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Differential antinociceptive effects induced by intrathecally administered endomorphin-1 and endomorphin-2 in the mouse

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Abstract

Two highly selective μ-opioid receptor agonists, endomorphin-1 and endomorphin-2, have been identified and postulated to be endogenous ligands for μ-opioid receptors. Intrathecal (i.t.) administration of endomorphin-1 and endomorphin-2 at doses from 0.039 to 5 nmol dose-dependently produced antinociception with the paw-withdrawal test. The paw-withdrawal inhibition rapidly reached its peak at 1 min, rapidly declined and returned to the pre-injection levels in 20 min. The inhibition of the paw-withdrawal responses to endomorphin-1 and endomorphin-2 at a dose of 5 nmol observed at 1 and 5 min after injection was blocked by pretreatment with a non-selective opioid receptor antagonist naloxone (1 mg/kg, s.c.). The antinociceptive effect of endomorphin-2 was more sensitive to the μ_1-opioid receptor antagonist, naloxonazine than that of endomorphin-1. The endomorphin-2-induced paw-withdrawal inhibition at both 1 and 5 min after injection was blocked by pretreatment with κ-opioid receptor antagonist nor-binaltorphimine (10 mg/kg, s.c.) or the δ_2-opioid receptor antagonist naltriben (0.6 mg/kg, s.c.) but not the δ_1-opioid receptor antagonist 7-benzylidine naltrexone (BNTX) (0.6 mg/kg s.c.). In contrast, the paw-withdrawal inhibition induced by endomorphin-1 observed at both 1 and 5 min after injection was not blocked by naloxonazine (35 mg/kg, s.c.), nor-binaltorphimine (10 mg/kg, s.c.), naltriben (0.6 mg/kg, s.c.) or BNTX (0.6 mg/kg s.c.). The endomorphin-2-induced paw-withdrawal inhibition was blocked by the pretreatment with an antiserum against dynorphin A-(1-17) or [Met^5]enkephalin, but not by antiserum against dynorphin B-(1-13). Pretreatment with these antisera did not affect the endomorphin-1-induced paw-withdrawal inhibition. Our results indicate that endomorphin-2 given i.t. produces its antinociceptive effects via the stimulation of μ_1-opioid receptors (naloxonazine-sensitive site) in the spinal cord. The antinociception induced by endomophin-2 contains additional components, which are mediated by the release of dynorphin A-(1-17) and [Met^5]enkephalin which subsequently act on κ-opioid receptors and δ_2-opioid receptors to produce antinociception.

Keywords: Endomorphin; Antinociceptive effect; μ-Opioid receptor agonist; (Mouse)

1. Introduction

Endomorphin-1 and endomorphin-2 are two recently discovered endogenous peptides which selectively activate μ-opioid receptors (Zadina et al., 1997; Goldberg et al., 1998; Gong et al., 1998; Hosohata et al., 1998). Immunoreactivities to these peptides are localized in many areas of the central nervous system involved in pain processing, including the dorsal horn of the spinal cord, trigeminal nucleus, and the periaqueductal gray (Martin-Schild et al., 1997, 1998, 1999; Pierce et al., 1998). In the spinal cord, endomorphin-like immunoreactivity is found

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in the superficial laminae of the dorsal horn, where endomorphin-2 is co-localized with substance P and calcitonin gene-related peptide. Moreover, dorsal rhizotomy or capsaicin treatment abolishes endomorphin-2-immumoreactivity in the dorsal horn (Martin-Schild et al., 1998; Pierce et al., 1998). Thus, it is likely that endomorphins are present in terminals of primary sensory afferents (Martin-Schild et al., 1997, 1998, 1999; Pierce et al., 1998). Some differences have been noted for the distribution of endomorphin-1 and endomorphin-2 in the brain and spinal cord. Endomorphin-1 is seen more than endomorphin-2 in the brain, whereas endomorphin-2 dominates in the spinal cord (Martin-Schild et al., 1999). Both of these peptides display high affinity and selectivity for the μ-opioid receptor in vivo and in vitro. Neither endomorphin had appreciable affinity for δ- and κ-opioid receptors (Zadina et al., 1997). Distinct pharmacological properties of endomorphins have been reported in both electrophysiological (Chapman et al., 1997) and behavioral experiments (Zadina et al., 1997; Stone et al., 1997; Goldberg et al., 1998; Tseng et al., 2000). The antinoception induced by endomorphin-1 and endomorphin-2 given intrathecally (i.t.) or intracerebroventricularly (i.c.v.) is selectively blocked by pretreatment with the non-selective opioid receptor antagonist, naloxone or the μ-opioid receptor antagonist, β-funaltrexamine (Stone et al., 1997; Zadina et al., 1997; Goldberg et al., 1998), indicating that they are mediated by the stimulation of μ-opioid receptors. Furthermore, pretreatment with the μ_1-opioid receptor antagonist, naloxonazine, attenuates the antinociception induced by i.t. administered endomorphin-2 but not by endomorphin-1, suggesting that endomorphin-2-induced antinociception may be mediated by the stimulation of μ_1-opioid receptors (Sakurada et al., 1999, 2000a).

Although antinociceptive effects induced by both endomorphin-1 and endomorphin-2 are mediated by the stimulation of μ-opioid receptors, some differential antinociceptive effects induced by endomorphin-1 and endomorphin-2 have been noted. The antinociception induced by endomorphin-2, but not by endomorphin-1, is blocked by pretreatment with antiserum against dynorphin A-(1-17) and by the κ-opioid receptor antagonist nor-binaltorphimine, indicating that endomomorphin-2 appears to stimulate a different subtype of μ-opioid receptor, which subsequently induces the release of dynorphins that act on κ-opioid receptors to produce antinociception (Tseng et al., 2000). Intrathecal pretreatment with antiserum against dynorphin A-(1-17) or [Met^5]enkephalin blocks the antinociception induced by supraspinally administered endomorphin-2 but not endomorphin-1, indicating that endomorphin-2 but not endomophin-1 given supraspinally releases both dynorphins and [Met^5]enkephalin from the spinal cord to produce antinociception (Ohsawa et al., 2000). The present study was conducted to determine whether there are any differential actions of endomorphin-1 and endomorphin-2 administered on production of antinociception in the mouse. We found that antinociceptive effects induced by both endomorphin-1 and endomorphin-2 given i.t. are initially mediated by the stimulation of μ-opioid receptors, the endomorphin-2-induced antinociception contains an additional component, that is mediated by the release of dynorphin A-(1-17) and [Met^5]enkephalin acting on κ- and δ-opioid receptors, respectively.

2. Methods

2.1. Animal

Adult male ddY mice weighing 22–25 g were housed in a light- and temperature-controlled room (light on 0900–2100 h; 24 °C) and had free access to food and water. The experiments were performed with the approval of the Committee of Animal Experiments at Tohoku Pharmaceutical University.

2.2. Drugs

Endomorphin-1 and endomorphin-2 were synthesized in our laboratory. Endomorphins were prepared by the Fmoc strategy using a Shimadzu PSSM-8 peptide synthesizer (Kyoto, Japan) in our laboratory. After cleavage the protected amino acid residues with TFA/ethandithiol/thioanisole (90:5:5 v/v), the crude peptides were purifed with a Gilson HPLC systems (Gilson, France) using the following conditions; a reverse-phase column was Cosmosil C_{18} (Nacalai Tesque, Osaka, Japan), size 20×250 mm; the mobile phase A was 0.1% TFA/H_2O and the mobile phase B was 0.1% TFA/70% acetonitrile; the gradient program of the solvent was from B 0% to B 60% for 45 min; the flow rate was 10 ml/min; the peptide was detected with the UV monitor at 210 and 270 nm. Finally, purified endomorphins were characterized by amino acid analysis (Hitachi L-8500, Ibaraki, Japan) and electrospray mass spectrometry (ThermQuest Finnigan TSQ-700, San Jose, USA). The purity of the peptides was more than 99%. Naloxone, nor-binaltorphimine (Portoghese et al., 1988; Horan et al., 1992), naltriben (Sofuoglu et al., 1991) and 7-benzylidine naltrexone hydrochloride (BNTX) were purchased from Research Biochemical International (Natick, MA, USA), and [D-Ala^2, MePhe^4, Gly(ol)^5]enkephalin (DAMGO) was from Sigma (St. Louis, MO, USA). Endomorphins and DAMGO were dissolved in sterile artificial cerebrospinal fluid (CSF) containing 7.4 g NaCl, 0.19 g KCl, 0.19 g $MgCl_2$, 0.14 g $CaCl_2$/1000 ml. Naloxonazine (35 mg/kg, s.c.) was dissolved in saline and injected s.c. in a volume of 0.1 ml/10 g body weight 24 h prior to testing. Under these conditions, naloxonazine (35 mg/kg, s.c.) antagonizes μ_1-mediated antinociception. Naloxone was administered 10 min before administration of endomorphins and 5 min before DAMGO. Naltriben or BNTX was administered 25 min before administration of

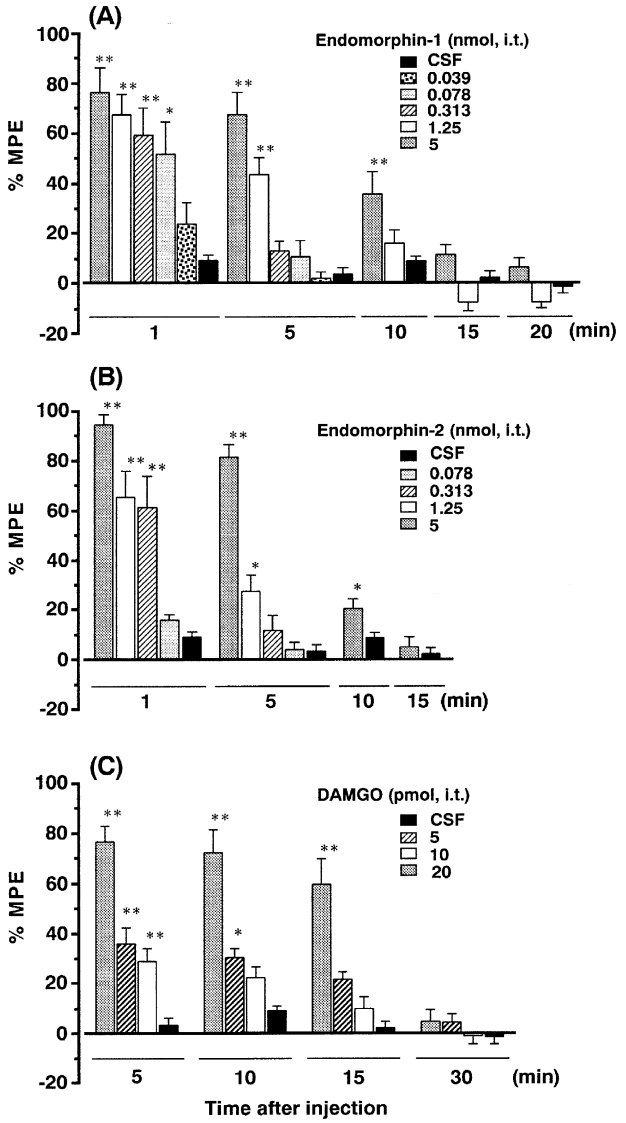

Fig. 1. Time course of i.t. administered endomorphin-1 (A), endomorphin-2 (B) and DAMGO (C) in the mouse paw withdrawal test. Antinociception was expressed as percent of maximum possible effect (% MPE) = 100 × (post-drug responsive latency − pre-drug responsive latency)/(10.0 − pre-drug responsive latency). Each column represents the mean ± S.E.M. for 10 mice. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the respective value in the CSF-control group.

endomorphins and 20 min before DAMGO. The antisera against dynorphin A-(1-17) and dynorphin B-(1-13) were obtained from Cosmobio Chem (Tokyo, Japan). The antisera against [Met^5]enkephalin and [Leu^5]enkephalin were obtained from Chemicon International (Temecula, CA, USA).

2.3. Procedure for intrathecal injection

Various doses of endomorphin-1 and endomorphin-2 were injected according to the procedure of Hylden and Wilcox (1980). The drugs were delivered slowly in an injection volume of 2 μl/mouse using a 29-gauge needle connected to a Hamilton microsyringe inserted directly between L5 and L6.

2.4. Assessment of nociceptive threshold

The antinociceptive activity of opioid peptides against the response to a thermal stimulus was assessed by the mouse paw withdrawal test. Antinociceptive thresholds were determined by an automated tail-flick unit (BM kiki, Tokyo). Mice were adapted to the testing environment for at least 1 h before any stimulation. Each animal was restrained with a soft cloth to reduce visual stimuli and the radiant heat source was positioned under the glass floor directly beneath the hind paw. The heat stimulus intensity was determined by the reaction time of the removal of the

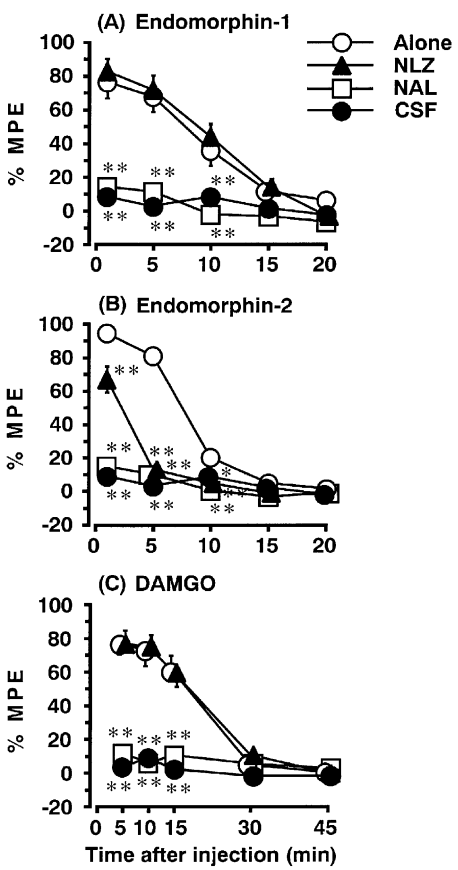

Fig. 2. Effects of naloxone (NAL) and naloxonazine (NLZ) on i.t. endomorphin-1, endomorphin-2 and DAMGO-induced antinociception in the paw withdrawal test. Naloxone was administered 10 min before i.t. administration of endomorphin-1 (5 nmol), endomorphin-2 (5 nmol) and 5 min before i.t. administration of DAMGO (20 pmol). Naloxonazine (35 mg/kg, s.c.) was administered 24 h before i.t. administration of opioids. Each data point represents the mean ± S.E.M. for 10 mice. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with each agonist alone.

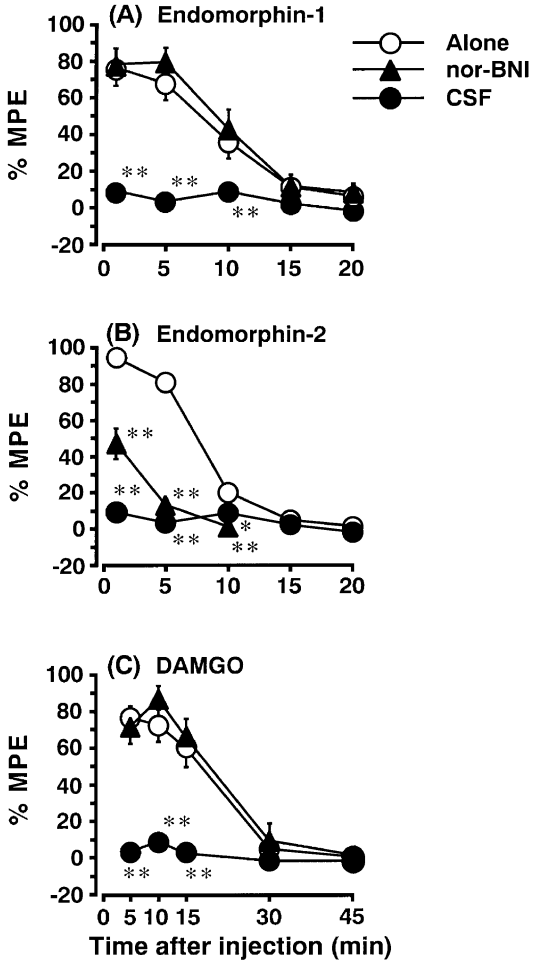

Fig. 3. Effects of nor-binaltorphimine (nor-BNI) on i.t. endomorphin-1, endomorphin-2 and DAMGO-induced antinociception in the paw withdrawal test. Nor-BNI (10 mg/kg) was administered s.c. 24 h before i.t. administration of endomorphin-1 (5 nmol), endomorphin-2 (5 nmol) and DAMGO (20 pmol). Each data point represents the mean ± S.E.M. for 10 mice. $^{**}P < 0.01$ compared with each agonist alone (open column).

paw from a source of noxious radiant heat. The intensity of the light beam was adjusted so that baseline reaction time was 2–4 s. The light beam was focused on the same plantar spot of the hind paw in all animals. To prevent tissue damage, trials were terminated automatically if the mouse did not lift the paw within 10 s. The mean threshold of the control response before the injection was determined by a total of two consecutive measurements each separated by 10 min. No animal was used more than once. To prevent experimenter bias, observers were uninformed of the dose of the endomorphins and DAMGO being injected, and were uninformed of whether each opioid receptor antagonist was given as pretreatment when modification of each agonist-induced antinociception was investigated. After determination of pre-drug values, animals were injected. Antinociceptive activity for each animal was calculated with the following equation and represented as percent of maximum possible effect (%MPE) = (P2 − P1/10 − P1) × 100, where P1 and P2 are pre-drug and post-drug responsive time (in seconds), respectively.

2.5. Data analysis and statistics

Statistical significance of the data was estimated with a mixed two-factor analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A level of probability of 0.05 or less was accepted as significant. The ED_{50} values and their 95% confidence limits (95% CL) for the antinociceptive effect of endomorphins and DAMGO were computed according to the method of Litchfield and Wilcoxon (1949) with Programs 11 and 47 of the Pharmacological calculations system of Tallarida and Murray (1987).

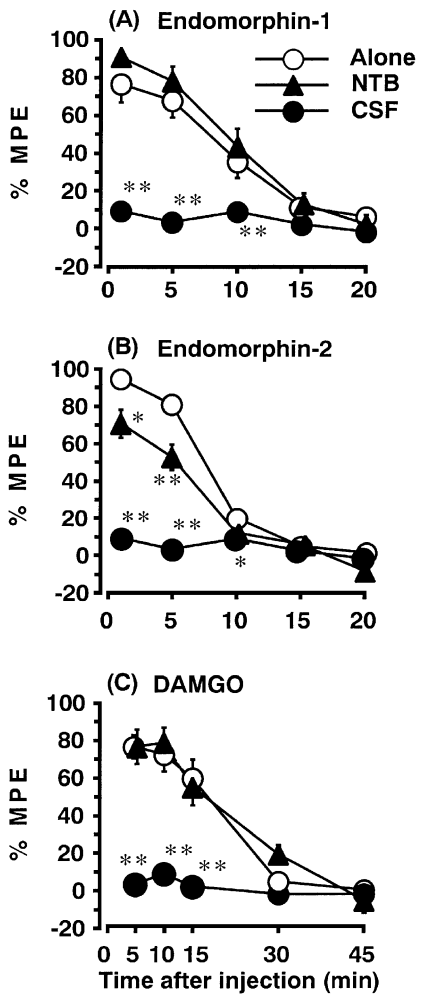

Fig. 4. Effects of naltriben (NTB) on i.t. endomorphin-1, endomorphin-2 and DAMGO-induced antinociception in the paw withdrawal test. Naltriben (0.6 mg/kg) was administered s.c. 25 min before i.t. administration of endomorphin-1 (5 nmol) and endomorphin-2 (5 nmol) and 20 min before i.t. administration of DAMGO (20 pmol). Each data point represents the mean ± S.E.M. for 10 mice. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with each agonist alone.

3. Results

3.1. Time course of paw-withdrawal responses to i.t.-administered endomorphin-1 and endomorphin-2

Groups of mice were injected i.t. with CSF and different doses of endomorphin-1 and endomorphin-2, and the paw-withdrawal responses were measured 1, 5, 10, 15 and 20 min after injection. The i.t.-injection of endomorphin-1 and endomorphin-2 at doses of 0.039–5.0 nmol caused a dose-dependent increase in intensity and duration of the inhibition of the paw-withdrawal response. The inhibition reached its peak rapidly 1 min after injection, rapidly declined, and returned to the preinjection levels in 20 min (Fig. 1). Endomorphin-1 and -2 at 1 min post-injection produced dose-dependent antinociception with ED_{50} values 0.25 (0.05–1.20) and 0.34 (0.10–1.57) nmol, respectively. The ED_{50} values for endomorphin-1 and -2 for paw-withdrawal inhibition observed at 5 min after i.t. injection were 1.95 (0.54–7.07) and 1.90 (0.68–5.32) nmol, respectively. The ED_{50} value for DAMGO given i.t. was 14.0 (8.52–22.99) pmol observed at the 5–10 min after injection (Fig. 1).

3.2. Effect of s.c. pretreatment with naloxone, naloxonazine, nor-binaltorphimine, naltriben or BNTX on inhibition of the paw-withdrawal responses induced by i.t. administered endomorphin-1 and endomorphin-2

The effects of pretreatment with naloxone, nor-binaltorphimine or naltriben given s.c. on the paw-withdrawal inhibition induced by endomorphin-1 or endomorphin-2 given i.t. at 1, 5, 10 and 15 min after injection were determined. A dose of 5 nmol of endomorphin-1 and endomorphin-2 was chosen for the studies. Intrathecal injection of a dose 5 nmol of endomorphin-1 and endomorphin-2 at 1 or 5 min after injection produced 76.3 ± 9.9% and 94.1 ± 3.9% or 67.4 ± 8.9% and 76.3 ± 7.7% MPE, respectively, of paw-withdrawal inhibition. The inhibition of the paw-withdrawal response induced by endomorphin-1 and endomorphin-2 are markedly blocked by the pretreatment with naloxone (1 mg/kg, s.c.) (Fig. 2). The same pretreatment also completely blocked the paw-withdrawal inhibition induced by DAMGO (20 pmol, i.t.) (Fig. 2). Endomorphin-1 and DAMGO-induced antinociception were insensitive to naloxonazine (35 mg/kg, s.c.), whereas endomorphin-2 responses at 1 min were significantly antagonized and at 5 min was completely blocked by a dose (35 mg/kg, s.c.) of the μ_1-opioid receptor antagonist. The inhibition of the paw-withdrawal response induced by

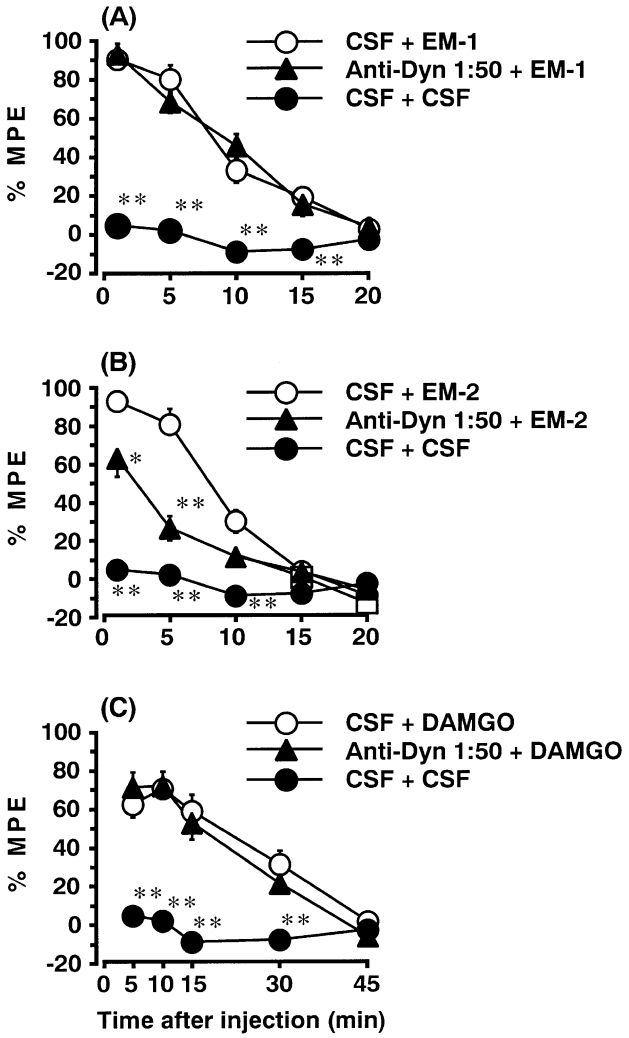

Fig. 5. Effects of antiserum against dynorphin A(1-17) on i.t. endomorphin-1, endomorphin-2 and DAMGO-induced antinociception in the paw withdrawal test. Dynorphin A(1-17) (Anti-Dyn) antiserum was administered i.t. 19 min before i.t. administration endomorphin-1 (EM-1, 5 nmol) and endomorphin-2 (EM-2, 5 nmol) and i.t. 10 min before i.t. administration of DAMGO (20 pmol). First and second administrations were done in 4 and 2 μl volume, respectively. Antinociceptive effect induced by each peptide was measured after second administration. Each data point represents the mean ± S.E.M. for 10 mice. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with each agonist alone.

Table 1
Effect of BNTX on endomorphin-1, endomorphin-2 and DAMGO-induced antinociception in the paw-withdrawal test

	1 min	5 min	10 min
Endomorphin-1	76.28 ± 9.93	67.44 ± 8.93	
+ BNTX	88.76 ± 5.51	68.19 ± 7.68	
Endomorphin-2	94.08 ± 3.88	80.81 ± 5.30	
+ BNTX	92.03 ± 7.41	64.36 ± 10.13	
DAMGO		76.47 ± 6.34	72.19 ± 9.01
+ BNTX		60.73 ± 7.47	61.42 ± 8.78

BNTX (0.6 mg/kg) was administered s.c. 25 min before i.t. administration of endomorphin-1 (5 nmol) and endomorphin-2 (5 nmol), and 20 min before i.t. administration of DAMGO (20 pmol). Each value represents the mean ± S.E.M. for 10 mice.

endomorphin-2 (5 nmol, i.t.) at 1 and 5 min after i.t. injection was significantly blocked by pretreatment with nor-binaltorphimine (10 mg/kg, s.c.) and by naltriben (0.6 mg/kg, s.c.) (Figs. 3 and 4). By contrast, the paw-withdrawal inhibition induced by endomorphin-1 (5 nmol, i.t.) and DAMGO (20 pmol, i.t.) was not blocked by nor-binaltorphimine (10 mg/kg, s.c.) or naltriben (0.6 mg/kg, s.c.) (Figs. 3 and 4). s.c. Pretreatment with BNTX was ineffective against the paw-withdrawal inhibition induced by endomorphins (5 nmol, i.t.) or DAMGO (20 pmol, i.t.) (Table 1).

3.3. Effect of i.t. pretreatment with antisera to dynorphin A-(1-17), dynorphin B-(1-13), [Met^5]enkephalin or [Leu^5]enkephalin on inhibition of the paw-withdrawal response induced by i.t.-administered endomorphin-1 and endomorphin-2

I.t. pretreatment with antiserum against dynorphin A-(1-17) attenuated the paw-withdrawal inhibition induced by endomorphin-2-induced antinociception in a dose-dependent manner (Fig. 5). However, the paw-withdrawal inhibition induced by endomorphin-2 was not affected by antisera against dynorphin B-(1-13) or [Leu^5]enkephalin (Table 2). Moreover, i.t. pretreatment with antiserum against [Met^5]enkephalin partially but significantly attenuated the antinociception induced by endomorphin-2, but not endomorphin-1 (Fig. 6). The same pretreatment with these antisera did not affect the paw-withdrawal inhibition induced by endomorphin-1 or DAMGO (Fig. 6).

Table 2
Effect of pretreatment with dynorphin B-(1-13) and Leu-enkephalin antisera on endomorphin-1, endomorphin-2 and DAMGO-induced antinociception in the paw-withdrawal test

	1 min	5 min	10 min
Endomorphin-1			
+ CSF	90.30 ± 5.36	80.09 ± 7.10	
+ Dynorphin B-(1-13) antiserum	88.92 ± 8.95	67.31 ± 14.15	
+ Leu-enkephalin antiserum	90.14 ± 6.47	78.05 ± 7.78	
Endomorphin-2			
+ CSF	92.58 ± 4.51	80.64 ± 8.59	
+ Dynorphin B-(1-13) antiserum	87.27 ± 8.33	76.59 ± 10.63	
+ Leu-enkephalin antiserum	91.14 ± 6.11	79.47 ± 8.67	
DAMGO			
+ CSF		62.56 ± 6.90	70.29 ± 5.33
+ Dynorphin B-(1-13) antiserum		73.61 ± 7.78	74.45 ± 7.63
+ Leu-enkephalin antiserum		68.47 ± 9.94	64.23 ± 9.12

The antisera against dynorphin B-(1-13) and Leu-enkephalin was preadministered i.t. 19 min prior to i.t. administration of endomorphin-1 (5 nmol), endomorphin-2 (5 nmol) and DAMGO (20 pmol). First and second administrations were done in 4 and 2 μl volume, separately. Antinociceptive effect induced by each peptide was measured after second administration. Each value represents the mean ± S.E.M. for 10 mice.

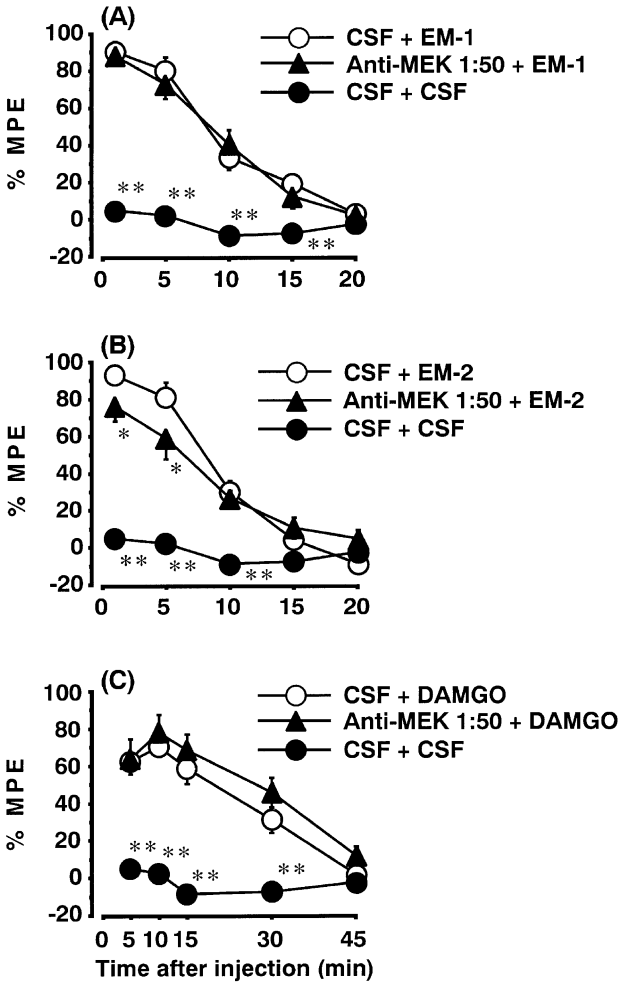

Fig. 6. Effects of antiserum against Met-enkephalin on i.t. endomorphin-1, endomorphin-2 and DAMGO-induced antinociception in the paw withdrawal test. Met-enkephalin antiserum (Anti-MEK) was administered i.t. 19 min before i.t. administration endomorphin-1 (EM-1, 5 nmol) and endomorphin-2 (EM-2, 5 nmol) and i.t. 10 min before administration of DAMGO (20 pmol). First and second administrations were done in 4 and 2 μl volume, respectively. Antinociceptive effect induced by each peptide was measured after second administration. Each data point represents the mean ± S.E.M. for 10 mice. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with each agonist alone.

4. Discussion

Endomorphin-1 and endomorphin-2 given i.t. are about equipotent in inhibiting the tail pressure (Sakurada et al., 1999) and the paw withdrawal responses. The peak effects appeared rapidly 1 min after i.t. administration, declined rapidly, and returned to the control level in 15 min. The rapid onset of antinociception after i.t. administration of endomorphins are in agreement with those of Stone et al.

who reported a rapid onset and short duration of the hot-water tail-flick inhibition induced by 10 nmol of endomorphin-1 and endomorphin-2 (Stone et al., 1997).

We found in the present study that inhibition of the paw-withdrawal response induced by endomorphin-1 or endomorphin-2 observed at 1 and 5 min after i.t. injection is blocked by pretreatment with naloxone, indicating that the antinociception induced by both endomorphin-1 and endomorphin-2 given spinally is mediated by the stimulation of opioid receptors. The finding is consistent with the previous studies showing that endomorphin-1 and endomorphin-2 compete for μ-opioid receptors but have no appreciable affinity for either δ- or κ-opioid receptors in vitro and are found to produce potent and prolonged antinociceptive activity that is reversible by naloxone and the selective μ-opioid receptor antagonist β-funaltrexamine (Takemori et al., 1981; Zadina et al., 1997; Stone et al., 1997; Goldberg et al., 1998). Naloxonazine selectively antagonizes μ_1-antinociception (Ling et al., 1986; Heyman et al., 1988; Paul et al., 1989; Pick et al., 1991). Recent behavioral pharmacological studies suggest that the presence of μ_1-opioid receptors sensitive to naloxonazine in spinal sites as assayed with the formalin test, hot-plate, tail-pressure and tail-flick tests (Sato et al., 1999; Sakurada et al., 1999, 2000a, 2000b). Autoradiographic studies show that μ_1- and μ_2-opioid receptor subtypes are localized in spinal and supraspinal structures involved in the modulation of nociception (Moskowitz and Goodman, 1985). A reasonable dose of naloxonazine to selectively block μ_1-opioid receptors in mice is 35 mg/kg, s.c. (Ling et al., 1986). The antinociceptive activity of endomorphin-2 at 1 min after i.t. injection was significantly antagonized and at 5 min was completely blocked by pretreatment with this dose of the antagonist. This antagonistic response is similar to that by pretreatment with nor-binaltorphimine or with antiserum against dynorphin A-(1-17).

The paw-withdrawal inhibition induced by endomorphin-2 observed at 1 or 5 min, after injection was blocked by pretreatment with the κ-opioid receptor antagonist nor-binaltorphimine (10 mg/kg, s.c.) and the δ_2-opioid receptor antagonist naltriben (0.6 mg/kg, s.c.) but not δ_1-opioid receptor antagonist BNTX (0.6 mg/kg s.c.). By contrast, the antinociception induced by endomorphin-1 at 1 or 5 min or DAMGO at 10 min after injection was not blocked by pretreatment with nor-binaltorphimine, naltriben or BNTX. These findings are different from previously reported data antinociception by supraspinally administered endomorphin-2 was not attenuated by i.c.v. co-administration of δ_1-opioid receptor and δ_2-opioid receptor antagonists (Tseng et al., 2000). Our results indicate that the antinociception induced by intrathecal administered endomorphin-2, but not endomorphin-1 or DAMGO, involves κ- and δ_2-opioid receptors for the production of antinociception.

Endomorphin-2 has a very low affinity for δ- and κ-opioid receptors in the opioid receptor binding assays (Zadina et al., 1997). It is not likely that the endomorphin-2-induced antinociception is mediated by direct stimulation of δ- and κ-opioid receptors. It is more likely that the effects of endomorphin-2 are mediated by the release of [Met^5]-enkephalin and dynorphins, the endogenous ligands for the δ- and κ-opioid receptors. We found that i.t. pretreatment with antiserum against dynorphin A-(1-17) or [Met^5]enkephalin significantly attenuated the antinociception induced by i.t. administered endomorphin-2, whereas i.c.v. pretreatment with antiserum against [Met^5]-enkephalin was ineffective on antinociception of i.c.v. endomorphin-2 (Tseng et al., 2000). However, i.t. pretreatment with antiserum against dynorphin B-(1-13) did not block endomorphin-2-induced antinociception, indicating that the effect is selective to the release of dynorphin A-(1-17). Thus antinociception induced by spinally administered endomorphin-2 also is mediated in part by the release of dynorphin A-(1-17) and [Met^5]enkephalin acting on κ-opioid and δ-opioid receptors in the spinal cord. Our findings are similar to the previous reports that antinociception induced by supraspinally administered endomorphin-2 was attenuated by i.c.v. pretreatment with nor-binaltorphimine (Tseng et al., 2000) and by i.t. pretreatment with nor-binaltorphimine and δ-opioid receptor antagonists (Ohsawa et al., 2000). We have also shown that i.t. administered endomorphin-2, but not endomorphin-1 or DAMGO, is sensitive to antagonism by naloxonazine (Sakurada et al., 1999, 2000a) and 3-methoxynaltrexone (Sakurada et al., 2000a). Taken together, the previous and present results show that different μ-opioid receptor-selective agonists can interact differently with the μ-opioid receptor and produce different downstream effects. The mechanisms of these differential effects are unclear at this point. Modulation of enkephalin release by morphine and other opioids has been shown to occur in other tissues, such as the ileum (Xu et al., 1989) and may be dependent on variations in receptor-G-protein-effector coupling (Wang and Gintzler, 1997). A single gene encoding the mouse μ-opioid receptor (MOR-1) has been cloned (Reisine and Bell, 1993; Uhl et al., 1994) and seven splice variants have been identified (Bare et al., 1994; Zimprich et al., 1995; Ohsawa et al., 2000; Pan et al., 2000) with different immunohistochemical distributions (Pan et al., 1999; Abbadie et al., 2000). Different physical states of the receptor, such as dimerization (Jordan and Devi., 1999) also have been demonstrated. These or other, as yet undiscovered subtypes, splice variants or physical states of the receptor may contribute to the differences observed in the present and previous studies. Regardless, the present results support the idea that different μ-opioid receptor-selective agonists can induce antinociception by different mechanisms.

In conclusion, endomorphin-1 and endomorphin-2 given spinally dose-dependently produce their antinociception via simulation of μ-opioid receptors. However, the antinociception by endomorphin-2 contains additional components that are naloxonazine sensitive and are mediated

by the release of dynorphin A-(1-17) and [Met^5]enkephalin acting on κ- and δ-opioid receptors.

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