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Review article

Antagonists of excitatory opioid receptor functions enhance morphine's analgesic potency and attenuate opioid tolerance/dependence liability

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Abstract

Recent preclinical and clinical studies have demonstrated that cotreatments with extremely low doses of opioid receptor antagonists can markedly enhance the efficacy and specificity of morphine and related opioid analgesics. Our correlative studies of the cotreatment of nociceptive types of dorsal-root ganglion neurons in vitro and mice in vivo with morphine plus specific opioid receptor antagonists have shown that antagonism of Gs-coupled excitatory opioid receptor functions by cotreatment with ultra-low doses of clinically available opioid antagonists, e.g. naloxone and naltrexone, markedly enhances morphine's antinociceptive potency and simultaneously attenuates opioid tolerance and dependence. These preclinical studies in vitro and in vivo provide cellular mechanisms that can readily account for the unexpected enhancement of morphine's analgesic potency in recent clinical studies of post-surgical pain patients cotreated with morphine plus low doses of naloxone or nalmefene. The striking consistency of these multidisciplinary studies on nociceptive neurons in culture, behavioral assays on mice and clinical trials on post-surgical pain patients indicates that clinical treatment of pain can, indeed, be significantly improved by administering morphine or other conventional opioid analgesics together with appropriately low doses of an excitatory opioid receptor antagonist. © 2000 International Association for the Study of Pain. Published by Elsevier Science B.V.

Keywords: Excitatory vs inhibitory opioid receptors; Excitatory opioid receptor antagonists; Ultra-low-dose naltrexone; Opioid analgesia enhancement; Opioid tolerance/dependence attenuation

1. Introduction

Systemic administration of opioid analgesics such as morphine remains the most effective means of alleviating severe pain across a wide range of conditions (Yaksh, 1997; Portenoy and Payne, 1997). However, their clinical use has been limited by undesirable side-effects that occur in substantial proportions of patients, e.g. tolerance, dependence, respiratory depression, nausea, pruritus, constipation, cognitive impairment and other aversive reactions (Jaffe and Martin, 1990; Cherny, 1996; Portenoy and Payne, 1997). Millions of people suffer needlessly from agonizing pain because physicians have been reluctant to use 'high-risk' opioids. These serious problems in utilizing opioid analgesics to treat pain have stimulated extensive studies to develop non-opioid analgesics with potencies comparable to those of opioids but without their undesirable side-effects (e.g. Merskey, 1997; Portenoy and Payne, 1997; Bannon et al., 1998). During the past decade our laboratory has carried out studies of the cotreatment of nociceptive types of sensory neurons in vitro and mice in vivo with morphine plus selective antagonists of a subset of opioid receptors that are coupled to an excitatory second-messenger system (Crain and Shen, 1990, 1992a, 1996a, 1998a,b; Shen and Crain, 1989, 1990a, 1992, 1994a) (see below). Correlation of these in vitro and in vivo results has demonstrated that direct competitive antagonism of Gs-coupled excitatory opioid receptor functions by cotreatment with extremely low doses of clinically available opioid antagonists, e.g. naloxone and naltrexone, markedly enhances morphine's analgesic potency and simultaneously attenuates opioid tolerance and dependence (Crain and Shen, 1995a, 1998b; Shen and Crain, 1997). Furthermore, recent clinical studies of post-surgical pain patients cotreated with morphine plus ultra-low doses of naloxone or nalmefene (a 6-methylene analog of naltrexone, Hahn et al., 1975) have demonstrated significant enhancement of morphine's analgesic potency (Gan et al., 1997; Joshi et al., 1999), as predicted by our preclinical studies in vitro and in vivo.

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2. Preclinical studies demonstrating that ultra-low-doses of opioid antagonists enhance opioid analgesia and attenuate tolerance/dependence by selectively antagonizing excitatory opioid receptor functions

2.1. In vitro studies

Electrophysiologic studies of opioid effects on nociceptive types of dorsal root ganglion (DRG) neurons isolated in culture have suggested that inhibitory Gi/Go-coupled opioid receptor-mediated effects (e.g. shortening of the Ca^{2+}-dependent component of the action potential duration (APD) and inhibition of transmitter release) provide a useful cellular model of opioid analgesia (North, 1986). By contrast, excitatory Gs-coupled opioid receptor-mediated effects, e.g. prolongation of the APD (Crain et al., 1988; Crain and Shen, 1990; 1992a,b; Shen and Crain, 1989, 1990a,b; 1994b), and stimulation of transmitter release (Suarez-Roca and Maixner, 1993) have been demonstrated by tests with remarkably low concentrations (<pM–nM) of morphine and many other μ, δ and *k* opioid alkaloid or peptide agonists on these same DRG neurons (Fig. 1). We have proposed that these directly evoked excitatory opioid functions may provide insights into mechanisms underlying opioid hyperalgesia (Crain and Shen, 1990, 1991; Shen and Crain, 1989, 1994b) and 'anti-analgesia' (Fujimoto and Rady, 1989; Crain and Shen, 1992b, 1998b; Arts et al., 1993; Shen and Crain, 1994a). The excitatory effects of most opioid agonists have been generally overlooked because they are often masked by the inhibitory effects elicited by opioids when administered at high concentrations (ca. μM) comparable to the systemic levels required to produce analgesia in vivo. Furthermore, directly evoked opioid excitatory effects on isolated neurons are clearly distinct from excitatory effects elicited by opioids in synaptic networks of the CNS which are mediated primarily by disinhibitory mechanisms via activation of inhibitory opioid receptors on GABA-ergic and other non-opioid interneurons (e.g. Zieglgansberger et al., 1979; Tortella, 1988; Pan, 1998).

We recently characterized a group of opioid alkaloids and peptides that at remarkably low concentrations have selective antagonist actions on excitatory, but not inhibitory, opioid receptor-mediated functions in DRG neurons in culture. This group includes specific opioid receptor antagonists, e.g. naloxone (NLX), naltrexone (NTX) and diprenorphine (Gonzalez and Brogden, 1988; Jaffe and Martin, 1990), as well as potent opioid analgesics, e.g. etorphine (Blane et al., 1967; Blane and Robbie, 1970), dihydroetorphine (Bentley and Hardy, 1967; Qin, 1993) and biphalin (Horan et al., 1993). At extremely low (pM) concentrations, all of these diverse opioids selectively antagonize excitatory

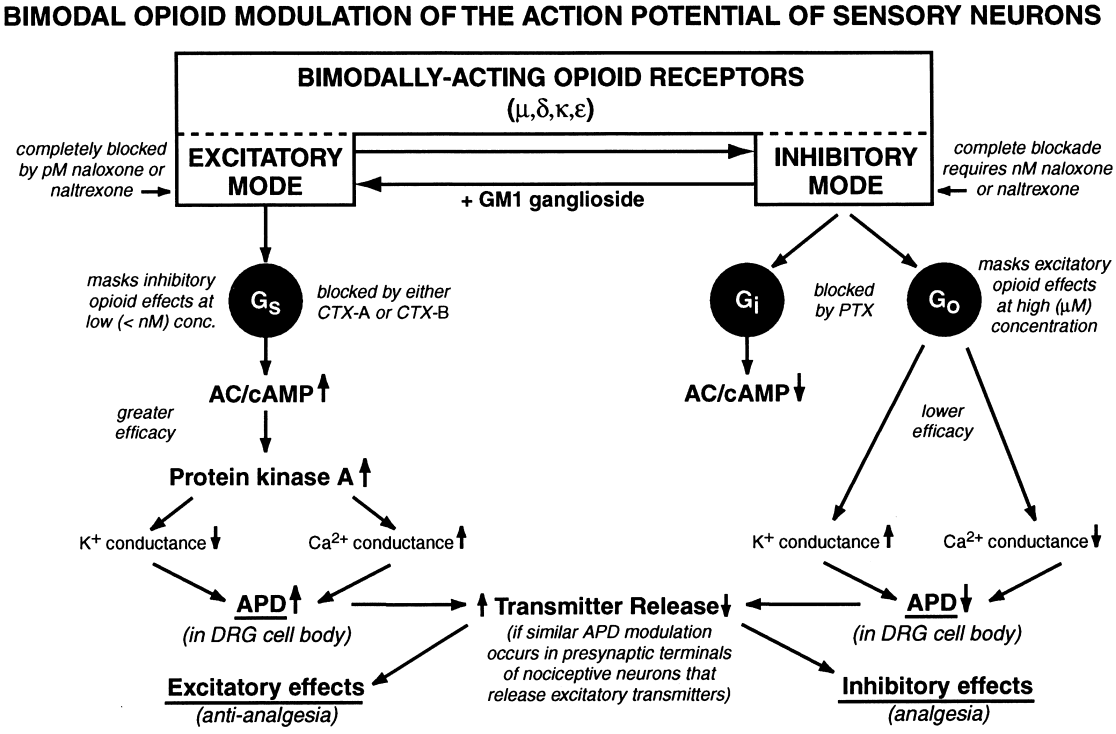

Fig. 1. Acute bimodal opioid modulation of the action potential of nociceptive DRG neurons appears to be mediated by activation of GM1-regulated, interconvertible opioid receptors that can occur either in a Gi/Go-coupled inhibitory mode (right) or in a Gs-coupled excitatory mode (left) (see text). Note sharply contrasting linkages of these Gi/Go- versus Gs-coupled receptors to K^+ and Ca^{2+} conductances, which control APD and transmitter release in presynaptic terminals of sensory neurons involved in opioid analgesic systems. AC, adenyl cyclase; cAMP, cyclic AMP; CTX, cholera toxin; PTX, pertussis toxin. Selective blockade of excitatory opioid effects in DRG neurons by cotreatment with pM naloxone or naltrexone attenuates the 'anti-analgesic' effects of morphine and other bimodally-acting opioid agonists and thereby enhances the 'analgesic' efficacy of these opioid agonists (From: Crain and Shen, 1998a).

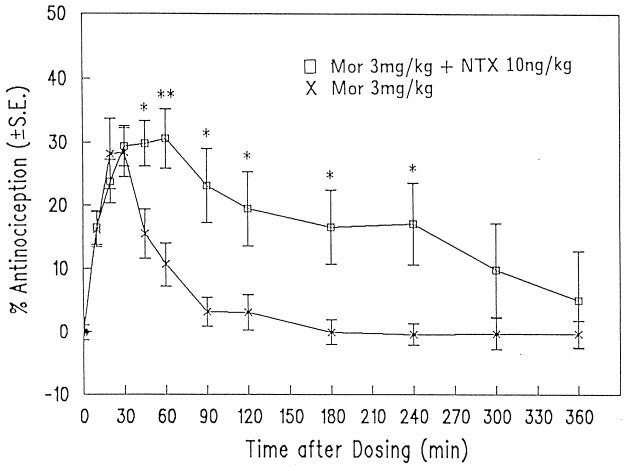

Fig. 2. Cotreatment of mice with ultra-low dose NTX (10 ng/kg) markedly enhances morphine's antinociceptive potency, in contrast to the characteristic attenuation of morphine analgesia by higher doses of NTX. (Injection of 10 ng/kg NTX alone did not elicit analgesic effects.) Time-effect curves show that the antinociception resulting from cotreatment with morphine (3 mg/kg, i.p.) plus a 300 000-fold lower dose of NTX is prolonged for > 2 h after the effects of morphine alone are no longer detectable ($n = 20$ for each curve). Hot-water (55°C)-immersion tail-flick tests. Note: asterisks indicate statistically significant differences between Mor versus Mor plus NTX time points: $^{**}P < 0.01$, $^{*}P < 0.05$ (From: Shen and Crain, 1997).

(APD-prolonging) effects elicited by pM–nM morphine or other μ, δ or *k* opioid agonists and unmask inhibitory (APD-shortening) effects which generally require much higher (μM) concentrations of morphine or other bimodally acting (excitatory/inhibitory) opioid agonists (Crain and Shen, 1995a,b; Shen and Crain, 1994a, 1995).

Etorphine is well known as a potent opioid analgesic (ca. 1000-fold more effective than morphine) (Blane et al., 1967; Blane and Robbie, 1970; Qin, 1993), whereas NLX or NTX antagonize opioid analgesia and are utilized clinically to counteract opioid overdoses (Gonzalez and Brogden, 1988; Jaffe and Martin, 1990). Etorphine has potent agonist action on inhibitory Gi/Go-coupled opioid receptor functions (Shen and Crain, 1994a), whereas NTX is an antagonist at these receptors. However, electrophysiologic studies on DRG neurons in culture show that *both* alkaloids have, in addition, a previously unrecognized selective antagonist action on excitatory Gs-coupled opioid receptor functions when administered at 1000-fold lower concentrations than are required to elicit their characteristic effects on inhibitory opioid receptor functions (Shen and Crain, 1994a; Crain and Shen, 1995b).

Excitatory opioid receptor-mediated effects on DRG neurons in culture are selectively blocked by low concentrations of the A fraction of CTX (Shen and Crain, 1990a) (which ADP-ribosylates the stimulatory regulatory protein, Gs), whereas inhibitory opioid receptor-mediated effects are selectively blocked by pertussis toxin (PTX) (which ADP-ribosylates the inhibitory regulatory proteins, Gi and Go) (Shen and Crain, 1989; Crain and Shen, 1990; Gintzler and Xu, 1991; Cruciani et al., 1993). These and related results suggested that activation of Gs-coupled opioid receptors on sensory neurons elicits stimulatory effects via an adenylate cyclase (AC)/cyclic AMP/protein kinase A (PKA)-mediated transduction system which thereby attenuates inhibitory effects mediated by concomitant activation of Gi/Go-coupled opioid receptors on these cells (Crain and Shen, 1990, 1992a, 1998b; Cruciani et al., 1993) (Fig. 1). Furthermore, recent studies by Wu et al. (1997, 1998) with cloned opioid receptors transfected into non-opioid cell lines have shown that opioid receptors can be rapidly interconverted between inhibitory Gi/Go-coupled and excitatory Gs-coupled modes following physiologic alterations in the concentration of a specific glycolipid, GM1 ganglioside (Fig. 1; see also Fig. 5). GM1 is abundantly distributed on the surface of neuronal cell membranes and is synthesized by a cAMP/PKA-dependent glycosyltransferase (Dawson et al., 1983; Scheideler and Dawson, 1986; see below). This dynamic plasticity of GM1-regulated opioid receptors provides a unique cellular mechanism that may underlie modulation of opioid analgesia, tolerance and dependence (Crain and Shen, 1998a,b and see below), as well as opioid receptor-mediated functions involved in brain-reward circuits (Gardner and Lowinson, 1993; Schulteis and Koob, 1996).

Our in vitro studies showed that cellular signs of tolerance and dependence in chronic μM morphine- or D-Ala-D-Leu-enkephalin-exposed DRG neurons are due to sustained activation of supersensitized excitatory opioid receptor functions (Crain and Shen, 1992a; see Section 4 and Fig. 5). These tolerance/dependence effects on DRG neurons could be prevented by chronic cotreatment with selective blockers of excitatory opioid receptor functions: (1) pM concentrations of NTX or other antagonists of excitatory opioid receptors (Crain and Shen, 1995a; Shen and Crain, 1994a); (2) cholera toxin-B subunit which selectively binds to GM1 ganglioside on neuronal cell membranes and blocks a putative allosteric GM1 ganglioside site on Gs-coupled excitatory opioid receptors (Shen and Crain, 1990b, 1992; Shen et al., 1991; see also Crain and Shen, 1992b, 1998a,b; Wu et al., 1995, 1997, 1998).

It should also be emphasized that Gs-coupled excitatory opioid receptors appear to become progressively *sensitized* during chronic exposure of DRG neurons to bimodally-acting opioid agonists (Crain and Shen, 1992a; Shen and Crain, 1992a). This is in sharp contrast to the marked *desensitization* that occurs during sustained agonist exposure of Gs-coupled β-adrenergic receptors (Freedman and Lefkowitz, 1996), some types of Gi/Go-coupled opioid receptors (Aghajanian, 1978; Harris and Williams, 1991; Freedman and Lefkowitz, 1996), and many other G-protein-coupled receptors (Chuang et al., 1996).

2.2. In vivo studies

These studies on DRG neurons in vitro were confirmed by in vivo assays showing that acute cotreatment of mice (i.p.)

with morphine (1–3 mg/kg) plus a remarkably low dose of NTX (10–100 ng/kg) does, in fact, markedly enhance the magnitude and duration of antinociceptive effects of morphine, as measured by hot-water-immersion tail-flick assays (Fig. 2; Crain and Shen, 1995a; Shen and Crain, 1997). Furthermore, chronic cotreatment of mice with morphine (30–50 mg/kg) plus relatively low doses of NTX (10 μg/kg, i.p. or higher oral dosage due to low oral bioavailability of NTX (Shen and Crain, 1997); see also Section 3.4) sharply attenuates development of tolerance and NLX-precipitated withdrawal-jumping in antinociceptive and physical-dependence assays (Fig. 3; Crain and Shen, 1995a; Shen and Crain, 1997).

Interestingly, cotreatment with a subanalgesic dose of etorphine (10 ng/kg) is equally effective as NTX in enhancing morphine's antinociceptive potency (Shen and Crain, 1997, Fig. 9) and attenuating withdrawal-jumping after chronic exposure (Fig. 3B). The similar effects of co-treatment with ultra-low doses of etorphine or NTX in enhancing morphine's antinociceptive potency and attenuating its tolerance/dependence liability, in contrast to their opposite effects when administered alone at higher conventional clinical doses, would be difficult to account for without recognizing the shared selective high-affinity antagonist action of both alkaloids on excitatory opioid receptor functions, as revealed by in vitro studies on DRG neurons (see above; Shen and Crain, 1994a; Crain and Shen, 1995a,b).

Many clinical reports have noted the unexpected and paradoxical observation that administration of relatively low doses (ca. 10 μg/kg) of NLX resulted in analgesia (e.g. Levine et al., 1979; Schmidt et al., 1985; Taiwo et al., 1989) or *enhanced*, rather than attenuated, the analgesic effects of morphine or other opioid agonists (e.g. Bergman et al., 1988; Levine et al., 1988; see reviews in Gillman and Lichtigfeld, 1985; 1989; Holmes and Fujimoto, 1993; Crain and Shen, 1995a). Correlative studies in animals have suggested that low-dose NLX may selectively block a putative endogenous opioid system that is antagonistic to analgesia (Gillman and Lichtigfeld, 1985; 1989; see also Kayser and Guilbaud, 1981; Kayser et al., 1986; Vaccarino et al., 1989; Poulos et al., 1990) or an endogenous dynorphin ''antianalgesic system'' (Fujimoto and Rady, 1989; Holmes and Fujimoto, 1993). Low-dose NLX may also elicit analgesia by blocking specific κ opioid-mediated hyperalgesic systems in the central nervous system (Wu et al., 1983; see also Hamann and Martin, 1992; 1994; Parvini et al., 1993; Hamann and Sloan 1994). Alternatively, it has been proposed that low-dose NLX may enhance release of endogenous opioid peptides by blocking presynaptic autoinhibition of enkephalin release (Ueda et al., 1986). Our studies suggest that lower doses of NLX and NTX (ca. 1–100 ng/kg) may be even more effective because they selectively block activation by endogenous as well as exogenous opioid agonists of higher-affinity Gs-coupled excitatory opioid receptors mediating anti-analgesia without concomitantly attenuating inhibitory Gi/Go-coupled opioid receptors

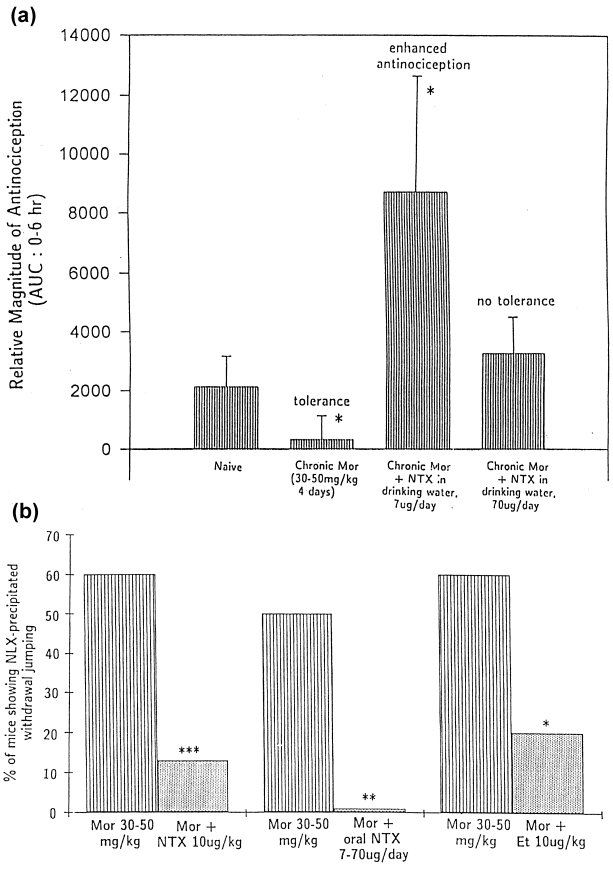

Fig. 3. Chronic cotreatment with morphine in mice plus low-dose NTX or etorphine attenuates development of tolerance and dependence. (A) Chronic treatment with morphine (30–50 mg/kg, i.p.) in mice maintained for 4 days on low-dose NTX, administered ad libitum in the drinking water, attenuates tolerance and enhances morphine's antinociceptive potency. Histogram bars represent areas under the time-effect curves illustrated in Shen and Crain (1997), Fig. 10. Asterisk indicates statistically significant difference from naïve control group: $P < 0.05$. (B) Chronic cotreatment of mice (i.p.) for 4 days with morphine plus low-dose NTX ($n = 30$) or etorphine ($n = 20$) attenuates development of dependence (in contrast to control Mor alone groups: $n = 30$ and $n = 20$, respectively). Note the particularly dramatic blockade of naloxone-precipitated withdrawal jumping in chronic morphine-treated mice receiving low-dose NTX in their drinking water at 7 μg/day ($n = 10$) as well as at 70 μg/day ($n = 10$) (bar represents data pooled from both groups) in contrast to control Mor alone group ($n = 10$). Asterisks indicate statistical difference from control Mor alone group: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ (Modified from: Shen and Crain, 1997).

mediating analgesia (Crain and Shen, 1995a). Our results are in good agreement with a study by Holmes and Fujimoto (1993) demonstrating enhancement of intrathecal morphine antinociception in mice by cotreatment with similarly low doses (1 ng/kg, i.p.) of either NLX or nalmefene and evidence of Quock et al. (1993) of enhanced kappa opioid-induced antinociception (using U50,488H; 3 mg/kg, s.c.) by cotreatment with even lower doses of NLX (1–10 pg/kg, s.c.). However, neither of these studies, nor the others noted above, addressed possible concomitant attenuation of the development of morphine tolerance or dependence during sustained cotreatment with morphine,

or other opioid agonists, plus low doses of opioid antagonists (Crain and Shen, 1996b).

3. Cotreatment of pain patients with low doses of opioid antagonists enhances analgesic potency of morphine and other opioid agonists

3.1. Low-dose naloxone enhances pentazocine analgesia

The first systematic clinical study to determine if a low dose of an opioid receptor antagonist could enhance the analgesic potency of an opioid agonist was carried out by Levine et al. (1988). This clinical study utilized cotreatment with a low dose of NLX plus a common opioid analgesic for the treatment of pain following tooth-extraction surgery (>100 patients; double-blinded). The analgesic potency of a 60 mg dose of the *k* opioid agonist, pentazocine (ca. 1 mg/kg) was markedly increased by cotreatment (i.v.) with 0.4 mg NLX (ca.6 μg/kg) in pain evaluation tests made at 50 min and at 3 h after dosing. As noted by Levine et al. (1988), ''The combination of low-dose naloxone and pentazocine produced significantly greater analgesia [ca. 2-fold] than...high-dose morphine [15 mg] administered alone.... Since the half-life of naloxone in humans is only 60 min, the prolonged (up to 3 h) analgesia produced by low-dose naloxone plus pentazocine in humans suggests that significantly lower doses of naloxone than those employed in this study may be sufficient to potentiate pentazocine analgesia.'' Although comparative cotreatment tests carried out by Levine et al. (1988) with 8 mg morphine plus 0.4 mg NLX resulted in blockage of morphine's analgesic effect in these dental patients, we believe that this failure to enhance morphine's analgesic potency was due to the use of too high a dose of NLX. Cotreatment with lower doses of NLX would probably have enhanced morphine analgesia by selectively antagonizing morphine's excitatory, but not inhibitory, receptor-mediated functions as occurs in mice (Crain and Shen, 1995a; Shen and Crain, 1997). On the other hand, this dose of NLX (0.4 mg) was evidently effective in enhancing pentazocine's analgesic potency because NLX has weaker antagonist potency at *k*, in contrast to μ, inhibitory opioid receptors.

3.2. Low-dose naloxone enhances morphine analgesia

An even more compelling clinical study was carried out by Gan et al. (1997) on 60 post-hysterectomy patients (double-blinded). Low-dose NLX infusion (0.25 μg/kg/h, i.v.) during a 24-h test period significantly reduced the cumulative patient-controlled morphine usage (i.v. via a PCA machine) from about 60 mg down to 40 mg. Furthermore, the differences between the cumulative morphine use in the NLX-cotreated versus placebo groups began to occur only after 4–8 h and became increasingly prominent by 20–24 h (Fig. 4). These data suggest that the patients using morphine alone were becoming progressively *tolerant* to the analgesic effects of morphine during the 24-h test period, in contrast to the stable rate of morphine usage by the patients receiving low-dose NLX cotreatment. On the other hand, no opioid-sparing effect, and slight attenuation of morphine analgesia, was observed in another group of these patients infused with a 4-fold higher dose of NLX, as had been reported in previous clinical trials on post-surgery patients using 1 μg/kg/h NLX cotreatments (Johnson et al., 1988; Wright et al., 1992). In addition to demonstrating that low-dose NLX cotreatment enhanced morphine's analgesic potency and attenuated development of tolerance, the study of Gan et al. (1997) also showed that several aversive hyperexcitability side-effects of the morphine treatment, e.g. nausea, vomiting and pruritus, were reduced from about 55–80% down to 20–45%. The ''surprising and intriguing opioid-sparing effect seen with low-dose naloxone'' by Gan et al. (1997) was predicted by our preclinical studies in vitro and in vivo demonstrating that cotreatment with appropriately low-doses of NLX or NTX results in sustained enhancement of morphine's antinociceptive potency by selectively antagonizing its excitatory anti-analgesic side-effects (Crain and Shen, 1995a, 1998b; Shen and Crain, 1997).

3.3. Low-dose naltrexone enhances codeine analgesia

Low-dose NTX is a particularly attractive agent for cotreatment with morphine or other opioid agonists in chronic pain patients because of its well-established effectiveness as an opioid receptor antagonist with a long duration of action (ca. 24 h) (Martin, 1984) and with no significant toxicity even when administered orally, once a day, for several *years*, at doses of 1 mg/kg (Verebey et al., 1976; Gonzalez and Brogden, 1988; O'Brien, 1997). This high NTX dosage, which has been used for long-term maintenance treatment of large numbers of opioid and alcohol addicts (Martin, 1984; Washton et al., 1984; Kleber et al., 1985; Gonzalez and Brogden, 1988), blocks all of the inhibitory, as well as excitatory, opioid receptor functions in central and peripheral neurons so that even large doses of morphine are ineffective in eliciting analgesia, as well as euphoria. By contrast, selective blockade of excitatory opioid receptor functions occurs in mice cotreated i.p. with morphine plus much lower doses of NTX, e.g. <0.1 μg/kg, or higher levels of NTX administered orally (ad libitum) via the drinking water supplied to these mice (Section 2.2; Crain and Shen, 1995a; Shen and Crain, 1997). Initial studies on the analgesic efficacy of cotreatment with oral low-dose NTX, as an alternative to i.v. low-dose NLX, have recently been carried out by thermal pain-threshold tests on 80 normal human volunteers. A Thermal Sensory Analyzer (Medoc Ltd. Advanced Medical Systems) was utilized to apply an electronically controlled series of stimuli via a variable temperature thermode to the index finger. This trial demonstrated that acute oral cotreatment with a weak dose of codeine plus a low dose of NTX

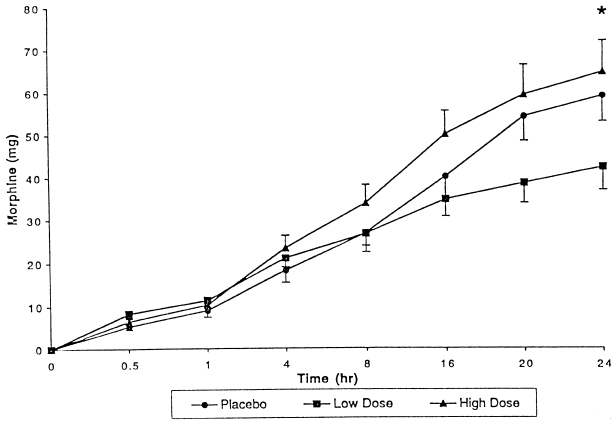

Fig. 4. Opioid-sparing effects of a continuous i.v. infusion of low-dose naloxone together with patient-administered i.v. morphine sulfate during 24-h treatment after abdominal hysterectomy. The cumulative morphine dose is plotted versus time (mean ± SEM). * $P < 0.05$ for low-dose plotted versus placebo or high-dose regimens. Low dose: 0.25 μg/kg/h naloxone; high dose: 1 μg/kg/h naloxone % saline (From: Gan et al., 1997).

resulted in a >2-fold increase in the analgesic effect, at 2 h after drug administration, as compared to double-blinded tests on the same group of subjects given codeine plus placebo (J.C. Arezzo, R. Kroop et al. (1999) in prep.).

3.4. Low-dose nalmefene enhances morphine analgesia

Low-dose nalmefene is another useful opioid antagonist for enhancing the analgesic potency of morphine or other opioid agonists because of its even longer duration of action and higher potency than NTX when administered orally (Gal et al., 1986) as well as i.v. (Dixon et al., 1986; Gal et al., 1986; Kaplan and Marx, 1993). Moreover, an oral dose of nalmefene has a systemic bioavailability of about 40%, whereas oral NTX availability may be much lower, ca. 5–10% (Meyer et al., 1984; Gal et al., 1986; Gonzalez and Brogden, 1988). Comparative antinociceptive tail-flick and withdrawal-jumping physical-dependence assays of nalmefene versus NTX in mice showed that nalmefene (i.p.) is effective at significantly lower doses than NTX in enhancing morphine's analgesic potency and attenuating dependence after chronic cotreatment with morphine (Crain and Shen, 1996c, in prep.). Furthermore, a clinical study with ultra-low dose nalmefene was recently carried out by Joshi et al. (1999) on 120 post-hysterectomy (and related abdominal-surgery) patients (double-blinded). A single injection of a remarkably low dose of nalmefene (15 or 25 μg, i.v.) was administered shortly after surgery to two groups of 40 patients followed by patient-controlled morphine usage for a 24-h test period. Patient visual-analog assessments at 24 h indicated that the groups cotreated with 15 or 25 μg nalmefene plus morphine reported significantly lower scores of pain severity: 3.5 and 2.3, respectively, versus 5.5 for the group treated with morphine plus placebo (Table 1). Moreover, the cotreated patients reported significantly higher scores for pain relief: 8 and 9 versus 6 in the control group. The total consumption of morphine during the 24-h study period was, however, similar in the three groups of patients. Further studies will be required to determine if the single injection of nalmefene used in this study did, in fact, provide effective antagonist action on excitatory opioid receptor functions during the entire 24-h test period. More frequent injections of nalmefene, e.g. at 8 h intervals, and lower doses may result in greater enhancement of morphine's analgesic potency and a reduction in cumulative patient-controlled morphine usage. Cotreatment with low-dose nalmefene also attenuated other hyperexcitability side-effects of morphine, e.g. nausea, vomiting and pruritus, as previously reported by Gan et al. (1997) during cotreatment with low-dose NLX.

3.5. Consonance of preclinical and clinical evidence that cotreatment with low doses of opioid antagonists enhances morphine analgesia

The results of the study by Joshi et al. (1999) demonstrating marked enhancement of morphine analgesia by cotreatment with a single i.v. injection of the long-acting opioid antagonist nalmefene at 0.2 μg/kg during a 24-h test period are in good agreement with the study by Gan et al. (1997) showing that continuous i.v. perfusion of the short-acting antagonist NLX at 0.25 μg/kg/h also enhanced morphine analgesia during a 24-h test period. Furthermore, both of these clinical studies are remarkably consonant with our preclinical studies demonstrating that cotreatment of mice with a single i.p. injection of NTX at 0.001–0.1 μg/kg markedly enhances both the magnitude and duration of the analgesia elicited by a single dose of morphine during 6-h test periods (Crain and Shen, 1995a; Shen and Crain, 1997).

All of these clinical and preclinical results with low-dose NLX, NTX or nalmefene cotreatments can be readily accounted for by selective antagonism of morphine's excitatory effects mediated by Gs-coupled opioid receptor functions (Crain and Shen, 1995a, 1998b). By contrast, these results are unexpected and 'paradoxical' when viewed from the standpoint of traditional opioid pharmacology concepts (e.g. Uhl et al., 1994; Reisine and Pasternak, 1997) which fail to acknowledge that morphine and most opioid alkaloid and peptide agonists can activate opioid receptors distributed in both excitatory Gs-coupled as well as inhibitory Gi/Go-coupled modes on nociceptive neurons (Crain and Shen, 1998a, b). The striking consistency of these multi-disciplinary studies on nociceptive neurons in culture, behavioral assays on mice and clinical trials on post-surgical pain patients (and experimental thermal pain in human volunteers), provides encouraging evidence that clinical treatment of pain can, indeed, be significantly improved by administering morphine or other conventional opioid analgesics together with appropriately low doses of an excitatory opioid receptor antagonist, e.g. NLX, NTX or nalmefene.

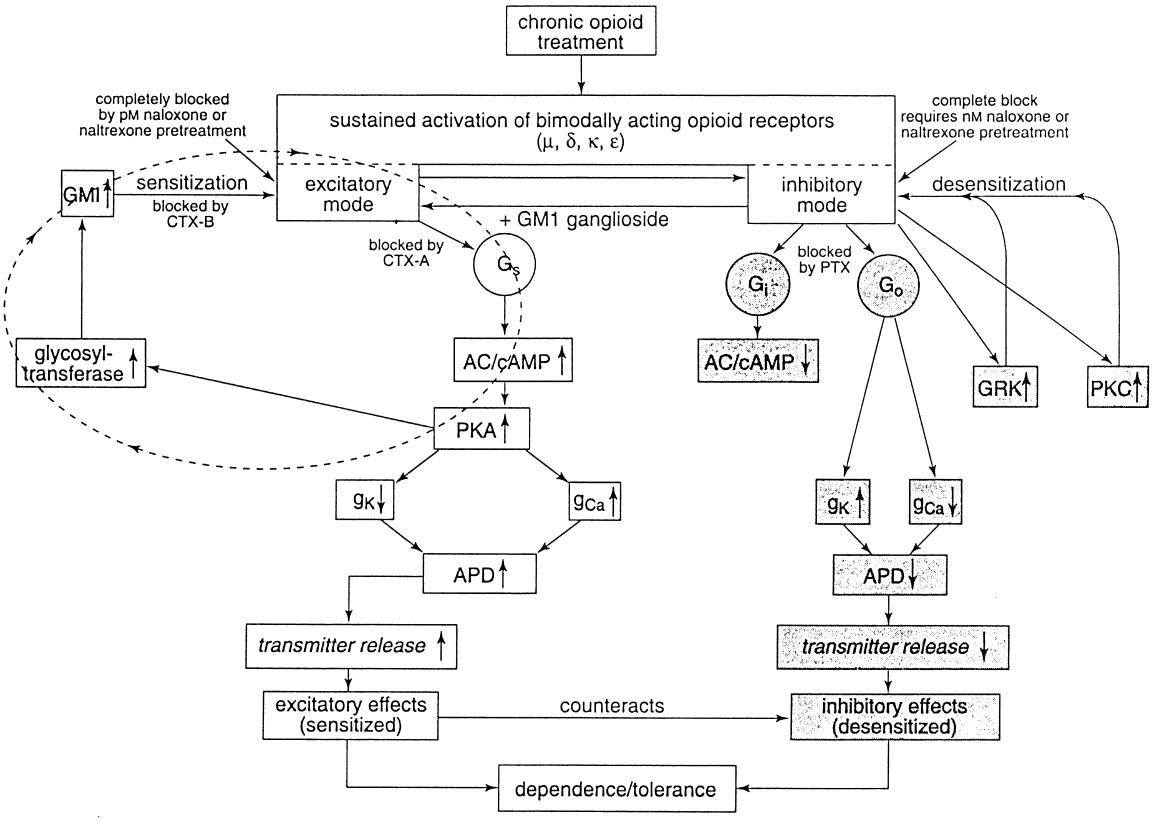

Fig. 5. Complex effects of chronic opioid treatment of nociceptive DRG neurons resulting from sustained activation of opioid receptors that can occur either in a Gi/Go-coupled inhibitory mode (right) or a Gs-coupled excitatory mode (left) depending on the GM1 level in these treated neurons. The positive-feedback phosphorylation cycle involving adenylate cyclase (AC), cAMP, protein kinase A (PKA) and GM1, might mediate sustained sensitization of excitatory Gs-coupled opioid receptor functions, resulting in tolerance and dependence during chronic opioid treatment. By contrast, inhibitory Gi/Go-coupled opioid receptor functions might be progressively desensitized by the activation of G protein-coupled receptor kinases (GRK) and arrestins, as well as by activation of protein kinase C (PKC), thereby contributing to the development of tolerance. Sustained opioid-induced activation of PKC could also increase the activity of Ca^{2+} channels, providing an additional mechanism for modulating opioid tolerance or dependence (see references in Crain and Shen, 1998b). CTX-A, CTX-B: A and B subunits of cholera toxin; g_k, g_{ca}: K^+ and Ca^{2+} conductances (From: Crain and Shen, 1998b).

3.6. Safety of cotreatment with morphine plus low-doses of opioid antagonists

Cotreatments with morphine plus low doses of opioid antagonists appear to be intrinsically safe. A major uncertainty lies in the possibility that low doses of opioid antagonists may not only enhance morphine's analgesic potency but might also increase morphine's depressant effects on the

Table 1
The need for treatment of nausea, vomiting, and pain over the 24-h study period and the 24-h patient assessments in the three treatment groups[a]

	Placebo	Nalmefene (15 μg)	Nalmefene (25 μg)
Treatment of side effects			
Antiemetic therapy	25 (62.5)	13 (33.3)[b]	13 (32.5)[b]
Antipruritic therapy	9 (22.5)	2 (5.1)[b]	2 (5.0)[b]
Total (24 h) morphine dose (mg)	50 ± 25	45 ± 24	56 ± 26
24-h patient assessments			
Recalled being nauseated	33 (82.5)	19 (48.7)[b]	17 (42.5)[b]
Remembered vomiting	3 (7.5)	4 (10.3)	6 (15.0)
Remembered complaining of itching	24 (60.0)	2 (5.1)[b]	2 (5.0)[b]
Pain severity VAS (cm) [c]	5.5 ± 2.9	3.5 ± 2.4[b]	2.3 ± 2.5[b]
Pain relief VAS (cm) [d]	6 ± 3	8 ± 2[b]	9 ± 2[b]

[a] Values are numbers (percentages) or mean ± SD. VAS, visual analog score.
[b] $P < 0.05$ compared with placebo group.
[c] No pain = 0.
[d] Complete pain relief = 10 (Modified from Joshi et al., 1999).

respiratory system. However, preclinical studies in mice indicate that even when low-dose NTX is cotreated with extremely high doses of morphine (30–50 mg/kg for several days) this combination ''markedly enhanced the antinociceptive (inhibitory) potency of morphine…[but] did not appear to enhance morphine's depressant effects on respiratory neurons as evidenced by the absence of any lethal or abnormal distress signs in these groups of mice'' (Shen and Crain, 1997). Furthermore, cotreatment of postsurgical pain patients with morphine (ca. 40–50 mg/day via PCA) plus low-dose naloxone perfusion (i.v.) or single low doses of nalmefene (i.v.) significantly enhanced the analgesic potency of morphine but did not result in any detectable increase in signs of respiratory depression (Gan et al., 1997; Joshi et al., 1999; see Sections 3.2 and 3.4).

Application of these cotreatment procedures with low-dose opioid antagonist plus morphine to chronic pain patients that have become dependent on prior use of opioid analgesics will, of course, require careful monitoring to avoid possible transitory opioid withdrawal symptoms that might be elicited by administration of low doses of opioid antagonists. Such side-effects should, however, be far less aversive than those often elicited during the clinical transition procedures commonly used prior to initiation of high-dose NTX (1 mg/kg) maintenance treatment of acutely detoxified opiate addicts (e.g. Kleber et al., 1985; Gonzalez and Brogden, 1988; O'Brien, 1997).

4. Modulation of opioid tolerance and dependence by Gs-coupled, GM1 ganglioside-regulated opioid receptor functions

In addition to providing a remarkably simple and clinically safe method to acutely enhance the analgesic potency of morphine and other conventional opioid analgesics, cotreatment with low-dose NLX or NTX can markedly attenuate tolerance/dependence liability during chronic opioid administration. As noted above, blockade of sustained activation of excitatory opioid receptor functions in chronic μM morphine-treated DRG neurons in culture by cotreatment with either pM NTX (Crain and Shen, 1995a) or cholera toxin-B fraction (Shen and Crain, 1992) prevents development of the usual tolerance to the inhibitory (APD-shortening) effects of morphine and supersensitivity to the excitatory (APD-prolonging) effects of extremely low concentrations of acutely applied opioid agonists, as well as nM NLX, on these treated DRG neurons (Crain and Shen, 1992a, 1995c; Fig. 5). These in vitro studies indicate that sustained activation of Gs-coupled, GM1-regulated excitatory opioid receptor functions is required for the development of cellular manifestations of tolerance and physical dependence (Shen and Crain, 1992; Crain and Shen, 1998a; b).

Our in vitro studies on DRG neurons led us to propose (Crain and Shen, 1992a) that opioid tolerance/dependence is mediated not only by up-regulation of the well-known Gs/AC/cAMP/PKA second-messenger system (Sharma and Nirenberg, 1975, 1977; Makman et al., 1988; Terwilliger et al., 1991; Cruciani et al., 1993; Avidor-Reiss et al., 1995), but also by elevation of GM1 ganglioside following activation of the cAMP/PKA-dependent glycosyltransferase that synthesizes GM1 (McLawhon et al., 1981; Dawson et al., 1983; Scheideler and Dawson, 1986; Wu et al., 1995). Coordination of these processes provides a positive-feedback phosphorylation cycle (Fig. 5) that could amplify the sensitivity of GM1-regulated, Gs-coupled excitatory opioid receptors to extremely low levels of *endogenous* opioids (Crain and Shen, 1992a). This cellular mechanism may account for the protracted dependence (measured by acute NLX-precipitated excitatory effects) observed for *months* after withdrawal of chronic *exogenous* opioids from DRG neurons in long-term cultures (Crain and Shen, 1995c) as well as in vivo (e.g. Goldberg and Schuster, 1969; Martin, 1984). Elevation of GM1 by sustained activation of Gs-coupled opioid receptor functions (Wu et al., 1995) in DRG neurons may have two major effects: (1) increased conversion of opioid receptors from the inhibitory Gi/Go-coupled mode (Wu et al., 1997, 1998) (Fig. 5), thereby providing increased *numbers* of receptors in the excitatory Gs-coupled mode; and (2) increased *efficacy* of coupling of Gs-coupled opioid receptors to the AC/cAMP transducer system resulting in supersensitized excitatory opioid receptors (Crain and Shen, 1992a, b, 1998a). As noted above, these in vitro studies have been confirmed in vivo by demonstrating that selective blockade of excitatory opioid receptor functions during chronic cotreatment of mice with morphine plus low-dose NTX does, in fact, prevent development of tolerance as well as dependence (Crain and Shen, 1995a; Shen and Crain, 1997; Fig. 3). Furthermore, the clinical study by Gan et al. (1997) provides preliminary evidence that sustained cotreatment of post-surgical pain patients with low-dose NLX plus morphine may, indeed, attenuate development of opioid tolerance (Fig. 4). Longer-term clinical trials of chronic pain patients cotreated with morphine plus low-dose NTX or nalmefene will be required to determine the degree to which selective antagonists of excitatory opioid receptor functions can, in fact, reliably attenuate opioid tolerance and dependence liability in humans as well as in mice.

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