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Research Report

Increased expression of cannabinoid receptor 1 in the nucleus accumbens core in a rat model with morphine withdrawal

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ABSTRACT

Relapse is a major clinical problem and remains a major challenge in the treatment of drug addiction. There is strong evidence that the endocannabinoid system of the nucleus accumben core (NAcc) is involved in drug-seeking behavior, as well as in the mechanisms that underlie relapse to drug use. To reveal the mechanism that underlies this finding, we examined the expression pattern of the cannabinoid receptor 1 (CB1-R) in the NAcc of SD rats that had been undergoing morphine withdrawal (MW) for 1 day, 3 days and 3 weeks (acute, latent and chronic phases, respectively). Morphine exposure induced conditioned place preference (CPP) in rats. Significant increase of CB1-R expression in NAcc was observed in animals in the 1 day, 3 days and 3 weeks morphine withdrawal compare to the control group. Immunofluorescence labeling showed axonal fibers or terminals by fluorescence microscope observation. Immunoelectron microscopy detection showed silver-gold particles located in the presynaptic membranes that mainly give rise to symmetrical synapses. Quantitative electron microscopy showed an increase in number of CB1-R-positive terminals in the morphine withdrawal groups and the number of immunogold particles was significantly increased at these inhibitory terminals. We also confirmed that infusions of the CB1-R antagonist rimonabant into the NAcc attenuated the CPP during morphine withdrawal. Our present data have thus indicated that increasing pattern of CB1-R expression in the NAcc during above morphine withdrawal phases, which might underlie the relapse associated drug seeking behavior after morphine withdrawal.

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1. Introduction

Opiates as a drug class represent the single largest contribution to illicit drug-related mortality and morbidity worldwide and remain a major clinical problem for drug treatment (Darke, 2007). With repeated use, opiates cause long-lasting chemical and neuronal adaptations in the brain. These changes produce tolerance, physical dependence and can ultimately result in the disease state known as addiction (Christie, 2008). Relapse is a major clinical problem and remains a major challenge in the treatment of addictions (Brown and Lawrence, 2009), which is exacerbated by drug craving behavior during the withdrawal phase.

Endocannabinoids are released from the postsynaptic neurons in an activity-dependent manner and bind to presynaptic cannabinoid receptor 1 (CB1-R), thereby suppressing the release of exciting and inhibiting transmitters from the presynaptic terminals (Demuth and Molleman, 2006; Freund et al., 2003; Mackie, 2008; Wilson and Nicoll, 2002). There is strong evidence that the endocannabinoid system is involved in drug-seeking behavior, as well as in the mechanisms that underlie relapse to drug use (Justinova et al., 2009). Increasing studies demonstrated that the cannabinoid system participates in the relapse and manipulations of the endocannabinoid system offered one of the pharmacotherapeutic treatments for addiction and relapse (Justinova et al., 2009; Fattore et al., 2011; Fang et al., 2011). However, the mechanisms that underlie the modulatory role of cannabinoids on drug seeking remain poorly understood. High levels of CB1 receptors are present in brain regions that are thought to have a key role in relapse-like behavior and conditioning processes in laboratory animals; these regions include the prefrontal cortex, amygdala, nucleus accumbens, striatum and hippocampus (Kalivas and McFarland, 2003). In both human addicts and animal-reinstatement models, a return to drug use can be precipitated by stimulus such as environmental stimulus (Meil and See, 1997; McFarland and Ettenberg, 1997), pharmacological stimulus (De Vries et al.,1998), and stressors (Ahmed and Koob, 1997; Shaham et al., 1997). These three different modes of stimuli engage distinct neural circuits. Interestingly, all three modes converge on the anterior cingulated cortex and have a final common output through the core of the nucleus accumbens (NAcc) (Kalivas and McFarland, 2003). It has been reported that glutamatergic projection from the prefrontal areas to the nucleus accumbens has an important role in relapse behavior (Kalivas and McFarland, 2003; De Vries and Schoffelmeer, 2005). An important role for CB1-R in the NAcc in drug-seeking behavior has been established in many studies. Alvarez-Jaimes et al. (2008) found that infusing a CB1-R antagonist into the NAcc could reduce cue-induced heroin-seeking behavior. Xi et al. (2006) also demonstrated that intra-NAC administration of the CB1 receptor antagonist AM251 inhibits cocaine-induced reinstatement.

Chronic exposure to morphine has been proven to cause changes in the expression of CB1-R in the brain (Fattore et al., 2007; Gonzalez et al., 2002; Rubino et al., 1997). However, few investigations have focused on the quantity and distribution of CB1-R in the NAcc during the different phases of morphine withdrawal (MW).

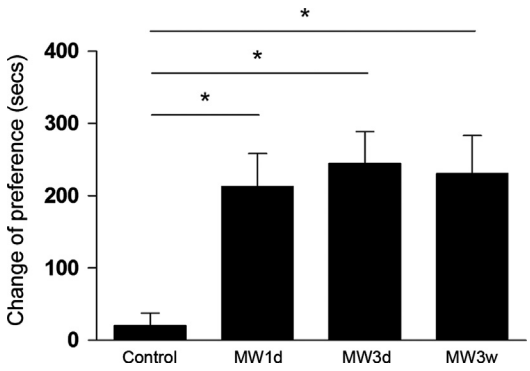

Fig. 1 – Conditioned place preference (CPP) results of models. Rats at different time points of morphine withdrawal (MW) and control rats were tested after 5 days of scheduled conditioning. The change of preference for the drug-paired compartment of the MW groups ($n=12$ for each group) was noticeably more intense than that of the control group ($n=12$). Significant differences between MW groups and control group were found, but there was no significant difference among the MW groups. Statistics were calculated using Tukey's multiple comparison tests. $^{*}p<0.05$ vs control group.

Using the MW model we examined the expression of CB1-R in the NAcc by western blot (WB), immunofluorescence (IF) and immunoelectron microscopy (IEM). More CB1-R was expressed in the rats representing the three phases of MW than in the control group. The most dramatic change in CB1-R expression occurred in the presynaptic membrane of symmetric synapses. Intracranial injection of rimonabant into the NAcc was able to attenuate the conditioned place preference (CPP) during MW. Our results suggest that the increase in CB1-R at the presynaptic membrane of symmetric synapses in MW phases may underlie the relapse associated drug seeking behavior.

2. Results

2.1. *Morphine withdrawal rats showed persistent conditioned place preference*

The rats showed no preference between the two chambers during the pre-exposure phase, and the mean time spent in the two chambers was similar to each other. After the conditioning phase, the rats in the different groups were tested for the change of preference for drug-paired side: the control group (20.2 ± 17.2 s), morphine withdrawal 1 day (MW1d) group (212.9 ± 45.7 s), morphine withdrawal 3 days (MW3d) group (244.6 ± 44.2 s) and morphine withdrawal 3 weeks (MW3w) group (230.4 ± 52.7 s) (Fig. 1). Tukey's multiple comparison test revealed that MW models showed CPP, compared to control group ($p<0.05$). No significant effect was observed among the MW groups ($p>0.05$).

2.2. *CB1-R expression increased in MW models*

As shown in Fig. 2A, the expression of the CB1-R increased markedly in the NAcc of the MW groups compared to the