Differential Mechanisms Mediating Descending Pain Controls for Antinociception Induced by Supraspinally Administered Endomorphin-1 and Endomorphin-2 in the Mouse

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ABSTRACT

We have previously demonstrated that both endomorphin-1 and endomorphin-2 produce their antinociception by the stimulation of \(\mu\)-opioid receptors. However, the antinociception induced by endomorphin-2 contains an additional component, which is mediated by the release of dynorphin A (1-17) acting on \(\delta\)-opioid receptors. These studies were done to determine whether the antinociception induced by endomorphin-1 and endomorphin-2 given supraspinally was mediated by the activation of different descending pain control pathways in the mouse. Specific receptor antagonists or antisera against endogenous opioid peptides were injected intrathecally to block the receptors or bind the released endogenous opioid peptides, and endomorphin-1 or endomorphin-2 was then administered i.c.v. to activate the descending pain control systems to produce antinociception. The tail-flick response was used as antinociceptive test. The blockade of the \(\alpha_2\)-adrenoceptors and 5-hydroxytryptamine receptors in the spinal cord by i.t. injection of yohimbine and methysergide, respectively, inhibited the antinociception induced by i.c.v.-administered endomorphin-1 and endomorphin-2. However, the antinociception induced by endomorphin-2 was inhibited by i.t. pretreatment with \(\delta\)-opioid receptor antagonist naltriben or \(\kappa\)-opioid receptor antagonist nor-binaltorphimine, but not by the \(\mu\)-opioid receptor antagonist \(\alpha\)-Phe-Cys-Tyr-\(\delta\)-Try-Orn-Thr-Pen-Thr-NH\(_2\) or the \(\delta\)-opioid receptor antagonist 7-benzylidene naltrexamine. Intrathecal pretreatment with antiserum against Met-encephalin attenuated the antinociception induced by i.c.v.-administered endomorphin-2, but not endomorphin-1. Furthermore, i.t. pretreatment with antiserum against dynorphin A (1-17) also inhibited the antinociception induced by i.c.v.-administered endomorphin-2, but not endomorphin-1. Intrathecal pretreatment with antiserum against Leu-enkephalin or \(\beta\)-endorphin did not inhibit i.c.v.-administered endomorphin-1 or endomorphin-2-induced antinociception. The results indicate that, like other \(\mu\)-opioid receptor agonists, morphine, and [\(\alpha\)-Ala\(_2\),N-Me-Phe\(_4\), Gly\(_2\)-ol]-enkephalin, endomorphin-1 and endomorphin-2 given i.c.v. produce antinociception by activating spinopelvic noradrenergic and serotonergic pathways for producing antinociception. However, the antinociception induced by endomorphin-2 given i.c.v. also contains other components, which are mediated by the release of Met-encephalin and dynorphin A (1-17) acting on opioid \(\delta\) and \(\kappa\) receptors, respectively, in the spinal cord.

Recently, two new peptides, endomorphin-1 and endomorphin-2, have been isolated from mammalian brain. These peptides activate \(\mu\)-opioid receptors with high affinity and selectivity, raising the possibility that they are two endogenous \(\mu\)-opioid receptor ligands (Zadina et al., 1997). Neither compound has appreciable affinities for \(\delta\) and \(\kappa\)-opioid receptors. Endomorphins are found in the regions of the brain and spinal cord that are also rich in \(\mu\)-opioid receptors (Martin-Schild et al., 1997, 1998, 1999; Zadina et al., 1997; Pierce et al., 1998; Schreff et al., 1998). Intrathecal (i.t.) or i.c.v. injection of endomorphins produces potent analgesia, which is inhibited by the pretreatment with \(\mu\)-opioid receptor antagonists, naloxone, and \(\beta\)-fenaltrexamine (\(\beta\)-FNA) (Stone et al., 1997; Tseng et al., 2000). Endomorphin-1 and endomorphin-2 given i.c.v. produce no or little antinociception in \(\mu\)-opioid receptor knockout mice and in \(\mu\)-opioid receptor-deficient CXBK mice (Tseng et al., 1998; Mizoguchi et al., 1999). In [\({\text{35S}}\)]guanosine-5'-O-(3-thio)triphosphate binding assays, both endomorphin-1 and endomorphin-2 compete with \(\mu\)- and \(\delta\)-opioid receptor sites potently (Goldberg et al., 1998). Neither compound has appreciable affinities for \(\delta\) and \(\kappa\)-opioid receptors. Endomorphins are found in the regions of the brain and spinal cord that are also rich in \(\mu\)-opioid receptors (Martin-Schild et al., 1997, 1998, 1999; Zadina et al., 1997; Pierce et al., 1998; Schreff et al., 1998). Intrathecal (i.t.) or i.c.v. injection of endomorphins produces potent analgesia, which is inhibited by the pretreatment with \(\mu\)-opioid receptor antagonists, naloxone, and \(\beta\)-fenaltrexamine (\(\beta\)-FNA) (Stone et al., 1997; Tseng et al., 2000). Endomorphin-1 and endomorphin-2 given i.c.v. produce no or little antinociception in \(\mu\)-opioid receptor knockout mice and in \(\mu\)-opioid receptor-deficient CXBK mice (Tseng et al., 1998; Mizoguchi et al., 1999). In [\({\text{35S}}\)]guanosine-5'-O-(3-thio)triphosphate binding assays, both endomorphin-1 and endomorphin-2 compete with \(\mu\)- and \(\delta\)-opioid receptor sites potently (Goldberg et al., 1998).
riphosphatase (GTPγS)-binding assay, neither endomorphin-1 nor endomorphin-2 produces any activation of G-protein in the spinal cord (Narita et al., 1998) and in the pons/medulla (Mizoguchi et al., 1999) membrane obtained from the μ-opioid receptor knockout mice. These findings indicate that μ-opioid receptors play an essential role in mediating endomorphin-induced antinociception and G-protein activation.

We have previously demonstrated that both endomorphin-1 and endomorphin-2 given supraspinally produce their antinociception by the stimulation of μ-opioid receptors. However, the antinociception induced by endomorphin-2 given supraspinally contains an additional component, which is mediated by the release of dynorphin A (1-17) acting on κ-opioid receptors (Tseng et al., 2000). This is supported by the finding that antinociception induced by i.c.v.-administered endomorphin-1 or endomorphin-2 is inhibited by i.c.v. pretreatment with μ-opioid receptor antagonist β-FNA. However, the antinociception induced by endomorphin-2, but not endomorphin-1 is inhibited by the pretreatment with κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI) and antiserum against dynorphin A (1-17) (Tseng et al., 2000). This finding seems to suggest that the antinociception induced by endomorphin-1 and endomorphin-2 may be mediated by the activation of different descending pain control pathways. These studies were then performed to analyze the descending pain control pathways activated by endomorphin-1 and endomorphin-2. Specific receptor antagonists or antisera against endogenous opioid peptides were injected i.t. to inhibit the receptors or bind the released endogenous opioid peptides, and endomorphin-1 and endomorphin-2 were then administered i.c.v. to activate the descending pain control systems to produce antinociception.

### Materials and Methods

**Animals.** Male ICR mice weighing 25 to 30 g (Charles River Breeding Laboratories, Wilmington, MA) were used for the studies. 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. Animals were used only once in all experiments.

**Drugs and Antisera.** Endomorphin-1 (Tyr-Pro-Arg-Phe-NH₂), endomorphin-2 (Tyr-Pro-Phe-Phe-NH₂) (Zadina et al., 1997), naltrexone (NTB) (Portoghese et al., 1992), b-2-hydroxytryptamine (BNTX) (Portoghese, 1991), and nor-BNI were synthesized in H. Nagase’s laboratory (Basic Research Laboratories, Kamakura, Japan). The other drugs used were d-Phe-Cys-Tyr-d-Tyr-Orn-Thr-Phe-Thr-NH₂ (CTOP) (Peninsula Laboratory International, Belmont, CA), yohimbine (Sigma Chemical Co., St. Louis, MO), and methysergide (Research Biochemicals International, Natick, MA). The antisera against dynorphin A (1-17), Met-enkephalin, Leu-enkephalin, and β-endorphin were produced by immunization of male New Zealand White rabbits according to the method described previously and the potencies and the cross-immunoreactivities of these antisera have been characterized (Tseng and Collins, 1993; Tseng et al., 2000).

**Assessment of Antinociceptive Response.** Antinociceptive response was determined with the tail-flick test (D’Amour and Smith, 1941). For the measurement of the latency of the tail-flick response, mice were gently held with one hand with the tail positioned in the apparatus (model TF6; EM DIE Instrument Co., Maidens, VA) for radiant heat stimulation. The tail-flick response was elicited by applying radiant heat to the dorsal surface of the tail. The intensity of the heat stimulus in the tail-flick test was adjusted so that the animal flicked its tail within 3 to 5 s. The latency of the tail-flick response was measured before (T₀) and at various times after (Tᵢ) i.c.v. injections of endomorphins. The inhibition of the tail-flick response to endomorphins was expressed as a percentage of the maximum possible effect (%MPE), which was calculated as \[ \frac{(T₀ - Tᵢ)(T₀ - T₂)}{100} \]

### Results

**Effect of i.t. Pretreatment with Yohimbine or Methysergide on Inhibition of Tail-Flick Response Induced by I.c.v.-Administered Endomorphin-1 or Endomorphin-2.** Groups of mice were injected i.t. with various doses of yohimbine, methysergide, or saline 10 min before i.c.v. injection of various doses of endomorphin-1, endomorphin-2, or saline and the tail-flick responses were measured every 5 min after i.c.v. injection for 20 min. Intracerebroventricular injection of endomorphin-1 at a dose of 16.4 nmol or endomorphin-2 at a dose of 35 nmol increased the inhibition of the tail-flick response in mice injected with saline. The inhibition reached its peak 5 min after injection, rapidly declined, and returned to the preinjection level 20 min after injection. The duration of the tail-flick inhibition induced by endomorphin-1 appeared to be longer than that of endomorphin-2 (Figs. 1 and 2).

Intrathecal pretreatment with yohimbine at doses from 0.026 to 2.6 nmol dose dependently attenuated the inhibition of tail-flick responses induced by i.c.v.-administered endomorphin-1 (16.4 nmol) or endomorphin-2 (35 nmol) (Fig. 1). Intrathecal pretreatment with methysergide at doses from 0.021 to 2.1 nmol also dose dependently attenuated the inhibition of the tail-flick responses induced by i.c.v.-adminis-
tered endomorphin-1 (16.4 nmol) or endomorphin-2 (35 nmol) (Fig. 2).

Effects of i.t. Pretreatment with CTOP, nor-BNI, BNTX, or NTB on Inhibition of Tail-Flick Response Induced by i.e.v. Administration of Endomorphin-1 or Endomorphin-2. Intrathecal pretreatment with nor-BNI at doses from 0.66 to 6.6 nmol for 24 h dose dependently attenuated the inhibition of the tail-flick responses induced by i.e.v.-administered endomorphin-2 (35 nmol) (Fig. 4). However, i.t. pretreatment with nor-BNI at 6.6 nmol did not affect the inhibition of the tail-flick response induced by endomorphin-1 (16.4 nmol) (Fig. 3). Intrathecal pretreatment with NTB at doses from 1.9 to 18.8 nmol dose dependently attenuated the tail-flick inhibition induced by endomorphin-2 (35 nmol i.c.v.) (Fig. 4). However, i.t. pretreatment with NTB (18.8 nmol) did not affect the inhibition of the tail-flick response induced by endomorphin-1 (16.4 nmol) (Fig. 3). Intrathecal pretreatment with BNTX (2.0 nmol) or CTOP (47 pmol) did not antagonize the inhibition of the tail-flick response induced by endomorphin-1 or endomorphin-2 (35 nmol) (Figs. 3 and 4).

Effects of i.t. Pretreatment with Antisera to Dynorphin A (1-17), Met-Enkephalin, Leu-Enkephalin, or β-Endorphin on Inhibition of Tail-Flick Response Induced by Endomorphin-1 or Endomorphin-2. Dynorphin A (1-17) and Met-enkephalin have been proposed to be the endogenous opioid ligand for κ- and δ-opioid receptors, respectively. The finding that antinociception induced by endomorphin-2 was inhibited by the δ-opioid receptor antag-
narcotic NTB and the κ-opioid receptor antagonist nor-BNI suggests that endomorphin-2 may release dynorphins and Met-enkephalin, which subsequently act on κ- and δ-opioid receptor, respectively, to produce antinociception. The effects of i.t. pretreatment with an antiserum against dynorphin A (1-17), Met-enkephalin, or other endogenous opioid peptides on the tail-flick inhibition induced by endomorphin-1 and endomorphin-2 were studied. Intrathecal pretreatment with an antiserum against dynorphin A (1-17) at doses from 10 to 100 μg for 1 h dose dependently attenuated the tail-flick inhibition induced by endomorphin-2 (35 nmol) (Fig. 6). However, i.t. pretreatment with an antiserum against dynorphin A (1-17) 100 μg, which significantly attenuated the tail-flick inhibition induced by endomorphin-2, did not affect the tail-flick inhibition induced by endomorphin-1 (16.4 nmol) (Fig. 5). Moreover, i.t. pretreatment with an antiserum against Met-enkephalin at doses from 10 to 100 μg for 1 h dose dependently attenuated the tail-flick inhibition induced by endomorphin-2 (35 nmol) (Fig. 6). The same treatment did not affect the tail-flick inhibition induced by endomorphin-1 (16.4 nmol) (Fig. 5). The tail-flick inhibition induced by endomorphin-1 (16.4 nmol) or endomorphin-2 (35 nmol) was not affected by i.t. pretreatment with an antiserum against Leu-enkephalin or β-endorphin (Figs. 5 and 6).

Discussion

The activation of spinopetal descending pain control systems by opioid receptor agonists plays a major role in opioid-induced antinociception. Multiple descending pain control pathways are involved in antinociception induced by the stimulation of various opioid agonists given supraspinally (Tseng, 1995; Narita and Tseng, 1998). The antinociception induced by μ-opioid receptor agonists such as morphine and [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO) given supraspinally is mediated by the release of noradrenaline and 5-HT, which act on α₂-adrenoceptors and 5-HT receptors, respectively, in the spinal cord (Tseng and Tang, 1990; Tseng and Collins, 1991b), whereas the antinociception induced by κ-opioid receptor agonists such as U50,488H and bremazocine given supraspinally is mediated by the release of dynorphin A (1-17) acting on κ-opioid receptors (Tseng and Collins, 1993). The antinociception induced by β-endorphin given supraspinally is mediated by the release of Met-enkephalin acting on δ₂-opioid receptors (Tseng, 1995; Narita and Tseng, 1998).

We have previously demonstrated that the inhibition of the tail-flick response induced by either endomorphin-1 or endomorphin-2 given i.c.v. is inhibited by i.c.v pretreatment with
the selective μ-opioid receptor antagonist β-FNA, but not by the δ-opioid receptor antagonist NTB or the δ-opioid receptor antagonist NTB, indicating that the antinociception induced by endomorphin-1 and endomorphin-2 is mediated selectively by the stimulation of μ-opioid receptors (Tseng et al., 2000). Thus, endomorphin-1 and endomorphin-2 given supraspinally will be expected to use the same descending pain control pathways as that of other μ-opioid agonists such as morphine and DAMGO for producing antinociception. Indeed, we found that the inhibition of α2-adrenoceptors and 5-HT receptors by i.t. treatment with yohimbine and methysergide, respectively, effectively inhibited the antinociception induced by i.c.v.-administered endomorphin-1 and endomorphin-2. Our results indicate that, like morphine and DAMGO, endomorphin-1 and endomorphin-2 activate the spinopetal noradrenergic and serotonergic systems and release of noradrenaline and 5-HT acting on α2-adrenoceptors and 5-HT-receptors, respectively, in the spinal cord for producing antinociception.

In addition to the monoaminergic descending pain control systems, which are activated by endomorphin-1 and endomorphin-2, two additional opioidergic descending pathways were found to be involved in antinociception induced by i.c.v.-administered endomorphin-2, but not by endomorphin-1. We found that i.t. pretreatment with the δ-opioid receptor antagonist NTB or the κ-opioid receptor antagonist nor-BNI attenuated the antinociception produced by i.c.v.-administered endomorphin-2. The effect appears to be due to the specific inhibition of δ-opioid and κ-opioid receptors because i.t. pretreatment with the μ-opioid receptor antagonist CTOP or the δ-opioid receptor antagonist BNTX did not inhibit antinociception induced by endomorphin-2. Because the opioid δ-opioid and κ-opioid receptors are the receptors for endogenous ligands Met-enkephalin and dynorphins, respectively, it is then expected that the effects be mediated by the release of Met-enkephalin and dynorphin A (1-17). We found that i.t. pretreatment with an antiserum against Met-enkephalin or dynorphin A (1-17) significantly attenuated the antinociception induced by endomorphin-2. However, i.t. pretreatment with antiserum against β-endorphin or Leu-enkephalin did not affect the antinociception induced by i.c.v.-administered endomorphin-2. Thus, antinociception induced by supraspinally administered endomorphin-2 also is mediated in part by the releases of Met-enkephalin and dynorphin A (1-17) acting on opioid δ- and κ-receptors in the spinal cord.

The mechanism underlying the activation of descending dynorphinergic systems is not clear. We have recently proposed that endomorphin-1 produces antinociception by stimulating one subtype of μ-opioid receptors, like morphine or DAMGO, whereas endomorphin-2 has an additional component that initially stimulates a different subtype of μ-opioid receptors, and subsequently induces the release of dynorphins acting on κ-opioid receptors for producing antinociception (Tseng et al., 2000). Activation of κ-opioid receptors by the i.c.v. injection of κ-opioid receptor agonists U50,488H or bremazocine has been reported to release the dynorphin A, which subsequently acts on κ-receptors in the spinal cord for producing antinociception (Tseng and Collins, 1993). It seems likely that the activation of κ-opioid receptors followed by the release of dynorphin A induced by endomorphin-2 at the supraspinal site activates the descending dynorphinergic systems. Alternatively, endomorphin-2 may activate undescended pain control pathway, which induces the release of dynorphin A (1-17) and stimulation of κ-opioid receptors in the spinal cord for producing antinociception. Although, the detailed relationships between the activation of supraspinal κ-opioid receptors and release of dynorphin A in the spinal cord are not clear, these results strongly support the hypothesis that antinociception produced by i.c.v.-administered endomorphin-2 may involve the release of dynorphin A (1-17) either in the supraspinal or spinal sites.

We found for the first time that endomorphin-2 given supraspinally released Met-enkephalin from the spinal cord. This Met-enkephalin-releasing effect of endomorphin-2 is thought to be initially mediated by the stimulation of μ-opioid receptors. This view is supported by our previous findings that endomorphin-2 does not produce any antinociception in μ-opioid receptor knockout mice (Mizoguchi et al., 1999) and the blockade of supraspinal μ-opioid receptors by i.c.v. pretreatment with selective μ-opioid receptor antagonist β-FNA blocks completely i.c.v.-administered endomorphin-2-induced antinociception (Tseng et al., 2000). However, it is possible that a different subtype of μ-opioid receptors activated by endomorphin-2 is involved in the release of Met-enkephalin and activation of descending Met-enkephalinergic systems. Further studies are needed to clarify these possibilities.

Different subtypes of μ-opioid receptors have been discovered. There are six distinct μ-opioid receptors, which are generated from alternative splicing (Bare et al., 1994; Zimmersch et al., 1995; Pan et al., 1999). The anatomical distributions of these μ-opioid receptors are different. MOR-1 and MOR-1C are derived from the same gene, their markedly different immunohistochemical distributions implicated region-specific processing (Pan et al., 1999). Relative expression of MOR-1D and MOR-1E to MOR-1C varied from region to region (Pan et al., 1999). The differential effects of endomorphin-1 and endomorphin-2 in activating descending pain control pathways for antinociception may be mediated by the stimulation of a different subtype of μ-opioid receptors.

Several other opioid receptor agonists, such as etorphine, bremazocine, and β-endorphin given supraspinally also re-

Fig. 6. Effects of i.t. pretreatment with various doses of antiserum against dynorphin A (1-17) (10, 30, and 100 μg), Met-enkephalin (10, 30, and 100 μg), or 100 μg Leu-enkephalin or β-endorphin on inhibition of the tail-flick response induced by i.c.v.-administered endomorphin-2 (35 nmol). Antiserum against dynorphin-A (1-17), Met-enkephalin, Leu-enkephalin, or β-endorphin was injected 60 min before the injection of endomorphin-2. The tail-flick response was measured 5 min after the injection of endomorphin-2. Each column represents the mean with S.E. for 10 mice. *P < .05 compared with the normal rabbit serum (NRM)-injected control.
lease Met-enkephalin and activation of δ-opioid receptors in the spinal cord. The antinociception induced by these opioids has been postulated to be mediated entirely or at least in part by the stimulation of putative ε-opioid receptors (Tseng et al., 1985, 1986, 1997; Tseng, 1986, 1995; Tseng and Collins, 1991a,b, 1993; Suh et al., 1992; Tseng and Huang, 1992; Tseng and Wang, 1992; Xu et al., 1992). However, whether these opioids also stimulate the subtype of μ-opioid receptors stimulated by endomorphin-2 for the release of Met-enkephalin needs to be further evaluated.

In conclusion, antinociception induced by endomorphin-1 and endomorphin-2 is mediated by the release of noradrenaline and 5-HT acting on α2-adrenoceptors and 5-HT receptors, respectively, in the spinal cord. However, the antinociception induced by endomorphin-2 also contains additional components, which are mediated by the releases of Met-enkephalin and dynorphin A (1-17) acting on δ-opioid receptors, respectively, in the spinal cord.

References


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