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Antagonism of κ opioid receptor in the nucleus accumbens prevents the depressive-like behaviors following prolonged morphine abstinence

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HIGHLIGHTS

- Prolonged morphine abstinence induced depressive-like behaviors in mice.
- Prodynorphin increased in the nucleus accumbens (NAc) after morphine abstinence.
- Intra-NAc injection of norBNI blocked morphine abstinence-induced behavioral deficits.

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ABSTRACT

The association between morphine withdrawal and depressive-like symptoms is well documented, however, the role of dynorphin/κ opioid receptor system and the underlying neural substrates have not been fully understood. In the present study, we found that four weeks morphine abstinence after a chronic escalating morphine regimen significantly induced depressive-like behaviors in mice. Prodynorphin mRNA and protein levels were increased in the nucleus accumbens (NAc) after four weeks of morphine withdrawal. Local injection of κ opioid receptor antagonist nor-Binaltorphimine (norBNI) in the NAc significantly blocked the expression of depressive-like behaviors without influencing general locomotor activity. Thus, the present study extends previous findings by showing that prolonged morphine withdrawal-induced depressive-like behaviors are regulated by dynorphin/κ opioid receptor system, and shed light on the κ opioid receptor antagonists as potential therapeutic agents for the treatment of depressive-like behaviors induced by opiate withdrawal.

1. Introduction

Epidemiological reports have indicated a high rate of comorbidity between opiate addiction and psychiatric disorders [1]. Drug abstinent people are commonly suffered from depression, anger and anxiety [2–4]. These neuropsychiatric disorders, in particular depression, induced by opiate withdrawal, have been shown to trigger craving and relapse [5,6]. Consistent with the human reports, animal studies also indicated that abstinent mice developed depressive-like deficits [7–10]. These findings provided direct links between opiate withdrawal and depressive-like behaviors, however, the molecular mechanism underlying opiate withdrawal-induced depression remains unclear.

The κ opioid system is comprised of κ opioid receptor and the endogenous ligands dynorphins [11]. There is increasing evidence showing that activation of κ opioid receptor produces depressive-like disorders in human and rodents [12–19], whereas blockade of κ opioid receptor produces antidepressive-like effects [20–23]. Chronic exposure to cocaine, morphine, heroin and alcohol have been shown to be associated with increased dynorphins expression in different brain regions, such as striatum, NAc and amygdala, and potentiated endogenous signaling through κ opi-

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oid receptor [24–30]. Importantly, recent studies demonstrated that the dynorphin/κ opioid receptor system was involved in mediating anxiety- and depressive-like behaviors after prolonged withdrawal from ethanol or cocaine [10,29], since κ opioid receptor antagonist norBNI attenuated the anxiety- and depressive-like behaviors observed during protracted withdrawal. To the best of our knowledge, the role of dynorphin/κ opioid receptor system in depressive-like behaviors induced by morphine withdrawal has not been fully understood.

NAc, receiving dopaminergic projections arising from ventral tegmental area (VTA), is critically involved in reward and depression [31–35]. Disruption of dopamine (DA) function within NAc caused anhedonia, a core feature of major depressive disorder [36]. Muschamp et al. (2011) observed that activation of the transcription factor cAMP response element binding protein (CREB) within the NAc shell produced anhedonia [37], which was consistent with former findings showing that CREB-mediated induction of dynorphins in the NAc triggered depressive-like behaviors [38]. There is substantial evidence implicating that NAc is involved in the depressive-like effects of dynorphins [39,40]. Together, these findings suggest that NAc is embedded within brain circuits that regulate depression.

We hypothesize that dynorphin/κ opioid receptor system in the NAc plays a crucial role in prolonged morphine abstinence-induced depressive-like behaviors, and the plasticity of the prodynorphin gene expression in the NAc contributes to the negative affective states. Here, we first utilized a chronic escalating morphine regimen and rodent models of depression to verify prolonged morphine withdrawal-induced depressive-like behaviors. We then quantified prodynorphin mRNA and protein levels in the NAc after prolonged morphine withdrawal. Finally, the ability of NAc microinjected with κ opioid receptor antagonist norBNI to block the expression of depressive-like behaviors was examined.

2. Methods and materials

2.1. Materials

Morphine sulfate (Qinghai Pharmaceutical General Factory, China) was prepared in saline (0.9% sodium chloride) and injected at a volume of 10 ml/kg. norBNI was purchased from Abcam (Abcam, England). Naloxone was purchased from Sigma–Aldrich (Sigma-Aldrich, USA). Anti-prodynorphin antibody was purchased from Abcam (Abcam, England). Anti-actin antibody was purchased from Sigma–Aldrich (Sigma–Aldrich, USA). HRP-goat anti-rabbit IgG antibody and HRP-goat anti-mouse IgG antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). The primers of prodynorphin and glyceraldehyde-3-phosphate dehydrogenase (gapdh) were synthesized by Genewiz (Genewiz, China).

2.2. Animals

Male C57BL/6J mice weighing 20–26 g (6–9 weeks old) were obtained from the Experimental Animal Center, Fudan University (Shanghai, China). Mice were housed in groups with eight/cage (cage size: 37 cm × 20 cm) in a temperature controlled room (24 ± 2 °C) on a 12 h light/12 h dark cycle (lights on at 7:00 AM). Mice were allowed free access to food and water in their home cages throughout the experiments. All experimental procedures and protocols were approved by the National Institutes of Health Guide for the Care and Use of Laboratory.

To minimize the basal stress, saline-treated mice and morphine-treated mice were housed in different cages, and the mice were moved to the testing room 24 h before behavioral tests, and handled twice a day for 3 days before behavioral tests.

2.3. Cannula implantation and microinjection

Mice were anesthetized using sodium pentobarbital (70 mg/kg, i.p.) under aseptic conditions, and a stereotaxic instrument with nonpuncture ear bars (Narishige, Japan) was used. For NAc infusion, 26-gauge guide cannula was implanted in the NAc (anteroposterior: +1.3 mm; mediolateral: ±1 mm; dorsoventral: −2.5 mm). Implanted cannula was cemented to two anchoring screws on the skull. Bilateral microinfusions were made through 33-gauge dummy cannula (Plastics One, USA) that extended 2 mm beyond the tip of the guide cannula to prevent blockage [41], which was connected to a 10 μl microsyringe mounted in the microinfusion pump (Harvard Apparatus, USA). The rate of infusion is 200 nl/min. After injection, each mouse was given an additional 2 min for drug diffusion. norBNI was dissolved in PBS (5 μg/μl, 0.5 μl/side) and was bilaterally microinjected into the NAc 24 h before testing [42,43].

2.4. Morphine dependence and development of abstinence

Mice were given with increasing doses of morphine (20, 40, 60, 80, 100 mg/kg, s.c.) or saline twice a day for five days, followed by a single 100 mg/kg injection on day six [8,9]. Four hours after the last morphine or saline injection, mice were treated with naloxone (3 mg/kg, i.p.) to precipitate withdrawal syndromes. The somatic signs of withdrawal were quantified for 30 min by direct observation. Jumping was recorded as the number of event occurred. Body tremor, blepharoptosis and chew behaviors were recorded as the number of 5 min intervals (maximal score: 6).

After chronic treatment, mice were maintained drug free. One, two, and four weeks after the last injection, the forced swim test and the sucrose preference test were undertaken to evaluate the depressive-like behaviors.

2.5. Behavioral experiments

2.5.1. Forced swim test

Forced swim test was performed according to a modified version of the paradigm [44]. Briefly, mice were placed in a cylinder of water (23–25 °C in temperature; 12 cm in diameter; 25 cm in height) for 6 min. The depth of water was set to prevent animals from touching the bottom and the wall with their hind limbs. Animal behaviors were videotaped from the side. The immobility time of each animal spent during the last 4 min was counted by an observer blind of the animal treatments. Immobility was defined as floating or remaining motionless.

2.5.2. Sucrose preference test

Sucrose preference test was performed according to the previous studies [45]. Briefly, mice were provided with two bottles of tap water with one containing 1% sucrose for 24 h. Both of the sucrose and water intake volume were measured. To prevent location preference, the bottle position was changed every 12 h [46]. Sucrose preference was calculated as: sucrose solution volume/(sucrose solution volume + water volume) × 100%, with control data normalized to 100%.

2.5.3. Locomotor activity

In brief, animals were placed into the locomotor chambers (Shanghai Jiliang Software Technology, China) equipped with infrared video recorder. Activity was monitored for one hour, and the data of moving distance were analyzed using locomotor activity analysis software.