European Journal of Pharmacology 609 (2009) 51–57

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

Chronic morphine administration induces over-expression of aldolase C with reduction of CREB phosphorylation in the mouse hippocampus

Hai-Yu Yang [a], Xiao-Ping Pu [a,b,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, 100191, PR China
[b] Department of Molecular and Cellular Pharmacology, School of Pharmaceutical Science, Peking University, 38 Xueyuan Road, Haidian District, Beijing 100191, PR China

ARTICLE INFO

Article history:
Received 18 January 2009
Received in revised form 27 February 2009
Accepted 2 March 2009
Available online 14 March 2009

Keywords:
Morphine dependence
Aldolase C
CREB phosphorylation

ABSTRACT

In recent studies, alterations in the activity and expression of metabolic enzymes, such as those involved in glycolysis, have been detected in morphine-dependent patients and animals. Increasing evidence demonstrates that the hippocampus is an important brain region associated with morphine dependence, but the molecular events occurring in the hippocampus following chronic exposure to morphine are poorly understood. Aldolase C is the brain-specific isoform of fructose-1, 6-bisphosphate aldolase which is a glycolytic enzyme catalyzing reactions in the glycolytic, gluconeogenic, and fructose metabolic pathways. Using Western blot and immunofluorescence assays, we found the expression of aldolase C was markedly increased in the mouse hippocampus following chronic morphine treatment. Naloxone pretreatment before morphine administration suppressed withdrawal jumping, weight loss, and overexpression of aldolase C. CREB is a transcription factor regulated through phosphorylation on Ser133, which is known to play a key role in the mechanism of morphine dependence. When detecting the expression of phosphorylated CREB (p-CREB) in the mouse hippocampus using Western blot and immunohistochemistry, we found CREB phosphorylation was clearly decreased following chronic morphine treatment. Interestingly, laser-confocal microscopy showed that overexpression of aldolase C in mouse hippocampal neurons was concomitant with the decreased immunoreactivity of p-CREB. The results suggest potential links between the morphine-induced alteration of aldolase C and the regulation of CREB phosphorylation, a possible mechanism of morphine dependence.

1. Introduction

In recent studies, alterations of the activity and expression of enzymes in cell metabolism, such as glycolysis, were detected in morphine-dependent patients and animals, indicating that a disturbance of glycometabolism may be involved in the molecular events of morphine dependence (Sharma et al., 2003; Xiang et al., 2006; Serres et al., 2005; Chen et al., 2007). Aldolase catalyzes a step of the glycolytic pathway, the aldolhydrolysis of fructose-1, 6-biphosphate into dihydroxyacetone phosphate and glycerol-3-phosphate, and the two substrates play key roles in producing ATP through the tricarboxylic acid cycle and other energy metabolism pathways. Vertebrate aldolases exist as three isozymes with different tissue distribution and kinetics: A, B, and C. Aldolase C is the brain-specific isoform of fructose-1, 6-bisphosphate aldolase (Lebherz and Rutter, 1969). Recently, several proteomics studies have reported altered expression levels of aldolase C in different brain regions of chronic morphine-administered animals (Li et al., 2006; Kim et al., 2005).

Increasing evidence demonstrates that the hippocampus is an important region associated with morphine dependence (Nestler, 2002; Morón et al., 2007). The rodent hippocampus is functionally involved in morphine-induced conditioned place preference (Rezayof et al., 2003; Zarrindast et al., 2006) and withdrawal behavior (Done et al., 1992; Lu et al., 2000; Dong et al., 2008). One recent study found downregulation of some energy metabolism enzymes, lower ATP levels, and an impaired ability to convert glucose into ATP in the mouse hippocampus following chronic morphine treatment, which indicates that an abnormality in hippocampal energy metabolism may contribute to morphine dependence (Chen et al., 2007). However, whether morphine treatment induces alterations in the expression of aldolase C in the hippocampus remains unclear.

Modulation of transcription factors such as the cAMP response element binding protein (CREB) is an important mechanism underlying the development of morphine dependence (Williams et al., 2001; Deisseroth et al., 1996). CREB is regulated through phosphorylation of Ser133, which modulates the transcription of genes containing cAMP response elements (CRE) in their promoters (Lonze and Ginty, 2002). Evidence suggests that chronic morphine administration induces changes in the expression and function of CREB in several brain regions such as the nucleus accumbens and locus coeruleus, which may contribute to withdrawal behaviors and neural adaptations associated with morphine dependence (Guitart

* Corresponding author. Department of Molecular and Cellular Pharmacology, School of Pharmaceutical Science, Peking University, 38 Xueyuan Road, Haidian District, Beijing 100191, PR China. Tel./fax: +86 10 8280 2431.
E-mail address: pxp123@bjmu.edu.cn (X.-P. Pu).

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.03.028

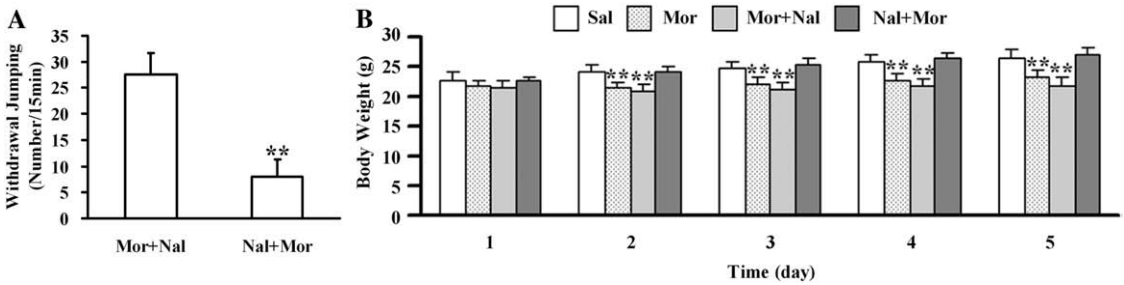

Fig. 1. A: Effects of naloxone pretreatment before morphine administration on withdrawal jumping behavior in mice. $^{**}P<0.01$ ($n=8$), vs. Mor + Nal. B: Effects of chronic morphine administration on body weight. The weight of each animal was measured before morphine or saline injection for 5 days. Sal: a control group receiving saline; Mor: a morphine-treated group; Mor + Nal: Morphine + Naloxone, a naloxone-precipitated withdrawal group received a single injection of naloxone (10 mg/kg, i.p.) 1 h after the last morphine injection on day 5; Nal + Mor: Naloxone + Morphine, a naloxone-pretreated morphine group received a single injection of naloxone (10 mg/kg, i.p.) 30 min before each morphine injection, as described in Section 2.1. $^{**}P<0.01$ ($n=8$), vs. Sal. Data represent means ± S.E.M ($n=8$; analyzed using Student's *t*-test).

et al., 1992; Lane-Ladd et al., 1997; Shaw-Lutchman et al., 2002). However, few studies have reported regulation of CREB phosphorylation in the hippocampus associated with morphine dependence.

In this study, we used Western blot and immunofluorescence to evaluate the effect of morphine on the regulation of aldolase C in the mouse hippocampus. In addition, we examined the expression of phosphorylated CREB (p-CREB) by Western blot and immunohistochemistry, hypothesizing that chronic morphine administration would cause alterations in CREB phosphorylation. Finally, we examined the co-expression of aldolase C and p-CREB in hippocampal neurons using laser-scanning confocal microscopy in order to study changes in aldolase C expression associated with changes in the regulation of morphine-mediated CREB phosphorylation.

2. Materials and methods

2.1. Animals and treatment

Thirty-two male ICR mice (20–25 g; Experimental Animal Center of Peking University Health Science Center, Beijing, China) were maintained in a colony room at 24 ± 2 °C on a 12 h light/dark cycle with food and water available freely. One week after arrival, mice were randomly divided into the following 4 groups, containing 8 mice per group: a control group receiving saline (Sal), a morphine-treated group (Mor), a naloxone-precipitated withdrawal group (Mor + Nal), and a naloxone-pretreated morphine group (Nal + Mor) as described previously (Chen et al., 2007). In morphine-treated animals, morphine hydrochloride (Qinghai Pharmaceutical Factory, China) was injected subcutaneously twice daily at 12 h intervals (8:00 and 20:00) for 4 days with increasing doses on each day (20, 30, 40, 50 mg/kg). On day 5, all animals received a single subcutaneous injection of morphine (10 mg/kg) at 8:00. The naloxone-precipitated withdrawal group received a single injection of naloxone (10 mg/kg, i.p.; Beijing Four Ring Pharmaceutical Technology Co., China) 1 h after the last morphine injection on day 5 (Crain and Shen, 1995). The naloxone-pretreated animals received a single injection of naloxone (10 mg/kg, i.p.) 30 min before each morphine injection. Immediately after the naloxone injection, each mouse in the last two groups was placed in an acrylglass box (30 × 30 × 40 cm) and the number of jumps was recorded over the next 15 min. The animals in control group and morphine-treated group were killed by decapitation 1 h after the last saline or morphine injection (Chen et al., 2007).

The body weight of all animals was measured before any injection each day. After the withdrawal behavior experiments, 4 mice per group were used for Western blot analysis and immunofluorescence or immunohistochemical staining. All experiments were performed in strict accordance with the principles and guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Aldolase C and p-CREB Western blot

For Western blot analysis, 4 mice per group were decapitated following the withdrawal behavior experiment, their brains were quickly removed and their hippocampi were rapidly dissected out on an ice-cold glass Petri dish. Samples were immediately weighed, each group's samples were pooled, and samples were individually homogenized with a supersound homogenizer in 10 vol. of ice-cold 80 mM Tris–HCl buffer (pH 7.4) containing 150 mM NaCl, 2 mM EDTA, 0.4 mM DTT and 0.1% SDS. The homogenates were centrifuged at 20,000 × *g* at 4 °C for 1 h and supernatants were collected. Protein concentration was determined by BCA protein assay. Equal amount of proteins for each group were separated in a 12.5% SDS-PAGE gel and electrophoretically transferred to PVDF (Millipore, Bedford, MA), blocked, and probed overnight at 4 °C with goat polyclonal anti-aldolase C antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) or rabbit monoclonal anti-p-CREB antibody (1:400; Cell Signaling Technology, Beverly, MA). Rabbit polyclonal anti-β-actin antibody (1:1000; Santa Cruz) was used as a protein loading control. Peroxidase-conjugated secondary antibodies (1:5000; Beijing ZhongShan GoldenBridge Biotechnology Co., China) were added and developed with enhanced chemiluminescence and exposed to X-film (Kodak, Rochester, NY). After film scanning, the integrated optical density for each band was quantified using Gel-Pro software (Media Cybernetics, USA). Relative variations between the bands of the experimental samples and the control samples were calculated in the same image. The Western blot experiments were

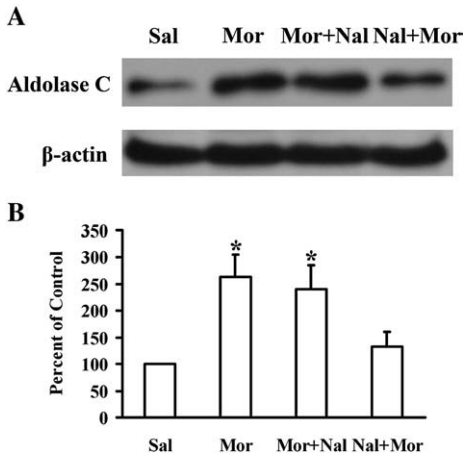

Fig. 2. The effect of morphine administration on the expression of aldolase C in the mouse hippocampus analyzed using Western blot. A: Representative Western blot for aldolase C. B: Quantitative estimation of the bands. Sal: a control group receiving saline; Mor: a morphine-treated group; Mor + Nal: a naloxone-precipitated withdrawal group; Nal + Mor: a naloxone-pretreated morphine group. Data were expressed as a percentage of control and values represent mean ± S.E.M ($n=3$) of three independent experiments. Differences in image density were analyzed by Student's *t*-test. $^{*}P<0.05$, vs. Sal.