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Neuroscience Letters 350 (2003) 93–96

Neuroscience Letters

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Nocistatin potentiates electroacupuncture antinociceptive effects and reverses chronic tolerance to electroacupuncture in mice

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Received 12 May 2003; received in revised form 15 July 2003; accepted 16 July 2003

Abstract

Nocistatin (NST) and nociception/orphanin FQ (OFQ) are peptides derived from the same precursor that play opposing roles in pain modulation. OFQ antagonizes morphine analgesia and electroacupuncture (EA)-induced antinociceptive effect. The present study investigates whether NST potentiates EA-induced antinociceptive effect and reverses chronic tolerance to EA in mice. Injection of NST (0.5, 5.0 and 50.0 ng) intracerebroventricularly had no effect on basal thermal latency, but produced a dose-dependent potentiation of EA-induced antinociceptive effect in mice with the maximum response at 5.0 ng. NST (5.0 ng) partly reversed chronic tolerance to EA. These results suggest that NST in the brain might play roles in EA-induced antinociceptive effect and the development of chronic tolerance to EA in mice.

Keywords: Nocistatin; Electroacupuncture; Analgesia; Nociception; Tolerance

Acupuncture has been used in China and other Asian countries for more than 2000 years. It is very effective in the treatment of many diseases and has few side effects. However, tolerance to repeated applications has been reported. Researchers are striving to solve these problems. Many neurotransmitters, including peptides, are known to be involved in acupuncture-induced analgesia and tolerance, among which is nociception/orphanin FQ (OFQ). OFQ is an endogenous agonist for the orphan opioid receptor-like 1 (ORL_1) receptor [4,6]. Intracerebroventricularly (i.c.v.) OFQ could antagonize opioid-mediated stress-induced antinociception, as well as μ-, κ- and δ-receptor-mediated opioid antinociception in mice [5]. Our previous studies showed that i.c.v. injection of OFQ produced antagonism to 100 Hz electroacupuncture (EA)-induced antinociception, and i.c.v. antisense oligonucleotide to OFQ receptors produced a marked augmentation of EA in rats. In addition, i.c.v. OFQ antibody could partly reverse chronic tolerance to EA in rats [12,13].

Nocistatin (NST) is a bioactive peptide derived from the same precursor as OFQ [7] and plays important roles in the regulation of pain transmission in the central nervous system (CNS) in rats [16,19]. Administration of NST (i.t.) attenuated allodynia and hyperalgesia caused by i.t. injection of OFQ [15]. In mice, similar data indicated that endogenous OFQ acted as a modulator of morphine analgesia and tolerance. For example, when morphine was given to morphine-tolerant mice, OFQ receptor gene knockout mice showed only 50% reduction in the peripheral antinociception of morphine compared with wild-type mice, suggesting that OFQ or its receptor facilitated the development of morphine tolerance [14]. In mice exposed to acute as well as chronic treatment with morphine, i.c.v. ORL_1 antagonist [Nphe1]NC(1–13)NH_2 strongly potentiated the antinociceptive effect of i.c.v. morphine [9]. Our recent work found that endogenous opioids were involved in EA-induced antinociception in mice [3] as in rats. So, if OFQ plays an important role in antinociception and tolerance induced by EA in mice, and NST is a functional antagonist to OFQ, then NST should potentiate EA-induced antinociception and reverse tolerance to EA, respectively. This idea was examined in the present study.

Male C57BL/6J mice weighing 20–25 g were provided by the Animal Department of our university. They were housed four per cage under natural light-dark cycle with food pellets and water ad libitum according to University

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0304-3940/03/$ - see front matter © 2003 Published by Elsevier Ireland Ltd.
doi:10.1016/S0304-3940(03)00863-2

Guidelines for Animal Care and Use adapted from the National Institutes of Health (USA).

Experiments were performed in a temperature-controlled room (22 ± 1°C). Mice were kept in a specially designed polyethylene holder, with the hind legs and tail exposed. Thermal latency was assessed using the radiant heat tail flick latency (TFL) as described previously [3]. Focused light from a projection bulb was applied to the tail 2–3 cm from the tip and the TFL was measured. The intensity of thermal stimulus was adjusted by changing the voltage of electricity to obtain a basal latency within the range of 3.5–5.5 s. To avoid tissue damage, a cut-off limit of 10 s was used. The mean of three consecutive measures at 5 min interval was taken as the basal TFL.

EA was applied as described previously [3]. Two stainless-steel needles (0.4 mm diameter, 4 mm length) were inserted in each hind leg, one at the Zusanli acupoint (ST 36) and another at the Sanyinjiao acupoint (SP 6). Square waves generated from a Han's Acupoint Nerve Stimulator (LH series, manufactured in our university) were applied to both legs simultaneously. The frequency was 100 Hz and the pulse duration was 0.2 ms. The intensity of stimulation was increased in a stepwise manner (0.5 mA increments between 1.0 and 2.0 mA) each lasting for 10 min. TFL was measured every 10 min during the EA for 30 min. The percent increase of TFL for the three assessments was averaged as a measure of EA-induced antinociception and was calculated as follows: TFL (%) = (latency after EA − basal latency)/basal latency × 100%.

To develop EA chronic tolerance, EA was applied once daily for 6 days consecutively. The thermal latency was examined at the end of EA to evaluate the EA-induced antinociceptive effect. The antinociceptive effect decreased as the duration of EA increased. This condition was termed chronic tolerance to EA [13].

Data were expressed as mean ± SEM. One- or two-way analyzes of variance (ANOVA) was used where appropriate, followed by Newman–Keuls post-hoc test. $P < 0.05$ was taken as the significant difference level.

NST (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in normal saline (NS). Twenty minutes prior to EA, NST or NS was injected free-hand i.c.v. using a 10-μl Hamilton syringe according to the procedure of Haley and McCormick [1]. The doses of NST were 0.5, 5.0, 50.0 ng/mouse in a volume of 5 μl. To observe the effect of NST on basal thermal latency, TFL was measured at 10 min intervals for a total of 60 min after injection. The results are shown in Fig. 1. Compared with the NS group, three NST groups remained stable in TFLs, suggesting that i.c.v. NST produced neither antinociception nor hyperalgesia in mice.

The effect of i.c.v. NST on EA-induced antinociception is shown in Fig. 2. Mice were randomly divided into four groups receiving i.c.v. injection of NS or NST at different doses 20 min prior to EA. NST produced a dose-dependent

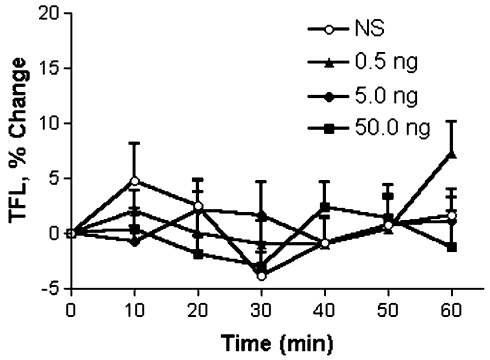

Fig. 1. Effect of i.c.v. NST on basal TFL in mice. No significant difference between groups was observed ($P > 0.05$). $n = 7–8$.

potentiation of EA-induced antinociception with a maximal effect at 5.0 ng ($P < 0.05$ compared with NS group).

A reversal effect of i.c.v. NST on chronic tolerance to EA was also observed. Twenty mice were randomly divided into two groups. Each was given EA once daily for 6 days consecutively. The analgesic effect decreased gradually from days 1 to 6, a significant difference was observed between days 1 and 6 ($P < 0.01$), indicating the development of chronic tolerance to EA. At day 7, 20 min after i.c.v. injection of NST (5.0 ng) or NS, another session of EA was applied. The results were shown in Fig. 3. A significant reversal effect of EA-induced tolerance was observed in the NST group compared with the NS group ($P < 0.01$).

The main finding of the present study was that NST could potentiate EA-induced antinociception and partly reverse chronic EA chronic tolerance in mice. This suggests that endogenous NST might play roles in the modulation of EA-induced antinociception and tolerance. The underlying mechanisms are not clear, but the antagonistic effect of NST to OFQ might be an important component.

Many reports have indicated that OFQ is an anti-opioid peptide in the CNS [5], where it antagonized opioid antinociception and EA-induced antinociception. In the rat tail-flick test, i.c.v. injection of OFQ elicited a significant

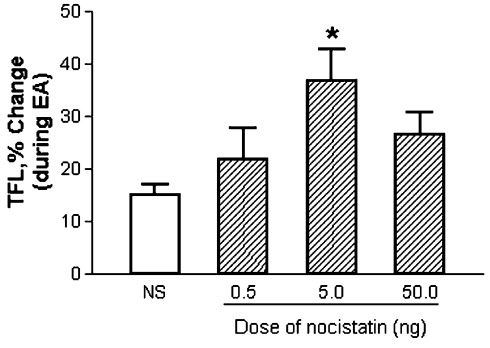

Fig. 2. Effect of nocistatin on 100 Hz EA-induced antinociception in mice. Mice were injected with NST (0.5, 5.0 and 50.0 ng i.c.v.) (hatched columns) or normal saline (NS) (blank columns) 20 min before EA. The TFL was measured every 10 min during EA for 30 min. Data are expressed as means ± SEM. *$P < 0.05$ compared with the NS group by one-way ANOVA followed by Newman–Keuls post hoc test. $n = 10$.

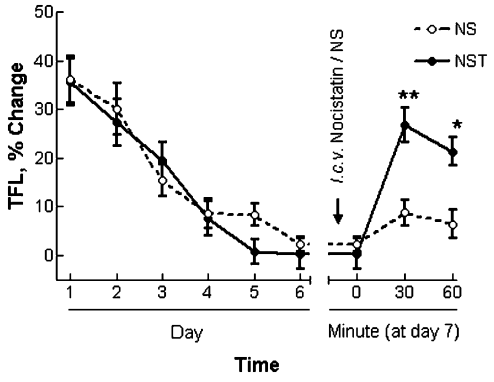

Fig. 3. Reversal effect of NST on chronic tolerance to EA-induced antinociception in mice. Chronic tolerance developed as EA (1.0, 1.5, or 2.0 mA, 10 min) was given once daily for 6 days. At day 7, mice were injected with nocistatin (5.0 ng) or NS prior to receiving another session of EA 20 min after injection. For the first 6 days of EA, there was no significant difference between the two groups. At day 7, the EA-induced antinociception in the NST-treated group was much higher compared with the NS group. $^*P < 0.05$ compared with EA plus NS group. $n = 10$.

decrease in thermal latency which was abolished by pretreatment with antisense oligonucleotides to the OFQ receptor. Combined i.c.v. injection of OFQ with the μ-, κ- or δ-opioid receptor agonists, endomorphine-1, U-50,488H or DSLET attenuated μ- and κ- but not δ-receptor mediated antinociception [18]. When used in combination with OFQ, EA-induced antinociception decreased dose-dependently. When the synthesis of OFQ receptor was blocked with repeated i.c.v. injections of antisense oligonucleotide, EA-induced antinociception increased significantly [19].

The distribution of NST-like immunoreactivity is almost identical to that of OFQ-like immunoreactivity. Anti-NST antibodies potentiates OFQ-induced allodynia in mice [8].

Endogenous OFQ might be involved in the development of EA chronic tolerance. Tian et al. demonstrated that i.c.v. injection of antibody against OFQ could partly reverse the chronic tolerance to EA in rats [13]. They also reported that i.c.v. injection of antibody against OFQ could convert EA low responders into high responders [12].

Existing evidence supports the contention that NST antagonizes OFQ. Okuda-Ashitaka et al. reported that NST existed in the CNS and could attenuate allodynia or hyperalgesia evoked by OFQ in mice [7]. The OFQ content in some regions of the rat brain increased after induction of morphine tolerance [16]. Further, NST could reverse the antagonistic effect induced by OFQ to morphine antinociception in rats [17] and partly reverse acute and chronic morphine tolerance [10].

The potentiation of NST to EA-induced antinociception was dependent on the dosage. It is not clear why potentiation of NST on EA-induced antinociception disappeared at the dose of 50 ng. This result is similar to that obtained from reversal of NST on chronic morphine tolerance in rats [10].

Of course, the interaction of NST with peptides other than OFQ cannot be excluded. For example, the anti-opioid peptide cholecystokinin octapeptide has a significant role in antagonizing opioid antinociception and EA-induced antinociception [11].

When acupuncture is applied for a prolonged period of time such as in the treatment of chronic pain, the analgesic effects will decrease. The current results may help in finding a useful method to improve the therapeutic effects of acupuncture in the treatment of chronic diseases when ‘acupuncture tolerance’ occurs [2,12].

Acknowledgements

The work was supported by grants from the National Basic Research Program of China (G1999054000 to Y.W.), the National Natural Science Foundation of China (30170319 to Y.W. and 39830160 to J.S.H), and the Scientific and Technological Program of the Bureau of Education, Jiangxi Province, China (C.H.). The authors thank Dr Richard A. Collins (Hong Kong DNA Chips Ltd) for his help in preparing the manuscript.

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