Dose NTX Attenuates Withdrawal Jumping Behavior. *Acute physical dependence assays.* Three to 4 hr after the administration of a high dose of morphine (100 mg/kg, s.c.), injection of NLX (10 mg/kg, i.p.) evoked characteristic withdrawal jumping behavior. About 67% of these treated mice ($n = 30$) showed 5–100 robust jumps during a 15-min test period ($n = 30$; Fig. 4), whereas jumping behavior was observed in only 10–20% of untreated mice. On the other hand, after cotreatment of mice with a 10,000-fold lower dose of NTX (10 μg/kg) administered 15 min prior to and together with 100 mg of morphine per kg, the incidence of NLX-precipitated jumping behavior was markedly reduced to only 23% of the treated animals ($n = 30$; Fig. 4). The mice were routinely pretreated with NTX to ensure antagonist binding to excitatory opioid receptors prior to their possible long-lasting activation by morphine. An

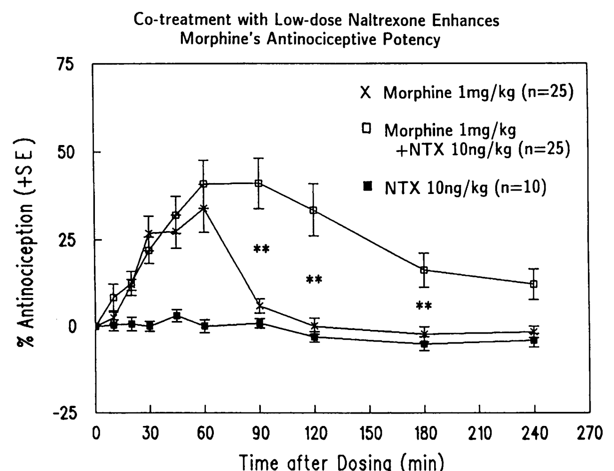

FIG. 3. Cotreatment of mice with low-dose NTX enhances morphine's antinociceptive potency, in contrast to the characteristic attenuation of morphine analgesia by higher doses of NTX (see text). Injection of 10 ng of NTX per kg alone did not elicit analgesic effects. Shown are time–response curves for morphine plus a 100,000-fold lower dose of NTX (i.p.) in a warm-water (55°C) tail-flick test. Twenty-five mice were used per dosing group (10 animals for NTX alone). **, Statistically significant differences between individual Mor vs. Mor plus NTX time points; $P < 0.01$.

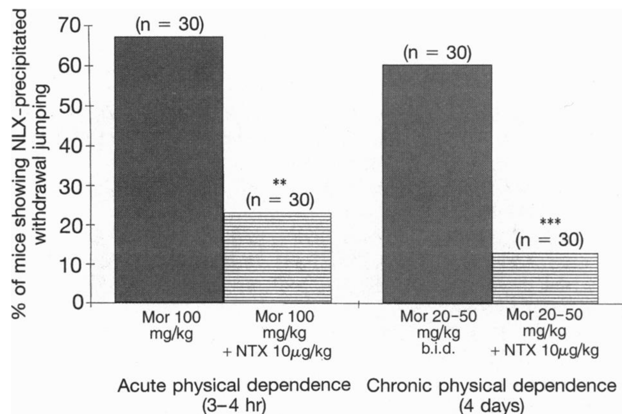

FIG. 4. Cotreatment of mice with low-dose NTX attenuates acute and chronic morphine (Mor) dependence. **, Statistically significant difference from control Mor alone group: $P < 0.01$; ***, $P < 0.001$. Additional injections of NTX (10 μg/kg, s.c.) were made at 15 min prior to all injections of morphine plus NTX (s.c.). Antinociceptive tail-flick tests on naive mice (as in Fig. 3) showed that cotreatment with 20–100 mg of morphine per kg plus 10 μg of NTX per kg did not significantly attenuate the analgesic effect of these doses of morphine injected alone (see text).

additional injection of NTX (10 μg/kg, s.c.) was made 2 hr after administration of morphine plus NTX, because this antagonist has been reported to have a much shorter duration of action in mice, in contrast to humans (52, 55).

Antinociceptive tail-flick tests on naive mice were made in order to show that this effect of 10 μg of NTX per kg was mediated primarily by blocking excitatory, rather than inhibitory, opioid receptor functions. Cotreatment of mice with 100 mg of morphine per kg plus 10 μg of NTX per kg (i.p.) did not significantly attenuate the potent (supramaximal) analgesic effect of 100 mg of morphine per kg injected alone. In both groups of treated mice, tail-flick latencies rapidly increased to the peak cutoff value of 10 s.

Chronic physical dependence and tolerance assays. Characteristic naloxone-precipitated withdrawal jumping was also elicited in mice treated twice daily with progressively increasing doses of morphine: 20, 30, 40, and 50 mg/kg (s.c.) and challenged on the fifth day with 10 mg of NLX per kg (i.p.). About 60% of the treated mice showed stereotyped jumping as observed in the acute dependence tests ($n = 30$; Fig. 4). By contrast, after cotreatment of mice with 10 μg of NTX per kg (s.c.) administered 15 min prior to and together with each of the morphine injections indicated above, NLX-precipitated jumping occurred in only 13% of the mice ($n = 30$; Fig. 4). Tail-flick assays on naive mice showed that cotreatment with 20 mg of morphine per kg plus 10 μg of NTX per kg did not significantly attenuate the analgesic effect of 20 mg of morphine per kg injected alone. In similar chronic cotreatment tests using a 10-fold lower dose of NTX (1 μg/kg), withdrawal jumping was still markedly attenuated from 60% down to 30% of the mice ($n = 30$; data not shown). These results demonstrate that chronic cotreatment with morphine plus 50,000- to 5000-fold lower doses of NTX significantly decreased development of physical dependence.

Tail-flick assays on some of these chronic cotreated mice at 1 day after drug withdrawal showed that opioid tolerance was also partially attenuated. Acute injection of 1 mg of morphine per kg resulted in a much larger degree of antinociception in chronic morphine plus 10 ng of NTX per kg cotreated mice (17% ± 3%, $n = 10$; time to peak effect at 30 min), as compared to chronic morphine-treated mice (3% ± 2% at 30 min, $n = 10$; peak effect of 7% ± 1% at 60 min) (data not shown).

DISCUSSION

Enhancement of Analgesic Potency of Morphine or Other Bimodally Acting Opioid Agonists by Low Doses of NLX or NTX. The selective antagonism by low picomolar concentrations of NLX or NTX of the excitatory effects of morphine in nociceptive DRG neurons in culture and the concomitant unmasking of potent inhibitory effects of morphine are remarkably consonant with our behavioral tail-flick assays in mice, demonstrating that the antinociceptive potency of morphine (1 mg/kg) is significantly enhanced by cotreatment with an ultra-low (10 ng/kg) dose of NTX. These studies provide insight into cellular mechanisms that may underlie many of the paradoxical analgesic effects of these opioid antagonists *in vivo.* The much longer duration of antinociceptive action of morphine plus 10 ng of NTX per kg vs. morphine alone may be due to blockade of the antianalgesic effects (see below) resulting from cAMP second-messenger-mediated processes initiated by morphine activation of putative excitatory opioid receptors on nociceptive neurons (31–37, 56–58). These long-lasting higher-efficacy excitatory opioid receptor-mediated activities would normally mask inhibitory opioid receptor-mediated analgesic effects elicited by the low levels of morphine remaining in the central nervous system at >1 hr after injection.

The results of the present study are in good agreement with a previous report that an unexpectedly low intrathecal dose of NLX (0.1 fg) in mice antagonizes the antianalgesic effects of endogenous dynorphin A (22). Our *in vitro* and *in vivo* studies may also help to account for reports of paradoxical enhancement by NLX of morphine analgesia in humans (5, 10) and rats (59), buprenorphine analgesia in humans and animals (6, 60), and pentazocine analgesia in humans and rats (4). Levine *et al.* (4) found that cotreatment of rats with 5 μg of pentazocine per kg plus 50 ng of NLX per kg significantly increased the antinociceptive potency of pentazocine.

Paradoxical Analgesia Induced by NLX or NTX. Selective blockade of tonic excitatory, but not inhibitory, opioid receptor-mediated activity by NLX or NTX provides a simple mechanism that may underlie the paradoxical analgesia induced by these opioid antagonists, especially at low doses, in humans (1–3) and animals (4, 8–15, 18–21, 23–27). In adult rats subjected to persistent pain related to arthritic inflammation (13, 14) or to peripheral neuropathy (62), single injections of 3–10 μg of NLX per kg (i.v.) elicited a "significant paradoxical antinociceptive effect," which has been more difficult to detect in normal adult animals (see, however, ref. 4). Endogenous opioid peptide levels are markedly elevated in these abnormal rats (61) and may result in increased tonic opioid activities. Under these conditions, NLX-induced blockade of excitatory, but not inhibitory, opioid receptor functions may elicit a more prominent increase in analgesia. Similarly, analgesia resulting from daily injections of NLX developed only when rats were stressed by daily "hot-plate" tests (18, 20).

Attenuation of Tolerance/Dependence in DRG Cultures and *in Vivo* After Chronic Cotreatment with Morphine and Low-Dose NLX or NTX. The present study demonstrates that cotreatment of mice with morphine and low-dose NTX facilitates opioid antinociception and attenuates development of withdrawal symptoms in chronic, as well as acute, physical dependence assays. These *in vivo* results were predicted on the basis of our *in vitro* studies showing that chronic cotreatment of DRG neurons with micromolar morphine plus picomolar NLX or NTX prevented development of excitatory supersensitivity to nanomolar NLX or femtomolar dynorphin A-(1–13). It should be emphasized that the low levels of NTX used in these cotreatment paradigms *in vivo* and *in vitro* were designed to preferentially block sustained activation of excitatory opioid receptor functions (postulated to mediate tolerance/dependence: refs. 34, 50, 51) while minimizing antagonist action at inhibitory opioid receptors that mediate antinociception. This is in sharp contrast to previous animal and

human studies in which chronic NLX or NTX was shown to prevent morphine-induced physical dependence only at high doses that simultaneously block opioid analgesia (ref. 62; see reviews in refs. 53, 54, and 63). The present *in vivo* results are in good agreement with our previous evidence that the cellular manifestations of tolerance and dependence in chronic morphine- or [D-Ala^2,D-Leu^5]enkephalin-treated DRG neurons in culture can be prevented by cotreatment with cholera toxin B subunit (which selectively blocks GM1 ganglioside-regulated excitatory opioid receptor functions; ref. 50; see also refs. 37, 47, 57, 64, and 65) or picomolar etorphine (which directly antagonizes excitatory, but not inhibitory, opioid receptors; refs. 35 and 39). Our studies suggest that ultra-low-dose NTX treatment may selectively block sensitization of excitatory opioid receptor functions during chronic exposure to bimodally acting endogenous as well as exogenous opioid agonists and thereby attenuate *protracted* as well as acute dependence syndromes (34, 49–51).

Note Added in Proof. Following completion of the present study, we became aware of a paper by Holmes and Fujimoto (66) that demonstrates enhancement of intrathecal morphine antinociception in mice by cotreatment with remarkably low doses (1 ng/kg) of either NLX or nalmefene (a NTX-related opioid antagonist). These results provide further support for our *in vitro* and *in vivo* evidence that NLX and NTX act as potent selective antagonists of excitatory opioid receptor functions.

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