Dynorphinergic Mechanism Mediating Endomorphin-2-Induced Antianalgesia in the Mouse Spinal Cord

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ABSTRACT
We have previously demonstrated that both endomorphin-1 (EM-1) and endomorphin-2 (EM-2) at high doses (1.75–35 nmol) given intrathecally (i.t.) or intracerebroventricularly produce antinociception by stimulation of μ-opioid receptors. Now, we report that EM-2 at small doses (0.05–1.75 nmol), which injected alone did not produce antinociception, produces antinociception against opioid agonist-induced antinociception. The tail-flick (TF) response was used to test the antinociception in male CD-1 mice. Intrathecal pretreatment with EM-2 (0.02–1.75 nmol) 45 min before i.t. morphine (3.0 nmol) injection dose dependently attenuated morphone-induced TF inhibition. On the other hand, a similar dose of EM-1 (1.64 nmol) failed to produce any antinociceptive effect. The EM-2 (1.75 nmol)-produced anti-analgesia against morphine-induced TF inhibition was blocked by i.t. pretreatment with the μ-opioid antagonist naloxone or 3-methoxynaltrexone, but not δ-opioid receptor antagonist naltrindole, κ-opioid receptor antagonist nor-binaltorphimine, or N-methyl-D-aspartate (NMDA) receptor antagonist MK-801. The EM-2-induced antianalgesic effect against morphine-induced TF inhibition was blocked by i.t. pretreatment with antisense oligodeoxynucleotides against exon-8 of β3-endorphin receptor clone inhibits the antinociception induced by EM-1 (Wu et al., 2001). Intrathecal pretreatment with selective μ-opioid receptor antagonist [D-Ala2,N-Me-Phe4,Gly-ol5]-enkephalin (1 μg) failed to produce antianalgesia is not mediated by the release of [Met]-enkephalin, [Leu]-enkephalin, β-endorphin, or cholecystokinin, nor does it involve κ- or δ-opioid or NMDA receptors in the spinal cord.

Endogenous opioid tetrapeptides endomorphin-1 (EM-1) and endomorphin-2 (EM-2) have been found to be highly selective for μ-opioid receptors (Zadina et al., 1997). Both EM-1 and EM-2 potently compete with μ-opioid receptor binding with no appreciable affinity with δ- and κ-opioid receptors and selectively activate μ-opioid receptor-mediated G-proteins with [35S]guanosine 5′-O-(3-thio)triphosphate binding (Goldberg et al., 1998; Narita et al., 1998, 2000; Monory et al., 2000). The increases of the [35S]guanosine 5′-O-(3-thio)triphosphate binding and antinociceptive effects induced by both EM-1 and EM-2 are selectively blocked by the pretreatment with selective μ-opioid receptor antagonist β-funaltrexamine or d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH2, indicating that the effects are mediated by the stimulation of μ-opioid receptors.

However, recent studies indicate that the antinociceptive effects induced by EM-1 and EM-2 are mediated by the stimulation of different subtypes of μ-opioid receptors. Pretreatment with μ-opioid receptor antagonist 3-methoxynaltrexone selectively attenuated EM-2, but not EM-1-induced antinociception, whereas β-funaltrexamine inhibits both (Sakurada et al., 2000). There is an asymmetric cross-tolerance between EM-1 and EM-2, where mice made acutely tolerant to EM-1 are not cross-tolerant to EM-2, but mice made acutely tolerant to EM-2 cause partial cross-tolerance to EM-1 (Wu et al., 2001). Intrathecal pretreatment with antisense oligodeoxynucleotides against exon-8 of μ-opioid receptor clone inhibits the antinociception induced by EM-1.
but not EM-2 (Wu et al., 2002b). These findings strongly support the view that EM-1 and EM-2 stimulate different subtypes of μ-opioid receptors to produce their pharmacological functions.

Dynorphin A(1-17) (Dyn) has been shown to process both antinociceptive and pronociceptive actions (for review, see Millan, 1999). We found in our previous studies that the TF inhibition induced by EM-2, but not EM-1 or other μ-opioid agonists given i.c.v. or i.t., is attenuated by the pretreatment with antisera against Dyn or κ-opioid antagonist nor-binaltorphimine (nor-BNI), indicating that the EM-2-induced antinociception is mediated in part by the release of Dyn acting on κ-opioid receptors (Tseng et al., 2000; Ohsawa et al., 2001). Early publications have provided evidence that one of the antianalgesic actions against morphine- and other opioid-induced analgesia is mediated by the release of Dyn (Fujimoto et al., 1990; Arts et al., 1992; Holmes and Fujimoto, 1992; Aksu et al., 1993). In the present study, we have demonstrated another aspect of the action of EM-2. Pretreatment with a subanalgesic dose of EM-2 attenuates the antinociception induced by subsequent injection of morphine or other opioids. We propose that the anti-analgesia of EM-2 is mediated by the stimulation of a subtype of μ-opioid receptors and the release of Dyn. Experiments were performed to demonstrate that this antianalgesia induced by EM-2 is selectively blocked by the pretreatment with the selective μ-opioid receptor antagonist 3-methoxynaltrexone and antisera against Dyn. We also determined whether this EM-2-induced antianalgesia is selective against antinociception induced by μ-opioid agonists or is generalized to δ- or κ-opioid agonists.

Materials and Methods

Animals. Male CD-1 mice weighing 25 to 30 g (Charles River Laboratories, 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. The animals were 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 Antinociception. Antinociceptive responses were measured with the (TF) test (D’Amour and Smith, 1941). To measure the latency of the tail-flick (TF) response, mice were gently held with the tail put on the apparatus (model TF6; 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 maximum possible effect (%MPE), which was calculated as \[\%\text{MPE} = \frac{(T_1 - T_2)/T_1 \times 100}{T_2 - T_0} \times 100\]. T_0 and T_2 were the TF latencies before and after i.t. injection of morphine, respectively, and T_1 was 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) Groups of mice were administered i.t. with 1.64 nmol of EM-1 or 1.75 nmol of EM-2, and TF responses were measured at different times after injection for 2 h. Other groups of mice were pretreated i.t. with 1.64 nmol of EM-1, 1.75 nmol of EM-2, or vehicle 45 min before i.t. morphine (3.0 nmol) injection, and the TF responses were then measured every 5 to 10 min after injection for 1 h. 2) Determine the time course and the dose-response relationship of EM-2 pretreatment for the development of anti-analgesia against morphine-induced antinociception. Groups of mice were pretreated i.t. with EM-2 (1.64 nmol) for different times (15–120 min) or different doses (0.02–17.5 nmol) of EM-2 for 45 min before i.t. administration of morphine and the TF responses were measured at different times thereafter. 3) Determine the type of receptors involved in EM-2-induced antianalgesia against morphine-induced TF inhibition. Mice were pretreated i.t. with the selective opioid antagonist 3-methoxynaltrexone (6.4 pmol) 25 min (Sakurada et al., 2000), δ-opioid receptor antagonist naltrexolrindole (NTI, 11.1 nmol) 10 min (Mizoguchi et al., 1995; Wu et al., 2002a), κ-opioid receptor antagonist nor-BNI (6.6 nmol) 24 h (Tseng et al., 1997; Ohsawa et al., 2001), nonselective opioid-receptor antagonist naloxone (5.5–55 pmol) 10 min, or NMDA receptor antagonist MK-801 (10 nmol) 20 min (Gardell et al., 2002) before i.t. injection of EM-2 (1.75 nmol). Morphine (3.0 nmol) was then injected i.t. 45 min after EM-2 injection, and TF responses were measured at different times thereafter. 4) Determine the type of endogenous neuropetides involved in EM-2-induced antianalgesia against morphine-induced TF inhibition. Groups of mice were pretreated i.t. with antisera against Dyn, cholecystokinin-8s (CCK-8s), β-endorphin, [Leu^5]-enkephalin, [Met^5]-enkephalin, or nor-β-endorphin serum (NRS) 1 h before i.t. injection of EM-2 and morphine was then injected i.t. 45 min thereafter. The TF responses were then measured at different times after morphine injection. The doses of antisera against Dyn were 50 to 300 μg and 200 μg for other antisera. The doses of antisera used have been shown previously to be sufficient for their specific effects (Tseng and Huang, 1992; Xu and Tseng, 1997; Ohsawa et al., 2001; Wu et al., 2002a). Finally, 5) determine whether the EM-2-induced antianalgesia is selective against opioid μ-agonists or is generalized to δ- and κ-agonists. Groups of mice were injected i.t. with EM-2 (1.75 nmol) 45 min before i.t. administration of DAMGO (0.02 nmol), EM-1 (16.4 nmol), EM-2 (35 nmol), deltorphin II (6.4 nmol), or U50,488H (123.2 nmol), and the TF response were measured at different times after injection.

Drugs and Antisera. Endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH₂) were purchased from Calbiochem (La Jolla, CA). NTI and nor-BNI were obtained from National Institute of Drug Abuse (Baltimore, MD). U50,488H, naloxone, 3-methoxynaltrexone, and MK-801 were purchased from Sigma-Aldrich (St. Louis, MO). [D-Ala²,N-Me-Phe³,Gly-ol⁴]-enkephalin (DAMGO) and deltorphin II were purchased from Phoenix Pharmaceuticals (Belmont, CA). The EM-1 and EM-2 for i.t. injection were dissolved in 0.9% saline containing 10% hydroxypropyl-β-cyclodextrin, U50,488H, 3-methoxynaltrexone, NTI, nor-BNI, and MK-801 were dissolved in 0.9% saline. The DAMGO was dissolved in 0.9% saline containing 0.01% of Triton X-100 and deltorphin II was dissolved in 0.9% saline containing 1% dimethyl sulfosiloxane. The antisera against dynorphin A(1-17), β-endorphin, [Leu^5]-enkephalin, [Met^5]-enkephalin, and CCK-8s were produced by immunization of male New Zealand White rabbits according to the method described in previous publications, and the potencies and cross-immunoreactivity of these antisera have been characterized (Tseng and Huang, 1992; Tseng and Collin, 1993; Wu et al., 2002a).

Statistical Analysis. The antinociceptive responses, %MPE, were presented as the mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett’s post-test, two-way ANOVA followed by Bonferroni 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 of EM-2-induced antianalgesia. The GraphPad Prism software was used to perform the statistics (version 3.0; GraphPad Software Inc., San Diego, CA).

Results

Tail-Flick Responses after Intrathecal Injection of 1.64 nmol of EM-1 or 1.75 nmol of EM-2. Groups of mice were injected i.t. with EM-1 (1.64 nmol), EM-2 (1.75 nmol), or...
vehicle, and the TF response were measured at different times after injection. EM-1 and EM-2 at such a dose produced a short and weak TF inhibition (23 and 15 %MPE, respectively) at 5 min and returned to vehicle control levels in 10 to 15 min for the rest 2 h of measurement times (Fig. 1).

**Effect of Intrathecal Pretreatment with 1.64 nmol of EM-1 or 1.75 nmol of EM-2 on the TF Inhibition Induced by Intrathecal-Administered Morphine (3.0 nmol).** Groups of mice were pretreated i.t. with 1.64 nmol of EM-1, 1.75 nmol of EM-2, or vehicle 45 min before i.t. injection of morphine (3.0 nmol), and the TF responses were measured at various times after injection. The morphine-induced TF inhibition was attenuated by i.t. pretreatment with EM-2, but not EM-1 or vehicle (Fig. 2).

**Effects of Different Times of Pretreatment with EM-2 Given Intrathecal on the TF Inhibition Induced by Intrathecal-Administered Morphine.** Groups of mice were pretreated i.t. with EM-2 (1.75 nmol) at various times before i.t. injection of morphine (3.0 nmol), and the TF inhibition was measured 15 min after the injection. Other groups of mice pretreated i.t. with vehicle served as controls. The i.t. administration of morphine (3.0 nmol) produced 77 to 90 %MPE of the maximum TF inhibition in mice pretreated i.t. for different times with vehicle. Intrathecal pretreatment with EM-2 time dependently attenuated the TF inhibition induced by i.t. morphine; the attenuation of the morphine-induced TF inhibition developed slowly, reached the lowest level (18.6 ± 5.0 %MPE) at 45 min and returned to control level at 90 or 120 min (Fig. 3A). Pretreatment time of 45 min for EM-2 was then used for the following experiments.

**Effects of Intrathecal Pretreatment with Different Doses of EM-2 on the TF Inhibition Induced by Intrathecal-Administered Morphine.** Groups of mice were pretreated i.t. with different doses of EM-2 (0.02–17.5 nmol) 45 min before i.t. injection with morphine (3.0 nmol), and the TF response was measured 15 min after injection. EM-2 at 0.05 to 1.75 nmol dose dependently attenuated the TF inhibition induced by i.t. morphine (Fig. 3B). The ED$_{50}$ for EM-2 to produce antianalgesia was estimated to be 0.06 nmol (95% confidence interval, 0.02–0.15 nmol). Pretreatment dose of EM-2 at 1.75 nmol was found to produce a maximum inhibition and was therefore used for the following experiments.

**Effect of Intrathecal Pretreatment with Naloxone, 3-Methoxynaltrexone, NTI, nor-BNI, and MK-801 on the EM-2-Produced Antianalgesia against Morphine-Induced TF Inhibition.** The experiments were designed to determine whether the antianalgesic effect of EM-2 against i.t. morphine-induced TF inhibition is mediated by the stimulation of a subtype of $\mu$-opioid receptor and to determine whether NMDA receptors, $\delta$- and $\kappa$-opioid receptors are involved in EM-2-induced antianalgesia. Intrathecal pretreatment with a nonselective $\mu$-opioid receptor antagonist naloxone (5.5–55 pmol) given 10 min before EM-2 administration blocked the antianalgesia produced by i.t. EM-2 pretreatment and dose dependently restored the sensitivity to morphine (3 nmol) for producing TF inhibition (Fig. 4). Similarly, i.t. pretreatment with a selective $\mu$-opioid receptor antagonist 3-methoxynaltrexone (6.4 pmol) given 25 min before EM-2 administration significantly blocked the antianalgesic effect of EM-2 and enhanced the morphine-induced (3.0 nmol) TF inhibition (87 %MPE). On the other hand, i.t. pretreatment with the selective $\delta$-opioid receptor antagonist NTI (11.1 nmol), $\kappa$-opioid antagonist nor-BNI (6.6 nmol), or NMDA receptor antagonist MK-801 (10 nmol) did not affect the attenuation of the morphine-induced TF inhibition produced by EM-2 compared with vehicle-pretreated groups (Fig. 5).

**Effects of Intrathecal Pretreatment with Antisera against Dyn, $\beta$-Endorphin, [Met]-Enkephalin, [Leu]-Enkephalin, or CCK-8s on the Attenuation of Morphine-Induced TF Inhibition Produced by EM-2 Pretreatment.** Antiserum against Dyn and other neuropeptides were used to determine whether Dyn or other neuropeptides is involved in mediating the EM-2 produced anti-analgesia against i.t.-morphine-induced antinociception. Intrathecal pretreatment with different doses (50–300 $\mu$g) of antiserum...
against Dyn dose dependently restored the sensitivity to morphine and enhanced the morphine-induced TF inhibition in mice pretreated i.t. with EM-2 (Fig. 6; Table 1). However, i.t. pretreatment with antiserum (200 μg each) against β-endorphin, [Met]-enkephalin, [Leu]-enkephalin, or CCK-8s did not alter the morphine-induced TF inhibition in mice pretreated with EM-2. Intrathecal pretreatment with NRS also showed no effect on EM-2-induced antianalgesia against morphine-induced TF inhibition (Table 1).

Effects of Intrathecal Pretreatment with EM-2 on the TF Inhibition Induced by μ-, δ-, and κ-Opioid Agonists. Groups of mice were pretreated i.t. with EM-2 (1.75 nmol) or vehicle 45 min before i.t. injection of morphine (3.0 nmol) alone as negative control. The TF inhibition (%MPE) was measured 15 min after morphine injection. Each group represents the mean and the vertical bar represents the S.E.M. with 8 to 11 mice in each group. The one-way ANOVA followed by Dunnett’s post-test was used to test the difference between groups. For groups of mice pretreated with different doses of EM-2 versus vehicle pretreated mice, F = 12.70, *p < 0.001.
EM-2 at Subantinociceptive Doses Produces Antianalgesia against Morphine-Induced Antinociception.

We have previously demonstrated that EM-2 at high doses (35 nmol) given i.t. produces antinociception, which is mediated by the stimulation of µ-opioid receptors (Ohsawa et al., 2001). This is evidenced by the findings that the TF inhibition induced by EM-2 given i.t. is blocked by the pretreatment with selective µ-opioid receptor antagonist β-funaltrexamine, or δ-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH₂ (Goldberg et al., 1998; Ohsawa et al., 2001). We demonstrated in the present studies that EM-2 at small doses (0.05–1.75 nmol), which given i.t. alone did not produce any appreciable antinociception, but attenuated the antinociception induced by i.t.-administered morphine. This antinociception of EM-2 against morphine-induced antinociception developed slowly, reached a maximal peak at 45 min, and returned to control level 90 to 120 min after EM-2 pretreatment.

The Antinociception Produced by EM-2 Pretreatment Is Mediated by the Stimulation of a Subtype of µ-Opioid Receptor. Both EM-1 and EM-2 are highly selective ligands for µ-receptors with high affinity. However, we found in the present study that only pretreatment with EM-2, but not EM-1 at the comparable pharmacological potency dose, produced antinociception against morphine-induced antinociception. The finding indicates that the EM-2-induced antinociception is mediated by the stimulation of a subtype of µ-opioid receptor, which is only sensitive to the EM-2, but not EM-1. This view is further supported by the finding that pretreatment with 3-methoxynaltrexone blocked the antinociceptive effect of EM-2 but not EM-1. 3-Methoxynaltrexone is a selective µ-opioid receptor antagonist, which blocks selectively the antinociception induced by EM-2, but not EM-1 or morphine (Sakurada et al., 2000). The presence of different subtypes of µ-opioid receptors for EM-1 and EM-2 to perform their pharmacological actions can also be obtained from our previous studies of antinociceptive effects induced by high doses of EM-1 and EM-2. There is an asymmetric cross-tolerance between EM-1 and EM-2 for producing antinociception. Mice or rats made acutely tolerant to EM-1 are not

**Discussion**

**EM-2 at Subantinociceptive Doses Produces Antinociception against Morphine-Induced Antinociception.**
cross-tolerant to EM-2, whereas mice or rats made tolerant to EM-2 are partially cross-tolerant to EM-1 (Wu et al., 2001; Hung et al., 2002). Intrathecal pretreatment with antisense oligodeoxynucleotides against exon-1 or -4 of μ-opioid receptor clone is about equally effective in attenuating the antinociception induced by EM-1 and EM-2. On the other hand, pretreatment with antisense oligodeoxynucleotides against exon-8 of μ-opioid receptor clone attenuates the antinociception induced by EM-1, but not EM-2 (Wu et al., 2002b). Thus, distinct subtypes of μ-opioid receptors, which reflect different splice variants of exons of MOR-1, are involved in pharmacological responses induced by EM-1 and EM-2.

The μ-opioid receptors have been pharmacologically further classified into μ1 and μ2-opioid receptors by Pasternak and colleagues (Pick et al., 1991; Pasternak, 1993). The proposed subtype of μ-opioid receptors for EM-2 to produce antianalgesia is speculated to belong to pharmacologically identified μ1- rather than μ2-opioid receptors. This view is supported by the findings obtained from the antinociceptive study of EM-1 and EM-2 in mice. Pretreatment of mice with μ1-opioid receptor antagonist naloxonazine is more effective in blocking the antinociception induced by i.t.-administered EM-2 than EM-1, indicating that the antinociceptive effect induced by EM-2 is mediated by the stimulation of μ1- rather than μ2-opioid receptors. Interestingly, pretreatment with antiserum against Dyn or κ-opioid antagonist nor-BNI blocks the antinociception induced by i.t.-administered EM-2, but not EM-1. Thus, EM-2 at high doses stimulates μ1-opioid receptors to induce the release of Dyn acting on κ-opioid receptors for the production of antinociception (Ohsawa et al., 2001; Sakurada et al., 2001).

δ- or κ-opioid Receptors or NMDA Receptors Are Not Involved in EM-2-Produced Antianalgesia. Pretreatment with δ-opioid receptor antagonist NTI or κ-opioid receptor antagonist nor-BNI did not affect morphine-induced antinociception in EM-2-pretreated mice, indicating that δ- and κ-opioid receptors may not be involved in EM-2-induced antianalgesia. Spinal administration of dynorphin produces a long-lasting allodynia, which cannot be blocked by pretreatment with nonselective opioid antagonist naltrexone but blocked by pretreatment with NMDA receptor antagonist MK-801 (Vanderah et al., 1996; Laughlin et al., 1997). However, Tang et al. (2000) demonstrate that dynorphin A elicits an increase in intracellular calcium level through a nonopioid and non-NMDA mechanism, which subsequently modulate NMDA receptor activity. Our results showed that neither nor-BNI nor MK-801 blocked EM-2-induced antianalgesia against morphine-induced antinociception, indicating that Dyn released after EM-2 administration may act via nonopioid and non-NMDA receptor systems to produce its antianalgesic effect.

The Antinociception Induced by EM-2 Is Mediated by the Release of Dyn, but Not β-Endorphin, [Met]-Enkephalin, [Leu]-Enkephalin, or Cholecystokinin. We found in the present study that i.t. pretreatment with anti-serum against Dyn, which neutralizes Dyn action, blocked the EM-2-produced anti-analgesia and reestablished the antinociception induced by i.t.-administered morphine. The finding indicates that EM-2 may release Dyn to produce the antianalgesic effect. We have recently found that EM-2 perforated i.t. for 3 min at (5–50 nmol) dose dependently increases the release of immunoreactive Dyn in the spinal perfusates measured with ELISA in urethane-anesthetized rats. The increase of the release of Dyn induced by EM-2 is also blocked by naltrexone (Leitermann et al., 2003). Thus, our studies with antiserum against Dyn clearly indicate that Dyn may be involved in EM-2-induced antianalgesia against morphine-induced antinociception.

Previously, Holmes and Fujimoto (1993) found that i.t. injection with a small dose of naltrexone (5 pg) or β-funaltrexamine (0.25 ng) enhances the i.t.-administered morphine-induced antinociception. Also, i.t. pretreatment with anti-serum against Dyn, which neutralizes Dyn action, also enhances morphine-induced antinociception. The findings indicate that there is a tonic release of a μ-opioid ligand, which induces the release of Dyn to antagonize i.t. morphine-induced antinociception. Dyn function as an antianalgesic modulator to oppose opioid-induced antinociception seems to be specific to Dyn. Other dynorphins such as dynorphin A(1-8), dynorphin A(2-17), dynorphin B, and α- and β-neoendorphin lack such antianalgesic activity (Rady et al., 1991). We speculate that this endogenous μ-opioid ligand to induce Dyn release for producing antianalgesia is EM-2, which is densely located in the dorsal horn of the spinal cord.

We found in the present study that pretreatment with antiserum against β-endorphin, [Met]-enkephalin or [Leu]-enkephalin did not affect the EM-2-induced antianalgesia against morphine-induced antinociception. The results indicate that β-endorphin, [Met]-enkephalin and [Leu]-enkephalin may not be involved in EM-2-induced antianalgesia.

Cholecystokinin has been shown to be a pronociceptive and antiopioid ligand, which attenuates the antinociceptive properties of μ-opioid receptor agonists (Millan, 2002). Blockade of the action of CCK, especial CCKB receptor, potentiatates morphine-induced antinociception (Singh et al., 1996; McNally, 1999). However, our result clearly demonstrated that CCK was not involved in the EM-2-induced antianalgesia, which was evidenced by our finding that the pretreatment of antiserum against CCK could not restore morphine-induced antinociception.

The EM-2 Pretreatment Nonselectively Attenuates the Antinociception Induced by Opioid μ, δ, or κ-Agonists. The question remains whether the EM-2-induced antianalgesia is selective against antinociception induced by μ-opioid agonists or can be generalized to δ- and κ-opioid agonists. We found that pretreatment with EM-2 attenuated the TF inhibition induced by δ-opioid receptor agonist deltorphin II and κ-opioid receptor agonist U50,488H, indicating that the EM-2-induced antianalgesia can also be generalized to the antinociception induced by δ- and κ-opioid receptor agonists. Because Dyn has been proposed to be an endogenous ligand for κ-opioid receptors, the blockade of κ-opioid receptor by nor-BNI should have blocked the EM-2-induced antianalgesia. Because we found that the blockade of the κ-opioid receptors by nor-BNI pretreatment did not affect the EM-2-induced antianalgesia (Fig. 5), the Dyn released by EM-2 does not act on κ-opioid for producing antianalgesia. This view is consistent with the study by Wang et al. (2001) of dynorphin-mediated chronic neuropathic pain. They reported that pronociceptive action of dynorphins for the maintenance of neuropathic pain is mediated by the activation of nonopioid receptors.

It is concluded that i.t. pretreatment with low dose of EM-2 stimulates a subtype of μ-opioid receptor to elicit the release
of Dyn. The release of Dyn by EM-2 pretreatment subsequently elicits an antianalgesic effect against μ-, δ-, or κ-opioid agonist-induced antinociception.

References


