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Attenuation of Cocaine-Induced Locomotor Activity in Male and Female Mice by Active Immunization

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Abstract

Background and objectives—Immunotherapy for drug addiction is being investigated in several laboratories but most studies are conducted in animals of one sex. Yet, women show heightened immune responses and are more likely to develop autoimmune diseases than men. The purpose of this study was to compare the effects of an active anti-cocaine vaccine, succinyl-norcocaine conjugated to keyhole limpet hemocyanin, for its ability to elicit antibodies and alter cocaine-induced ambulatory activity in male versus female mice.

Methods—Male and female BALB/c mice were vaccinated (n=44) or served as non-vaccinated controls (n=34). Three weeks after initial vaccination, a booster was given. Ambulatory activity induced by cocaine (20 mg/kg) was assessed at 7-wk and plasma obtained at 8-wk to assess antibody levels.

Results—High antibody titers were produced in mice of both sexes. The vaccine reduced ambulatory activity cocaine-induced but this effect was greater in female compared to male mice.

Discussion and conclusions—The efficacy of this anti-cocaine vaccine is demonstrated in mice of both sexes but its functional consequences are greater in females than males.

Scientific significance—Results point to the importance of testing animals of both sexes in studies of immunotherapies for addiction.

Keywords

sex differences; vaccine; addiction; cocaine antibodies

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Introduction

Cocaine addiction is a serious problem in the United States but there are no approved pharmacotherapies (1). A common approach to developing medications for addiction is to test if an agent alters the behavioral effects of the abused drug (2, 3). Agents are often chosen because they target neuropharmacological mechanisms affected by the abused drug. Rather than selecting agents because they block or mimic effects of the abused drug in brain, we, and others are developing immunotherapies for addiction (4–6). An effective addiction vaccine should produce antibodies that sequester the drug in circulation, if ingested, to slow drug entry into the brain. Slower drug entry would reduce the drug's reinforcing effects (7–9) and presumably help the recovering addict maintain abstinence. We previously provided clinical support for the efficacy of a cocaine vaccine by showing it reduced cocaine-positive urines, particularly in addicts who produced high antibody levels (10, 11).

There was a great deal of variability in antibody responses among the subjects in the Martell studies and some participants produced very low levels of antibodies. We believe that this vaccine, made from succinyl norcocaine (SNC) and conjugated to cholera toxin B (12), could be improved by altering the carrier protein to which the SNC hapten is attached (4). Our recent work testing vaccines for methamphetamine (13) and morphine (14) utilized keyhole limpet hemocyanin (KLH) as the carrier protein and these vaccines blocked conditioned effects of the targeted drugs in rodents. KLH is likely to improve upon the immunogenicity of cholera toxin B. It can cause activation and maturation of monocyte derived dendritic cells for antigen presentation via signaling through the mannose receptor and eliciting high interleukin 12 production (15, 16). Thus, we constructed a new cocaine conjugate vaccine, SNC-KLH, and tested it in mice.

A second purpose of this study is to include assessments of the cocaine vaccine in mice of both sexes. This is important for vaccine treatment development in general because, relative to men, women show greater antiviral, inflammatory, and cellular immune responses, and are more likely to develop autoimmune diseases (17). For example, a candidate herpes simplex virus (HSV) vaccine is more efficacious in women than men (18). Sex hormones can affect vaccine responses; ovariectomized female mice exhibit less protection against HSV infection than those given progesterone and 17 β -estradiol (19). Further, gender should be considered in the design and application of anti-drug vaccines because male and female cocaine addicts differ in the course of addiction (20, 21) and in response to traditional treatments (22). Although there are more adult male cocaine users than females, this gender gap is closing among adolescent cocaine users (23) suggesting a possible increased prevalence of cocaine addiction among women in the future. A vaccine treatment may work well in females, but not males, but could be abandoned from further tests if only male subjects are evaluated. Or, women may show a more efficacious response (17) suggesting a lower amount be used to avoid potential adverse side-effects. Thus, we tested the ability of active immunization with the anti-cocaine vaccine, SNC-KLH, to alter the acute locomotor activating effects of cocaine in male and female BALB/c mice.

Methods

Animals and housing

Male and female BALB/c mice were housed in groups of five by sex in standard, shoebox cages under temperature- and humidity-controlled conditions. Food and water were available *ad libitum* in a colony maintained on a 12:12 light/dark cycle with lights on at 0600. All procedures were approved by the Institutional Animal Care and Use Committee in accordance with regulations of the Animal Welfare Act.

Mice were either administered the SNC-KLH vaccine as described below or served as non-vaccinated controls. The sample sizes per sex and vaccine group are as follows: 10 control females; 24 control males; 15 SNC-KLH females; and 29 SNC-KLH male mice.

Drug

Cocaine HCl (Research Triangle Institute, Research Triangle, NC) was dissolved in isotonic saline and prepared as salt base. A dose of 20 mg/kg was used because we find it increases ambulatory activity without inducing stereotypy.

SNC-KLH vaccine

Succinyl norcocaine (SNC) was prepared as described previously (12). The SNC-KLH vaccine was prepared according to the method we used previously to synthesize a 6-succinyl morphine-KLH conjugate vaccine (14).

Immunization and anti-cocaine determination

The vaccine (100 µg/mouse) plus 1.5 mg of alum (Sigma) was administered (IM) into the thigh of each hind leg (80–100 µl per site). A booster of the same amount was given at 3-wk. At 8-wks, blood was collected and titers of specific anti-cocaine IgG antibodies were determined using enzyme-linked immunosorbent assay (ELISA). Antibody titer levels were defined by the dilution of serum calculated to give an optical density of 1.0 (background in the ELISA was less than 0.2 for all samples at these dilutions). Sex differences in antibody titers were compared by t-test.

Locomotor activity

At 7-wks, mice were tested for effects of cocaine on ambulatory activity using an automated system of 15 apparatuses (Opto-M3; Columbus Instruments; Columbus, OH). Mice were placed individually into an acrylic test cage (17.5" L X 17.5" W X 8" H) located between parallel bars of infrared sensors placed 1" above the floor. Numbers of beam breaks were tabulated and relayed to a computer and data stored in 10-min time blocks. The first 60-min of the test session was used to habituate the mice to the apparatus with the last 10-min block utilized as baseline (non-drug). After habituation, mice were injected with cocaine (20 mg/kg; IP) and placed back into the apparatus for another 60-min. This session length was chosen because IP cocaine administration in mice leads to peak brain values within 5-min and has a half-life of 16-min in plasma and brain (24).

Assessments of cocaine-induced locomotor activity were determined by tabulating ambulatory counts per 10-min time period across the session. The activity levels from the final 10-min time block of the habituation session were used as co-variate in the analysis of the cocaine-induced activity levels. That is, the data were analyzed with 2 X 2 X 6 ANOCOVA representing between group factors of Sex and Vaccine with repeated measures on Time. Significance level was set at $P < 0.05$. Data are presented as mean ($\pm$ S.E.M.).

Results

Anti-Cocaine antibody levels

Antibody titers in serum did not differ between female ($117.8 \times 10^{-3} \pm 21.4 \times 10^{-3}$) and male ($143.7 \times 10^{-3} \pm 18.3 \times 10^{-3}$) mice at 8-wk after the initial immunization, $P > 0.10$.

Locomotor activity

Ambulatory activity counts during the 60-min habituation period did not differ by Sex or Vaccine group nor was the interaction significant, P 's > 0.10 . There was a significant Time effect, $F(5,370) = 14.78$; $P < 0.0001$. Activity levels decreased across this period (data not shown). The ambulatory activity counts during the final 10-min segment of the habituation period are shown on the left side of Fig. 1 and were used as a co-variate in the analysis of the cocaine-induced activity level data.

Ambulatory activity levels after cocaine administration are shown in Fig. 1 by 10-min time blocks in control and vaccinated, female and male mice. Ambulatory activity induced by cocaine differed across the session as supported by the significant Time effect, $F(5,370) = 2.47$; $P < 0.05$. Activity levels were significantly less in female versus male mice as supported by the significant Sex effect, $F(1,74) = 18.17$; $P < 0.0001$. Mice vaccinated with SNC-KLH showed lower levels of ambulatory activity as supported by the significant Vaccine effect, $F(1,74) = 5.19$; $P < 0.05$. In addition, the effect of Time showed a significant two-way interaction with Vaccine, $F(5,370) = 4.63$; $P < 0.00005$, and a significant three-way interaction with Vaccine and Sex, $F(5,370) = 2.42$; $P < 0.05$. As seen in Fig 1, these interactions with Time may reflect that the greatest differences between vaccine groups of both sexes were seen earlier in the session (i.e., 20–40 min time blocks), particularly for females, compared to later in the session (50–60 min time blocks).

Inspection of the data in Fig. 1 shows that, relative to the habituation baseline, cocaine administration led to a decrease in activity levels in female mice that were vaccinated with SNC-KLH. In contrast, male mice exhibited increased ambulation in response to cocaine administration but this level was lower among male mice that were vaccinated with SNC-KLH. Because the main effect of Sex was significant, we explored the role of variable on the functional effect of the vaccine by performing analyses separately in male and female mice. The Vaccine effect was significant among female mice, $F(1,23) = 6.04$; $P < 0.02$, but not among male mice, $P > 0.10$.

Discussion

The results of this study show that mice vaccinated with SNC-KLH produce antibodies against cocaine and exhibit attenuated cocaine-induced locomotor activity. Antibody titers do not differ between mice of both sexes. However, while the overall vaccine effect on locomotor activity was significant, separate analyses by Sex reveals that much of the significance is carried by female mice. This is seen despite the fact that there are almost twice as many male mice per Vaccine group than female mice. The interaction of Vaccine and Sex is not significant but this likely reflects that the vaccine decreased activity levels in both sexes. Overall, the results of this preliminary study point to the importance of testing potential vaccines for addiction in animals of both sexes.

While our prediction that female mice would show heightened immune responses relative to male mice did not hold (17), we did not anticipate that cocaine-induced locomotor activity would be lower in females compared to males. Much previous research conducted with rodents of both sexes shows the opposite – higher cocaine-induced activity levels in females versus males – most likely due to gonadal hormone effects (25–29). Indeed, female rats in estrus (29) or those that were ovariectomized and received hormone replacement with either estrogen alone or combined with progesterone, but not progesterone alone (27), exhibit enhanced cocaine-induced activity levels. Female rats in estrus also show enhanced responding for cocaine under a progressive ratio schedule of self-administration (30). In the present study, female mice were cycling freely and estrous stage was not assessed. It would be important to control for gonadal hormone status in future studies that assess immunization to cocaine.

Among most strains of mice and rats, females show greater activational effects of cocaine than males. Why the direction of this sex difference differs in some strains of mice, including the BALB/c strain (31, 32) that we employed in this study and in our prior work with vaccines (13), is not clear. However, the functional effect of the SNC-KLH vaccine was greater in female mice despite the fact that there was no sex difference in immune response. This suggests that females may be the preferred sex to use when examining potential vaccine treatments with this strain, a commonly employed strain among immunology researchers. It would be of interest to determine whether the SNC-KLH vaccine shows similar sex-dependent functional effects in other strains of rodents particularly those that show the more typical sex-dependent pattern of responsivity to cocaine.

A number of researchers have tested effects of passive or active immunization against cocaine in rodents but each study was conducted with rodents of one sex. Antibodies from active immunization with anti-cocaine vaccines reduced the conditioned rewarding, analgesic, and discriminative stimulus effects of cocaine in female rats (33, 34) and decreased the locomotor effects of cocaine in male rats (35, 36). Active and passive immunizations against cocaine in male rats also attenuated cocaine self-administration and blocked the reinstatement of this behavior in rats (12, 37). The results of the present study show the utility of testing potential anti-cocaine vaccines in animals of both sexes. Future studies should test the ability of cocaine (or other anti-drug) vaccines on a number of

behavioral procedures using a wider range of doses in both males and females as well as investigate the contribution of gonadal hormones.

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FIGURE 1.

Mean ($\pm$ SEM) ambulatory counts for vaccinated (Vax; closed symbols) and non-vaccinated (control; open symbols), female (circles) and male (squares) mice are shown by 10-min intervals after a cocaine (20 mg/kg) injection. The -10 point is the mean ambulatory counts during the last 10-min of a 60-min habituation session. Cocaine-induced ambulatory activity levels are decreased by the SNC-KLH vaccine and are lower in female versus male mice. See text for statistics.