Cannabinoid CB1 receptor antagonist rimonabant attenuates reinstatement of ketamine conditioned place preference in rats

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A B S T R A C T
Recent evidence suggests that cannabinoid CB1 receptors may represent effective targets for therapeutic agents used to treat cocaine and heroin relapse. However, the role of cannabinoid CB1 receptors in the potential treatment for other drugs of abuse is still largely unknown. The present study was conducted to determine whether cannabinoid CB1 receptors play a similar role in relapse to ketamine abuse. To establish a ketamine reinstatement model in the conditioned place preference paradigm, rats were trained to develop place preference conditioned by ketamine, which was subsequently extinguished through daily exposure to the test chambers in the absence of ketamine. On the day following the last extinction session, four groups of rats were injected with ketamine (1, 5, 10 and 15 mg/kg, i.p.) to reestablish previously extinguished conditioned place preference. To investigate the effects of rimonabant, a cannabinoid CB1 receptor antagonist, on reinstatement of ketamine-induced place preference, different doses of rimonabant (0.1, 0.5 and 3 mg/kg, i.p.) were injected 30 min prior to the ketamine (5 and 15 mg/kg, i.p.) priming injection in a separate group of rats. To determine whether rimonabant itself produced conditioned place preference or place aversion, rats were trained for conditioned place preference or place aversion with rimonabant (0, 0.1, 0.5, 3.0 mg/kg, i.p.). While ketamine priming injections reinstated extinguished place preference, rimonabant administration significantly attenuated the reinstatement of ketamine-induced place preference in a dose-dependent manner. Importantly, rimonabant itself did not produce conditioned place preference or place aversion. Since the reinstatement effects of ketamine administration were inhibited by rimonabant, these findings suggest that a cannabinoid CB1 receptor antagonist may be useful in preventing relapse to ketamine abuse.

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1. Introduction

Ketamine, also known as “special K,” has been used recreationally in many countries over the past decade (Curran and Morgan, 2000; Fang et al., 2006). Ketamine is an anesthetic derivative of phencyclidine that has dissociative, analgesic, and psychotomimetic properties (Wolf and Winstock, 2006). Ample evidence from animal research has shown strong similarities between ketamine and other addictive drugs in a wide range of behavioral paradigms, including self-administration (Marquis et al., 1989; Winger et al., 1989), locomotor sensitization (Meyer and Phillips, 2003; Trujillo et al., 2007; Uchihashi et al., 1993; Wiley et al., 2007), drug discrimination (Grant et al., 1991; Shelton and Balster, 1994), and conditioned place preference (CPP) (Suzuki et al., 2000). Ketamine, which functions as an N-methyl-D-aspartate (NMDA) receptor antagonist, can also influence various behavioral effects of other addictive drugs. For instance, ketamine has been shown to prevent morphine-induced place preference (Suzuki et al., 2000) and analgesic tolerance (Shimoyama et al., 1996; Trujillo and Akil, 1994). Ketamine can also suppress rapid tolerance of ethanol or barbiturates (Khanna et al., 1998; Khanna et al., 2002; Khanna et al., 1997). When given in combination with ethanol, ketamine potentiated ethanol-induced locomotion (Meyer and Phillips, 2003) and neutralized ethanol’s anxiolytic-like effect (Silvestre et al., 2002).

The conditioned place preference model has been used widely to assess the rewarding effect of addictive drugs (Tzschentke, 1998, 2007). Although the vast majority of research using this model has concentrated on the development and expression of drug-induced place preference, a growing number of place preference studies incorporate extinction and reinstatement phases. Extinction of previously established drug-induced place preference can be accomplished with either repeated place preference testing without any drug exposure or by pairing the two environments of the test apparatus with saline injections. Drug priming injections (Mueller
et al., 2002; Mueller and Stewart, 2000), presentation of stress (Lu et al., 2001, 2002; Zhao et al., 2007), and conditioned fear stimuli (Sanchez and Sorg, 2001) can then reinstate the extinguished place preference. Although ketamine has been shown to produce significant conditioned place preference in mice (Suzuki et al., 2000), extinction and reinstatement of ketamine-induced place preference has not been tested experimentally. The primary goal of the current study was to characterize the extinction and reinstatement of ketamine conditioned place preference. A cannabinoid CB1 receptor antagonist, rimonabant, was also used to determine the effect of cannabinoid CB1 receptor blockade on the reinstatement of ketamine conditioned place preference following extinction.

2. Materials and methods

2.1. Animals and drugs

A total of 100 male Sprague–Dawley rats (200–220 g) obtained from the animal laboratory at Guiyang Medical College were used in the experiments. The animals were housed at a room temperature of 22±2 °C with a 12 h light–dark cycle and allowed to habituate for seven days prior to the experiment. Food and water were available ad libitum. The animals weighed 250–300 g at the start of the experiment. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The procedures were approved by the local Committee for Animal Use and Protection.

The drugs used in the present study were ketamine hydrochloride (Shanghai First Biochem. & Pharma. Co., Ltd. Shanghai, China) and rimonabant (Xinxiang Crude Medicinal Drugs Co., Jiangsu, China). Ketamine hydrochloride was diluted to 10 mg/ml with saline. Rimonabant was dissolved with saline containing 1% Tween 80.

2.2. Conditioned place preference procedure

The conditioned place preference system (Shanghai Jiliang Soft Co. Shanghai, China) was computer controlled and consisted of three PVC compartments. Two of the compartments were identical in size (L×W×H: 30 cm × 30 cm × 50 cm) with different colors and floor textures. The black compartment had a textured floor and the white compartment had a smooth floor. The middle compartment was a 12 cm × 30 cm × 50 cm (L×W×H) grey tunnel.

The conditioning procedure for ketamine-induced place preference was modified from previous studies (Calcagnetti and Schechter, 1993; Lu et al., 2005; Suzuki et al., 1999). In order to determine compartment bias, rats were initially placed in the middle chamber and permitted to move freely through the apparatus for a period of 15 min during the pre-conditioning period. Animals that spent more than 150 s in one compartment compared to the other were considered to have compartment bias and excluded from the study. During the conditioning trial, each rat was treated for 8 consecutive sessions with alternate injections of either ketamine (10 mg/kg, i.p.) or saline (1 ml/kg, i.p.). Rats were confined to the white compartment for 45 min immediately after ketamine administration and to the black compartment after saline injection. After 8 consecutive sessions, rats were re-tested for ketamine (post-conditioning) conditioned place preference by being allowed free access to both the white and black chambers for 15 min. Rats in the control group received saline injections throughout the conditioning and post-conditioning phases.

In order to exclude the possibility that rimonabant produced place preference or place aversion, five groups of rats received place preference training with different doses of rimonabant (saline group, 0, 0.1, 0.5, and 3.0 mg/kg) administered in a similar fashion to the ketamine. Briefly, animals underwent 8 consecutive sessions in which they received alternating injections of either rimonabant or saline (1 ml/kg, i.p.) (De Vries et al., 2001). After 8 consecutive sessions, rats were re-tested for place preference or place aversion induced by the rimonabant. The control rats received saline injections throughout the conditioning and post-conditioning phases.

2.3. Extinction and reinstatement of ketamine conditioned place preference

During the ketamine conditioned place preference extinction phase, rats were injected with saline and confined to the previous ketamine- or saline-paired compartment for 45 min a day for 28 days (Lu et al., 2000; Wang et al., 2006). Immediately following the last extinction session, rats were injected with ketamine (1, 5, 10 and 15 mg/kg) to reinstate extinguished place preference conditioned by ketamine. To investigate the effect of rimonabant on the reinstatement...

Fig. 1. Acquisition, extinction and reinstatement of ketamine conditioned place preference: (A) Acquisition of ketamine conditioned place preference. Data are depicted as the time spent (mean ± SEM) in the drug-paired chamber during the pre-conditioning and post-conditioning phases of conditioned place preference. *Different from pre-conditioning, \(P<0.05\); #Different from the saline group, \(P<0.05\). (B) Extinction of ketamine conditioned place preference. Data are depicted as the conditioned place preference scores (mean ± SEM) during the pre- and post-extinction of conditioned place preference. *Different from pre-extinction, \(P<0.05\). (C) Reinstatement of ketamine conditioned place preference. Data are depicted as the place preference scores (mean ± SEM). *Different from 1 mg/kg ketamine group within post-priming, \(P<0.05\); #Different from the pre-priming, \(P<0.05\).
of ketamine conditioned place preference, rimonabant (0, 0.1, 0.5, 3.0 mg/kg) was administered 30 min prior to the priming injection of ketamine.

2.4. Data analysis and statistics

The place preference score was defined as the time spent in the drug-paired chamber during the post-conditioning (pre-extinction), post-extinction (pre-priming) or post-priming session minus the time spent in the drug-paired chamber during the pre-conditioning session.

The data were expressed as means ± SEM. The statistical analysis was performed using two-way analysis of variance (ANOVA) with the place preference scores as the dependent factor. When appropriate, post-hoc comparison of means was carried out with the Tukey test for multiple comparisons. A linear regression was used to assess the correlation between the reinstatement of ketamine place preference by different doses of the drug, and the effects of different doses of rimonabant on the reinstatement of ketamine place preference. The level of statistical significance was set at \( P<0.05 \).

3. Results

3.1. Acquisition, extinction and reinstatement of ketamine conditioned place preference

After the 8 conditioning sessions with ketamine, rats demonstrated significant place preference for the ketamine-paired chamber. As shown in Fig. 1A, ANOVA analysis revealed that there was a significant effect of treatment and phase (Treatment: \( F_{1,155}=54.2, P<0.001 \); Phase: \( F_{1,155}=57.6, P<0.001 \); Treatment×Phase: \( F_{1,155}=61.3, P<0.001 \)). Post hoc analysis showed that there were significant differences in the time spent in the drug-paired chamber between the pre- and post-conditioning periods in ketamine group (\( P<0.001 \)). Ketamine place preference was significantly extinguished after 28 extinction sessions. Ketamine-induced place preference was reinstated by a single injection of ketamine. As shown in Fig. 1B, there were significant differences in the place preference scores before (pre-extinction) and after (post-extinction) the extinction phase (\( P<0.01 \)). As shown in Fig. 1C, ANOVA analysis revealed that there were significant differences for place preference scores between pre-priming and post-priming treatment (\( F_{1, 24}=60.6, P<0.001 \)). Also, ketamine significantly reinstated extinguished conditioned place preference in a dose-dependent manner (\( r=0.97 \), \( P<0.05 \)). There were significant differences for place preference score between pre- and post-priming in the groups at the doses of 5, 10 and 15 mg/kg, but not at 1 mg/kg, ketamine.

3.2. Effects of rimonabant on drug-primed reinstatement of ketamine conditioned place preference

The reinstatement of conditioned place preference induced by priming injections of ketamine was significantly attenuated by pretreatment with rimonabant. As shown in Fig. 2B, the ANOVA analysis revealed that there were significant effects of rimonabant treatment (pre-priming and post-priming) on place preference scores across groups (\( F_{1, 30}=18.0, P<0.001 \)). The reinstatement of extinguished ketamine conditioned place preference primed by 5 mg/kg ketamine was dose-dependently attenuated by pretreatment with rimonabant (\( r=0.934, P<0.05 \)) (Fig. 2B). The reinstatement of extinguished ketamine conditioned place preference was attenuated by rimonabant at doses of 0.5 and 3 mg/kg (\( P<0.01 \)), but not at a dose of 0.1 mg/kg.

To explore whether rimonabant attenuated the reinstatement of ketamine place preference induced by a higher dose of ketamine, extinguished place preference was reinstated by ketamine at a dose of 15 mg/kg (Fig. 2C). As shown in Fig. 2C, the ANOVA analysis revealed that there were significant effects of rimonabant treatment on place preference score across groups (\( F_{1, 124}=129.06, P<0.001 \)). The reinstatement of extinguished ketamine place preference primed by 15 mg/kg ketamine was attenuated by pretreatment with rimonabant in a dose-dependent manner (\( r=0.91, P<0.05 \)). The reinstatement of extinguished ketamine place preference was attenuated by rimonabant at doses of 0.5 and 3 mg/kg (\( P<0.001 \)), but not at a dose of 0.1 mg/kg.
3.3. Rimonabant conditioned place preference or conditioned place aversion

To determine whether rimonabant itself could produce place preference or place aversion, naive rats were trained with different doses of rimonabant during the conditioning phase. As shown in Fig. 3, there were no significant differences between the time spent in the drug- and saline-paired chambers across all tested doses of rimonabant (saline and 0.1, 0.5, 3.0 mg/kg rimonabant) (P>0.05). These findings indicate that rimonabant itself does not have any rewarding or aversive properties.

4. Discussion

The current study was conducted to experimentally verify the extinction and reinstatement of ketamine-induced place preference in rats and to determine the effect of rimonabant, a cannabinoid CB1 receptor antagonist, on the reinstatement of ketamine-induced place preference. The major findings are (1) rats expressed significant place preference conditioned by ketamine; (2) their place preference to ketamine was extinguished following 28 days of extinction and this extinguished place preference response was reinstated by priming injections of ketamine across a wide range of doses; (3) the reinstatement of ketamine conditioned place preference was dose-dependently attenuated by rimonabant, which itself failed to induce either conditioned place preference or place aversion in the animals. Taken together, these findings demonstrate that the establishment, extinction and reinstatement of ketamine conditioned place preference response can be induced in rats, and that cannabinoid CB1 receptors play an important role in the reinstatement of ketamine conditioned place preference.

The extinction and reinstatement of ketamine conditioned place preference has not been previously demonstrated, despite the fact that a growing number of studies have shown a similar pattern of extinction and reinstatement of conditioned place preference induced by a wide range of other drugs, including cocaine (Mueller and Stewart, 2000; Parker et al., 2004), morphine (Lu et al., 2002; Mueller et al., 2002), ethanol (Cunningham et al., 1998), and nicotine (Biala and Budzynska, 2006). Only one report has demonstrated that administration of ketamine was sufficient to induce conditioned place preference (Suzuki et al., 2000) in mice. Our findings confirmed that ketamine produces conditioned place preference in rats, and further we provide the first demonstration that ketamine conditioned place preference can be extinguished in rats, and then dose-dependently reinstated by priming injections of the drug.

The mechanisms underlying the role of cannabinoid CB1 receptors in the reinstatement of ketamine conditioned place preference are likely associated with the distribution of these receptors in the brain and the interaction between the endogenous cannabinoid system and other neurotransmitters, such as dopamine and glutamate. High levels of cannabinoid CB1 receptors are present in brain regions that are thought to play a key role in relapse-like behavior and conditioning processes, including prefrontal cortex, amygdala, nucleus accumbens, striatum, ventral tegmental area, and hippocampus (Everitt et al., 1999; Kalivas and McFarland, 2003; Tanda et al., 1997). Cannabinoid CB1 receptor activation has been shown consistently to influence dopaminergic transmission (Di Chiara and Imperato, 1988). For example, extracellular dopamine levels were increased in the shell of the nucleus accumbens in rats trained to self-administer the cannabinoid CB1 receptor agonist, WIN, 55 212-2 (Fadda et al., 2006). In contrast, rimonabant, given systemically, can reduce increased dopamine levels from the same region induced by novel and highly palatable food (Mellis et al., 2007). In addition, cannabinoids have been shown to mediate presynaptic inhibition of glutamatergic transmission in various brain regions, including hippocampus (Ohno-Shosaku et al., 2002; Shen et al., 1996), substantia nigra pars reticulata (Szabo et al., 2000), striatum (Gerdeman and Lovinger, 2001; Huang et al., 2002), VTA (Mellis et al., 2004), and amygdala (Azad et al., 2003). A recent report has demonstrated that transient release of endogenous cannabinoids can be induced by postsynaptic dopamine neurons within the VTA in a Ca2+ and CB1 receptor-dependent fashion and that they act retrogradely and inhibit glutamatergic release from the presynaptic terminals (Mellis et al., 2004). Thus, blockade of the reinstatement of ketamine conditioned place preference by rimonabant is possibly via the alteration of glutamatergic transmission in the brain. Certainly, further studies are necessary to explore the critical role of the dopaminergic system in the reinstatement of ketamine conditioned place preference.

The possibility of any potential behavioral effect of rimonabant interfering with the place preference effect of ketamine was also addressed in the present study. Our present findings demonstrate that neither place preference nor place aversion was evident in the animals repeatedly treated with rimonabant. Since ketamine can function as a discriminative stimulus (De Vry and Jentzsch, 2003; Narita et al., 2001), it is rather possible that the inhibitory effect of rimonabant on the conditioning effect of ketamine is indeed caused by its suppression on the discriminative effect, instead of place preference of ketamine. However, previous studies have demonstrated that rimonabant did not affect the discriminative stimulus effects of cocaine (Filip et al., 2006) or nicotine (Le Foll and Goldberg, 2004). Thus, while the question of whether rimonabant affects the discriminative stimulus effect of ketamine remains unknown, it seems unlikely to be the case in present study.

In conclusion, re-exposure to ketamine reinstated extinguished ketamine conditioned place preference, which was, however, blocked by the cannabinoid CB1 receptor antagonist rimonabant. Recent findings suggest that cannabinoid CB1 receptors may be a novel target for a new class of therapeutic agents used to treat cocaine and heroin addiction (De Vries et al., 2003; De Vries et al., 2001; De Vries and Schoffelmeer, 2005). Like these studies, our findings also suggest that the cannabinoid CB1 receptor plays a role in relapse to ketamine abuse, and thus that cannabinoid CB1 receptor antagonists may be useful in the treatment of ketamine addiction.

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References


