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Effects of active anti-methamphetamine vaccination on intravenous self-administration in rats

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ABSTRACT

Background: D-Methamphetamine (METH) addiction is a serious public health concern for which successful treatment remains elusive. Immunopharmacotherapy has been shown to attenuate locomotor and thermoregulatory effects of METH. The current study investigated whether active vaccination against METH could alter intravenous METH self-administration in rats.

Methods: Male Sprague-Dawley rats (Experiment 1: $N = 24$; Experiment 2: $N = 18$) were vaccinated with either a control keyhole-limpet hemocyanin conjugate vaccine (KLH) or a candidate anti-METH vaccine (MH6-KLH) or. Effects of vaccination on the acquisition of METH self-administration under two dose conditions (0.05, 0.1 mg/kg/inf) and post-acquisition dose-substitution (0, 0.01, 0.05, 0.20 mg/kg/inf, Experiment 1; 0.01, 0.05, 0.10, 0.15 mg/kg/inf, Experiment 2) during steady-state responding were investigated. Plasma METH concentrations were determined 30 min after an acute challenge dose of 3.2 mg/kg METH.

Results: Active vaccination inhibited the acquisition of METH self-administration under the 0.1 mg/kg/inf dose condition, with 66% of the MH6-KLH-vaccinated rats compared to 100% of the controls reaching criteria, and produced transient and dose-dependent effects on self-administration during the maintenance phase. Under the 0.05 mg/kg/inf dose condition, MH6-KLH-vaccinated rats initially self-administered more METH than controls, but then self-administration decreased across the acquisition phase relative to controls; a subsequent dose–response assessment confirmed that MH6-KLH-vaccinated rats failed to acquire METH self-administration. Finally, plasma METH concentrations were higher in MH6-KLH-vaccinated rats compared to controls after an acute METH challenge, and these were positively correlated with antibody titers.

Conclusions: These data demonstrate that active immunopharmacotherapy for METH attenuates the acquisition of METH self-administration.

1. Introduction

D-Methamphetamine (METH) abuse and addiction continues to be a serious public health concern for which successful treatment remains elusive (SAMHSA, 2010). Behavioral therapy shows moderate success (Roll et al., 2006), but maintaining long-term abstinence is a challenge for recovering addicts. Pharmacotherapies also have limited efficacy for treating METH addiction (Karila et al., 2010; Vocci and Appel, 2007), and additional approaches are needed. Immunopharmacotherapy has shown promise as a treatment for drug addiction in recent years (Gentry et al., 2009; Janda and Treweek, 2012; LeSage et al., 2006b; Moreno and Janda, 2009; Shen et al., 2013). In outline, conjugate vaccines stimulate drug-specific antibodies that sequester drug molecules in the blood stream, thereby reducing distribution to the brain.

Clinical trials for vaccines against cocaine (Haney et al., 2010; Kosten et al., 2002; Martell et al., 2009) and nicotine (Cornuz et al., 2008; Hatsukami et al., 2011) were advanced due to promising pharmacokinetic and behavioral results from preclinical studies. Anti-nicotine vaccines generate nicotine-specific antibodies (Cerny et al., 2002; de Villiers et al., 2004; Pentel et al., 2000), reduce brain nicotine concentrations (de Villiers et al., 2004; Pentel et al., 2000), and

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delay nicotine elimination (Keyler et al., 1999, 2005). Similar pharmacokinetic results exist for anti-cocaine vaccines (Carrera et al., 1995). Behavioral effects of drugs such as cocaine (Carrera et al., 1995, 2001, 2000; Kantak et al., 2001, 2000; Wee et al., 2012) and nicotine (Carrera et al., 2004; LeSage et al., 2006a; Lindblom et al., 2002; Roiko et al., 2008) are likewise attenuated by vaccination. Kantak et al. (2001) reported that vaccination decreased cocaine intravenous self-administration (IVSA) in rats by about 30%. Wee et al. (2012) showed that vaccination reduced cocaine IVSA in rats across several phases of the self-administration procedure, including a dose–response assessment, progressive ratio schedule, extinction and reinstatement. (LeSage et al. 2006a) reported that 70% of controls while only 36% of vaccinated rats acquired nicotine IVSA, and that by the end of the study vaccination reduced the amount of nicotine self-administered by 38%. Collectively, these successes prompted the development of vaccines capable of opposing the actions of METH.

An initial study found no effect of an active anti-METH vaccine on METH-induced locomotor activity (Byrnes-Blake et al., 2001) but recent findings show that active vaccination with the MH6-KLH conjugate vaccine (Moreno et al., 2011) blocks METH-induced locomotor and thermoregulatory disruptions in rats (Miller et al., 2013), and another vaccine alters METH-induced locomotion in mice (Shen et al., 2013). Lastly, active vaccination altered METH IVSA in rats; that is, vaccinated rats initially self-administered *more* METH than controls, but then self-administration decreased to a level indistinguishable from controls as the response requirement progressively increased across sessions (Duryee et al., 2009).

The current study investigated the effects of an anti-METH vaccine on METH IVSA, with a primary focus on the *acquisition* of self-administration. Since drug dependence is a minority outcome for most humans who sample a given drug (Anthony et al., 1994; Schramm-Sapyta et al., 2009), prevention of the establishment of a compulsive use pattern is important to model pre-clinically. Although anti-drug vaccine investigators frequently assume that broad spectrum vaccination of, e.g., adolescents is unimaginable, the approval and acceptance of a vaccine against human papilloma virus (Constantine et al., 2007; Shi et al., 2007) shows such views are unduly pessimistic. Preclinical investigators should determine what is biologically possible rather than fail to do so based on suppositions about what might be approved as an eventual treatment. We have previously shown that the MH6-KLH conjugate vaccine is capable of sequestering METH in the blood compartment of the rat while decreasing brain levels and that actively vaccinated rats are protected from thermoregulatory and locomotor effects of METH (Miller et al., 2013). Consequently, rats were not lever trained prior to self-administration sessions, the response requirement remained constant throughout the study, and two different training doses were used (unlike the Duryee et al. study). Effect of vaccination across a range of METH doses during the maintenance phase of self-administration was investigated, along with an assessment of antibody titers and plasma METH concentrations at the end of the study.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (Experiment 1: $N = 24$; Experiment 2: $N = 18$; Charles River, NY, USA) weighing ~250 g on arrival were group housed in clear shoebox cages in a vivarium with a 12:12 reverse light-dark cycle. Food pellets and water were available ad libitum in the vivarium. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and under protocols approved by the Institutional Animal Care and Use Committee (IACUC) of The Scripps Research Institute.

2.2. Drug and hapten

D-*Methamphetamine* HCl (provided by RTI under contract to the National Institute on Drug Abuse) was dissolved in sterile saline and administered intravenously in a volume of 0.1 ml per infusion. Doses are expressed as the salt.

Methamphetamine hapten (MH6) was coupled with the KLH (control) carrier protein and administered (100 μg per innoculation) in formulation with the Sigma Adjuvant System® as previously reported (Miller et al., 2013).

2.3. Equipment

Standard self-administration chambers (MED Associates, St. Albans, VT, USA; Model ENV-007) equipped with 2 response levers and cue lights, pellet magazine, and drug infusion pump (Med Associates Model ENV-045) were used. Each chamber was enclosed in a sound-attenuating box and all equipment was controlled by MED-PC IV software.

2.4. Vaccination procedure

For vaccination, either MH6-KLH or KLH (control) were added to adjuvant to create 100 μg/0.5 ml vaccine for each rat, which was administered across 3 sites (0.2 ml s.c. in the nape; 0.2 ml s.c. in the left hind quadricep/flank; 0.1 ml i.p.). Rats were vaccinated during weeks 0, 2, and 5 (Experiment 1) and weeks 0, 2, 5, 9, and 13 (Experiment 2). The vaccination schedule was designed to match that used in a prior report from our laboratory (Miller et al., 2012). As such, a vaccination is typically administered during week 9. In Experiment 1 of the current study, however, the week 9 vaccination was not administered because it coincided with the dose–response assessment. However, an additional vaccination was administered (during week 13) in Experiment 2 because the self-administration conditions ran 6 weeks longer than in Experiment 1; vaccine administration occurred between the acquisition and maintenance phases for that reason. Vaccinations administered during the acquisition were administered after self-administration sessions. A summary of experimental conditions is shown in Table 1.

2.5. Surgery

Chronic intravenous catheters were surgically implanted into all rats as described in (Aarde et al., 2015a; Creehan et al., 2015; Miller et al., 2012). There were 4 days of surgical recovery prior to starting self-administration sessions; for the first 3 days, cephazolin (0.4 g/ml; 2.0 ml/kg s.c.; once daily) and flunixin (2.5 mg/ml; 2.0 ml/kg s.c.; once daily) were administered. Catheters were flushed with sterile physiological saline containing either timentin (before sessions; 0.1 g/ml; 0.2–0.3 ml/rat) or heparin (after sessions; 10 USP units/ml; 0.2–0.3 ml/rat). Catheter patency was checked weekly after the session by administering 0.2–0.3 ml of the ultra-short-acting barbiturate anesthetic Brevital sodium (1% methohexital sodium, Eli Lilly, Indianapolis, IN) through the catheter. Rats with patent catheters exhibit prominent signs of anesthesia (pronounced loss of muscle tone) within 3 s of the Brevital injection. Failure to produce loss of muscle tone was considered a sign of a faulty catheter. In those cases, the faulty catheter was surgically removed and a new catheter was implanted into the left jugular vein; after recovery, the rats resumed self-administration sessions.

2.6. Immunologic assays

Blood samples were collected from the tail vein during weeks 18 and 20, and from the heart during exsanguination on week 22 (Experiment 2 only). Samples were placed on ice to prevent clotting and then centrifuged at 10,000 × *g* for 15 min; the plasma was extracted and then stored at −80 °C until further processing.

Antibody titers were assessed by enzyme-linked immunosorbent assay (ELISA) as previously described (Moreno et al., 2011) using MH6-BSA conjugates as coating antigens. Titers were calculated as the dilution corresponding to an absorbance reading 50% of the maximal value from the plot of absorbance versus log dilution.

METH concentrations in terminal blood samples were assessed using high-throughput liquid chromatography with tandem mass spectrometric detection (LC–MS/MS) at the Scripps Center for Metabolomics and Mass Spectrometry. For this assessment, blood samples were obtained 30 min after a 3.2 mg/kg (s.c.) METH challenge during week 22 (Experiment 2 only), and were prepared for LC–MS/MS using a trichloroacetic acid-based extraction described in (Hendrickson et al., 2006) to dissociate drug from antibody.

2.7. Experiments

In both experiments, rats were trained to self-administer METH according to a fixed ratio (FR1) schedule of reinforcement. That is, every response on the left lever activated the drug infusion pump for 4 s, and delivered either 0.10 (Experiment 1) or 0.05 (Experiment 2) mg/kg/inf METH. After the lever press, a 20-s time-out ensued with the illumination of the stimulus light above the lever. Responses on either lever had no programmed consequences during the time-out. Sessions were conducted within the first 3 h of the dark phase, at ambient temperature ~23 °C.