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A vaccine against methamphetamine attenuates its behavioral effects in mice

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ABSTRACT

Background: Vaccines have treatment potential for methamphetamine (MA) addiction. We tested whether a conjugate vaccine against MA (succinyl-methamphetamine-keyhole limpet hemocyanin carrier protein; SMA-KLH) would generate MA antibodies and alter MA-induced behaviors.
Methods: Mice were injected with SMA-KLH and received booster administrations 3 and 20 weeks later. Serum antibody titers reached peak levels by 4–6 weeks, remained at a modest level through 18 weeks, peaked again at 22 weeks after the second boost, and were still elevated at 35 weeks. At 7 weeks, groups of vaccinated and non-vaccinated mice were administered one of three MA doses (1, 2 or 3 mg/kg) to assess locomotor activity.
Results: Non-vaccinated mice showed dose-dependent effects of MA with hypolocomotion at the lowest dose and elevated activity levels at the highest dose. Both dose effects were reduced in SMA-KLH groups, particularly low dose-induced hypolocomotion at later times post MA administration. Separate groups of vaccinated and non-vaccinated mice were trained in MA place conditioning at 30 weeks with either 0 (vehicle) or 0.5 mg/kg MA. Although times spent in the MA-paired side did not differ between groups on test vs. baseline sessions, SMA-KLH mice conditioned with MA showed reduced conditioned approach behaviors and decreased conditioned activity levels compared to control groups.
Conclusion: These data suggest SMA-KLH attenuates the ability of MA to support place conditioning and reduces or delays its locomotor effects. Overall, results support SMA-KLH as a candidate MA vaccine.

1. Introduction

Methamphetamine (MA) abuse has grown at alarming rates in the United States over the past two decades and is spreading across Southeast and East Asia (Gonzales et al., 2010; McKetin et al., 2008). Currently, there are no FDA approved medications for treating MA addiction. The highly addictive effects of MA likely relates to its known central and peripheral sympathomimetic effects (Darke et al., 2008; Fowler et al., 2007; Volkow et al., 2010) and to its ability to release multiple neurotransmitters, including dopamine (DA), norepinephrine (NE), serotonin (5-HT), histamine, and gamma-aminobutyric acid (GABA), from synaptic vesicles (Sulzer et al., 2005). Due to this multiplicity of effects, MA is much less likely than other drugs of abuse to be treated effectively with specific pharmacological antagonists or substitute agonists.

An increasing appealing approach to drug addiction treatment is to use conjugate drug vaccines to induce specific antibody blockade of abused drugs. Conjugate vaccines developed against cocaine and nicotine has progressed to clinical trials (Hatsukami et al., 2005; Martell et al., 2009). Data from these trials suggest that many patients may not produce a sufficient antibody response, but of those who do, drug use is reduced and abstinence rates can be quite good (Hatsukami et al., 2011; Martell et al., 2009; Maurer and Bachmann, 2007). An anti-MA vaccine could also be a viable treatment approach for this addiction. Several laboratories have been working on evaluating the best composition for an MA vaccine. For example, Janda's group has recently evaluated three MA-KLH conjugates vaccines that generated substantial antibody titers with good affinity (Moreno et al., 2011). There are several choices in vaccine construction, including hapten design, selection of the carrier protein, the chemical positioning of a linker between the target antigen and the carrier protein, and selection of the adjuvant (Byrnes-Blake et al., 2001; Moreno et al., 2011; Peterson et al., 2007).

In theory, an anti-MA vaccine would generate antibodies that bind to MA so that when MA is subsequently introduced into the bloodstream, those antibodies would bind to it and form antibody-MA complexes within the circulatory system. Such complexes

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should be too large to readily cross the blood–brain barrier and therefore would reduce the rate or amount of MA entry into the brain. Antibody-bound drug would then be slowly released from antibody binding in the equilibrium state as residual-free drug to be metabolized and eliminated. Reduction in either rate or amount of MA entering the brain should attenuate its behavioral effects, including its ability to be rewarding. Here, we report on the effects of one such construct, succinyl-methamphetamine-keyhole limpet hemocyanin (SMA-KLH), in mice.

We assessed the ability of SMA-KLH to generate antibodies in mice by measuring serum titers across a 35-week period. In addition, we tested the functional effects of SMA-KLH by examining whether vaccinated mice would show attenuated behaviors induced by MA administration. Two behavioral assays were chosen based on known actions of MA and other psychostimulants in mice and rats. These were locomotor activity and conditioned place preference (CPP). MA and other psychostimulants, such as cocaine, increase locomotor activity at moderate doses and can induce stereotypic responses, such as sniffing, head bobbing, and other in-place activities, at higher doses (Antoniou et al., 1998; Brien et al., 1978; Ellinwood and Balster, 1974; Kuczenski and Segal, 1989). However, at very low doses, cocaine and amphetamine can cause hypolocomotion in rats and mice (George, 1989, 1990) and we recently confirmed this observation with MA in mice (Kitahama and Valatx, 1979; Singh et al., 2012). CPP is a procedure that has been used to assess the rewarding properties of many drugs (Bardo and Bevins, 2000; Carr et al., 1989; Schechter and Calcagnetti, 1993; Tzschentke, 1998). In CPP, drug administrations are paired with a distinct context while vehicle is paired with a different context. After several conditioning trials, the animal is allowed access to both contexts and the degree to which it approaches and spends more time in the drug-paired context is thought to measure drug reward.

2. Methods

2.1. Animals and housing

Female BALB/c mice were bred in the Houston VAMC vivarium from mice originally purchased from Charles River Laboratories (Wilmington, MA). Mice were 8 weeks of age and weighed approximately 20 g at the start of the study. They were group-housed (5 per cage) under temperature- and humidity-controlled conditions with a 12:12 h light/dark cycle (lights on from 0600). Food and water were available ad libitum. Procedures were approved by the Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (1996).

2.2. Drug

(+) Methamphetamine hydrochloride (MA; Research Triangle Institute, Research Triangle, NC) was dissolved in PBS and prepared as salt base. MA was administered at a dose of 1, 2 or 3 mg/kg (IP) in a volume of 5 mL/kg for the locomotor study. A dose of 0.5 mg/kg (SC) in a volume of 4 mL/kg was used for the conditioned place preference (CPP) study. These doses were chosen based on previous studies (Brien et al., 1978; Itzhak and Ali, 2002; Shabani et al., 2011b; Wheeler et al., 2009).

2.3. Generation of a carrier protein/methamphetamine conjugate

The hapten, succinyl methamphetamine (SMA), was prepared by refluxing a solution of methamphetamine HCl, succinic anhydride, and triethyl amine in CH_2Cl_2 for several hours. The solution was washed with 10% HCl and saturated NaCl, dried over anhydrous Na_2SO_4 and the solvent removed on a rotary evaporator. Thin layer chromatography (Analtech Uniplate) using 5% methanol in CH_2Cl_2 showed no starting material. Conjugation to keyhole limpet hemocyanin (KLH) (Thermo Fisher Scientific, Houston, TX) or fish gelatin (FG) involved first making a solution of 10 mg KLH or FG in 1-mL phosphate-buffered saline (PBS; pH 7.4). Then, a solution of sulfoNHS and EDC in 0.5 mL PBS was prepared and a solution of SMA in 10 μL DMSO was added. After several hours of stirring at room temperature, this solution was added to the KLH or FG solution. The pH was adjusted to ~7.5 with NaOH and stirring continued overnight at room temperature. The conjugate protein solution was passed through an NAP-25 column (GE HealthCare, Fairfield, CT) equilibrated with PBS and 2.5 mL was collected, giving a final concentration of KLH or FG conjugate of 4 mg/mL. Hapten structure was confirmed by NMR and conjugation conditions to carrier proteins were selected by maximum ELISA responses with anti-methamphetamine antisera.

2.4. Immunization, blood collection, and behavioral testing schedules

Mice were administered (SC) a vaccine consisting of 200-μl PBS containing 100-μg of SMA-KLH and 50-μg of monophosphoryl lipid A (MPL; Sigma; St. Louis, MO). They received a booster injection with the same vaccine formula twice, once at 3 weeks and again at 20 weeks after the initial injection. Blood was collected at weeks 2, 4, 6, 8, 12, 22, 24, 26, 35 and 40 after initial immunization and allowed to clot at room temperature for 2 h. Samples were centrifuged (4000 rpm for 15 m) and sera collected and stored at −80 °C until ELISAs were performed (see below). The locomotor study was conducted at week 7 and the conditioned place preference (CPP) study from weeks 31 to 33 as shown in the timeline in Fig. 1.

2.5. Determination of serum anti-MA titers by ELISA

To measure specific anti-MA antibody, ELISA plates (Immulon 2HB, Daigger, Vernon Hills, IL) were coated overnight in carbonate buffer (0.05 M; pH 9.6) using SMA conjugated to FG, a heterologous carrier protein. Background antibody binding to the carrier alone (which was very low in most samples) was subtracted from every sample to ensure that the results reflected antibodies specific for the hapten, MA.

Pooled ($n = 30$) or individual serum samples were added to plates in three fold serial dilutions starting at 1:5000 or 1:15,000 in PBS-tween (0.1%) and incubated for 2 h. After washing with PBS-Tween, goat anti-mouse IgG conjugated to horseradish peroxidase (Southern Biotech, Birmingham, AL) was then incubated in the plates for 30 min. The plates were again washed and then substrate (Tetramethylbenzidine, Sigma, St. Louis, MO) was incubated in the plates for 45 min. Reactions were stopped with 1 M HCl. The optical density (O.D.) was measured on a microplate reader (LabX, Canadian; ON, Canada).

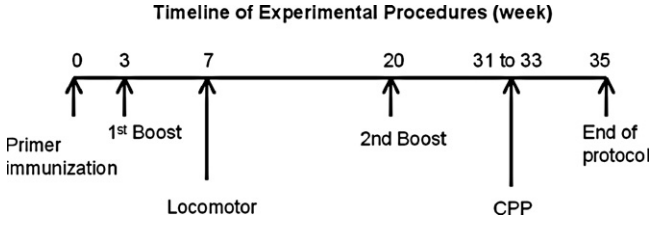

Fig. 1. The timeline of the vaccine administrations and behavioral tests is presented.