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FORUM MINIREVIEW

Recent Advances in the Search for the μ-Opioidergic System

Endomorphin-Induced Motivational Effect: Differential Mechanism of Endomorphin-1 and Endomorphin-2

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ABSTRACT—The newly discovered endogenous μ-opioid receptor (MOP-R) ligands endomorphin-1 (EM-1) and -2 (EM-2) exhibit the highest specificity and affinity for the MOP-R of any endogenous substance so far described in the mammalian nervous system. This review focuses on differential mechanism of the motivational effects induced by EM-1 and EM-2. In the [^{35}S]GTPγS binding assay, either EM-1 or EM-2 causes a concentration-dependent G-protein activation in brain membrane of normal mice, whereas neither EM-1 nor EM-2 produces any activation of G-protein in membranes obtained from the MOP-R knockout mice. These results provide direct evidence at the molecular level that both EMs act on the MOP-R as the endogenous MOP-R agonists. Based on the conditioned place preference paradigm in mice, EM-1 given intracerebroventriculally produced a dose-related place preference. This effect was abolished by pretreatment with the MOP-R antagonist β-funaltrexamine (FNA) but not the δ-opioid receptor (DOP-R) antagonist naltrindole and the κ-opioid receptor (KOP-R) antagonist nor-bialtorphimine (BNI). Unlike EM-1, EM-2 exhibited a place aversion. The aversive effect was inhibited by not only β-FNA but also nor-BNI. Place aversion produced by EM-2 was also attenuated by pretreatment with an antiserum against an endogenous KOP-R ligand dynorphin A(1 – 17). These findings indicate that EM-1 may produce its rewarding effect via MOP-Rs. Furthermore, the aversive effect induced by EM-2 may be associated with the stimulation of the EM-1-insensitive MOP-R subtype and necessarily activate an endogenous KOPergic system in the mouse brain.

Keywords: Endomorphin-1, Endomorphin-2, Motivational effect, μ-Opioid receptor, κ-Opioid receptor

Discovery of two new endogenous μ-opioid receptor (MOP-R) ligands, endomorphin-1 (EM-1) and -2 (EM-2)

Based upon pharmacological, behavioral and biochemical studies on opioids, opioid receptors have been classified into three types of MOP-R, δ-opioid receptor (DOP-R) and κ-opioid receptor (KOP-R). Recently, two new endogenous MOP-R ligands, which are called EM-1 (Tyr-Pro-Trp-Phe-NH_2) and EM-2 (Tyr-Pro-Phe-Phe-NH_2), were isolated first from bovine brain and later from human cortex (1, 2). These peptides differ structurally from previously described endogenous opioid peptides (such as endorphins, enkephalins and dynorphins, which all have the amino-terminal amino-acid sequence Tyr-Gly-Gly-Phe) in their N-terminal (Tyr-Pro) sequence, C-terminal amidation and tetrapeptide length.

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Binding properties of EM-1 and EM-2 for opioid receptors

At first, we examined the binding properties of EM-1 and EM-2 for MOP-, DOP- and KOP-R in membranes obtained from mouse brain. The [^{3}H]DAMGO binding was displaced by EM-1 and EM-2 in a concentration-dependent manner. The IC_{50} values of EM-1 and EM-2 were almost the same (Fig. 1). In contrast, either [^{3}H]DPDPE or [^{3}H]U69593 bindings were not affected by both EM-1 and EM-2. We previously demonstrated that the antinociceptive actions produced by intracerebroventricular injection of either EM-1 or EM-2 were significantly reduced in heterozygous MOP-R knockout mice and virtually abolished in homozygous MOP-R knockout mice. Thus, the evidence described so far indicates that EM-1 and EM-2 are natural selective ligands for the MOP-R.

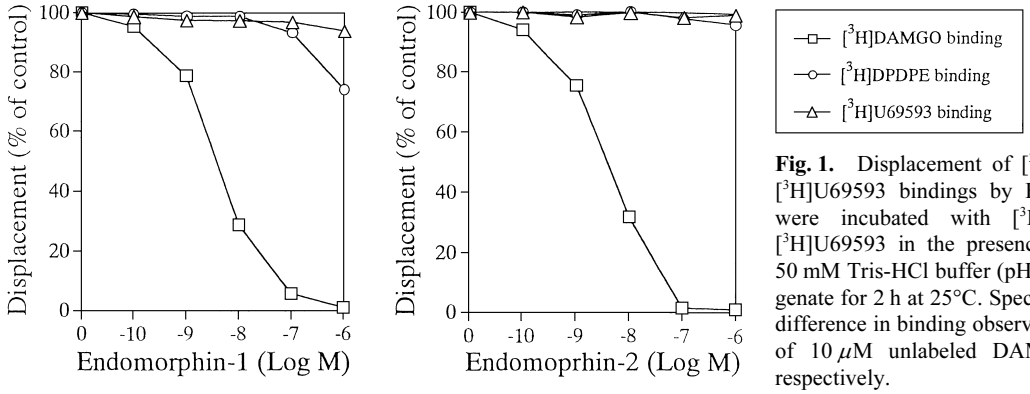


Fig. 1. Displacement of [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69593 bindings by EM-1 and EM-2. Membranes were incubated with [^{3}H]DAMGO, [^{3}H]DPDPE or [^{3}H]U69593 in the presence of EMs at 1 – 1000 nM in 50 mM Tris-HCl buffer (pH 7.4) and the membrane homogenate for 2 h at 25°C. Specific binding was defined as the difference in binding observed in the absence and presence of 10 μM unlabeled DAMGO, DPDPE and U69593, respectively.

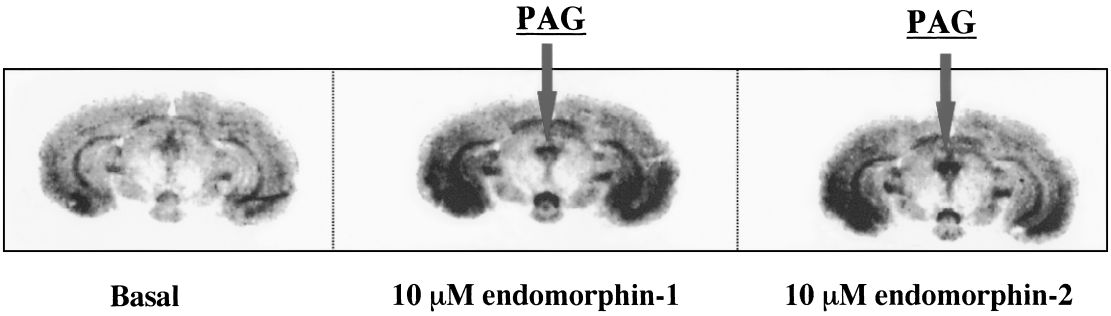


Fig. 2. Autoradiograms depicting endomorphin-stimulated [^{35}S]GTPγS binding at the level of the mouse periaqueductal gray (PAG). Sections were incubated with 2 mM GDP and then [^{35}S]GTPγS (40 pM) with 2 mM GDP and 10 μM EMs. Basal binding was assessed in the absence of EMs. EM-1 and EM-2 stimulated high levels of [^{35}S]GTPγS binding in the PAG area as compared to basal.

Effect of EMs on G-protein activation in the brain

We next examined the effect of EMs on G-protein activation in the brain (3). The ability of MOP-R agonists to activate G-proteins in the brain of mice was examined by monitoring the binding to membranes of the non-hydrolyzable analog of GTP, [^{35}S]GTPγS. Both EM-1 and EM-2 (0.001 to 10 μM) produced concentration-dependent increases in [^{35}S]GTPγS binding to pons/medulla membranes obtained from normal type of mice. The synthetic selective MOP-R agonist DAMGO and the prototype of MOP-R agonists morphine also stimulated [^{35}S]GTPγS binding in a concentration-dependent manner. In vitro [^{35}S]GTPγS autoradiography has also been performed in coronal sections of the midbrain of mice to map the anatomical distribution of EM-stimulated [^{35}S]GTPγS binding. Autoradiograms depicting EM-stimulated [^{35}S]GTPγS binding at the level of the mouse midbrain are illustrated in Fig. 2. EM-1 and EM-2 produce similar anatomical distributions of activated G-proteins. Both EM-1 and EM-2 stimulate high levels of [^{35}S]GTPγS binding in the periaqueductal gray as compared to the basal.

Knockout mice with MOP-R gene deletions have been successfully developed by homologous recombination. These mice display profound gene-dose-dependent reductions in morphine analgesia. The availability of transgenic MOP-R knockout mice allows us to determine the extent to which the MOP-R gene products are necessary for the expression of physiological actions by these newly discovered opioid peptides and alkaloids. We have investigated the effects of EM-1 and EM-2 on [^{35}S]GTPγS binding in the brain obtained from mice expressing normal (wild-type), half-normal (heterozygous), and absent (homozygous) MOP-R complements (3). In heterozygous MOP-R gene knockout mice, [^{35}S]GTPγS binding stimulated by either EM-1, EM-2, DAMGO or morphine was markedly decreased to about half of the stimulation observed in wild-type mice. In homozygous knockout mice, no stimulation of [^{35}S]GTPγS binding stimulated by either EM-1, EM-2, DAMGO or morphine could be detected.

Motivational effects produced by selective MOP-, DOP- and KOP-R agonists

The place conditioning procedure is used to evaluate the motivational properties, such as rewarding or aversive effects, of drugs. This was introduced in the early 1980s to compensate for methodological and interpretive difficulties associated with the self-administration technique, the conventional method for assessing reinforcing properties of

drugs (4). The place conditioning (conditioned place preference, CPP) paradigm has become the most frequently used method and its use has been reported more frequently than the self-administration paradigm (5). In this procedure, a test apparatus consisting of at least two differently coloured compartments of a shuttle box is used, so that an animal is confined to one compartment after being injected with a drug. In a subsequent session, the animal is injected with vehicle and placed in the other compartment. This procedure is repeated several times so that the animal learns to associate the cues of one distinct compartment of the test apparatus with the central effects of the test drug. A day after these conditioning sessions, the animal is placed in the test apparatus without any confinements. The animal will have the opportunity to move freely around the different compartments and the relative amount of different compartments entered and the relative amount of time spent in these compartments is measured. This technique only requires that the animals perform a simple operation to approach or avoid the place paired with the drug. If the drug experience has a positive effect, it is presumed that subjects will spend more time in the place associated with the drug experience.

Several investigators have demonstrated that the reinforcing effects of MOP-R agonists can be evaluated by the conditioned place preference paradigm (5 – 7). Morphine produces place preference with doses ranging from 0.08 to 10 mg/kg in rats. Other MOP-R agonists, such as codeine, dihydrocodeine, and sufentanyl, also produce dose-related preferences for the drug-associated place in rats. Furthermore, i.c.v. administration of the specific MOP-R agonist DAMGO and the selective DOP-R agonist DPDPE also produces a dose-related conditioned place preference in rats. Utilizing mice, our laboratory successfully found that i.c.v. treatment of morphine and DAMGO displays a dose-related conditioned place preference (5). Mice treated i.c.v. with either a preferring D_1OP-R agonist DPDPE or a selective D_2OP-R agonist [D-Ala^2]deltorphin II manifest a dose-related conditioned place preference (8). These findings indicate that besides MOP-R, DOP-R also mediate rewarding effects.

By contrast, KOP-R agonists, such as U50,488H, U69593, and the metabolically stable dynorphin-like peptide E-2078 induce a significant place aversion in rats (9). In good agreement with previous reports using rats, we confirmed by using mice that either U50,488H or E-2078 induce a significant place aversion (10). Consequently, these findings suggest that mice are also suitable for investigating opioid-induced place preference or aversion.

Place preference produced by EM-1

EM-1 (1 – 30 nmol/mouse) was injected into the lateral cerebral ventricle of mice. One day before the beginning of the drug or saline injection, mice had been anesthetized with ether and a 2-mm double-needle attached to a 25-ml Hamilton microsyringe was inserted into the unilateral injection site; as a result, simply a hole for the injection was made in the skull. The unilateral injection site was approximately 2 mm from either side of the midline between the anterior roots of the ears. The head of the mouse was held against a V-shaped holder and the drugs were injected into the hole. We found that EM-1 given i.c.v. induced a dose-dependent place preference. The EM-1 (17 nmol/mouse, i.c.v.)-induced place preference was abolished by β-funaltrexamine (FNA) (10 mg/kg, s.c.), a MOP-R antagosist. However, pretreatment with either naltrindole (NTI) (1 mg/kg, s.c.), a selective DOP-R antagonist, and nor-binaltorphimine (BNI) (3 mg/kg, s.c.), a specific KOP-R antagonist, failed to affect the EM-1-induced place preference. These opioid receptor antagosists alone did not produce either preference or aversion of the drug-associated place.

Place aversion produced by EM-2

Unlike EM-1, EM-2 (1, 5.6 and 10 nmol/mouse, i.c.v.) induced a bell-shaped dose-response curve. Although lower (1 nmol/mouse) and higher (10 nmol/mouse) doses of EM-2 did not produce a conditioned place aversion, a significant conditioning aversion was observed only at the dose of 5.6 nmol/mouse.

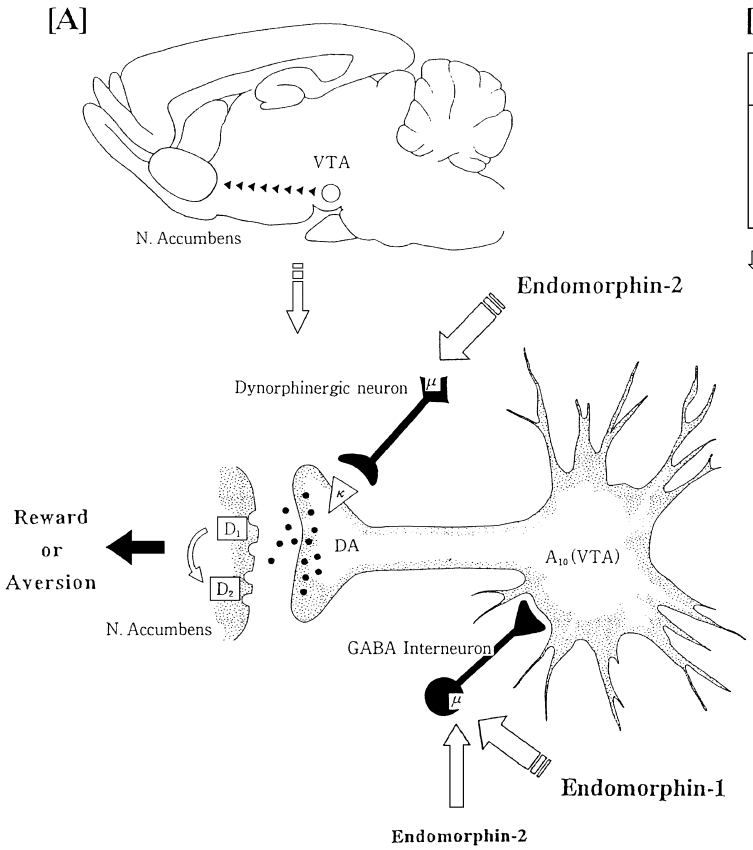
The EM-2 (5.6 nmol/mouse, i.c.v.)-induced place aversion was abolished by β-FNA (10 mg/kg, s.c.). In contrast, NTI (1 mg/kg, s.c.) had no effect on the EM-2-induced place aversion. It should be noted that the EM-2 (1, 5.6 and 10 nmol/mouse, i.c.v.)-induced place aversion can be significantly antagonized by pretreatment with nor-BNI (3 mg/kg, s.c.).

It is of interest to note that i.c.v. pretreatment with an antiserum against dynorphin A(1 – 17), an endogenous KOP-R ligand, significantly attenuated the place aversion induced by EM-2 (5.6 nmol/mouse, i.c.v.). Pretreatment with vehicle or antiserum against dynorphin A(1 – 17) alone did not produce either preference or aversion of the drug-associated place.

Concluding remarks

Several investigators have demonstrated that either MOP- or DOP-R ligands produce place preference, whereas KOP-R ligands produce place aversion. In the present study, i.c.v. administration of EM-1 produced a MOP-R antagonist-reversible place preference. Unlike EM-1, EM-2 given i.c.v. produced a significant place aversion. Furthermore, the effect of EM-2 was also attenuated by pretreatment with an antiserum against an endogenous KOP-R ligand dynorphin A(1 – 17).

Recent molecular studies have indicated the existence



[B] Motivational effects

	β-FNA (μ)	NTI (δ)	Nor-BNI (κ)	Dynorphin A (1-17) antibody
Endomorphin-1	⇩	±	±	N.T.
Endomorphin-2	⇩	±	⇩	⇩

⇩, decrease; ±, not change; N.T., not test

Fig. 3. Motivational effects by endogenous μ-opioid ligands, EMs. EM-1 produces the rewarding effect, whereas EM-2 at the lower doses causes the aversive effect. A: A hypothetical model for the modulation of the mesolimbic dopaminergic system by EM-1 and EM-2. VTA: vental tegmental area, DA: dopamine. B: Summary for the effects of the MOP-, DOP- and KOP-R antagonists and antibody against the endogenous KOP ligand dynorphin A(1 – 17) on the motivational effects by EM-1 and EM-2.

of at least ten exons on the MOP-R gene (11, 12). It is likely that the existence of several exons in genes encoding MOP-R can provide scope for additional diversity by virtue of splicing events. In fact, the mapping study on MOP-R using antisense oligodeoxynucleotides against identified exons provides possible evidence that distinct MOP-R isoforms may differently mediate a number of opioid-induced pharmacological actions (13).

Although further investigation would be needed, we propose that EM-2 stimulates a different subtype of MOP-R from the EM-1-sensitive receptors, which subsequently induces the release of dynorphins to express the aversive effect (14, 15 and summarized in Fig. 3).

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