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ORIGINAL ARTICLE

Clavulanic acid enhances glutamate transporter subtype I (GLT-1) expression and decreases reinforcing efficacy of cocaine in mice

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Abstract The β-lactam antibiotic ceftriaxone (CTX) reduces cocaine reinforcement and relapse in preclinical assays through a mechanism involving activation of glutamate transporter subtype 1 (GLT-1). However, its poor brain penetrability and intravenous administration route may limit its therapeutic utility for indications related to CNS diseases. An alternative is clavulanic acid (CA), a structural analog of CTX that retains the β-lactam core required for GLT-1 activity but displays enhanced brain penetrability and oral activity relative to CTX. Here, we tested the hypothesis that CA (1, 10 mg/kg ip) would enhance GLT-1 expression and decrease cocaine self-administration (SA) in mice, but at lower doses than CTX. Experiments revealed that GLT-1 transporter expression in the nucleus accumbens of mice treated with repeated CA (1, 10 mg/kg) was enhanced relative to saline-treated mice. Repeated CA treatment (1 mg/kg) reduced the reinforcing efficacy of cocaine (0.56 mg/kg/inf) in mice maintained on a progressive-ratio (PR) schedule of reinforcement but did not affect acquisition of cocaine SA under fixed-ratio responding or acquisition or retention of learning. These findings suggest that the β-lactamase inhibitor CA can activate the cellular glutamate reuptake system in the brain reward circuit and reduce cocaine's reinforcing efficacy at 100-fold lower doses than CTX.

Keywords Clavulanic acid · Cocaine · Self-administration · Glutamate · GLT-1 · Ceftriaxone · β-Lactamase inhibitor · Reinforcing

Introduction

Ceftriaxone (CTX) improves symptoms of CNS diseases in multiple animal models, including amyotrophic lateral sclerosis, multiple sclerosis, stroke, seizure, Huntington's disease, depression, and drug addiction (Rothstein et al. 2005; Lipski et al. 2007; Mineur et al. 2007; Miller et al. 2008, Sari et al. 2009; Knackstedt et al. 2010; Rawls et al. 2010a, b; Ward et al. 2011; Rao and Sari 2012). The mechanism of CTX involves enhancement of cellular glutamate uptake through activation of glutamate transporter subtype I (GLT-1), an astrocytic transporter responsible for the majority of cellular glutamate reuptake in the mammalian brain (Rothstein et al. 2005; Lipski et al. 2007; Miller et al. 2008; Kovalevich et al. 2012). In the context of drugs of abuse, previous work has demonstrated that CTX reduces: analgesic tolerance, physical dependence, and conditioned place preference (CPP) resulting from chronic morphine exposure (Rawls et al. 2010a, b; Schroeder et al. 2014; Shen et al. 2014); relapse to heroin seeking (Shen et al. 2014); relapse to cocaine seeking (Sari et al. 2009; Knackstedt et al. 2010); reinforcing and motivational effects of cocaine in mice; locomotor sensitization to cocaine or amphetamine (Rasmussen et al. 2011; Sondheimer and Knackstedt 2011; Tallarida et al. 2013); methamphetamine-induced CPP

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(Abulseoud et al. 2012); and alcohol consumption (Rao and Sari 2012; Sari et al. 2013).

Despite its promising preclinical efficacy, CTX possesses unfavorable pharmacokinetic and pharmacodynamic properties (e.g. poor oral bioavailability, limited blood–brain barrier penetrability, antibacterial activity) that may limit its therapeutic utility as a glutamate-based CNS medication. An attractive alternative to CTX is FDA-approved clavulanic acid (CA), which is normally administered in combination with amoxicillin (Augmentin) to overcome resistance in bacteria that secrete β-lactamase enzymes that otherwise inactivate most penicillins. CA is structurally similar to CTX with both compounds having a central β-lactam pharmacophore required for activation of GLT-1 transporters (Rothstein et al. 2005). The potential advantage of CA relative to CTX is a more desirable therapeutic profile, including greater oral bioavailability (64–75 %), enhanced brain penetrability evident from a cerebrospinal fluid/plasma ratio of around 0.25 in patients with intact meninges, and negligible antibacterial activity (Bolton et al. 1986; Nakagawa et al. 1994; Kim et al. 2009). Based on evidence that CTX reduces cocaine self-administration (SA) in rats and mice, and structural similarities between CTX and CA and enhanced brain penetrability of CA, we hypothesized that CA would reduce the reinforcing strength of cocaine in a mouse model of SA and increase GLT-1 transporter expression in the nucleus accumbens, but at lower doses than CTX.

Materials and methods

Animals

Male C57Bl/6 mice from Harlan Laboratories (Indianapolis, Indiana, USA) were used. All animal use procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Temple University Institutional Animal Care and Use Committee. Mice were housed in controlled environment with constant airflow, temperature (21–23 °C), on a reversed 12 h light/dark cycle. Mice had ad libitum food and water access except where noted.

Chemicals

Ceftriaxone sodium (CTX) and potassium clavulanate (CA) were injected intraperitoneally (ip). Doses of CTX (200 mg/kg) and CA (1, 10 mg/kg) were based on prior work (Rothstein et al. 2005; Trantham-Davidson et al. 2012; Sanna et al. 2013; Schroeder et al. 2014). Dosing schedules for CTX and CA utilized repeated injections because consistent evidence across multiple laboratories shows that CTX has to be given repeatedly, for at least 5 days, and at a dose of 200 mg/kg, to detect significant efficacy in animal models of CNS diseases (Rothstein et al. 2005). For comparative purposes, CA was administered under the same schedule, and its dose of 10 mg/kg was estimated from prior in vivo work (Sanna et al. 2013; Schroeder et al. 2014). Cocaine hydrochloride was generously provided by the National Institute on Drug Abuse (NIDA). All drugs were dissolved in sterile, physiological saline.

GLT-1 expression studies in nucleus accumbens

Mice were injected with CA (1, 10 mg/kg ip) or saline (ip) for 7 days for analysis of GLT-1 expression in the nucleus accumbens using previously described methods (Rasmussen et al. 2011; Parikh et al. 2014). Twenty-four h after the last injection, mice were anesthetized with isoflurane and decapitated. Brains were removed and the nucleus accumbens was dissected. Tissue was homogenized in lysis buffer (50 mmol/L Tris–HCl, pH 7.5, 150 mmol/L NaCl, 0.5 % NP-40, 1:200 protease inhibitor cocktail; Calbiochem, San Diego, CA, USA). Samples were then centrifuged at 13,000×*g* at 4 °C for 5 min. Supernatant was collected and stored at −80 °C until analysis. Protein concentration of each sample was determined via Bradford assay. In each lane twenty μg of protein from a single animal was loaded onto pre-cast midi-gel (4–12 % Bis–Tris; Invitrogen, Carlsbad, CA, USA) and separated by electrophoresis for 60 min at 60 V and transferred onto nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). Membranes were blocked in 5 % non-fat milk in 1x tris-buffered saline, 0.1 % Tween-20 for 30 min. 120 min incubation in GLT-1 primary antibody (1:20,000; Millipore) was followed by wash with 1× Tris-buffered saline, 0.1 % Tween-20 and 1-h incubation in appropriate anti-mouse secondary antibody (1:10,000; Thermo Scientific, Lafayette, CO, USA) for 60 min. All incubation was done at room temperature (23 °C). Membranes were developed with enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Densitometry was conducted using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and normalized to loading control with α-tubulin.

Operant responding protocol

Cocaine SA experiments and learning experiments were performed in mouse operant conditioning chambers (Med-Associates, St Albans, VT, USA) that were tailored for intravenous cocaine or liquid food administration contingent on nose-poke responding (Ward et al. 2009). Nose-poke responses in the active hole resulted in either a cocaine infusion (0.56 mg/kg/inf) in SA experiments or

presentation of 50 % vanilla-flavored Ensure in the learning experiments. Responses in the inactive hole had no consequences. Each reward was paired with a light stimulus and tone cue. 0.1 mL of heparinized-saline solution (0.9 %) was used to flush the catheters in order to check for blockade and leaks before each SA session. In addition, 0.1 mL infusion of solution containing methohexital sodium (1 mg/mL) was used to check catheter patency every 5 days, or as needed.

Fixed-ratio (FR-1) cocaine SA experiments

To study effects of repeated CA treatment on the acquisition of cocaine SA, mice were surgically implanted with chronic indwelling back-mounted jugular catheter (Thomsen and Caine 2005). Afternoon treatment with CA (1 or 10 mg/kg, ip) or saline (ip) began 3 days following surgery. Following 3 days of CA or saline injections, mice were allowed to self-administer cocaine (0.56 mg/kg/inf) under a fixed-ratio 1 (FR-1) reinforcement schedule during 2-h daily morning sessions for 10 days. Animals continued to receive CA or saline in the afternoon following each cocaine SA session. The number of reinforcers obtained per session was determined for the duration of the study.

Progressive-ratio (PR) cocaine SA experiments

To study effects of repeated CA treatment on the reinforcing strength of cocaine, and the motivation to self-administer cocaine, mice with no prior experience with the SA procedure were implanted with catheters and trained to self-administer cocaine (0.56 mg/kg/inf) under a FR-1 schedule. After successful acquisition of SA under the FR-1 schedule, mice were switched to respond for the same dose of cocaine under a progressive ratio (PR) reinforcement schedule. The following progression requirements for reinforcement were implemented as described by Richardson and Roberts (1996) (Richardson and Roberts 1996): 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145 etc. Treatment with CA (1 mg/kg ip) or saline (ip) was initiated once mice met the criteria for stable responding, which was defined as three consecutive days of responding without upward or downward variance of three reinforcers received. All injections were administered in the afternoon, and SA sessions were executed in the morning for 7 days. The number of reinforcers obtained during the daily 4-h sessions was recorded for the duration of the study.

Learning experiments

To study effects of CTX and CA on learning and memory, mice were treated with CTX (100, 200 mg/kg ip), CA (1, 10 mg/kg ip) or saline (ip) for 10 days prior testing in a two session food-reinforced operant paradigm (Foley et al. 2008; Walker et al. 2011). Mice were food restricted after the last drug administration for 24 h before the beginning of the learning and memory task. At the start of the session, a house light illuminated the chamber, and mice were presented with a tone on a variable-time schedule (mean of 45 s, range 4–132 s), with the tone remaining on for 6 s or until a nose-poke response occurred in the center nose-poke hole. If a nose-poke response occurred, the mouse was presented with a dipper filled with 50 % ensure through the center nose-poke hole, and the tone was turned off. Each session lasted for 2 h or until 20 reinforced nose pokes were recorded. After the first session, mice were fed 1.5 g of food and returned to their cages. On the second day, the same procedure was repeated to measure retention of the previously learned response. Adjusted latency, defined as the latency to obtain 10 rewards after obtaining the first reward, was recorded on both day 1 (acquisition) and day 2 (retention).

Statistical analysis

Protein expression data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc analysis. SA data were analyzed by two-way (treatment × day) ANOVA followed by a Bonferroni test. Learning data were analyzed by one-way ANOVA followed by a Bonferroni test. Values of $p < 0.05$ were considered statistically significant. GraphPad Prism 6 software was used for statistical analyses.

Results

Effect of CA on GLT-1 transporter protein expression in the nucleus accumbens

Data presented in Fig. 1 shows the effects of repeated CA (1, 10 mg/kg × 7 days) on GLT-1 transporter expression. One-way ANOVA revealed a significant main effect of treatment [$F_{(2,14)} = 14.59$, $p < 0.001$] (Fig. 1). Post hoc analysis revealed that GLT-1 transporter expression in mice treated with CA (10 mg/kg) for 7 days was increased relative to saline-treated controls ($p < 0.001$). Repeated treatment with a tenfold lower dose of CA (1 mg/kg × 7 days) also enhanced GLT-1 transporter expression compared to saline-treated controls ($p < 0.01$).

Effects of CA on the acquisition and reinforcing strength of cocaine

Data presented in Fig. 2 shows the effects of repeated CA treatment (1, 10 mg/kg) on the acquisition of cocaine SA

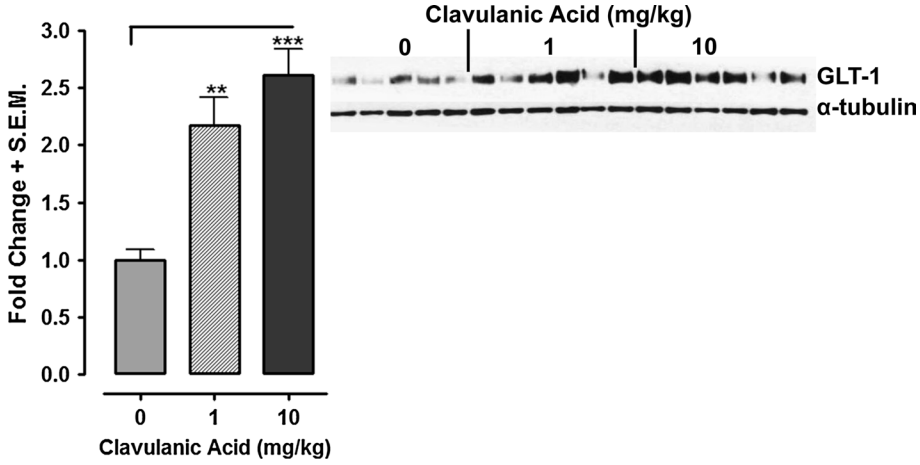

Fig. 1 Clavulanic acid (CA) increases GLT-1 transporter protein expression in the nucleus accumbens. Mice were treated with CA (1, 10 mg/kg ip) or saline (ip) for 7 days followed by western blot analysis. Each *band* represents a protein sample from a single animal. Data (normalized to α-tubulin loading control) are expressed as fold-change (±SEM) compared to saline-treated group. $N = 5$–6 mice/group. $^{**}p < 0.01$ or $^{***}p < 0.001$ compared to saline (i.e., 0 mg/kg CA)

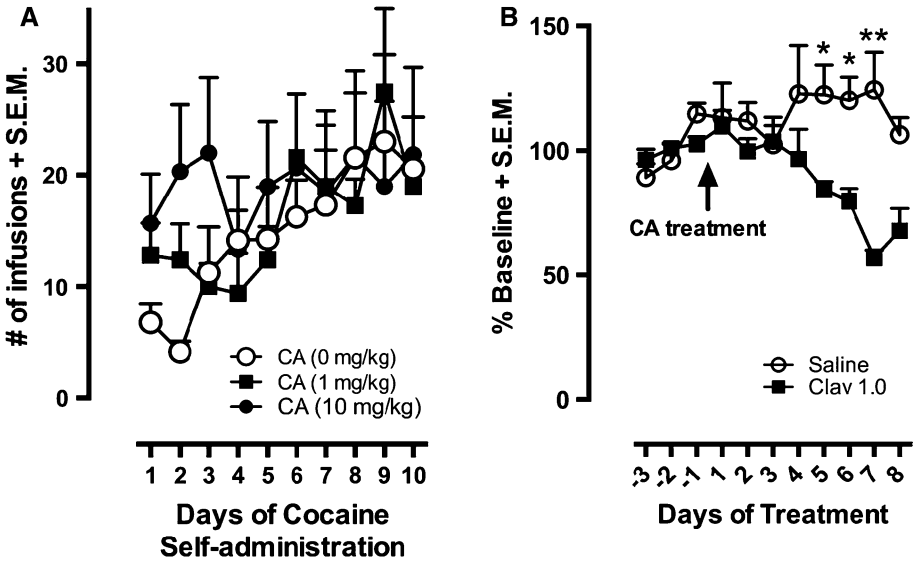

Fig. 2 CA reduces motivation but not acquisition of cocaine SA. **a** Acquisition (FR-1 schedule): mice pretreated with CA (1, 10 mg/kg ip) or saline (ip) for 3 days were allowed to self-administer cocaine (0.56 mg/kg/inf, FR-1 schedule) for 10 days during which CA or saline injections were continued. Data ($N = 6$–8 mice/group) are expressed as number of infusions (±SEM) for each daily SA session. **b** Motivation (PR-1 schedule): mice were trained on a PR-1 reinforcement schedule for 7 days following successful acquisition of SA under the FR-1 schedule. Treatment with CA (1 mg/kg ip) or saline (ip) was initiated once mice met the criteria for stable responding, which was defined as three consecutive days of responding without upward or downward variance of three reinforcers received. Data ($N = 4$–6 mice/group) are expressed as %baseline (±SEM) cocaine infusions. $^{*}p < 0.05$ or $^{**}p < 0.01$ compared to saline (i.e., 0 mg/kg CA)

under a FR-1 reinforcement schedule (Fig. 2a) and on the motivation of mice to self-administer cocaine under a PR reinforcement schedule (Fig. 2b). For FR-1 experiments, two-way ANOVA indicated a significant effect of time [$F_{(9,150)} = 2.963$, $p < 0.01$] but not treatment [$F_{(2,150)} = 2.899$, $p > 0.05$], indicating that the ability of mice to acquire cocaine was not significantly different in mice treated with CA (1, 10 mg/kg) or saline.

For PR-1 experiments (Fig. 2b), a new group of mice was allowed to self-administer cocaine under a PR schedule. Two-way ANOVA indicated a significant effect of treatment [$F_{(1,47)} = 26.50$, $p < 0.0001$] and a significant interaction [$F_{(6,47)} = 3.113$, $p < 0.05$]. The baseline breaking point in the saline treatment group was 9.4 ± 1.05 (mean ± SEM) and in the Clavulanic acid treated group was 13.1 ± 0.63 (mean ± SEM). Bonferroni analysis indicated that the percent baseline of responding for mice treated with CA (1 mg/kg) was significantly lower than saline-treated mice on days 5 ($p < 0.05$), 6 ($p < 0.05$) and 7 ($p < 0.001$).

CA and CTX do not affect learning in mice

Data presented in Fig. 3 shows effects of CA treatment (1, 10 mg/kg) and CTX treatment (100, 200 mg/kg) on food-motivated instrumental learning and memory retention. One-way ANOVA showed no significant effect of CA and CTX on either the acquisition of instrumental learning [$F_{(5,64)} = 1.586$, $p > 0.05$] or the retention of the learned behavior [$F_{(5,64)} = 1.818$, $p > 0.05$].

Discussion

The β-lactam antibiotic CTX is also a GLT-1 transport activator that can antagonize addictive effects of cocaine in laboratory animals; however, clavulanic acid (CA), a structurally-related β-lactamase inhibitor with more attractive pharmacodynamics and pharmacokinetic properties, has not been investigated in this context. We now report for the first time that CA, at 100-fold lower doses than CTX, can enhance GLT-1 transporter protein expression and reduce the reinforcing strength of cocaine in mice. Multiple laboratories, including our own, have shown that CTX increases cellular glutamate reuptake and decreases extracellular glutamate in the brain through a mechanism involving increased GLT-1 transporter expression (Rothstein et al. 2005; Lipski et al. 2007; Miller et al. 2008; Knackstedt et al. 2010; Rasmussen et al. 2011; Trantham-Davidson et al. 2012; Parikh et al. 2014). The mechanism of CTX requires the presence of a central β-lactam core since GLT-1 expression is not enhanced by antibiotics lacking this structural feature (Rothstein et al. 2005). Because CA also has a central β-lactam core, we hypothesized that it, too, would enhance GLT-1 transporter expression, and that is indeed what we found, thereby offering further evidence that β-lactam-containing compounds can increase cellular glutamate reuptake.

The therapeutic utility of CTX as a glutamate-based CNS medication is hindered by its lack of oral bioavailability that necessitates cumbersome parenteral administration, risk of antibiotic resistance during chronic administration regimens, and poor CNS penetrability requiring frequent administration of high doses to achieve CNS efficacy, thereby increasing the risk of adverse effects including toxicity and diarrhea (Rothstein et al. 2005). In fact, detecting significant increases in GLT-1 expression in the brains of rats or mice following systemic CTX administration typically requires at least 5 days of injections at a dose of 200 mg/kg (Rothstein et al. 2005), which is equivalent to a dose of 13 g/day for a typical adult patient and at least sixfold higher than the maximal dose (2 g/day) typically administered to human patients. Assuming a linear, allometric relationship in CTX dose for a mouse-to-human scale-up, plasma levels of CTX are clearly greater under our conditions than those levels achieved by a therapeutic dose in humans.

Thus, an important result from our neurochemical experiments was that CA increased GLT-1 transporter expression at 100-fold lower doses than CTX. The greater relative potency of CA compared to CTX is likely related to a more favorable pharmacokinetic profile, including enhanced brain penetrability evident from a cerebrospinal fluid/plasma ratio of around 0.25 in patients with intact meninges (Bolton et al. 1986; Nakagawa et al. 1994). Since CA also has negligible antibacterial activity, the risk of antibiotic resistance developing during a chronic CA regimen, which would almost surely be required for the management of CNS diseases such as drug addiction, epilepsy, and depression, is minimal.

In behavioral experiments CA reduced cocaine SA in mice maintained under a progressive-ratio (PR) reinforcement schedule. PR provides a measure of the reinforcing efficacy of a self-administered drug and is different from a fixed-ratio (FR) schedule. Under FR conditions, cocaine

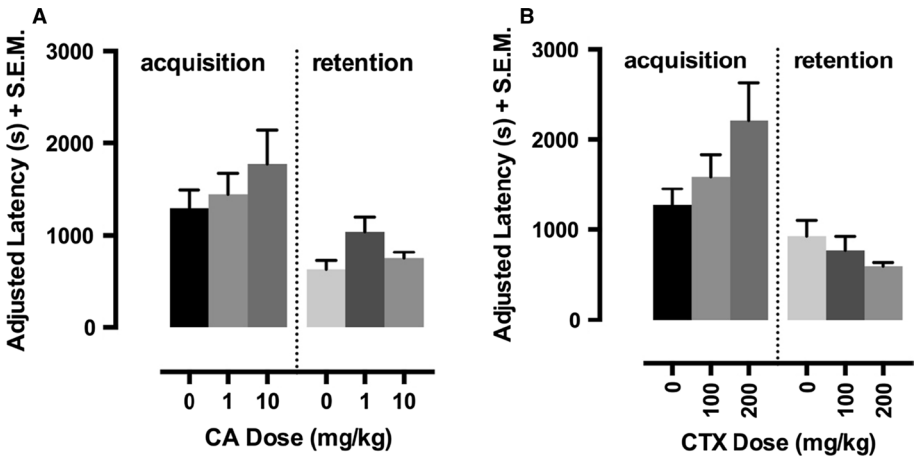

Fig. 3 CTX or CA treatment does not significantly affect acquisition or retention of learning in mice. Mice were treated with CA (0, 1, 10 mg/kg ip) (**a**) or CTX (0, 100, 200 mg/kg ip) (**b**) for 10 days prior to testing for acquisition of learning followed by retention testing on the succeeding day. Data ($N = 12$ mice/group) are expressed as adjusted latency (s) (±SEM)

is delivered each time following a pre-selected number of responses by the animal. Under a PR schedule, the response requirement to deliver cocaine escalates according to a logarithmic progression until the animal fails to complete a response requirement, or ratio. The index of evaluation in a PR schedule is the highest ratio of responses that an animal completes to obtain a single infusion of cocaine, called the break point, which provides information about motivation to self-administer the drug (Arnold and Roberts 1997; Gardner 2000). Decreased motivation is detected as a drop in break point, and increased motivation is detected as increased break point. In our study CA reduced cocaine intake under a progressive ratio (PR) schedule, and this reduction in 'break point' suggests that CA reduced the motivation of mice to respond for cocaine. The most parsimonious explanation for the ability of CA to reduce cocaine's reinforcing efficacy is enhancement of GLT-1 transporter expression, which would lead to normalization of glutamate dysfunction in the brain reward circuit of cocaine self-administering mice (Miller et al. 2008; Knackstedt et al. 2010; Trantham-Davidson et al. 2012). Our protein expression data showing that GLT-1 transporter expression in the nucleus accumbens is enhanced in a dose-dependent manner by a 7-day CA treatment regimen supports this explanation. In effect, we demonstrated a temporal correlation between neurochemical and behavioral effects of CA (i.e., 7-day CA treatment increased GLT-1 expression and reduced cocaine's reinforcing efficacy). Our present results concur with published data showing that repeated CTX treatment reduces cocaine SA under a PR reinforcement schedule (Ward et al. 2011). Furthermore, mice treated with a chronic cocaine regimen display reduced GLT-1 transporter expression that is normalized by CTX treatment (Kovalevich et al. 2012). More broadly, our results with CA provide further evidence that GLT-1 upregulation can attenuate relapse to cocaine seeking (Sari et al. 2009; Knackstedt et al. 2010; Trantham-Davidson et al. 2012).

Interestingly, CA did not significantly alter the ability of mice to acquire cocaine under FR-responding. This outcome with CA is not wholly consistent with CTX, which reduces acquisition of cocaine SA in mice but not rats (Sondheimer and Knackstedt 2011; Ward et al. 2011). These inconsistencies between CA and CTX in cocaine SA acquisition experiments suggest that the two β-lactam-containing compounds act through mechanisms that may not be entirely identical. One example is the increase in dopamine transmission produced by CA (Kost et al. 2011; Sanna et al. 2013). Some direct dopamine agonists display a profile similar to CA in that they decrease the breaking points under PR conditions but increase, or have no effect, on cocaine SA under FR reinforcement schedules (Depoortere et al. 1993). Hence, one explanation is that CA can both increase dopamine transmission and enhance glutamate reuptake, and that the enhanced dopamine transmission during acquisition of cocaine counteracts, or masks, the influence of decreased glutamate transmission. Close scrutiny of our FR results revealed an initial, but non-significant, increase in the number of reinforcers earned on day 2 in mice treated with 10 mg/kg CA. Interestingly, the same initial rise in cocaine responding was observed in CTX treated mice (Ward et al. 2011). We attribute this effect to 3 days of CA treatment prior to the first SA session, which may have produced a modest increase in GLT-1 expression and partial enhancement of glutamate reuptake that initially reduced cocaine efficacy. As a result, mice treated with CA may have required a higher number of cocaine infusions to reach the same level of affective state produced by cocaine as did CA-naïve mice (Knackstedt et al. 2010).

Given that glutamate signaling contributes to learning and memory, we conducted learning experiments with CA and CTX using a food-motivated operant procedure to determine if the efficacy of CA in reducing the motivation to self-administer cocaine was due in part to a general disruption of learning. No significant differences in time taken to learn a behavior (acquisition) and to repeat the learned behavior after 24 h (retention) was observed between drug-naïve mice and mice treated with either repeated CA or CTX. The lack of effect of CA and CTX on acquisition and retention of learning is in agreement with findings by Karaman and colleagues (2013) who demonstrated using the Morris water maze paradigm that chronic CTX treatment did not impact learning and memory. A study using the novel object recognition paradigm has demonstrated memory impairment in rats treated with CTX (Matos-Ocasio et al. 2014). The different behavioral tasks may trigger the recruitment of different brain regions, which may be responsible for the inconsistent results (Morris 1984).

In conclusion, we provide the first evidence that the β-lactamase inhibitor CA can enhance GLT-1 transporter expression in the brain. Furthermore, we provide evidence that a β-lactamase inhibitor can reduce the reinforcing strength of cocaine in a standard mouse SA assay. This evidence is consistent with recent data showing that a β-lactamase inhibitor reduces the rewarding effects of morphine in a rat conditioned place preference assay (Schroeder et al. 2014). Importantly, CA produces these effects at 100-fold lower doses than the β-lactam antibiotic CTX. These findings point toward studying the β-lactamase inhibitor CA as an alternative to CTX as a glutamate-based medication.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

Abulseoud OA, Miller JD, Wu J, Choi D-S, Holschneider DP (2012) Ceftriaxone upregulates the glutamate transporter in medial prefrontal cortex and blocks reinstatement of methamphetamine seeking in a condition place preference paradigm. Brain Res 1456:14–21

Arnold JM, Roberts DC (1997) A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. Pharmacol Biochem Behav 57:441–447

Bolton G, Allen G, Davies B, Filer C, Jeffery D (1986) The disposition of clavulanic acid in man. Xenobiotica 16:853–863

Depoortere RY, Li DH, Lane JD, Emmett-Oglesby MW (1993) Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. Pharmacol Biochem Behav 45:539–548

Foley JJ, Raffa RB, Walker EA (2008) Effects of chemotherapeutic agents 5-fluorouracil and methotrexate alone and combined in a mouse model of learning and memory. Psychopharmacology 199:527–538

Gardner EL (2000) What we have learned about addiction from animal models of drug self-administration. Am J Addict 9:285–313

Karaman I, Kizilay-Ozfidan G, Karadag CH, Ulugol A (2013) Lack of effect of ceftriaxone, a GLT-1 transporter activator, on spatial memory in mice. Pharmacol Biochem Behav 108:61–65

Kim DJ, King JA, Zuccarelli L, Ferris CF, Koppel GA, Snowdon CT, Ahn CH (2009) Clavulanic acid: a competitive inhibitor of beta-lactamases with novel anxiolytic-like activity and minimal side effects. Pharmacol Biochem Behav 93:112–120

Knackstedt LA, Melendez RI, Kalivas PW (2010) Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. Biol Psychiatry 67:81–84

Kost GC, Selvaraj S, Lee YB, Kim DJ, Ahn C-H, Singh BB (2011) Clavulanic acid increases dopamine release in neuronal cells through a mechanism involving enhanced vesicle trafficking. Neurosci Lett 504:170–175

Kovalevich J, Corley G, Yen W, Rawls SM, Langford D (2012) Cocaine-induced loss of white matter proteins in the adult mouse nucleus accumbens is attenuated by administration of a β-lactam antibiotic during cocaine withdrawal. Am J Pathol 181:1921–1927

Lipski J, Wan C, Bai J, Pi R, Li D, Donnelly D (2007) Neuroprotective potential of ceftriaxone in in vitro models of stroke. Neuroscience 146:617–629

Matos-Ocasio F, Hernández-López A, Thompson KJ (2014) Ceftriaxone, a GLT-1 transporter activator, disrupts hippocampal learning in rats. Pharmacol Biochem Behav 122:118–121

Miller BR, Dorner JL, Shou M, Sari Y, Barton SJ, Sengelaub DR, Kennedy RT, Rebec GV (2008) Up-regulation of GLT1 expression increases glutamate uptake and attenuates the Huntington's disease phenotype in the R6/2 mouse. Neuroscience 153:329–337

Mineur YS, Picciotto MR, Sanacora G (2007) Antidepressant-like effects of ceftriaxone in male C57BL/6 J mice. Biol Psychiatry 61:250–252

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11:47–60

Nakagawa H, Yamada M, Tokiyoshi K, Miyawaki Y, Kanayama T (1994) Penetration of potassium clavulanate/ticarcillin sodium into cerebrospinal fluid in neurosurgical patients. Jpn J Antibiot 47:93–101

Parikh V, Naughton SX, Shi X, Kelley LK, Yegla B, Tallarida CS, Rawls SM, Unterwald EM (2014) Cocaine-induced neuroadaptations in the dorsal striatum: glutamate dynamics and behavioral sensitization. Neurochem Int 75:54–65

Rao P, Sari Y (2012) Glutamate transporter 1: target for the treatment of alcohol dependence. Curr Med Chem 19:5148

Rasmussen B, Unterwald EM, Rawls SM (2011) Glutamate transporter subtype 1 (GLT-1) activator ceftriaxone attenuates amphetamine-induced hyperactivity and behavioral sensitization in rats. Drug Alcohol Depend 118:484–488

Rawls SM, Karaca F, Madhani I, Bhojani V, Martinez RL, Abou-Gharbia M, Raffa RB (2010a) β-lactamase inhibitors display anti-seizure properties in an invertebrate assay. Neuroscience 169:1800–1804

Rawls SM, Zielinski M, Patel H, Sacavage S, Baron DA, Patel D (2010b) Beta-lactam antibiotic reduces morphine analgesic tolerance in rats through GLT-1 transporter activation. Drug Alcohol Depend 107:261–263

Richardson NR, Roberts DC (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods 66:1–11

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Hoberg MD, Vidensky S, Chung DS (2005) β-Lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 433:73–77

Sanna F, Melis MR, Angioni L, Argiolas A (2013) Clavulanic acid induces penile erection and yawning in male rats: comparison with apomorphine. Pharmacol Biochem Behav 103:750–755

Sari Y, Smith KD, Ali PK, Rebec GV (2009) Upregulation of GLT1 attenuates cue-induced reinstatement of cocaine-seeking behavior in rats. J Neurosci 29:9239–9243

Sari Y, Sreemantula SN, Lee MR, Choi D-S (2013) Ceftriaxone treatment affects the levels of GLT1 and ENT1 as well as ethanol intake in alcohol-preferring rats. J Mol Neurosci 51:779–787

Schroeder JA, Tolman NG, McKenna FF, Watkins KL, Passeri SM, Hsu AH, Shinn BR, Rawls SM (2014) Clavulanic acid reduces rewarding, hyperthermic and locomotor-sensitizing effects of morphine in rats: a new indication for an old drug? Drug Alcohol Depend 142:41–45

Shen H-W, Scofield MD, Boger H, Hensley M, Kalivas PW (2014) Synaptic glutamate spillover due to impaired glutamate uptake mediates heroin relapse. J Neurosci 34:5649–5657

Sondheimer I, Knackstedt LA (2011) Ceftriaxone prevents the induction of cocaine sensitization and produces enduring attenuation of cue-and cocaine-primed reinstatement of cocaine-seeking. Behav Brain Res 225:252–258

Tallarida CS, Corley G, Kovalevich J, Yen W, Langford D, Rawls SM (2013) Ceftriaxone attenuates locomotor activity induced by acute and repeated cocaine exposure in mice. Neurosci Lett 556:155–159

Thomsen M, Caine SB (2005) Chronic intravenous drug self-administration in rats and mice. Curr Protoc Neurosci Chapter 9:Unit 9.20

Trantham-Davidson H, LaLumiere RT, Reissner KJ, Kalivas PW, Knackstedt LA (2012) Ceftriaxone normalizes nucleus accumbens synaptic transmission, glutamate transport, and export following cocaine self-administration and extinction training. J Neurosci 32:12406–12410

Walker EA, Foley JJ, Clark-Vetri R, Raffa RB (2011) Effects of repeated administration of chemotherapeutic agents tamoxifen, methotrexate, and 5-fluorouracil on the acquisition and retention of a learned response in mice. Psychopharmacology 217:539–548

Ward SJ, Rosenberg M, Dykstra LA, Walker EA (2009) The CB1 antagonist rimonabant (SR141716) blocks cue-induced reinstatement of cocaine seeking and other context and extinction phenomena predictive of relapse. Drug Alcohol Depend 105:248–255

Ward SJ, Rasmussen BA, Corley G, Henry C, Kim JK, Walker EA, Rawls SM (2011) β-lactam antibiotic decreases acquisition of and motivation to respond for cocaine, but not sweet food, in C57Bl/6 mice. Behav Pharmacol 22:370