Neurochem Res (2008) 33:2070–2077
DOI 10.1007/s11064-008-9686-9

ORIGINAL PAPER

Distinct Responses of DREAM to Electroacupuncture Stimulation with Different Frequencies During Physiological and Inflammatory Conditions in Rats

Yi Li · Ying Zhang · Ji-Sheng Han · Yun Wang

Accepted: 25 March 2008 / Published online: 11 April 2008

Abstract Our previous results indicated that dynorphin in the spinal dorsal horn mediates the analgesic effect of high frequency electroacupuncture stimulation (EAS). Here we report that the transcriptional repressor downstream regulatory element antagonist modulator (DREAM) of dynorphin precursor-preprodynorphin (PPD) may participate in this process. In normal rats, 100 Hz, but not 2 Hz EAS triggered the nuclear export and membrane translocation of DREAM concomitantly with the upregulation of PPD mRNA in the dorsal horn. In inflammatory rats, both 2 and 100 Hz EAS alleviated thermal and mechanical hypersensitivity and caused the nuclear export and membrane translocation of DREAM, but only 100 Hz EAS enhanced the mRNA level of PPD and DREAM. These results suggest the role of DREAM in the dorsal horn in the regulation of PPD gene expression by EAS is frequency dependent, and DREAM may exert different roles in different frequency EAS under physiological and inflammatory conditions.

Keywords DREAM · Preprodynorphin · Electroacupuncture · Frequency · Spinal dorsal horn · Inflammation

Introduction

Peripherally applied electrical stimulation has been postulated to accelerate the release of endogenous opioid peptides [1], resulting in significant analgesia. This effect was shown to be frequency-dependent, with low frequency (2 Hz) electroacupuncture (EA) increasing the release of an array of opioid peptides, including enkephalin, β-endorphin and endomorphin to interact with the μ- and δ-opioid receptors in the CNS, and high frequency (100 Hz) stimulation increasing the release of dynorphin to interact with the κ-opioid receptor in the spinal cord [2–4].

Several groups characterized the transcriptional control of the preprodynorphin (PPD) gene, and found that CREB, *c-fos* and *c-Jun* all participated in the transcriptional regulation of PPD [5]. In 1999, a new calcium-dependent transcriptional repressor, downstream regulatory element antagonist modulator (DREAM) was identified [6]. DREAM can bind specifically to the downstream regulatory element (DRE) of the PPD gene, and represses gene expression [7]. DREAM contains four EF-hand motifs, and upon stimulation by Ca^{2+}, its ability to bind the DRE and the related repressor function are prevented [6].

DREAM appears to be a protein with multiple functions. Because DREAM is able to bind both calcium and presenilins, it is denoted as calsenilin [8]. DREAM also belongs to a group of structurally and functionally related Ca^{2+}-binding proteins (KChIP-1 to -4) that interact with voltage-dependent K^+ channels of the Kv4 class, which modulate potassium currents in a Ca^{2+}-dependent manner in the plasma membrane. KChIP-3 corresponds to DREAM [9].

Knockout of DREAM results in attenuation of pain responses in various pain models without major defects in

Special issue article in honor of Dr. Ji-Sheng Han.

Y. Li · Y. Zhang · J.-S. Han · Y. Wang (✉)
Neuroscience Research Institute & Department of Neurobiology, The Key Laboratory for Neuroscience of the Ministry of Education and of Health, Peking University, 38 Xueyuan Road, Beijing 100083, People's Republic of China
e-mail: wangy66@bjmu.edu.cn

motor function or learning and memory. Mice lacking DREAM have elevated levels of PPD mRNA and dynorphin A peptides in the spinal cord, and the reduction of pain behaviors in *dream-/-* mice was shown to be mediated through dynorphin-selective κ-opioid receptors [10]. These findings identify DREAM as a critical molecule for pain modulation, and thus, the hypothesis "no DREAM, no pain" was developed [11].

Based on the above evidence, PPD is likely a link between DREAM and 100 Hz EAS, and thus, in the present study, we investigate whether DREAM participates in the upregulation of PPD induced by high frequency electroacupuncture stimulation (EAS) in normal and inflammatory rats. Elucidation of the mechanism of how the central nervous system recognizes peripheral stimulation will allow us to understand the basis of the therapeutic effect of peripheral electroacupuncture and promote the clinic application of electroacupuncture. On the other hand, to study the function of DREAM in vivo is very important to elucidate the physiological functions of this transcription factor.

Experimental Procedure

Animals

Male Sprague-Dawley rats weighing 200–250 g were supplied by the Animal Center of Peking University Health Science Center. They were housed in climate-controlled rooms on a 12 h light–dark cycle with free access to food and water. The animals were acclimated for 5 days prior to the start of any experimental procedures. All experimental procedures conformed to the Animal Care and Use Committee of Peking University guidelines and all efforts were made to minimize discomfort to the animals.

For the study of normal rats, rats were randomly divided into the following four groups, (1) Naïve, untreated group; (2) Needling, subject to the same manipulation as the EAS group without electrical stimulation; (3) 2 Hz EAS, treated with 2 Hz EAS; (4) 100 Hz EAS, treated with 100 Hz EAS. Rats of groups (3) and (4) received EA treatment once every 2 days, for a total of two treatments.

For the study of inflammatory rats, rats were randomly divided into the following four groups, (1) Complete Freund's adjuvant (CFA), only received CFA injection without other treatment; (2) Needling, subject to the same manipulation as the EAS group without electrical stimulation; (3) 2 Hz EAS, treated with 2 Hz EAS; (4) 100 Hz EAS, treated with 100 Hz EAS. All rats received CFA injection, and rats in groups (3) and (4) received EA treatment at one day and four days post CFA injection.

CFA-induced Inflammatory Pain Model and Behavioral Test

About 100 μl CFA (Sigma-Aldrich, St Louis, MO, USA) was injected into the plantar surface of the left hind paw of rats under ether anesthesia [12]. Classical signs of acute inflammation including edema, redness, and heat were observed.

Assessment of Hypersensitivity

Thermal hyperalgesia was evaluated using radiant heat testing. Briefly, the animals were allowed to become accustomed to the environment for 20 min before testing. Then, the plantar surface of a hind paw was exposed to a beam of radiant heat through a transparent perspex surface. A cut-off time of 30 s was enforced to prevent tissue damage. The paw withdrawal latency was recorded and averaged over three trials at 5 min intervals.

Mechanical allodynia was tested by Von Frey filaments [13]. The paw withdrawal threshold was calculated using the Up–down method.

Electroacupuncture Stimulation

Two stainless steel needles, 0.25 mm in diameter, were inserted into each hind leg, one in the Zusanli point (S36, 5 mm lateral to the anterior tubercle of the tibia), and the other in the Sanyinjiao point (Sp6, 3 mm proximal to the medial malleolus, at the posterior border of the tibia). Square waves of EAS generated from a Han's Acupoint Nerve Stimulator (HANS, manufactured at the Health Science Center, Peking University) were applied to both legs simultaneously. The frequency of EAS was 2 Hz with a pulse width of 0.6 ms and 100 Hz with a pulse width of 0.2 ms. The intensity was increased in a stepwise manner at (0.5–1.0–1.5) mA, each lasting for 10 min.

Protein Extraction

After anesthesia, the lumbar enlargement of the spinal dorsal horn of rats was dissected out, frozen in liquid nitrogen, and stored at −80°C until analysis.

Extraction of Nuclear Protein

The samples were homogenized in ice-cold buffer A [10 mM Hepes-NaOH (pH 7.8), 15 mM KCl, 1 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 1 mM PMSF, 1 μg/μl Leupeptin], and vortexed for 10 s with 10% NP-40 (the volume of which was 10% of buffer A). The homogenate was then centrifuged at 10,000*g* for 20 sec at 4°C. The resulting pellet was dissolved in ice-cold buffer B [20 mM

Hepes-NaOH (pH 7.9), 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA, 25% glycerol (V/V), 0.5 mM DTT, 1 mM PMSF, 1 μg/μl Leupeptin], and centrifuged at 12,000*g* for 4 min at 4°C. The resulting supernatant was the nuclear fraction.

Extraction of Membrane Protein

The samples were homogenized in ice-cold RIPA buffer [50 mM Tris (pH 7.5), 250 mM NaCl, 10 mM EDTA, 0.5% NP-40, 1 μg/ml Leupeptin, 1 mM PMSF, 4 mM NaF], and the homogenate was centrifuged at 500*g* for 10 min at 4°C. The resulting pellet was removed and the supernatant (total protein extracts) was further centrifuged at 25,000*g* for 60 min. The resulting pellet was dissolved in ice-cold RIPA buffer containing 0.1% (v/v) Triton X-100 and the solution was the membrane fraction.

Concentration of protein was measured with a BCA assay kit (Pierce Biotechnology, Rockford, IL, USA).

Western Blotting

Equivalent amounts (50 μg) of protein preparations for each sample were denatured, subjected to SDS-PAGE using 12% running gels, and transferred to nitrocellulose membranes. After blocking with TBST [10 mM Tris–HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween 20] containing 5% non-fat dried milk, for 1 h at room temperature, the membranes were incubated with a polyclonal DREAM antibody (1:300, Santa Cruz Biotechnology, CA, USA) or monoclonal β-actin antibody (1:2000, Sigma, USA) overnight at 4°C. The membranes were then incubated with HRP-conjugated goat anti-rabbit or mouse secondary antibody (1:2000, Jackson Laboratories, West Grove, PA, USA) for 1 h at room temperature. Between incubations, the membranes were washed three times in TBST for 10 min. Finally, the blots were examined using a chemiluminescence kit (Santa Cruz, Biotechnology, CA, USA).

RNA Isolation and Real-time RT-PCR

We performed real-time quantitative RT-PCR for PPD and DREAM mRNA. Total RNA were isolated from the spinal dorsal horn of rats with Trizol Reagent (Invitrogen, Carlsbad, CA, USA; 1 ml/100 mg tissue) according to the instructions of the manufacturer. Reverse transcription was performed with 2 μg of total RNA with the MMLV-reverse transcriptase (Progema, Madison, WI, USA). Real-time PCR was performed using a SYBR premix kit (TOYOBO, QPK-201T), and running it for 35 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 30 s. After it was normalized by quantification of the housekeeping GAPDH gene, the mRNA levels were expressed as ratios compared with the Naïve control or CFA control. Primers for the real-time RT-PCR are as follows, GAPDH, forward 5′-AGTGCCAG CCTCGTCTCATAG-3′ and reverse 5′-CGTTGAACTTG CCGTGGGTAG-3′; PPD, forward 5′-TTCATCCTCCTC TGCTTATTC-3′, and reverse 5′-CCCTTGGTAGTGGT AGTTTAG-3′; DREAM, forward 5′-AGATGCCACCACC TATGC-3′ and reverse 5′-TTGATGTCGTAGAGATTG AAGG-3′.

Statistical Analysis

Data are presented as mean ± SEM. Differences between groups were compared using either the Student's *t*-test or one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test. The criterion for statistical significance was $P < 0.05$.

Results

Effect of 2 and 100 Hz EAS on DREAM and PPD Expression in Spinal Dorsal Horn of Normal Rats

As DREAM has been reported to be a transcriptional repressor of PPD for pain modulation in the spinal cord [10], and our previous results indicate that DREAM was mainly distributed in the membrane, few in nuclear and almost no in cytoplasma fractions of the spinal cord dorsal horn [15], thus we first evaluated the effect of different frequencies of EAS on the protein level of DREAM in the nuclear and membrane fraction of the spinal dorsal horn of normal rats. As shown in Fig. 1, 100 Hz, but not 2 Hz EAS reduced the expression of DREAM in the nuclear fraction (Fig. 1A) and increased the expression of DREAM in the membrane fraction (Fig. 1B) compared with the Needling group. According to the quantification analysis, DREAM expression was significantly downregulated in the nuclear fraction ($P < 0.05$) (Fig. 1C), while it was significantly upregulated in the membrane fraction (Fig. 1D) after treatment with 100 Hz EAS ($P < 0.01$). Since DREAM was mainly distributed in the membrane, few in nuclear and almost no in cytoplasma fractions of the spinal cord dorsal horn, thus the summation of data from Fig. 1C and D representing the total amount of DREAM. The results indicate that no significant changes of the total amount of DREAM were observed following different treatments (Fig. 1E). To determine whether 100 Hz EAS affected the total expression level of DREAM, we performed real-time RT-PCR to detect the mRNA level of DREAM. Figure 1F shows that there were no significant changes in DREAM mRNA levels in the spinal dorsal horn after the treatment of both 2 and 100 Hz EAS, suggesting that 100 Hz EAS did not affect total expression level of DREAM, and it

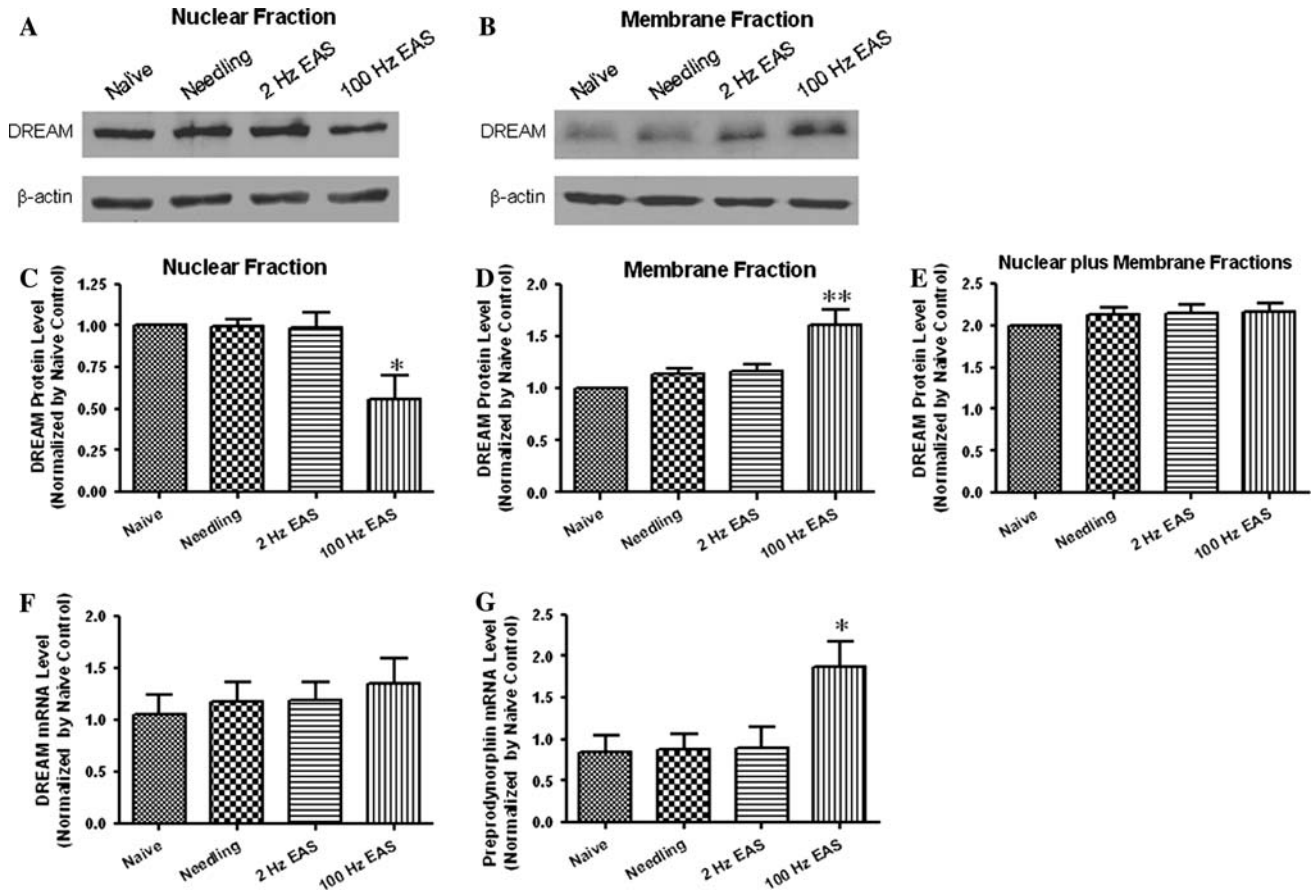

Fig. 1 Effect of EAS on DREAM and PPD expression in spinal dorsal horn of normal rats. (**A**) Representative Western blot results of the nuclear fraction of the spinal dorsal horn. The lower panel shows the loading control. (**C**) Quantification analysis of the optical density of these bands. Columns represent means ± SEM for four separate experiments. Data were normalized by Naïve control, one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test. $^{*}P < 0.05$, compared with the Needling group. (**B**) Representative Western blot results of the membrane fraction of the spinal dorsal horn. The lower panel shows the loading control. (**D**) Quantification analysis of the optical density of these bands. Columns represent means ± SEM for four separate experiments. Data were normalized by Naïve control, one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test. $^{**}P < 0.01$, compared with the Needling group. (**E**) Summation of the data of **C** and **D** which indicate the total amount of DREAM after different treatments. Columns represent means ± SEM for four separate experiments. Data were normalized by Naïve control. (**F**) Expression of DREAM mRNA after EA treatment. Quantification was by real-time RT-PCR. Columns represent means ± SEM for seven separate experiments. Data were normalized by Naïve control, one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test. (**G**) Expression of PPD mRNA after EA treatment. Quantification was by real-time RT-PCR. Columns represent means ± SEM for seven separate experiments. Data were normalized by Naïve control, one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test. $^{*}P < 0.05$, compared with the Needling group

could trigger the nuclear export of DREAM in the spinal dorsal horn. Figure 1G shows that only 100 Hz EA treatment causes the upregulation of PPD mRNA level, which is consistent with our previous results [4] and also matches the downregulation of DREAM expression in the nuclear fraction.

Effect of 2 and 100 Hz EAS on DREAM and PPD Expression in the Spinal Dorsal Horn of Inflammatory Rats

It has been reported that there are differences of electroacupuncture-induced analgesic effects in both normal and inflammatory rats [14]. Thus, we further tested the effect of different frequency EAS on DREAM and PPD expression in the rat spinal dorsal horn in the inflammatory pain model. We injected 100 μl CFA into the plantar surface of the left hind paw in rats, which resulted in considerable changes in hyperalgesia. At one day post injection, both the thermal latency and mechanical threshold of the left hind paw (injected side) significantly decreased and persisted until at least five days post injection (Fig. 2A and B). However, the thermal latency increased and showed obvious differences after EA treatment when compared with that of the Needling group without EA treatment (Fig. 2C). In addition, there was no difference between the

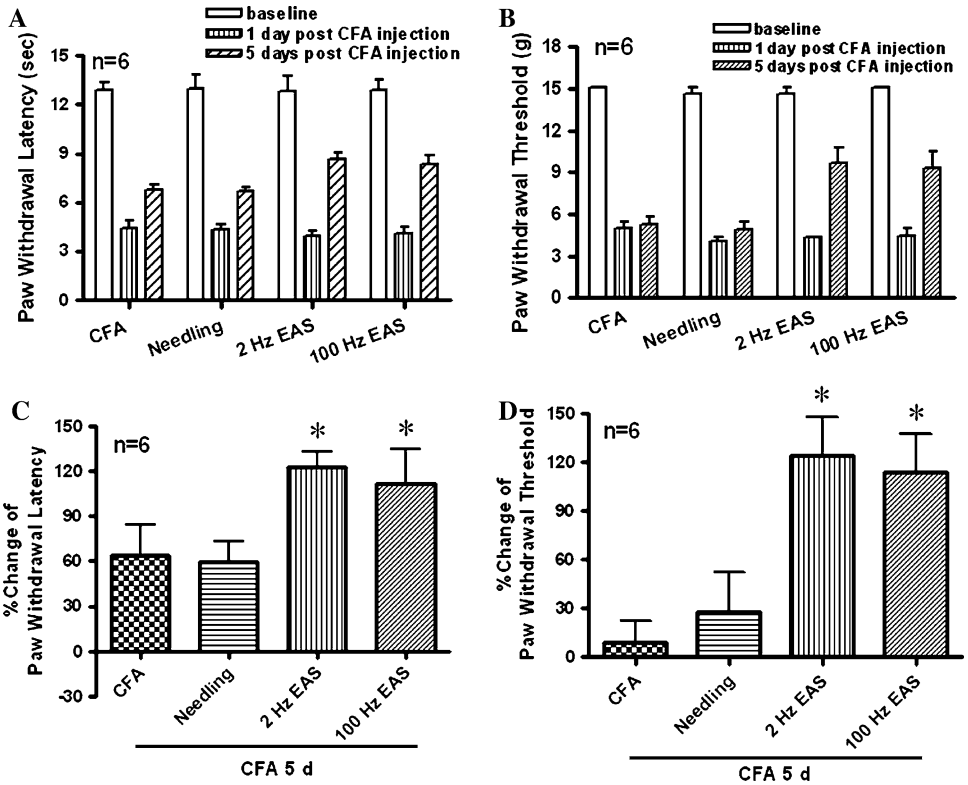

Fig. 2 Effect of EAS on CFA-induced thermal and mechanical hyperalgesia. (**A**) The paw withdrawal latency to thermal stimulation of left hind paw (CFA injected side) at different time points, including baseline, 1 and 5 days post CFA injection. (**B**) The paw withdrawal thresholds to mechanical stimulation of the left hind paw (CFA injection side) at different time points, including baseline, 1 and 5 days post CFA injection. (**C**) Effect of EAS on CFA-induced thermal hyperalgesia. Data are expressed as the percentage increase over baseline levels. $^*P < 0.05$, compared with the Needling group. (**D**) Effect of EAS on CFA-induced mechanical hyperalgesia. Data are expressed as the percentage increase over baseline levels. $^*P < 0.05$, compared with the Needling group

CFA group and the Needling group (Fig. 2C). They exhibited almost the same change in mechanical threshold with thermal latency after EA treatment (Fig. 2D). These results indicated that both 2 and 100 Hz EA treatments could significantly attenuate the inflammatory hyperalgesia. We then examined whether the expression level of DREAM in the nuclear and membrane fraction of the spinal dorsal horn changed under peripheral inflammation and different frequency EA treatments. Consistent with the previous results of our group [15], peripheral inflammation induced an upregulation of DREAM expression both in the nuclear and membrane fraction of the ipsilateral spinal dorsal horn until five days post injection (data not shown). To our surprise, both 2 and 100 Hz EAs could reduce this elevation in the nuclear fraction (Fig. 3A and B), while they enhanced the elevation in the membrane fraction, no significant change was observed in the needling alone group (Fig. 3C and D). The summation of the data from Fig. 3B and D indicates that both 2 and 100 Hz EAS could induce significant increases of the total amount of DREAM following inflammation, while no significant change was observed in the needling alone group (Fig. 3E). To better understand the changes in the DREAM expression pattern, we measured the mRNA level of DREAM to evaluate the total expression level of DREAM during peripheral inflammation and EA treatment. The results indicated that only the 100 Hz EA treatment could significant upregulate the total expression level of DREAM. There was no difference between the CFA group and the Needling group (Fig. 3F). To investigate changes in the expression of preprodynorphin in response to inflammation and EAS treatment, we used real-time RT-PCR to detect the expression of PPD mRNA. 100 Hz but not 2 Hz EA treatment induced a substantial upregulation of PPD mRNA in the ipsilateral spinal dorsal horn compared with the Needling group (Fig. 3G).

Discussion

The main findings of the present study are as follows (1) In the study of normal rats, no significant changes of total amount of DREAM were observed following different treatments, only 100 Hz EAS could trigger the nuclear export and membrane translocation of DREAM concomitantly with the upregulation of PPD mRNA in the dorsal horn compared with the Needling group (2 Hz EAS did not have this effect). (2) In the study of inflammatory rats, both 2 and 100 Hz EAS significantly increased mechanical withdrawal thresholds and paw withdrawal latency to heat stimulation of the left hind paw (CFA injected side) compared with the Needling group. An elevation of DREAM protein level in the nuclear and membrane fractions of the ipsilateral spinal dorsal horn was detected on 5 days post CFA injection, and both 2 and 100 Hz EAS could repress the elevation in the nuclear fraction, while enhancing the

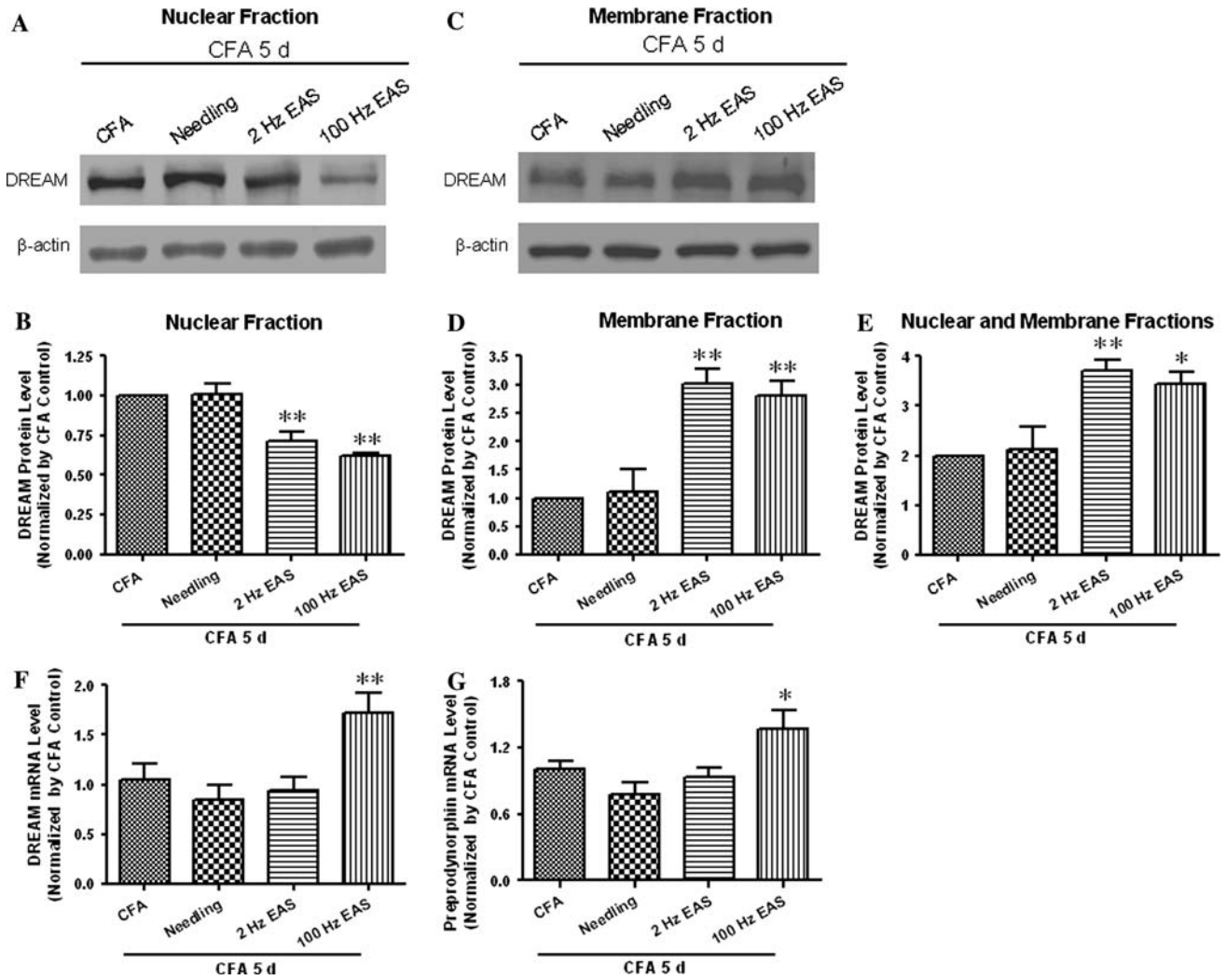

Fig. 3 Effect of EAS on DREAM and PPD expression in the spinal dorsal horn in inflammatory rats. (**A**) Representative Western blot results for the nuclear fraction from the ipsilateral spinal dorsal horn. The lower panel shows the loading control. (**B**) Quantification analysis of the optical density of these bands. Columns represent means ± SEM for three separate experiments. Data were normalized by the CFA control, one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test. $^{**}P < 0.01$, compared with the Needling group. (**C**) Representative Western blot results for the membrane fraction from the ipsilateral spinal dorsal horn. The lower panel shows the loading control. (**D**) Quantification analysis of the optical density of these bands. Columns represent means ± SEM for three separate experiments. Data were normalized by CFA control, one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test. $^{**}P < 0.01$, compared with the Needling group. (**E**) Summation of the data of **B** and **D** which indicate the total amount of DREAM after different treatments. Columns represent means ± SEM for three separate experiments. Data were normalized by the CFA control, one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the Needling group. (**F**) Expression of DREAM mRNA of the ipsilateral spinal dorsal horn after EA treatment. Quantification was by real-time RT-PCR. Columns represent means ± SEM for seven separate experiments. Data were normalized by the CFA control and ANOVA followed by the Newman-Keuls Multiple Comparison Test. $^{**}P < 0.01$, compared with the Needling group. (**G**) Expression of PPD mRNA of the ipsilateral spinal dorsal horn after EA treatment. Quantification was by real-time RT-PCR. Data were normalized by the CFA control and ANOVA followed by the Newman-Keuls Multiple Comparison Test. $^{*}P < 0.05$, compared with the Needling group

elevation in the membrane fraction. However, a detectable increase of DREAM and PPD mRNA levels was observed only in the 100 Hz EAS group.

Our previous findings in normal rats showed that low and high frequency electroacupuncture (EA)-produced analgesia is mediated by different brain substrates and different opioid peptides. Likewise, 2 and 100 Hz EAS exert differential effects on opioid gene expression: while 2 Hz EAS induces a more extensive and intensive preproenkephalin (PPE) mRNA expression than 100 Hz EAS, it has no effect on preprodynorphin (PPD) mRNA expression, which is significantly increased by 100 Hz EAS [16]. About 2 and 100 Hz EAS induce markedly different spatial patterns of Fos expression in the rat brain

and as Fos and Jun proteins are involved in PPD rather than PPE gene transcription activated by EAS, this suggests that there are distinct neuronal pathways underlying EAS of different frequencies [17]. In the present study, the response pattern of the new transcriptional factor DREAM was also observed in a frequency-dependent manner with EAS. In normal rats, after 100 Hz, but not 2 Hz EA treatment, although the mRNA level of DREAM was not changed, the expression level of DREAM in the nuclear fraction of the spinal dorsal horn decreased, suggesting that 100 Hz EA treatment specifically promoted the nuclear export of DREAM. Moreover, we also found that PPD gene expression level was upregulated upon 100 Hz EAS. As DREAM can bind specifically to the downstream regulatory element (DRE) of the PPD gene and represses gene expression, we speculate that nuclear export of DREAM triggered by 100 Hz EAS may release the repression of PPD gene expression, and elevate the PPD mRNA level. This hypothesis needs to be further investigated.

Consistent with the results in normal rats, in the study of inflammatory rats, 100 Hz EAS still could repress the elevation of DREAM in the nuclear fraction of the ipsilateral spinal dorsal horn, indicating the nuclear export of DREAM after 100 Hz EAS. However, only 100 Hz EAS could induce the upregulation of PPD gene expression, suggesting that even in inflammatory conditions, the paired expression patterns between DREAM and PPD remain, suggesting that the upregulation of the PPD gene triggered by 100 Hz EAS may be mediated at least partly by the nuclear export of DREAM.

Although the nuclear export of DREAM and an increase in PPD gene expression induced by 100 Hz EAS was well correlated both in normal rats and inflammatory rats, the present study provides evidence that DREAM might exert different roles with different frequency EAS under physiological and inflammatory conditions: (1) In normal rats, only 100 Hz EAS triggered the nuclear export and membrane translocation of DREAM. (2) In inflammatory pain rats, both 2 and 100 Hz EAS could repress the elevation in the nuclear fraction, while enhancing the elevation in the membrane fraction. However, a detectable increase of DREAM and PPD mRNA level was observed only in the 100 Hz EAS group, suggesting that although 2 Hz EAS can trigger the nuclear export and membrane translocation of DREAM, it cannot enhance the synthesis of DREAM like that of 100 Hz EAS. The nuclear export of DREAM triggered by 2 Hz EAS in the inflammatory model was impaired with the enhancement of PPD gene expression, suggesting that nuclear export of DREAM induced by different frequency EAS may exert different mechanisms for pain relief. As we mentioned above, although 2 Hz EAS induced more extensive and intensive preproenkephalin (PPE) mRNA expression than 100 Hz EAS, it had no effect on preprodynorphin (PPD) mRNA expression which was significantly increased by 100 Hz EA stimulation. It is known that DREAM can repress the gene expression of, for example, PPD, c-Fos, the apoptotic gene Hrk but not PPE, and thus, the downstream regulator of nuclear export of DREAM triggered by 2 Hz EAS needs to be further verified.

Interestingly, both frequencies of EAS could trigger an upregulation of DREAM in membrane extracts of the ipsilateral spinal dorsal horn. As we know that Kv4.2 mediates most of the A-type currents in dorsal horn neurons, DREAM may augment A-type currents via increased surface channel density, slower inactivation and faster recovery from the inactivation of Kv4 channels [9]. DREAM can induce a dramatic redistribution of Kv4.2, from the intrinsic endoplasmic reticulum to the cell surface [18]. Upregulation of DREAM in the cytomembrane by inflammatory pain may reduce the excitability of dorsal horn neurons by affecting the biophysical and molecular characteristics of Kv4. From this point of view, DREAM may inhibit the hyperactivity of dorsal horn neurons during inflammatory pain, while different frequency EAS could enhance the inhibitory effect of DREAM by potentiating the upregulation of DREAM in the membrane during inflammation.

In summary, in normal conditions, gene expression of PPD might be regulated by DREAM only by high frequency electroacupuncture stimulation; while in inflammatory conditions, DREAM might play multiple roles in the spinal cord in pain modulation in addition to regulation of PPD gene expression during both low and high frequency EAS.

Acknowledgements This work was supported by grants from the National Natural Science Foundation of China (30330230 and 30470550); a grant from the Beijing Natural Science Foundation (5052019), the grant for Outstanding Young Teacher of Higher Academic School to YW from the Ministry of Education, China [2001-182] and a grant from State Administration of Traditional Chinese Medicine of the People's Republic of China (04-05JP04) to YW.

References

1. Goldberg IE, Rossi GC, Letchworth SR et al (1998) Pharmacological characterization of endomorphin-1 and endomorphin-2 in mouse brain. J Pharmacol Exp Ther 286:1007–1013
2. Han JS (1993) Acupuncture and stimulation produced analgesia. In: Herz A (ed) Opioid II (handbook of experimental pharmacology). Springer, Berlin, pp 105–125
3. Han JS, Wang Q (1992) Mobilization of specific neuropeptides by peripheral stimulation of identified frequencies. News Physiol Sci 7:176–180
4. Han JS, Xie GX, Ding XZ et al (1984) High and low frequency electroacupuncture analgesia are mediated by different opioid peptides. Pain Suppl 2:543

5. Messersmith DJ, Kim DJ, Iadarola MJ (1998) Transcription factor regulation of prodynorphin gene expression following rat hindpaw inflammation. Brain Res Mol Brain Res 53:260–269
6. Carrion AM, Link WA, Ledo F et al (1999) DREAM is a Ca^{2+}-regulated transcriptional repressor. Nature 398:80–84
7. Carrion AM, Mellstrom B, Naranjo JR (1998) Protein kinase A-dependent derepression of the human prodynorphin gene via differential binding to an intragenic silencer element. Mol Cell Biol 18:6921–6929
8. Buxbaum JD, Choi EK, Luo Y et al (1998) Calsenilin: a calcium-binding protein that interacts with the presenilins and regulates the levels of a presenilin fragment. Nat Med 4:1177–1181
9. An WF, Bowlby MR, Betty M et al (2000) Modulation of A-type potassium channels by a family of calcium sensors. Nature 403:553–556
10. Cheng HY, Pitcher GM, Laviolette SR et al (2002) DREAM is a critical transcriptional repressor for pain modulation. Cell 108:31–43
11. Costigan M, Woolf CJ (2002) No DREAM, no pain. Closing the spinal gate. Cell 108:297–300
12. Yang YR, He Y, Zhang Y et al (2007) Activation of cyclin-dependent kinase 5 (Cdk5) in primary sensory and dorsal horn neurons by peripheral inflammation contributes to heat hyperalgesia. Pain 127:109–120
13. Chaplan SR, Bach FW, Pogrel JW et al (1994) Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 53:55–63
14. Sekido R, Ishimaru K, Sakita M (2003) Differences of electro-acupuncture-induced analgesic effect in normal and inflammatory conditions in rats. Am J Chin Med 31:955–965
15. Zhang Y, Li Y, Yang YR et al (2007) Distribution of downstream regulatory element antagonist modulator (DREAM) in rat spinal cord and upregulation of its expression during inflammatory pain. Neurochem Res 32:1592–1599
16. Guo HF, Tian J, Wang X et al (1996) Brain substrates activated by electroacupuncture of different frequencies (I): comparative study on the expression of oncogene c-fos and genes coding for three opioid peptides. Brain Res Mol Brain Res 43:157–166
17. Guo HF, Tian J, Wang X et al (1996) Brain substrates activated by electroacupuncture (EA) of different frequencies (II): role of Fos/Jun proteins in EA-induced transcription of preproenkephalin and preprodynorphin genes. Brain Res Mol Brain Res 43:167–173
18. Shibata R, Misonou H, Campomanes CR et al (2003) A fundamental role for KChIPs in determining the molecular properties and trafficking of Kv4.2 potassium channels. J Biol Chem 278:36445–36454