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β -lactam antibiotic decreases acquisition of and motivation to respond for cocaine, but not sweet food, in C57Bl/6 mice

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Abstract

No medication is approved to treat cocaine addiction, but mounting evidence suggests glutamate-directed approaches may reduce cocaine dependence and relapse. We tested the hypotheses that the glutamate transporter subtype 1 (GLT-1) activator ceftriaxone disrupts acquisition of cocaine self-administration, motivation to self-administer cocaine, and conditioned place preference in mice. Repeated ceftriaxone (200 mg/kg) reduced the ability of mice to acquire cocaine and the motivation to self-administer cocaine following successful acquisition without affecting acquisition of or motivation for sweet food. Repeated ceftriaxone had no effect on cocaine-conditioned place preference. These results suggest that a β -lactam antibiotic reduces the direct reinforcing strength of cocaine without producing non-specific deficits in conditioned learning processes.

Keywords

cocaine; glutamate; GLT-1; ceftriaxone; self-administration; place preference; β -lactam; addiction; mouse

Introduction

Although the ability of cocaine to increase extracellular dopamine in the nucleus accumbens contributes to its primary reinforcing effects, glutamatergic involvement in cocaine reinforcement has been a more recent focus (Knackstedt and Kalivas, 2009). Evidence suggests pharmacological approaches targeting substrates within the glutamate system disrupts cocaine reinforcement and relapse (Schmidt and Pierce, 2010). One promising but poorly understood target is glutamate transporter subtype 1 (GLT-1), a predominantly astrocytic transporter that mediates about 90% of forebrain glutamate uptake (Robinson, 1998). Treatment with the β -lactam antibiotic ceftriaxone (CTX) increases GLT-1 activity and reduces extracellular glutamate levels in the nucleus accumbens of conscious rats (Rothstein et al., 2005, Rasmussen et al., 2011). CTX also attenuates the development of

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opioid tolerance and physical dependence in rats and abstinence-induced withdrawal from cocaine in planarians (Rawls et al., 2008). The present experiments tested whether repeated CTX administration altered acquisition of cocaine self-administration (SA) in drug-naïve male C57Bl/6 mice, or motivation to self-administer in mice that already acquired cocaine SA behavior. To determine the specificity of these effects, we examined CTX treatment on palatable food SA in a separate group. Lastly, we tested whether CTX altered the conditioned rewarding properties of cocaine using the cocaine conditioned place preference (CPP) paradigm.

Methods

Subjects

Experimentally naïve male C57Bl/6 mice (***SUPPLIER???) were used. All animal use procedures were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Temple University Institutional Animal Care and Use Committee.

Apparatus

Food and cocaine SA experiments were conducted in standard mouse operant conditioning chambers (Med Associates, Georgia, VT, USA) outfitted for liquid food or intravenous reinforcer delivery contingent upon nose-poke responding (Ward et al., 2009).

Procedure

Behavioral sessions were run in the morning and saline or CTX injections were administered in the evening.

Cocaine self-administration—To study the effect of CTX treatment on acquisition of cocaine SA, mice were implanted with chronic indwelling back-mounted jugular cannulae (Caine et al., 1999). The cannula lines were maintained by daily heparin treatment, and only animals with patent cannulae throughout the experiments were included in the final data analysis. After 2 days of recovery from surgery, treatment with CTX (100, 200 mg/kg, i.p.) or saline began each afternoon. Following 3 days of treatment, mice were allowed access to self-administer 0.56 mg/kg/inf cocaine under an fixed ratio (FR) 1 schedule during daily 2 h morning sessions for 10 days while continuing to receive daily afternoon CTX or saline injections. During drug availability, the fan and house light were on, and the active nose-poke hole was illuminated by the internal amber stimulus light. Initiation of drug delivery was signaled by the simultaneous illumination of the cue light above the nose-poke hole and onset of a 1 s tone. A 20-s timeout period was imposed following each nose-poke response in the active hole, during which the internal stimulus light was turned off. Responses in the inactive nose-poke hole were recorded but not reinforced.

To study the effect of CTX treatment on motivation to self-administer cocaine, a separate group of mice were implanted and trained to self-administer cocaine under a FR-1 schedule of reinforcement. Following FR-1 responding, mice were given access to 0.56 mg/kg/inf cocaine under a progressive ratio (PR) schedule of reinforcement. The following progression of response requirements for reinforcement within each session was used: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145 *etc.* (Richardson and Roberts, 1996). Break point was defined as the number of infusions obtained during the daily sessions, which were set to a maximum of 4 h, but were terminated once 20 min elapsed with no active-hole nose pokes. Stable responding under the PR schedule was defined as three days of baseline responding where the number of reinforcers earned differed by no more than three, with no upward or

downward trends. Once stable responding was achieved in non-treated mice, break points were re-determined following repeated saline and CTX (200 mg/kg, i.p.) treatment.

Place conditioning—To study the effect of CTX treatment on acquisition of palatable food SA and motivation to self-administer palatable food, two additional groups of mice were trained to respond for 50% vanilla-flavored Ensure under FR-1 and PR schedule of reinforcement during daily 1-h sessions. Treatment with CTX or saline was identical to that for the cocaine studies.

CPP chambers were comprised of two compartments divided by a removable partition. Mice were pretested during a single 30-min session with the partition removed, and then equally assigned to either a saline (saline/cocaine) or CTX (CTX/cocaine) treatment group. The pretest and pilot experiments demonstrated that the chambers did not produce side-bias. Mice were then treated for 5 days prior to CPP testing with saline or CTX (200 mg/kg, i.p.). Drug or saline treatment continued during daily 30 min conditioning session; in a biased design, cocaine (10, 20 mg/kg, i.p.) was administered with confinement in the non-preferred side while saline was administered on alternating days in the preferred side for a total of 6 conditioning trials. The day after the final pairing a 30-min test was performed. To test for the effect of CTX on the re-expression of CPP, the saline/cocaine groups were then administered either saline (saline/cocaine/saline) or CTX at 200 mg/kg (saline/cocaine/CTX) for 6 subsequent days followed by a 30 min retest. Preference scores were calculated for each mouse by subtracting their pre-conditioning score from their test or retest score.

Statistical analysis

Data were analyzed using one-way or two-way analysis of variance (ANOVA) (GraphPad Prism 4.0).

Results

Saline-treated mice readily acquired cocaine SA under an FR 1 schedule and this behavior was significantly affected by treatment with 200 but not 100 mg/kg CTX (Figure 1A). Two-way ANOVA show significant main effects of Treatment [$F_{(2,161)} = 61.87$, $p < 0.01$] and Time [$F_{(9,161)} = 3.71$, $p < 0.01$] and a significant interaction [$F_{(18,161)} = 8.49$, $p < 0.01$]. Bonferroni posttest revealed a significant effect of 200 mg/kg CTX on day 1 and again on days 7-10. Interestingly, CTX increased initial responding on day 1, followed by a decrease in responding on days 7-10. Conversely, 200 mg/kg CTX treatment had no significant effect on acquisition of 50% Ensure SA (Figure 1B). A separate group of saline-treated mice also self-administered cocaine under the PR schedule of reinforcement, and this effect was attenuated by treatment with 200 mg/kg CTX (Figure 1C). Repeated measures one-way ANOVA revealed a significant effect of treatment [$F_{(6,24)} = 13.54$, $p < 0.0001$], and Bonferroni posttest revealed that all CTX treatment days were significantly different from saline treatment ($p < 0.05$). As with acquisition, 200 mg/kg CTX treatment had no significant effect on motivation to self-administer 50% Ensure (Figure 1D). CTX treatment had no effect on non-reinforced responses in the inactive nose-poke hole on any SA procedure (data not shown).

In the CPP procedure, both 10 and 20 mg/kg cocaine induced a preference that was not significantly altered by CTX pretreatment (Figure 2A). In the saline-treated group, CPP induced by cocaine at 10 mg/kg was no longer present when tested again 7 days later, and this decline was not altered by the CTX treatment following the first test (data not shown). In contrast, cocaine CPP induced by cocaine at 20 mg/kg was increased on the retest one week later, and this increase was also not reversed following CTX administration (Figure 2B).

Discussion

The GLT-1 activator CTX attenuated acquisition of cocaine SA in a dose-dependent manner and reduced PR responding in cocaine-trained mice, thus supporting a role for glutamate in the motivation to self-administer cocaine (Paterson and Markou, 2005; Xi et al., 2010). GLT-1 upregulation by CTX treatment prevents reinstatement of cocaine seeking behavior (Sari et al., 2009; Knackstedt et al., 2010). Our results expand upon those findings by demonstrating that CTX treatment blocks actual cocaine intake under both FR1 and PR schedules of reinforcement. One interesting result from the cocaine SA acquisition study was the initial, dose-dependent increase in the number of reinforcers earned in the CTX-treated mice (Figure 1A). We interpret this finding as an initial burst in responding made by the mice in order to surmount the blunted reinforcing efficacy of cocaine produced by 3 days of CTX administration, similar to the pattern of SA that may be seen initially in rodents responding at peri-threshold cocaine doses. One reason may be that cocaine-induced increases in nucleus accumbens glutamate levels are only partially blocked by 3 days of CTX treatment as GLT-1-mediated glutamate uptake is beginning to increase (Smith et al., 1995; Knackstedt et al., 2010). As continued CTX treatment further enhances GLT-1 activity and more effectively inhibits cocaine-induced glutamate levels, subsequent responding across days decreases as the reinforcing efficacy of cocaine is blocked. It is unlikely that the initial burst in responding was related to analgesic properties of CTX because prior work has shown that CTX, while blocking the development of analgesic and hypothermic tolerance to opioids, lacks intrinsic antinociceptive efficacy (Rawls et al., 2010). Future experiments incorporating SA of a range of cocaine doses will lead to an even firmer interpretation of the present data, determining for example whether CTX produces a rightward and/or downward (or leftward) shift in the cocaine dose response curve.

The present data also suggest that the CTX effect is specific to the reinforcing properties of cocaine as opposed to a non-specific deficit in conditioned learning processes, in that CTX-treated mice acquired palatable food SA and cocaine CPP. Furthermore, the lower reinforcing efficacy of cocaine following CTX treatment was probably not due to an impairment of the acute rewarding properties of cocaine, since acquisition of cocaine CPP was not modified in CTX-treated mice. This difference in CTX effects on SA versus CPP is not surprising since the behavioral responses evaluated in these two paradigms are not equivalent (Aguilar, 2009; Bardo and Bevins, 2000; Camarini et al., 2011). While CPP evaluates the expression of indirect reward, SA is used to directly study the reinforcing properties of a drug, and these data add to a wider literature demonstrating such a distinction. Taken together, the present results suggest that therapeutic approaches which enhance GLT-1 activity may be effective at treating cocaine abuse by decreasing the reinforcing strength of cocaine.

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Fig. 1.

The effect of CTX treatment on acquisition of cocaine (0.56 mg/kg/inf) (1A) or vanilla Ensure (50%) (1B) self-administration, and on motivation to self-administer cocaine (0.56 mg/kg/inf) (1C) or vanilla Ensure (50%) (1D). $n=5-8$ /group; bars are S.E.M. * indicates significance from vehicle as determined by Bonferroni posttest.

Fig. 2.

The effect of CTX treatment (200 mg/kg) on CPP induced by cocaine (COC) at 10 or 20 mg/kg (2A) and the effect of subsequent CTX treatment on re-expression as described in Methods (2B). $n=9-12$ /group for test, 4-5/group for retest; bars are S.E.M.