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Intrathecal Treatment With 6-Hydroxydopamine or 5,7-Dihydroxytryptamine Blocks the Antinociception Induced by Endomorphin-1 and Endomorphin-2 Given Intracerebroventricularly in the Mouse

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Abstract. The involvement of spinopetal noradrenergic and serotonergic systems in antinociception induced by endomorphin-1 (EM-1) and endomorphin-2 (EM-2) given supraspinally or spinally were investigated in male CD-1 mice. Groups of mice were pretreated intrathecally (i.t.) with 6-hydroxydopamine (6-OHDA, 20 μ g) or 5,7-dihydroxytryptamine (5,7-DHT, 50 μ g) for 3 days before intracerebroventricular (i.c.v.) or i.t. injection of different doses of EM-1 or EM-2, and the tail-flick response was measured for antinociceptive effects. I.t. pretreatment with 6-OHDA for 3 days, which markedly depleted noradrenaline (NA) contents by more than 90%, but not serotonin (5-HT) in the spinal cord, completely abolished the antinociception induced by i.c.v.-administered EM-1 or EM-2. Intrathecal pretreatment with 5,7-DHT for 3 days, which markedly reduced 5-HT contents by more than 92%, but only reduced NA by 14–25% in the spinal cord, also markedly attenuated the antinociception induced by i.c.v.-administered EM-1 or EM-2. However, the antinociception induced by i.t.-administered EM-1 or EM-2 was not affected in either 6-OHDA or 5,7-DHT pretreated mice. It is concluded that NA and 5-HT in the spinal cord are involved in the antinociception induced by supraspinally, but not spinally administered EM-1 and EM-2.

Keywords: endomorphin, noradrenaline, serotonin, 6-hydroxydopamine, 5,7-dihydroxytryptamine

Introduction

Endomorphin-1 (EM-1) and endomorphin-2 (EM-2) are two tetrapeptides isolated from the bovine frontal cortex (1) and human brain (2) and are proposed to be the endogenous ligands for μ -opioid receptors. Receptor-binding assays and immunocytochemical studies reveal that EM-1 and EM-2 are highly selective for μ -opioid receptors and are localized in the regions of the brain and the spinal cord that are abundant in μ -opioid receptors (3–10). The release of EM-2 from the dorsal horn of the spinal cord by electrical stimulation and the inhibition of the electrical activity of spinal substantia

gelatinosa neurons or rostral ventrolateral medulla in vitro by EM-1 and EM-2 have been demonstrated (3, 10–12). Like other μ -opioid receptor agonists, EM-1 or EM-2 activates μ -opioid receptors mediated G-protein activation only in wild type mice, but not in μ -opioid receptor knockout mice (13). Intracerebroventricular (i.c.v.) or intrathecal (i.t.) injection of EM-1 or EM-2 produces potent antinociception that is selectively blocked by μ -opioid receptor antagonists, naloxone, β -funaltrexamine, or CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂) (1, 14–16). The antinociception induced by EM-1 or EM-2 is significantly attenuated in heterozygous knockout mice and virtually abolished in homozygous knockout mice (13). These findings clearly support the notion that EM-1 and EM-2 are indeed

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endogenous opioid ligands and the antinociception induced by these two peptides are mediated by the stimulation of μ -opioid receptors (17, 18).

The antinociception induced by μ -opioid agonists such as morphine or DAMGO (D-Ala²-N-methyl-phe⁴-Gly-ol⁵) given i.c.v. is mediated by the activation of spinopetal noradrenergic and serotonergic systems to release noradrenaline (NA) and serotonin (5-hydroxytryptamine, 5-HT). The released noradrenaline and 5-HT subsequently act on α_2 -adrenoceptors and 5-HT receptors, respectively, in the spinal cord to produce antinociception. This view for the antinociception of morphine is supported by the findings that depletion of NA and 5-HT in the spinal cord induced by i.t. pretreatment with 6-hydroxydopamine (6-OHDA) and 5,7-dihydroxytryptamine (5,7-DHT), respectively, attenuates the antinociception induced by morphine administered supraspinally (19–22). Also the blockade of the α_2 -adrenoceptor and 5-HT receptors in the spinal cord by i.t. treatment with yohimbine and methysergide blocks the antinociception induced by morphine given supraspinally (23). Since EM-1 and EM-2 are two endogenous ligands selectively for μ -opioid receptors, the neuronal mechanisms involved in activation of the descending system to produce antinociception will be expected to be similar to that of morphine. The present study was then designed to determine the effects of i.t. pretreatment with 6-OHDA and 5,7-DHT to deplete NA and 5-HT, respectively, on the antinociception induced by supraspinally administered EM-1 and EM-2.

Materials and Methods

Animals

Male CD-1 mice (Charles River Breeding Laboratory, Wilmington, MA), weighing 25–30 g were used. Animals were housed five per group in a room maintained at $22 \pm 0.5^\circ\text{C}$ with an alternating 12-h light-dark cycle. Food and water were available ad libitum. Animals were used only once.

Drugs

EM-1 (Tyr-Pro-Trp-Phe-NH₂) and EM-2 (Tyr-Pro-Phe-Phe-NH₂) were purchased from Calbiochem Corp. (La Jolla, CA, USA). The peptides were dissolved in sterile saline solution (0.9% NaCl solution) containing 10% hydroxypropyl- β -cyclodextrin for i.c.v. injection. 6-OHDA and 5,7-DHT (Sigma Chemical Company, St. Louis, MO, USA) were dissolved in 0.9% sterile saline containing 1 mg/ml (0.1%) ascorbic acid (Sigma Chemical Co.) to prevent them from being oxidized. Desipramine (Sigma Chemical Co.) was dissolved in 0.9% saline.

Assessment of the antinociceptive response

The antinociceptive response was assessed with the thermal tail-flick test (24). Mice were gently held with the tail positioned in the tail-flick apparatus (Model TF6; EMDIE Instrument Co., Maidens, VA, USA) for radiant heat stimulation of the dorsal surface of the tail. The intensity of the heat stimulus was adjusted to cause the animal to flick its tail within 3 to 4 s as the baseline of the tail-flick latency. After measuring the latency, different groups of mice were injected i.c.v. or i.t. with EM-1, EM-2, or vehicle; and tail-flick responses were then measured at different times after injection. The data were expressed as “percent maximum possible effect (%MPE)”, which was 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, and T_2 was the cutoff time that was set at 10 s to minimize tissue damage.

Protocols for drug administration

The neurotoxins, 6-OHDA (20 μg) and 5,7-DHT (50 μg), were injected i.t. to degenerate noradrenergic and serotonergic terminals in the spinal cord. Mice pretreated with 5,7-DHT were also injected intraperitoneally (i.p.) with 25 mg/kg of desipramine. The use of desipramine is to block the non-specific uptake of 5,7-DHT into noradrenergic terminals (25). The i.c.v. injection (4 μl) was performed according to the procedure described by Haley and McCormick (26), and i.t. injection (5 μl) was performed according to the procedure of Hylden and Wilcox (27) using a 25- μl Hamilton syringe with a 30-gauge needle.

Two sets of experiments were designed to determine the role of spinopetal noradrenergic and serotonergic systems in antinociception induced by EM-1 or EM-2 given supraspinally or spinally. Groups of mice were pretreated i.t. with 6-OHDA, 5,7-DHT with desipramine, or vehicle 3 days prior to i.c.v. or i.t. second injection of various doses of EM-1 or EM-2; and the tail-flick test was performed at 2.5, 5, 7.5, 10, 15, and 20 min thereafter. Mice were then sacrificed immediately after the antinociceptive tests and the spinal cord excised for measuring the contents of NA and 5-HT. To establish dose-response curves, at least four doses were used with 8 to 15 mice at each dose. For the calculation of the ED₅₀ values for EM-1- and EM-2-induced tail-flick inhibition, the antinociception was assessed using the peak effect, which occurred at either 2.5 or 5 min after injection.

Neurochemical analysis

Mice were killed by decapitation. A cut was made at the lumbar spine region. The spinal cord was then quickly pushed out by inserting a pipette tip connected to a 10-ml glass syringe prefilled with cold saline into the

lumbar end of the spinal cord. The spinal cord containing thoracic and lumbar regions was weighed and homogenized in 0.2 M perchloric acid solution, which also contains 100 μ M EDTA (ethylenediaminetetraacetic acid) (1 ml/200 mg of spinal tissue). The sample was centrifuged at $20,000 \times g$ for 15 min at 0°C . The clear supernatant was filtered using 0.45- μ m membrane filters for the assay of monoamines. The contents of noradrenaline and 5-HT in the spinal extract were determined by HPLC-ECD (high performance liquid chromatograph-electrochemical detector) methods as described by Sasa and Blank (28) and Ishikawa and McGaugh (29). A 10- μ l sample solution was injected

onto a reverse-phase column (C_{18} , 5 μ m, 3.0 mm inside diameter $\times$ 150 mm) with a guard pre-column having an amperometric detector (Eicom, Kyoto). The mobile phase (0.5 ml/min) was 15% (v/v) methanol containing 50 mM sodium acetate, 35 mM citric acid, 0.74 mM 1-octanesulfonic acid, and 13.4 μ M EDTA.

Statistical analyses

The data were expressed as the mean with S.E.M. The maximal %MPE was used to graph dose-response curves for EM-1 and EM-2. A two-way ANOVA followed by Bonferroni test was used to indicate the significance among groups for the time course of

Fig. 1. Time course of changes in the tail-flick inhibition induced by i.c.v.-injected endomorphin-1 (EM-1, A) and endomorphin-2 (EM-2, B) and i.t.-injected EM-1 (C) and EM-2 (D). Groups of mice were injected i.t. with vehicle (5 μ l) for 3 days and then i.c.v.- or i.t.-injected with different doses of EM-1, EM-2, or vehicle given i.c.v. (4 μ l) or i.t. (5 μ l), and tail-flick responses were measured at different times after injection. Each value represents the mean $\pm$ S.E.M. with 10 to 16 mice per group. A two-way ANOVA followed by Bonferroni test was used to test the difference among groups. * $P < 0.05$, ** $P < 0.01$ compared with the vehicle-treated group.

Fig. 2. Effects of i.t. pretreatment with 6-OHDA (A) and 5,7-DHT (B) on dose-response curves for the inhibition of the tail-flick response induced by i.c.v. administered endomorphin-1 (EM-1) or endomorphin-2 (EM-2). Groups of mice were pretreated with vehicle or 20 μ g of 6-OHDA for 3 days and followed by i.c.v. injection with various doses of EM-1 or EM-2 (A). Groups of mice were pretreated i.t. with vehicle or 50 μ g of 5,7-DHT (after desipramine) for 3 days and then injected with various doses of EM-1 or EM-2 given i.c.v. (B). Tail-flick responses were measured. Each value represents the mean $\pm$ S.E.M. with 8 to 13 mice per group. An F-test was used to analyze the statistical significance between dose-response curves. ** P < 0.01 compared with the vehicle-pretreated group. F value = 73.7 for 6-OHDA, i.t. / EM-1, i.c.v. vs Vehicle, i.t. / EM-1, i.c.v.; F value = 30.9 for 6-OHDA, i.t. / EM-2, i.c.v. vs Vehicle, i.t. / EM-2, i.c.v.; F value = 13.2 for 5,7-DHT, i.t. / EM-1, i.c.v. vs Vehicle, i.t. / EM-1, i.c.v.; F value = 9.2 for 5,7-DHT, i.t. / EM-2, i.c.v. vs Vehicle, i.t. / EM-2, i.c.v.

Table 1. Effects of i.t. pretreatment with 20 μ g of 6-OHDA and 50 μ g of 5,7-DHT (after desipramine) on ED₅₀ values for EM-1 and EM-2 given i.c.v. on the inhibition of the tail-flick response

i.t./i.c.v. ^a	ED ₅₀ (95% CI) nmol	Ratio (treatment/vehicle)
Vehicle/EM-1	6.8 (5.4 – 8.7)	
6-OHDA/EM-1	>280	>20
Vehicle/EM-2	12.9 (9.1 – 18.3)	
6-OHDA/EM-2	>280	>20
Vehicle/EM-1	5.9 (3.9 – 9.0)	
5,7-DHT/EM-1	30.6 (14.8 – 63.4)	5.2
Vehicle/EM-2	8.7 (5.4 – 14.1)	
5,7-DHT/EM-2	30.6 (21.3 – 43.9)	3.5

^aGroups of mice were pretreated with vehicle, 6-OHDA, or 5,7-DHT given i.t. for 3 days followed by a second injection with endomorphin-1 (EM-1) or endomorphin-2 (EM-2) given i.c.v.

changes. GraphPad Prism software (version 3.0; GraphPad Software, San Diego, CA, USA) was used to calculate dose-response curves, ED₅₀ values, and their confidence intervals. An F-test was used to analyze the significance of differences between dose response curves. A one-way ANOVA followed by Bonferroni test was used to determine the significance of the difference in spinal biogenic concentrations between the vehicle-pretreated group and neurotoxin-pretreated group.

Results

Effects of i.t. pretreatment with 6-OHDA or 5,7-DHT on the tail-flick inhibition induced by i.c.v.-administered EM-1 or EM-2

I.c.v. injection of EM-1 at doses of 3.3 – 16.4 nmol (Figs. 1A, 2A, and 2B) and EM-2 at doses of 3.5 – 35 nmol (Figs. 1B, 2A and 2B) dose-dependently produced the inhibition of the tail-flick response in mice pretreated i.t. with vehicle. The inhibition of the tail-flick response induced by EM-1 and EM-2 developed rapidly, reached their peaks at 5 min, declined rapidly, and returned to the pre-injection level in 20 min. A dose of 16.4 nmol of EM-1 or 35 nmol of EM-2 produced about 80%MPE at 5 min after injection. I.t. pretreatment with 6-OHDA completely abolished the tail-flick inhibition induced by EM-1 and EM-2. EM-1 or EM-2 even at high doses failed to produce any tail-flick inhibition (Fig. 2A). Likewise, i.t. pretreatment with 5,7-DHT also caused a marked attenuation of the tail-flick inhibition induced by EM-1 or EM-2, and the dose-response curves for EM-1 and EM-2 to inhibit the tail-flick response were shifted to the right by 5.2- and 3.5-fold, respectively (Fig. 2B, Table 1). I.t. pretreatment with 6-OHDA and 5,7-DHT did not alter the base line tail-flick latencies in mice injected with saline; the tail-flick latencies measured 5 min after i.c.v. injection of saline in mice pretreated with vehicle, 6-OHDA,

Fig. 3. Effects of i.t. treatment with 6-OHDA (A) and 5,7-DHT (B) on dose-response curves for the inhibition of the tail-flick response induced by i.t.-administered endomorphin-1 (EM-1) or endomorphin-2 (EM-2). Groups of mice were pretreated with vehicle or 20 μ g of 6-OHDA for 3 days and followed by i.t. injection with various doses of EM-1 or EM-2 (A). Groups of mice were pretreated i.t. with vehicle or 50 μ g of 5,7-DHT (after desipramine) for 3 days and then injected with various doses of EM-1 or EM-2 given i.t. (B). Tail-flick responses were measured. Each value represents the mean $\pm$ S.E.M. with 8 to 15 mice per group. An F-test was used to analyze the statistical significance between dose-response curves. F value = 0.07 for 6-OHDA, i.t. / EM-1, i.c.v. vs Vehicle, i.t. / EM-1, i.c.v.; F value = 0.1 for 6-OHDA, i.t. / EM-2, i.t. vs Vehicle, i.t. / EM-2, i.t.; F value = 0.16 for 5,7-DHT, i.t. / EM-1, i.t. vs Vehicle, i.t. / EM-1, i.t.; F value = 0.9 for 5,7-DHT, i.t. / EM-2, i.t. vs Vehicle, i.t. / EM-2, i.t.

Table 2. Effects of i.t. pretreatment with 20 μ g of 6-OHDA and 50 μ g of 5,7-DHT (after desipramine) on ED₅₀ values for EM-1 and EM-2 given i.t. on the inhibition of the tail-flick response

i.t./i.t. ^a	ED ₅₀ (95% CI) nmol	Ratio (treatment/vehicle)
Vehicle/EM-1	5.1 (3.5 – 7.3)	0.9
6-OHDA/EM-1	4.6 (3.1 – 6.9)	
Vehicle/EM-2	12.1 (7.7 – 19.0)	1.1
6-OHDA/EM-2	13.8 (8.5 – 22.5)	
Vehicle/EM-1	5.0 (3.6 – 7.0)	1.1
5,7-DHT/EM-1	5.6 (4.3 – 7.3)	
Vehicle/EM-2	8.2 (5.7 – 11.8)	1.4
5,7-DHT/EM-2	11.2 (7.9 – 15.9)	

^aGroups of mice were pretreated with vehicle, 6-OHDA, or 5,7-DHT given i.t. for 3 days followed by a second injection with endomorphin-1 (EM-1) or endomorphin-2 (EM-2) given i.t.

vehicle with desipramine, and 5,7-DHT with desipramine were 3.3 ± 0.05 , 3.3 ± 0.07 , 3.3 ± 0.08 , and 3.2 ± 0.06 s ($n = 40$ for each group), respectively.

Effects of i.t. pretreatment with 6-OHDA or 5,7-DHT on the tail-flick inhibition induced by i.t.-administered EM-1 or EM-2

I.t. injection of EM-1 at doses of 3.3 – 16.4 nmol and EM-2 at doses of 3.5 – 35.0 nmol dose-dependently

produced the inhibition of the tail-flick response in mice pretreated i.t. with vehicle. The inhibition of the tail-flick response induced by EM-1 and EM-2 developed rapidly, reached their peaks at 5 min, declined rapidly, and returned to the pre-injection level in 20 min. A dose of 16.4 nmol of EM-1 or 35 nmol of EM-2 produced about 80%MPE at 5 min after injection (Fig. 1: C and D). I.t. pretreatment with 6-OHDA or 5,7-DHT did not affect the tail-flick inhibition induced by i.t.-administered EM-1 or EM-2 (Fig. 3: A and B). The ED₅₀ values for EM-1 and EM-2 to inhibit the tail-flick response were not changed in mice pretreated i.t. with 6-OHDA or 5,7-DHT (Table 2).

Effects of i.t. pretreatment with 6-OHDA and 5,7-DHT on NA and 5-HT contents in the spinal cord

Acute i.c.v. injection of EM-1 (3.3 to 16.4 nmol) or EM-2 (3.5 to 70 nmol) even at high doses did not cause any significant change of the steady state level of NA or 5-HT of the spinal cord in mice pretreated with vehicle for 3 days. Compared to vehicle-pretreated mice, i.t. pretreatment with 6-OHDA markedly depleted NA contents in the spinal cord by more than 90%, but did not cause any change of 5-HT contents of the spinal cord in mice acutely injected with different doses of EM-1 and EM-2 (Table 3). I.t. pretreatment with 5,7-DHT with desipramine for 3 days markedly reduced 5-

Table 3. Effects of i.t. pretreatment with vehicle or 20 µg of 6-OHDA on spinal biogenic amine concentrations in mice 3 days before treated with i.c.v. vehicle, EM-1, or EM-2 at various concentrations

i.t./i.c.v. ^a		Spinal biogenic amine concentration (ng/g)	
		NA	5-HT
Vehicle/Vehicle		556 ± 35.4	1195 ± 51.9
Vehicle/EM-1	6.6 nmol	544 ± 27.0	1027 ± 42.6
	13.1 nmol	555 ± 36.5	1117 ± 78.2
	16.4 nmol	540 ± 51.2	1100 ± 97.4
6-OHDA/EM-1	6.6 nmol	51.4 ± 13.7***	1184 ± 85.5
	13.1 nmol	30.0 ± 5.3***	1088 ± 64.0
	16.4 nmol	44.9 ± 15.2***	1104 ± 54.1
Vehicle/EM-2	7.0 nmol	508 ± 64.5	1156 ± 82.0
	17.5 nmol	515 ± 45.0	1078 ± 91.5
	35.0 nmol	571 ± 49.9	1050 ± 44.0
6-OHDA/EM-2	7.0 nmol	69.8 ± 12.3***	1077 ± 58.3
	17.5 nmol	45.3 ± 11.3***	1054 ± 40.4
	35.0 nmol	44.7 ± 12.0***	1113 ± 32.8

^aGroups of mice were pretreated with vehicle or 6-OHDA given i.t. for 3 days followed by a second injection with vehicle, endomorphin-1 (EM-1), or endomorphin-2 (EM-2) given i.c.v. Each value represents the mean ± S.E.M. with 8 to 15 mice. ****P*<0.001, compared with the vehicle/EM group, by one-way ANOVA followed by Bonferroni test.

Table 4. Effects of i.t. pretreatment with vehicle or 50 µg of 5,7-DHT (after desipramine) on spinal biogenic amine concentrations in mice treated with i.c.v. EM-1 or EM-2 at various concentrations

i.t./i.c.v. ^a		Spinal biogenic amine concentration (ng/g)	
		NA	5-HT
Vehicle/Vehicle		556 ± 35.4	1195 ± 51.9
Vehicle/EM-1	6.6 nmol	463 ± 43.5	1011 ± 41.1
	13.1 nmol	538 ± 18.1	1226 ± 63.3
	16.4 nmol	523 ± 29.7	1085 ± 38.0
5,7-DHT/EM-1	6.6 nmol	360 ± 28.8	79.4 ± 17.5***
	13.1 nmol	431 ± 36.6	90.6 ± 18.8***
	16.4 nmol	402 ± 24.5	70.1 ± 13.7***
Vehicle/EM-2	7.0 nmol	489 ± 41.1	1160 ± 50.2
	17.5 nmol	579 ± 39.9	1237 ± 74.8
	35.0 nmol	532 ± 40.8	1042 ± 29.4
5,7-DHT/EM-2	7.0 nmol	423 ± 28.3	103.4 ± 18.9***
	17.5 nmol	399 ± 26.0*	85.8 ± 13.6***
	35.0 nmol	430 ± 46.6	113.4 ± 16.0***

^aGroups of mice were pretreated with vehicle or 5,7-DHT given i.t. for 3 days followed by a second injection with vehicle, endomorphin-1 (EM-1) or endomorphin-2 (EM-2) given i.c.v. Each value represents the mean ± S.E.M. with 8 to 12 mice. **P*<0.05, ****P*<0.001, compared with the vehicle/EM group, by one-way ANOVA followed by Bonferroni test.

HT contents in the spinal cord by more than 92% in mice acutely injected with different doses of EM-1 or EM-2, while NA contents in the spinal cord was only reduced by 14% to 25% (Table 4).

Discussion

EM-1 at doses of 3.3 to 16.4 nmol or EM-2 at doses of 3.5 to 35.0 nmol given supraspinally or spinally dose-dependently induced the antinociception with the tail-

flick test in mice pretreated with vehicle. The results of the present studies in time course and the potencies are consistent with our previous findings (30, 31).

EM-1 and EM-2 are two endogenous opioid receptor ligands for μ -opioid receptors (1). The antinociceptive effects induced by EM-1 or EM-2 given supraspinally by i.c.v. administration are mediated by the stimulation of μ -opioid receptors. This view is evidenced by the findings that the antinociception induced by EM-1 or EM-2 is selectively blocked by the pretreatment with the μ -opioid receptor antagonists, naloxone, β -funaltrexamine, or CTOP (4, 8, 14, 16, 17).

It has been well documented that the antinociception with the tail-flick test induced by other μ -opioid agonists such as morphine or DAMGO given supraspinally is mediated by the activation of spinopetal noradrenergic and serotonergic systems and involve the releases of noradrenaline and 5-HT acting on α_2 -adrenoceptors and 5-HT receptors, respectively, in the spinal cord (32 – 34). This view is supported by the finding that i.t. pretreatment with 6-OHDA or DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine) to deplete noradrenaline or i.t. pretreatment with 5,7-DHT to deplete 5-HT effectively attenuates the antinociception induced by morphine given supraspinally (20 – 22). Also the blockade of α_2 -adrenoceptors and 5-HT receptors by i.t. treatment with the α_2 -adrenoceptor blocker yohimbine and 5-HT receptor blocker methysergide effectively blocks the antinociception induced by morphine given supraspinally (23, 32, 34). Since EM-1 and EM-2 are two selective endogenous opioid ligands of the μ type, the neural mechanisms for the production of antinociception will be expected to be the same as that of other μ -opioid agonists such as morphine or DAMGO. We found in the present studies that i.t. pretreatment with 6-OHDA for 3 days, which effectively depleted NA by more than 90%, but not 5-HT, completely abolished the antinociception induced by i.c.v.-administered EM-1 or EM-2. The result strongly indicates that the release of NA in the spinal cord plays an essential role in the inhibition of the tail-inhibition induced by supraspinally administered EM-1 and EM-2. I.t. pretreatment with 5,7-DHT for 3 days, which depleted 5-HT by more than 92%, but also reduced NA by 14% to 25%, attenuated the antinociception induced by i.c.v. administration of EM-1 or EM-2 by 5.2- or 3.5-fold. The results of our study indicate that the spinopetal noradrenergic pathway is more important than the serotonergic pathway in mediating the antinociception activated by supraspinally-administered EM-1 and EM-2. However, 5-HT in the spinal cord may also be involved in the tail-flick inhibition induced by supraspinally administered EM-1 and EM-2. We have previously demonstrated that the

blockade of either α_2 -adrenoceptors by i.t. treatment with yohimbine or 5-HT receptors by i.t. pretreatment with methysergide effectively blocks the antinociception induced by i.c.v.-administered EM-1 or EM-2 (30). More studies are needed to determine the relative importance of these two pathways in the spinal cord involved in supraspinally-administered EM-1 and EM-2-induced antinociception.

In addition to the monoaminergic descending pain control systems, which are activated by EM-1 and EM-2, we have previously reported that two additional opioidergic pathways are also involved in antinociception induced by supraspinally-administered EM-2, but not EM-1 (17). This antinociception activated by EM-2 supraspinally is mediated in part by the release of Met-enkephalin and dynorphin A(1 – 17) acting on DOP2-R and KOP-R, respectively, in the spinal cord. Since we found in the present study that depletion of noradrenaline and 5-HT by 6-OHDA and 5,7-DHT pretreatment, respectively, completely abolished the antinociception induced by EM-2 given supraspinally, it is most likely that these additional opioidergic pathways play a minor role in the overall supraspinal EM-2-induced antinociception.

Like the other μ -opioid receptor agonists morphine or DAMGO, EM-1 or EM-2 given spinally produces its antinociception by direct stimulation of μ -opioid receptors located on the dorsal horn of the spinal cord. This view is supported by the finding that the antinociception induced by i.t.-administered EM-1 or EM-2 is blocked by the i.t. pretreatment with μ -opioid receptor antagonist β -funaltrexamine or CTOP (31). We found in the present studies that the same i.t. pretreatment with 6-OHDA or 5,7-DHT did not affect the antinociception induced by i.t.-administered EM-1 and EM-2. The result clearly indicates that NA and 5-HT in the spinal cord are not involved in the antinociception induced by spinally-administered EM-1 and EM-2.

It is concluded that EM-1 and EM-2 given supraspinally activate spinopetal noradrenergic and serotonergic systems and release NA and 5-HT in the spinal cord for the production of antinociception. On the other hand, NA and 5-HT in the spinal cord are not involved in the antinociception induced by spinally administered EM-1 and EM-2.

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