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(-)-Stepholidine reduces cue-induced reinstatement of cocaine seeking and cocaine self-administration in rats

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ABSTRACT

Background: Dopamine receptors are implicated in cocaine reward and seeking. We hypothesize that (-)-stepholidine, a dopamine D1/D2/D3 multi-receptor agent, would be effective in reducing cocaine reward and seeking in an animal model. We investigated the effects of (-)-stepholidine in cue-induced reinstatement of cocaine seeking and cocaine self-administration (reward).

Methods: Cue-induced reinstatement experiment: Rats were trained to press a lever reinforced by cocaine (1 mg/kg/injection) for 15 consecutive daily sessions, after which the response was extinguished by withholding cocaine and cocaine-paired cues (light and pump activation). This was followed by a cue-induced reinstatement test where subjects were exposed to two cocaine cue presentations and presses on the active lever produced cues. Subjects were treated with one of four (-)-stepholidine doses prior to the reinstatement test. Cocaine self-administration (reward) experiment: Rats were trained to self-administer cocaine under a progressive ratio schedule of reinforcement. After stable breakpoints were established, rats were injected with four doses of (-)-stepholidine prior to testing; each dose was injected prior to a separate test session with no-treatment sessions intervening to re-establish break points.

Results: (-)-Stepholidine significantly reduced cue-induced reinstatement of cocaine seeking in a dose-related manner. Additionally, (-)-stepholidine significantly reduced break points for cocaine reward. (-)-Stepholidine did not significantly affect locomotor activity.

Conclusions: (-)-Stepholidine reduces cue-induced reinstatement of cocaine seeking and cocaine reward, suggesting that it may be useful in treating relapse in cocaine addiction.

1. Introduction

Drug use is a prevalent problem in the United States; in 2016 roughly 1.9 million people aged 12 or older were current users of cocaine (Center for Behavioral Health Statistics and Quality, 2017). Cocaine addiction is classified as a maladaptive cycle of use (often binge-like), abstinence and relapse. A major obstacle in the treatment of cocaine addiction is the prevention of relapse, itself caused by craving often induced by cocaine-associated cues (Childress et al., 1988; Tiffany, 1990; O'Brien et al., 1998).

Cocaine produces its rewarding effects through its capacity to increase dopamine neurotransmission in the mesolimbic system. It is thought that this mechanism plays a critical role in its abuse liability. Furthermore, cocaine associated cues, which instigate feelings of craving and increase the incentive motivation for drug, similarly increase dopamine neurotransmission in the mesolimbic system (Gratton and Wise, 1994; Kiyatkin, 1993). The increased dopamine transmission enhances stimulation of dopamine receptors in terminal regions that contribute to craving. Hence, from a pharmacological approach, effective cocaine addiction treatment could target mesolimbic dopamine receptors, disrupt cue-induced stimulation of these receptors and reduce cue-induced relapse.

Focus on individual dopamine receptor targets in treatment of cocaine addiction has yielded a range of results. Selective D1 receptor antagonists, although successful in reducing cue- and context-induced reinstatement of cocaine seeking (Alleweireldt et al., 2002; Crombag

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et al., 2002), have a limited therapeutic application in humans as their repeated administration can increase blood pressure and produce sedation, anhedonia and motor incapacitation (Haney et al., 2001). Focus on the D3 receptor has suggested its potential as a target in reducing cocaine reward: under low fixed ratio schedules (Gál and Gyertyán, 2003; Song et al., 2012) and under a progressive ratio (PR) schedule of reinforcement, D3 receptor antagonists have been shown to reduce cocaine self-administration (Galaj et al., 2014). Selective D3 antagonists have likewise been found to reduce cue-induced reinstatement of cocaine seeking (Gilbert et al., 2005; Galaj et al., 2014; Song et al., 2014). However, in early tests, currently available D3 antagonists have been shown to raise blood pressure (Appel et al., 2015) and potentiate the hypertensive effects of cocaine (Asico et al., 1998; Luippold et al., 2001). Taken together, these results show the utility of individual dopamine receptor therapy in their ability to reduce incentive motivational states integral to drug craving/seeking and to reduce drug reward, but also the limitations of currently available compounds. Thus, it is necessary to continue to search for dopamine receptor targeting compounds that might prove effective. One possible direction is toward compounds that can target multiple dopamine receptors.

Dopamine D1 and D3 receptors are co-localized to the same medium spiny neurons within the nucleus accumbens (Le Moine and Bloch, 1996; Ridray et al., 1998; Schwartz et al., 1998), a dopamine terminal region strongly implicated in cocaine reward and addiction-related behaviors. Interestingly, the anatomical and biochemical interplay of D1 and D3 receptors can result in the formation of complex heteromers producing functionally interactive states of these receptors (Fiorentini et al., 2008; Marcellino et al., 2008; Perreault et al., 2014). It might prove an interesting line of research to investigate compounds that can target these heteromers. We found that co-treatment with a D1 receptor partial agonist and D3 receptor antagonist produced effects on cocaine seeking and reward that far exceeded the therapeutic benefits of the individual compounds without reducing natural rewards or motoric ability (Galaj et al., 2016). These results suggest that compounds acting as both a D1 receptor partial agonist and a D3 receptor antagonist may hold potential as cocaine addiction treatments.

Tetrahydroprotoberberine (THPB) alkaloids adhere to a D1/D2/D3 multi-receptor pharmacological profile. They fall into two main subgroups that enact either similar action (e.g., tetrahydropalmatine, a D1/D2/D3 antagonist) or opposite action (e.g., isocorypalmine, a D1 partial agonist and D2/D3 antagonist). Tetrahydropalmatine and isocorypalmine have displayed efficacy in attenuating cocaine reward and reinstatement in cocaine addiction models (Mantsch et al., 2007; Xi et al., 2007). (-)-Stepholidine is a member of the THPB family that maintains similar dopamine receptor polypharmacology; its role in cocaine addiction constitutes the focus of this study.

(-)-Stepholidine is a naturally occurring compound in the Chinese herb *Stephania intermedia* that displays affinity for dopamine D1, D2 and D3 receptors. While (-)-stepholidine has been consistently reported to exhibit antagonist activity at D2 and D3 receptors in vitro, intrinsic activity at the D1 receptor appears to be more controversial; for example, being reported as a D1 partial agonist (Mo et al., 2007), D1 full agonist (Hicks et al., 2018) and D1 antagonist (Meade et al., 2015). Differences in the assay conditions (e.g., receptor reserve/level) and the transfected cells used may account for these differences in reported D1 intrinsic activity (Hicks et al., 2018). (-)-Stepholidine has been found to inhibit heroin-induced reinstatement (Ma et al., 2014), attenuate heroin self-administration and reduce cue-induced reinstatement of heroin seeking (Yue et al., 2014) with minimal effects on motoric activity. The effect of (-)-stepholidine on cocaine self-administration and reinstatement has not yet been investigated. In the present study we tested whether (-)-stepholidine would reduce cocaine self-administration/reward and cue-induced reinstatement of a cocaine seeking response.

2. Materials and methods

2.1. Subjects

The housing conditions and care of the animals were consistent with specifications in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 2011). All experiments were approved by the Queens College Institutional Animal Care and Use Committee.

Subjects consisted of male Long Evans rats, weighing 350–450 g at the start of the experiments, obtained from our in-house colony; male and female breeders were purchased from Charles River Laboratories (Kingston, NY, US). All animals were individually housed in a temperature-controlled environment (70 °F) and had free access to food and water under a 12 h light/dark cycle. All experiments were conducted during the animals' active periods (dark cycle).

2.2. Surgery

Surgeries were conducted under atropine sulfate [0.54 μg/0.1 mL of distilled water, intraperitoneal (IP) injection] and sodium pentobarbital (65 mg/kg, IP) anesthesia. Surgical sites were cleaned and pruned of fur. An incision (2 cm) was made ventral to the right mandible. The jugular vein was isolated, cleaned and opened with a vessel dilator, allowing for insertion of the silastic catheter (Dow Corning, Midland, MI) to a position just short of the right atrium. The inserted catheter was secured to the vein with surgical sutures and the distal portion passed subcutaneously to the back of the neck and exited through an incision made on the scalp. A bent 22-gauge stainless-steel cannula (25 mm, 90° angle with plastic connector piece attached) was inserted into the exposed tip of the catheter such that 10 mm of the cannula was sheathed. Five stainless-steel screws were implanted in the skull around the cannula, secured in place by an arm of the stereotaxic apparatus, and submerged in dental acrylic until only the distal half of the plastic connector piece and tip of the cannula were exposed (attachment site for drug line). The rats were given an intravenous (IV) injection of Gentamicin (4.0 mg/kg/0.05 mL) after surgery and daily thereafter for one week, receiving an IV injection of heparin saline solution (200 U/mL) after surgery and daily thereafter. Animals were given 3 days to recover after surgery before initiating self-administration training.

2.3. Apparatus

2.3.1. Self-administration chambers

Intravenous self-administration chambers were equipped with two retractable levers (3.5 × 2.0 cm, 8 cm apart) with corresponding lights (2-W bulb, 10 cm above each lever). Chambers consisted of a transparent plastic door and opposing wall, left and right aluminum walls and a transparent plastic ceiling with a centered hole (3 cm diameter). The floors were made of aluminum rods, spaced 1.0 cm apart. Levers were embedded in the right aluminum wall (26 × 26 cm) and required a force of 0.09 N to register activity. Each chamber was encased within a sound-attenuating box equipped with a house light, ventilating fan and infusion pump (Razel, 3.33 rpm) that held a 20-mL syringe.

2.3.2. Food operant conditioning chambers

Food operant chambers were equipped with two levers (2.5 cm x 2.5 cm, 11.4 cm apart) with corresponding lights (2-W bulb, 5.0 cm above each lever). Chambers (20.3 cm x 30.5 cm x 20.3 cm) consisted of a transparent plastic door and opposing wall, left and right aluminum walls, and a transparent plastic ceiling. The floors were made of aluminum rods, spaced 2.0 cm apart. Levers were embedded in the right aluminum wall and connected to a food dispenser that dropped one food pellet into a square food dish (5.0 cm x 5.0 cm) centered between the two levers. Each chamber was encased within a sound-attenuating box (36.8 cm x 56.0 cm x 33.0 cm) equipped with a