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Effects of corticotrophin on pain behavior and BDNF, CRF levels in frontal cortex of rats suffering from chronic pain

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KEY WORDS brain-derived neurotrophic factor; corticotropin-releasing hormone; chronic pain; corticotropin; rats

ABSTRACT

AIM: To investigate the effects of corticotrophin (Cor) on corticotropin-releasing factor (CRF), brain-derived neurotrophic factor (BDNF), and its functional receptor trkB in the frontal cortex of complete Freud's adjuvant (CFA)-induced arthritic rats. **METHODS**: The chronic pain rat model was modified and pain behaviour scores were assessed. BDNF-immunoreactivity (IR), trkB-IR, and CRF mRNA-positive neurons were measured by immunohistochemistry and *in situ* hybridization methods. **RESULTS**: Compared with control rats, pain behavior scores, BDNF-IR, CRF mRNA-positive, trkB-IR, and BDNF/CRF mRNA double-labeling neurons in the contralateral frontal cortex of the arthritic rats increased significantly at 24 h after injection of CFA ($P < 0.05$), and these effects were decreased markedly by ip injection of Cor ($P < 0.05$). The decrease in pain behavior and BDNF-IR, CRF mRNA levels in frontal cortex of arthritic rats due to Cor were partly prevented by adrenalectomy (ADX). **CONCLUSION**: The increment in BDNF and CRF levels in the contralateral frontal cortex of arthritic rats may be inhibited by corticotrophin.

INTRODUCTION

Recent studies showed that infusion of exogenous brain-derived neurotrophic factor (BDNF) into the adult rat midbrain and dorsal raphe produced analgesia[1,2], which was perhaps caused by its modulation of neuropeptides related to chronic pain such as β-endorphin, substance P (SP), neuropeptide Y (NPY), and calcium gene-related peptide (CGRP)[3]. Furthermore, peripheral inflammation induced by complete Freud's adjuvant (CFA) also increased expression of both BDNF mRNA and BDNF-immunoreactivity (IR) in the dorsal root ganglia and spinal cord of the adjuvant-induced arthritic (AIA) rats, and this increment was mediated by nerve growth factor (NGF)[4,5]. Corticotropin-releasing factor (CRF) is the major hypophysiotropic factor regulating basal stress-induced release of corticotropin (Cor), β-endorphin, and other pro-opiomelanocortin-derived peptides from the pituitary[6]. Recently it was reported that CRF produced analgesia in humans and rats[7], and CRF mRNA coexisted with BDNF in the hypothalamus of the stressed rats, suggesting that BDNF may modulate the expression of CRF[8]. Stressful conditions also activate endogenous analgesic systems, so it seems that BDNF can affect the expression of CRF in the frontal cortex during chronic pain, the frontal cortex being the somatosensory center modulating sensory inputs (ie, pain). Cor has effects on pain responsiveness in formalin test of rats[9,10], and the release of Cor is affected by CRF. Thus may be some relation among BDNF, CRF mRNA, and Cor in the frontal cortex during chronic pain conditions.

In our present study, we evaluated pain behavior scores, the immunoreactivity changes of BDNF and trkB with immunohistochemistry, CRF mRNA-positive neurons with *in situ* hybridization and BDNF/CRF mRNA double-labeling neurons with double-labeling methods in the frontal cortex of chronically algesic rats. The function of Cor was also studied.

MATERIALS AND METHODS

Models The chronic pain arthritic rat model was established according to Butler's method with some modification[11]. In brief, Wistar rats ($n = 60$, both sexes, 200 g ± 25 g, grade Ⅱ from the Center of Experimental Animals of Third Military Medical University) were injected sc with CFA 150 μL containing 15 g/L killed tu-

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berclebacillus in the right ankles.

Immunohistochemistry The CFA injected rats were allowed to survive for 4 h, 24 h, or 7 d ($n = 15$, 4 or 5 rats at each time point). Twenty-four hours after CFA injection, the rats were ip injected with Cor 25 IU/kg or 12.5 IU/kg respectively. And some of the arthritic rats were adrenalectomised 5 to 7 d before ip Cor. At the end of the experiment, the rats were anesthetised deeply and perfused through the aorta with normal saline solution and 4 % paraformaldehyde (4 ℃) successively. The frontal cortex was dissected out and post-fixed in 4 % paraformaldehyde for 2 h, then was cryoprotected in 30 % sucrose at 4 ℃. The frontal cortex was cut at 40 μm thickness and collected in phosphate-buffered saline (PBS) and the sections were immunostained with an affinity-purified rabbit antibody to recombinant human BDNF (1∶500) or trkB (1∶500 both from Santa Cruz Biotechnology, Inc) with the avidin-biotin peroxidase complex (1∶100, ABC kit from Vector Labs of Oxford University, England) and visualized by daminobenzidin (from Sigma).

***In situ* hybridization** The hybridization procedure was according to the method of Bloch *et al*[12]. The probe was digitoxin labeled antisense cRNA (0.5 μL/mL, from Department of Embryology of our university). And the concentration of anti-Dig antibody was 1∶1000. The sections were stained with NBT/BCIP (400 mg/L and 200 mg/L) for 12 h at 4 ℃ in darkness. No signals were detected in control sections. In the present study, neurons which had grain density of at least 5 times higher than the background density were considered to show positive expression. Some of the NBT/BCIP stained sections were double labeled with BDNF immunohistochemistry, the procedure of which was the same as described before.

Statistics For calculating the BDNF-IR, trkB-IR, CRF mRNA-positive, and BDNF/CRF mRNA double-labeling neurons, 5 sections were selected randomly from every group and objectively assessed with a computer-assisted image analyzer (Image Pro Plus, Media Cybernetics, Silver Spring, USA), and an average number of neurons from the contralateral frontal cortex were got for that group to be expressed as $\bar{x} \pm s$. Significance between each group was examined with PDA-2 software.

RESULTS

Effect of Cor on pain behavior scores The ankles of rats appeared red and swollen 2 – 4 h after injection of adjuvant, and apparent pain behavior scores, including reduction of mobility and withdrawal ability, and the increasing of joint stiffness and toe retraction, were also observed at that time. Twenty-four hours after injection, the swelling and pain behavior scores reached a peak point and remained at a high level for 1 wk or so, then declined during 9 and 10 wk. However, the weight of arthritic rats kept on elevating (data not shown). The reduction in mobility and withdrawal scores were inhibited when the arthritic rats received ip of Cor. And the effects of Cor were attenuated by ADX (Tab 1).

Effects of corticotrophin on BDNF, trkB, CRF mRNA and double-labeling neurons in frontal cortex Four hours after injection of CFA, the numbers of BDNF-IR neurons and CRF mRNA-positive neurons in the contralateral frontal cortex increased by 40.96 % ($P < 0.05$) and 35.71 % ($P < 0.05$) respectively, while those of ipsilateral frontal cortex had no marked changes. And the increment reached the peak point at 24 h, the number of BDNF-IR neurons and CRF mRNA-positive neurons increased by 166.33 % ($P < 0.01$, Fig 1B) and 92.50 % ($P < 0.01$, Fig 1F) respectively, and were still on a high level at d 7. The number of trkB-IR, BDNF/CRF mRNA double-labeling neurons were increased by 87.92 % ($P < 0.05$) and

Tab 1. Pain behavior scores of arthritic rats. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control. $^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* AIA 24 h. $^{g}P > 0.05$, $^{h}P < 0.05$ *vs* Cor (25 IU/kg) ip + AIA 24 h. **RMS: reduction of mobility scores; RWS: reduction of withdrawal scores; JS: joint stiffness; WT: withdrawal of toes.**

	RMS	RWS	JS	WT
Control	0	0	0	0
AIA 24 h	4.80 ± 0.41^{c}	5.41 ± 0.34^{c}	3.04 ± 0.51^{b}	2.45 ± 0.33^{b}
Cor (25 IU/kg) ip + AIA 24 h	3.43 ± 0.53^{e}	2.84 ± 0.43^{f}	3.20 ± 0.33^{d}	2.42 ± 0.21^{d}
Cor (25 IU/kg) ip + AIA 24 h + ADX	4.23 ± 0.32^{h}	3.84 ± 0.21^{h}	3.24 ± 0.44^{g}	2.80 ± 0.31^{g}

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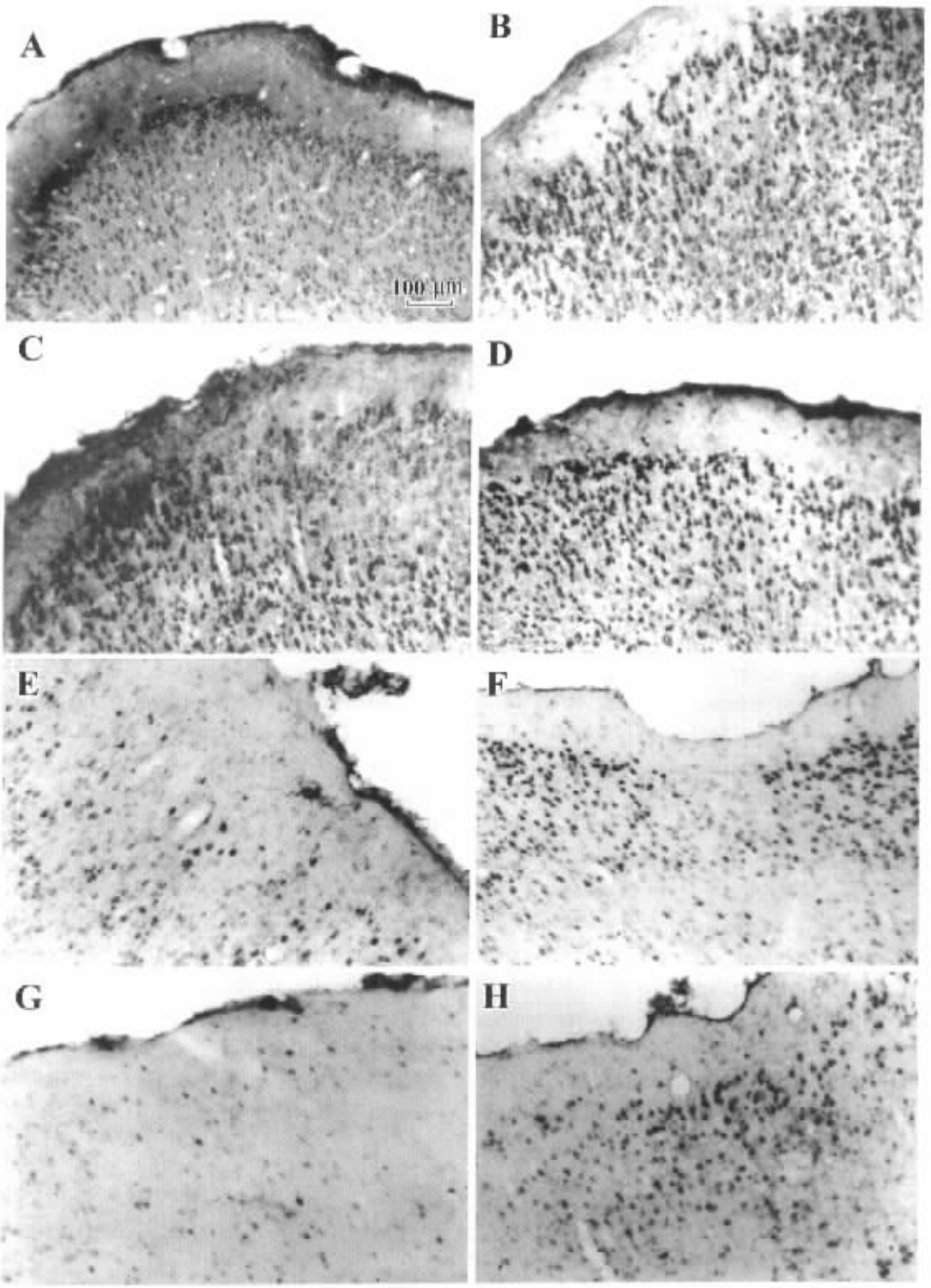

Fig 1. Immunohistochemistry of BDNF in the contralateral frontal cortex of rats (A-D, DAB-H_2O_2 stain, × 75) A) Control, B) AIA 24 h, C) Cor 25 IU/kg + AIA 24 h, D) Cor 25 IU/kg + AIA 24 h Dig-CRF-cRNA *in situ* hybridization of rat contralateral frontal cortex (NBT/BCIP stain × 75), E) Control, F) AIA 24 h, G) Cor 25 IU/kg + AIA 24 h. H) Cor 25 IU/kg + AIA 24 h.

105.01 % ($P < 0.01$) 24 h after injection of CFA and remained at a high level even on d 7. Among BDNF-IR neurons, about 14 % were BDNF/CRF mRNA double-labeling neurons. However, this incremental tendency of BDNF-IR, trkB-IR, CRF mRNA-positive neurons and BDNF/CRF mRNA double labeling neurons at 24 h was inhibited significantly by large dosage of Cor (25 IU/kg weight, ip), the number of BDNF-IR, trkB-IR, CRF mRNA-positive neurons and BDNF/CRF mRNA double-labeling neurons were decreased by 31.41 % ($P < 0.05$), 35.02 % ($P < 0.05$), 34.08 % ($P < 0.05$), and 39.66 % ($P < 0.05$) respectively (Fig 1C, G), while the effect with smaller dosage of Cor (12.5 IU/kg weight) was not marked. When the adjuvant-induced arthritic rats were adrenalectomised, the number of BDNF-IR, CRF mRNA-positive neurons and BDNF/CRF mRNA double-labeling neurons were increased by 29.65 % ($P < 0.05$), 47.75 % ($P < 0.05$) and 79.41 % ($P < 0.05$) respectively (Tab 2, Fig 1D, H).

DISCUSSION

Our present study showed that sc injection of CFA increased pain behavior scores, BDNF-IR, CRF mRNA-positive neurons, and BDNF/CRF mRNA double-labeling neurons in contralateral frontal cortex of the rats. BDNF are widely distributed in the brain of adult rats, especially in the hippocampus, cortex, and cerebullar, and mRNA of BDNF and trkB are mainly expressed in the hippocampus and cortex[13], suggesting that hippocampus and cortex are the main areas synthesizing BDNF. It has been reported that exogenous BDNF produces analgesia in the formalin test[3], and BDNF-IR and BDNF mRNA levels increased in the dorsal root ganglia and spinal cord when the rats suffered from inflammatory conditions[4,5], suggesting that both exogenous and endogenous BDNF were involved in modulation of chronic pain. The increment of BDNF-IR neurons in contralateral hippocampus, which participates in the modulation of chronic pain, may be caused by increased synthesis or anterograde transportation of BDNF from other areas such as cortex. The analgesia due to exogenous BDNF is supposed to stimulate the synthesis of some peptides related to chronic pain such as SP, CGRP and NPY[3]. It is well documented that BDNF can stimulate the synthesis of peptides in cortex and hippocampus[14], it can affect the neuropeptides in the nervous system at the gene level. This was confirmed by the existence of BDNF/CRF mRNA double-labeling neurons in the hypothalamus of the stressed rats. The evidence that BDNF affects CRF at the gene level during chronic pain in our study is that the BDNF/CRF mRNA double-labeling neurons exist in the frontal cortex and increase when rats are sc injected with CFA.

However, the increasing pain behavior scores of AIA rats were inhibited by large dosage of Cor, so were the increasing BDNF-IR, CRF mRNA-positive neurons, BDNF/CRF mRNA double-labeling neurons in contralateral frontal cortex of AIA rats. This effect of different dosage of Cor has been reported in our previous study on formalin test in rats[9,10].

The effect of Cor was attenuated when the AIA rats were adrenalectomised, which showed that adrenal was critical to the function of Cor. After adrenalectomy (ADX), with no negative feedback of Cor in the hypothalamus-pituitary-adrenal axis, the CRF was consumed and the level of CRF decreased, and the antinociceptive effect of β-endorphine stimulated by CRF also decreased[15]. Furthermore, considering that BDNF produces a significant increment in the number of neurons expressing *c*-fos and NOS[16], BDNF seems to play an

Tab 2. Effect of Cor on the number of BDNF-IR, trkB-IR, CRF mRNA-positive neurons, and BDNF/CRF mRNA double-labeling neurons in contralateral frontal cortex of arthritic rats. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* **control.** $^{d}P > 0.05$, $^{e}P < 0.05$ *vs* **AIA 24 h.** $^{g}P > 0.05$, $^{h}P < 0.05$ *vs* **Cor 25 IU/kg + AIA 24 h.**

	BDNF-IR	TrkB-IR	CRF mRNA	BDNF/CRF mRNA
Control	50 ± 6	98 ± 8	105 ± 9	7.4 ± 1.4
AIA 4 h	70 ± 5^{b}	106 ± 15^{a}	143 ± 9^{b}	10.8 ± 1.0^{a}
24 h	133 ± 13^{c}	185 ± 10^{b}	202 ± 15^{c}	15.1 ± 2.4^{c}
7 d	121 ± 10^{c}	163 ± 8^{b}	181 ± 9^{c}	13.0 ± 1.8^{b}
Cor (12.5 IU/kg) + AIA 24 h	119 ± 12^{e}	157 ± 9^{e}	199 ± 16^{e}	14.5 ± 1.8^{e}
Cor (25 IU/kg) + AIA 24 h	91 ± 9^{d}	120 ± 9^{d}	133 ± 10^{d}	9.1 ± 1.5^{d}
Cor (25 IU/kg) + AIA 24 h + ADX	118 ± 11^{h}	142 ± 10^{g}	189 ± 16^{h}	16.4 ± 1.4^{g}

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important part in chronic pain following peripheral inflammation. BDNF and CRF in the frontal cortex of the AIA rats are involved in the modulation of chronic pain, and BDNF seems to influence the expression of CRF. Cor inhibits the levels of BDNF, CRF mRNA in the frontal cortex in a dose-dependent manner, and adrenal is critical to the function of corticotrophin.

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促肾上腺皮质激素对慢性痛大鼠痛行为、额叶皮层内脑源性神经营养因子和促肾上腺皮质激素释放因子水平的影响

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关键词 脑源性神经营养因子；促肾上腺皮质素释放激素；慢性痛；促肾上腺皮质激素；大鼠

目的：研究促肾上腺皮质激素对佐剂性关节炎大鼠额叶大脑皮层中 CRF、BDNF 及其功能性受体 trkB 的影响。**方法**：痛行为评分法，BDNF、trkB 的免疫组化法及 CRF 的原位杂交法。**结果**：在注射完全福氏佐剂后 24 h，大鼠的痛行为评分明显增高，同时对侧额叶皮层的 BDNF、trkB 免疫活性神经元、CRF mRNA 阳性神经元和 BDNF/CRFmRNA 双染神经元数均明显升高，腹腔注射促肾上腺皮质激素后能明显抑制该效应，摘除双侧肾上腺后，促肾上腺皮质激素的抑制效应可被显著翻转。**结论**：肾上腺的促肾上腺皮质激素可抑制关节炎大鼠对侧额叶皮层内 BDNF 和 CRF 水平的升高而介导镇痛作用。

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