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Research report

Expression of c-Fos in the rat central amygdala accompanies the acquisition but not expression of conditioned place aversion induced by withdrawal from acute morphine dependence

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Abstract

Conditioned reinforcement is hypothesized to be critically involved in drug addiction as a factor contributing to compulsive drug use and relapse. The present study focused on the neurobiology involved in the acquisition and expression of conditioned reinforcing effects of morphine withdrawal employing a conditioned place aversion (CPA) paradigm in acute-dependent rats. Expression of c-Fos in the amygdala (the central nucleus, CeA; the medial nucleus, MeA; the basolateral nucleus, BLA) following naloxone-precipitated withdrawal and the CPA test was examined using a range of naloxone doses (0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 mg/kg). Naloxone dose-dependently produced CPA in rats given a single morphine exposure. In CeA, but not MeA with high-level constitutive neuronal activity, the naloxone-induced modification in c-Fos immunoreactivity following morphine pretreatment exhibited a dose-dependent pattern similar to that seen in the behavioral study. On the other hand, none of the three amygdaloid nuclei examined including CeA, MeA and BLA showed notable sensitivity of c-Fos to the conditioned withdrawal stimulus. These results suggest that CeA may play a role in the negative affective aspect of withdrawal from acute dependence, and in part suggest that the acquisition and expression of CPA may involve different neurobiological mechanisms.

Keywords: Morphine; Acute dependence; Withdrawal; Acquisition of conditioned place aversion; Expression of conditioned place aversion; Amygdala; c-Fos

1. Introduction

Drug addiction is a chronic relapsing disorder characterized by compulsive drug seeking and use [28]. Among the factors that may contribute to loss of control over drug intake and relapse after periods of abstinence is conditioned reinforcement [14,21]. Previously neutral stimuli can acquire motivational significance by being associated with the positive affective states of drug taking itself or the negative affective states of withdrawal both in human and in animals. These include the drug-taking environment or even internal cues through classical conditioning processes. Re-exposure to these conditioned stimuli can provide the motivation for continued drug use and relapse after abstention [14,21]. A better understanding of the neurobiology involved in the acquisition and expression of the conditioned reinforcing effects will provide information for a rational basis for developing the pharmacotherapies of drug addiction.

Conditioned place aversion (CPA) serves as a highly sensitive measure reflective of the negative motivational aspect of opiate withdrawal and an index of conditioned affective withdrawal. It is widely used to explore the neurobiological mechanisms underlying the withdrawal aversion in chronic-dependent animals focusing on the acquisition phase of conditioning. In recent years, this behavioral paradigm has

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been established in animals subjected to acute dependence [1,2,16].

Accumulating evidence suggests that the amygdala may be involved in the acquisition of CPA in chronic-dependent animals [8,9,11,23,26,27]. We previously reported that the centromedial amygdala of rats receiving a single morphine exposure displayed increased c-Fos expression following naloxone-precipitated withdrawal that could produce CPA with a dose of naloxone of 0.5 mg/kg, suggesting a possible involvement of this brain area in the negative motivational component of withdrawal from acute morphine dependence [13]. The first purpose of the present study was to confirm the correlation between the acquisition of CPA and c-Fos expression in the centromedial amygdala in acutely dependent animals using a range of naloxone doses.

Compared with the acquisition, the situation for the expression of conditioned withdrawal would more closely approximate the clinical condition wherein any conditioned associations between opiate withdrawal and environmental stimuli would have been formed. Although numerous studies have explored the neurobiological basis for aversive aspects of withdrawal employing the CPA paradigm, relatively little work has been performed to clarify the mechanism underlying the expression of CPA after the conditioned aversion has been established. To our knowledge, there are only two reports about such investigations employing animals chronically dependent on morphine; one by Schulteis et al. [21] who found that clonidine blocked the acquisition but not expression of CPA; the other by Maldonado et al. [15] who found that γ-hydroxybutyric acid, a metabolite of γ-aminobutyric acid (GABA), blocked both the acquisition and expression of CPA. It has been demonstrated that conditioned stimuli can induce the expression of the Fos protein or c-Fos gene in specific regions of the brain [4,5,10,20,24,25]. The second purpose of the present study was to determine whether variation in c-Fos expression within the amygdala accompanies the expression of CPA in rats undergoing a single morphine exposure by employing c-Fos immunohistochemistry.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Charles River, Japan; initial weight 200–230 g) were housed two or three per cage. The room temperature was kept at 23 ± 1 °C, and a 12 h light–dark cycle was maintained throughout the experiment. Food and water were available ad libitum. The experimental protocol was conducted according to the Guidelines of the Ethics Review Committee for Animal Experimentation of Okayama University Medical School. The rats were adapted to handling every day for 1 week prior to the experiment.

2.2. Drugs

Both morphine hydrochloride (Takeda Chemical Industries Ltd.) and naloxone hydrochloride (Sigma) were dissolved in 0.9% sodium chloride solution and injected subcutaneously at a volume of 1 ml/kg.

2.3. Conditioned place aversion

The method to establish CPA has been described elsewhere [13]. Briefly, the apparatus consisted of two chambers (42 cm × 30 cm × 29 cm) with different floors that were covered with wire mesh and check-pattern sandpaper squares, respectively. The distinctive tactile stimuli served as the conditioning cues. The animals experienced a pre-conditioning habituation to the apparatus and those showing initial bias for either compartment were eliminated from the study. On the first day of the conditioning procedure, all rats were injected with saline and 5 min later were confined to one side of the apparatus, either the mesh-floor chamber or the sandpaper-floor chamber in a counterbalanced manner, for 30 min. This chamber will be referred to as the "nontreatment-paired chamber". On Day 2, rats were injected with 10 mg/kg of morphine and then returned to their home cages. On Day 3 (24 h after morphine administration), rats were given saline or one of five doses of naloxone (0.02, 0.05, 0.1, 0.2 or 0.5 mg/kg) (n = 7–12) and 5 min later, placed in the chamber opposite to that on Day 1 for 30 min. This chamber will be referred to as the "treatment-paired chamber". Forty-eight hours after the conditioning trial, all rats were allowed to freely explore the entire apparatus for 15 min and the amount of time spent in each chamber was measured. CPA scores representing the time spent in the treatment-paired chamber minus the time spent in the nontreatment-paired chamber during the place preference test were calculated.

2.4. Expression of c-Fos following the CPA test

Two hours after the end of the behavior test, the animals were deeply anesthetized with sodium pentobarbital (200 mg/kg, i.p.) and perfused transcardially with 200 ml of 0.9% saline followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). In our preliminary experiment, the morphine-exposed rats showing a clear CPA had exhibited no notable modification of c-Fos expression in the amygdala following a 60 min interval between the beginning of CPA test and animal sacrifice. So, an interval of 2 h was employed here according to a report where such a time interval had been suggested to be sufficient for morphine-conditioned place cues to induce reliable increase of Fos expression in some brain regions including the amygdala [10]. The brains were removed and coronal slices (40 μm) were cut consecutively through the amygdala from −2.12 to −3.14 mm posterior to the bregma [17] with a vibrating microtome. Every third section was collected and processed for c-Fos immunohistochemistry. A representative schematic diagram of the amygdala is shown in Fig. 1.

2.5. Expression of c-Fos following naloxone-precipitated withdrawal

To examine c-Fos expression induced by naloxone-precipitated withdrawal, separate groups of rats (n = 4) were employed and they experienced the same conditioning trial as described above. They were pretreated with morphine or saline on the second day of the conditioning procedure. Then on Day 3, the morphine-pretreated rats were given saline or one of six doses of naloxone (0.02, 0.05, 0.1, 0.2, 0.5 or 1.0 mg/kg) and those rats pretreated with saline received