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Acute antinociceptive tolerance and unidirectional cross-tolerance to endomorphin-1 and endomorphin-2 given intraventricularly in the rat

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Abstract

The effect of the pretreatment with endomorphin-1 or endomorphin-2 given into anterior 4th ventricle (i.vt.) on the antinociception with the tail-flick test induced by subsequent intraventricular (i.vt.) injection of endomorphin-1 or endomorphin-2were studied in rats. The i.vt. pretreatment with 30 nmol of endomorphin-1 or 60 nmol of endomorphin-2 developed an antinociceptive tolerance to the subsequently challenging dose of i.vt.-administered endomorphin-1 or endomorphin-2, respectively, with different time courses. The endomorphin-1-induced antinociceptive tolerance reached a maximal level at 2 h, recovered slowly in 24 h after the pretreatment with endomorphin-1, whereas endomorphin-2-induced antinociceptive tolerance developed in 1 h and recovered in 4 h. Rats made tolerant to endomorphin-1 by i.vt. pretreatment with endomorphin-1 exhibited nearly no cross-tolerance to endomorphin-2 to produce antinociception. On the other hand, rats made tolerant to endomorphin-2 exhibited a complete cross-tolerance to endomorphin-1 to produce antinociception. We propose that different degrees of receptor endocytosis for receptor inactivation after stimulation of μ-opioid receptors by endomorphin-1 and endomorphin-2 and/or two separate subtypes of μ-opioid receptors are involved in the antinociception induced by endomorphin-1 and endomorphin-2.

Keywords: Antinociception; Tail-flick test; Endomorphin-1; Endomorphin-2; Tolerance; Cross-tolerance

1. Introduction

Endomorphin-1 and endomorphin-2 are two tetrapeptides isolated from the bovine frontal cortex (Zadina et al., 1997) and human brain (Hackler et al., 1997) and proposed to be the endogenous ligand for μ-opioid receptors. Receptor-binding assays and immunocytochemical studies reveal that endomorphin-1 and endomorphin-2 are highly selective for μ-opioid receptors and widely localized in the regions of the brain and the spinal cord that are abundant in μ-opioid receptors (Goldberg et al., 1998; Martin-Schild et al., 1997, 1998, 1999; Shreff et al., 1998; Pierce et al., 1998; Wu et al., 1999). The release of endomorphin-2 from the dorsal horn of the spinal cord by electrical stimulation and the inhibition of the electrical activity of rostral ventrolateral medullar or spinal substantia gelatonosa neurons in vitro by endomorphin-1 and endomorhin-2 have been demonstrated (Chu et al., 1999; Williums et al., 1999; Wu et al., 1999). Like other μ-opioid receptor agonists, endomorphin-1 or endomorphin-2 activates μ-opioid receptor-mediated G-protein activation only in wild-type mice, but not in μ-opioid receptor knockout mice (Mizoguchi et al., 1999). Intracerebroventricular (i.c.v.) or intrathecal (i.t.) injection of endomorphin-1 or endomorphin-2 produces potent antinociception and the antinociception is blocked only by μ-opioid receptor antagonists, naloxone, β-funaltrexamine or CTOP (Zadina et al. 1997; Stone et al., 1997; Narita et al., 1998; Tseng et al. 2000). The antinociception induced by endomorphin-1 or endomorphin-2 is significantly attenuated in heterozygous knockout mice and virtually abolished in homozygous knockout mice (Mizoguchi et al., 1999). These findings clearly support the notion that endomorphin-1 and endomorphin-2 are indeed endogenous opioid ligands and the antinociception induced by these two peptides are mediated by the stimulation of μ-opioid receptors.

However, differential antinociceptive effects induced by endomorphin-1 and endomorphin-2 have been reported. Earlier, Fisher and Undem (1999) reported that naloxone blocks the effects of endomorphin-1, but not endomorphin-2. Sakurada et al. (1999, 2000) reported that μ1-opioid receptor antagonist, naloxonazine or morphine-6 β glucuronide antag-

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onist, 3-methoxynaltrexone is more effective in blocking the antinociception induced by endomorphin-2 than endomorphin-1 given i.c.v. or i.t. in mice. In addition, the descending pain control systems activated by endomorphin-1 and endomorphin-2 given supraspinally to produce antinonociception appear to be different. Pretreatment i.t. with antiserum against dynorphin A(1-17) or Met-enkephalin or κ-opioid receptor antagonist nor-binaltorphimine or δ_2-opioid receptor antagonist naltriben blocks the antinociception induced by endomorphin-2, but not endomorphin-1 given supraspinally (Ohsawa et al., 2000). The findings indicate that two different subtypes of μ-opioid receptors are involved in endomorphin-1- and endomorphin-2-induced antinociception.

We have previously reported that mice made tolerance to endomorphin-1 by i.c.v. pretreatment with endomorphin-1 exhibit little cross-tolerance to endomorphin-2 to produce antinociception. On the other hand, mice made tolerance to endomorphin-2 exhibit a partial cross-tolerance to endomorphin-1 to produce antinociception (Wu et al., 2001). We now report a similar phenomenon of the antinociceptive tolerance between endomorphin-1 and endomorphin-2 given i.vt. in rats.

2. Materials and methods

2.1. Animals

Male CD-1 rats (Charles River Breeding Laboratory, Wilmington, MA), weighing 300–350 g at the time of surgery, were used. Animals were housed two per group in a room maintained at 22 ± 0.5 °C with an alternating 12-h light–dark cycle. Food and water were available ad libitum. Each animal was used only once.

2.2. Procedures for the intraventricular drug injection and the tail-flick analgesiometric test

Rats were implanted stereotaxically with a guide cannula for intraventricular injection (i.vt.) into fourth ventricle. Rats were pretreated with methylatropine (5 mg/kg, i.p.), anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and then mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). A stainless-steel guide cannula, which was exactly 12 mm in length constructed from a 24-gauge thin-wall stainless-steel tubing (Samll Parts, Miami, FL) was positioned stereotaxically 0.3 mm anterior to the interaural point, 0.0 mm to the midline and inserted 3.0 mm down from the surface of the skull (Paxinos and Watson, 1998). The guide cannula was affixed to the skull with three stainless-steel screws (no. 0.8, 3.2 mm) and acrylic dental cement. Each guide cannula was then fitted with a 30-gauge stainless-steel stylet to keep it patent and free of debris. After a recovery period of 4 days, animals, which were without motor defects and with normal tail-flick latency, were used for the experiments.

The antinociceptive response was assessed with the thermal tail-flick test (Dewey and Harris, 1975). For measurement of the latency of the tail-flick response, the rat was held with one hand with the tail positioned in the tail-flick apparatus (Model TF6, EMDIE Instrument, 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 was adjusted so the animal flicked its tail within 3 to 4 s. After measuring the rat's baseline tail-flick latency, endomorphin-1 or endomorphin-2 was injected into the anterior fourth ventricular space and the tail-flick responses were then measured at different times after injection. The coordinates of anterior fourth ventricular space for drug injection was +0.3 mm from the interaural, 0.0 mm from the midline and 6.5 mm down from the surface of the skull (Paxinos and Watson, 1998). The doses of endomorphin-1 and endomorphin-2 for the i.vt. pretreatment were determined to be 30 and 60 nmol, respectively, based on the results obtained from the preliminary studies which indicated that endomorphin-1 and endomorphin-2 at these doses produced 90% to 100% of antinociception and the similar duration of antinociception. The first experiment was to determine the time course of the development and the recovery of the acute antinociceptive tolerance to endomorphin-1 and endomorphin-2. Groups of rats were pretreated with 30 and 60 nmol of endomorphin-1 and endomorphin-2, respectively, given i.vt. for different times before challenged given i.vt. with the same doses of endomorphin-1 and endomorphin-2, and the tail-flick responses were measured different times after each injection. The second experiment was to determine the antinociceptive tolerance and cross-tolerance to endomorphin-1 and endomorphin-2. Groups of rats were i.vt. pretreated with 30 and 60 nmol of endomorphin-1 and endomorphin-2, respectively, and injected i.vt. with a series of increasing doses of endomorphin-1 (1.64, 3.28, 11.48,

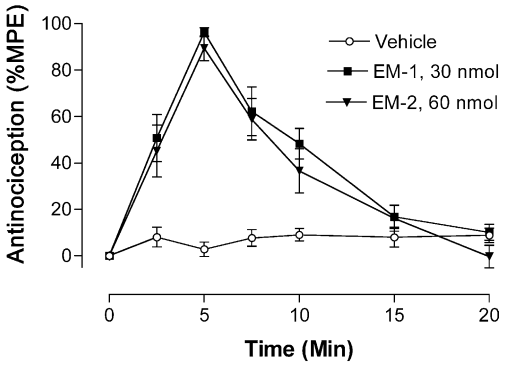

Fig. 1. Time course of changes of the tail-flick response to i.vt. administered endomorphin-1, endomorphin-2, and saline vehicle. Groups of rats were injected i.vt. with endomorphin-1 (30 nmol), endomorphin-2(60 nmol) and vehicle (10 μl) and the tail-flick responses were measured after each injection. Each value represents the mean, and the vertical bar represents the ± SEM with 9 to10 rats/group.

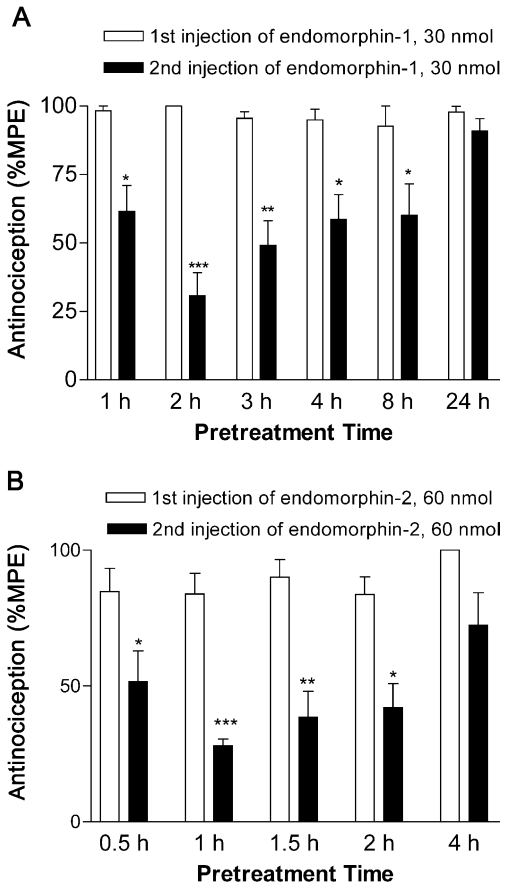

Fig. 2. Time course of the i.vt. pretreatment (first injection) with endomorphin-1 (A) and endomorphin-2 (B) on the inhibition of tail-flick response induced by i.vt. injection (second injection) of endomorphin-1 and endomorphin-2, respectively. Groups of rats were pretreated with endomorphin-1 (30 nmol) 1, 2, 3, 4, 8 or 24 h before i.vt. injection of endomorphin-1 (30 nmol), and the tail-flick responses were then measured after injection. (B) Groups of rats were pretreated i.vt. with endomorphin-2 (60 nmol) 0.5,1, 1.5, 2, or 4 h before i.vt. injection of endomorphin-2 (60 nmol), and tail-flick responses were then measured after injection. Each column represents the mean, and the vertical bar represents the SEM. with 7 to 10 rats/group. Student pair t test was used to determine the significant difference of % MPE between the first- and second-injected groups. *, $p<0.05$, **, $p<0.001$, ***, $p<0.0005$.

32.8 and 114.8 nmol) and endomorphin-2 (1.75, 3.50, 12.25, 35.0 and 122.5 nmol), respectively, and the tail-flick response was measured at 5 min after each injection. The final cumulative doses for endomorphin-1 and endomorphin-2 were 1.64, 4.92, 16.4, 49.2, and 164 nmol and 1.75, 5.25, 17.5, 52.5, and 175 nmol, respectively.

2.3. Drugs

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) were purchased from Calbiochem (La Jolla, CA). The peptides were dissolved in 10% hydroxypropyl-beta-cyclodextrin in sterile saline solution (0.9% NaCl solution) for i.vt. injection. Injection volume for each i.vt. injection was 10 μl.

2.4. Statistical analysis

The data of antinociception was expressed as "percent maximum possible effect (% MPE)", which is calculated as $[(T_1 - T_0)/(T_2 - T_0)] \times 100$. T_0 and T_1 were pre-drug and post-drug latency, respectively, whereas T_2 was the cutoff time that was set at 10 s to minimize tissue damage. The GraphPad Prism software (version 3.0, GraphPad Software, San Diego, CA) was used to calculate dose–response curves, ED_{50} values and their confidence intervals. Paired student t-test was used to analyze the statistical significant difference of the antinociception induced by endomorphin-1 or endomorphin-2.

3. Results

3.1. Time courses of the tail-flick response to i.vt. administration of endomorphin-1 and endomorphin-2

Groups of rats were injected i.vt. with endomorphin-1 (30 nmol), endomorphin-2 (60 nmol) or saline and the tail-flick response was measured 2.5, 5, 7.5, 10, 15 and 20 min after injection. Intraventricular injection of endomorphin-1 or endomorphin-2 produced inhibition of the tail-flick response. The inhibition of the tail-flick response after

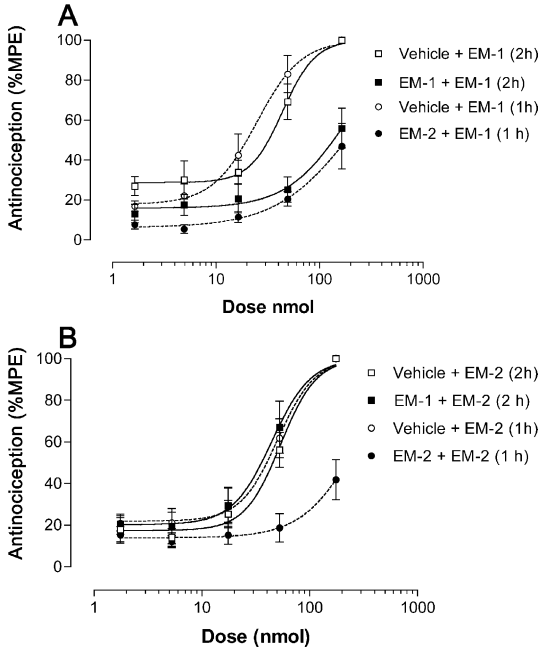

Fig. 3. Dose–response curves for the inhibition of the tail-flick response induced by i.vt. administered endomorphin-1 (EM-1; A) and endomorphin-2 (EM-2; B) in rats pretreated i.vt. with endomorphin-1, endomorphin-2, or vehicle. Groups of rats were pretreated i.vt. with endomorphin-1 (30 nmol) for 2 h, endomorphin-2 (60 nmol) for 1 h, and vehicle for 1 or 2 h before being injected i.vt. with increasing doses of endomorphin-1 (A) or endomorphin-2 (B) every 5 min. The tail-flick response was measured every 5 min after each injection. Each point represents the mean, and the vertical bar represents SEM with 6 to 10 rats/group.

Table 1
The antinociceptive potencies (ED_{50}) and the slope functions of the dose–response curves for i.vt. administered endomorphin-1 in rats pretreated with vehicle, endomorphin-1, or endomorphin-2

Pretreatment	Pretreatment time (h)	ED_{50} for endomorphin-1 (nmol) (95% CI)	Hill slope (95% CI)	Tolerance ratio[a]
Vehicle	2	44.1 (32.1–60.6)	2.7 (−0.3–5.8)	
Endomorphin-1	2	176.1[b] (110.7–280.2)	1.5 (0.0–3.0)	4.0
Vehicle	1	24.8 (17.1–36.0)	2.0 (0.8–3.2)	
Endomorphin-2	1	205.1[b] (126.9–331.3)	1.2 (0.2–2.1)	8.2

Groups of rats were pretreated with vehicle, endomorphin-1 (30 nmol), or endomorphin-2 (60 nmol) prior to the i.vt. injection of endomorphin-1 and the tail-flick responses were measured 5 min after injection. ED_{50} values and the slope functions were calculated based on the data shown in Fig. 2A.
CI, confidence interval.
[a] Tolerance ratio=(ED_{50} of endomorphin group)/(ED_{50} of vehicle group).
[b] $p<0.0001$, the ED_{50} of endomorphin compared with that of vehicle group.

i.vt. injection of endomorphin-1 or endomorphin-2 developed rapidly, reached its peaks at 5 min, gradually declined, and returned to the pre-injection level 20 min after injection. Injection of saline vehicle did not show any change in the latency of the tail-flick response (Fig. 1).

3.2. Time-courses of the development of acute antinociceptive tolerance to i.vt. injection of endomorphin-1 or endomorphin-2 after i.vt. pretreatment with endomorphin-1 or endomorphin-2, respectively

Groups of rats were first pretreated with endomorphin-1 (30 nmol) or endomorphin-2 (60 nmol) given i.vt. for various times before i.vt. injection of the same dose of endomorphin-1 or endomorphin-2, respectively. The tail-flick response was measured every 5 min after each injection. The first i.vt. injection of endomorphin-1 or endomorphin-2 produced consistently 85% to 100% MPE of the maximum tail-flick inhibition. The inhibition of the tail-flick response induced by endomorphin-1 or endomorphin-2 attenuated time-dependently by i.vt. pretreatment with endomorphin-1 or endomorphin-2, respectively. The attenuation of the endomorphin-1-induced tail-flick inhibition developed slowly, reached a maximal level at 2 h, and returned slowly to control level in 24 h. On the other hand, the attenuation of the endomorphin-2-induced tail-flick inhibition developed rather rapidly, reached a maximal level at 1 h, and returned to control level in 4 h (Fig. 2A and B). The 2 h of pretreatment times for endomorphin-1 and 1 h for endomorphin-2 were then used for the following experiments.

3.3. Effects of the i.vt. pretreatment with endomorphin-1 or endomorphin-2 on the tail-flick inhibition induced by endomorphin-1 or endomorphin-2 given i.vt.

Endomorphin-1 at doses between 1.64 and 164 nmol and endomorphin-2 at doses between 1.74 and 175 nmol given i.vt. dose-dependently inhibited the tail-flick response in rats pretreated with saline vehicle for 2 and 1 h, respectively. The endomorphin-1 was found to be about 1.2- to 2.0-fold more potent than endomorphin-2 in inhibiting tail-flick response. The i.vt. pretreatment with 30 nmol of endomorphin-1 for 2 h attenuated markedly the tail-flick inhibition induced by endomorphin-1, and the dose–response curve was shifted to the right by fourfold compared with that of rats pretreated with saline (Fig. 3A, Table 1). Similarly, the i.vt. pretreatment with 60 nmol of endomorphin-2 for 1 h attenuated markedly the tail-flick inhibition induced by endomorphin-2, and the dose–response curve was shifted to the right by 5.3-fold compared with that of rats pretreated with saline vehicle (Fig 3B, Table 2). The slope functions of dose–response curves for endomorphin-1- and endomorphin-2-induced tail-flick inhibition were also decreased after

Table 2
The antinociceptive potencies (ED_{50}) and the slope functions of the dose–response curves for i.vt. administered endomorphin-2 in rats pretreated with vehicle, endomorphin-1, or endomorphin-2

Pretreatment	Pretreatment time (h)	ED_{50} for endomorphin-2 (nmol) (95% CI)	Hill slope (95% CI)	Tolerance ratio[a]
Vehicle	2	53.7 (42.8–67.4)	2.6 (0.5–4.8)	
Endomorphin-1	2	44.5 (30.3–65.2)	2.4 (0.1–4.7)	0.8
Vehicle	1	50.4 (38.4–66.2)	2.5 (0.3–4.7)	
Endomorphin-2	1	267.3[b] (128.3–556.9)	1.7 (−0.8–4.3)	5.3

Groups of rats were pretreated i.vt. with vehicle, endomorphin-1 (30 nmol), or endomorphin-2 (60 nmol) prior to i.vt. injection of endomorphin-2 and the tail-flick responses were measured 5 min after injection. ED_{50} values and slope functions for endomorphin-2-induced antinociception were calculated based on the data shown in Fig. 2B.
CI, confidence interval.
[a] Tolerance ratio=(ED_{50} of endomorphin group)/(ED_{50} of vehicle group).
[b] $p<0.0001$, the ED_{50} of endomorphin compared with that of vehicle group.

the pretreatment with endomorphin-1and endomorphin-2, respectively.

The i.vt. pretreatment with 60 nmol of endomorphin-2 for 1 h markedly attenuated the tail-flick inhibition induced by endomorphin-1, and the dose–response curve for the endomorphin-1-induced tail-flick inhibition was shifted to the right by 8.2-fold compared with that of rats pretreated with vehicle for 1 h (Fig. 3A, Table 1). However, the i.vt. pretreatment with 30 nmol of endomorphin-1 for 2 h did not cause any significant change of the tail-flick inhibition induced by endomorphin-2; the ED_{50} value and the slope function of the curve for endomorphin-2-induced tail-flick inhibition was not affected by the pretreatment with endomorphin-1(Fig. 3B, Table 2).

4. Discussion

The antinociception induced by endomorphin-1 and endomorphin-2 has been previously demonstrated to be selectively mediated by the stimulation of μ-opioid receptors. This is supported by the findings that antinociception with the tail-flick test induced by endomorphin-1 and endomorphin-2 given supraspinally or spinally is blocked by the pretreatment with μ-opioid receptor antagonists β-funaltrexamine or CTOP, but not by δ-opioid receptor antagonists naltrindol or κ-opioid receptor antagonist norbinaltrophimine (Zadina et al., 1997; Goldberg et al., 1998; Stone et al., 1997; Tseng et al. 2000; Ohsawa et al., 2001). Endomorphin-1 or endomorphin-2 given spinally fail to induce any antinociception in μ-opioid receptor knockout mice (Mizoguchi et al., 1999).

Like other μ-opioid receptor agonists, the i.vt. pretreatment with a dose of endomorphin-1 or endomorphin-2 induced an acute antinociceptive tolerance to the subsequently challenging doses of i.vt.-administered endomorphin-1 or endomorphin-2, respectively. The results of our findings in rats are consistent with our previous report performed in mice (Wu et al., 2001), which demonstrates that intracerebroventricular pretreatment with endomorphin-1 or endomorphin-2 attenuates the antinociception induced by i.c.v.-administered endomorphin-1 or endomorphin-2, respectively. We also found in the present study in the rat that the antinociceptive tolerance caused by endomorphin-1 appeared to develop much slowly than that caused by endomorphin-2. The endomorphin-1-induced tolerance reached the maximal level at 2 h, recovered slowly in 24 h after the pretreatment with endomorphin-1, whereas endomorphin-2-induced antinociceptive tolerance developed in 1 h and recovered in 4 h. The different time courses in the development of the antinociceptive tolerance to endomorphin-1 and endomorphin-2 found in the present study in the rat are also consistent to the results found in the mouse (Wu et al., 2001). However, rats appear to recover from antinociceptive tolerance to endomorphin-1 and endomorphin-2 slower than that of mice. It is unlikely that the different time courses of the development of acute antinociceptive tolerance to endomorphin-1 and endomorphin-2 are due to different degrees of receptors stimulation by these two peptides. Both peptides at the doses used produced about the same duration of action and the same magnitude of peak effect. μ-Opioid receptor is one of many G-protein-coupled receptors which undergoes agonist-induced endocytosis. Such endocytosis has been implicated in the process of the downregulation and desensitization of receptors. It is possible that differences in time courses of the development and recovery of antinociceptive tolerance to endomorphin-1 and endomorphin-2 may be due to differences in the degree of receptor endocytosis induced by endomorphin-1 relative to endomorphin-2.

We found in the present study that rats made tolerant to endomorphin-1 by i.vt. pretreatment with endomorphin-1 exhibited nearly no cross-tolerance to endomorphin-2 to produce antinociception. On the other hand, rats made tolerant to endomorphin-2 exhibited a complete cross-tolerance to endomorphin-1 to produce antinociception. This unidirectional cross-tolerance between endomorphin-1 and endomorphin-2 found in the rat is consistent with our previous study in the mouse (Wu et al., 2001). Thus, the present finding adds an additional evidence to support the view proposed in our previous reports that different neuronal mechanisms are involved in antinociception induced by endomorphin-1 and endomorphin-2 (Tseng et al., 2000). We propose that two different subtypes of μ-opioid receptors are involved in antinociception induced by endomorphin-1 and endomorphin-2. One μ-subtype is stimulated by both endomorphin-1 and endomorphin-2 and another μ-subtype is solely stimulated by endomorphin-2. Thus, pretreatment with endomorphin-2 still attenuates the antinociception induced by endomorphin-1; however, pretreatment with endomorphin-1 is unable to attenuate the antinociception induced by endomorphin-2. Molecular studies in μ-opioid receptor cloning have found that there are at least seven isoforms of μ-opioid receptor RNAs (Pasternak, 2001a, b). However, it remains to be established that significant amounts of functional receptor protein forms with different sequences are expressed from these mRNAs and inserted appropriately into the plasma membrane of neurons in pathways regulating nociception.

Different subtypes of μ-opioid receptors, which are involved in endomorphin-1 and endomorphin-2-induced antinociception, have been reported. Pretreatment with μ1-opioid receptor antagonist naloxonazine is more effective in antagonizing the antinociception induced by endomorphin-2 than endomorphin-1. Also pretreatment with 3-methoxynaltrexone, a morphine-6 β-glucuronide antagonist, blocks the antinociception induced by endomorphin-2, but not endomorphin-1 (Sakurada et al., 1999, 2000). The descending pain control systems activated by endomorphin-1 and endomorphin-2 given supraspinally

for producing antinociception are also different. The antinociception induced by endomorphin-1 and endomorphin-2 is mediated by the activation of descending noradrenergic and serotonergic systems in the spinal cord. However, the antinociception induced by supraspinally administered endomorphin-2 contains an additional component, which is mediated by the release dynorphin A (1–17) acting on the κ-opioid receptors and the release of Met-enkephalin acting on δ_2-opioid receptors in the spinal cord (Ohsawa et al., 2000, 2001; Tseng et al., 2000). This view is evidenced by the findings that the pretreatment with antiserum against dynorphin A (1–17) or Met-enkephalin, or κ-opioid receptor antagonist norbinaltorphimine or δ_2-opioid receptor antagonist naltriben blocks the antinociception induced by endomorphin-2, but not endomorphin-1. These findings indicate that the antinociception induced by endomorphin-1 and endomorphin-2 is mediated by the stimulation of different subtypes of μ-opioid receptors.

It is concluded that i.vt. pretreatment of rats with endomorphin-1 and endomorphin-2 develops with different time courses the antinociceptive tolerance to endomorphin-1 and endomorphin-2, respectively. Rats made tolerant to endomorphin-2 exhibit an antinociceptive cross-tolerance to endomorphin-1, whereas rats made tolerance to endomorphin-1 show no cross-tolerance to endomorphin-2. We propose that different degree of endocytosis of μ-opioid receptors stimulated by endomorphin-1 relative to endomorphin-2 and/or two separate subtypes of μ-opioid receptors are involved in endomorphin-1 and endomorphin-2-induced antinociception.

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