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Brain Research 851 (1999) 290–296

BRAIN RESEARCH INTERACTIVE

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Interactive report

Suppression of morphine withdrawal by electroacupuncture in rats: dynorphin and κ-opioid receptor implicated

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Accepted 8 September 1999

Abstract

Our previous work has demonstrated that 100-Hz electroacupuncture (EA) or 100-Hz transcutaneous electrical nerve stimulation (TENS) was very effective in ameliorating the morphine withdrawal syndrome in rats and humans. The mechanism was obscure. (1) Rats were made dependent on morphine by repeated morphine injections (5–140 mg/kg, s.c., twice a day) for eight days. They were then given 100-Hz EA for 30 min 24 h after the last injection of morphine. A marked increase in tail flick latency (TFL) was observed. This effect of 100-Hz EA could be blocked by naloxone (NX) at 20 mg/kg, but not at 1 mg/kg, suggesting that 100-Hz EA-induced analgesia observed in morphine-dependent rats is mediated by κ-opioid receptors. (2) A significant decrease of the concentration of dynorphin A (1–17) immunoreactivity (-ir) was observed in the spinal perfusate in morphine-dependent rats, that could be brought back to normal level by 100-Hz EA. (3) 100-Hz EA was very effective in suppressing NX-precipitated morphine withdrawal syndrome. This effect of EA could be prevented by intrathecal administration of nor-BNI (2.5 μg/20 μl), a κ-opioid receptor antagonist, or dynorphin A (1–13) antibodies (25 μg/20 μl) administered 10 min prior to EA. In conclusion, while the steady-state spinal dynorphin release is low in morphine-dependent rats, it can be activated by 100-Hz EA stimulation, which may be responsible for eliciting an analgesic effect and ameliorating morphine withdrawal syndrome, most probably via interacting with κ-opioid receptor at spinal level.

Keywords: Morphine withdrawal; Electroacupuncture; Analgesia; Dynorphin; κ-opioid receptor

1. Introduction

Dynorphin A (1–17) is an endogenous opioid peptide distributed throughout the central nervous system [1,2], suggesting that it might serve multiple regulatory functions [3]. Our previous work has demonstrated that analgesia induced by 100-Hz electroacupuncture (EA) or 100-Hz transcutaneous electrical nerve stimulation (TENS) was produced by accelerating the release of dynorphin from the spinal cord of the rats [4] and humans [5]. We have also shown that 100-Hz EA was capable of suppressing the morphine withdrawal syndrome in rats [6] and 100-Hz TENS was very effective in ameliorating the withdrawal syndrome in heroin addicts [7]. Although it has been reported that the dynorphin content in the spinal cord was decreased during morphine-dependent and protracted abstinence [8], there were no published data dealing with the issue whether the release of spinal dynorphin is altered during morphine dependent in rats. The first purpose of the current study was to investigate whether 100-Hz EA would induce an analgesic effect in morphine-dependent rats as was the case in normal rats, and whether the effect was mediated by dynorphin via the κ-opioid receptor in spinal cord. The second purpose was to determine whether 100-Hz EA would increase the release of the spinal dynorphin in morphine-dependent rats, and whether the dynorphin is responsible for suppressing the morphine withdrawal syndrome via activation of κ-opioid receptor in the spinal cord. In brief, the aim of this study was to clarify a possible mechanism for the suppression of morphine withdrawal syndrome by 100-Hz EA stimulation in rats.

2. Materials and methods

2.1. Animals and experimental design

Adult male Wistar rats weighting 250–300 g were obtained from the Laboratory Animal Center, Beijing Med-

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0006-8993/99/$ - see front matter
PII: S0006-8993(99)02069-7

ical University in batches, with food and water ad libitum in the home cage. Three batches of rats were used during the whole study. The first batch of 32 rats were randomly divided into four groups (one control group and three morphine-dependent groups) of eight rats. They were given 100-Hz EA stimulation to induce an analgesic effect. The second batch of 26 rats were randomly divided into three groups, one normal control ($n = 8$) and two morphine-dependent group ($n = 9$), and were subjected to spinal perfusion 5 h and 24 h after the last morphine injection. The third batch 39 morphine-dependent rats were randomly divided into five groups of 7–8 rats. The rats were implanted with an intrathecal catheter to inject various kinds of drugs prior to 100-Hz EA simulation for the observation of the drug effects on naloxone (NX)-precipitated withdrawal syndrome.

2.2. The rat model for induction of morphine dependence

The rats were injected subcutaneously with morphine hydrochloride in normal saline twice a day (8:00 h and 20:00 h) at the following doses per injection: 5 mg/kg on the first day, followed by 10, 20, 40, 80, 100, 120 and 140 mg/kg from day 2 through day 8. The injection volume was 2 ml/kg. The control group of rats was given a subcutaneous injection of normal saline, with the same injection volume of 2 ml/kg. The rats were then given 100-Hz EA 24 h after the last injection of morphine to assess the efficacy of EA analgesia and the effect of EA on spinal dynorphin release 5 h and 24 h after the last injection of morphine. In experiments for the observation of the effect of EA on NX-precipitated withdrawal, EA (30 min) was given 6 h after the last morphine injection on day 8, followed by a challenging dose of NX (1 mg/kg, i.p.) administered immediately after the end of EA.

2.3. Nociceptive test and EA stimulation

The nociceptive threshold of the rat was measured by tail flick latency (TFL) elicited by radiant heat [9,10]. The rats were partially restrained in plastic holders. The tail and the hind legs were protruding naturally. Focused light from a projection bulb (8–12 W, adjustable) was applied to the junction between the middle and the lower 1/3 of the tail through an aperture of 6 mm diameter, and the TFL was recorded by an automatic electronic timer. At the beginning of the experiment, TFL was assessed three times at 5-min intervals and the mean value from the first three assessments was taken as the basal pain threshold, usually within the range of 4–6 s. The values of subsequent measurements were expressed as percentage changes of the baseline level. An elevation over 150% of the basal TFL was taken as a cut-off limit to avoid unnecessary skin damage.

Two stainless-steel needles of 0.25 mm diameter and 5 mm in length were inserted at two sites on the hind legs: one at the 'Zusanli' point (St_{36}) near the knee joint (5 mm lateral to the anterior tubercle of tibia) and the other at the 'Sanyinjiao' point (Sp_6) near ankle joint (at the level of the superior border of the medial melleolus, between the posterior border of the tibia and the anterior border of the Achilles tendon). Bidirectional square wave electrical pulses (0.2 ms duration, 100 Hz), designated as electroacupuncture (EA) were given for a total of 30 min. The intensity of the stimulation was increased stepwise from 1 mA to 2 mA, up to 3 mA, with each step lasting for 10 min. The TFL was measured every 10 min for a total of 30 min, and continued for another 30 min after the ending of the EA. EA was temporarily switched off during the measurement of TFL.

2.4. Intrathecal (i.t.) catheterization and injection

Rats were anesthetized with chloral hydrate (350 mg/kg, i.p.) and were implanted with PE-10 tubing by a procedure modified from Yaksh and Rudy. A 12.5-cm length PE-10 tubing that had a knot 7.5 cm from the tip was inserted into the spinal subarachnoid space through an incision made on the atlanto-occipital membrane. The tip of the tubing lay in the region of the lumbar enlargement. The rats were allowed to recover for 24 h, and were injected via the PE-10 tubing. Drugs were dissolved in sterile 0.9% NaCl (normal saline, NS) and injected in 10 μl volume, and the tubing was flushed with 10 μl NS after drug injection. The animals in control group were i.t. administered with 20 μl NS. The i.t. injection was completed within 30 s.

2.5. Perfusion of the subarachnoid space of the spinal cord and radioimmunoassay of dynorphin A (1–17) immunoreactivity

The procedure of anesthesia and inserting a PE-10 tubing into the subarachnoid space was the same as that described above. In order to collect the perfusate, another piece of polyethylene tubing (PE-50) was inserted 1 cm into the cisterna and fixed in situ. At the end of the surgical procedure, artificial cerebrospinal fluid (ACSF) containing captopril (1 μM) and bestatin (1 μM) was perfused at a rate of 1.0 ml/30 min with a constant speed push–pull pump (Pharmacia, Sweden). Aliquots of spinal perfusate (1.0 ml) were collected in polyethylene tubes prior to, during and immediately after the EA stimulation (as described in the nociceptive test), respectively. The perfusate was kept in an ice–water bath and then heated for 10 min in a 100°C water bath. After cooling, the perfusate was centrifuged at 10 000 rpm for 10 min. Supernatants were dried in a lyophilizer and kept at −20°C.

The residue of the lyophilized spinal perfusate was reconstituted with 250 μl H_2O for the radioimmunoassay (RIA) of dynorphin A (1–17). ^{125}I-dynorphin RIA kits were bought from the Phoenix Pharmaceutical Company,

USA. The dynorphin A (1–17) antiserum showed 1.0% cross-reactivity with dynorphin A (1–13), but showed no detectable cross-reactivity with dynorphin A (1–8), dynorphin B, β-endorphin (human), α-neo-endorphin or leu-enkephalin. The non-specific binding of this RIA kit was rather high (24.4%), compared to the total binding of 27.8%, but the standard curve of dynorphin A (1–17) vs. B/B_0 (%) was linear within the range of detection, with IC_{50} at 11.35 pg/tube. To polyethylene test tubes, 100 μl standards (or samples) and 100 μl dynorphin A (1–17) antiserum (1:6500) were added and incubated for 24 h at 4°C after sufficient mix. ^{125}I-dynorphin A (1–17) (100 μl, 8500 cpm) was then added and incubate for another 24 h at 4°C. Free labeled peptide was separated using goat anti-rabbit IgG and normal rabbit serum. The reaction mixtures were centrifuged for 20 min at 3500 rpm, 4°C. The residues were counted using micro-γ-counter (Beckman).

2.6. Scoring of withdrawal signs

The morphine-dependent rats equipped with i.t. catheters were randomly divided into five groups, one blank control group and four i.t. injection groups. The i.t. injection groups were given NS, normal rabbit serum IgG (NRS), nor-BNI 2.5 μg [11] or dynorphin A (1–13) antiserum IgG raised in rabbit (Dyn-AS, 25 μg) [12], respectively. Stainless steel needles were inserted into the acupoints of the rats in the blank control group and they remained in situ without electrical stimulation for 30 min. The rats in the other four groups received 100-Hz EA stimulation (as described in the session of nociceptive test) 10 min after the i.t. injection. In order to observe the NX-precipitate morphine withdrawal, all rats were administered with NX (1 mg/kg, i.p.) at the termination of 30 min EA stimulation. The rats were then placed individually in plastic cages and observed for any exhibition of withdrawal symptoms. The observer was 'blind' to the drug treatment procedures. The withdrawal symptoms were monitored for 3×15 min and scored according to the weighting factors described by Neal and Sparber [13]. In short, signs of mild withdrawal (rearing, screech) were assigned a score of 1. Score 2 was given to withdrawal signs including wet-dog shakes, teeth-chattering, escape attempts, writhing and ejaculation. The body weight change of the rats during withdrawal was measured before and 45 min after the NX injection, that is, weight loss (g) = initial body weight − body weight measured 45 min after the NX challenge.

2.7. Chemicals

Nor-binaltorphimine (nor-BNI) and naloxone HCl were obtained from Sigma Inc. Rabbit-anti dynorphin A (1–13) antiserum IgG was a gift from Dr. Avram Goldstein of the Addiction Research Foundation, Palo Alto, CA, USA, showing 100% cross-reactivity to dynorphin A (1–17). Its cross-reactivity with α-neo-endorphin was less than 0.01%, and it had no cross-reactivity with β-endprhin or leu-enkephalin. The ^{125}I-dynorphin A (1–17) RIA kit were bought from the Phoenix Pharmaceutical Company, USA. Morphine HCl is a product of Qinghai Drug House (China). They were dissolved in sterile NS immediately before use.

2.8. Data analysis

Data obtained from EA analgesic test, radioimmunoassay of dynorphin A (1–17) and weight loss of the NX-precipitated withdrawal rats were expressed as means ± SEM, and analyzed using ANOVA followed by a Newman–Kuels test. Data for scoring of NX-induced withdrawal signs were evaluated using the median test of the non-parametric Kruskal–Wallis ANOVA followed by the Mann–Whitney U-test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. 100-Hz EA-induced analgesia in morphine-dependent rats

The basal TFL of the rats was 4.96 ± 0.13 s in the NS control group ($n = 8$) and 3.76 ± 0.14 s in morphine-dependent group ($n = 24$), tested 24 h after the last morphine injection. The latter was markedly lower than the former (100:76, $P < 0.001$). The morphine-dependent rats were randomly divided into three groups of eight rats and given injection of NS or NX (at 1 and 20 mg/kg, s.c.), respectively. Ten minutes after the injection, all the three groups of morphine-dependent rats as well as the NS control rats received 100-Hz EA as described in Section 2. The results are shown in Fig. 1. The TFL in the control group showed an increase of 49 ± 14.8% during EA, and 95 ± 15.4% after EA, that were significantly higher than the basal level. It was interesting to note (Fig. 1) that EA was still effective in producing an analgesic effect in morphine-dependent rats. The apparent overshoot of the EA-induced analgesia in terms of percent change in the NS control group (column 3 significantly higher than column 1, $P < 0.01$) was due to the lowering of the basal tail flick latency. This effect of EA analgesia could be significantly attenuated by NX at 20 mg/kg (column 7 and 8), but not at 1 mg /kg (column 5 and 6).

3.2. The effect of 100-Hz EA on immunoreactive dynorphin A (1–17) concentration in the spinal perfusate of morphine-dependent rats

As shown in Fig. 2, the concentration of dynorphin A immunoreactivity (-ir) in spinal cord perfusate of the nor-

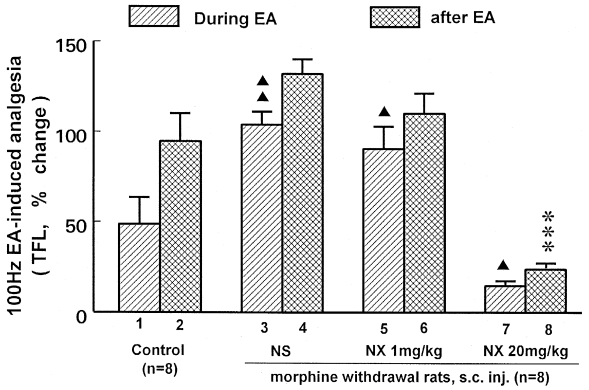

Fig. 1. 100-Hz electroacupuncture (EA)-induced analgesia in morphine withdrawal rats and its blockade by naloxone (NX). NS control rats or morphine withdrawal rats were given 100-Hz EA stimulation for 30 min, and the percentage increase in tail flick latency (TFL) was measured every 10 min for a total of 60 min. The mean percent increases in TFL in the three measurements during EA or after EA were calculated to represent the degree of EA analgesia. Data were analyzed using ANOVA followed by Newman–Keuls test. *** represent $P < 0.001$, compared with columns 2, 4, 6; ▲ and ▲▲ represent $P < 0.05$ and $P < 0.01$, compared with column 1.

mal control rats was 17.1 ± 2.4 pg/ml. In morphine-dependent rats tested 5 h or 24 h after the last morphine injection, the dynorphin-ir decreased significantly ($P < 0.05$) to a level corresponding to 30% or 44% of the normal control group respectively. 100-Hz EA produced an increase of the dynorphin A-ir reaching or approaching the normal control level (100% or 75% respectively) during the period of EA stimulation, and an overshoot 31% or 38% higher than the normal control level after 100-Hz EA ($P < 0.001$). In short, while spinal dynorphin release was significantly suppressed in morphine-dependent rats, 100-Hz EA could effectively accelerate its release.

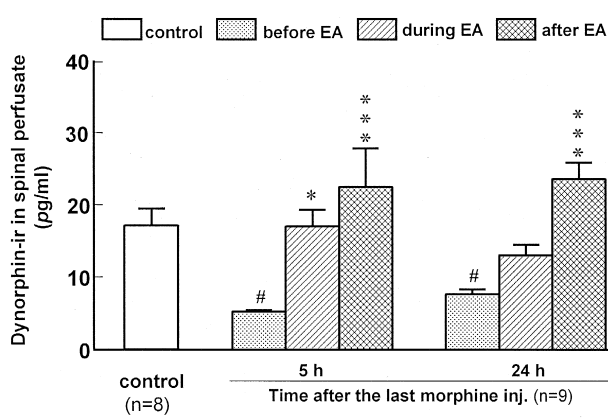

Fig. 2. Effect of 100-Hz electroacupuncture (EA) on dynorphin A immunoreactivity (-ir) concentration in spinal perfusate of morphine dependent and withdrawal rats ($n = 9$). The dynorphin-ir in spinal perfusate of normal saline (NS) control rats: 17.1 ± 2.4 pg/ml ($n = 8$). Data was analyzed using ANOVA followed by Newman–Keuls test. # indicates $P < 0.05$, compared with control group; * $P < 0.05$, *** $P < 0.001$, compared with the corresponding 'before EA' group.

3.3. The influence of i.t. nor-BNI or dynorphin A (1–13) antiserum on the effect of 100-Hz EA in suppressing NX-precipitated morphine withdrawal signs

Experiments were performed to test the hypothesis that the therapeutic effect of 100-Hz EA in suppressing withdrawal syndrome is mediated by spinal dynorphin via κ-opioid receptors. If this is true, then the effect of 100-Hz EA should be blocked by the antibodies against dynorphin (Dyn-AS) or by the antagonist against κ-opioid receptor (nor-BNI) administered intrathecally. NS and IgG obtained from normal rabbit serum (NRS) were used as control. The results are shown in Figs. 3 and 4. Seven of the eight signs, i.e., wet-dog-shakes, escape attempts, teeth chattering, writhing, ejaculation, rearing and screech could be counted, and were depicted in Fig. 3B–H. Their total scores were composed and shown in Fig. 3A. The nalox-

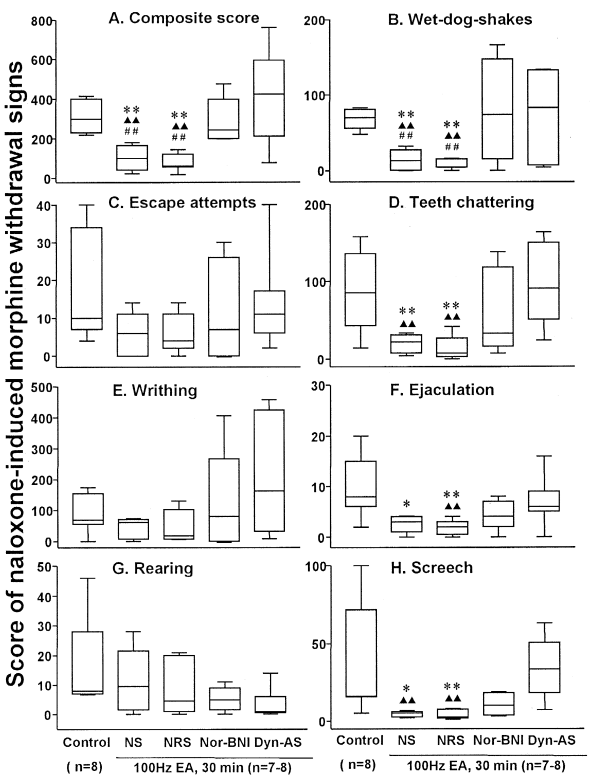

Fig. 3. Effect of the intrathecal (i.t.) nor-BNI or dynorphin A (1–13) antibodies 10 min prior to 100-Hz EA on naloxone (NX, 1 mg/kg, i.p.)-precipitated withdrawal syndrome in rats ($n = 7–8$). Data in (A) were expressed as composite score, determined by counting the score of seven (B)–(H) observed withdrawal signs during the 45-min period of NX-precipitated withdrawal. Data were expressed as median values, with the boxes showing 25% and 75% range, and the bars showing the whole range. Significance of difference between groups was statistically evaluated by the non-parametric Kruskal–Wallis ANOVA followed by the Mann–Whitney U-test. Administered via i.t. injections were normal saline (NS, 20 μl), normal rabbit serum IgG (NRS, 25 μg), nor-binaltorphimine (nor-BNI, 2.5 μg) and dynophin A (1–13) antiserum IgG raised in rabbit (Dyn-AS, 25 μg), respectively. * $P < 0.05$, ** $P < 0.01$, compared with NS control group; # $P < 0.05$, ## $P < 0.01$, compared with nor-BNI group; ▲ $P < 0.05$, ▲▲ $P < 0.01$, compared with Dyn-AS group.

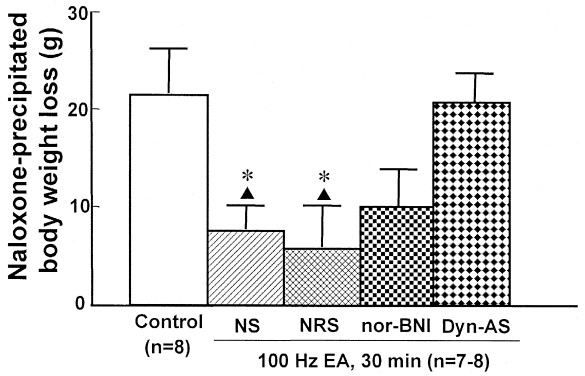

Fig. 4. Changes of body weight (gram) in 45 min after the injection of naloxone, 1 mg/kg, i.p. Data were expressed as means ± S.E.M., and analyzed with ANOVA followed by Newman–Keuls test. Other legends are same as in Fig. 3.

one-induced body weight loss could be measured in grams and was shown separately in Fig. 4. It is obvious from Fig. 3A that 100-Hz EA produced a 60–70% suppression of the withdrawal syndrome (see NS control group and NRS control group), an effect almost completely prevented by i.t. injection of IgG against dynorphin (Dyn-AS) or the antagonist against κ-opioid receptor (nor-BNI). The naloxone-precipitated body weight loss was markedly suppressed by EA, an effect completely reversed by dynorphin antibodies, but not by normal rabbit serum IgG (Fig. 4). In summary, the efficacy of EA in suppressing withdrawal syndrome was most obvious ($P < 0.05$, $P < 0.01$) in five out of eight parameters, including wet-dog-shakes, teeth chattering, ejaculation, screech and body weight loss, and was not so obvious in two of the eight signs, i.e., escape attempts and writhing. No such trend was observed in the scores for rearing.

4. Discussion

Previous studies have shown that 100-Hz EA or TENS can be used for the treatment of opiate dependence with considerable success both in animals [6] and in humans [7]. This would naturally lead to several questions of theoretical interest.

4.1. Would the effect of EA remain in morphine-dependent rats?

One could postulate that animals rendered tolerant to and dependent on morphine would inevitably show a tolerance to exogenous opiates or decreased sensitivity to endogenous opioids. Since EA is known to produce an analgesic effect by accelerating the release of endogenous opioid peptides [4,5], the effect of EA should be expected to be decreased or abolished in opiate tolerant-dependent rats. With this question in mind we tested the efficacy of 100-Hz EA analgesia in morphine-dependent rats. It was not possible to test the analgesic effect of EA 5 h after the last morphine injection, since the TFL was very high (over cut-off limit) at that time. So it was tested 24 h after the last morphine injection when the basal TFL was approaching or lower than normal. The results showed that the effect of 100-Hz EA analgesia was still existing, if not potentiated (Fig. 1). This seemingly paradoxical phenomenon may be explained in at least two ways. Since morphine is a relatively specific μ-receptor agonist, a rat made tolerant to morphine would be cross-tolerant to μ-agonists but not to κ-agonists. It is thus understandable that the κ-specific agonist U50488 [14] and dynorphin A [15] remain effective as analgesic in morphine tolerant animals. It is also obvious that the analgesic effect of 100-Hz EA remains unaffected in morphine-dependent rats since it is mediated by dynorphin [4,12] which is known to be a κ-selective agonist [16]. In accordance with this was the finding that the analgesic effect of 100-Hz EA observed in morphine-dependent rats could be blocked only by a high dose of NX (Fig. 1). Goldstein et al. have pointed out that the effect of dynorphin A (1–13) on the contraction of guinea pig ileum can be blocked completely by naloxone, but the apparent efficiency of naloxone is 1/13th that for blockade of leu-enkephalin or normorphine [17]. We have shown in an in vivo study that the naloxone ID_{50} in blocking the analgesic effect induced by i.t. dynorphin was 14.3 times that for blocking the effect of morphine, and 30 times that for blocking the effect of the highly selective μ-agonist morphiceptin [18]. A similar magnitude of difference was found in the naloxone blockade of analgesia induced by EA of different frequencies, i.e., the IC_{50} for blocking 100-Hz EA (dynorphin-mediated) analgesia was 24 mg/kg, as compared to 1.02 mg/kg for blocking 15-Hz EA (enkephalin-mediated) analgesia, respectively [19]. Another factor deserving consideration is that there is an up-regulation of brain and spinal cord κ-opioid receptors in morphine tolerant-dependent animals [20,21], that is certainly in favor of 100-Hz EA analgesia.

4.2. What is the status of spinal dynorphin release in the morphine-dependent rats

It has been generally accepted that a high concentration of exogenously administered opiate in the body would induce a negative feedback control on the expression and release of endogenous opioid peptides. Rattan et al. [8] have made a detailed survey on the effect of morphine tolerance, dependence and abstinence on immunoreactive dynorphin A (1–13) levels in discrete brain regions and the spinal cord. They found a significant decrease in the spinal cord tissue content of dynorphin A (1–13) immunoreactivity (ir) in morphine-dependent and abstinent rat. To our knowledge there is so far no data dealing with the extent of dynorphin release in morphine-dependent and

protracted abstinence rats. In the present study the dynorphin A-ir in spinal perfusate was measured and was taken as an estimate of the spinal release of dynorphin A. We found a 70% and 56% reduction of dynorphin A-ir in the spinal perfusate of morphine-dependent rats 5 h and 24 h after the last morphine injection (Fig. 2).

What then is the physiological implication of a significant lowering of spinal dynorphin release? Its involvement in the development of hyperalgesia is a matter of interest. It has been well established that hyperalgesia is a consistent sign of the withdrawal syndrome, which has been associated with an increase in the spinal excitatory amino acid release [22,23]. Since spinal dynorphin has been shown to have an analgesic effect [12], a lowering of spinal dynorphin release might also contribute to the 24% decrease of the pain threshold observed in the morphine-dependent rats (24 h after the last morphine injection).

4.3. Is EA still effective in morphine-dependent animals to accelerate the release of dynorphin in the spinal cord

The relation between dynorphin and opiate dependent has long been an issue of scientific interest. Wen et al. reported a suppression of heroin withdrawal syndrome by dynorphin A (1–17) in 1982 [24] and dynorphin A (1–13) in 1992 [25]. Another study revealed that the site of action for dynorphin to attenuate withdrawal in morphine-dependent rats is located in the spinal cord rather than in the brain [26]. Therefore the key issue for the clarification of the mechanisms of 100-Hz EA to suppress opiate withdrawal lies on the ability of 100-Hz EA to accelerate the spinal dynorphin release. Results shown in Fig. 2 clearly demonstrate a robust effect of 100-Hz EA to increase the dynorphin concentration in spinal perfusate. Taking the dynorphin A-ir level in the normal control rats as 100%, 100-Hz EA for 30 min can change the dynorphine concentration from an extremely low level (30% or 44% of the normal control 5 h or 24 h after the last morphine injection) to a level 31% or 38% over that of the control after the termination of EA (an increase of 337% or 214%, $P < 0.001$, compared to the baseline level of the morphine-dependent or withdrawal rats). The data shown in the original article of Fei et al. [4] indicated that 100-Hz EA for 30 min produced a 200% increase of the dynorphin A-ir in spinal perfusate in the naive rats. The percentage change of EA-induced increase of spinal dynorphin A-ir in morphine-dependent and withdrawal rats ($+337\%$ and $+214\%$) was more or less of the same magnitude as that of normal rats, although the absolute amount of dynorphin release may still be less marked in the dependent and withdrawal rats owing to the significantly lowered baseline level.

Since the effect of EA is mediated by multiple neurochemical substrates [27], it would be critical to sort out the role played by the dynorphin A/κ-opioid receptor system in this complex system. To do so we used specific antibodies to prevent dynorphin from receptor binding, and the selective κ-receptor antagonist nor-BNI to block the κ-receptors, and see to what extent the effect of 100-Hz EA can be prevented or attenuated. The results shown in Figs. 3 and 4 indicate that five out of eight withdrawal signs were markedly suppressed by 100-Hz EA, that were in turn significantly reversed by the administration of dynorphin A antibody or nor-BNI. The findings suggest that spinal cord dynorphin and κ-receptors play important roles in ameliorating most, if not all of the morphine withdrawal signs.

In conclusion, the findings of the present study provide new evidence supporting the clinical efficacy of 100-Hz EA or TENS for treating morphine withdrawal syndrome [7].

Acknowledgements

The authors wish to thank Dr. Avram Goldstein for his generous help with the dynorphin A antiserum IgG. This work was supported by NIH grant DA 03983, USA, to Ji-Sheng Han, and a grant (39570682) from the National Natural Science Foundation of China to Liu-Zhen Wu.

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