

Analgesic effect of endomorphin-1

LI Zheng-Hong¹, SHAN Li-Dong, JIANG Xing-Hong, GUO Shi-Yu, YU Guang-Di, HISAMITSU Tadashi², YIN Qi-Zhang³ (Laboratory of Neurobiology, School of Medicine, Soochow University, Suzhou 215007, China; ²Department of Physiology, School of Medicine, Showa University, Tokyo 142, Japan)

KEY WORDS endomorphins; analgesia; mu opioid receptors; drug tolerance; naloxone; cyprodime

ABSTRACT

AIM: To study the analgesic effect of endomorphin-1 (EM-1). **METHODS:** The experiment was performed in rats and mice to study the analgesic effect of intraperitoneal (ip) injection of EM-1 with tail stimulation-vocalization test, writhing test, adjuvant arthritis, and neuropathic pain model and to compare it with the analgesic effects produced by intracerebroventricular (icv) and intrathecal (it) administrations. **RESULTS:** 1) EM-1 raised the pain threshold dose-dependently in tail stimulation-vocalization test in rats and inhibited the writhing responses induced by ip acetic acid in mice. EM-1 also decreased the hyperalgesia in both adjuvant arthritis and neuropathic pain model. 2) The analgesic effect induced by central (icv and it) administration of EM-1 was faster and more powerful than that induced by peripheral (ip) administration. 3) The analgesic effect of EM-1 was reversed by naloxone (opioid receptor antagonist), as well as by cyprodime (μ -opioid receptor selective antagonist). Repeated administrations of EM-1 induced tolerance. **CONCLUSION:** EM-1 had a definite analgesic effect and the analgesic effect of EM-1 was mediated by central μ -opioid receptor.

INTRODUCTION

Two potent endogenous opioid peptides, endomorphin-1 (EM-1) and endomorphin-2 (EM-2), which are selective agonists for the μ -opioid receptor, have been recently isolated from bovine^[1] and human^[2] brain. These

endomorphins have been shown to produce analgesia and inhibit the electric stimulation-induced contraction of guinea pig ileum, which is consistent with the action of μ -opioid receptor agonists^[1]. Immunoreactivity of EM-1 and EM-2 has been found in the central nervous system (CNS), in regions rich in μ -opioid receptor^[1,3-5]. Furthermore, both the peptides bound with high affinity to the μ -opioid receptor^[1] and displayed no activity in μ -opioid receptor knock-out mice^[6], which suggests that these peptides induce antinociception via μ -opioid receptor-specific mechanism.

Until now the analgesic effect of EM was measured principally with tail-flick test, when administered intrathecally (it) or intracerebroventricularly (icv) to mice^[1,7]. However, it is well known that morphine elicits analgesic effect not only by central (icv, it) administration, but also by peripheral (intravenous or intraperitoneal) administration. The present study was, therefore, undertaken to investigate the analgesic effect of intraperitoneal (ip) administration of EM-1 with different nociceptive tests and compare it with that induced by it and icv administrations, and the mechanism of analgesia was analyzed.

MATERIALS AND METHODS

Animals and drugs All experiments were carried out on male Wistar rats weighing 180-250 g and Kunming mice weighing 18-22 g, which were provided by the Experimental Animal Center, School of Medicine Soochow University (Grade II, Certificate No 98001).

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂) was synthesized by Peptide Institute Co, Japan. Naloxone hydrochloride (lot 990627) was produced in Hunan Yiqiao Pharmaceutical Co, morphine hydrochloride (lot 991203) from Shenyang First Pharmaceutical Factory. Killed *Mycobacterium tuberculosis* (lot 980601) was purchased from Shanghai Institute of Biological Products. Cyprodime was purchased from Sigma Chemical Co. All the drugs, when necessary, were dissolved in 0.9 %

¹Now in Department of Physiology, Bengbu Medical College, Bengbu 233003, China.

³Correspondence to Prof YIN Qi-Zhang.

Phn 86-512-520-7982. Fax 86-512-520-7982.

E-mail: yinqz@pub.sz.jsinfo.net

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normal saline.

Nociceptive tests Analgesic effect of EM-1 for the acute somatic pain was measured using tail stimulation-vocalization test in rats⁽⁸⁾. The tail was stimulated with an incremental current (mA) until the vocalization (taken as threshold) was elicited. To minimize the potential tissue damage, a cut-off current of 1.0 mA was employed. The measurements were taken 3 times at 5 min intervals and their mean was used for calculation.

The effect of EM-1 was estimated in a time-course profile. Measurements were taken before the EM-1 administration and again at 15, 30, 60, 90, and 120 min after injection. Normal saline was injected as negative control and morphine as positive control. The experiments were also designed to find out whether the non-selective antagonist of opioid receptor, naloxone, and the selective antagonist of μ -opioid receptor, cyprodime, could reverse the analgesic effect of EM-1. Naloxone (1 mg/kg, ip) and cyprodime (30 μ g in 10 μ L, icv) were injected, respectively, 10 min before EM-1 administration.

The analgesic effect of EM-1 for visceral pain was examined with writhing test in mice⁽⁹⁾, which was carried out at 15, 30, 60, 90, and 120 min after injection of EM-1. The effect was evaluated by counting the number of writhing responses during 10 min after ip 0.2 mL of 0.06% acetic acid, and the inhibition rate was calculated as compared with control group.

The analgesic effect of EM-1 on inflammatory pain was studied with adjuvant arthritis in rats⁽¹⁰⁾. Freund's complete adjuvant 0.1 mL with killed *Mycobacterium tuberculosis* (10 g/L) was injected subcutaneously in the right ankle. Rats injected with Freund's adjuvant displayed impaired motor activity and local inflammation in the ankle, and the hyperalgesia was observed by radiant heat-withdrawal test. The analgesic effect of EM-1 was examined 1 week (acute phase) and 4 weeks (chronic phase) thereafter.

Neuropathic pain model was also used to evaluate the analgesic effect of EM-1. The rats were anesthetized and the right sciatic nerve was partly (1/3 - 1/2) tied according to the method of Seltzer *et al*⁽¹¹⁾. The observation of the analgesic effect of EM-1 was similar to the protocol used in the case of adjuvant arthritis, eg. at 1 week (acute phase) and 4 weeks (chronic phase) after operation.

Injection techniques Intraperitoneal injection was carried out conventionally. Intrathecal injection was performed through catheter inserted 7 d before using the

method of Yaksh and Rudy⁽¹²⁾. An incision was made at the base of the skull to expose the atlanto-occipital membrane. A slit was made in the membrane to insert a PE-10 tubing catheter, which was then passed caudally 7.5 cm in the subarachnoid space to lie at the lumbar enlargement. Animals exhibiting any signs of neurological motor deficit were discarded. Intracerebroventricular injection was performed through cannula, targeted to the left ventricle, implanted 3 d before experiment.

Observation of tolerance To observe the development of tolerance of EM-1-induced analgesia, rats were given intraperitoneally at a dose of 25 μ g \cdot kg⁻¹ \cdot d⁻¹, once a day, for 9 d. On d 0, 1, 3, 5, 7, and 9 after the beginning of injection and on d 3, 6, 9, and 12 after the cessation of injection, the analgesia effect of EM-1 was evaluated using tail stimulation-vocalization test in order to observe the development and the recovery of tolerance.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared by *t* test.

RESULTS

Analgesic effect of EM-1 as revealed by tail stimulation-vocalization test Intraperitoneal injection of EM-1 at different doses (12.5, 25, and 50 μ g/kg) resulted in a dose-dependent increase of vocalization threshold, with a peak effect at 30 min after injection. The time-course study indicated that the duration of effect was prolonged as the dose of EM-1 increased. The analgesia persisted for 120 min in the case of 50 μ g/kg, showing the similar time course of morphine (3 mg/kg), while no analgesic effect was observed after injection of normal saline (Fig 1).

A similar dose-dependent analgesic effect was observed when EM-1 was injected intrathecally or intracerebroventricularly, but the peak effect was advanced and appeared at 15 min after injection and the intensity of analgesia was greater than that observed in the case of intraperitoneal injection and reached statistical significance ($P < 0.01$) at dose of 50 μ g/kg (Fig 2).

The analgesic effect of EM-1 was effectively reversed by opioid receptor antagonist, naloxone, as well as by μ -opioid receptor selective antagonist, cyprodime (Tab 1).

Analgesic effect of EM-1 in writhing test After injection of EM-1 the number of writhing responses during 10 min after acetic acid injection was inhibited and the inhibition rate was 40.1%, 26.8%, and 23.9%

at 15, 30, and 60 min, respectively (Tab 2).

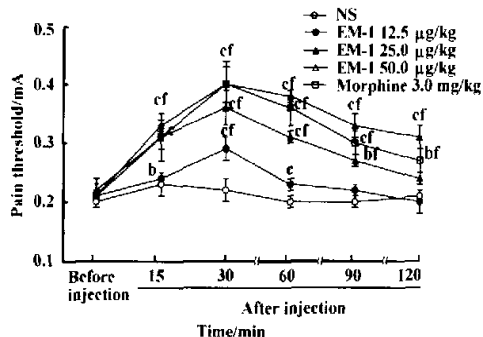


Fig 1. Analgesic effect of intraperitoneal injection of EM-1 in tail stimulation-vocalization test. $n = 7 - 9$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs before injection. $^dP < 0.05$, $^eP < 0.01$ vs NS.

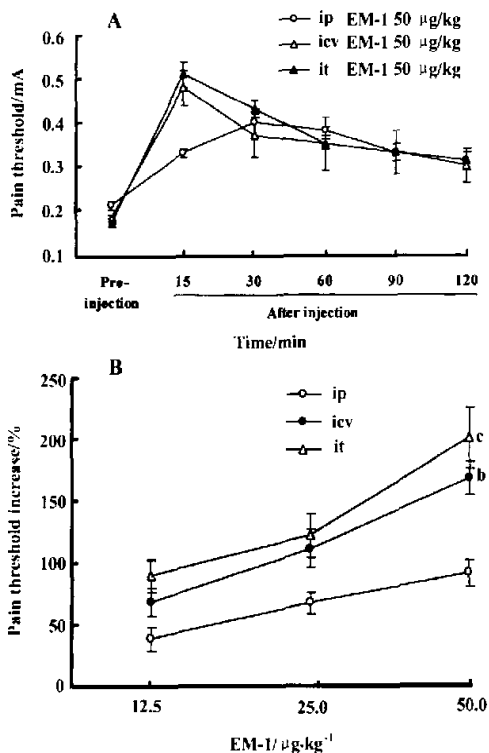


Fig 2. Comparison of peak analgesic effect of EM-1 among three different routes of injection in tail stimulation-vocalization test. A: Time course comparison; B: Intensity comparison. $n = 6 - 9$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs ip group.

The analgesic effect of EM-1 on adjuvant

arthritis rats One week (acute phase) and 4 weeks (chronic phase) after inoculation of adjuvant, local thermal hyperalgesia was observed as usual. At these time points intraperitoneal injection of EM-1 (50 µg/kg) prolonged withdrawal latencies (Tab 3).

The analgesic effect of EM-1 on neuropathic pain in rats Just as in adjuvant arthritis, after ligation of sciatic nerve thermal hyperalgesia developed at 1 week and 4 weeks after operation. At these two time points intraperitoneal injection of EM-1 (50 µg/kg) prolonged withdrawal latencies greatly (Tab 3).

Tolerance development Repeated injections of EM-1 resulted in a gradual reduction of analgesic effect. At first day of injection the pain threshold was increased by 72 % ± 14 %, while on 3, 5, 7, and 9 d of repeated injections the increase of pain threshold was decreased gradually (Fig 3). The results indicated that the tolerance was developed.

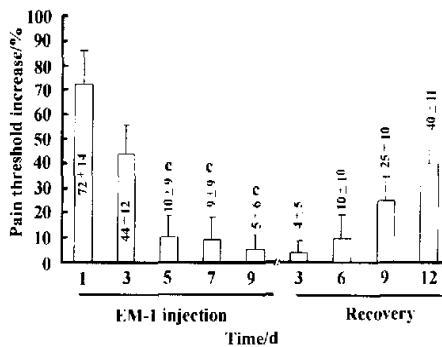


Fig 3. Development of tolerance of analgesia induced by EM-1 (25 µg·kg⁻¹·d⁻¹, ip) and its recovery in tail stimulation-vocalization test. $n = 6$ rats. $\bar{x} \pm s$. $^cP < 0.01$ vs 1 d.

DISCUSSION

In the majority of previous studies the analgesia of EM-1 was demonstrated principally in tail-flick test by central (icv and it) administration^[1,7,13-15]. In the present study, the similar dose-dependent analgesia was also obtained by peripheral (ip) administration, but the peak analgesic effect appeared later in time course and less in intensity than that induced by central (icv and it) administration. Both the later appearance and more weak intensity of peak analgesic effect by peripheral administration suggest that the analgesia induced by EM-1 is central in nature.

Tab 1. Reversal of EM-1 (50 µg/kg)-induced analgesia by naloxone (1 mg/kg, ip) and cyprodime (30 µg, icv) in tail stimulation-vocalization test. n=6 rats. $\bar{x} \pm s$. ^bP < 0.05, ^cP < 0.01 vs NS. ^eP < 0.05, ^fP < 0.01 vs EM-1 + NS.

| | Before injection | Pain threshold/ mA | | | | |
|------------------|------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | | 15 | 30 | After injection/min | | 120 |
| | | | | 60 | 90 | |
| NS | 0.204 ± 0.011 | 0.225 ± 0.018 | 0.217 ± 0.021 | 0.196 ± 0.007 | 0.197 ± 0.012 | 0.207 ± 0.009 |
| Naloxone | 0.192 ± 0.010 | 0.200 ± 0.013 | 0.179 ± 0.008 | 0.188 ± 0.009 | 0.194 ± 0.005 | 0.203 ± 0.011 |
| Em-1 + NS | 0.209 ± 0.016 | 0.332 ± 0.020 ^c | 0.42 ± 0.03 ^c | 0.379 ± 0.022 ^e | 0.310 ± 0.019 ^b | 0.305 ± 0.006 ^c |
| Em-1 + Naloxone | 0.21 ± 0.03 | 0.27 ± 0.07 | 0.26 ± 0.05 ^f | 0.261 ± 0.017 ^f | 0.23 ± 0.03 ^e | 0.28 ± 0.06 |
| NS | 0.177 ± 0.005 | 0.236 ± 0.019 | 0.22 ± 0.03 | 0.214 ± 0.021 | 0.207 ± 0.012 | 0.204 ± 0.010 |
| Cyprodime | 0.183 ± 0.014 | 0.201 ± 0.007 | 0.190 ± 0.005 | 0.187 ± 0.010 | 0.183 ± 0.009 | 0.192 ± 0.008 |
| Em-1 + NS | 0.191 ± 0.011 | 0.51 ± 0.06 ^e | 0.37 ± 0.04 ^e | 0.35 ± 0.06 ^b | 0.33 ± 0.05 ^b | 0.30 ± 0.04 ^b |
| Em-1 + Cyprodime | 0.198 ± 0.021 | 0.221 ± 0.016 ^f | 0.215 ± 0.017 ^f | 0.198 ± 0.010 ^f | 0.200 ± 0.010 ^e | 0.215 ± 0.006 ^e |

Tab 2. Inhibitory effect of EM-1 (50 µg/kg, ip) on writhing responses in mice. n=10. $\bar{x} \pm s$. ^bP < 0.05, ^cP < 0.01 vs NS control group.

| Time/min | NS | EM-1 | | Morphine | |
|----------|-----------------|-------------------------|--------------|-------------------------|--------------|
| | Writhing number | Writhing number | Inhibition/% | Writhing number | Inhibition/% |
| 15 | 29.7 ± 2.1 | 17.8 ± 0.8 ^e | 40.1 | 15.5 ± 2.0 ^c | 47.8 |
| 30 | 25.7 ± 2.3 | 18.8 ± 1.4 ^b | 26.8 | 13.0 ± 1.6 ^c | 49.4 |
| 60 | 27.6 ± 1.1 | 21.0 ± 2.3 ^b | 23.9 | 13.5 ± 1.3 ^c | 51.1 |
| 90 | 27.4 ± 1.8 | 23.5 ± 1.3 | 14.2 | 15.5 ± 1.5 ^c | 43.4 |
| 120 | 26.4 ± 1.4 | 25.1 ± 2.5 | 4.9 | 16.1 ± 1.5 ^c | 39.0 |

Tab 3. Analgesic effect of EM-1 (50 µg/kg, ip) on hyperalgesia in adjuvant arthritis test and in neuropathic pain model. n=6 rats. $\bar{x} \pm s$. ^cP < 0.01 vs control.

| | Baseline | Latency/s | | | |
|---------------------------|--------------|------------------|------------------------|------------------|------------------------|
| | | Acute phase | | Chronic phase | |
| | | Before injection | After injection | Before injection | After injection |
| Adjuvant arthritis | | | | | |
| Control | 14.5 ± 0.8 | 3.2 ± 0.8 | 5.4 ± 0.5 | 4.5 ± 1.0 | 4.3 ± 1.0 |
| EM-1 | 14.95 ± 0.12 | 3.8 ± 0.7 | 9.4 ± 1.4 ^c | 4.5 ± 0.6 | 9.1 ± 0.9 ^c |
| Neuropathic pain | | | | | |
| Control | 15.00 ± 0.00 | 3.27 ± 0.20 | 3.42 ± 0.16 | 3.49 ± 0.27 | 3.77 ± 0.26 |
| EM-1 | 15.00 ± 0.00 | 3.06 ± 0.17 | 7.1 ± 0.4 ^c | 3.41 ± 0.18 | 7.2 ± 0.5 ^c |

In the field of analgesia study, it is recognized that the results of experiments vary with the nociceptive methods¹⁶⁻¹⁹, and it would be reasonable to use different nociceptive tests in order to obtain a full aspect of evaluation. In the present experiment, the analgesia of EM-1 was observed in different nociceptive tests. The tail stimulation-vocalization test represents acute skin pain, while writhing test, the visceral pain. Adjuvant arthritis is one kind of inflammatory pain. Ligation of sciatic nerve elicits neuropathic pain. We used electric stimulus in tail stimulation and chemical stimulus (acetic acid) in

writhing test. In arthritis and neuropathic pain the pain threshold was measured with thermal stimulation. Therefore, experimental results indicated that EM-1 induced analgesic effects, not only on acute somatic and visceral pain induced by different physico-chemical stimulations, but also on the acute and chronic hyperalgesia in inflammation and neuropathic pain as well.

It was often mentioned that the analgesia induced by EM was naloxone-reversible^{11,14}. In the present study, the analgesia of EM-1 was reversed not only by opioid receptor antagonist, naloxone, but also by selective

μ -opioid receptor antagonist, cyprodime. This result is concordant with the reversal of EM-induced analgesia by another μ -opioid receptor antagonist, β -funaltrexamine^[1]. Moreover, repeated injections of EM-1 could result in tolerance. All these results suggested that the analgesia of EM-1 was mediated by μ -opioid receptor.

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内吗啡肽-1的镇痛作用

李正红¹, 单立冬, 蒋星红, 郭试瑜, 俞光第, 久光 正², 印其章³ (苏州大学医学院神经生物学研究室, 苏州 215007, 中国; ²昭和大学医学部第一生理学教室, 东京 142, 日本)

关键词 内吗啡肽类; 镇痛; μ 阿片受体; 药物耐受性; 纳洛酮; cyprodime

目的: 研究内吗啡肽-1(EM-1)的镇痛作用。 **方法:** 采用电刺激鼠尾-嘶叫法、扭体法、佐剂性关节炎以及神经源性疼痛等多种疼痛模型, 观察腹腔注射 EM-1 的镇痛作用, 并和脊髓蛛网膜下腔注射和侧脑室注射 EM-1 的镇痛作用进行比较。 **结果:** 1)EM-1 能剂量依赖地提高大鼠电刺激鼠尾-嘶叫法的痛阈; 能抑制醋酸引起的小鼠扭体反应; 在佐剂性关节炎所致的炎症性痛觉过敏及坐骨神经部分结扎所引起的神经源性痛觉过敏中, EM-1 也有镇痛作用。 2)中枢给 EM-1 的镇痛作用比外周给药出现得较快, 而且较强。 3)阿片受体拮抗剂纳洛酮能翻转 EM-1 的镇痛作用; μ -阿片受体选择性拮抗剂 cyprodime 也能翻转 EM-1 的镇痛作用; 反复给予 EM-1, 其镇痛效应逐渐减弱, 即产生耐受。 **结论:** EM-1 具有确切的镇痛作用, 其镇痛作用由中枢 μ 阿片受体介导。

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