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REVIEW ARTICLE

Endomorphin-1 and Endomorphin-2: Involvement of Endogenous μ-Opioid Receptor Ligands in Analgesia, Antinociceptive Tolerance, Antianalgesia, and Hyperalgesia

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Endomorphin-1 (EM-1) and endomorphin-2 (EM-2) are endogenous ligands for μ-opioid receptors. Both EM-1 and EM-2, given supraspinally or spinally, produce potent antinociception (analgesia) in mice and rats, measured by the thermal tail-flick response. The antinociception produced by either EM-1 or EM-2 is mediated by the stimulation of μ-opioid receptors, but not by δ- or κ-opioid receptors. EM-1 or EM-2 given supraspinally stimulates primarily μ-opioid receptors and subsequently releases spinipetal noradrenaline and serotonin, acting on α_2-adrenoceptors and serotonin receptors in the spinal cord for producing antinociception. However, the antinociception produced by EM-2, but not by EM-1, also contains an additional component, which is mediated by the release of dynorphin A_{1-17} and Met-enkephalin acting on κ-opioid receptors and δ_2-receptors, respectively, in the spinal cord for producing antinociception. Pretreatment with EM-1 or EM-2, given supraspinally or spinally, attenuates the antinociception (antinociceptive tolerance) produced by EM-1 or EM-2, respectively. Pretreatment with EM-2 attenuates the antinociception produced by EM-1; however, pretreatment with EM-1 does not attenuate the antinociception produced by EM-2 (asymmetric cross-tolerance). The antinociception produced by (−)-morphine given into the ventral periaqueductal gray is attenuated by pretreatment with a subanalgesic dose of EM-1 or EM-2 given into the ventral periaqueductal gray in rats (antianalgesia). The antianalgesia produced by EM-2, but not by EM-1, is mediated by the release of dynorphin A_{1-17}, which antagonizes the analgesic response to (−)-morphine. EM-2, but not EM-1, given into the centromedial amygdala decreases the tail-flick latencies (hyperalgesia) in rats. The hyperalgesia induced by EM-2 from centromedial amygdala is mediated by the release of dynorphin A_{1-17} acting on N-methyl-D-aspartate receptors. It is therefore proposed that there are two separate subtypes of μ-opioid receptors: μ and μ′. The μ-opioid receptors are stimulated by both EM-1 and EM-2, (−)-morphine, and [D-Ala^2,NMePhe^4,Gly^5-ol]enkephalin, and blocked by D-Pro^2-endomorphin-1. The μ′-opioid receptors are stimulated by EM-2 but not by EM-1, and blocked by D-Pro^2-endomorphin-2, naloxonazine, and 3-methoxynaltrexone. However, both subtypes of μ-opioid receptors are commonly blocked by β-funaltrexamine, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2, and (−)-naloxone.

1. Introduction

Since the initial demonstration of μ-opioid receptors more than 35 years ago, investigators have searched for their endogenous ligands. The search led to the discovery of enkephalins, endorphins, and dynorphins in the 1970s[1–5]; yet they have either low selectivity or low efficacy for the μ-opioid receptors.[6,7] Enkephalins are endogenous ligands for δ-opioid receptors, and dynorphin A_{1-17} is an endogenous ligand for κ-opioid receptors.[8,9] Although β-endorphin is an endogenous ligand for ε-opioid receptors,[10–12] it also binds equally well to μ- and δ-opioid receptors with high affinity.[13] Thus, many investigators believe that these peptides are not the endogenous ligands for μ-opioid receptors due to their selectivity profiles.

Later, two new peptides, endomorphin-1 (EM-1, Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (EM-2, Tyr-Pro-Phe-Phe-NH_2), have been isolated from mammalian brain and found to activate μ-opioid receptors with high affinity and selectivity, raising the possibility that they are two endogenous μ-opioid receptor ligands.[7] In opioid receptor binding assays, both EM-1 and EM-2 compete with μ-opioid receptor sites potently.[14] Neither compound has appreciable affinities

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for δ- and κ-opioid receptors. Endomorphins (EMs) were found in the brain and spinal cord regions, which are also rich in μ-opioid receptors.[7,15–19] Both EM-1 and EM-2 also induce μ-opioid receptor-mediated G protein activation by increasing the binding of [^{35}S] guanosine 5′-O-(3-thio)triphosphate, which is selectively blocked by the μ-opioid receptor antagonists β-funaltrexamine and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP), but not by δ-opioid receptor antagonist naltrindole or κ-opioid receptor antagonist norbinaltorphimine.[20,21] In addition, neither EM-1 nor EM-2 induces any G protein activation in the membrane preparation obtained from μ-opioid receptor clone (MOR-1) knockout mice.[22,23] The specific action of EM-1 and EM-2 in stimulating the μ-opioid receptor found *in vitro* is consistent with the *in vivo* antinociceptive studies in mice. Both EM-1 and EM-2 given intracerebroventricularly or intrathecally produce potent antinociception, which is blocked by pretreatment with CTOP or β-funaltrexamine.[14,24,25] EM-1 or EM-2 does not produce any antinociception in MOR-1 knockout mice or in μ-opioid receptor-deficient CXBK mice, indicating that μ-opioid receptors play an essential role in mediating EM-induced antinociception.[14,22,23]

Recent studies indicate that different subtypes of μ-opioid receptors are involved in the antinociception induced by EM-1 and EM-2. Similar to (−)-morphine or [D-Ala^2,NMePhe^4,Gly^5-ol] enkephalin (DAMGO), EM-1 stimulates one subtype of μ-opioid receptors, whereas EM-2 stimulates another subtype of μ-opioid receptors that are involved in the release of dynorphin A_{1-17} acting on κ-opioid receptors and Met-enkephalin acting on δ_2-opioid receptors for producing antinociception.[24,25] This view is supported by the findings that pretreatment with the μ_1-receptor antagonist naloxonazine or 3-methoxynaltrexone blocks the antinociception induced by EM-2 more effectively than that produced by EM-1.[26,27] Spinal pretreatment with antisense oligodeoxynucleotides against exon-1, -4, or -8 of MOR-1 to knockdown different isoforms of the μ-opioid receptor differentially attenuates the antinociception induced by EM-1 and EM-2.[28] These findings strongly indicate that different subtypes of μ-opioid receptors are involved in the pharmacological actions produced by EM-1 and EM-2. These two different subtypes of μ-opioid receptors are, therefore, tentatively designated as μ- and μ′-opioid receptors (Table 1). The present review depicts the differential neural mechanisms involved in the antinociception, acute antinociceptive tolerance, as well as antianalgesia and hyperalgesia produced by EM-1 and EM-2.

2. Antinociception (analgesia) produced by EM-1 and EM-2

2.1. Differential antinociception produced by EM-1 and EM-2 given intracerebroventricularly in mice

EM-1 at a dose of 3.3–16.4 nmol or EM-2 at a dose of 1.6–3.5 nmol, given intracerebroventricularly dose dependently, inhibits the tail-flick response in male CD-1 mice (antinociception). The antinociceptive effect induced by EM-1 or EM-2 reaches its peak 5 minutes after injection, declines rapidly, and returns to the pre-injection level 20 minutes after injection. Duration of the tail-flick inhibition induced by EM-1 appears to be longer than that induced by EM-2. In addition, the 50% effective dose of EM-2 for inhibiting the tail-flick response is about 3.3-fold higher than that of EM-1. The slope of the dose–response curve of EM-2 for inhibiting the tail-flick response is significantly steeper than that of EM-1. This difference in slope functions suggests that these two peptides may produce antinociception by different modes of action.[24]

Table 1 Pharmacology of the subtypes of μ-opioid receptor

Subtypes	Endogenous ligands	Agonists	Antagonists
μ	Endomorphin-1 Endomorphin-2	(−)-Morphine DAMGO	D-Pro^2-endomorphin-1 β-FNA (−)-Naloxone CTOP
μ′	Endomorphin-2		D-Pro^2-endomorphin-2 Naloxonazine 3-Methoxynaltrexone β-FNA (−)-Naloxone CTOP

β-FNA = β-funaltrexamine; CTOP = D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; DAMGO = [D-Ala^2,NMePhe^4,Gly^5-ol]encephalin; EM = endomorphin.

The original description of EMs reveals that both compounds have a profound μ selectivity.[7] Both EMs compete for μ-binding sites over 1000-fold more effectively than for either δ- or κ-opioid receptors.[7] Goldberg et al[14] also confirm that both EM-1 and EM-2 compete for both μ_1- and μ_2-opioid receptor sites potently, but have no appreciable affinity for either δ- or κ-opioid receptors. Inhibition of the tail-flick and hot-plate responses produced by either EM-1 or EM-2 (given supraspinally) is blocked completely by the selective μ-opioid receptor antagonist β-funaltrexamine, but not by the δ_1-opioid receptor antagonist 7-benzylidenenaltrexone or the δ_2-opioid receptor antagonist naltriben.[24] The findings are consistent with the view that these two EMs are selective ligands for μ-opioid receptors and that the antinociception induced by EM-1 and EM-2 is mediated by the selective stimulation of μ-opioid receptors, but not by that of δ_1- or δ_2-opioid receptors. However, the antinociception induced by EM-2, but not by EM-1, is also partially blocked by pretreatment with the κ-opioid receptor antagonist norbinaltorphimine, indicating that the antinociception induced by EM-2, but not by EM-1, is produced in part by κ-opioid receptor activation. Because EM-2 has a very low affinity for κ-opioid receptors in *in vitro* ligand-binding assays, it is unlikely that EM-2-induced antinociception is mediated by direct stimulation of κ-opioid receptors. It is most likely that EM-2 produces its antinociception by the release of dynorphin A_{1-17}, which subsequently acts on κ-opioid receptors. This is evidenced by the finding that pretreatment of mice with an antiserum against dynorphin A_{1-17}, which binds the released dynorphin A_{1-17}, attenuates the antinociception induced by EM-2. However, pretreatment with norbinaltorphimine or the antiserum against dynorphin A_{1-17} even at high doses blocks the antinociception induced by EM-2 only partially and not completely, suggesting that EM-2-induced antinociception is mediated, in part, by a κ-minergic mechanism.[24]

2.2. Differential mechanisms mediating descending pain controls for antinociception produced by supraspinally administered EM-1 and EM-2 in mice

Activation of spinipetal descending pain control systems by opioid receptor agonists plays a major role in the antinociceptive effects produced by stimulation of various opioid agonists given supraspinally. These antinociceptive effects involve multiple descending pain control pathways. The antinociception induced by μ-opioid receptor agonists such as (−)-morphine and DAMGO given supraspinally is mediated by the release of noradrenaline and serotonin (5-HT) acting on α_2-adrenoceptors and 5-HT receptors, respectively, in the spinal cord,[29,30] 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.[31] The antinociception induced by β-endorphin given supraspinally is mediated by the release of Met-enkephalin acting on δ_2-opioid receptors.[11,12]

Inasmuch as the antinociception induced by either EM-1 or EM-2 given supraspinally is mediated by the stimulation of μ-opioid receptors,[24] both EM-1 and EM-2 given supraspinally will also use the same descending pain control pathways as that of other

μ-opioid agonists, such as (−)-morphine and DAMGO, for producing antinociception. Indeed, inhibition of α_2-adrenoceptors and 5-HT receptors in the spinal cord by intrathecal treatment with yohimbine and methysergide, respectively, effectively inhibits the antinociception induced by supraspinally administered EM-1 and EM-2. Similar to (−)-morphine and DAMGO, EM-1 and EM-2 activate the spinipetal noradrenergic and serotonergic systems and the release of noradrenaline, 5-HT acting on α_2-adrenoceptors, and 5-HT receptor in the spinal cord for producing antinociception.[32]

Besides the monoaminergic descending pain control systems, which are activated by EM-1 and EM-2, two additional opioidergic descending pathways are also involved in antinociception induced by supraspinally administered EM-2, but not by EM-1. This is evidenced by the finding that spinal pretreatment with the δ_2-opioid receptor antagonist naltriben or the κ-opioid receptor antagonist norbinaltorphimine attenuates the antinociception produced by supraspinally administered EM-2. Because δ_2- and κ-opioid receptors are the receptors for endogenous ligands Met-enkephalin and dynorphins, respectively, it is expected that the effects are mediated by the release of Met-enkephalin and dynorphin A_{1-17}. Indeed, spinal pretreatment with an antiserum against Met-enkephalin or dynorphin A_{1-17} given intrathecally significantly attenuates the antinociception induced by EM-2. By contrast, spinal pretreatment with an antiserum against β-endorphin or Leu-enkephalin does not affect the antinociception induced by supraspinally administered EM-2. Thus, antinociception induced by supraspinally administered EM-2 contains additional components, which are mediated by the release of Met-enkephalin and dynorphin A_{1-17} acting on δ_2- and κ-opioid receptors, respectively, in the spinal cord.[32] Pharmacological findings of EM-2 on the release of Met-enkephalin for producing antinociception are in line with the biochemical finding that EM-2, but not EM-1 given intraventricularly, increases the release of immunoreactive Met-enkephalin in the spinal perfusates in male CD rats. The increased release of Met-enkephalin from the spinal cord induced by EM-2 is blocked by μ-opioid receptor antagonist CTOP.[33] Figure 1 illustrates the μ-opioid receptor-mediated spinipetal descending pain control systems activated by EM-1 and EM-2 for producing antinociception.

2.3. Differential antinociception induced by spinally administered EM-1 and EM-2 in mice

EM-1 or EM-2 at a dose of 0.04-5 nmol given into the intrathecal space of the spinal cord dose dependently produces antinociception (analgesia), measured with the thermal tail-flick or paw-withdrawal test in mice.[25,26] The antinociception reaches its peak 5 minutes after injection, rapidly declines, and returns to the preinjection level 20 minutes after injection. The duration of the antinociception induced by EM-1 and EM-2 given spinally is about the same, but EM-1 is about two-fold more potent than EM-2.[25] The antinociception induced by either EM-1 or EM-2 given spinally can completely be blocked by spinal pretreatment with the μ-opioid receptor antagonist CTOP or (−)-naloxone, indicating that the antinociception induced by EM-1 and EM-2 is also mediated by the stimulation of μ-opioid receptors in the spinal cord.[25,26,34] Both EM-1 and EM-2 do not activate G-proteins in the spinal cord of the μ-opioid receptor knockout mice.[20,22] However, the antinociception induced by spinally administered EM-2, but not by EM-1, contains additional components, which are mediated by the release of dynorphin A_{1-17} and Met-enkephalin in the spinal cord. This view is supported by the finding that spinal pretreatment with an antiserum against dynorphin A_{1-17} or Met-enkephalin attenuates the antinociception induced by EM-2 given spinally. In addition, spinal pretreatment with the κ-opioid receptor antagonist norbinaltorphimine and δ_2-opioid receptor antagonist naltriben blocks the antinociception

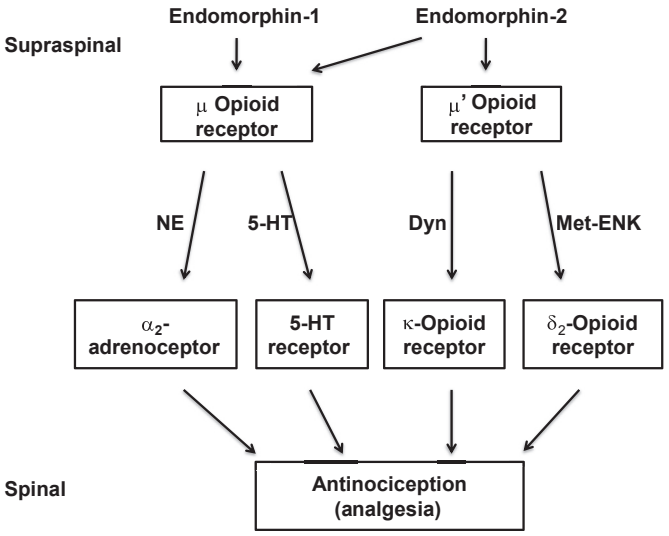

Figure 1 Schematic representation of two separate spinipetal descending pain control systems stimulated by endomorphin-1 and endomorphin-2 for producing antinociception. Endomorphin-1 and endomorphin-2 given supraspinally stimulates one subtype of μ-opioid receptors to induce the release of noradrenaline and 5-HT acting on α_2-adrenoceptors and 5-HT receptors, respectively, in the spinal cord for the production of antinociception. Endomorphin-2 given supraspinally also stimulates another subtype of μ-opioid receptors and/or μ′-opioid receptors, to induce the release of dynorphin A_{1-17} and Met-enkephalin acting on κ- and δ_2-opioid receptors, respectively, in the spinal cord for producing antinociception. Dyn = dynorphin A_{1-17}; Met-enk = Met-enkephalin; NE = norepinephrine; 5-HT = serotonin.

induced by EM-2 given spinally.[25,26] Thus, μ′-opioid receptor activation by EM-2 induces the release of dynorphin A_{1-17} and Met-enkephalin, which subsequently act on κ- and δ_2-opioid receptors, respectively, for the production of antinociception (Figure 1).[25,27]

Systemic pretreatment with the μ_1-opioid receptor antagonist naloxonazine attenuates the antinociception induced by EM-2, but not by EM-1 given spinally or supraspinally, indicating that the antinociception induced by EM-2 is mediated by the stimulation of different subtypes of μ-opioid receptors.[26,35] Spinal treatment with a low dose of D-Pro^2-endomorphin-1 (0.1 pmol) markedly attenuates the tail-flick inhibition induced by EM-1 (16.4 nmol), but not by EM-2 (35 nmol) given intrathecally, whereas spinal treatment with a low dose of D-Pro^2-endophalin-2 (16.4 nmol) attenuates the tail-flick inhibition induced by EM-2 (35 nmol) and, to a much lesser extent, by EM-1 (16.4 nmol) given intrathecally.[36] Pretreatment with different antisense oligodeoxynucleotides against a different G-protein subunit is also useful to differentiate between antinociceptive effects induced by EM-1 and EM-2. Spinal pretreatment with antisense oligodeoxynucleotides against the G-protein subunit $Gi\alpha_2$ protein attenuates the antinociception induced by spinally administered EM-2, but not by EM-1, while spinal pretreatment with antisense oligodeoxynucleotides against the G-protein subunit of $Gi\alpha_1$, $Gi\alpha_3$, or $Gz\alpha$ does not affect the antinociception induced by either EM-1 or EM-2.[37] Thus, the observed differential antinociceptive actions induced by EM-1 and EM-2 are mediated by the stimulation of different subtypes of μ-opioid receptors.

3. Acute antinociceptive tolerance to EM-1 and EM-2

3.1. Acute antinociceptive tolerance and asymmetric cross-tolerance to EM-1 and EM-2 given intracerebroventricularly in mice

Pretreatment with a high dose of the μ-opioid receptor agonist attenuates the antinociception produced by the subsequently

administered μ-opioid agonist. This phenomenon has been defined as acute antinociceptive tolerance. Similar to other μ-opioid agonists, pretreatment with a high dose of EM-1 (30 nmol) or EM-2 (70 nmol) injected intracerebroventricularly produces antinociceptive tolerance to the subsequent administration of EM-1 or EM-2, respectively, in male CD-1 mice, measured by the tail-flick test.[38] Acute antinociceptive tolerance caused by EM-1 appears to develop at a much slower rate than that caused by EM-2. EM-1-induced antinociceptive tolerance reaches the maximal level at 2 hours and recovers to the control level 3–4 hours after the pretreatment with EM-1, whereas EM-2-induced antinociceptive tolerance develops in 1 hour and recovers to the control level in 90 minutes to 2 hours. Pretreatment with EM-1 (30 nmol) for 2 hours produces a three-fold shift of the dose–response curve to the right for EM-1-produced antinociception. Similarly, 1-hour pretreatment with EM-2 (70 nmol) causes a 3.9-fold shift in the dose–response curve to the right for EM-2-produced antinociception. In cross-tolerance studies, pretreatment with EM-2 (70 nmol) causes a 2.3-fold shift of the dose–response curve to the right for EM-1-produced antinociception, whereas pretreatment with EM-1 (30 nmol) causes no change in the dose–response curve for EM-2-produced antinociception. Thus, mice acutely made tolerant to EM-1 are not cross-tolerant to EM-2, although mice made tolerant to EM-2 are partially cross-tolerant to EM-1; thus, an asymmetric cross-tolerance occurs. Pretreatment with DAMGO (0.03 nmol), a highly selective μ-opioid receptor agonist, for 3 hours given intracerebroventricularly attenuates markedly the antinociception induced by EM-1 and DAMGO, but not by EM-2. This finding supports the notion that two separate subtypes of μ-opioid receptors, μ and μ′, are involved in the antinociceptive tolerance to EM-1 and EM-2. One subtype of μ-opioid receptors is stimulated by DAMGO, EM-1, and EM-2, and another subtype is stimulated solely by EM-2. Thus, pretreatment with EM-2 still attenuates the antinociception induced by EM-1; however, pretreatment with EM-1 is unable to attenuate the antinociception induced by EM-2. Mice made tolerant to DAMGO show cross-tolerance to EM-1, but not to EM-2. EM-1 and DAMGO may act on the same subtype of μ-receptor, whereas EM-2 acts on another subtype of μ-receptor for producing antinociception.[38]

3.2. Acute antinociceptive tolerance and asymmetric cross-tolerance to EM-1 and EM-2 given intraventricularly in rats

Pretreatment with EM-1 (30 nmol) or EM-2 (60 nmol) given into the anterior fourth ventricle develops antinociceptive tolerance to the subsequently challenging dose of EM-1 or EM-2, respectively, in male CD-1 rats, measured by the tail-flick test.[39] EM-1-induced antinociceptive tolerance reaches a maximal level at 2 hours and recovers slowly in 24 hours after the pretreatment with EM-1, whereas EM-2-induced antinociceptive tolerance develops in 1 hour and recovers to the control level in 4 hours. Pretreatment with EM-1 (30 nmol) for 2 hours attenuates markedly the antinociception induced by EM-1, and the dose–response curve is shifted four-fold to the right compared with that of rats pretreated with saline. Pretreatment with EM-2 (60 nmol) for 1 hour attenuates markedly the antinociception produced by intraventricularly administered EM-2, and the dose–response curve for EM-2 is shifted 5.3-fold to the right. In cross-tolerance studies, rats made tolerant to EM-1 by pretreatment with EM-1 exhibit nearly no cross-tolerance to EM-2 to produce antinociception. On the other hand, rats made tolerant to EM-2 exhibits a complete cross-tolerance to EM-1 to produce antinociception. The findings of the study in rats[39] are consistent with the finding in mice[38] and indicate that two separate subtype of μ-opioid receptor are involved in the antinociception induced by EM-1 and EM-2.

3.3. Acute antinociceptive tolerance and asymmetric cross-tolerance to EM-1 and EM-2 given spinally in mice

Pretreatment of mice with a high dose of EM-1 (32.7 nmol) given intrathecally for 1.5 hours produces 5.3- and 2.4-fold shifts of the dose–response curves to the right for EM-1- and EM-2-induced antinociception, respectively; by contrast, pretreatment with EM-2 (70 nmol) given intrathecally for 1 hour causes 4.3- and 4.5-fold shifts of the curve to the right for EM-2- and EM-1-induced antinociception, respectively. Thus, mice made antinociceptive tolerant to EM-1 given spinally are only partially cross-tolerant to EM-2, and those made antinociceptive tolerant to EM-2 given spinally are completely cross-tolerant to EM-1. Thus, antinociceptive effects induced by EM-1 and EM-2 given spinally are mediated by the stimulation of two different subtypes of μ-opioid receptors, μ and μ′, in the spinal cord of mice; the μ subtype of μ-opioid receptor is stimulated by both EM-1 and EM-2, and the μ′ subtype is stimulated only by EM-2.[40]

4. Antianalgesia induced by EM-1 and EM-2 against (−)-morphine produced analgesia

4.1. Differential mechanisms of antianalgesia induced by EM-1 and EM-2 given into the ventral periaqueductal gray against (−)-morphine-produced analgesia in rats

Pretreatment with a small dose of EM-2 (1.7–7.0 nmol) or EM-1 (3.5–28 nmol), given into ventral periaqueductal gray (vPAG) for 45 minutes dose dependently, attenuates the tail-flick inhibition produced by (−)-morphine (9 nmol) given into vPAG in male CD rats. This phenomenon has been defined as antianalgesia. Attenuation of (−)-morphine-produced tail-flick inhibition, induced by EM-2 or EM-1 pretreatment, is then blocked or reversed by pretreatment with the μ-opioid antagonist (−)-naloxone, but not by nonopioid (+)-naloxone, indicating that they are mediated by the stimulation of μ-opioid receptors. However, pretreatment with a morphine-6β-glucuronide-sensitive μ-opioid receptor antagonist 3-methoxynaltrexone selectively blocks EM-2- but not EM-1-induced antianalgesia. In addition, pretreatment with dynorphin $A_{1–17}$ antiserum to bind the endogenous dynorphin $A_{1–17}$ blocks only EM-2- but not EM-1-induced antianalgesia. Pretreatment with other types of antisera, such as an antiserum against β-endorphin, Met-enkephalin, Leu-enkephalin, substance P, or cholecystokinin, or with other opioid receptor antagonists, such as the δ-opioid receptor antagonist naltrindole (2.2 nmol) or the κ-opioid receptor antagonist norbinaltorphimine (6.6 nmol), does not affect EM-2-induced antianalgesia. Thus, EM-2 selectively releases dynorphin $A_{1–17}$ by stimulation of a novel subtype of μ-opioid receptors in the vPAG to induce antianalgesia against (−)-morphine-produced analgesia, whereas the antianalgesia induced by EM-1 is mediated by the stimulation of another subtype of μ-opioid receptors.[41]

4.2. Dynorphinergic mechanism mediating the antianalgesia induced by EM-2, but not by EM-1, in the mouse spinal cord

Pretreatment with a small dose of EM-2 (0.05–1.75 nmol), given into the intrathecal space of the spinal cord for 45 minutes prior to an intrathecal injection of (−)-morphine (3.0 nmol) dose dependently, attenuates (−)-morphine-induced tail-flick inhibition in male CD-1 mice. By contrast, pretreatment with a similar dose of EM-1 (1.64 nmol) fails to produce any antianalgesic effect. The EM-2 (1.75 nmol)-produced antianalgesia against (−)-morphine-induced analgesia is blocked by spinal pretreatment with the μ-opioid antagonist (−)-naloxone or 3-methoxynaltrexone, but not with the δ-opioid receptor antagonist naltrindole, κ-opioid receptor

antagonist norbinaltorphimine, or N-methyl-D-aspartate (NMDA) receptor antagonist MK-801. The EM-2-induced antianalgesic effect against (−)-morphine-induced analgesia is also blocked by spinal pretreatment with an antiserum against dynorphin A_{1-17}, but not with β-endorphin, Met-enkephalin, Leu-enkephalin, or cholecystokinin antiserum. Thus, EM-2 treatment at a subanalgesic dose stimulates a subtype of μ-opioid receptors and subsequently induces the release of dynorphin A_{1-17} to produce antianalgesic effects against (−)-morphine-produced antinociception. EM-2-induced 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.[42] Pharmacological findings of EM-2 on the release of dynorphin A_{1-17} for producing antianalgesia are in line with the biochemical finding that EM-2 (15—50 nmol) injected into the spinal perfusate dose dependently increases the release of immunoreactive dynorphin A_{1-17} in the spinal perfusates of anesthetized rats. By contrast, EM-1 produces a slight increase only at a high dose (50 nmol). The increased release of dynorphin A_{1-17} from the spinal cord induced by EM-2 is blocked by the μ-opioid receptor antagonist (−)-naloxone or 3-methoxynaltrexone.[43] The cellular mechanism of EM-1-induced antianalgesia against (−)-morphine-induced analgesia is not clear.

Thus, both analgesia and antianalgesia produced by EM-2 are mediated by the release of dynorphin A_{1-17}. Dynorphin A_{1-17} released by EM-2 appears to produce biphasic effects—analgesia,[24,25,35] and antianalgesia[41,42]; an initial release of dynorphin A_{1-17} produces analgesia, which is mediated by the stimulation of κ-opioid receptors, whereas a delayed release of dynorphin A_{1-17} induces antianalgesia, which is not mediated by the stimulation of κ-opioid receptor or NMDA receptor mechanism.[41]

5. Paradoxical hyperalgesia induced by EM-2, but not by EM-1, microinjected into the centromedial amygdala of rats

The amygdala plays a central role in the interaction of sensory information, especially pain-related behavior.[44] Endogenous κ-opioids involved in stress-induced analgesia are probably produced within the amygdala complex, especially the central amygdaloid nucleus and stria terminalis.[45] The central amygdaloid nucleus is an important site for pain perception and analgesia produced by opioids through the projection to the periaqueductal gray.[46] The central amygdaloid nucleus receives neuronal inputs from the spinal cord dorsal horn and parabrachial nucleus.[47] The spinopontoamygdaloid pathway has been shown to specially transmit nociceptive information.[48] In addition, this amygdaloid nucleus contains endogenous opioid peptides and all their opioid receptors, including EM-1, EM-2, and μ-opioid receptors.[17] The nociceptive threshold is increased following the microinjection of (−)-morphine and other μ-opioid agonists into the central and basolateral nucleus.[49,50] Furthermore, lesions placed in the amygdala reduce the magnitude of systemic (−)-morphine analgesia.[51] Analgesia induced by (−)-morphine, elicited from the basolateral amygdala, is mediated by μ-opioid receptors, but not by δ- or κ-opioid receptors.[52] Thus, the central amygdala may play an important role in both descending pain facilitating and pain inhibitory pathways.[53]

Microinjection of EM-2 (8.7—35.0 nmol), given into the centromedial amygdala time and dose dependently, decreases the tail-flick latencies (hyperalgesia) in male CD rats. By contrast, EM-1 (8—32.6 nmol) given into the same site does not cause any change of the tail-flick latency. However, EM-2 or EM-1 given into the basolateral site of amygdala does not affect the tail-flick latency. The decrease of the tail-flick latencies (hyperalgesia) induced by EM-2 is reversed by pretreatment with the antiserum against dynorphin A_{1-17}. EM-2-induced hyperalgesia is also blocked by the EM-2 selective μ-opioid receptor antagonist 3-methoxynaltrexone and by the NMDA receptor antagonist MK-801, but not by the μ-opioid receptor antagonist norbinaltorphimine. Thus, EM-2, but not EM-1, given into the centromedial amygdala stimulates a 3-methoxynaltrexone-sensitive μ-opioid receptor subtype to induce the release of dynorphin A_{1-17}, which then acts on the NMDA receptor, but not on the μ-opioid receptor for producing hyperalgesia.[54] This conclusion is further supported by the additional finding that dynorphin A_{1-17} itself, given into the centromedial amygdala, also causes a decrease in the tail-flick latency, which is similarly blocked by the NMDA receptor antagonist MK-801 (30 nmol), but not by the κ-opioid receptor antagonist norbinaltorphimine (6.6 nmol).[54]

Thus, EM-2 can induce either analgesia or hyperalgesia depending on the brain sites into which it is injected. EM-2 microinjected into the centromedial amygdala, but not into the basolateral amygdala, induces hyperalgesia. The hyperalgesia induced by EM-2 is mediated by the stimulation of a selective μ-opioid receptor subtype μ′, which subsequently induces the release of dynorphin A_{1-17} acting on NMDA receptors, but not on κ-opioid receptors.

References

1. Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature (Lond)* 1975;**258**:577—80.
2. Bradbury AF, Smyth DF, Snell CR, Birdsall NJM, Hulme EC. C-fragment of lipotropin has a high affinity for brain opiate receptors. *Nature (Lond)* 1976;**260**:793—5.
3. Li CH, Chung D. Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. *Proc Natl Acad Sci U S A* 1976;**73**:1145—8.
4. Goldstein A, Tachibana S, Lowney LI, Hunkapiller M, Hood L. Dynorphin-(1-13), an extraordinarily potent opioid peptide. *Proc Natl Acad Sci U S A* 1979;**76**:6666—70.
5. Goldstein A, Fischli W, Lowney LI, Hunkapiller M, Hood L. Porcine pituitary dynorphin: complete amino acid sequence of the biologically active heptadecapeptide. *Proc Natl Acad Sci U S A* 1981;**78**:7219—23.
6. Corbett AD, Paterson SJ, Kosterlitz HW. Selectivity of ligands for opioid receptors. In: Herz A, editor. *Opioids I, handbook of experimental pharmacology*, vol. 104/I. New York: Springer-Verlag; 1993. p. 645—79.
7. Zadina JE, Hackler L, Ge L-J, Kastin AJ. A potent and selective endogenous agonist for the μ-opiate receptor. *Nature (Lond)* 1997;**386**:499—502.
8. Knapp RJ, Vaughn LK, Yamamura HI. Selective ligands for μ and δ opioid receptors. In: Tseng LF, editor. *The pharmacology of opioid peptides*. Chur, Switzerland: Harwood Academic Publishers; 1995. p. 1—28.
9. Nock B. κ and ε opioid receptor binding. In: Tseng LF, editor. *The pharmacology of opioid peptides*. Chur, Switzerland: Harwood Academic Publishers; 1995. p. 29—56.
10. Tseng LF. Mechanisms of β-endorphin-induced antinociception. In: Tseng LF, editor. *The pharmacology of opioid peptides*. Chur, Switzerland: Harwood Academic Publishers; 1995. p. 249—69.
11. Tseng LF. Evidence for ε-opioid receptor-mediated β-endorphin-induced analgesia. *Trends Pharmacol Sci* 2001;**22**:623—30.
12. Narita M, Tseng LF. Evidence for the existence of β-endorphin-sensitive "ε-opioid receptor" in the brain: the mechanisms of ε-mediated antinociception. *Jpn J Pharmacol* 1998;**76**:233—53.
13. Reisine T. Review: neurotransmitter receptors: V, opiate receptors. *Neuropharmacology* 1995;**34**:463—72.
14. Goldberg IE, Rosi GC, Letchworth SR, Mathis JP, Ryan-Moro J, Leventhal L, Su W, et al. Pharmacological characterization of endomorphin-1 and endomorphin-2 in mouse brain. *J Pharmacol Exp Ther* 1998;**286**:1007—13.
15. Martin-Schild S, Zadina JE, Gerall AA, Vigh S, Kastin AJ. Localization of endomorphin-2-like immunoreactivity in the rat medulla and spinal cord. *Peptides* 1997;**18**:1641—9.
16. Martin-Schild S, Gerall AA, Kastin AJ, Zadina JE. Endomorphin-2 is an endogenous opioid in the primary sensory afferent fibers. *Peptides* 1998;**19**:1783—9.
17. Martin-Schild S, Gerall AA, Kastin AJ, Zadina JE. Differential distribution of endomorphin 1- and endomorphin 2-like immunoreactivities in the CNS of the rodent. *J Comp Neurol* 1999;**405**:450—71.
18. Pierce TL, Grahek MD, Wessendorf MW. Immunoreactivity for endomorphin-2 occurs in primary afferents in rats and monkey. *Neuroreport* 1998;**9**:385—9.
19. Schreff M, Schulz S, Wiborny D, Höllt V. Immunofluorescent identification of endomorphin-2-contraining nerve fibers and terminals in the rat brain and spinal cord. *Neuroreport* 1998;**9**:1031—4.

20. Narita M, Mizoguchi H, Oji GS, Tseng EL, Suganuma C, Nagase H, Tseng LF. Characterization of newly selective endogenous μ-opioid receptor agonist endomorphin-1 and endomorphin-2 on [^{35}S]GTPγS binding in mouse spinal cord. *Eur J Pharmacol* 1998;**351**:383–7.
21. Narita M, Mizoguchi H, Narita M, Dun NJ, Hwang BH, Endoh T, Suzuki T, et al. G-protein activation by endomorphins in the mouse periaqueductal gray matter. *J Biomed Sci* 2000;**7**:221–5.
22. Mizoguchi H, Narita M, Oji DE, Suganuma C, Nagase H, Sora I, Uhl GR, et al. μ-Opioid receptor gene-dose dependent reductions in G-protein activation in the pons/medulla and antinociception induced by endomorphins in μ-opioid receptor knockout mice. *Neuroscience* 1999;**94**:203–7.
23. Narita M, Mizoguchi H, Sora I, Uhl GR, Tseng LF. Absence of G-protein activation by endomorphin-1 and -2 in the spinal cord of μ-opioid receptor knockout mice. *Br J Pharmacol* 1999;**126**:451–6.
24. Tseng LF, Narita M, Suganuma C, Mizoguchi H, Ohasawa H, Nagase H, Kampine JP. Differential antinociceptive effects of endomorphin-1 and endomorphin-2 in the mouse. *J Pharmacol Exp Ther* 2000;**292**:576–83.
25. Ohsawa M, Mizoguchi H, Narita M, Nagase H, Kampine JP, Tseng LF. Differential antinociception induced by spinally administered endomorphin-1 and endomorphin-2 in the mouse. *J Pharmacol Exp Ther* 2001;**298**:592–7.
26. Sakurada S, Hayashi T, Yuhki M, Fujimura T, Maruyama K, Yonezawa A, Sakurada C, et al. Differential antagonism of endomorphin-1 and endomorphin-2 spinal antinociception by naloxonazine and 3-methoxynaltrexone. *Brain Res* 2000;**88**:1–8.
27. Sakurada S, Hayashi T, Yuhki M, Orito T, Zadina JE, Kastin AJ, Fujimura T, et al. Differential antinociceptive effects induced by intrathecally administered endomorphin-1 and endomorphin-2 in the mouse. *Eur J Pharmacol* 2001;**427**:203–10.
28. Wu HE, Mizoguchi H, Terashvili M, Leitermann RJ, Hung KC, Fujimoto JM, Tseng LF. Spinal pretreatment with antisense oligodeoxynucleotides against exon-1, -2 or -8 of μ-opioid receptor clone leads to differential loss of spinal endomorphin-1- and endomorphin-2-induced antinociception in the mouse. *J Pharmacol Exp Ther* 2002;**303**:867–73.
29. Tseng LF, Tang R. Differential mechanisms mediate β-endorphin- and morphine-induced inhibition of the tail-flick response in rats. *J Pharmacol Exp Ther* 1990;**252**:546–51.
30. Tseng LF, Collins KA. Different mechanisms mediating tail-flick inhibition induced by β-endorphin, DAMGO and morphine from Rob and GiA in anesthetized rat. *J Pharmacol Exp Ther* 1991;**257**:530–8.
31. Tseng LF, Collins KA. Spinal involvement of both dynorphin A and Met-enkephalin in the antinociception induced by intracerebroventricularly administered bremazocine but not morphine in the mouse. *J Pharmacol Exp Ther* 1993;**266**:1430–8.
32. Ohsawa M, Mizoguchi H, Narita M, Chu M, Nagase H, Tseng LF. Differential mechanisms mediating descending pain controls for antinociception induced by supraspinally-administered endomorphin-1 and endomorphin-2 in the mouse. *J Pharmacol Exp Ther* 2000;**294**:1106–11.
33. Ohsawa M, Ohsawa M, Mizoguchi H, Narita M, Kawai K, Nagase H, Narita M, et al. Release of immunoreactive [Met^5]enkephalin from the spinal cord by intraventricular injection of endomorphin-1, but not endomorphin-2 in the anesthetized rats. *Neurosci Lett* 2001;**316**:1–4.
34. Stone LS, Fairbanks CA, Laughlin TM, Nguyen HO, Bushy TM, Wessendorf MW, Wilcox GL. Spinal analgesic actions of the new endogenous opioid peptides endomorphin-1 and -2. *Neuroreport* 1997;**8**:3131–5.
35. Sakudara S, Zadina JE, Kastin AJ, Katsuyama S, Fujimura T, Murayama K, Yuki M, et al. Differential involvement of μ-opioid receptor subtypes in endomorphin-1- and endomorphin-2-induced antinociception. *Eur J Pharmacol* 1999;**372**:25–30.
36. Hung KC, Wu HE, Mizoguchi H, Sakurada S, Okayama T, Fujimura T, Murayama K, et al. D-Pro^2-endomorphin-1 and D-Pro^2-endomorphin-2 respectively attenuate the antinociception induced by endomorphin-1 and endomorphin-2 given intrathecally in the mouse. *J Pharmacol Exp Ther* 2002;**303**:874–9.
37. Sanchez-Blazquez P, Rodriguez-Diaz M, Deantonio I, Garzon J. Endomorphin-1 and endomorphin-2 show differences in their activation of μ opioid receptor regulated G-proteins in supraspinal antinociception in mice. *J Pharmacol Exp Ther* 1999;**291**:12–8.
38. Wu HE, Hung KC, Mizoguchi H, Fujimoto JM, Tseng LF. Acute antinociceptive tolerance and asymmetric cross-tolerance between endomorphin-1 and endomorphin-2 given intracerebroventricularly in the mouse. *J Pharmacol Exp Ther* 2001;**299**:1120–5.
39. Hung KC, Wu HE, Mizoguchi H, Tseng LF. Acute antinociceptive tolerance and unidirectional cross-tolerance to endomorphin-1 and endomorphin-2 given intraventricularly in the rat. *Eur J Pharmacol* 2002;**448**:169–74.
40. Wu HE, Darpolor M, Nagase H, Tseng LF. Acute antinociceptive tolerance and partial cross-tolerance to endomorphin-1 and endomorphin-2 given intrathecally in the mouse. *Neurosci Lett* 2003;**348**:139–42.
41. Terashvili M, Wu H, Leitermann RJ, Sun H, Clithero AD, Tseng LF. Differential mechanisms of antianalgesia induced by endomorphin-1 and endomorphin-2 in the ventral periaqueductal gray of the rat. *J Pharmacol Exp Ther* 2005;**312**:1257–65.
42. Wu HE, Sun HS, Darpolar M, Leitermann J, Kampine JP, Tseng LF. Dynorphinergic mechanism mediating endomorphin-2-induced antianalgesia in the mouse spinal cord. *J Pharmacol Exp Ther* 2003;**307**:1135–41.
43. Leitermann RJ, Terashvili M, Mizoguchi H, Wu HE, Chen F, Clithero A, Tseng LF. Increased release of immunoreactive dynorphin A_{1-17} from the spinal cord after intrathecal treatment with endomorphin-2 in anesthetized rats. *Eur J Pharmacol* 2004;**504**:177–83.
44. Pikanen A, Savander V, LeDoux JL. Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends Neurosci* 1997;**20**:517–23.
45. Da Costa Gomez TM, Behbehani MM. An electrophysiological characterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: the role of opioid receptors. *Brain Res* 1995;**689**:21–31.
46. Finnegan TF, Chen SR, Pan HL. Effects of the μ opioid on excitatory and inhibitory synaptic inputs to periaqueductal gray-projecting neurons in the amygdala. *J Pharmacol Exp Ther* 2004;**312**:441–8.
47. Burstein R, Potrebic S. Retrograde labeling of neurons in the spinal cord that directly to the amygdala or the orbital cortex in the rat. *J Comp Neurol* 1993;**335**:469–85.
48. Bester H, Chapman V, Besson JM, Bernard JF. Physiological properties of the lamina I spinoparabrachial neurons in the rat. *J Physiol* 2000;**83**:2239–59.
49. Helmstetter FJ, Bellgowan PS, Teshner SA. Inhibition of the tail-flick reflex following microinjection of morphine into the amygdala. *NeuroReport* 1993;**4**:471–4.
50. Rodgers RJ. Influence of intra-amygdaloid opiate injections on shock thresholds, tail-flick latencies and open field behaviour in rats. *Brain Res* 1978;**153**:211–390.
51. Manning BH, Mayer DJ. The central nuclei of the amygdala contributes to the production of morphine antinociception in the tail-flick test. *J Neurosci* 1995;**15**:8199–213.
52. Helmstetter FJ, Bellgowan PSF, Poore LH. Microinfusion of mu but not delta or kappa opioid agonists into the basolateral amygdala results in inhibition of the tail-flick reflex in pentobarbital-anesthetized rats. *J Pharmacol Exp Ther* 1995;**275**:381–8.
53. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci* 1984;**7**:309–38.
54. Terashvili M, Wu HE, Schwasinger E, Tseng LF. Paradoxical hyperalgesia induced by μ-opioid agonist endomorphin-2, but not endomorphin-1, microinjected into the centromedial amygdala of the rat. *Eur J Pharmacol* 2007;**554**:137–44.