A Methamphetamine Vaccine Attenuates Methamphetamine-Induced Disruptions in Thermoregulation and Activity in Rats

Michelle L. Miller, Amira Y. Moreno, Shawn M. Aarde, Kevin M. Creehan, Sophia A. Vandewater, Brittani D. Vaillancourt, M. Jerry Wright Jr., Kim D. Janda, and Michael A. Taffe

Background: There are no approved pharmacotherapies for d-methamphetamine (METH) addiction and existing therapies have limited efficacy. Advances in using immunotherapeutic approaches for cocaine and nicotine addiction have stimulated interest in creating a similar approach for METH addiction. This study investigated whether active vaccination against METH could potentially attenuate responses to METH in vivo.

Methods: Male Sprague Dawley rats ($n = 32$) received a four-boost series with one of three candidate anti-METH vaccines (MH2[R], MH6, and MH7) or a control keyhole limpet hemocyanin conjugate vaccine. Effects of METH on rectal temperature and wheel activity at 27°C ambient temperature were determined. The most efficacious vaccine, MH6, was then contrasted with keyhole limpet hemocyanin conjugate vaccine in a subsequent experiment ($n = 16$), wherein radiotelemetry determined home cage locomotor activity and body temperature at 23°C ambient temperature.

Results: The MH6 vaccine produced high antibody titers with nanomolar affinity for METH and sequestered METH in the periphery of rats. In experiment 1, the thermoregulatory and psychomotor responses produced by METH at 27°C were blocked in the MH6 group. In experiment 2, METH-induced decreases in body temperature and locomotor activity at 23°C were also attenuated in the MH6 group. A pharmacokinetic study in experiment 2 showed that MH6-vaccinated rats had higher METH serum concentrations, yet lower brain METH concentrations, than control rats, and METH concentrations correlated with individual antibody titer.

Conclusions: These data demonstrate that active immunopharmacotherapy provides functional protection against physiological and behavioral disruptions induced by METH.

Key Words: Activity, d-methamphetamine, drug addiction, immunopharmacotherapy, stimulants, thermoregulation

D-methamphetamine (METH) addiction is a growing public health concern, but effective treatments are lacking. Pharmacotherapies have limited success for treating drug addiction and often produce adverse side effects (1). Immunopharmacotherapy is a promising alternative (2–6). In the active immunization approach, vaccination stimulates the immune system to produce antibodies against the drug of abuse. The drug-recognizing antibodies sequester drug molecules in the blood stream, which reduces distribution to the brain, thereby reducing drug effects. To date, vaccines have been shown to effectively attenuate effects of drugs such as cocaine (7–12), nicotine (13–21), morphine and heroin (22,23), tetrahydrocannabinol (24), and phencyclidine (25,26). Clinical studies of anticocaine and antinicotine vaccines are ongoing (27–29) and have shown titer-dependent efficacy during abstinence (28–31), as well as reduced subjective ratings of pleasurable drug effects (28). This translational success has encouraged the development of anti-METH vaccines.

Efficacy of both passive and active anti-METH vaccines has been investigated in preclinical studies. Passive administration of monoclonal antibodies can reduce METH self-administration (32), reduce METH-induced locomotor activity (33–35), and impair METH discrimination in a drug discrimination paradigm (36). Although passive immunotherapy has the advantage of producing immediate and dose-dependent antagonist effects, it may be limited as a therapeutic approach. Monoclonal antibodies are expensive to manufacture and effects are transient, which complicates patient compliance.

Active vaccination offers an improved alternative because the immune system provides antibody protection across a long period of time. Although this protection can last for years to decades for microbe vaccination, at present efficacy for only weeks to months (after each boost) has been shown for drug vaccines. Active vaccination is more cost effective and requires minimal patient compliance; however, immune responses can vary and the resulting vaccine efficacy might differ among individuals. Prior preclinical investigations using active anti-METH vaccines are limited and show mixed results. Byrnes-Blake *et al.* (37) found no change in METH-induced locomotor activity in vaccinated rats, even though antibody titers reached significant levels. More recently, however, active vaccination was shown to transiently increase METH self-administration in a manner that might be interpreted as consistent with reduced brain penetrance of drug (38); unfortunately, no data on METH distribution were presented. This limited evidence, along with preclinical evaluations of cocaine and nicotine vaccines, suggests that a diversity of METH vaccines may be necessary to further

From the Committee on the Neurobiology of Addictive Disorders (MLM, SMA, KMC, SAV, BDV, MJW, MAT), and the Department of Chemistry (AYM, KDJ), Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California.

Address correspondence to Michael A. Taffe, Ph.D., The Scripps Research Institute, Committee on the Neurobiology of Addictive Disorders, SP30-2400, 10550 North Torrey Pines Road, La Jolla, CA 92037; E-mail: mtaffe@scripps.edu.

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basic understanding of antidrug vaccination biology and, ultimately, to ensure efficacy in a variant population of addicts.

Novel strategies for creating an active vaccination for METH have been explored in several laboratories (38–40). Most relevant to the current study, Moreno *et al.* (40) systematically generated a series of chemical structures to target the most stable conformation of METH using GIX+ mice. Following vaccination, three of six candidates (MH2[R], MH6, and MH7) generated elevated antibody titers and nanomolar (+)-METH affinity. The present study sought to determine whether any of the three anti-METH candidates alter METH-induced disruptions in the thermoregulatory and locomotor behavior of rats.

Methods and Materials

Experimental Design

There were two experiments in this investigation. Experiment 1 was an initial screen to determine which of three most promising candidate anti-METH vaccines from a previous study in mice (40) would confer effects consistent with the attenuation of METH's impact in vivo in rats. Experiment 1 therefore assessed rectal temperature values under a high ambient temperature condition ($T_A = 27 \pm 1°C$) to determine effects on METH-induced hyperthermia and locomotor activity as previously described (41,42). Experiment 2 focused on the vaccine to emerge from the first experiment as the most promising (MH6) to determine effects on METH-induced hypothermia under a typical laboratory ambient temperature condition ($T_A = 23 \pm 1°C$). This experiment used radiotelemetry devices for precise assessment of body temperature and locomotion under freely moving conditions in the standard shoebox style cages (43). Table 1 shows experimental conditions for both experiments.

Animals

Forty-eight male Sprague Dawley rats (experiment 1: $n = 32$, experiment 2: $n = 16$; Harlan, Livermore, California) weighing ~320 grams on arrival were group housed in clear shoebox cages (two to three per cage) in a vivarium with a 12:12-hour light-dark cycle. Food pellets and water were available ad libitum. Rats were 11 weeks old at the start of both experiments. All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and under protocols approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Drugs and Haptens

D-methamphetamine was dissolved in .9% sterile saline and administered subcutaneously (SC) for acute challenges. A constant injection volume of 1 mL/kg was used. D-methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and 4-methylmethcathinone (4-MMC) were provided by RTI (Research Triangle Park, North Carolina) under contract to National Institute on Drug Abuse; amphetamine (AMPH) was purchased from Sigma-Aldrich (St. Louis, Missouri).

Methamphetamine haptens (MH2[R], MH6, and MH7) were coupled with a keyhole limpet hemocyanin (KLH) carrier protein and in formulation with the Sigma Adjuvant System (Sigma-Aldrich) as previously reported (40).

Equipment

Standard activity wheels attached to clear shoebox cages were used (Model ENV-046; Med Associates, St. Albans, Vermont), and the number of wheel quarter rotations in each session was collected by MED-PC IV software (Med Associates) (experiment 1

Table 1. Chronological Summaries of the Experimental Procedures

Experiment 1				Experiment 2			
Week	Vaccine	Blood	METH (Doses)	Week	Vaccine	Blood	METH (Dose)
0	V (Prime)			0	V (Prime)		
1		B		1			
2	V (Boost 1)	B		2	V (Boost 1) Surgery	B	
3		B		3			
4		B		4		B	.0, 1.0, 5.6 mg/kg
5	V (Boost 2)	B		5	V (Boost 2)		
6		B		6		B	
7		B		7			.0, 1.0, 5.6 mg/kg
8		B		8		B	
9	V (Boost 3)	B		9	V (Boost 3)		
10		B		10		B	
11		B	1.0, 5.6 mg/kg	11			
12		B	1.0, 5.6 mg/kg	12		B	.0, .5, 1.0, 3.2, 5.6 mg/kg
				13			.0, .5, 1.0, 3.2, 5.6 mg/kg
				14		B	
				15			
				16		B	
				17			
				18		B	
				19			
				20		B	3.2 mg/kg

Chronological summaries of the experimental procedures are shown: vaccine administration, blood collection, acute methamphetamine challenges (doses), and surgery (experiment 2 only). Experiment 1 investigated effects of vaccination with MH2(R), MH6, MH7, and KLH (control) on rectal temperature and wheel activity in rats at $T_A = 27 \pm 1°C$. Experiment 2 investigated effects of vaccination with MH6 and KLH (control) on body temperature and locomotor activity in rats at $T_A = 23 \pm 1°C$.

B, blood collection; KLH, keyhole limpet hemocyanin ; METH, d-methamphetamine; V, vaccine administration.