Nonopioidergic Mechanism Mediating Morphine-Induced Antianalgesia in the Mouse Spinal Cord

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ABSTRACT

Intrathecal (i.t.) pretreatment with a low dose (0.3 nmol) of morphine causes an attenuation of i.t. morphine-produced analgesia; the phenomenon has been defined as morphine-induced antianalgesia. The opioid-produced antianalgesia was measured with the tail-flick (TF) test in male CD-1 mice. Intrathecal pretreatment with low dose (0.3 nmol) of morphine time dependently attenuated i.t. morphine-produced (3.0 nmol) TF inhibition and reached a maximal effect at 45 min. Intrathecal pretreatment with morphine (0.009–0.3 nmol) for 45 min also dose dependently attenuated morphine-produced TF inhibition. The i.t. morphine-induced antianalgesia was dose dependently blocked by the nonselective μ-opioid receptor antagonist (−)-naloxone and by its nonopioid enantiomer (+)-naloxone, but not by endomorphin-2-sensitive μ-opioid receptor antagonist 3-methoxynaltrexone. Blockade of δ-opioid receptors, κ-opioid receptors, and N-methyl-D-aspartate (NMDA) receptors by i.t. pretreatment with naltrindole, nor-binaltorphimine, and (−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801), respectively, did not affect the i.t. morphine-induced antianalgesia. Intrathecal pretreatment with antiserum against dynorphin A(1-17), [Leu]-enkephalin, [Met]-enkephalin, β-endorphin, cholecystokinin, or substance P also did not affect the i.t. morphine-induced antianalgesia. The i.t. morphine pretreatment also attenuated the TF inhibition produced by opioid μ agonist [d-Ala², N-Me-Phe⁶,Gly-ol³]-enkephalin, δ-agonist deltorphin II, and κ-agonist U50,488H. It is concluded that low doses (0.009–0.3 nmol) of morphine given i.t. activate an antianalgesic system to attenuate opioid μ, δ, and κ-agonist-produced antianalgesia. The morphine-induced antianalgesia is not mediated by the stimulation of opioid μ, δ, or κ-receptors or NMDA receptors. Neuropeptides such as dynorphin A(1-17), [Leu]-enkephalin, [Met]-enkephalin, β-endorphin, cholecystokinin, and substance P are not involved in this low-dose morphine-induced antianalgesia.

We have previously reported that intrathecal (i.t.) pretreatment with an endogenous μ-opioid peptide, endomorphin-2 (0.05–1.75 nmol), attenuates the antinociception produced by opioid agonists. The antianalgesic effect is caused by the release of dynorphin A(1-17) through the stimulation of a subtype of μ-opioid receptors. The unique features of this endomorphin-2-induced antianalgesic action are that there is a lag period before dynorphin A(1-17) is released, and the antianalgesic action of endomorphin-2 corresponds with the time course of dynorphin release (Leiterman et al., 2003; Wu et al., 2003). Furthermore, i.t. administered endomorphin-2 at larger doses (5.25–35 nmol) produces analgesia by itself through activation of spinal μ-opioid receptors (Ohsawa et al., 2001), whereas its delayed antianalgesic action is manifested more easily at small doses of endomorphin-2 by its ability to attenuate the analgesic action of other opioids administered after endomorphin-2 pretreatment.

There are indications from the literature that morphine may have an antianalgesic action. Pretreatment with a low dose of naloxone (0.00028 fmol) or dynorphin A antiserum given i.t. enhances the analgesic effect of intracerebroventricularly and i.t.-administered morphine (Fujimoto and Rady, 1989; Holmes and Fujimoto, 1993). Studies performed by Crain and Shen (1990, 1995, 2000) indicate that even though generally morphine has a depressant effect on action potential duration in mouse dorsal root ganglion preparations, very low doses (1 fmol–1 pmol) of morphine produce the opposite effect by prolonging the duration of the action potential, an excitatory potential. Recent studies also have shown that morphine and opioid compounds not only simply elicit

ABBREVIATIONS: TF, tail-flick; %MPE, percent maximum possible effect; NTI, naltrindole; nor-BNI, nor-binaltorphimine; NMDA, N-methyl-D-aspartate; MK-801, (−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate; DAMGO, [d-Ala²,N-Me-Phe⁶,Gly-ol³]-enkephalin; CCK, cholecystokinin; ANOVA, analysis of variance; CI, confidence interval; U50,488H, trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methane-sulfonate hydrate.
analgesia but also exert hyperalgesic effects, which may counteract antinoceception (Mao et al., 1995; Eisenach, 2000). One of the antianalgesic actions is mediated by a low dose (4.65 fmol) of dynorphin (Fujimoto et al., 1990; Holmes and Fujimoto, 1993) and the stimulation of a nonopioid system, which promotes the release of excitatory neurotransmitters from primary afferent neurons (Vanderah et al., 1996, 2001). Although these studies in the literature are suggestive, none show a direct antianalgesic effect for i.t.-administered morphine. Furthermore, these studies indicate that the excitatory actions of morphine occur rapidly after opioid administration.

High doses of morphine given i.t., intracerebroventricularly, or systemically produces analgesia. The present study was designed to see whether morphine can be shown to have an antianalgesic action and if so, whether it occurs through the mediation of dynorphin A(1-17) and whether this action is rapidly evident or occurs after a latent period as with endomorphin-2-induced antianalgesia. We now report that pretreatment with a low dose (0.3 nmol) of morphine attenuates the analgesia produced by subsequent injection of morphine or other opioids. Unlike the antianalgesia produced by endomorphin-2, which is mediated by stimulation of a novel subtype of μ-opioid receptors and subsequent release of dynorphin, the antianalgesia induced by low doses (0.009–0.3 nmol) of morphine is not mediated by μ-, δ-, or κ-opioid receptors and is not mediated by the release of dynorphin.

**Materials and Methods**

**Animals.** Male CD-1 mice weighing 25 to 30 g (Charles River Laboratories, Inc., Wilmington, MA) were used. Animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light/dark cycle. Food and water were available ad libitum. Each animal was used only once. All experiments were approved by and conformed to the guidelines of the Animal Care Committee of the Medical College of Wisconsin.

**Assessment of Analgesia.** Analgesic responses were measured with the tail-flick (TF) test (D’Amour and Smith, 1941). To measure the latency of the TF response, mice were gently held with the tail put on the apparatus (model TFP; EMDIE Instrument Co., Maidens, VA). The TF response was elicited by applying radiant heat to the dorsal surface of the tail. The intensity of the heat stimulus was set to provide a predrug TF response time of 3 to 4 s. The inhibition of the TF response was expressed as percent maximal possible effect (%MPE), which was calculated as \((\frac{T_0 - T_r}{T_0 - T_p}) \times 100\), where \(T_0\) and \(T_r\) are the TF latencies before and after i.t. injection of morphine, respectively, and \(T_p\) is the cutoff time, which was set at 10 s.

**Experimental Protocols.** Intrathecal injection was performed according to the procedure of Hylden and Wilcox (1980), using a 25-μl Hamilton syringe with a 30-gauge needle. The injection volume was 5 μl. The following experiments were performed. 1) The timecourse and the dose-response relationships of morphine pretreatment for the development of antianalgesia against morphine-produced antinociception were determined. Groups of mice were pretreated i.t. with low dose (0.3 nmol) of morphine for different times (15–120 min) or different doses (0.003–3 nmol) 45 min before i.t. administration of morphine (3.0 nmol), and the TF responses were measured at different times thereafter. 2) The type of opioid receptors involved in morphine-induced antianalgesia was determined based on the initial presumption that μ-opioid receptors were involved. Groups of mice were pretreated i.t. with the μ-opioid receptor antagonist 3-methoxynaltrexone (6.4 pmol), which antagonizes endomorphin-2, heroin, and morphine-6β-glucuronide-produced analgesia but not morphine, 25 min (Brown et al., 1997; Sakurada et al., 2000); δ-opioid receptor antagonist naltrindole (NTI, 11.1 or 22.3 nmol), 10 min (Mizuguchi et al., 1995; Wu et al., 2003); κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI, 6.6 or 39.6 nmol) 24 h (Tseng et al., 1997; Ohawa et al., 2001; Wu et al., 2003); nonselective opioid-receptor antagonist (+)-naloxone (0.03–27.5 pmol), 10 min; the nonopioid-receptor antagonist (+)-naloxone (0.28–55 pmol), 10 min; or NMDA receptor antagonist MK-801 (10 nmol), 20 min (Wu et al., 2003) before i.t. injection of morphine (0.3 nmol). An analgesic dose of morphine (3.0 nmol) was then injected i.t. 45 min after pretreatment with a low antianalgesic dose (0.3 nmol) of morphine, and TF responses were measured at different times thereafter.

**Drugs and Antisera.** Morphine sulfate, (+)-naloxone enantiomer (+)-naloxone, NTI, and nor-BNI were obtained from National Institute on Drug Abuse (Baltimore, MD). U50,488H, (+)-naloxone, 3-methoxynaltrexone, and MK-801 were purchased from Sigma-Aldrich (St. Louis, MO). DAMGO and deltorphin II were purchased from Phoenix Pharmaceuticals (Belmont, CA). Morphine, U50,488H, (+)-naloxone, (+)-naloxone, 3-methoxynaltrexone, NTI, nor-BNI, and MK-801 were dissolved in 0.9% saline. DAMGO was dissolved in 0.9% saline containing 0.01% Triton X-100, and deltorphin II was dissolved in 0.9% saline containing 10% hydroxypropyl-β-cyclodextrin. The antiserum against dynorphin A(1-17), β-endorphin, [Leu]-enkephalin, [Met]-enkephalin, substance P, or normal rabbit serum 1 h before i.t. injection of morphine (0.3 nmol) followed by i.t. morphine (3.0 nmol) 45 min thereafter. The TF responses were then measured. The doses of antiserum used have been shown previously to be sufficient for their specific effects (Tseng and Huang, 1992; Xu and Tseng, 1997; Ohawa et al., 2001; Wu et al., 2002). 4) Determine whether the morphine-induced antianalgesia is selective against opioid μ-agonists or is generalized to δ- and κ-agonists. Groups of mice were injected i.t. with morphine (0.3 nmol) 45 min before i.t. administration of [−Ala2,−N-Me-Phe4,Gly-ol2]-enkephalin (DAMGO) (0.02 nmol), deltorphin II (12.8 nmol), or U50,488H (123.2 nmol), and the TF response was measured at different times thereafter.

**Statistical Analysis.** The analgesic responses, %MPE, were presented as the mean ± S.E.M. The Kolmogorov-Smirnov test was used to test the normality of the %MPE data. If the data presented were not normally distributed, then a transformation of the log transform on fraction of baseline latency of TF test was performed before using analysis of variance (ANOVA) or Student’s t test. One-way ANOVA followed by Dunnett’s post test, two-way ANOVA followed by Bonferroni’s post tests, or Student’s t test was used to test the differences between groups. Nonlinear regression model was used to fit the dose-response curve and calculates the ED50 value and 95% confidence interval (CI) of morphine-induced antianalgesia. GraphPad Prism software was used to perform the statistics (version 4.0; GraphPad Software, Inc., San Diego, CA).
Results

Tail-Flick Responses after i.t. Injection of 0.3 nmol of Morphine. Two groups of mice were injected i.t. with morphine (0.3 nmol) or vehicle, and the TF responses were measured at different times after injection. Morphine at such a dose produced a weak TF inhibition (28% MPE) at 15 min and returned to vehicle control levels in 40 to 60 min for the remaining 2-h measurement times (Fig. 1).

Effects of Different Times and Doses of Pretreatment with Morphine Given i.t. on the TF Inhibition Produced by i.t.-Administered Morphine. Groups of mice were pretreated i.t. with morphine (0.3 nmol) at various times before i.t. injection of morphine (3.0 nmol), and the TF response was measured 15 min thereafter. Other groups of mice pretreated i.t. with vehicle served as controls. The i.t. administration of morphine (3.0 nmol) produced 84 to 95% MPE of TF inhibition in mice pretreated i.t. with vehicle for different times. Intrathecal pretreatment with a low dose (0.3 nmol) of morphine time dependently attenuated the TF inhibition produced by i.t. morphine (3.0 nmol); the attenuation of the morphine-produced TF inhibition developed slowly in 15 to 30 min, reached a maximal attenuation (22.1 ± 8.1% MPE) at 45 min, remained markedly attenuated at 60 min, and returned to control values at 90 to 120 min (Fig. 2A). Pretreatment time of 45 min for morphine was then used for the following experiments.

Groups of mice were pretreated i.t. with different doses (0.003–3.0 nmol) of morphine 45 min before i.t. administration of morphine (3.0 nmol), and the TF response was measured at 15 min thereafter. Low doses (0.009–0.3 nmol) of morphine pretreatment dose dependently attenuated the TF inhibition produced by i.t.-administered morphine (Fig. 2B). The ED50 for morphine to induce antianalgesia against morphine-produced TF inhibition was estimated to be 0.076 nmol (95% CI, 0.04–0.16 nmol). Morphine at a dose of 0.3 nmol for the pretreatment was found to produce a maximum attenuation of morphine-produced analgesia and was therefore used for the following experiments.

Effect of i.t. Pretreatment with (−)-Naloxone and (+)-Naloxone on the i.t. Morphine-Induced Antianalgesia. The nonselective µ-opioid receptor antagonist (−)-naloxone and its nonopioid enantiomer (+)-naloxone were used to determine whether the antianalgesic effect induced by morphine pretreatment is mediated by the stimulation of µ-opioid receptors. Groups of mice were pretreated i.t. with (−)-naloxone (2.75 pmol) for different times before i.t. morphine (0.3 nmol) injection and followed by morphine (3 nmol) injected i.t. 45 min thereafter. Pretreatment with (−)-naloxone given i.t. time dependently and effectively restored the attenuation of morphine-produced TF inhibition induced by a low dose (0.3 nmol) of morphine pretreatment. Intrathecal pre-

Fig. 1. TF responses after i.t. administration of morphine or vehicle. Groups of mice were injected i.t. with morphine (0.3 nmol) or vehicle (5 µl), and the TF responses were then measured at different times thereafter. Each point represents the mean, and the vertical bar represents the S.E.M. of %MPE with seven to 10 mice in each group. The repeated measure two-way ANOVA followed by Bonferroni’s post test was used to test the difference between groups. The F interaction, treatment, time = 13.03, 18.59, 14.55; *, p < 0.05.

Fig. 2. Effect of different pretreatment times (A) and doses (B) with morphine given i.t. on the TF inhibition produced by i.t.-administered morphine. A, groups of mice were pretreated i.t. (5 µl each) with morphine (0.3 nmol) or vehicle 15, 30, 45, 60, 90, or 120 min before i.t. administration of morphine (3.0 nmol). B, groups of mice were pretreated i.t. with morphine (0.003–3.0 nmol) or vehicle 45 min before i.t. injection of morphine (3.0 nmol). The TF response (%MPE) was measured 15 min after morphine (3.0 nmol) administration. Each column represents the mean, and the vertical bar represents the S.E.M. with eight to 10 mice in each group. Two-way ANOVA followed by Bonferroni’s post test (A) or one-way ANOVA followed by Dunnett’s post test (B) was used to test the difference between groups. The F interaction, treatment, time = 11.85, 175, 17.73; F = 32.68; *, p < 0.001.
treatment with (−)-naloxone for 10 or 30 min effectively blocked the i.t. morphine-induced antianalgesia. The blocking effect of (−)-naloxone declined in 45 min and completely dissipated after 60 to 120 min of pretreatment (Fig. 3A). Intrathecal pretreatment with (−)-naloxone (0.28–2.75 pmol) 10 min before i.t. morphine (0.3 nmol) pretreatment dose dependently restored the attenuation of the i.t. morphine (3.0 nmol)-produced TF inhibition and reached its maximal effect at 2.75 pmol (Fig. 3B). Intrathecal pretreatment with (−)-naloxone (2.75 pmol) alone 55 min before morphine (3.0 nmol) challenge did not affect the morphine-produced TF inhibition (90.7 ± 4.4% MPE).

Intrathecal pretreatment with (+)-naloxone (0.28–55.0 pmol) 10 min before i.t. pretreatment with morphine (0.3 nmol) also dose dependently restored the attenuation of the i.t. morphine-produced TF inhibition and reached its maximal reversal at a dose of 55 pmol of (+)-naloxone pretreatment (Fig. 4). Because this finding is extremely interesting, the effect of (+)-naloxone pretreatment on endomorphin-2 (1.75 nmol)-induced antianalgesia against i.t. morphine-produced TF inhibition was also studied. Pretreatment with (+)-naloxone (55 pmol) given i.t. did not inhibit the endomorphin-2-induced (1.75 nmol) antianalgesic effect against morphine-produced TF inhibition. The TF inhibition (% MPE, mean ± S.E.M.) produced by morphine (3.0 nmol) was 43.9 ± 3.4 and 33.1 ± 6.3 (n = 8–10) after (+)-naloxone and saline vehicle pretreatment, respectively, 10 min before endomorphin-2 (1.75 nmol) pretreatment.

The ED_{50} values for i.t. (−)-naloxone (0.03–55.0 pmol) and (+)-naloxone (0.28–55.0 pmol) to inhibit morphine (0.3 nmol)-induced antianalgesia were estimated to be 0.21 pmol (95% CI, 0.04–1.17 pmol) and 1.79 pmol (95% CI, 0.68–4.71 pmol), respectively. Thus, (−)-naloxone is about 8.5-fold more potent than (+)-naloxone in blocking the morphine-induced antianalgesia.

**Effect of i.t. Pretreatment with 3-Methoxynaltrexone, NTI, nor-BNI, or MK-801 on i.t. Morphine-Induced Antianalgesia.** We have previously demonstrated that pretreatment with 3-methoxynaltrexone, but not NTI, nor-BNI, or MK-801, effectively blocks the endomorphin-2-induced antianalgesia (Wu et al., 2003). The effects of these antagonists on the morphine-induced antianalgesia were then studied. Intrathecal pretreatment with the endomorphin-2 sensitive μ-opioid receptor antagonist 3-methoxynaltrexone (6.4 pmol) 25 min before i.t. morphine (0.3 nmol) administration did not affect the antianalgesic effect of morphine. Intrathecal pretreatment with the selective δ-opioid receptor antagonist NTI (11.1 or 22.3 nmol), κ-opi-
oid antagonist nor-BNI (6.6 or 39.6 nmol), or NMDA receptor antagonist MK-801 (10 nmol) also did not affect the attenuation of the i.t. morphine (3.0 nmol)-produced TF inhibition induced by i.t. morphine (0.3 nmol) pretreatment compared with i.t. vehicle-pretreated groups (Table 1).

Effects of i.t. Pretreatment with Antiserum against Dynorphin A(1-17), [Leu]-Enkephalin, [Met]-Enkephalin, β-Endorphin, CCK-8s, or Substance P on i.t. Morphine (0.3 nmol)-Induced Antianalgesia. Antiserum against endogenous opioid peptides or other neuropeptides were used to determine whether they were involved in mediating i.t. morphine-induced analgesia. Intrathecal pretreatment with a low dose (0.3 nmol) of morphine attenuated the i.t. morphine-produced TF inhibition to 27.8% MPE from 77.8% MPE in mice pretreated i.t. with normal rabbit serum. Morphine-induced antianalgesia was not affected by i.t. pretreatment with antiserum against dynorphin A(1-17), [Met]-enkephalin, [Leu]-enkephalin, β-endorphin, CCK-8s, or substance P (Table 2).

Effects of i.t. Pretreatment with a Low Dose (0.3 nmol) of Morphine on the TF Inhibition Produced by μ-, δ-, and κ-Opioid Agonists. Groups of mice were pretreated i.t. with a low dose (0.3 nmol) of morphine 45 min before i.t. injection of the μ-opioid receptor agonist DAMGO (0.02 nmol), δ-opioid receptor agonist deltorphin II (12.8 nmol), or κ-opioid receptor agonist U50,488H (12.3 nmol), and the TF response was measured 10 min after injection. Mice pretreated i.t. with vehicle served as control. Intrathecal pretreatment with a low dose (0.3 nmol) of morphine attenuated the TF inhibition produced by DAMGO, deltorphin II, or U50,488H (Fig. 5).

**Discussion**

Pretreatment with Low Doses (0.09–0.3 nmol) of Morphine Induces Antianalgesia. We found in the present studies that pretreatment with low doses (0.09–0.3 nmol) of morphine dose dependently attenuated the analgesia produced by i.t.-administered morphine (3.0 nmol). Thus, morphine is at least 11-fold more potent to induce antianalgesia than to produce analgesia; the ED_{50} value for morphine-induced antianalgesia is 0.076 nmol and for morphine-produced TF inhibition is 0.87 nmol (Suh and Tseng, 1988). Because morphine at such low doses (0.3 nmol and lower) given alone produced slight or no analgesia, it is unlikely that the antianalgesic effect induced by low doses of morphine is due to the desensitization of μ-opioid receptors after small doses of morphine pretreatment.

The Antianalgesia Induced by Low Doses of Morphine Pretreatment Is Mainly Mediated by the Stimulation of a Non-μ-Opioid Receptor. We found that both the nonselective μ-opioid receptor antagonist (−)-naloxone and its nonopoid enantiomer (+)-naloxone were effective in blocking the antianalgesia induced by low doses of morphine pretreatment. The enantiomer (+)-naloxone is at least 3 to 4 orders of magnitude less potent than its enantiomer (−)-naloxone in blocking the μ-opioid receptor in [3H]naloxone receptor binding, ruling out the possibility that (+)-naloxone might bind the μ-opioid receptors (Iijima et al., 1978). The lack of stereospecific action between (+)- and (−)-naloxone in blocking low doses of morphine-induced antianalgesia strongly indicates that a non-μ-opioid receptor is involved in the morphine-induced antianalgesia. However, the finding that the nonselective opioid receptor antagonist (−)-naloxone was about 8.5-fold more potent than (+)-naloxone in blocking the morphine-induced antianalgesia leads us to speculate that μ-opioid receptors may also be involved in morphine-induced antianalgesia. More study is needed to clarify this possibility.

**Comparison of Antianalgesia Induced by Morphine and Endomorphin-2 Pretreatment.** Both low doses of endomorphin-2 (0.18–1.75 nmol) and morphine (0.09–0.3 nmol) induce antianalgesia with a similar time course, whereas larger doses of these two drugs produce analgesia (Ohsawa et al., 2001). In addition, the antianalgesia induced by a low dose of endomorphin-2 or morphine is effectively

<table>
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<tr>
<th>Antiserum (n)</th>
<th>Morphine (0.3 nmol) i.t. Pretreatment</th>
<th>TF Inhibition (%MPE) by i.t. Morphine (3 nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rabbit serum (9)</td>
<td>No</td>
<td>77.79 ± 8.09</td>
</tr>
<tr>
<td>Normal rabbit serum (8)</td>
<td>Yes</td>
<td>27.78 ± 3.56</td>
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<tr>
<td>A/S against dynorphin A (1-17) (8)</td>
<td>Yes</td>
<td>26.55 ± 4.97</td>
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<tr>
<td>A/S against [Leu]-enkephalin (8)</td>
<td>Yes</td>
<td>40.73 ± 6.01</td>
</tr>
<tr>
<td>A/S against [Met]-enkephalin (9)</td>
<td>Yes</td>
<td>27.46 ± 4.04</td>
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<tr>
<td>A/S against β-endorphin (7)</td>
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<td>22.65 ± 5.37</td>
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<tr>
<td>A/S against substance P (8)</td>
<td>Yes</td>
<td>38.80 ± 6.11</td>
</tr>
</tbody>
</table>

A/S, antiserum.
study, i.t. pretreatment with antiserum against dynorphin A (1-17) did not affect the antianalgesia induced by a low dose of morphine, indicating that endogenous dynorphin A(1-17) is not involved in morphine-induced antianalgesia.

Dynorphin A (1-17) has been shown to exert both antinociceptive and nociceptive actions (Millan, 1999). Spinal dynorphin A(1-17) has been shown to be a mediator of antianalgesia against i.t.-administered morphine-produced antinociception. The antianalgesic effects can be blocked by i.t. pretreatment with naloxone or nor-BNI or antiserum against dynorphin A(1-17) (Fujimoto et al., 1990; Aksu et al., 1993; Holmes and Fujimoto, 1993). The spinal dynorphin then activates ascending neuronal circuits to the brain and then through a descending neuronal circuit activates spinal CCK, [Leu]-enkephalin, and NMDA receptors (Wang et al., 1994; Rady et al., 1998, 2001). In these studies, the antianalgesia elicited by spinal dynorphin has quick onset and peak action, which occurs in 5 min. However, we found in the present study that a low dose (0.3 nmol) of morphine-induced antianalgesia developed gradually and reached its peak effect after 45 min, and the pretreatment of antiserum against dynorphin A(1-17) did not block the morphine-induced antianalgesia. We also demonstrated that antiserum against [Leu]-enkephalin or CCK did not block the morphine-induced antianalgesia. Thus, the present antianalgesia system does not involve the quick-acting dynorphin system. Similar to the antianalgesia induced by a low dose of endomorphin-2, the antianalgesia induced by a low dose of morphine was also not affected by the pretreatment with antiserum against [Met]-enkephalin and β-endorphin, indicating that these two opioid neuropeptides are not involved in the morphine-induced antianalgesia. Substance P has been shown to be a nociceptive neurotransmitter, which subsequently activates postsynaptic neurokinin-1 receptors in the spinal cord (Salter and Henry, 1991; Malmberg and Yaksh, 1992). However, our results clearly indicated that substance P is not involved in the morphine-induced antianalgesia, because pretreatment with antiserum against substance P failed to affect the morphine-induced antianalgesia.

**Δ- or κ-Opioid Receptors or NMDA Receptors Are Not Involved in Morphine-Induced Antianalgesia.** Previous studies have shown that activation of κ-opioid receptors specifically antagonize μ-opioid receptor-mediated antianalgesia, which can be blocked by κ-opioid receptor antagonist nor-BNI (Holmes and Fujimoto, 1993; Pan et al., 1997). The δ-opioid receptor and NMDA receptor are involved in spinal dynorphin-mediated antianalgesia, because pretreatment with δ-opioid receptor antagonist naltrindone or NMDA receptor antagonist MK-801 given i.t. blocks the spinal dynorphin-produced antianalgesia (Rady et al., 2001). Similar to endomorphin-2-induced antianalgesia (Wu et al., 2003), we found in the present study that pretreatment with δ-opioid receptor antagonist NTI, κ-opioid receptor antagonist nor-BNI or NMDA receptor antagonist MK-801 did not affect morphine-induced antianalgesia, indicating that δ- and κ-opioid and NMDA receptors are not involved in morphine-induced antianalgesia.

**Morphine Pretreatment Nonselectively Attenuates the Analgesia Produced by μ-, δ-, and κ-Opioid Agonists.** The question remains whether the morphine-induced antianalgesia is selective against antinociception produced by μ-opioid agonists or can be generalized to δ- and κ-opioid
agonists as well. We found that pretreatment with a low dose (0.3 nmol) of morphine attenuated the TP inhibition produced by μ-opioid agonist DAMGO, δ-opioid receptor agonist deltorphin II, or κ-opioid receptor agonist U50,488H, indicating that the morphine-induced antianalgesia is nonselective and is generalized to attenuate the analgesia produced by μ-, δ-, and κ-opioid agonists. Similarly, we have previously demonstrated that the endomorphin-2-induced antianalgesia is also nonselective and attenuates the analgesia produced by μ-, δ-, and κ-opioid receptor agonists (Wu et al., 2003).

The antianalgesic effect found in the present study of low doses of morphine suggests the existence of a different system, which is not mediated by any known dynorphin systems. The fact that (+)-naloxone antagonizes the antianalgesia might be useful in designing a morphine regimen in which the analgesic action of morphine could be enhanced to provide a therapeutic advantage such as enhanced analgesia with less potential for development of tolerance. Also, even as there are different opioid receptors involved in analgesia, there seems to be several mechanisms by which antianalgesia may be induced. Further study of these antianalgesic systems should lead to a better understanding of the homeostatic mechanisms involved in certain modalities of pain control.

It is concluded that i.t. pretreatment with low doses (0.09–0.3 nmol) of morphine stimulates a nonopioid receptor system to elicit an antianalgesic effect against μ-, δ-, or κ-opioid agonist-produced analgesia. Dynorphin A(1-17), [Leu]-enkephalin, [Met]-enkephalin, β-endorphin, cholecystokinin, substance P, and NMDA receptors are not involved in mediating this morphine-induced antianalgesia. Our results obtained from the present studies imply that the phenomenon of the acute nociceptive tolerance to morphine, which occurs after pretreatment with morphine may be in part mediated by the activation of this nonopioid antianalgesic mechanism of morphine.

References


