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Research report

Levo-tetrahydropalmatine inhibits the acquisition of ketamine-induced conditioned place preference by regulating the expression of ERK and CREB phosphorylation in rats

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HIGHLIGHTS

- The drug *l*-THP attenuated the acquisition of ketamine-induced CPP in rats.
- *l*-THP prevented the increase in ERK phosphorylation in the Hip and CPu of the rats exhibiting CPP.
- *l*-THP decreased the phosphorylation of CREB in the Hip and CPu of the rats exhibiting CPP.
- *l*-THP may be a potential medicine for the treatment of ketamine addiction.

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ABSTRACT

Levo-tetrahydropalmatine (*l*-THP) is an alkaloid purified from the Chinese herbs *Corydalis* and *Stephania* and has been used in many traditional Chinese herbal preparations for its sedative, analgesic and hypnotic properties. Previous studies demonstrated that *l*-THP has antagonistic activity on dopamine receptors; thus, it may have potential therapeutic effects on drug abuse. However, whether *l*-THP affects ketamine-induced conditioned place preference (CPP) remains unclear. Therefore, the present study was designed to evaluate the effects of *l*-THP on the rewarding behavior of ketamine through CPP. Results revealed that ketamine (5, 10 and 15 mg/kg) induced CPP in rats. Furthermore, Ketamine (10 mg/kg) promoted the phosphorylation of extracellular-regulated kinase (ERK) and cAMP responsive element binding protein (CREB) in the hippocampus (Hip) and caudate putamen (CPu), but not in the prefrontal cortex (PFc). *l*-THP (20 mg/kg) co-administered with ketamine during conditioning inhibited the acquisition of ketamine-induced CPP in rats. Furthermore, *l*-THP (20 mg/kg) prevented the enhanced phosphorylation of ERK and CREB in CPu and Hip. These results suggest that *l*-THP has potential therapeutic effects on ketamine-induced CPP. The underlying molecular mechanism may be related to its inhibitory effect on ERK and CREB phosphorylation in Hip and CPu. The present data supports the potential use of *l*-THP for the treatment of ketamine addiction.

1. Introduction

Ketamine is commonly used in surgery for its anesthetic, sedative, and analgesic properties [1]. Over the past decade, ketamine has emerged as a recreational drug [2], and the abuse of ketamine is increasing year by year. Long-term use of ketamine produced persistent changes in behavior and in brain structure associated with learning and memory [3]. Memories of the learned association between cues and the rewarding properties of abused drugs are

Abbreviations: *l*-THP, levo-tetrahydropalmatine; ERK, extracellular-regulated kinase; CREB, cAMP responsive element binding protein; Hip, hippocampus; CPu, caudate putamen; PFc, prefrontal cortex; CPP, conditioned place preference; PCP, phencyclidine; NMDA, *N*-methyl-D-aspartate.

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difficult to extinguish and this contributes significantly to the high propensity to relapse [4]. Previous studies demonstrated that the mesolimbic dopamine system significantly affects drug-induced behavioral and neuronal changes [5]. The neural circuitry of drug-seeking behavior involves the prefrontal cortex (PFc), caudate putamen (CPu) and the nucleus accumbens (NAc) [6]. Ketamine can improve the release of dopamine in the NAc and activate dopamine D_1 receptors in the PFc by reducing glutamatergic inhibition of dopaminergic transmission and reducing dopaminergic re-uptake [7,8]. Ketamine is similar to other addictive drugs in terms of affecting behavior, such as locomotor sensitization [8,9], self-administration [10] and conditioned place preference (CPP) [11], which is a standard test of the rewarding/addictive properties of drugs [12,13]. Therefore, the dopaminergic system is increasingly recognized as a therapeutic target for drug addiction yet there is still no medication currently available for treating ketamine addiction in clinical practice.

Tetrahydropalmatine (THP) is a major active ingredient of *Corydalis ambigua* and *Stephania tetranada*; it has sedative, neuroleptic, and analgesic effects [14]. Pharmacological research has revealed that *l*-THP is an antagonist of dopamine D_1 and D_2 receptors; *l*-THP is more closely associated with dopamine D_1 receptors than with D_2 receptors [15]. *l*-THP also affects dopamine D_3 receptors [16,17]. These profiles of *l*-THP suggest that it may have potential therapeutic effects on drug addiction [18]. *l*-THP can attenuate methamphetamine-induced locomotor sensitization [19], CPP [20], oxycodone-induced CPP [21], and cocaine self-administration [17]. However, the effect of *l*-THP on the rewarding behavior of ketamine in rats and its underlying molecular mechanisms remains unclear.

ERK is a kinase that plays important roles in second messenger signaling pathways and is implicated in playing a role in the development of addiction to various drugs, including cocaine and methamphetamine. The phosphorylation of ERK is enhanced by addictive drugs [22,23], and this process is related to methamphetamine-induced rewarding behavior [24]. Furthermore, the inhibition of ERK phosphorylation attenuates cocaine-induced CPP [25]. CREB regulates gene expression in cell signaling pathways [26] and is also closely related to drug addiction. Morphine enhances CREB phosphorylation in the hippocampus (Hip) of mice exhibiting CPP [27]. Thus, ERK and CREB phosphorylation may be significantly related to drug-abuse properties. Nevertheless, studies have yet to clarify the mechanism by which *l*-THP modulates the development of ketamine-induced CPP and the relevance of the activation of ERK and CREB phosphorylation in the brain of rats.

In the present study, a CPP model was used to determine the effects of *l*-THP on the rewarding behavior of ketamine. The relationship between rewarding behaviors and changes in ERK and CREB phosphorylation in CPu, Hip and PFc in Sprague-Dawley (SD) rats was investigated.

2. Materials & methods

2.1. Animals

Male SD rats (200–220 g) were purchased from the Academy of Military Medical Sciences (AMMS, China). All the animals were maintained on 12 h/12 h light/dark cycle at 22 ± 2 °C and 55 ± 5% humidity with food and water ad libitum, and were acclimatized for 1 week. The rats were handled daily for 1 week to adapt to the situation before treatment. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Shanxi Medical University and accordance with the guidelines of National Institute of Health (NIH) guideline (NIH publication NO. 8023. Revised 1978).

2.2. Reagents

l-THP (99.00%) was purchased from the Sigma-Aldrich Inc. (USA) and it was dissolved in distilled water. Ketamine hydrochloride was obtained from Hengrui pharmaceutical factory (Jiang Xi, RP China). Rabbit anti-P-ERK antibody and rabbit anti-ERK antibody were acquired from Cell Signaling Technology (USA). Goat anti-rabbit IgG antibody was purchased from Boster Company (Wuhan, RP China). Rabbit anti-CREB and rabbit anti-P-CREB antibody were procured from Abcam (Cambridge, UK). The volume of intraperitoneal (i.p.) injection was 10.0 ml/kg.

2.3. Experimental design

2.3.1. Ketamine addiction experiment

In this experiment, different doses of ketamine were administered to reveal the rewarding effect of ketamine. The rats were divided into four groups randomly (n = 7 per group) consisting of (i) a control group treated with saline alone; (ii) a group treated with ketamine of 5 mg/kg; (iii) a group treated with ketamine of 10 mg/kg; (iv) a group treated with ketamine of 15 mg/kg.

2.3.2. l-*THP therapeutic experiment*

In this experiment, the dose of ketamine was 10 mg/kg; different doses of *l*-THP were treated in combination to study the therapeutic effect of *l*-THP. The rats were divided into four groups randomly (n = 6 per group) consisting of (a) a control group treated with saline alone; (b) a group treated with ketamine of 10 mg/kg; (c) a group treated with ketamine of 10 mg/kg in combination with *l*-THP of 10 mg/kg; (d) a group treated with ketamine of 10 mg/kg in combination with *l*-THP of 20 mg/kg. *l*-THP was administered 30 min before ketamine was administered.

2.4. CPP apparatus

The CPP apparatus (JLBeHv, China) consists of two equal-sized compartments (L * W * H: 30 cm * 30 cm * 40 cm) with a 7 cm * 10 cm sliding door in the center of the base. One compartment had 2 cm wide black and white horizontal stripes and a wire-mesh floor, whereas the other compartment had 2 cm wide black and white vertical stripes and a bar metal grid rod floor [28]. The time that the rats spent in each compartment were recorded by infrared monitoring system.

2.5. Conditioned place preference procedure

The ketamine-induced CPP was examined by a three-phase CPP procedure similar to that described before [29,30] with modifications (Fig. 1A). Briefly, the experiment consisted of pre-test phase (1 day); conditioning training phase (10 days), and post-test phase (1 day). For pre-test phase (the 1th day), each rat was drug free and was placed in the compartment randomly with free access to the entire CPP apparatus for 15 min. The time of rat spent in each compartment was recorded. The rat who spends over 600 s of the 15 min in either compartment were excluded from further analysis (10% of all animals). For conditioning training phase (from the 2th day to the 11th day), ketamine was paired with the non-preferred side (horizontal stripes compartment). On the 2th day, Group (i) received an i.p. saline injection and was confined in the vertical stripes compartment for 40 min; meanwhile, Groups (ii)–(iv) received different doses of ketamine (5 mg/kg, 10 mg/kg, and 15 mg/kg) injection (i.p.) respectively and they were confined in the horizontal stripes compartment for 40 min. All the groups received an i.p. saline injection and were confined in the vertical stripes compartment for 40 min on the 3th day. Groups (ii)–(iv)