FORUM MINIREVIEW

Recent Advances in the Search for the μ -Opioidergic System Differential Antinociceptive Effects Induced by Intrathecally- Administered Endomorphin-1 and Endomorphin-2 in Mice

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ABSTRACT—Two highly selective μ -opioid receptor (MOP-R) agonists, endomorphin-1 (EM-1) and endomorphin-2 (EM-2), have been identified and postulated to be endogenous ligands for MOP-R. Experiments were designed to determine the involvement of subtypes of MOP-R on the antinociceptive effects of EM-1 or EM-2 using the paw withdrawal test. The intrathecal (i.t.) injection of EM-1 and EM-2 produced dose-dependent antinociception in mice 1 min after the injection. Subcutaneous (s.c.) pretreatment with naloxonazine (NLZ), a selective MOP1-R antagonist, dose-dependently antagonized the antinociceptive effect of EMs. The antinociceptive effect of EM-2 was more sensitive to NLZ than that of EM-1. The selective heroin/morphine-6 β -glucuronide antagonist 3-methoxynaltrexone (3-MNT) blocked EM-2-induced antinociception, but not EM-1-induced antinociception. The dose-response curve of EM-2 was shifted threefold to the right by pretreatment with s.c. 3-MNT at a dosage of 0.25 mg/kg. EM-2-induced antinociception was attenuated by pretreatment with s.c. nor-binaltorphimine and naltrindole, whereas the effect of EM-1 was not affected. Moreover, the antinociceptive effect of EM-2 was attenuated by i.t. pretreatment with antisera against dynorphin A(1–17) or methionine-enkephalin. These results suggest that EM-2-induced antinociception may be mediated by the subtype of MOP-R, which is sensitive to NLZ and 3-MNT, and by subsequent release of dynorphin A(1–17) and methionine-enkephalin in the spinal cord.

Keywords: Endomorphin, μ -Opioid receptor, Naloxonazine, 3-Methoxynaltrexone, Dynorphin A(1–17)

Endomorphin-1 (EM-1: Tyr-Pro-Trp-Phe-NH₂) and endomorphin-2 (EM-2: Tyr-Pro-Phe-Phe-NH₂) are recently postulated mammalian endogenous peptides, which selectively activate μ -opioid receptor (MOP-R) (1). Both peptides have much the same pharmacological profiles, antinociception (against heat and mechanical stimuli and inflammatory and neuropathic pain), vasodepressor activity, decreased mean arterial pressure, inhibited gastrointestinal transit, and so on (1–7). Furthermore, immunoreactivity for EM-1 and EM-2 is widely and densely distributed throughout human and rat brain, whereas EM-2-immunoreactivity is present in the superficial laminae of the spinal cord dorsal horn where primary afferent nociceptors terminate. These findings indicate that EM-2 physiologically regulates nociceptive information in the spinal cord (8–10).

MOP-R subtypes

There is biochemical and pharmacological evidence supporting the existence of MOP-R subtypes (11–13) which are localized in spinal and supraspinal structures involved in the modulation of nociception (14). At least two MOP-R subtypes have been proposed: MOP1-R and MOP2-R (15). β -Funaltrexamine irreversibly antagonizes the antinociception which is mediated by both MOP1- and MOP2-Rs in supraspinal and spinal sites, whereas naloxonazine (NLZ) selectively antagonizes the MOP1-R-mediated antinociception (16). It has been suggested that these receptor subtypes play different physiological roles; MOP1-Rs mediate supraspinal antinociception, whereas MOP2-Rs mediate spinal antinociception.

Recent reports suggest that heroin and morphine-6 β -glucuronide in the family of MOs activate a novel third subtype of MOP-R, which distinguishes these ligands from morphine. 3-Methoxynaltrexone (3-MNT), which selectively displaces ³H-morphine-6 β -glucuronide binding, antago-

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Table 1. ID₅₀ values for s.c. naloxonazine on EM-1, EM-2 and DAMGO in the mouse paw-withdrawal test

	ID ₅₀ values (mg/kg, s.c.)
EM-1	48 (39.91 – 57.73)
EM-2	12 (9.65 – 14.93)
DAMGO	60 (51.58 – 69.79)

Naloxonazine were administered s.c. 24 h before i.t. administration of EM-1, EM-2 and DAMGO.

nizes the antinociceptive effects of heroin and morphine-6 β -glucuronide without interfering with MOP-R (morphine and DAMGO), δ -opioid receptor (DOP-R) or κ -opioid receptor (KOP-R) agonist-induced antinociception (17, 18).

Inhibition of EM-1- and EM-2-induced antinociception by NLZ and 3-MNT

The antinociceptive effect of EM-2 was completely blocked by pretreatment with NLZ at the dosage of 10.4 or 35 mg/kg (s.c.) that shows the selectivity to MOP1-R in the paw withdrawal test (19), suggesting that EM-2 may be a selective agonist for MOP1-R. Pretreatment with NLZ at the dosage of 35 mg/kg (s.c.) did not block the antinociception induced by EM-1, whereas higher doses of NLZ (52.5 or 65.6 mg/kg, s.c.) significantly attenuated EM-1-induced antinociception, indicating that at high dosage, NLZ may lose its selectivity for MOP1-Rs (Table 1) (19).

A recent study has suggested that morphine-6 β -glucuronide acts through a novel subtype of MOP-R that distinguishes this ligand from morphine (17, 18). Pretreatment with 0.25 mg/kg of 3-MNT, the selective antagonist for morphine-6 β -glucuronide, markedly attenuated the antinociception induced by i.t. injected EM-2, but not EM-1. The present results indicate that EM-2 activates MOP-R, which is selective for morphine-6 β -glucuronide, and is pharmacologically distinguishable from morphine, DAMGO and EM-1.

Effect of s.c. pretreatment with naloxone, nor-binaltorphimine, naltriben or BNTX on the antinociception induced by i.t. administered EM-1 and EM-2

The effects of pretreatment with naloxone, a non selective opioid receptor antagonist, nor-binaltorphimine, a selective KOP-R antagonist, naltriben, a selective DOP2-R antagonist, or 7-benzylidene naltrexamine (BNTX), a selective DOP1-R antagonist, given s.c. were determined on the antinociception induced by i.t.-administered EM-1 and EM-2. The antinociception induced by i.t. EM-1 and EM-2 are markedly blocked by the pretreatment with naloxone (1 mg/kg, s.c.). The antinociception induced by EM-2 given i.t. was also blocked by pretreatment with nor-binaltor-

phimine (10 mg/kg, s.c.) and naltriben (0.6 mg/kg, s.c.) (20). On the contrary, antinociception induced by EM-1 was not affected by nor-binaltorphimine or naltriben. Pretreatment with BNTX (0.6 mg/kg, s.c.) was ineffective against antinociception induced by both EM-1 and EM-2. These findings are not consistent with a previous report that antinociception induced by i.t. administered EM-2 was not attenuated by pretreatment with i.t. administration of DOP1-R and DOP2-R antagonists in the tail-flick test (21). The reason for this discrepancy is unclear, but may be related to the different nociceptive assay methods. Our results indicate that the antinociception induced by both EM-1 and EM-2 given spinally is mediated by the stimulation of MOP-Rs. Moreover, KOP-R and DOP2-R are also involved in the antinociception induced by EM-2, but not EM-1. EM-2 has postulated an endogenous MOP-R agonist, but not a DOP-R and KOP-R agonist, because EM-2 has a high affinity for only a MOP-R in the opioid receptors binding assay. These evidences suggest that EM-2 may indirectly stimulate the DOP-R and KOP-R, by the release of endogenous opioid peptide activating those receptors.

Effect of i.t. pretreatment with antisera to dynorphin A(1 – 17), dynorphin B(1 – 13), methionine-enkephalin or leucine-enkephalin on the antinociception induced by i.t.-administered EM-1 and EM-2

Pretreatment with antiserum against dynorphin A(1 – 17) significantly attenuated the antinociception induced by EM-2, but not by EM-1. Moreover, i.t. pretreatment with antiserum against methionine-enkephalin partially attenuated the antinociception induced by EM-2. These results indicate that the antinociception induced by spinally-administered EM-2 is mediated in part by the release of dynorphin A(1 – 17) and methionine-enkephalin acting on KOP-R and DOP-R in the spinal cord, respectively.

Previous reports suggest that EM-1 and EM-2 produce potent antinociception via stimulation of MOP-Rs in the spinal cord. In contrast with EM-1, EM-2 may stimulate additional antinociceptive mechanisms which produce the release of dynorphin A(1 – 17) and methionine-enkephalin in spinal cord by activation of NLZ and 3-MNT sensitive MOP-R.

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