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Effective active vaccination against methamphetamine in female rats

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ABSTRACT

Background: Immunotherapies directed against methamphetamine (MA) abuse have shown success in rodent models, however only a limited number of studies have investigated active vaccination in female mice and none in female rats. It is critical to determine if potential immunotherapeutic strategies generalize across sex, particularly for drugs that may produce significant sex-differences on behavioral or physiological endpoints.
Methods: Female Wistar rats were initially vaccinated with keyhole-limpet hemocyanin (KLH) or an anti-methamphetamine-KLH conjugate (MH6-KLH) three times over five weeks and implanted with radiotelemetry devices to assess locomotor activity and body temperature responses to MA. Rats were first exposed to MA via vapor inhalation (100 mg/mL in propylene glycol) and then by injection (0.25–1.0 mg/kg, i.p.) and vapor after a final vaccine boost.
Results: The MH6-KLH vaccine generated an increase in antibody titers across the initial 6-week, 3 immunization protocol and a restoration of titer after a week 14 booster. Locomotor stimulation induced by 0.25 mg/kg MA, i.p, in the KLH group was prevented in the MH6-KLH group. MH6-KLH animals also exhibited an attenuated locomotor stimulation produced by 0.5 mg/kg MA, i.p. No group differences in locomotion induced by vapor inhalation of MA were observed and body temperature was not differentially affected by MA across the groups, most likely because vapor inhalation of MA that produced similar locomotor stimulation resulted in ~10-fold higher plasma MA levels.
Conclusions: This study confirms the efficacy of the MH6-KLH vaccine in attenuating the effects of MA in female rats.

1. Introduction

D-methamphetamine (MA) addiction is a public health problem; however, there are currently no medications approved for the treatment of addiction and abuse disorders involving MA. Immunopharmacotherapy, specifically through active immunization, has been utilized as a method for the sequestration of drug molecules from the brain and for the reduction of drug effects. By eliciting high-affinity drug-specific antibodies, anti-drug vaccines have been developed as potential therapeutics (see Kosten and Domingo, 2013; Ohia-Nwoko et al., 2016; Skolnick, 2015; Zalewska-Kaszubska, 2015 for review), for psychomotor stimulants such as MA and cocaine (Kosten et al., 2014) and even recently emerged designer cathinones, such as alpha-pyrrolidinopentiophenone and 3,4-methylenedioxypyrovalerone (Nguyen et al., 2016c). Multiple reports followed a seminal demonstration that an anti-cocaine vaccine produced an attenuation of cocaine-stimulated locomotor increases in male Wistar rats (Carrera et al., 1995). There have been at least four programs investigating active anti-MA vaccines that have provided evidence of *in vivo* efficacy of anti-MA vaccine in animal models (Byrnes-Blake et al., 2001; Duryee et al., 2009; Miller et al., 2015; Miller et al., 2013b; Ruedi-Bettschen et al., 2013; Shen et al., 2013). Specifically these studies have shown that vaccination is effective against MA-induced suppression of food-maintained responding in male Sprague-Dawley rats (Ruedi-Bettschen et al., 2013), produces initially increased self-administration of MA followed by extinction in lever-trained male rats (Duryee et al., 2009; Miller et al., 2015) and delays the acquisition of self-administration in behaviorally naïve male rats (Miller et al., 2015). Evidence for efficacy against locomotor stimulant effects of MA is mixed, with vaccines attenuating effects of MA in wheel activity and spontaneous locomotion in rats (Miller et al., 2013b) and mice (Shen et al., 2013) at some doses and a failure to protect against locomotor activating effects of 3 mg/kg MA, i.p., in male rats (Byrnes-Blake et al., 2001) reported.

Determining the behavioral or physiological targets that are most

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likely to be affected by anti-MA vaccination in laboratory models would enhance the prospects for successful clinical trials. Sex differences are one potential critical issue which may affect vaccine effectiveness, yet these have not been well delineated in anti-drug vaccine development efforts. Women may be at differential risk for drug dependence, for example, methamphetamine dependence starts earlier in women (Rawson et al., 2005), MDMA dependence occurs more frequently in women (Bruno et al., 2009; Uosukainen et al., 2015) and discontinuation of cocaine is harder (DeVito et al., 2014). More generally, the escalation of substance use and resistance to treatment is higher in women (Westermeyer and Boedicker, 2000). Alternately, cocaine dependent women may respond favorable to an experimental drug where men do not (Fox et al., 2014). These observations reinforce the recent United States National Institutes of Health policy position identifying a need for additional sex-difference comparisons across biomedical domains (Clayton and Collins, 2014) and show that study of potential immunotherapies in female animal models are needed. Previous investigations of the effects of anti-MA vaccination found attenuation of locomotor stimulation and place conditioning in female BALB/c mice (Haile et al., 2015; Shen et al., 2013) but there have been no investigations of anti-MA vaccination in female rats.

This study was therefore undertaken to determine if the efficacy of the MH6-KLH vaccine (Miller et al., 2015; Miller et al., 2013b) extends to female rats. Experiments were conducted to determine potential protective effects against methamphetamine-induced stimulation of spontaneous locomotor activity after intraperitoneal injection. The vapor inhalation model was selected in addition to parenteral injection because humans who are dependent on methamphetamine use inhalation more than other routes of administration (Das-Douglas et al., 2008; Heinzerling et al., 2010; Wood et al., 2008) and this route is not commonly tested in nonhuman animal models.

2. Methods

2.1. Subjects

Female (N = 32) Wistar rats (Charles River, New York) were housed in humidity and temperature-controlled (23 ± 1 °C) vivaria on 12:12 h light:dark cycles. Rats entered the laboratory at 10 weeks of age. Vaccinated animals were 11 weeks of age on Week 0 of this study and the animals used for pharmacokinetics were 13 weeks of age at the start of those studies. Animals had ad libitum access to food and water in their home cages. All procedures were conducted under protocols approved by the Institutional Care and Use Committees of The Scripps Research Institute and in a manner consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Radiotransmitter implantation

Rats were anesthetized with an isoflurane/oxygen vapor mixture (isoflurane 5% induction, 1–3% maintenance), and sterile radiotelemetry transmitters (Data Sciences International; TA-F40) were implanted in the abdominal cavity through an incision along the abdominal midline posterior to the xyphoid space as previously reported (Aarde et al., 2015; Miller et al., 2013a; Wright et al., 2012). Absorbable sutures were used to close the abdominal muscle incision and the skin incision was closed with the tissue adhesive. A minimum of 7 days was allowed for surgical recovery prior to starting experiments. For the first three days of the recovery period, an antibiotic Cefazolin (Hikma Farmaceutica, Portugal; 0.4 mg/kg, i.m. in sterile water Day 1, s.c. Day 2–3) and an analgesic flunixin (FlunixiJect, Bimeda USA, Oakbrook Terrace, IL; 2.5 mg/kg, s.c. in saline) were administered daily.

2.3. Drugs and hapten

The *D*-methamphetamine HCl (MA) was obtained from the Drug Supply Program of the U.S. National Institute on Drug Abuse. MA was delivered in propylene glycol (PG) vehicle (at a concentration of 100 mg/mL) using e-cigarette-type cartridges for vapor inhalation sessions conducted in an exposure chamber as described below (Section 2.6). Four 10-s vapor puffs were delivered with 2-s intervals every 5 min that resulted in use of approximately 0.125 mL per 40 min exposure session. The chamber exhaust was vacuum controlled to 1 L air per minute during puffs to ensure that vapor entered the chamber on each device-triggering event. MA administered via intraperitoneal injection (0.25, 0.5, 1.0 mg/kg) was dissolved in physiological saline using an injection volume of 1 mL/kg. Dosing is expressed as the salt in all cases. MA doses were tested in each rat in a randomized order within the first vapor study and the i.p. study. The second vapor study, conducted a week after the i.p. study, began with all rats first exposed to 40 min of MA (100 mg/mL) inhalation. Next, the 20 min PG and MA (100 mg/mL) studies were conducted in a balanced order within the groups. There was a minimum 3–4 day interval between test days for all MA studies.

Methamphetamine hapten (MH6) was coupled with a keyhole limpet hemocyanin (KLH) carrier protein and administered (100 micrograms per immunization) in formulation with the Sigma Adjuvant System® as previously reported (Miller et al., 2013b).

2.4. Vaccination procedure

Rats (N = 8 per group) were vaccinated during weeks 0, 2, 5 and 14. For vaccination, MH6-KLH or KLH was added to adjuvant to create a 0.5 mL vaccine for each rat, which was administered across three sites (0.2 mL s.c. in the nape; 0.2 mL s.c. in the hind quadriceps; 0.1 mL i.p.).

2.5. Immunologic assays

For characterization of anti-MA antibody titers, rats were anesthetized with an isoflurane/oxygen vapor mixture (isoflurane 5% induction, 1–3% maintenance), and blood was collected from the jugular vein during weeks 1–6, 14, 20 and 30. Antibody titer was defined by the dilution required to achieve a 50% signal using enzyme-linked immunosorbent assay (ELISA) with a Biomek 4000 liquid handling robot. 96-well assay plates were coated with 25 µg/well MH6-BSA conjugate and blocked with skim milk. Twelve 1:1 rat plasma dilutions were added to the plate starting at 1:200 and allowed to incubate for 2 h. Following a wash step, goat anti-rat HRP IgG (SouthernBiotech) at 1:10,000 dilution was incubated in the plates for 18 h at 4 °C. After a second wash step the plates were developed using a 3,3′-5,5′-tetramethylbenzidine (TMB) substrate kit (Thermo Pierce) and 2 M H_2SO_4 as a stopping solution. The well absorbance values were read at 450 nm and normalized to the highest value for each sample in GraphPad Prism version 6, followed by curve fitting with log(inhibitor) vs. normalized response − variable slope to find the midpoint titer. One individual sample was unavailable in Week 6 and one animal was lost to the study prior to the Week 14, 20 and 30 time-points; the values were replaced with the group average for the time point for statistical analysis.

2.6. Inhalation apparatus

Sealed exposure chambers were modified from the 259 mm × 234 mm × 209 mm Allentown rat cage to regulate airflow and the delivery of vaporized drug to rats using e-cigarette type devices as has been previously described (Nguyen et al., 2016a; Nguyen et al., 2016b). A custom e-cigarette cartridge-triggering unit (Model SSV-1; La Jolla Alcohol Research, Inc; La Jolla CA) was controlled by MedPC IV software (MedAssociates, St Albans, VT) to deliver vapor puffs as scheduled. The chamber air was vacuum controlled by a chamber exhaust valve (i.e., a "pull" system) to flow room ambient air through an intake valve at 1 L per minute. This functioned to ensure that vapor filled the chamber on each device triggering event, i.e., the vapor