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Endomorphin-1 mediates 2 Hz but not 100 Hz electroacupuncture analgesia in the rat

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Abstract

This work was designed to elucidate the possible involvement of endogenous endomorphin-l (EM1) in analgesia induced by electroacupuncture of low or high frequencies. Taking radiant heat tail flick latency (TFL) as an indication of nociception, rats were subjected to intrathecal (i.t.) injection of 10 μl antiserum against EM1 (EM1-AS) or normal rabbit serum (NRS, as control) and then followed by 2 or 100 Hz electroacupuncture stimulation for 30 min. The analgesia induced by 2 Hz electroacupuncture was attenuated by i.t. injection of EM1-AS at 1:10 and 1:100 but not at 1:1000 dilution. No such suppressive effect was observed for 100 Hz EA analgesia when EM1-AS was injected i.t. at any dilutions. These results indicate that EM1 is involved in 2 Hz but not 100 Hz electroacupuncture analgesia at spinal level. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Endomorphin; μ-Opioid receptor; Microinjection of antiserum; Electroacupuncture; Frequency dependence

Endomorphin 1 and 2 (EM1, EM2) are two recently characterized peptides, which had been isolated and purified from bovine brain by Zadina et al. [18] in April 1997. Both of them display a potent opioid activity in vivo or in vitro and bind to μ-receptor with high affinity and selectivity. So they are regarded as the endogenous specific ligands for the μ-receptor [4,5,15,18].

Previous studies performed in our laboratory have demonstrated that electroacupuncture (EA) of different frequencies can trigger the release of different neuropeptides in the central nervous system (CNS). For example, low frequency EA accelerates the release of enkephalin and β-endorphin to activate δ/μ-receptors in CNS, whereas high frequency EA releases dynorphin to act on κ receptor in spinal cord [2,7,8]. The discovery of new members of the opioid peptide family gave rise to the questions whether they are involved in the mediation of electroacupuncture analgesia and what is the optimal frequency for their release.

This experiment was designed to elucidate the possible involvement of endogenous endomorphin-l (EM1) in analgesia induced by EA of low or high frequencies. We used the technique of microinjection of EM1 antiserum into the spinal subarachnoid space of the rat. Considering the high affinity and selectivity of the antibody-antigen reaction, it is believed that the biological activity of EM1 can be partially or completely abolished depending on the amount of EM1 antiserum injected, without affecting other structurally or functionally similar peptides. After EM1 antiserum injection, the changes of tail flick latency (TFL) were recorded in response to electroacupuncture stimulation and data were statistically analyzed to determine the role of EM1 in electroacupuncture analgesia at spinal level.

Rabbit-anti-Rat EM1 antiserum was provided by Phoenix Pharmaceuticals, Inc. USA (Rabbit No. 219–222), with the titre of 1:2500. Its cross-reactions to other opioid peptides and related peptides are less than 0.01%. Normal rabbit serum (NRS) was used as control. Chemicals were stored in the form of dry powder in −20°C refrigerator. Immediately before the experiment, chemicals were dissolved in ddH_2O and were diluted with sterile normal saline to a serious of concentration gradients as 1:1, 1:10, 1:100 and 1:1000. The reagents were kept in icy water bath before use.

Adult female Sprague–Dawley rats weighing 200–250 g were provided by the Animal Research Institute of Chinese

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Academy of Science. They were housed six in a cage with food pellets and water available ad libitum.

Rats were anesthetized with 10% chlorohydrate (350 mg/kg, i.p.). A PE-10 catheter was inserted into the subarachnoid space at the foramen magnum and threaded caudally 8.0 cm down the spinal cord, according to Yaksh and Rudy [17]. Two days were allowed for the animals to recover from the surgery. If any signs of the spinal cord or root damage such as paralysis and lameness were observed, the animal was discarded. After the determination of the basic tail flick latency, EM1-AS or NSR was injected intrathecally in a volume of 10 μl via the catheter within 1 min. The catheter was then filled with 10 μl of saline for flushing.

Thirty minutes after the i.t. injection, tail-flick latency was measured again, followed by electroacupuncture stimulation of 2 or 100 Hz for 30 min. Acupuncture stimulation from a 57-6D electric stimulator was given through stainless steel needles inserted bilaterally into the hind limbs at those acupoints corresponding to Zusanli and Sanyinjiao in man [12]. For 2 Hz EA, the pulse duration was 0.6 ms and the intensity of the electric current was 1 mA in the first 20 min and 2 mA in the last 10 min. For 100 Hz EA, the pulse duration was 0.2 ms and the intensity was increasing from 1 to 3 mA in three steps of 1 mA each. At the end of every 10 min, the EA stimulation was temporally stopped for the measurement of the changes of pain threshold. After the termination of EA, measurement of pain threshold continued for another 30 min. The test was performed in a blind manner.

Experiments were performed in a temperature-controlled room (20 ± 1°C). Nociceptive sensitivity was assessed using the radiant heat tail-flick assay. Rats were kept in a plastic restrainer with hindlimbs and tail extending. The animals were allowed 30 min to adapt the test situation. Focused light from a 12.5 W projection bulb was applied to the point between middle and lower 1/3 of the tail and the TFL was measured to the nearest 0.1 s. Values from the first three measurements, with an interval of 5 min, were averaged as the basal TFL, which was usually in the range of 4–6 s. TFL obtained in subsequent tests was expressed as percentage change from the basal level, with a cut-off limit of 150% in order to prevent possible tissue damage. In every

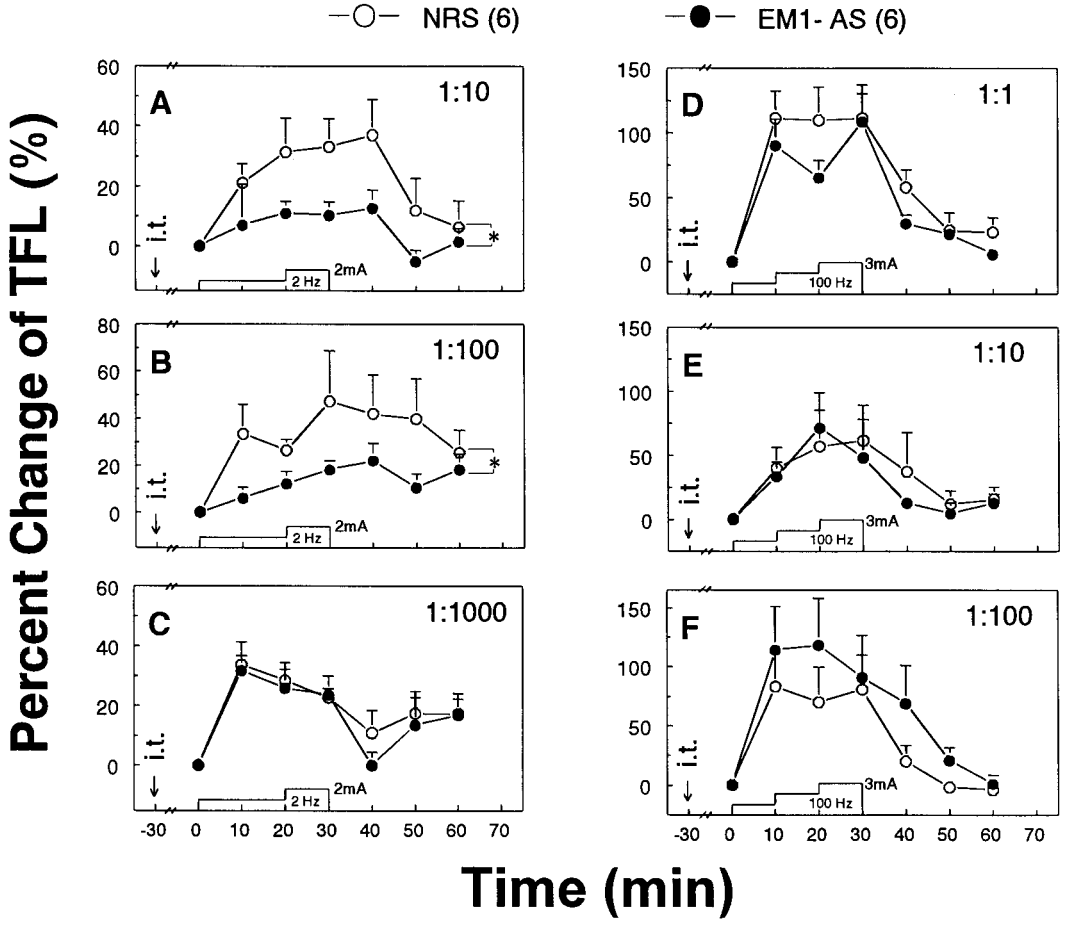

Fig. 1. Percent change of tail flick latency (TFL) induced by 2 Hz EA (A–C) and by 100 Hz EA (D–F), $n = 6$ in each group. Normal rabbit serum (NRS) or endomorphin-l antiserum (EM1-AS) was administered intrathecally 30 min prior to EA. Dilutions of EM1-AS are indicated in the up right corner of each table. TFL was measured during and after EA for 30 min each. *$P < 0.05$ compared between NRS and EM1-AS group by ANOVA.

tail flick test, we measured tail temperature with a skin thermometer (MGA-III 219, Shibaura Electronics Co., Ltd. Japan). If it increased more than 1°C (compared with the first measurement), the tail flick latency would be corrected by a coefficient of −0.25 s/°C [12].

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

Intrathecal injection of 1:10 and 1:100 times diluted EM1 antiserum resulted in a significant decrease of the effect of 2 Hz EA analgesia ($P < 0.01$, two-way ANOVA), whereas 1:1000 times diluted EM1 antiserum had no such effect, implying the participation of EM1 in the low frequency electroacupuncture analgesia.

One hundred Hertz electroacupuncture induced analgesia was not affected by the i.t. injection of EM1 antiserum at any dilution ranging from 1:100, 1:10 and 1:1, suggesting that EM1 does not play any important role in the analgesic effect elicited by high frequency electroacupuncture (Fig. 1).

To determine whether a neuropeptide participates in a given physiological activity, the most concise and convincing way is to apply the specific antagonist to its receptor and see if the activity is blocked. However, as the specific antagonist for endomorphin has not been worked out yet, another approach, microinjection of antibody against the peptide could be a reliable alternative [6,14]. EM1 antiserum injected into the subarachnoid space could bind specifically with the local EM1 and prevent it from receptor activation, thus attenuate its physiological effect [8,10,14].

Although EM1 and EM2 share many common properties both structurally and functionally, there are some differences in their pharmacological characters. The two endomorphins are equipotent supraspinally, but EM1 is more potent spinally [3]. The time courses of the pharmacological effects of EM1 and EM2 are very similar. Both of them reach their full analgesic effect 15–20 min after the i.t. administration, then diminished quickly. Tolerance developed rapidly after repeated injections, but the effect of EM1 remained longer than that of EM2 before tolerance was observed [15]. Considering the above-mentioned advantages of EM1, we took it as a candidate to begin our investigation with.

The results of this work shows that the analgesic effect of 2 Hz electroacupuncture could be substantially blocked by EM1-AS microinjection, implying the involvement of EM1 in mediating 2 Hz EA analgesia, or a possible acceleration of EM1 release during 2Hz EA. This is the first report concerning the effective measure for activating the function of EM1 in CNS in an in vivo set up. This result is consistent with the following findings: It was found that the analgesic effect of 2 Hz EA could be blocked by a small dose of naloxone (0.5 mg/kg, which is sufficient to block the μ-receptor, but not the κ receptor), implying that the analgesic effect of 2Hz EA is mediated by the μ-receptor [7]. In contrast, analgesic effect of 100 Hz EA could only be blocked by a large dose (20 mg/kg) of naloxone, indicating the involvement of κ receptor mediation in 100 Hz EA analgesia [7]. This hypothesis has been verified by the cross-tolerance study [1].

The finding of the present study provides a strong support to the hypothesis put forward from this lab that specific neuropeptides can be mobilized by identified frequencies [8]. Aside from enkephalin [2] and β-endorphin [16], endormorphinl is the third member of opioid peptide that can be mobilized by low frequency peripheral electrical stimulation. This is opposite to the generally believed hypothesis that central neuropeptides can be most effectively released by high, rather than low frequency stimulation [9]. However, in order to ascertain that spinal endomorphin-1 can be effectively released by 2 Hz EA, further neurochemical study should be performed. Work is in progress to measure the EM1 immunoreactivity in spinal perfusate of the rat before and after 2 Hz and 100 Hz EA stimulation.

It is interesting to note that immunoreactive EM2 has been characterized in primary afferents in rats and monkey [11] as well as in rat brain and spinal cord [10,13]. Similar studies concerning the existence of EM1 in these areas are waiting to be reported.

In conclusion, endormorphin seems to play an important role for mediating the 2 Hz but not 100 Hz electroacupuncture analgesia at spinal level of the rat.

Among the four currently available endogenous opioid peptides, three of them, namely endomorphins, enkephalins and β-endorphin can possibly be released by low frequency (2 Hz) peripheral stimulation whereas the dynorphin can only be released by high frequency (100 Hz) stimulation. These basic findings may be useful if one wants to make use of certain types of neuropeptides from the CNS for medical purpose.

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