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Positive allosteric modulation of mGluR5 receptors facilitates extinction of a cocaine contextual memory

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Abstract

Background—The perseverance of the motivational salience of drug-associated memories is an obstacle to the successful treatment of drug addiction, and is often a causative factor in triggering relapse.

Methods—The present study sought to determine if potentiation of type 5 metabotropic glutamate receptors (mGluR5), which are biochemically and structurally coupled to N-methyl-D-aspartate (NMDA) receptors, would facilitate the extinction of a cocaine-associated contextual memory as assessed by the conditioned place preference (CPP) paradigm in rats. Following the establishment of a cocaine CPP, rats were treated with the mGluR5 positive allosteric modulator 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB; 0.3, 3 and 30 mg/kg) prior to extinction test sessions. Additional groups of animals received 30 mg/kg CDPPB in combination with the mGluR5 antagonist 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine (MTEP, 1 mg/kg) or the NMDA receptor antagonist MK-801 (0.1 mg/kg).

Results—CDPPB dose-dependently facilitated the extinction of cocaine CPP, and these effects were not observed when animals were co-administered MTEP or MK-801. CDPPB failed to produce any evidence of neurotoxicity as assessed by FluoroJade C staining.

Conclusions—Positive allosteric modulation of mGluR5 function facilitates the extinction of a cocaine-associated contextual memory, which may represent a novel approach towards enhancing extinction learning in the context of drug addiction.

Keywords

cocaine; conditioned place preference; contextual memory; extinction learning; mGluR5

INTRODUCTION

The neural substrates of drug addiction have considerable overlap with those that underlie normal learning and memory processes (1,2). During the course of addiction, associations between drugs and specific environmental contexts becomes overly salient, and these drug-environment associations often lead to drug craving and relapse (3), and attempts at extinguishing the salience of drug conditioned cues by exposure therapy have been met with limited success primarily due to the context specificity of extinction (4,5). Since extinction is

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FINANCIAL DISCLOSURES

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an active learning process, it involves many of the neurobiological substrates that subserve normal learning and memory, including increased transmission and plasticity at central glutamatergic synapses. Pharmacological enhancement of glutamate transmission, for example with the N-methyl-D-aspartate (NMDA) receptor partial agonist D-cycloserine, facilitates the extinction of conditioned fear as well as drug-associated memories (6,7). Since type 5 metabotropic glutamate receptors (mGluR5) are structurally and biochemically linked to NMDA receptors and their activation increases NMDA receptor activity (8), we sought to determine if potentiation of mGluR5 receptor function by the systemically active mGluR5 positive allosteric modulator 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) (9) would facilitate extinction of a cocaine contextual memory as measured by the conditioned place preference (CPP) paradigm.

METHODS AND MATERIALS

Animals

All procedures were conducted with the approval of an Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (200–275 g, Harlan, Indianapolis, IN) were maintained on a 12 hour light-dark cycle (lights off at 7:00 am), and all experimentation was conducted during the dark phase. Rats were given ad libitum access to food and water throughout all procedures except during behavioral testing.

Apparatus

The CPP apparatus (Med Associates, St. Albans, VT) consisted of two adjacent conditioning compartments (20 × 16 × 21 cm) separated by a manual guillotine-type door. One of the conditioning compartments was equipped with vertical striped acrylic walls and a steel mesh floor, and the other was equipped with plain acrylic walls and a wire rod floor. Infrared photobeams monitored the animal's position in the apparatus and provided a measure of motor activity.

CPP Procedures

Animals were initially habituated to the testing apparatus during a single 20 min session with free access to both conditioning compartments. Animals were then subjected to a 20-min pre-conditioning test (Pre) in order to determine any initial preference for one of the conditioning compartments. Each animal was then assigned to receive cocaine (10 mg/kg) in the initially non-preferred compartment, and saline in the initially preferred compartment. Next, animals underwent place conditioning in twice-daily 20 min conditioning sessions. Saline conditioning sessions were conducted in the morning and cocaine conditioning sessions were conducted in the afternoon. Following 4 sequential conditioning days, animals were tested for place preference by allowing free access to both conditioning compartments for 20 min (Post). Next, animals underwent extinction testing, whereby they were administered vehicle or CDPPB (0.3, 3 or 30 mg/kg) 20 min prior to being placed in the CPP apparatus and allowed free access to both conditioning compartments for 20 min. Additional groups of animals received CDPPB in combination with the mGluR5 antagonist MTEP (1 mg/kg) or the NMDA receptor antagonist MK-801 (0.1 mg/kg), which were administered 10 min prior to CDPPB. Extinction tests were conducted daily for 5 consecutive days (i.e., E1, E2, E3, etc.).

Assessment of neurotoxicity

Since stimulation of mGluR5 receptors enhances NMDA receptor function, and excessive NMDA receptor activation can lead to neurotoxicity, we assessed potential effects of repeated administration of CDPPB on neurotoxicity and neurodegeneration using FluoroJade C staining (10). Rats were treated daily with CDPPB at a dose of 30 mg/kg for 5 consecutive days to

mimic the treatment regimen during extinction. As a positive control, separate animals received a single administration of kainic acid (10 mg/kg). One hr following the final administration of CDPPB or 2 days following kainic acid administration, rats were anesthetized and perfused transcardially with saline followed by 4% w/v paraformaldehyde. Following post-fixation, coronal 25 μ m brain sections through the ventromedial prefrontal cortex and CA3 region of the hippocampus were cut on a cryostat and mounted onto gelatin coated slides. Sections were stained with FluoroJade C (Chemicon, Temecula, CA) as described elsewhere (10). Sections were viewed under epifluorescence microscopy at 488 nm excitation using a Leica DMLB microscope equipped with a digital camera.

Drugs

3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) was custom synthesized by IQsynthesis (St. Louis, MO) according to (9). CDPPB was suspended in a vehicle consisting of 20% w/v 2-hydroxypropyl- β -cyclodextrin (Sigma-Aldrich, St. Louis, MO). 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride (MTEP, Alexis Biochemicals, San Diego, CA) was dissolved in 20% w/v 2-hydroxypropyl- β -cyclodextrin. MK-801 (Tocris Biosciences, Ellisville, MO), kainic acid (Tocris), and cocaine hydrochloride (Sigma-Aldrich) were dissolved in sterile 0.9% saline. CDPPB and its corresponding vehicle were administered via the subcutaneous route, while all other compounds were given via the intraperitoneal route. Injections were given in a volume of 1 ml/kg.

Data Analysis

Time spent in each conditioning compartment on each test day was converted to percent time spent in the initial non-preferred compartment as determined in the Pre test for each animal. CPP and locomotor data were analyzed by a mixed multivariate analysis of variance. Effects of treatment group were assessed using the Wilks' Lambda test statistic, and within-subjects effects across test sessions were assessed by repeated measures analysis of variance. For CPP data, multiple pairwise comparisons were performed for each treatment group at each extinction test session against Pre values. For locomotor data, multiple pairwise comparisons were performed for each treatment group at each extinction test against vehicle-treated animals during the same test session. All multiple pairwise comparisons utilized Sidak adjustments. Cocaine CPP was considered to be extinguished when the mean percent time spent in the non-preferred compartment for a particular treatment group during an extinction test was not statistically different from that observed during the Pre test. Data are presented as mean $\pm$ SEM, and the significance level was set at $p < 0.05$.

RESULTS

Conditioning with cocaine (10 mg/kg) produced a significant place preference in all groups, as evidenced by a significant increase in the percent time spent in the initial non-preferred side (Figures 1A and 1C). Cocaine CPP was observed to extinguished during the extinction test sessions as follows: vehicle (E5), 0.3 mg/kg CDPPB (E4), 3 mg/kg CDPPB (E1), 30 mg/kg CDPPB (E2), 30 mg/kg CDPPB + 1 mg/kg MTEP (E5), 30 mg/kg CDPPB + 0.1 mg/kg MK-801 (CPP was not extinguished after 5 days of extinction testing). Analysis of locomotor data (Figure 1B and 1D) revealed that only animals pre-treated with MK-801 exhibited changes in activity, which was increased on extinction test sessions E1-E5.

The effects of repeated administration of the 30 mg/kg dose of CDPPB on neuronal degeneration, as assessed by FluoroJade C staining, are shown in Figure 2. Neurotoxic effects of CDPPB were not observed in the ventromedial prefrontal cortex, CA3 region of the hippocampus, or other brain regions examined (not shown), as evidenced by a lack of

fluorescent staining in these regions. In contrast, fluorescent staining was observed in these regions in positive control animals treated with kainic acid.

DISCUSSION

We found that the systemically active mGluR5 positive allosteric modulator CDPPB dose-dependently facilitated the extinction of a cocaine contextual memory, as evidenced by fewer extinction test sessions required to reach extinction criteria. This effect appeared to be mediated by both mGluR5 and NMDA receptors, since the effect of the highest dose of CDPPB tested was reversed by co-administration of MTEP and MK-801, respectively. CDPPB did not influence locomotor activity, nor did it exhibit neurotoxic effects, as animals treated with this compound showed a lack of fluorescent staining with the neurodegeneration marker FluoroJade C (10). Thus, positive allosteric modulation of mGluR5 receptors may be a novel mechanism by which to facilitate the extinction of drug-associated contextual memories.

CDPPB potentiates the function of mGluR5 by 4- to 5-fold in the presence of glutamate by acting at an allosteric binding site separate from the orthosteric glutamate binding site (9,11). This enhancement of mGluR5 function indirectly increases NMDA receptor function (8), which may lead to increased synaptic plasticity and facilitated extinction learning, as has been previously shown with the NMDA partial agonist D-cycloserine (6,7). However, the possibility exists that NMDA receptor antagonism influenced extinction independently of the actions of CDPPB, since animals treated with CDPPB plus MK-801 showed slower rates of extinction as compared to vehicle-treated animals. Other alternative interpretations of the present data are that CDPPB inhibits the expression of cocaine CPP and/or disrupts the reconsolidation of drug-associated memories due to destabilization of contextual memory traces (12,13). However, given that mGluR5 positive allosteric modulators have pro-cognitive effects (14), enhance synaptic plasticity in the hippocampus (15), and increase performance in spatial learning tasks such as the Morris water maze (16), it is likely that CDPPB facilitates extinction of a cocaine CPP by enhancing extinction learning. The potential neuroanatomical regions that mediate this phenomenon need to be further investigated, and likely include the infralimbic prefrontal cortex (17,18), the basolateral amygdala (19), and the hippocampus (20), which mediate extinction learning, stimulus-reward learning, and drug-context associations, respectively.

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Figure 1.

Effects of the mGluR5 positive allosteric modulator CDPPB on the extinction of cocaine CPP and on locomotor activity. Data are presented as mean $\pm$ SEM. (A) CDPPB dose-dependently facilitated extinction of cocaine CPP, as evidenced by fewer extinction test sessions required to reach extinction criteria. A significant interaction between treatment group and test session [$F(30,458) = 1.70, p < .05$] was observed. Sample sizes are $n=21$ for vehicle, 0.3 and 3.0 mg/kg CDPPB, and $n=20$ for 30 mg/kg CDPPB. * denotes $p < 0.05$ vs. pre-test. (B) CDPPB had no significant effects on locomotor activity during extinction of CPP ($p > .5$). (C) The effects of 30 mg/kg CDPPB were reversed by pre-treatment with the mGluR5 antagonist MTEP or the NMDA antagonist MK-801. Sample sizes are $n=21$ for vehicle, $n=22$ for 30 mg/kg CDPPB + 1 mg/kg MTEP, and $n=21$ for 30 mg/kg CDPPB + 0.1 mg/kg MK-801. The vehicle-treated group in panels A and C are the same but are separated for visual clarity purposes. * denotes $p < .05$ vs. pre-test. (D) MK-801 increased locomotor activity during each of the five extinction trials, as evidenced by a significant interaction between treatment group and test session [$F(30,462) = 4.82, p < .001$]. * denotes $p < 0.05$ vs. vehicle-treated animals. Locomotor activity was not altered during extinction testing in MTEP pretreated animals. Pre, preconditioning test session; Post, post-conditioning test session; E1–E5, extinction test sessions 1–5.

Figure 2.

Lack of neurotoxic effects of repeated administration of 30 mg/kg CDPPB as assessed by FluoroJade C staining in the **(A)** ventromedial prefrontal cortex (CDPPB-vmPFC) and **(B)** CA3 region of the hippocampus (CDPPB-Hipp CA3). Animals treated with kainic acid (KA) as a positive control demonstrated significant neuronal degeneration as evidenced by FluoroJade C staining (arrows) in the **(C)** vmPFC (KA-vmPFC) and **(D)** CA3 region of the hippocampus (KA-Hipp CA3). Scale bar = 500 μ m.