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Paradoxical Effects of the Opioid Antagonist Naltrexone on Morphine Analgesia, Tolerance, and Reward in Rats

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ABSTRACT

Opioid agonists such as morphine have been found to exert excitatory and inhibitory receptor-mediated effects at low and high doses, respectively. Ultra-low doses of opioid antagonists (naloxone and naltrexone), which selectively inhibit the excitatory effects, have been reported to augment systemic morphine analgesia and inhibit the development of tolerance/physical dependence. This study investigated the site of action of the paradoxical effects of naltrexone and the generality of this effect. The potential of ultra-low doses of naltrexone to influence morphine-induced analgesia was investigated in tests of nociception. Administration of intrathecal (0.05 and 0.1 ng) or systemic (10 ng/kg i.p.) naltrexone augmented the antinociception produced by an acute submaximal dose of intrathecal (5 μg) or systemic (7.5 mg/kg i.p.) morphine in the tail-flick test. Chronic intrathecal (0.005 and 0.05 ng) or systemic (10 ng/kg) naltrexone combined with morphine (15 μg i.t.; 15 mg/kg i.p.) over a 7-day period inhibited the decline in morphine antinociception and prevented the loss of morphine potency. In animals rendered tolerant to intrathecal (15 μg) or systemic (15 mg/kg) morphine, administration of naltrexone (0.05 ng i.t.; 10 and 50 ng/kg i.p.) significantly restored the antinociceptive effect and potency of morphine. Thus, in ultra-low doses, naltrexone paradoxically enhances morphine analgesia and inhibits or reverses tolerance through a spinal action. The potential of naltrexone to influence morphine-induced reward was also investigated using a place preference paradigm. Systemic administration of ultra-low doses of naltrexone (16.7, 20.0, and 25.0 ng/kg) with morphine (1.0 mg/kg) extended the duration of the morphine-induced conditioned place preference. These effects of naltrexone on morphine-induced reward may have implications for chronic treatment with agonist-antagonist combinations.

Opioid drugs such as morphine are widely used in the treatment of severe pain; however, their chronic administration results in the development of tolerance to their analgesic effects, limiting their clinical usefulness in pain management. Although the mechanisms underlying the development of opioid tolerance are poorly understood, recent studies have suggested that alterations in the coupling of opioid receptors to G protein-linked effectors may play a significant role (Crain and Shen, 2000). Morphine and related agonists are recognized to produce their characteristic acute and chronic effects by activating spinal and supraspinal μ-, δ-, and κ-opioid receptors. Classically, morphine activates G_i protein-coupled μ-opioid receptors to inhibit adenylyl cyclase activity and decrease neuronal cAMP levels (Uhl et al., 1994). At the presynaptic level, μ-opioid receptor activation inhibits voltage-sensitive Ca^{2+} channels (Tallent et al., 1994) and reduces neurotransmitter release, whereas at the postsynaptic level, it opens potassium channels and hyperpolarizes neurons (North and Williams, 1983; Ikeda et al., 1995). The net result of these effects is inhibition of neuronal activity and the production of potent analgesia. These classical effects of morphine can be blocked by opioid receptor antagonists such as naloxone or naltrexone.

Recent studies suggest that at doses well below those producing neuronal inhibition, opioids exert stimulatory effects. Thus, in several tissue models, opioid receptors have been shown to stimulate adenylyl cyclase, promote calcium influx, and stimulate phosphoinositide hydrolysis (Smart and Lambert, 1996). In cultured dorsal root ganglion neurons, nanomolar concentrations of opioid agonists increase action potential duration, whereas micromolar concentrations produce the opposite effect (Chen et al., 1988; Shen and Crain, 1989). This dual action of opioids has been explained on the basis of a bimodal opioid receptor model. In this model, ultra-low doses (picomolar to nanomolar) of an agonist activate a G_s-coupled mode of the opioid receptor to activate adenylyl cyclase and increase neuronal excitability. These

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ABBREVIATIONS: CPP, conditioned place preference; MPE, maximum percent effect.

effects produce behavioral hyperalgesia (Crain and Shen, 2001) and are blocked by ultra-low doses of opioid receptor antagonists (Crain and Shen, 1995). In contrast, higher doses (micromolar) of opioids activate a G_i/G_o-coupled mode of the receptor to inhibit adenylyl cyclase activity and reduce neuronal excitability, effects that produce classical analgesia and are blocked by higher doses of antagonists. The bimodal model of morphine action has also been invoked to explain the development of opioid tolerance and physical dependence. According to this model, the predominance of the G_s-coupled mode of the μ-opioid receptor during chronic treatment opposes the analgesic response produced by activation of the G_i/G_o-coupled mode, compromises analgesic potency, and manifests as tolerance (Crain and Shen, 1990, 1992). In support of this concept, recent studies in mice have demonstrated that ultra-low doses of systemic naltrexone, which would selectively antagonize the stimulatory action of morphine, indeed augment morphine-induced analgesia and inhibit the development of tolerance/physical dependence (Shen and Crain, 1997).

The neural site at which ultra-low doses of opioid antagonists act to influence morphine analgesia and tolerance is unclear, but previous electrophysiological studies demonstrating the blockade of morphine-induced excitation in the dorsal root ganglion neurons suggest a spinal locus of action (Crain and Shen, 1990, 1995). These neurons project to the dorsal spinal cord via high-threshold afferent fibers that release neuropeptides (substance P and calcitonin gene-related peptide) in response to noxious input. Thus, in the present study, using the well established spinal opioid analgesia model (Yaksh and Rudy, 1976), we determined whether ultra-low-dose naltrexone influences morphine analgesia and tolerance at the spinal level. An important goal was to determine whether naltrexone also has the potential to reverse established morphine tolerance.

Although ultra-low doses of naltrexone enhance morphine analgesia and attenuate tolerance and physical dependence, the generality of this agonist-antagonist interaction is unclear. In addition to producing tolerance/physical dependence, opioids are well known to produce psychic dependence by acting on opioid receptors in the brain reward pathways. The rewarding effects of morphine are reflected in conditioned place preference experiments in animals and are effectively eliminated by treatment with opioid receptor antagonists (Olmstead and Franklin, 1997). However, the effects of ultra-low doses of opioid antagonists on the characteristic rewarding effects of morphine to our knowledge are not known. The overlap of the sites mediating both the supraspinal analgesic and rewarding effects of morphine (Le Magnen et al., 1980; Franklin, 1989, 1998) suggests that their rewarding effects may also be influenced by ultra-low doses of naltrexone. Thus, using a place preference-conditioning paradigm, we examined whether ultra-low doses of naltrexone influence the rewarding effects of systemic morphine.

Materials and Methods

All procedures were in accordance with the Animals for Research Act, the Guidelines of the Canadian Council of Animals Care, and the Queen's University Animal Care Committee.

Intrathecal Catheter Implantation and Drug Injection

All experiments were performed using adult male Sprague-Dawley rats (200–250 g) (Charles River Canada, Montreal, QB, Canada). Animals were housed in individual cages and allowed free access to food and water. Under halothane anesthesia (4%), animals were implanted with intrathecal catheters (Yaksh and Rudy, 1976; Powell et al., 1999). In brief, animals were placed prone in a stereotaxic frame, and the cisternal membrane was exposed. Polyethylene catheters (PE 10 tubing, 7.5 cm) were inserted through a small puncture in the membrane and threaded caudally to reach the lumbar enlargement of the spinal cord. The rostral end of the catheter was exteriorized at the top of the head and the wound closed with sutures. Animals were given 4 to 5 days to recover from surgery, and those displaying signs of motor dysfunction or paralysis were excluded from the study. Drugs were injected daily into the exteriorized portion of the catheter in a 10-μl volume, followed by 10 μl of 0.9% saline to flush the catheter.

Nociceptive Testing

To evaluate the animals' response to nociceptive stimuli, two spinal reflex tests were used (for details see Powell et al., 1999). The tail-flick test was used to measure the response to a thermal nociceptive stimulus. Radiant heat was applied to the base of the tail and the time latency for removal of the tail was recorded. The heat source was adjusted to yield a baseline of 2 to 3 s and a cut-off time of 10 s was used to prevent tissue damage. The paw pressure test was used to measure the response to a mechanical nociceptive stimulus. Using an air-filled inverted syringe, pressure was applied to the dorsal surface of the animal's hindpaw. The pressure at which the animal withdrew its hindpaw was recorded. A cut-off of 300 mm Hg was used to prevent tissue injury. Previous experience has demonstrated no significant interaction between these two tests (Loomis et al., 1985).

Induction of Spinal Morphine Tolerance

To induce morphine tolerance, animals were given injections of intrathecal morphine (15 μg) once daily between 10 and 11 AM for 7 days. Nociceptive testing was performed both before and 30 min after drug administration to determine baseline and drug-induced responses, respectively. Previous studies from our laboratory have shown that the peak antinociceptive effect of morphine occurs at 30 min after injection (Gouarderes et al., 2000). On day 8, cumulative morphine dose-response curves were obtained to determine the potency of acute morphine (Powell et al., 1999). To obtain these curves, animals were given increasing doses of morphine every 30 min, and nociceptive testing followed 30 min after each drug injection. This protocol was continued until a maximal antinociceptive response was obtained in each test. The ED_{50} values of morphine, an indicator of agonist potency, were calculated from each dose-response curve. A state of tolerance was indicated by a progressive decline in the antinociceptive effect of morphine over a 7-day period and an increase in ED_{50} value due to a rightward shift in the acute morphine dose-response curve.

Induction of Systemic Morphine Tolerance

To induce a state of systemic morphine tolerance, animals were given intraperitoneal injections of morphine (15 mg/kg) once daily for 7 days. Nociceptive testing was performed both before and 30 min after drug administration. Following the treatment period, on day 8, cumulative dose-response curves were constructed, and the ED_{50} values of morphine were determined, as described above.

Study 1: The Acute Effects of Naltrexone on Morphine Action

Spinal Morphine. To determine the effect of the opioid receptor antagonist naltrexone on the acute antinociceptive effects of morphine, naltrexone and morphine were given as a single coinjection. A

submaximal dose of intrathecal morphine (5 μg) was coinjected with ultra-low (0.05 and 0.1 ng) or high (1 μg) doses of naltrexone in drug-naive animals. Nociceptive testing was performed every 10 min following drug administration for the first hour and every 30 min for the following 2 h.

Systemic Morphine. A submaximal dose of morphine (7.5 mg/kg) was coinjected with an ultra-low (10 ng/kg) or high (2 mg/kg) dose of naltrexone in drug naive animals. Nociceptive testing was performed every 10 min after drug administration for the first hour and every 30 min for the following 2 h.

Study 2: The Effect of Naltrexone on the Development of Morphine Tolerance

Spinal Morphine. To determine the effects of naltrexone on the development of spinal morphine tolerance, naltrexone (0.005 and 0.05 ng) was coinjected with morphine (15 μg) once daily for 7 days. Nociceptive testing was performed daily and cumulative dose-response curves were generated on day 8, as described above. The action of naltrexone on the development of tolerance was assessed by examining its effect on the decline in magnitude of the antinociceptive effect of morphine over the 7-day treatment period and on the morphine ED_{50} values determined at the end of this period.

Systemic Morphine. To determine the ability of naltrexone to prevent the development of systemic morphine tolerance, naltrexone (10 ng/kg) was coinjected with morphine (15 mg/kg) once daily for 7 days. Nociceptive testing was performed daily and cumulative dose-response curves were generated on day 8, as described above.

Study 3: The Effect of Naltrexone on Established Morphine Tolerance

Spinal Morphine. To determine the ability of naltrexone to influence established tolerance, animals were first rendered tolerant to the antinociceptive effects of the agonist. Morphine (15 μg) was given once daily for 5 days to render the animals tolerant to its antinociceptive effects. On the following 5 days, naltrexone (0.05 ng) was given either alone or in combination with morphine. Morphine ED_{50} values were determined on day 11 from cumulative dose-response curves, as described above. The ability of naltrexone to reverse morphine tolerance was indicated by a recovery of morphine antinociception and agonist potency.

Systemic Morphine. Morphine was given once daily for 7 days (15 mg/kg) to induce a state of tolerance. On the following 7 days, naltrexone (10 ng/kg) was given alone or in combination with morphine. Morphine dose-response curves were generated on day 15, and acute morphine ED_{50} values were calculated, as described above.

Study 4: Conditioned Place Preference

Adult male Wistar rats (200–225 g) (Charles River Canada) were housed in pairs and allowed free access to food and water. Animals were pre-exposed to an experimental apparatus consisting of two distinctive (striped or plain walls, grid or mesh floor) compartments connected by a tunnel for three, 15-min sessions. During the 8-day conditioning period with the tunnel blocked, one compartment was paired with systemic morphine (0.01, 0.05, 0.1, 0.5, 1.0, and 2.0 mg/kg s.c.) and the other with vehicle, on alternate days. Each dose of morphine was administered to a separate group of animals. For the 15-min test session, animals were injected with saline, placed in the apparatus with the tunnel open, and observed for time spent in the drug-paired versus vehicle-paired compartment. In another set of experiments, designed to evaluate the time course of the rewarding effects of morphine (1.0 mg/kg), a range of delays (0, 30, 60, 90, or 120 min) was inserted between the time of injection and placement into the drug-paired compartment. The third experiment evaluated the ability of ultra-low doses of naltrexone (10.0, 16.7, 20.0, 25.0, and 200 ng/kg) to augment the nonsignificant place preference produced by morphine (1.0 mg/kg) injected 120 min prior to conditioning sessions. Naltrexone was coinjected with morphine. A final group received naltrexone (20 ng/kg) alone during conditioning sessions.

Drugs

Morphine was obtained from BDH Pharmaceuticals (Toronto, ON, Canada), and naltrexone was obtained from Sigma Chemical Co. (St. Louis, MO). All drugs were dissolved in physiological saline (0.9%).

Data Analysis

Tail-flick and paw pressure values were converted to a maximum percentage effect (MPE): MPE = 100 × [postdrug response − baseline response]/[cut-off value − baseline response]. Data are expressed as mean (± S.E.M.) in the figures. The ED_{50} values were determined using a nonlinear regressional analysis (Prism 2, GraphPad Software Inc., San Diego, CA). Statistical significance ($P < 0.05$) for analgesia and place-conditioning studies was determined using *t* tests or a one-way analysis of variance followed by a Student Newman-Keuls post hoc test for multiple comparisons between groups.

Results

Study 1: The Effect of Naltrexone on the Acute Action of Morphine

Spinal Morphine. The effects of naltrexone on the acute antinociceptive effect of a submaximal dose of morphine in the tail-flick test are represented in Fig. 1A. An acute submaximal injection of intrathecal morphine (5 μg) produced

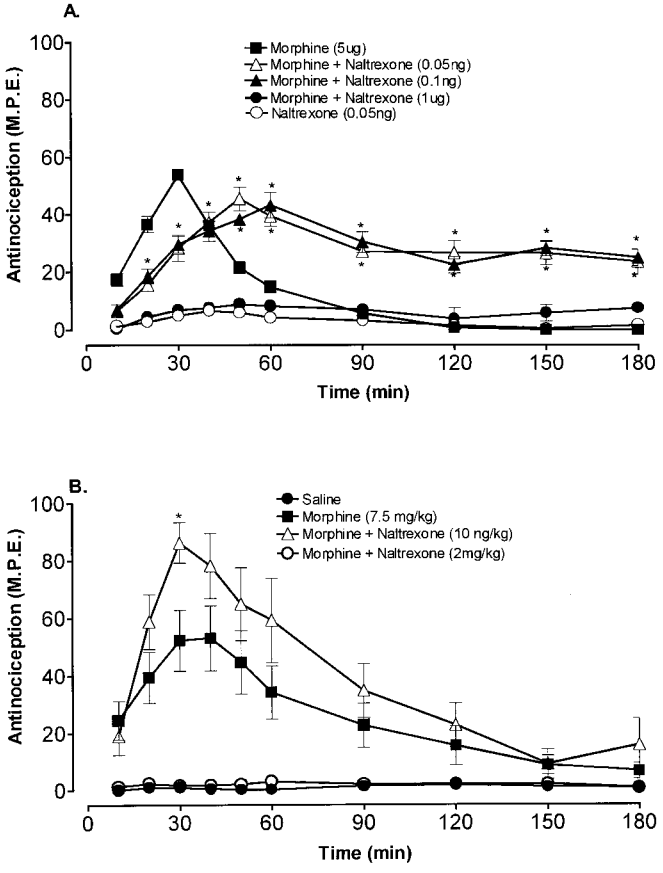

Fig. 1. Time course of the effects of spinal (A) and systemic (B) naltrexone on the acute antinociceptive actions of morphine in the tail-flick test. Morphine and naltrexone were administered as a single intrathecal (A) or intraperitoneal (B) injection. Nociceptive testing was performed every 10 min after injection for the first hour and every 30 min for the next 2 h. The data are presented as mean ± S.E.M. for five to seven animals. *, significant differences from the action of morphine alone ($P < 0.05$).

an antinociceptive effect that peaked at 30 min and rapidly returned to baseline levels by 90 min. Coinjection of morphine with the opioid antagonist naltrexone (1 μg) completely blocked this effect. However, coinjection of naltrexone (0.05 and 0.1 ng) at doses 10,000- and 20,000-fold lower doses than the antagonist dose not only prolonged the antinociceptive effect of morphine from 60 to 180 min following injection, but also delayed the peak response from 30 to 60 min. The animals showed a full recovery from this effect 24 h after injection (data not shown). Intrathecal administration of naltrexone alone (0.05 ng) did not produce an antinociceptive effect.

Systemic Morphine. The effects of naltrexone on the acute effects of systemic morphine in the tail-flick test are represented in Fig. 1B. Acute submaximal systemic morphine (7.5 mg/kg) produced an antinociceptive response that peaked at 30 min. Naltrexone (2 mg/kg) completely blocked this effect; however, ultra-low-dose naltrexone (10 ng/kg), a 200,000-fold lower dose, increased the peak antinociceptive effect of morphine at 30 min. The magnitude of the antinociceptive response observed with the morphine/naltrexone combination was significantly greater than that observed with morphine alone at 30 min. This response returned to baseline levels 150 min after injection.

Study 2: The Effect of Naltrexone on the Development of Morphine Tolerance

Spinal Morphine. The effect of naltrexone on the antinociceptive effect of chronic morphine is represented in the tail-flick test in Fig. 2A. Administration of morphine (15 μg) to drug-naive animals produced a maximal antinociceptive response on day 1. However, repeated daily administration of this dose resulted in a progressive decline of antinociception to baseline levels by day 4, reflecting the development of tolerance. Coadministration of naltrexone with morphine for 7 days dose dependently attenuated this decline. In groups receiving naltrexone (0.005 ng) with morphine, the antinociceptive effects elicited were significantly greater than those in the morphine group on days 4 and 5. However, in groups receiving a 10-fold higher dose of naltrexone (0.05 ng), the antinociceptive effects were maintained at a maximum level throughout the 7-day treatment period. Similar effects were also observed in the paw pressure test (Fig. 2B). Indeed, the antinociceptive responses obtained in the morphine/naltrexone (0.05 ng) group were significantly greater than the responses obtained in the morphine group on days 2 through 7. Administration of naltrexone (0.05 ng) for 7 days did not produce an antinociceptive effect in either test.

The morphine ED_{50} values obtained in the tail-flick and paw pressure test on day 8 from groups treated with morphine and naltrexone are represented in Table 1A. Chronic treatment with morphine resulted in a significant increase in the acute morphine ED_{50} values as compared with animals treated chronically with saline. These ED_{50} values increased approximately 5-fold, reflecting a substantial loss in agonist potency. Coadministration of naltrexone (0.005 ng) with morphine did not prevent this increase; however, a 10-fold higher dose of naltrexone (0.05 ng) completely blocked the increase in ED_{50} values. Indeed, the ED_{50} values obtained in the morphine/naltrexone (0.05 ng) group were not significantly different from those obtained in the saline group. Administration of naltrexone (0.05 ng) alone for 7 days did not significantly alter the morphine ED_{50} values from those obtained with chronic saline treatment.

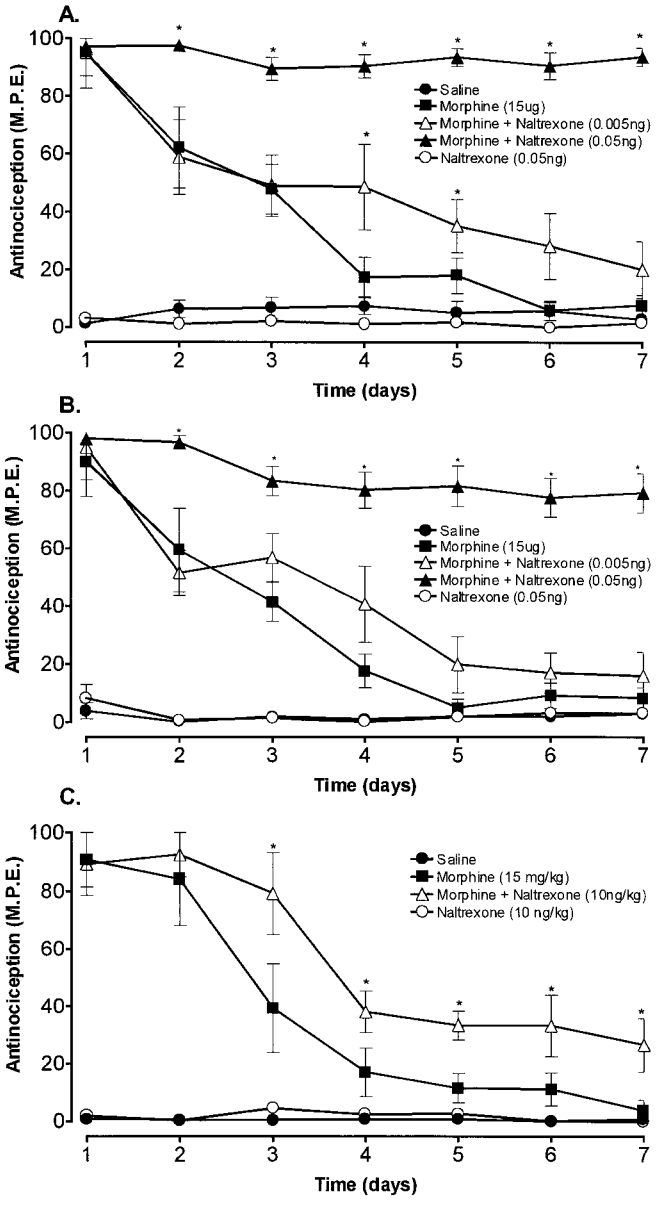

Fig. 2. Time course of the effects of spinal (A, B) and systemic (C) naltrexone on the development of tolerance to chronic morphine in the tail-flick (A, C) and paw pressure (B) test. Morphine and naltrexone were administered as a single intrathecal (A, B) or intraperitoneal (C) injection once daily for 7 days. Nociceptive testing was performed 30 min following each injection. The data are presented as mean ± S.E.M. for five to seven animals. *, significant differences from the action of morphine alone ($P < 0.05$).

Systemic Morphine. The effects of naltrexone on the chronic effects of systemic morphine in the tail-flick test are represented in Fig. 2C. Administration of morphine (15 mg/kg) produced a maximal antinociceptive response on day 1; however, this response declined to baseline levels by day 4, reflecting the development of tolerance. Coadministration of morphine with naltrexone (10 ng/kg) significantly attenuated this decline in morphine effect. In this treatment group, the antinociceptive effects elicited on days 3 through 7 were significantly greater than those in the morphine alone group. Table 1B shows the ED_{50} values of acute morphine obtained

TABLE 1

Effect of ultra-low dose naltrexone on the development of spinal (A) and systemic (B) morphine tolerance

Following the end of the 7-day chronic treatment period, cumulative dose-response curves to acute morphine were generated on day 8. ED_{50} values for morphine were derived from these curves.

Chronic Treatment	ED_{50} (mean ± S.E.M.)	
	Tail-Flick	Paw Pressure
	μg	
A. Spinal		
Saline	6.3 ± 0.5	8.1 ± 0.8
Morphine (15 μg)	33.3 ± 3.4*	41.2 ± 12.2*
Morphine/Naltrexone (0.005 ng)	35.6 ± 8.0*	48.7 ± 5.0*
Morphine/Naltrexone (0.05 ng)	6.0 ± 0.7	7.4 ± 0.7
Naltrexone (0.05 ng)	3.4 ± 0.5	5.4 ± 0.7
	mg/kg	
B. Systemic		
Saline	13.6 ± 1.8	
Morphine (15 mg/kg)	48.9 ± 4.5*	
Morphine/Naltrexone (10 ng/kg)	28.5 ± 3.8	
Naltrexone (10 ng/kg)	13.0 ± 1.7	

* Significant differences from saline group ($P < 0.001$).

in the tail-flick test in these animals on day 8. Administration of morphine for 7 days increased the ED_{50} value approximately 4-fold over that obtained in saline-treated animals, reflecting a significant loss of morphine potency. Administration of morphine and naltrexone (10 ng/kg) partially blocked the increase in ED_{50} value. The ED_{50} value obtained in this treatment group was significantly lower than that in the morphine group but remained significantly greater than the ED_{50} value obtained in the saline group. In the group receiving naltrexone alone (10 ng/kg) for 7 days, the ED_{50} value was not significantly different from that obtained in the saline group.

Study 3: Effect of Naltrexone on Established Morphine Tolerance

Spinal Morphine. The effects of naltrexone on established morphine tolerance in the tail-flick test are represented in Fig. 3A. Repeated daily administration of morphine once daily for 10 days resulted in a decline in the antinociceptive effects of morphine to baseline levels by day 5, reflecting the development of tolerance. Administration of naltrexone (0.05 ng) with morphine from days 6 to 10 produced a progressive recovery in the antinociceptive effect to approximately 70% of the original level by day 10. Similar effects were observed in the paw pressure test: for example, naltrexone (0.05 ng) restored the antinociceptive effect of morphine to approximately 50% of the original level by day 10 (Fig. 3B). Administration of saline or naltrexone alone on days 6 through 10 did not produce a recovery in morphine effect in either test.

The ED_{50} values of acute morphine obtained in the tail-flick and paw pressure test on day 11 are represented in Table 2A. Chronic administration of morphine (15 μg) for 10 days produced a 5-fold increase in the morphine ED_{50} values, reflecting a significant loss in opioid potency. However, administration of naltrexone (0.05 ng) with morphine on days 6 to 10 completely reversed the increase in ED_{50} values. In groups receiving saline only on days 6 to 10, the ED_{50} values remained 2- and 3-fold greater than those in the saline group in the tail-flick and paw pressure tests, respectively, suggesting that tolerance persists despite discontinuation of morphine treatment. Treatment with naltrexone alone on days 6 to 10 completely reversed the increase in ED_{50} in both tests. Thus, in tolerant animals, naltrexone has the potential to reverse the loss of morphine potency with and without coadministration of morphine.

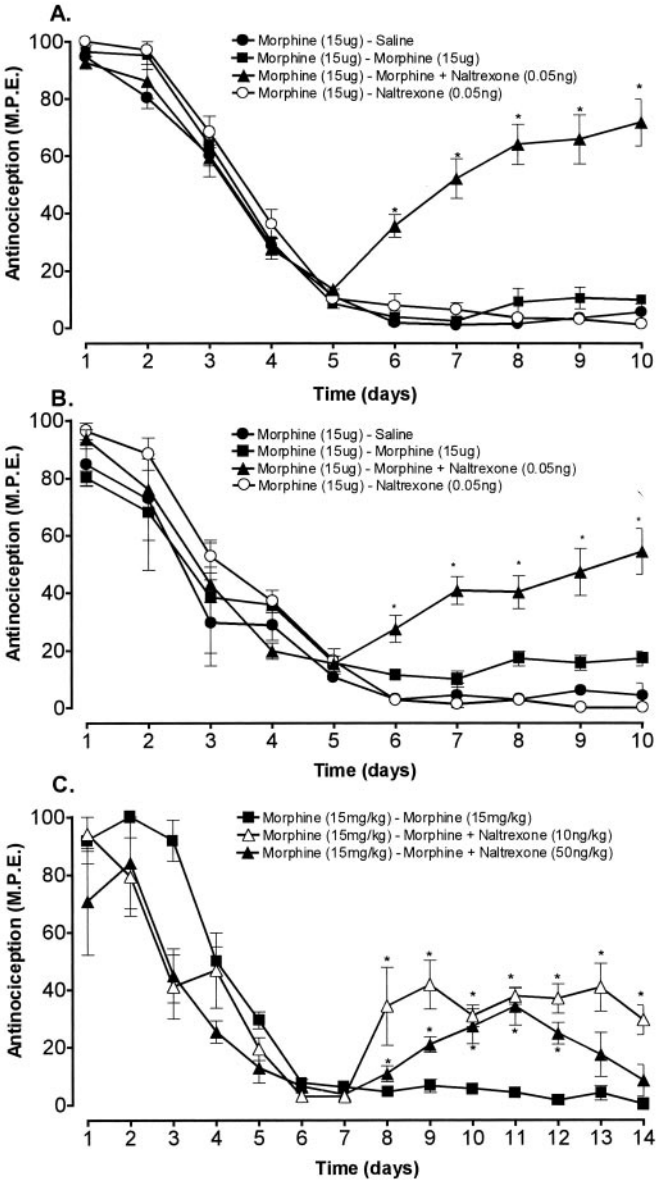

Fig. 3. Time course of the effects of spinal (A, B) and systemic (C) naltrexone on established morphine tolerance in the tail-flick (A, C) and paw pressure (B) test. Morphine and naltrexone were administered as a single intrathecal (A, B) or intraperitoneal (C) injection once daily. Nociceptive testing was performed 30 min following each injection. The data are presented as mean ± S.E.M. for five to seven animals. *, significant differences from the action of morphine alone ($P < 0.05$).

Systemic Morphine. The effects of naltrexone on established systemic morphine tolerance are shown in Fig. 3C. Administration of systemic morphine (15 mg/kg) for 14 days results in a decline in antinociceptive effects to baseline by day 7, reflecting the development of tolerance. Addition of naltrexone to morphine on days 8 to 14 restored morphine effect to approximately 40% of the original level. In groups receiving 10 ng/kg and 50 ng/kg of naltrexone with morphine, the antinociceptive effects elicited were significantly greater

TABLE 2

Effect of ultra-lose dose naltrexone on the reversal of spinal (A) and systemic (B) morphine tolerance

Following the end of the 10 or 14 day chronic treatment period, cumulative dose-response curves to acute morphine were generated on day 11 or 15, respectively. ED_{50} values for morphine were derived from these curves.

Chronic Treatment		ED_{50} (mean ± S.E.M.)	
		Tail-Flick	Paw Pressure
		μg	
A. Spinal			
Days 1–5	*Days 6–10*		
Saline	Saline	6.3 ± 0.5*	8.1 ± 0.8*
Morphine (15 μg)	Morphine (15 μg)	40.5 ± 4.6	48.0 ± 10.6
Morphine (15 μg)	Morphine/Naltrexone (0.05 ng)	9.1 ± 2.0*	16.5 ± 3.2*
Morphine (15 μg)	Saline	13.9 ± 1.6*	26.9 ± 10.6*
Morphine (15 μg)	Naltrexone (0.05 ng)	9.2 ± 3.7*	10.3 ± 4.5*
		mg/kg	
B. Systemic			
Days 1–7	*Days 8–14*		
Morphine (15 mg/kg)	Morphine (15 mg/kg)	65.9 ± 8.9	
Morphine (15 mg/kg)	Morphine/Naltrexone (10 ng/kg)	28.0 ± 0.7*	
Morphine (15 mg/kg)	Morphine/Naltrexone (50 ng/kg)	27.1 ± 4.1*	
Morphine (15 mg/kg)	Naltrexone (10 ng/kg)	6.4 ± 2.1*	

* Significant differences from morphine alone group ($P < 0.001$). Note: Saline ED_{50} values in Table 2A are the saline ED_{50} values in Table 1A.

than those in the morphine group on days 8 to 14 and 8 to 12, respectively. The ED_{50} values obtained from these groups on day 15 are represented in Table 2B. Administration of naltrexone with morphine on days 8 to 14, to animals previously receiving 7 days of morphine alone, partially reversed the increase in ED_{50} value observed with morphine alone. In the morphine/naltrexone (10 ng/kg and 50 ng/kg) groups, the ED_{50} values were significantly lower than those in the morphine group yet were also significantly greater than those in the saline group.

Study 4: Conditioned Place Preference (CPP)

Morphine produced a dose-dependent increase in the time spent in the drug-paired side from pre-exposure to test (Fig. 4A). The effect produced by morphine doses of 1.0 or 2.0 mg/kg was significant, and lower doses did not produce a significant effect. The strength of the CPP produced by morphine (1.0 mg/kg) decreased with an increasing delay from injection to placement in the drug-paired side (Fig. 4B); delays of 0, 30, 60, or 90 min resulted in a significant CPP, whereas a 120-min delay resulted in a nonsignificant effect. However, when naltrexone (16.7, 20.0, 25.0 ng/kg) was coinjected with morphine (1.0 mg/kg) at the 120-min delay, a significant CPP was seen (Fig. 4C). Higher (200.0 ng/kg) or lower (10.0 ng/kg) doses of naltrexone were ineffective, as was naltrexone alone (20 ng/kg).

Discussion

The results of this study show that ultra-low doses of naltrexone influence morphine analgesia and tolerance through a spinal action. These doses augmented acute morphine analgesia, whereas a high dose of naltrexone blocked analgesia. Ultra-low doses of naltrexone inhibited the development of morphine tolerance and partially restored morphine potency in animals previously showing tolerance. These effects of naltrexone were observed after intrathecal drug administration, suggesting that they are expressed at the spinal level. In the reward experiments, ultra-low doses of naltrexone extended the rewarding action of morphine in the conditioned place preference paradigm.

The paradoxical effects of opioid antagonists on pain sensitivity are thought to result from a bimodal G protein-coupled μ-opioid receptor. Its activity produces excitatory effects in response to ultra-low doses of agonist and inhibitory effects in response to high doses (Crain and Shen, 1995, 1998a). These excitatory and inhibitory effects are blocked by ultra-low (picomolar to nanomolar) and low (micromolar) doses of opioid antagonists, respectively. Shen and Crain (1997) have previously reported that in mice, intraperitoneal or orally administered ultra-low doses of naltrexone prevent the development of systemic morphine tolerance and physical dependence. The results of the present study show that in the rat, systemic ultra-low dose naltrexone indeed augments the effect of morphine in the tail-flick test and inhibits the development of tolerance. The present study shows for the first time that this unusual effect is expressed at the spinal level and is apparent in both thermal and mechanical models of nociception. The antitolerance effect was evident in both the time-effect relationship for the actions of morphine and in a quantitative measure of agonist potency, the ED_{50} value. Thus, ultra-low doses of naltrexone effectively prevented the decline of morphine effect observed over 7 days and inhibited the increase in morphine ED_{50} value. Remarkably, naltrexone also reversed established morphine tolerance, restoring the antinociceptive effect of morphine to 70% of the original level and the morphine ED_{50} values to those obtained in the saline-treated group.

Although both spinal and systemic ultra-low doses of naltrexone influenced the morphine antinociception, the profile of its action differed under these two conditions of administration. Systemic injection enhanced the peak effect of morphine, whereas spinal administration extended the duration of morphine's effect without significantly enhancing the peak response, although the latter was delayed by 30 min. A site synergism may have contributed to this difference; systemic naltrexone is likely to reach both spinal and supraspinal sites, and a synergistic interaction between these sites, with respect to morphine-naltrexone combination, may have augmented the peak effect. However, systemic naltrexone appeared to be less effective than intrathecal naltrexone in influencing the two indices of tolerance. Whereas intrathecal naltrexone completely blocked the increase in morphine

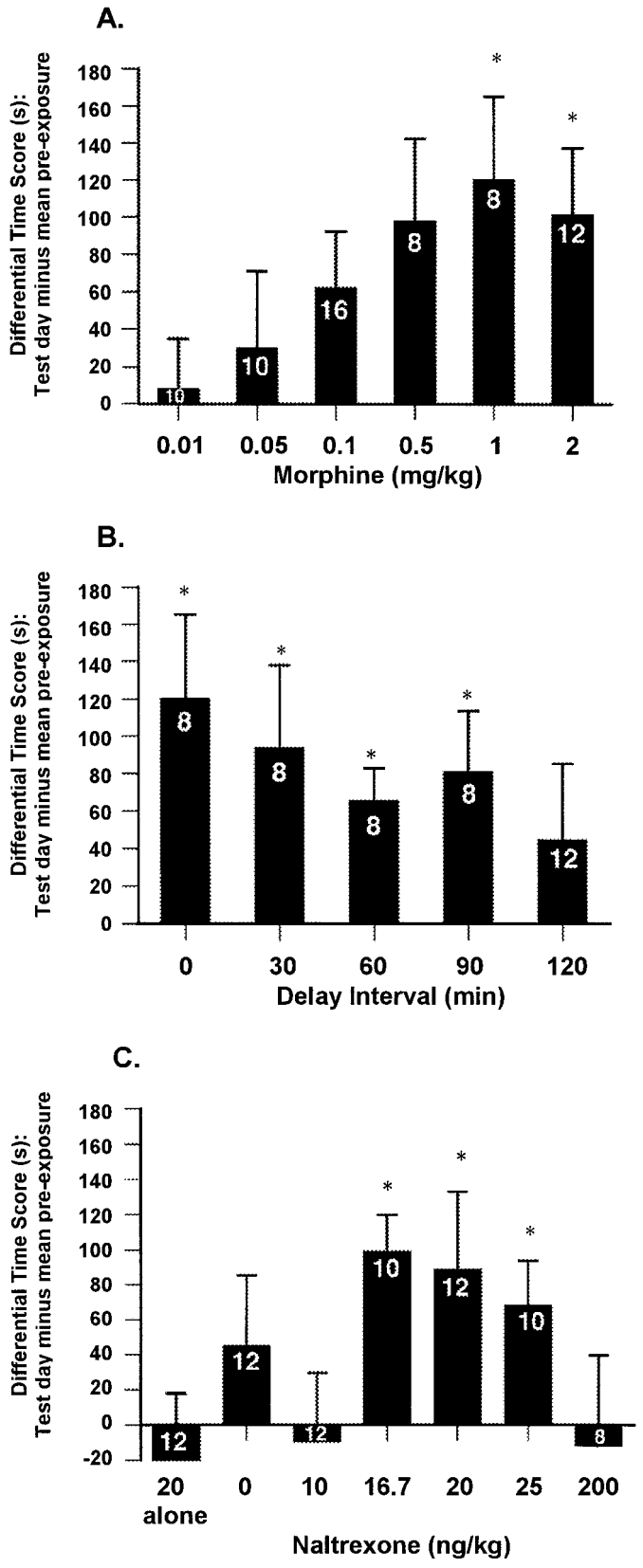

Fig. 4. CPP induced by morphine alone and in combination with ultra-low doses of naltrexone. A, CPP induced immediately following injection with a range of doses of subcutaneous morphine in independent groups. B, CPP induced following a range of delays between morphine injection and placement into the drug-paired compartment during conditioning. Note that the 0-min delay group is the 1.0 mg/kg group shown in Fig. 1A. C, CPP induced by injection of morphine and naltrexone followed by a 120-min delay before placement into the drug-paired side. Note that the 0 ng/kg dose group is the 120-min delay group from Fig. 1B. Morphine and naltrexone were given as a single injection. Numerals within each bar indicate group size. *, significant CPP in *t* tests comparing time spent in the drug-paired side during the test session with the mean time spent in that side during the pre-exposure sessions.

ED_{50} values associated with the development of tolerance and fully reversed the increase in ED_{50} value seen in tolerant animals, systemic naltrexone exerted these effects only partially. The greater effectiveness of intrathecally administered naltrexone may be related to direct drug delivery to spinal sites involved in the genesis of tolerance (Yaksh et al., 1988; Menard et al., 1996; Powell et al., 1999). These route-related differences notwithstanding, the results of this study demonstrate the potential of naltrexone to inhibit as well as reverse morphine tolerance.

The reversal of tolerance by naltrexone, however, was not immediate and multiple doses of the antagonist were required to restore morphine action in tolerant animals, implying a slow reversal of the mechanism contributing to opioid tolerance. Opioid tolerance has been suggested to result from the loss of agonist potency due to a latent activation of G_s-coupled opioid receptors by chronic morphine, a response that physiologically antagonizes the analgesic response. Crain and Shen (1998a,b) have postulated that this latent activation likely results from increases in GM1 ganglioside, a neuronal glycolipid that is thought to facilitate the conversion of opioid receptors from a G_i- to a G_s-coupled mode (Wu et al., 1997, 1998). Recent studies have shown that GM1 ganglioside levels are regulated by a cAMP/protein kinase A-dependent glycosyltransferase (Scheideler and Dawson, 1986) that can be activated following G_s-mediated increases in cAMP and protein kinase A (Crain and Shen, 1990, 1992). Thus, activation of G_s-coupled opioid receptors generates a positive feedback loop that increases the proportion of G_s-coupled receptors. Ultra-low doses of naltrexone likely inhibit the G_s-coupled receptor, block initiation of the feedback loop, and allow unopposed expression of the opioid effect. However, in opioid-tolerant animals, initiation of the feedback loop by chronic morphine likely results in high GM1 ganglioside levels and a very high proportion of G_s-coupled opioid receptors (Crain and Shen, 1998a,b). Thus, several doses of naltrexone may be required to decrease activity of the feedback loop and eventually reduce the large proportion of G_s-coupled receptors.

An alternate explanation is that naltrexone, by blocking an opioid autoreceptor, facilitates the release of endogenous opioids that in turn activate different opioid receptor types and thus influences tolerance (Ueda et al., 1986). This implies that such an autoreceptor has a very high affinity for naltrexone since its dose is 3×10^5 to 1.5×10^6 times lower than the dose of morphine producing analgesia. Recent evidence from molecular studies (Pasternak, 2001) has revealed at least seven different splice variants of the μ-opioid receptor and has identified specific exons important for receptor internalization and functional expression of morphine analgesia at spinal or supraspinal sites. Thus, the possibility of a receptor population that demonstrates very high affinity for naltrexone cannot be excluded. Interestingly, acute low-dose naltrexone did not produce analgesia, an effect that would be expected to follow from facilitated endogenous opioid release.

Alternatively, recent studies have demonstrated that heterodimeric μ- and δ-opioid receptors exist, and that μ agonists in the presence of δ antagonists show synergistic binding and enhanced effects (George et al., 2000; Gomes et al., 2000). Interestingly, preliminary data from our laboratory suggest that ultra-low doses of the selective δ-antagonist naltrindole share the naltrexone effect demonstrated in the study (Abul-Husn et al., 2001). Thus, the possibility that these effects are mediated by heterodimeric μ-/δ-receptors merits investigation in future studies.

An important question arising from previous studies and this study is whether ultra-low doses of naltrexone affect the reward system. Sites in the periaqueductal gray and the nucleus accumbens have been shown to mediate both opioid analgesia (Yeung et al., 1977; Yu and Han, 1989) and reward (Wise, 1989; Olmstead and Franklin, 1997). Given the overlap in supraspinal sites mediating analgesia and reward, it is likely that reward systems are similarly affected by ultra-low doses of naltrexone. The results of this study show that in the CPP paradigm, ultra-low doses of naltrexone in combination with systemic morphine produced a response that persisted beyond the effect of morphine alone. This effect is reminiscent of the action in analgesia experiments in which intrathecal naltrexone increased the duration of the agonist effect. Indeed, ultra-low doses of naltrexone significantly increased the ability of morphine to produce rewarding effects when the interval between the time of morphine injection and placement into the conditioning chamber was 2 h. Although the mechanisms underlying the action of ultra-low doses of naltrexone in this respect are not known, the action of this agent on reward may have implications for the use of naltrexone to modify the analgesic action of morphine. On the other hand, it should be noted that chronic cotreatment of mice with high doses of morphine plus ultra-low-dose naltrexone markedly attenuates physical dependence as manifested by naloxone-precipitated withdrawal jumping effects (Crain and Shen, 1995; Shen and Crain, 1997). Chronic cotreatment studies will be required to determine the degree to which the observed enhancement of morphine's rewarding effects following acute cotreatment with ultra-low-dose naltrexone may be correlated with a significant increase in drug dependence or abuse liability.

The results of this study, demonstrating the actions of a clinically used opioid antagonist on morphine analgesia, tolerance and reward, have implications for drug treatment of chronic pain and for drug abuse. With respect to the former, the established clinical acceptability of naltrexone and its ability to both inhibit and reverse tolerance, as demonstrated here, provides a rationale for combining these agents to minimize the loss of drug potency associated with chronic exposure to opioid drugs. Additionally, certain types of neuropathic pain are relatively insensitive to opioid drugs (Lee et al., 1995; Mao et al., 1995) but may respond to opioids in combination with ultra-low doses of naltrexone. With respect to drug abuse, the present findings suggest that the rewarding effects of morphine may be prolonged by combination with an ultra-low dose of naltrexone. It is likely that the eventual therapeutic advantages of combination treatments with opioids and ultra-low doses of naltrexone will outweigh the possible abuse liability of this drug combination.

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