Endomorphin and μ-opioid receptors in mouse brain mediate the analgesic effect induced by 2 Hz but not 100 Hz electroacupuncture stimulation

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Abstract

This work was designed to examine whether brain endomorphins (EM1 and EM2), the endogenous μ-opioid ligands, are involved in electroacupuncture (EA)-induced analgesia in the mice. C57BL/6J mice were given EA for 30 min and the effect of EA-induced analgesia was assessed by radiant heat tail flick latency (TFL). Intracerebroventricular (i.c.v.) injection of μ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Tyr-Orn-Thr-Pen-Thr-NH$_2$ (CTOP), or antiserum against EM1 or EM2 was performed to see whether EA analgesia could be blocked. The results showed that: (1) i.c.v. injection of CTOP at 25–100 ng dose-dependently antagonized the analgesia induced by EA of 2 Hz, but not 100 Hz. (2) Intracerebroventricular injection of EM1 antiserum (5 ml, 1:1 or 1:10 dilution) dose-dependently antagonized 2 Hz, but not 100 Hz EA analgesia. (3) EM2 antiserum showed similar effect at 1:1 dilution. The results are interpreted to mean that endogenously released EM1 and EM2 and the cerebral μ-receptors are involved in mediating 2 Hz but not 100 Hz EA analgesia in the mice. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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Endomorphin is a newly characterized opioid peptide with high selectivity for μ-opioid receptors [12,15,16,18]. The physiological functions of brain endomorphin and the proper method to trigger its release remain obscure. Peripherally applied electrical stimulation entitled 'electroacupuncture' (EA) has been claimed to accelerate the release of endogenous opioid peptides in rats and humans [3]. This effect was shown to be frequency-dependent, i.e. 2 Hz EA is highly effective in accelerating the release of central enkephalins and β-endorphin, whereas 100 Hz EA is specific for the release of dynorphin in the spinal cord [6]. Accordingly, 2 Hz EA analgesia has been shown to be mediated by μ and δ receptors, whereas 100 Hz EA analgesia is mediated by κ receptors [7,8]. These results were obtained mostly in the rat. It remains to be clarified (1) whether brain μ-opioid receptor is also responsible for 2 Hz EA analgesia in the mice; (2) if this proved to be true, then 2 Hz EA would likely to be effective to accelerate the release of brain endomorphin to exert an antinociceptive effect in mice. The results of the present study seem to show that the principles obtained from rats is likely to be applicable in mice.

Female C57BL/6J mice weighing 20–25 g were obtained from the animal center in Peking University. They were housed five in a cage with food pellets and water ad libitum. The selective μ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Tyr-Orn-Thr-Pen-Thr-NH$_2$ (CTOP) was purchased from Sigma Chemicals (USA), Antiserum against endomorphin-1 and -2 were provided by Phoenix Pharmaceuticals, Inc, USA (Rabbit No. 219–3 and 217–3). Their cross-reactions to other opioid peptides and related peptides are less than 0.01%. Normal rabbit serum provided by Phoenix Pharmaceuticals, Inc, USA was used as control. CTOP was dissolved in normal saline, the volume for intracerebroventricular (i.c.v.) injection was 5 ml/mouse. Lyophilized antiserum against endomorphin-1 and -2, respectively, were stored in −20°C refrigerator. Before the experiment, they were reconstituted with ddH$_2$O to the original volume (1:1) and were diluted with normal saline to a series of concentration gradients as 1:10, 1:100 and 1:1000. The volume of i.c.v. injection was fixed to 5 μl/mouse. Experiments were performed in a temperature-controlled room (20 ± 1°C).

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Nociceptive sensitivity was assessed using the radiant heat tail flick assay [14]. Two stainless-steel needles of 0.3 mm diameter, 3 mm length were inserted in each leg, one at the point ST36, 2 mm lateral to the anterior tubercle of tibia, and the other at the point SP6, 2 mm proximal to the upper border of medial melleolus, at the posterior border of the tibia [4]. Frequency was fixed by 2 Hz (pulse width 0.6 ms) or 100 Hz (pulse width 0.2 ms). Square waves generated from a Han's acupoint nerve stimulator (HANS, manufactured Neuroscience Research Institute, Peking University) were connected to the stainless steel needles in both legs simultaneously. The intensity of stimulation was increased according to the preset schedule of 1-2-2 mA, with each intensity lasting for 10 min. The average of the present increase of tail flick latency (TFL) obtained within the period of 30 min EA was taken as the effect of EA analgesia. Intracerebroventricular injections were performed according to the procedure of Haley and McCormick [4].

The experimental data were expressed as mean ± SEM. Group differences were tested by one-way analyses of variance (ANOVA) followed by Newman–Keuls post-hoc test. $P < 0.05$ was taken as the significant level of difference.

To observe the effect of CTOP on the 2 or 100 Hz electroacupuncture analgesia, mice were given i.c.v. injection of CTOP at doses of 12.5, 25, 50 and 100 ng, with normal saline as control, administered 10 min prior to EA. The results are shown in Fig. 1. 2 Hz of EA analgesia was attenuated dose-dependently by CTOP at the dose range of 25–100 ng $(P < 0.05)$ (Fig. 1A). In contrast, CTOP produced no significant effect on 100 Hz EA analgesia (Fig. 1B). In addition, CTOP per se is neither analgesic, nor hyperalgesia (data not shown).

Other mice were given i.c.v. injection of endomorphin1 (EM1)-antiserum (AS) at the dilution of 1:1, 1:10, 1:100 and 1:1000, with normal rabbit serum as control, administration 20 min prior to EA to observe the effect of microinjection of EM1-antiserum on 2 or 100 Hz EA analgesia. The results are shown in Fig. 2. The analgesic effect induced by 2 Hz EA was significantly attenuated by microinjection of EM1-antiserum at 1:1 and 1:10 dilutions $(P < 0.01)$ (Fig. 2A). No suppressive effects were observed for 100 Hz EA analgesia when EM1-antiserum was injected i.c.v. even at 1:1 dilutions (Fig. 2B).

Similar experiment was performed using EM2-antiserum instead of EM1-antiserum to observe the effect of microinjection of EM2-antiserum on 2 or 100 Hz EA analgesia. The results are shown in Fig. 3. The analgesic effect induced by 2 Hz EA was significantly attenuated by microinjection of EM2-antiserum at 1:1 dilution $(P < 0.05)$, but not at 1:10 dilution. No suppressive effects were observed for 100 Hz EA analgesia when EM2-antiserum were injected i.c.v. even at 1:1 dilutions.

The first finding of the present study is that 2 Hz EA-induced analgesia can be dose-dependently blocked by CTOP, the highly selective μ-opioid receptor antagonist, suggesting the involvement of μ-receptor in the mediation

of low frequency EA analgesia in the mice. Since the discovery of enkephalins [10] in 1975 and β-endorphin [11] in 1976, there two opioid peptides have been considered as the endogenous ligands for the μ-opioid receptor, although they have almost equal binding affinity with μ- and δ-opioid receptors [13]. The characterization of the μ-selective opioid peptides endomorphin1 (EM1) and endomorphin2 (EM2) by Zadina et al. [18] has opened a new area for opioid research. It has been reported that i.c.v. or intrathecal (i.t.) injection of EM produced potent analgesic effect which can be completely blocked by selective μ-antagonists [3]. There are several criteria, however, still missing to qualify EM as the endogenous μ-receptor agonist. The gene coding for EM is obscure, and evidence supporting the release of endogenous EM from intact central nervous system (CNS) is still lacking. Since pharmacological antagonist for EM is not available yet, we turned to try antibody microinjection approach. The basic idea was that the binding of the large molecule of antibody IgG to the endogenously released small peptide would prevent the latter from receptor activation by space blockade. This method has been used successfully for the study of the mechanisms of EA analgesia [5]. For example, 2 Hz EA analgesia can be blocked by the i.t. injection of antibodies against enkephalin but not dynorphin, and the reverse is true for the blockade of 100 Hz EA analgesia in the rat experiment
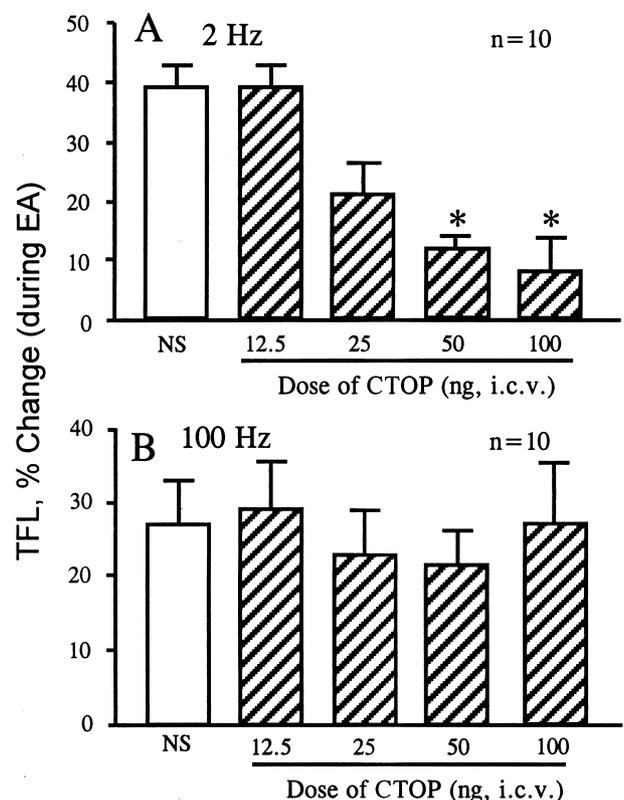

Fig. 1. (A) The effect of CTOP on 2 Hz EA-induced analgesia in mice. $*P < 0.05$, compared with normal saline (NS) group, ANOVA followed by Newman–Keuls post-hoc test. (B) The effect of CTOP on 100 Hz EA-induced analgesia in mice.
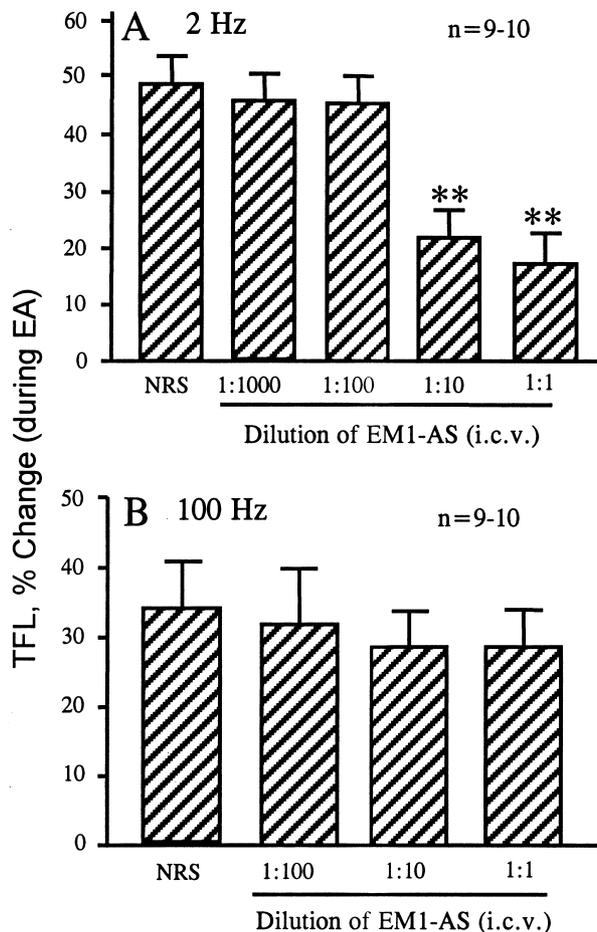
Fig. 2. (A) The effect of i.c.v. injection of EM1-antiserum (AS) on 2 Hz EA-induced analgesia in mice. **$P < 0.01$, compared with normal rabbit serum (NRS) group, ANOVA followed by Newman–Keuls post-hoc test. (B) The effect of EM1-AS on 100 Hz EA-induced analgesia in mice, $P > 0.05$.

Fig. 3. (A) The effect of EM2-AS on 2 Hz EA-induced analgesia in mice. *$P < 0.05$, compared with NRS group, ANOVA followed by Newman–Keuls post-hoc test. (B) The effect of EM2-AS on 100 Hz EA-induced analgesia in mice, $P > 0.05$.

[17], suggesting that 2 Hz EA accelerates the release of enkephalin and 100 Hz EA the dynorphin in the spinal cord. This notion has been amply supported by experiments using radioimmunoassay, cross tolerance and many other pharmacological approaches [1,2,8,17].

Results shown in Fig. 2 demonstrated clearly that i.c.v. injection of antiserum against EM1 dose (concentration)-dependently attenuated the analgesic effect induced by EA of 2 Hz but not 100 Hz. This result combining with the result shown in Fig. 1 strongly suggest that 2 Hz-, but not 100 Hz-EA accelerates the release of EM1 from the brain to interact with μ-opioid receptors to produce an analgesic effect. Similar result was obtained when EM2 antiserum were used instead of EM1 antiserum, but the result was considered statistically significant only when 1:1 dilution, but not 1:10 dilution of the antiserum is used. To our knowledge, this is the first report using antiserum against EM1 and EM2 for the study of the physiological functions of brain EM1 and EM2 in mice experiment. We have reported recently in the rat experiment that i.t. injection of antiserum against
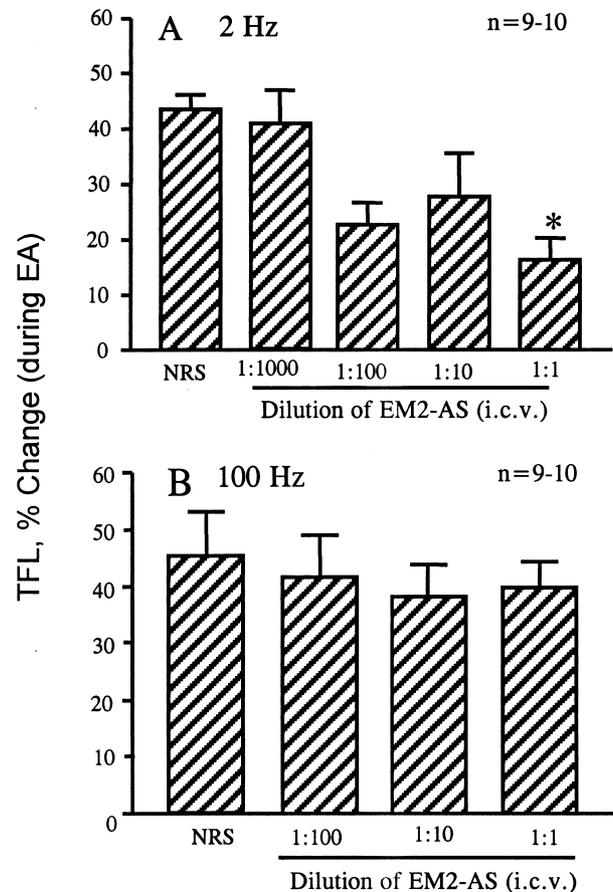
EM1 blocked 2 Hz EA analgesia at 1:100 dilution, but was unable to block 100 Hz EA analgesia even at 1:10 and 1:1 dilution [9], suggesting that EM in the rat spinal cord plays a similar role as it in the mouse brain. In conclusion, 2 Hz EA may accelerate the release of EM1 and EM2 in mouse brain, which may interact with μ-opioid receptor to produce an analgesic effect. The brain EM is not involved in the mediation of 100 Hz EA-induced analgesia in the mice. The results observed in mice are thus compatible to those obtained in rats and humans [7].

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