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Effects of ceftriaxone on conditioned nicotine reward in rats

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Abstract

Nicotine is the addictive compound in tobacco products which exerts psychosomatic effects that contribute to abuse and to low rates of abstinence in treatment-seeking smokers. At present, the most successful smoking cessation aide helps one in four individuals quit smoking at one-year post-cessation. New adjunctive therapies are needed to improve status of smoking-related public health crises, and β -lactam antibiotics are one class of potential therapies as they favorably augment extrasynaptic glutamate clearance. Our study used 2-chamber place conditioning to assess effects of ceftriaxone (CTX) on persistence of conditioned nicotine reward. Rats were conditioned to associate nicotine (0.4 mg/kg, s.c.) with one context and vehicle with an alternative context. After initial post-test, rats received either daily ceftriaxone (200 mg/kg, i.p.) or saline. All rats showed nicotine place preference during Post-Test 1. CTX-treated rats meeting extinction criterion by Post-Test 7 showed significantly reduced preference for the nicotine-paired context during Post-Test 2 compared to vehicle-treated rats. We interpret these data to support the further study of CTX as a smoking cessation aide. Our results suggest that CTX reduces persistence of conditioned nicotine reward and may be helpful for improving abstinence rates in a subset of treatment-seeking smokers.

Keywords

Ceftriaxone; conditioned place preference; extinction; glutamate; nicotine; reward; rat

Introduction

Nicotine is an ingredient in tobacco products that has rewarding and reinforcing properties which contribute to its abuse. Mechanistically, nicotine binds to nicotinic acetylcholine receptors (nAChRs) comprised homopentamerically ($\alpha 7$) and heteropentamerically (e.g., $\alpha 4\beta 2$) (Cooper et al. 1991; for review, Gotti et al. 2006). Desensitization and upregulation of

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$\beta 2$ subunit-containing nAChRs is evident following chronic nicotine use (Cosgrove et al. 2009; Fenster et al. 1997, 1999), and upregulation of $\beta 2$ subunit-containing nAChRs persists throughout and underlies symptoms of nicotine withdrawal (for review, Simmons and Gould 2014). The most effective smoking cessation aides, including varenicline and nicotine replacement therapies, bind in part to $\beta 2$ subunit-containing nAChRs to relieve symptoms emerging during nicotine withdrawal but only help about one in four treatment-seeking smokers successfully quit at one year post-cessation (Gonzales et al. 2006; Jorenby et al. 2006; Nides et al. 2006). While a half-century of research has rightfully been allocated to nAChR agonist-based therapies, alternative transmitter and peptide systems become dysregulated following chronic nicotine use, the receptors of which may be viable targets for pharmacotherapeutic intervention.

Chronic nicotine downregulates levels of astrocytic glutamate transporter 1 (GLT1; also known as excitatory amino acid transporter 2 [EAAT2]) within neuronal structures known to process reward and mediate relapse propensity (e.g., Knackstedt et al. 2009). Impaired extrasynaptic glutamate clearance can lead to neuronal death and is a pathophysiological hallmark of numerous central nervous system (CNS) diseases including substance use disorders. A seminal report found that ceftriaxone, a widely available β -lactam antibiotic, facilitates functional extrasynaptic glutamate clearance by upregulating membranous GLT1 (Rothstein et al. 2005). Throughout the recent decade, ceftriaxone (CTX) has been shown to reduce levels of self-administered nicotine (Sari et al. 2016), prevent tolerance to nicotine-associated analgesia (Schroeder et al. 2011), and attenuate drug-primed reinstatement of nicotine place preference (Alajaji et al. 2013). CTX thus appears to normalize dysregulated central glutamate transmission after chronic nicotine use and in turn normalize otherwise aberrant addiction-related behaviors.

The present study was conducted to investigate the potential therapeutic benefit of ceftriaxone by examining effects on persistence of conditioned nicotine reward. The results demonstrate that, while vehicle-treated control rats repeatedly show preference for a nicotine-paired context, CTX prevents repeated nicotine place preference in rats that fully extinguish by Post Test 7. These results suggest that CTX may facilitate reward devaluation or enhance extinction of a nicotine-paired context.

Methods

Subjects

Adult male Sprague-Dawley rats were purchased from Charles River Laboratories and adjusted to Temple University's rat vivarium for one week prior to beginning experiments. Rats were housed in a temperature- and humidity-controlled vivarium, were kept under a 12-hour : 12-hour light-dark cycle with lights turning on daily at 09:00h. Rats were pair-housed and given food and water without restriction except during place preference conditioning or testing sessions. All procedures were approved by Temple University's Institutional Animal Care and Use Committee.

Apparatus, groups and procedure

For place conditioning, a two-chamber apparatus with distinguishing visual and tactile cues was used. The experimental timeline can be found in Figure 1A. An initial 15-minute pre-test was conducted to establish baseline preference for each of two chambers. 24 h following pre-test, rats had 4 consecutive 20-minute conditioning days where contexts were paired either with sterile water (preferred side) or nicotine (0.4 mg/kg, s.c.; non-preferred side). For all rats, sterile water was given during the morning session (~10:00 – 12:00h) and nicotine was given during the afternoon session (~14:00PM – 16:00h). During conditioning, rats were confined to one of the two chambers. After conditioning, a series of 15-minute post-tests, spaced 72-h apart, were conducted. During post-tests, rats freely shuttled between contexts and amount of time within each context was quantified for analysis. After Post-Test 1, rats were divided into two treatment groups ($n=8$ /group): one group received CTX (200 mg/kg, i.p.) in the homecage ~2j following post-test while the other received saline (VEH) as control. The dose of CTX was selected on the basis of seminal work showing repeated administration at 200 mg/kg yields brain levels sufficient to upregulate GLT1 (Rothstein *et al.* 2005).

Analyses

For place conditioning, post-test data are expressed as CPP Score (post-test time [s] in nicotine-paired context minus pre-test time [s] in nicotine-paired context) as in prior reports (Gregg *et al.* 2016; Kim *et al.* 2016; Le Foll and Goldberg 2005). Independent samples t-tests were used to compare CPP Score between CTX and VEH groups for Post-Tests 1 and 2. Comparisons were made between all rats as well as on sub-populations that fully extinguished by Post Test 7. Additionally, comparisons were performed between each group's CPP Score and "0" – the point of no-change – during Post-Test 1 to assess for initial nicotine place preference. Four rats were omitted from analysis as their pre-test score showed 90% preference for one of two contexts. Rats were considered to have extinguished place preference when the CPP Score for Post-Tests 2+ was 20% of the CPP Score for Post-Test 1, similarly to the criterion used previously (e.g., Berry *et al.* 2012). Rats were ran during Post-Tests until the extinction criterion was reached, and the type I error rate (α) was established at 0.05.

Results

CTX and VEH groups showed significantly greater CPP Score compared to "0" during Post-Test 1, indicating a significant initial nicotine place preference (all $|t(5)| \geq 6.90$, $p < 0.01$; Figure 1B upper-right panel). During Post-Test 2, 50% of CTX-treated rats extinguished place preference whereas no VEH-treated rats reached extinction (Figure 1B left panel). Independent samples t-tests revealed no difference between CTX and VEH groups during Post-Test 1 ($|t(9)| = 1.18$, n.s.). During Post-Test 2, analyses on all rats revealed no significant difference in CPP Score ($|t(14)| = 0.12$, n.s.). Notably, in the subset of rats that eventually extinguished nicotine place preference, CTX treatment significantly reduced CPP Score relative to VEH-treated control rats ($|t(9)| = 2.33$, $p < 0.05$; Figure 1B bottom-right panel). All pre- and post-test CPP Scores can be found in Supplemental Table 1.

Discussion

In a seminal report detailing potential therapeutic utility of CTX for nicotine dependence, Alajaji and colleagues (2013) observed that CTX did not disrupt the development of nicotine place preference, an effect additionally observed by our research group (data not shown), but attenuated nicotine-primed reinstatement of place preference for a nicotine-paired context. The authors additionally found that CTX attenuated anxiety-like behavior and somatic withdrawal signs in tests of precipitated nicotine withdrawal in mice. Our results converge with these prior studies to endorse the utility of CTX to treat nicotine dependence by attenuating withdrawal signs, reducing relapse propensity and facilitating extinction of a nicotine-paired context. While our place conditioning apparatus tends to imbue a bias towards one side, we anticipate comparable results with unbiased place conditioning, as CTX is likely affecting the strength of nicotine preference, which can be established using either place conditioning design. From our results, it is clear that CTX is efficacious in some but not all rats. At present, we speculate that sensitivity to CTX treatment may be due to its augmentation of memranal GLT1 in structures that process reward. Determining neurobiological differences between “treatment-sensitive” and “treatment-resistant” sub-populations will be important to inform clinical study of putative β -lactam pharmacotherapies for smoking cessation in treatment-seeking smokers.

The ubiquitous effect of stimulant drugs on dysregulating glutamate transmission in brain reward- structures has yielded decades of intensive research. Only recently, however, are widely-available β -lactam antibiotics and β -lactamase inhibitors being considered for treating substance use disorders. Typically, CTX is prescribed (~25 mg/kg, i.v. for 10 days) for treating bacterial infection. Multiple preclinical reports show that 100–200 mg/kg CTX (i.p.) yields significant effects on augmenting glutamate neurochemistry and thus was selected as the single dose (e.g., Fischer et al. 2013; Gregg *et al.* 2016). The current state of knowledge supports that therapeutic effects of CTX in models of substance use disorder are likely attributed to the compound’s central and not peripheral effects (e.g., Rawls et al. 2007): 200 mg/kg in rats yields concentration in brain tissue of 3.5 μ M which is sufficient to upregulate GLT1 levels (Rothstein *et al.* 2005).

In addition to nicotine addiction, the efficacy of β -lactam pharmacotherapies have been tested in models of psychostimulant addictions, including to cocaine and synthetic cathinones, which are designer stimulant drugs that behave in mechanistically and subjectively comparable manners as cocaine (Baumann et al. 2013; Fischer *et al.* 2013; Kim et al. 2016; Simmons et al. 2016). Our study supports an impressive body of literature that is leading to clinical study of tolerated and non-addictive antibiotic adjunct therapies for substance use disorders including nicotine addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Alajaji M, Bowers MS, Knackstedt L, Damaj MI. Effects of the beta-lactam antibiotic ceftriaxone on nicotine withdrawal and nicotine-induced reinstatement of preference in mice. *Psychopharmacology (Berl)*. 2013; 228(3):419–426. [PubMed: 23503685]
- Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M..., et al. Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'bath salts' products. *Neuropsychopharmacology*. 2013; 38(4):552–562. [PubMed: 23072836]
- Berry JN, Neugebauer NM, Bardo MT. Reinstatement of methamphetamine conditioned place preference in nicotine-sensitized rats. *Behav Brain Res*. 2012; 235(2):158–165. [PubMed: 22884606]
- Cooper E, Couturier S, Ballivet M. Pentameric structure and subunit stoichiometry of a neuronal nicotinic acetylcholine receptor. *Nature*. 1991; 350(6315):235–238. [PubMed: 2005979]
- Cosgrove KP, Batis J, Bois F, Maciejewski PK, Esterlis I, Kloczynski T..., et al. beta2-Nicotinic acetylcholine receptor availability during acute and prolonged abstinence from tobacco smoking. *Arch Gen Psychiatry*. 2009; 66(6):666–676. [PubMed: 19487632]
- Fenster CP, Rains MF, Noerager B, Quick MW, Lester RA. Influence of subunit composition on desensitization of neuronal acetylcholine receptors at low concentrations of nicotine. *J Neurosci*. 1997; 17(15):5747–5759. [PubMed: 9221773]
- Fenster CP, Whitworth TL, Sheffield EB, Quick MW, Lester RA. Upregulation of surface alpha4beta2 nicotinic receptors is initiated by receptor desensitization after chronic exposure to nicotine. *J Neurosci*. 1999; 19(12):4804–4814. [PubMed: 10366615]
- Fischer KD, Houston AC, Rebec GV. Role of the major glutamate transporter GLT1 in nucleus accumbens core versus shell in cue-induced cocaine-seeking behavior. *J Neurosci*. 2013; 33(22):9319–9327. [PubMed: 23719800]
- Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB..., et al. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. *JAMA*. 2006; 296(1):47–55. [PubMed: 16820546]
- Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol Sci*. 2006; 27(9):482–491. [PubMed: 16876883]
- Gregg RA, Hicks C, Nayak SU, Tallarida CS, Nucero P, Smith GR..., et al. Synthetic cathinone MDPV downregulates glutamate transporter subtype I (GLT-1) and produces rewarding and locomotor-activating effects that are reduced by a GLT-1 activator. *Neuropharmacology*. 2016; 108:111–119. [PubMed: 27085607]
- Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE..., et al. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *JAMA*. 2006; 296(1):56–63. [PubMed: 16820547]
- Kim J, Connelly KL, Unterwald EM, Rawls SM. Chemokines and cocaine: CXCR4 receptor antagonist AMD3100 attenuates cocaine place preference and locomotor stimulation in rats. *Brain Behav Immun*. 2016a; doi: 10.1016/j.bbi.2016.08.015
- Kim J, John J, Langford D, Walker E, Ward S, Rawls SM. Clavulanic acid enhances glutamate transporter subtype I (GLT-1) expression and decreases reinforcing efficacy of cocaine in mice. *Amino Acids*. 2016b; 48(3):689–696. [PubMed: 26543027]
- Knackstedt LA, LaRowe S, Mardikian P, Malcolm R, Upadhyaya H, Hedden S..., et al. The role of cystine-glutamate exchange in nicotine dependence in rats and humans. *Biol Psychiatry*. 2009; 65(10):841–845. [PubMed: 19103434]
- Le Foll B, Goldberg SR. Nicotine induces conditioned place preferences over a large range of doses in rats. *Psychopharmacology (Berl)*. 2005; 178(4):481–492. [PubMed: 15765262]

- Nides M, Oncken C, Gonzales D, Rennard S, Watsky EJ, Anziano R..., et al. Smoking cessation with varenicline, a selective alpha4beta2 nicotinic receptor partial agonist: results from a 7-week, randomized, placebo- and bupropion-controlled trial with 1-year follow-up. *Arch Intern Med*. 2006; 166(15):1561–1568.
- Rawls SM, Tallarida R, Robinson W, Amin M. The beta-lactam antibiotic, ceftriaxone, attenuates morphine-evoked hyperthermia in rats. *Br J Pharmacol*. 2007; 151(7):1095–1102. [PubMed: 17592517]
- Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE..., et al. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature*. 2005; 433(7021):73–77. [PubMed: 15635412]
- Sari Y, Toalston JE, Rao PS, Bell RL. Effects of ceftriaxone on ethanol, nicotine or sucrose intake by alcohol-preferring (P) rats and its association with GLT-1 expression. *Neuroscience*. 2016; 326:117–125. [PubMed: 27060486]
- Schroeder JA, Quick KF, Landry PM, Rawls SM. Glutamate transporter activation enhances nicotine antinociception and attenuates nicotine analgesic tolerance. *Neuroreport*. 2011; 22(18):970–973. [PubMed: 22027514]
- Simmons SJ, Gould TJ. Involvement of neuronal beta2 subunit-containing nicotinic acetylcholine receptors in nicotine reward and withdrawal: implications for pharmacotherapies. *J Clin Pharm Ther*. 2014; 39(5):457–467. [PubMed: 24828779]
- Simmons SJ, Gregg RA, Tran FH, Mo L, von Weltin E, Barker DJ..., et al. Comparing rewarding and reinforcing properties between ‘bath salt’ 3,4-methylenedioxypyrovalerone (MDPV) and cocaine using ultrasonic vocalizations in rats. *Addict Biol*. 2016; doi: 10.1111/adb.12479

Figure 1.

(A) Timeline of place conditioning and subsequent Post-Test sessions. Arrows indicate when rats were injected either with ceftriaxone (CTX; 200 mg/kg, i.p.) or saline (VEH). (B) Ceftriaxone reduces persistence of conditioned nicotine reward in rats reaching extinction criterion ("Extinguished"). * $p < 0.05$ compared to Nic-Veh. n's=5–6/group.