L-Stepholidine, a natural dopamine receptor D1 agonist and D2 antagonist, inhibits heroin-induced reinstatement

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**Highlights**  
- Levo-tetrahydropalmatine attenuates heroin-induced reinstatement of heroin-seeking behavior.  
- Low doses of levo-tetrahydropalmatine do not affect locomotor activity.  

**Abstract**  
L-Stepholidine (l-SPD), an alkaloid extract of the Chinese herb Stephania intermedia, is the first compound known to exhibit mixed dopamine D1 receptor agonist/D2 antagonist properties and is a potential medication for the treatment of opiate addiction. The aim of the present study was to investigate the effects of pretreatment with l-SPD on heroin-seeking behavior induced by heroin priming. Male Sprague-Dawley rats were trained to self-administer heroin (0.05 mg/kg per infusion) under a fixed ratio 1 schedule for 12 consecutive days and nose-poke responding was extinguished for 12 days, after which reinstatement of drug seeking was induced by heroin priming. Pretreatment with l-SPD (2.5, 5.0 and 10.0 mg/kg, i.p.) inhibited the heroin-induced reinstatement of heroin-seeking behavior. Importantly, l-SPD did not affect locomotion, indicating that the observed effects of l-SPD on reinstatement are not the result of motor impairments. The present data suggested that l-SPD inhibits heroin-induced reinstatement and its potential for the treatment of heroin relapse.

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1. Introduction

Relapse to drug-seeking behavior is a primary manifestation of drug addiction, and reducing relapse is a clinical index of the success of interventions [19]. Relapse is modeled in rodents by measuring the reinstatement of drug-seeking behavior in animals that have undergone extinction training [6]. Drug-seeking can be induced by stimuli akin to those that elicit relapse in addicts, such as the presentation of drug-associated cues, stress or a single dose of the drug itself.

A large body of experimental evidence supports the hypothesis that the mesocorticolimbic dopamine (DA) system, which originates in the ventral tegmental area (VTA) and projects rostrally to the nucleus accumbens and the medial prefrontal cortex, plays an important neurobiological mediator of relapse to drug-seeking behavior [1,12,21,27,33]. The use of nonselective dopamine receptor agonists and antagonists in the treatment of addiction, however, has been met with very limited clinical success [22,31] and may be associated with debilitating side effects [8,32]. Recent investigation of drugs that target DA has focused on agents that function as partial agonists. For example, DA D1 receptor partial agonists SKF 38393 and SKF 75670 were shown to reverse cocaine-conditioned place preference and the behavioral stimulant effects of cocaine in mice and rodents [10,29]. In cocaine-trained animals, DA D1 receptor agonists attenuated the priming effects of cocaine [25]. Moreover, selective stimulation of DA D2 receptors mediates cocaine- and heroin-seeking behavior in reinstatement paradigms [4,11,25]. The available data suggest that activation of D2-like receptors is involved in heroin reinstatement [3] and the D2-like receptor antagonist risperidone attenuates cue-induced reinstatement of heroin seeking [13]. Importantly, several studies have indicated that DA partial agonists may be devoid of abuse liability and do not produce extrapyramidal side effects [23]. In fact, effective pharmacotherapy may require the use of drugs that...
target multiple DA receptor subtypes with differing potencies and/or intrinsic efficacies.

L-Stepholidine (L-SPD) was isolated from the Chinese herb Stephania intermedia and characterized as exerting dual action on brain DA receptors; L-SPD acts as a partial D1 receptor agonist and as a full D2 receptor antagonist [9,35,40]. Preliminary clinical trials and animal experiments suggest that L-SPD not only improves both the positive and negative symptoms of schizophrenia without producing significant extrapyramidal side effects but also alleviates functional cognitive impairment [2]. Several recent studies have shown that L-SPD blocks morphine-induced CPP [14,30] and inhibits amphetamine-induced DA neuron firing in the VTA [7]. This is also in agreement with an earlier study, which reported that L-THP, an analog of L-SPD, inhibited the drug-induced reinstatement of cocaine-seeking [16,17], the oxycodone-induced CPP [14] and heroin-induced reinstatement of heroin-seeking [36]. This phenomenon has also been reported for other THPBs [15]. Recently, our lab found that doses of 2.5, 5 and 10 mg/kg of L-SPD significantly decreased the number of active nose pokes under an fixed-ratio 1 schedule of heroin self-administration and reduced cue-induced reinstatement of heroin-seeking behavior without affecting locomotor activity in rats (in press). However, the effects of L-SPD on heroin-primed reinstatement of heroin seeking in animals have not been investigated.

Therefore, in the present study, we used an animal model of heroin-induced reinstatement of drug-seeking behavior to study whether L-SPD alters heroin-seeking behavior.

2. Materials and methods

2.1. Animals

The subjects were Male Sprague-Dawley rats (270–300 g, purchased from the Animal Center of the Tongji Medical College of Huazhong University of Science & Technology, Wuhan, China) that were housed individually in home cages in a temperature-controlled ventilated colony room with a reversed 12-h dark/light cycle (lights onset 21:00 h, offset 09:00 h). Food and water were freely available except when specified. All experimental procedures followed the guidelines of the Principles of Laboratory Animal Care (National Institutes of Health publication number 86–23, 1996).

2.2. Drugs

Diacetylmorphine HCl (heroin) was obtained from the Hubei Public Security Bureau and was dissolved in 0.9% NaCl. L-SPD was acquired from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). The L-SPD was 99.97% pure, as determined by HPLC. L-SPD was dissolved in 0.1 M H2SO4 and then diluted and adjusted to a pH of 5.0 with 0.1 M NaOH [7].

2.3. Surgery

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Atropine sulfate and penicillin B were given at the time of surgery. All rats were implanted with chronically indwelling i.v. catheters. A silicon catheter (3.5 cm in length, 0.58 mm in inner diameter, 0.91 mm in outer diameter; BPU-T30, Instech, Plymouth Meeting, PA, USA) was inserted into the right external jugular vein so that the tip reached the right atrium and was secured with thread. The other end of the catheter (10 cm, PE20) was passed s.c. through an incision on the back of the body, where it exited into a custom-made fluid connector fixed to a jacket. The catheters were flushed daily with 0.2 ml saline–heparin solution (25 U/ml heparin) to maintain catheter patency. Following catheter surgery, each rat was housed individually in its home cage and was allowed five to seven days of recovery during which it received a daily intravenous infusion of gentamicin (0.16 mg/kg) followed by 0.2 ml of a heparinized (1%) sterile saline solution to flush the antibiotic through the catheter.

2.4. Heroin self-administration

Forty-two rats were trained to self-administer heroin during daily 3 h sessions under a fixed ratio 1 schedule of reinforcement as previously described [37,38]. Rats received a single heroin infusion (0.05 mg/kg) following an active nose-poke. Each infusion was paired with a 5 s illumination of a light in combination with the noise of the infusion pump; together these stimuli served as a discrete conditioned cue paired with the drug infusion. Following infusions, a time-out period was imposed for 20 s, during which responding was recorded but produced no programmed consequences. Rats were put back into their individual home cages shortly after the session. An acquisition criterion required that subjects’ active nose-pokes vary by $\leq10\%$ over the course of three consecutive maintenance days. Rats not meeting the acquisition criterion were excluded from the experiment ($n=10$).

2.5. Extinction

After stable responding for i.v. heroin was established, rats underwent extinction training for 2 h daily without any lights and drugs for 12 consecutive days in the operant chamber. During the extinction sessions, responses on the active nose poke were recorded but had no program consequence. Extinction criterion was that subjects touch the active nose-poke <10% of the average responding on the active nose-poke during maintenance.

2.6. Heroin-induced reinstatement

After meeting this extinction criterion, the rats ($n=8$ in each group) were injected with L-SPD (2.5, 5.0 or 10.0 mg/kg) or vehicle 30 min prior to a heroin-induced reinstatement session in which all rats were injected with heroin (0.25 mg/kg s.c.) and then placed into the operant chamber for 2 h, during which time the nose pokes had no programmed consequences.

2.7. Locomotor test

Immediately after the heroin-induced reinstatement test, the rats were subjected to a locomotor activity test according to the method described previously [24]. The rats were tested for their locomotor responses using an automated photocell system (Animal Software & Instruments Co., Ltd., China) consisting of eight identical black Plexiglas chambers (43 x 43 x 35 cm) in light- and sound-controlled cubicles. Each chamber was equipped with a video camera on the top, which was interfaced with a computer to record the movement of the rats in the chambers. Rats ($n=8$ in each group) were then injected with L-SPD (0.0, 2.5, 5.0 or 10.0 mg/kg i.p.). Horizontal locomotor activities traveled were recorded for 2 h. Total distance traveled was recorded and analyzed as the measure of locomotion using MED Associates SOF-811 open-field activity software.

2.8. Statistical analysis

The data were expressed as the mean ± SEM. The differences in total active responses, inactive responses and locomotor activity were analyzed by one-way analyses of variance (ANOVA) followed