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D-serine facilitates the effectiveness of extinction to reduce drug-primed reinstatement of cocaine-induced conditioned place preference

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ABSTRACT

Addiction is a disease that is characterized by compulsive drug-seeking and use despite negative health and social consequences. One obstacle in treating addiction is a high susceptibility for relapse which persists despite prolonged periods of abstinence. Relapse can be triggered by drug predictive stimuli such as environmental context and drug associated cues, as well as the addictive drug itself. The conditioned place preference (CPP) behavioral model is a useful paradigm for studying the ability of these drug predictive stimuli to reinstate drug-seeking behavior. The present study investigated the dose-dependent effects of D-serine (10 mg/kg, 30 mg/kg and 100 mg/kg) on extinction training and drug-primed reinstatement in cocaine-conditioned rats. In the first experiment, D-serine had no effect on the acquisition or development of cocaine-induced locomotor sensitization or CPP. In the second experiment, D-serine treatment resulted in significantly decreased time spent in the drug-paired compartment following completion of an extinction protocol. A cocaine-primed reinstatement test indicated that the combination of extinction training along with D-serine treatment resulted in a significant reduction of drug-seeking behavior. The third experiment assessed D-serine's long-term effects to diminish drug-primed reinstatement. D-serine treatment given during extinction was effective in reducing drug-seeking for more than four weeks of abstinence after the last cocaine exposure. These findings demonstrate that D-serine may be an effective adjunct therapeutic agent along with cognitive behavioral therapy for the treatment of cocaine addiction.

This article is part of a Special Issue entitled 'Cognitive Enhancers'.

1. Introduction

Addiction can be defined as a psychological disease that is characterized by uncontrollable, compulsive drug seeking and drug use despite negative health and social consequences (Baler and Volkow, 2006). One obstacle for the treatment of addiction is the susceptibility to relapse which can persist several years despite prolonged periods of abstinence (O'Brien, 2003). The use of preclinical animal models such as self-administration, behavioral sensitization and conditioned place preference (O'Brien and Gardner, 2005) has allowed the mechanisms that underlie the priming of reinstatement behavior to be explored. The reinstatement of drug-seeking has been observed in rats exposed to addictive substances such as psychostimulants, nicotine, ethanol and opioids, and may be triggered by drug predictive stimuli such as environmental context, stress, drug-associated cues, as well as the addictive drug itself (Shaham and Miczek, 2003).

In the treatment of anxiety disorders, exposure therapy has been shown to be an effective treatment for reducing the frequency and intensity of episodes (Otto et al., 2004). The N-Methyl-D-aspartate (NMDA) receptor has been implicated as being involved in extinction learning (Falls et al., 1992), and several conditioned fear studies illustrate that antagonism of NMDA receptors during extinction impairs the effects of such training (Myers and Carlezon, 2010). In a complementary manner, enhancement of NMDA receptor activity with D-cycloserine, a partial agonist at the glycine site of the NMDA receptor, facilitates fear extinction (Walker et al., 2002). The translational success of this line of investigation from an understanding of preclinical mechanisms in animals to promising clinical results in humans has prompted a strong interest in using a similar rationale for the treatment of addiction, but the

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effectiveness of exposure therapy in this context has been unclear (Conklin and Tiffany, 2002).

Using a cocaine self-administration model, we have previously described a requirement for NMDA receptor activity during extinction training to reduce subsequent drug-primed reinstatement (Kelamangalath et al., 2007). In addition, we have examined the actions of D-serine, a full agonist at the glycine modulatory site of the NMDA receptor and its effects on cocaine-primed reinstatement. By employing sub-optimal extinction protocols in rats allowed either limited access (Kelamangalath et al., 2009) or extended access (Kelamangalath and Wagner, 2010) to cocaine self-administration, the enhancing effects of D-serine treatment during or immediately following extinction training resulted in reduced drug-primed reinstatement. This only occurred when D-serine is given in conjunction with extinction training; a finding also reported using D-cycloserine (Dhonnchadha et al., 2010).

D-cycloserine is effective in facilitating cocaine-induced conditioned place preference (CPP) in both rats and mice (Botreau and Stewart, 2006; Thanos et al., 2009). As is the case for self-administration, CPP behavior can be extinguished and reinstated following drug-priming, stress, or conditioned cues (Tzschentke, 2007). A significant feature of the CPP protocol is the practical advantage of being able to test relatively large numbers of animals that allow dose–response studies to be efficiently conducted. The present study was designed to investigate the dose-dependent effects of D-serine (10 mg/kg, 30 mg/kg and 100 mg/kg) on extinction and drug-primed reinstatement in cocaine-conditioned rats. When combined with extinction training, D-serine was effective in facilitating extinction and in reducing cocaine-primed reinstatement; an effect that persisted for more than 4 weeks. These results suggest that D-serine is a promising adjunct treatment to be combined with exposure therapy for the treatment of cocaine addiction.

2. Materials and methods

2.1. Drugs

D-serine was purchased from Sigma (St. Louis, MO, USA). Cocaine hydrochloride was obtained from NIDA (RTI International, NC, USA).

2.2. Animal maintenance

Sprague–Dawley male rats (Harlan, Indianapolis, IN, USA) were housed in pairs in clear plastic cages. They were maintained on a 12 h light/dark cycle (0700 h/1900 h) with food and water available ad libitum. Animals were allowed to acclimate to their home cages for one week and were habituated to handling for 3 days before behavioral testing began. Sessions were conducted daily between 0900 h and 1500 h. These studies were approved by the University of Georgia Institutional Animal Care and Use Committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3. Apparatus

Gosnell (2005) gave a detailed account of the chambers and its dimensions. Behavioral testing was carried out in 43.2 × 43.2 cm chambers with clear plastic walls and a solid smooth floor (Med Associates, St. Albans, VT, USA). Each chamber was housed in a sound-attenuating box equipped with two house lights (20 lx) and a ventilation fan. Two banks of 16 infrared photo beams and detectors detected horizontal activity. Activity Monitor software (Med Associates) was used to count beam breaks.

Conditioned place preference (CPP) testing occurred in a two compartment insert (Med Associates) that modified the open field chamber. The modified chamber was divided into two identical compartments (42.8 × 21.3 cm) separated by a black partition containing a guillotine door. When the guillotine door was removed, the rat had access to the entire chamber and was put in place to confine the animal's activity to one compartment during conditioning. The compartments differed by floor type (grid, wire mesh vs. rod, steel bars) and by ceiling color (transparent vs. black). The compartment with the rod-floor had the black ceiling which darkened that side of the chamber insert; preliminary studies indicated that this arrangement yielded an equal preference between compartments.

2.4. Experimental design

2.4.1. Experiment 1

Seymour and Wagner (2008) describe in detail the experimental design of our first set of experiments. Rats were tested in a Pretest session (protocol day 1) where each animal was placed in the light/grid compartment and allowed free access to both compartments of the CPP chamber for 15 min (Fig. 1A). The time spent in each compartment was analyzed to assure that none of the animals had a strong bias toward either side of the compartment. Any rat spending >65% of its time in either compartment was excluded from this unbiased CPP study. The following day the place preference inserts were removed and rats were placed in the center of the open field chamber for 30 min to monitor baseline activity (Activity, protocol day 2). An i.p. injection of either 0.9% saline ($n = 45$) or cocaine (10 mg/kg, $n = 34$) was given and the animal was placed back in the open field where activity was monitored for an additional 60 min.

Conditioning sessions began the next day (protocol days 3–6). Saline-paired and cocaine-paired animals were given either an i.p. injection of saline or 100 mg/kg of D-serine 1–2 h prior to conditioning sessions, in the home cage. In all, four groups were tested saline/saline ($n = 29$), saline/cocaine ($n = 24$), D-serine/saline ($n = 16$), and D-serine/cocaine ($n = 10$). Rats were placed in one of the two compartments for 15 min and then returned to their home cage. Four hours later, animals were injected with either saline or cocaine and confined to the opposite compartment for the second daily conditioning session. Following the completion of conditioning, a second drug-free place preference test was administered (Post Test, protocol day 7). One week later, the open field Challenge test (protocol day 13) was conducted in the same manner as the Activity test to assess sensitization.

2.4.2. Experiment 2

The protocol for Experiment 2 (Fig. 1B) did not include open field assessments of locomotor activity. Following the CPP Pretest, cocaine conditioning proceeded with saline and cocaine (15 mg/kg) pairings as previously described, except no home cage pretreatments occurred. Following the CPP Post Test, a passive extinction protocol (days 7–10) was carried out in which groups received their respective treatments of saline ($n = 10$), 10 mg/kg D-serine ($n = 6$), 30 mg/kg D-serine ($n = 7$), or 100 mg/kg of D-serine ($n = 14$) immediately before being confined to their former cocaine-paired compartment. As with during the conditioning phase, animals were also confined to the opposite compartment and given the vehicle treatment (saline) immediately prior to the second extinction session. Following the end of extinction training, a third CPP test was conducted (Extinction Test, protocol Day 11). Animals were placed in their saline-paired compartment and explored the CPP chamber for 15 min. The next day, all rats were given an i.p. injection of 10 mg/kg cocaine and again placed in their saline-paired compartment to explore the CPP chamber for 15 min (Reinstatement Test, protocol day 12).

2.4.3. Experiment 3

Experiment 3 followed the exact procedures of experiment 2 except some of the former rats were held abstinent in their home cage environment for an additional 30 days following the Reinstatement Test. On protocol day 43, a second reinstatement test (4 wk Reinstatement Test) was conducted. Once again, all animals received an i.p. injection of 10 mg/kg of cocaine immediately prior to being placed into the saline-paired compartment of the CPP chamber and allowed free access for 15 min. Two combined groups were analyzed in this experiment, a saline & 10 mg/kg group, $n = 11$; and a 30 & 100 mg/kg group, $n = 16$.

2.5. Statistical analysis

Statistics were done using SigmaStat software, version 3.1. One-way repeated measures (RM) ANOVAs were used to analyze time spent in the drug-paired compartment across multiple CPP tests and one-way ANOVAs were used to analyze between groups for the shift in preference, locomotor activity, and locomotor sensitization results. Post-hoc analysis used in all cases was Holm–Sidak.

3. Results

3.1. Experiment 1 (Fig. 1A) the effects of pretreatment with systemic D-serine on locomotor activity and the development of conditioned place preference or locomotor sensitization

3.1.1. D-serine pretreatment does not affect the response to a novel environment or cocaine

D-serine has undergone substantial testing as an antipsychotic agent in human trials (Tsai et al., 1998) and investigated for activity in several preclinical rodent models of schizophrenia (reviewed by Labrie et al. (2012)). However in some respects, relatively little characterization of the behavioral effects of D-serine has been reported in preclinical studies. For example, the measurement of