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

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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

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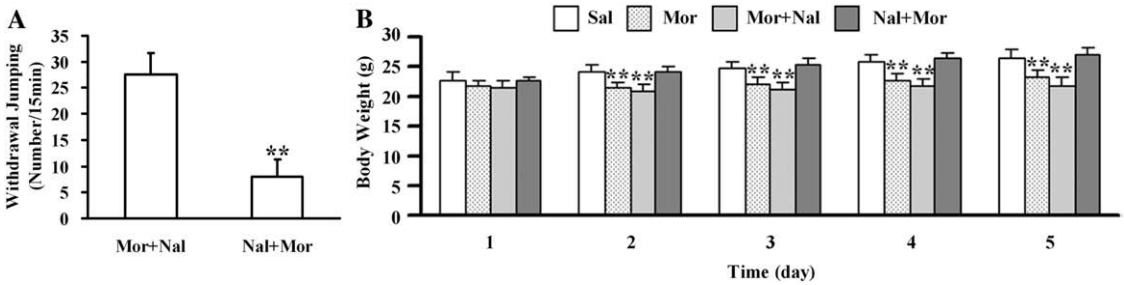

Fig. 1. A: Effects of naloxone pretreatment before morphine administration on withdrawal jumping behavior in mice. $^{**}P<0.01$ ($n=8$), vs. Mor + Nal. B: Effects of chronic morphine administration on body weight. The weight of each animal was measured before morphine or saline injection for 5 days. 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

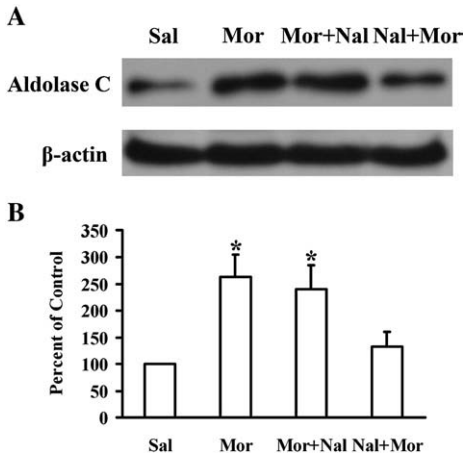

Fig. 2. The effect of morphine administration on the expression of aldolase C in the mouse hippocampus analyzed using Western blot. A: Representative Western blot for aldolase C. B: Quantitative estimation of the bands. Sal: a control group receiving saline; Mor: a morphine-treated group; Mor + Nal: 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.

repeated 3–4 times for each sample, with duplicate measurements for each blot. All assays were performed under conditions in which the densitometric signal intensity was linear with protein concentration, as determined by preliminary experiments.

2.3. Aldolase C immunofluorescence staining and confocal microscopy

Following the withdrawal behavior experiments, 4 mice per group were anaesthetized with ethylcarbamate (2 g/kg, i.p.) and perfused with 4% ice-cold paraformaldehyde in 0.1 M PBS (pH 7.4). Brains were removed and postfixed in the above solution at 4 °C overnight, then stored in 30% sucrose for at least 24 h for cryoprotection. Frozen sections were cut with a freezing microtome (Leica, Wetzlar, Germany) at 10 μm. The slices were treated for immunofluorescence staining to detect the expression of aldolase C, blocked, and probed overnight at 4 °C with goat anti-aldolase C antibody (1:100). Following three washes with PBS, the sections were incubated with FITC-conjugated antibody (1:300) for 1 h in the dark. After three additional PBS washes, the sections were stained with Hoechst 33342 (10 μg/ml; Sigma Chemical Co., St. Louis, MO) for 10 min, then coverslipped with 90% glycerol and 10% 0.1 M PBS (pH 8.0) as an antifading agent, mounted on glass slides, and subjected to fluorescence study. Finally, sections were observed with a TCS SP2 laser-scanning confocal microscope (Leica), using a Plan-Apochromat 40× oil immersion objective (1.4 numeric aperture). Negative controls were performed with the primary antibody omitted.

2.4. p-CREB immunohistochemistry

Immunostaining was performed on frozen sections with a Streptavidin–Peroxidase (SP) Kit (Beijing ZhongShan GoldenBridge Biotechnology Co.). The sections were microwaved in 0.01 M citrate buffer (pH 6.0) for 15 min, then blocked using 10% normal goat serum in 0.1 M PBS (pH 7.4) at room temperature for 1 h, and incubated with rabbit monoclonal anti-p-CREB antibody (1:100) at 4 °C overnight. After one 10-min incubation in 3% H_2O_2, sections were incubated with goat anti-rabbit secondary antibody (1:100) and biotin-streptavidin complex at room temperature for 30 min. All procedures were followed by three 5-min washes with PBS. Finally, these sections were stained with 3, 3′ diaminobenzidine (DAB) according to standard protocol for 3 min and restained with hematoxylin (3 mg/ml) for 10 s. Negative controls were prepared by omitting the primary antibody incubation. Conventional

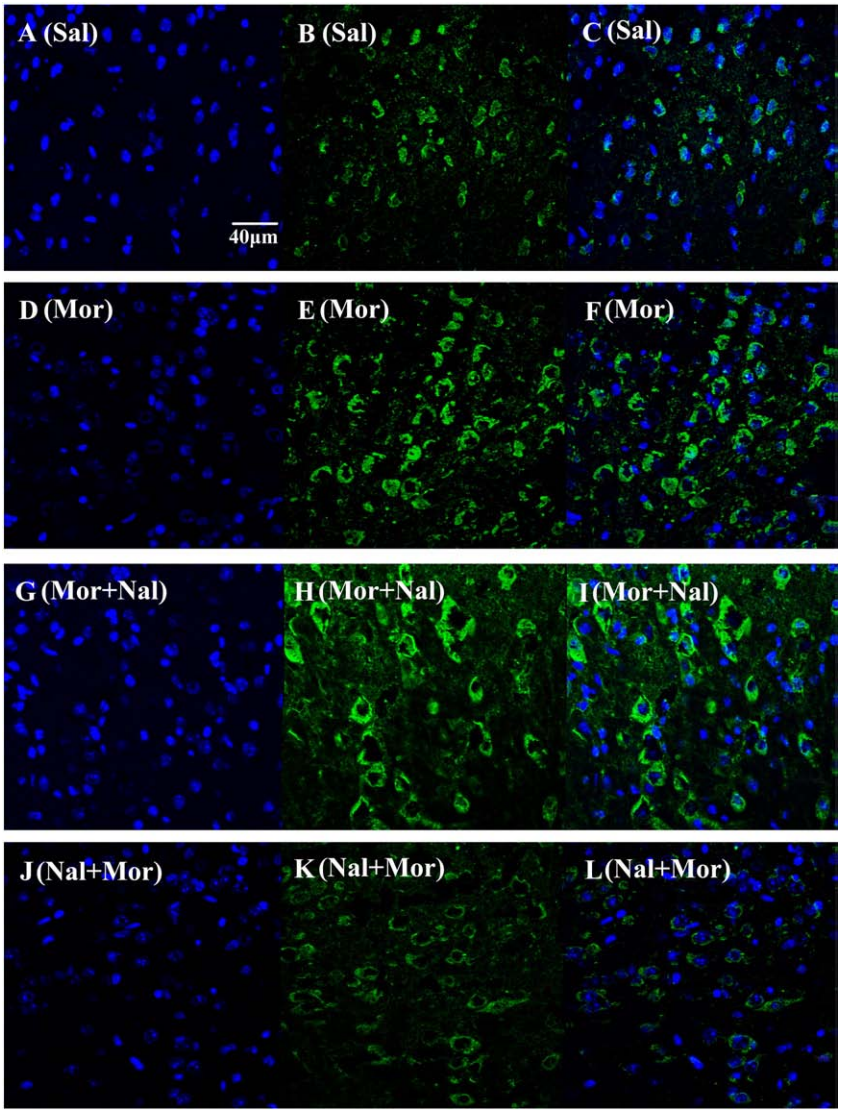

Fig. 3. The effect of morphine administration on the expression of aldolase C in the mouse hippocampus was analyzed using immunofluorescence. A, D, G, J: The staining of cell nuclei with Hoechst 33342 (blue). B, E, H, K: The immunostaining of aldolase C was mainly in the cytoplasm of neurons (green). C, F, I, L: The overlap in immunostaining of aldolase C and the staining of cell nuclei. A–C (Sal): a control group receiving saline; D–F (Mor): a morphine-treated group; G–I (Mor + Nal): a naloxone-precipitated withdrawal group; J–L (Nal + Mor): a naloxone-pretreated morphine group. Scale bar = 40 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

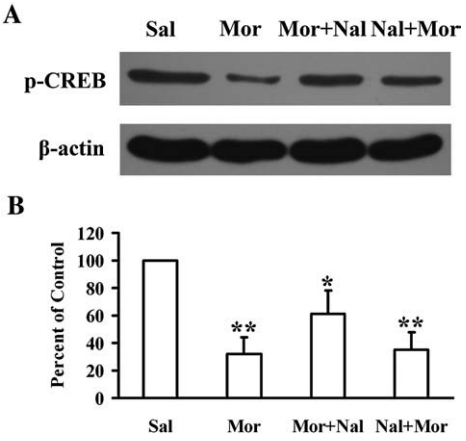

Fig. 4. The effects of morphine administration on the expression of p-CREB in the mouse hippocampus analyzed using Western blot. A: Representative Western blot for p-CREB. B: Quantitative estimation of the bands. Sal: a control group receiving saline; Mor: a morphine-treated group; Mor + Nal: a naloxone-precipitated withdrawal group; Nal + Mor: a naloxone-pretreated morphine group. Data were expressed as a percentage of the 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. *$P<0.05$, **$P<0.01$, vs. Sal.

images were obtained by a digital camera (Olympus, Tokyo, Japan) and processed using Adobe PhotoShop 8.0 software.

2.5. Double-immunofluorescence staining

The 10 μm frozen sections were treated for double immunofluorescence staining to detect the co-expression of aldolase C and p-CREB. After pre-incubation with 2% BSA plus 0.03% PBS-Triton at room temperature for 1 h, the sections were incubated with the primary antibodies (both 1:100) at 4 °C overnight. Following three washes with PBS, the sections were incubated with FITC-conjugated or TRITC-conjugated antibodies (both 1:300) for 1 h in the dark. After three additional PBS washes, the sections were coverslipped with 90% glycerol and 10% 0.1 M PBS (pH 8.0) as an antifading agent, and mounted on glass slides. A laser-scanning confocal microscope (Leica) was used to collect images from double-labeled specimens.

2.6. Statistical evaluation

All data are expressed as mean ± S.E.M. Statistical differences between treatment groups were analyzed by one-way ANOVA followed by a Student's *t*-test, with significance being defined as $P<0.05$.

3. Results

3.1. The assessment of morphine withdrawal

We found that naloxone precipitation induced robust withdrawal jumping in morphine-dependent mice (Fig. 1A). The effect was not observed with naloxone pretreatment before morphine administration in mice ($P<0.05$). To observe the effect of chronic morphine treatment on weight loss, the weight of each animal was measured before injection with morphine or saline each day. We found that mice treated with morphine showed significantly less weight gain than the control group from day 2 to day 5 (all $P<0.05$; Fig. 1B). However, mice treated with naloxone before morphine administration had no significant change in body weight compared to the control group (all $P>0.05$; Fig. 1B).

3.2. Increased expression of aldolase C in the mouse hippocampus after chronic morphine administration

The results of Western blot analyses showed that the aldolase C protein level was increased in the mouse hippocampus following chronic morphine treatment compared to the control group. However, the effects of morphine on the upregulation of aldolase C could be prevented by naloxone pretreatment before morphine administration (Fig. 2A and B). Likewise, immunofluorescence results showed that immunoreactivity of aldolase C increased in hippocampal neurons following chronic morphine treatment, particularly in the dentate gyrus and CA3 area (Fig. 3E); similar results were found in the naloxone-precipitated withdrawal group (Fig. 3H). Furthermore, fluorescence density within the hippocampus of the naloxone-precipitated withdrawal group was stronger than in the morphine-treated group (green).

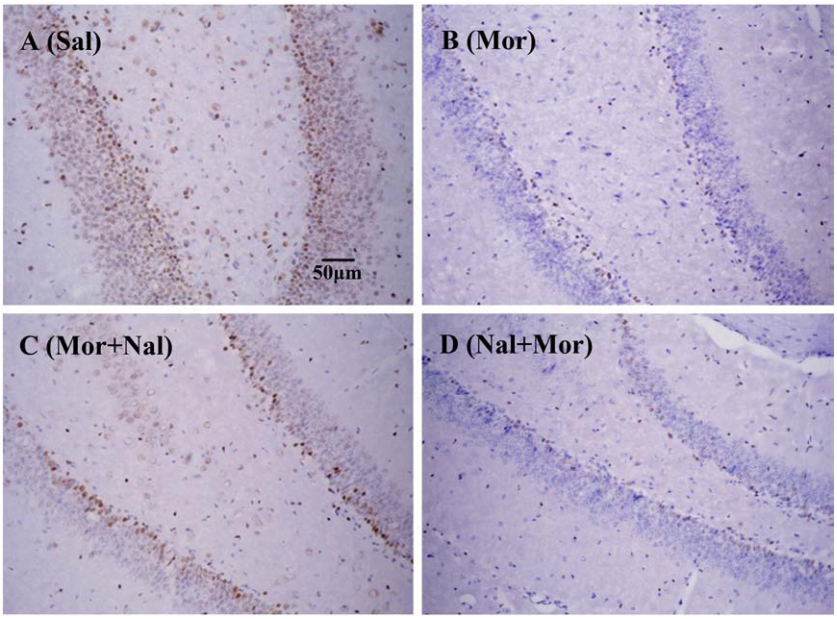

Fig. 5. The effects of morphine administration on the expression of p-CREB in the mouse hippocampus were analyzed using immunohistochemistry. The positive immunostaining of p-CREB was mainly in the nuclei of neurons (brown) and other cell nuclei were restained by hematoxylin (blue). A (Sal): a control group receiving saline; B (Mor): a morphine-treated group; C (Mor + Nal): a naloxone-precipitated withdrawal group; D (Nal + Mor): a naloxone-pretreated morphine group. Scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In contrast, the expression of aldolase C in mice pretreated with naloxone exhibited no difference to the control group (Fig. 3K and B).

3.3. Clear reduction of CREB phosphorylation in the mouse hippocampus after chronic morphine administration

Notably, Western blot results demonstrated that CREB phosphorylation was significantly decreased in the mouse hippocampus following chronic morphine treatment compared to the control group, but showed some increase after injection with naloxone to precipitate withdrawal compared to the morphine-treated group. However, the effects of morphine on the down-regulation of p-CREB could not be completely prevented by naloxone pretreatment prior to morphine administration (Fig. 4A and B). In agreement with p-CREB Western blot results, p-CREB immunohistochemistry results revealed clearly reduced immunostaining of p-CREB in the hippocampus after chronic morphine treatment (Fig. 5B), compared to the control group (Fig. 5A). In the naloxone-precipitated withdrawal group (Fig. 5C), the expression of p-CREB showed a slight increase compared to the morphine-treated group, but was lower than the control group. However, the reduction of p-CREB immunostaining in the hippocampus caused by chronic morphine treatment could not be completely prevented by naloxone pretreatment (Fig. 5D).

3.4. Overexpression of aldolase C with reduction of CREB phosphorylation in the mouse hippocampus after chronic morphine administration

To study the changes in aldolase C associated with changes in regulation of CREB phosphorylation, we examined the co-expression of aldolase C and p-CREB in hippocampal neurons using double immunofluorescence staining. As shown in Fig. 6A, the immunolocalization of aldolase C was mainly in the cytoplasm (green) while p-CREB was mainly in the nuclei (red) of neurons in the hippocampus in the control group. The results showed that, following chronic morphine treatment, the immunoreactivity of aldolase C was significantly increased with decreasing immunostaining of p-CREB in mice hippocampi (Fig. 6B). Similar results were found in the naloxone-precipitated withdrawal group (Fig. 6C). Furthermore, both fluorescence densities (green and red) in the naloxone-precipitated withdrawal group were stronger than in the morphine treatment group. Naloxone pretreatment before morphine administration inhibited the overexpression of aldolase C, but failed to prevent the decreasing immunoreactivity of p-CREB (Fig. 6D).

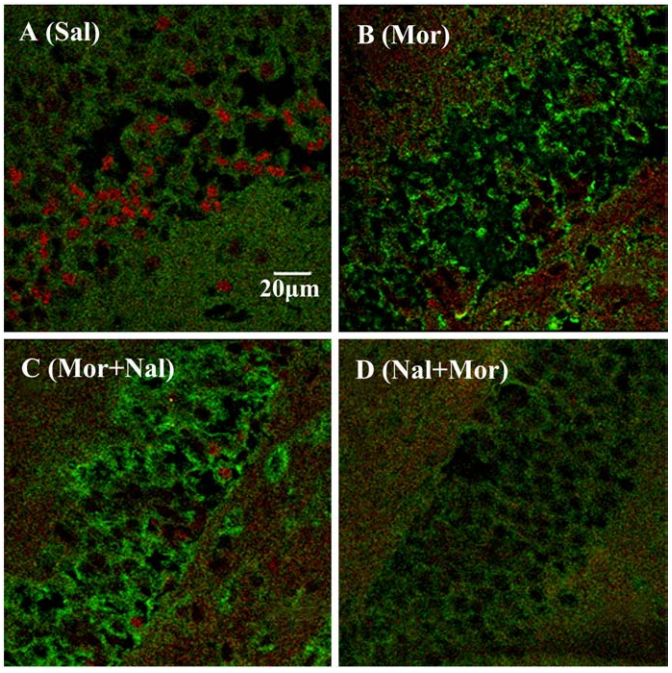

Fig. 6. The effects of morphine administration on co-expression of aldolase C and p-CREB in the mouse hippocampus were analyzed using double immunofluorescence staining. Immunostaining of aldolase C was mainly in the cytoplasm neurons (green). The immunostaining of p-CREB was mainly in the nuclei of neurons (red). A (Sal): a control group receiving saline; B (Mor): a morphine-treated group; C (Mor + Nal): a naloxone-precipitated withdrawal group; D (Nal + Mor): a naloxone-pretreated morphine group. Scale bar = 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Recently, several studies have measured the concentrations of glycolytic intermediates in rodent brains following chronic exposure to morphine using nuclear magnetic resonance spectroscopy. They found that the rate of glycolysis was increased and related metabolic enzymes were coordinately activated, which indicates that an abnormality of glycolysis may be involved in the molecular mechanism of morphine dependence (Sharma et al., 2003; Xiang et al., 2006; Serres et al., 2005). The hippocampus is recognized as an important region involved in morphine dependence (Nestler, 2002; Morón et al., 2007), but whether the abnormal expression of related glycolytic enzymes, such as aldolase C, in the hippocampus contribute to morphine dependence remains unclear. Previously, we found that the expression of aldolase C was increased in the rat hippocampus after chronic morphine treatment (Li Quan, unpublished data). In this study, we confirmed that chronic morphine administration induces overexpression of aldolase C in the mouse hippocampus. We presume that abnormal regulation of aldolase C induced by chronic morphine administration may be involved in a disturbance of glycometabolism in the hippocampus.

Among withdrawal behaviors in morphine-dependent rodents (especially mice), jumping is widely considered to be the most sensitive and reliable index, and is commonly used to test the state of morphine physical dependence. In our study, we observed violent withdrawal jumping by morphine-dependent mice following naloxone administration, together with a significantly lower body weight, which was consistent with previous studies (Guo et al., 2000; Kest et al., 2002). We also found that pretreatment with the opioid antagonist naloxone before morphine administration clearly suppressed withdrawal jumping, weight loss, and the overexpression of aldolase C. These findings indicate that the up-regulation of aldolase C induced by chronic morphine treatment may be mediated by opioid receptors and related to morphine withdrawal. Moreover, one study showed down-regulation of energy metabolism enzymes, lower ATP levels, and an impaired ability to convert glucose into ATP in the hippocampus of chronic morphine-treated mice (Chen et al., 2007). Several studies have reported that the administration of ATP and its metabolites, such as adenosine, can block naloxone-precipitated withdrawal behaviors in chronic morphine-dependent animals (Gomaa et al., 1989; Kaplan and Coyle, 1998). All of these results suggest that morphine withdrawal may involve the reduction of ATP production and that it may be related to an abnormality in hippocampal energy metabolism.

Increasing evidence is suggesting that aldolase C may exert other functions besides those characteristic of a glycolytic enzyme. Immunostaining of adult human and rodent brain tissues showed aldolase C was expressed in Purkinje cells in the cerebellum and may be related to cerebellar development (Ahn et al., 1994; Sugihara and Quy, 2007). In addition, high expression of aldolase C has been detected in the neonatal rat forebrain, suggesting involvement in early astrocyte differentiation (Staugaitis et al., 2001). Moreover, some studies have indicated that chronic morphine treatment can alter the birth of new neurons in the subgranular zone of rodent hippocampus, which sheds light on the functional links between morphine-induced alterations in neurogenesis and withdrawal behaviors (Kahn et al., 2005; Mandyam et al., 2004). In this study, we found that chronic morphine administration induced increased expression of aldolase C

in mice hippocampi, especially in the dentate gyrus and CA3 area. Thus, the question of whether increased expression of aldolase C is related to morphine-induced alteration of neurogenesis in the hippocampus requires further study.

While CREB is regulated through phosphorylation of Ser133, which plays a key role in the mechanism of morphine dependence (Maldonado, 1996), the molecular events occurring in the hippocampus following chronic exposure to morphine are not clearly understood. One study has demonstrated that prenatal morphine exposure could decrease CREB phosphorylation in the hippocampal CA1 subregion in young offspring (Yang et al., 2006). However, other studies have shown that morphine-induced conditioned place preference may involve the enhancement of CREB phosphorylation in several brain areas including the hippocampus (Gao et al., 2003; Gao et al., 2004). Recently, one study has demonstrated the regulation of ERK1/2 phosphorylation by acute and chronic morphine treatment, implying a role for CREB-dependent and Ets-like protein-1 (Elk-1)-dependent transcription (Ligeza et al., 2008).

In this study, we found CREB phosphorylation was clearly reduced in the mouse hippocampus after chronic morphine treatment, and could not be blocked by the application of naloxone before morphine administration. Also, a similar result was found by one recent study in which changes in expression of p-CREB in the mouse nucleus accumbens induced by the opioid agonist cocaine could not be prevented by naloxone pretreatment. Their data indicated that the mechanism of conditioned drug reward for cocaine does not rely on the endogenous opioid system, and that the related mechanism remained unclear (Walters et al., 2005). Preclinical studies have demonstrated that low doses of opioid antagonists such as naloxone could enhance opioid analgesia and attenuate tolerance/dependence by selectively antagonizing excitatory opioid receptor functions (Crain and Shen, 2000). If the reduction of CREB phosphorylation could not be completely prevented by naloxone pretreatment, it may be partly involved in the selective antagonizing effect of naloxone on opioid receptors. The detailed mechanism needs to be further confirmed.

Interestingly, we also found that high expression of aldolase C was concomitant with the reduction in CREB phosphorylation in mice hippocampal neurons following chronic morphine treatment. Regulation of CREB phosphorylation is known to play an important role in mediating morphine-induced changes in the cAMP signaling pathway and is closely associated with the development of dependence (Williams et al., 2001; Deisseroth et al., 1996). Also, several studies have demonstrated the effects of extracellular ATP on the activation of CREB (Zhang et al., 2004; Feng et al., 2004). One recent study showed lower ATP levels and an impaired ability to convert glucose into ATP in the mouse hippocampus following chronic morphine treatment (Chen et al., 2007). Thus, it would appear that inhibition of ATP production induced by chronic morphine administration may potentially contribute to the reduction of CREB phosphorylation in the hippocampus.

Moreover, we noted that CREB phosphorylation in the mouse hippocampus appeared to be enhanced after naloxone-precipitated withdrawal with higher expression of aldolase C. Aldolase C is a glycolytic enzyme catalyzing reactions in the glycolytic, gluconeogenic, and fructose metabolic pathways. One study has indicated that human aldolase C gene expression may be regulated through a cAMP-dependent signaling pathway (Buono et al., 2002). Therefore, we presume that the increased expression of aldolase C might be an energy compensation mechanism for the rapid production of ATP, resulting in the enhancement of CREB phosphorylation. These findings suggest potential links between the morphine-induced alteration of aldolase C and the regulation of CREB phosphorylation, which may provide a possible mechanism of morphine dependence. However, the question of whether the abnormal expression of aldolase C is directly related to changes in CREB phosphorylation in the hippocampus, and precisely what role it plays in morphine dependence, needs to be investigated further.

Acknowledgements

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