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Research article

Differential expression of endocannabinoid system-related genes in the dorsal hippocampus following expression and reinstatement of morphine conditioned place preference in mice

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HIGHLIGHTS

- Morphine CPP is associated with increased clearance and reduced receptors of eCB.
- Extinction of CPP is accompanied with recovery of eCB related genes expression.
- Reinstatement of CPP is associated with decreased MAGL and increased CB1R.

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ABSTRACT

The endocannabinoid signaling plays a critical role in mediating rewarding effects to morphine. The relative stability for the expression and reinstatement of morphine conditioned place preference (CPP) suggests the involvement of differential neuroadaptations in learned associations between environmental cues and morphine. Changes in gene expression in hippocampus through the endogenous cannabinoid system (eCB) may accompany and mediate the development of such neuroadaptations to repeated morphine stimulation. To test this possibility, we systematically compared the expression of eCB-related genes in the dorsal hippocampus following the expression, extinction, and reinstatement of morphine CPP using quantitative RT-PCR analyses. We found that expression of morphine CPP was associated with significant increases in mRNA expression for the primary clearance routes for anandamide (AEA) and 2-AG (fatty acid amide hydrolase [FAAH] and monoacylglycerol lipase [MAGL], respectively), but with reductions in cannabinoid 1 receptors (CB1R) and CB2R in dorsal hippocampus following the expression of CPP. However, our results indicated that decreased in MAGL and increased CB1R mRNA levels were accompanied with morphine CPP reinstatement. No significant changes in mRNA expression for enzymes involved in AEA and 2-AG biosynthesis (N-acylphosphatidylethanolamine phospholipase D [NAPEPLD] and diacylglycerol lipase-α/β [DAGLα/β], respectively) were found in all conditions. These results suggest that differential regulation of the synthesis and/or degradation of the eCB system contribute to the expression and reinstatement of morphine CPP.

1. Introduction

Drug addiction is a mental disorder that continues to exact enormous costs on society in terms of healthcare, family destruction, loss of productivity, and direct and indirect effects on our criminal justice system [10,13]. Inappropriate application of morphine could result in addiction. The mainstay treatment for opioid addiction is agonist substitution therapy with long-duration opioid agonists such as methadone or buprenorphine. Without treatment, most patients relapse to drug use again [24]. A major goal of current research is to better understand the neurobiology of opioid addiction so that more effective treatments can be developed.

Although the reward effect of addictive drugs is critically dependent on the mesolimbic dopamine system, the motivational and rewarding effects of opiates are less dopamine-dependent [22], and the endocannabinoid signaling contributes to the non-dopaminergic mechanisms [18]. The endocannabinoid system

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comprises G protein-coupled receptors and small neuromodulatory lipid ligands, as well as biosynthetic and metabolic enzymes for the synthesis and degradation of the ligands, respectively (for review see [18]). There are two subtypes of cannabinoid receptors have been cloned and characterized: cannabinoid 1 receptors (CB1R) and cannabinoid 2 receptors (CB2R) [12]. CB1Rs are found abundantly distributed in the adult brain, particularly mesocorticolimbic system, while CB2Rs are mainly located in the cells of the immune system [18]. CB1R have been shown to coordinate with reward-related associative learning and motivated behaviors of drug abuse to participate in the conditioned rewarding effects of opiates [12,17,18,21]. Although numerous brain regions likely are involved in associative learning aspects of drug exposure, the present study has focused on a crucial role of dorsal hippocampus. Previous studies have demonstrated that cannabinoid agonists impair whereas cannabinoid antagonists improve memory and plasticity, thus, hippocampal eCB signaling may play a prominent role in encoding contextual information required to form associations between contextual cues and drug-rewarding effects.

Repeated drug treatment leads to adaptations in hippocampal synaptic plasticity that may be involved in drug-context associations [20]. Previous studies have revealed that chronic morphine treatment and morphine-induced sensitization alter the hippocampal endocannabinoid levels [25,26], whereas inhibition of cannabinoid CB1 receptor attenuates the acquisition of morphine-induced conditioned place preference (CPP) along with extracellular signal-regulated kinase (ERK) signaling changes in the hippocampus [27]. Recently, cell type-specific plasticity in the hippocampus was found to be regulated by CB2 receptors [23]. However, the underlying alterations in hippocampal endocannabinoid system elicited by conditioned rewarding effect of morphine have not been well-documented.

In the present study, we have examined the expression of endocannabinoid system-related genes in the dorsal hippocampus following morphine- CPP. As different phases of CPP are associated with distinct adaptations in hippocampal synaptic plasticity that implicated in drug-context associations, we have compared the potential differences existing between expression and reinstatement of morphine CPP.

2. Materials and methods

2.1. Animals

Subjects were 8–12-week-old male C57BL/6J mice weighing 25 g–35 g were purchased from the Experimental Animal Center of Xi'an Jiaotong University. They were maintained on a 12 h light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) and habituated to the behavioral room for 1 week before the start of experiments. The animals were singly housed with food and water freely available. The room was maintained at a temperature of $22 \pm 2\,^{\circ}C$ and a humidity of $55 \pm 5\%$. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University.

2.2. Drug administration

Morphine hydrochloride (Shenyang, China) was dissolved in saline at the concentration of 1 mg/ml. For morphine CPP, mice were injected subcutaneously with daily escalating doses of morphine (5, 8, 10, and 15 mg/kg) or an equivalent volume of saline and then immediately placed in the conditioning chamber. Animals were sacrificed immediately after either morphine CPP expression (24 h after the final morphine injection), extinction (7 d after morphine treatment), or reinstatement (8 d after initial morphine treatment and 15 min after morphine reinstatement injection). Then, the dorsal hippocampi were dissected and stored at $-80\,^{\circ}C$ until use.

2.3. CPP apparatus and conditioning procedure

A two-chamber ($30 \times 16 \times 35$ cm, JLBeHv, Shanghai, China) apparatus was used in this study. The left chamber had a black background with a grid floor, and the right chamber had a white background with a mesh floor. The two chambers could be connected or isolated by opening or closing a guillotine door. The total amount of time spent in the chambers was recorded and analyzed by DigBehv software (JLBeHv, Shanghai, China).

All the mice were allowed to move freely between the two compartments for 15 min as preconditioning on days 1 and 2. On day 2, the time spent in each chamber was recorded, and the mice that spent more than 10 min in either chamber were excluded from the experiment.

After preconditioning, mice received eight conditioning sessions (four-morning and four-afternoon sessions) [3,20]. During conditioning, mice in the morphine CPP group received a subcutaneous injection of saline in the morning and were immediately confined to one chamber (saline-paired conditioning chamber) for 30 min. Four hours later, in the afternoon, mice in the morphine CPP group received a subcutaneous injection of morphine and were immediately confined to the other chamber (morphine-paired conditioning chamber) for 30 min. In contrast, mice in the saline CPP group received saline during both conditioning sessions. In addition, a morphine-unpaired group was included in which morphine was administered alternately in both conditioning chambers during the afternoon conditioning sessions. For the morphine unpaired control group, the location of morphine injection was alternated between days.

On the test day (24 h after final morphine injection), mice were allowed to freely explore the apparatus without injection for 15 min. The time spent in each chamber was recorded. Place preference scores were calculated by subtracting the time spent in the drug-paired side during preconditioning from the time spent in the drug-paired side during post-conditioning [7].

2.4. Extinction and reinstatement of morphine CPP

Extinction of morphine CPP commenced 24 h after morphine CPP. Mice in all groups received 10 extinction training sessions (five-morning and five-afternoon sessions), in which a subcutaneous injection of saline was administered and mice were confined to the saline-paired chamber for 30 min. During afternoon extinction training, mice received a second subcutaneous injection of saline and were confined to the morphine-paired chamber for 30 min. Testing of extinction occurred 24 h after the final conditioning session (7 d after morphine treatment); mice were given free access to the entire apparatus without any injection for 15 min. Preference scores were calculated in the same manner as described above (subtracting the time spent in the morphine-paired side during preconditioning from the time spent in the morphine-paired side during post-conditioning in extinction), and extinction of CPP was defined as a preference score that was <15% of the initial preference score [20]. As described above, a morphine-unpaired group was included in extinction experiments in which the mice received morphine in both chambers on alternating days during the training of morphine CPP, and extinction experiments were conducted in the same manner as described above.

The reinstatement of morphine CPP occurred 24 h after the CPP extinction test. Mice that showed extinction of morphine CPP or saline controls were treated with 5 mg/kg morphine and allowed to explore the chambers for 15 min. As before, a group of mice