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Sex differences in the effects of allopregnanolone on yohimbine-induced reinstatement of cocaine seeking in rats

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Abstract

Sex differences exist in several aspects of cocaine abuse, and recent research suggests that this may be due, in part, to differential sensitivity to stress. Women, compared to men, exhibit greater stress-induced cocaine craving and differential responses to both cocaine and stress during phases of the hormonal cycle. The goal of the present study was to compare male and female rats on the maintenance and extinction of cocaine seeking and on an animal model of stress-induced relapse by administering the pharmacological stressor yohimbine. An additional goal was to examine possible sex-specific treatment effects of the progesterone metabolite, allopregnanolone, on yohimbine-induced reinstatement. Male and female rats were trained to lever press for iv infusions of cocaine (0.4 mg/kg). Following a 14-day maintenance period, cocaine solutions were replaced with saline, and rats were allowed to extinguish lever pressing. Subsequently, rats were administered saline, yohimbine (2.5 mg/kg), or allopregnanolone (15 mg/kg) + yohimbine (2.5 mg/kg) priming injections on separate days using a within-subjects reinstatement procedure. The results indicated that females were more resistant to extinction than male rats and that both groups reinstated cocaine seeking following injections of yohimbine; however, female rats responded more than males to yohimbine-priming injections. Additionally, allopregnanolone blocked yohimbine's potentiating effect on responding in females but not males. These results suggest that females may be more sensitive than males to stress-induced reinstatement of cocaine-seeking behavior, and the progesterone metabolite, allopregnanolone, offers protection against this vulnerability to relapse.

Keywords

Sex differences; Relapse; Stress; Yohimbine; Cocaine; Allopregnanolone

1. Introduction

There are numerous examples of sex differences in cocaine abuse. Women, compared to men, are more likely to initiate cocaine use at an earlier age (Chen and Kandel 2002), and they have greater difficulty abstaining from cocaine than males (Ignjatova and Raleva 2009; Kosten et al. 1993). Research also indicates the presence of sex differences in stress reactivity, a major vulnerability factor implicated in drug abuse liability (Sinha 2008) and a leading cause of relapse (Sinha 2007; 2009; Sinha and Li 2007). Indeed, females exhibit greater levels of craving

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for cocaine and other drugs of abuse than males following the presentation of stressful stimuli (for review see Fox and Sinha 2009).

Recent findings have further implicated estrogen and progesterone in mediating responses to both cocaine and stress in women. This research indicates that the effects of these hormones are opposite in direction; estrogen facilitates, while progesterone and its metabolites attenuate these responses. For example, during the menstrual cycle, when endogenous estrogen levels are at their highest (i.e., follicular phase), women reported an increase in cocaine-induced positive subjective effects, while progesterone treatment prevented this facilitation (for reviews see Evans 2007; Terner and de Wit 2006). Similar findings have been reported with female animals, and in these studies estrogen treatment facilitated, while progesterone or allopregnanolone treatment attenuated the acquisition (Hu and Becker 2008; Jackson et al. 2006; Lynch et al. 2001), escalation (Larson et al. 2007) and reinstatement ((Anker et al. 2009; Anker et al. 2007; Feltenstein et al. 2009; Jackson et al. 2006); Larson and Carroll 2007; Larson et al. 2005) of cocaine-seeking behavior in female rats.

Progesterone is also involved in modulating craving elicited by stressful stimuli in women. In a study conducted by Sinha et al. (2007), women were separated into three groups that had high, medium, or low levels of circulating PROG, corresponding to the midluteal, early luteal, and follicular phases of their menstrual cycle, respectively. Women reported less stress-induced cocaine craving when they had high (midluteal) or moderate (luteal), compared with low (follicular) levels of circulating progesterone. The anxiolytic effects of progesterone may be attributed to its rapid metabolism into allopregnanolone, a more potent anxiolytic (Russell et al. 2008). However, little is known regarding how allopregnanolone affects stress as it relates to cocaine addiction. It is also not known whether this effect applies to males.

The purpose of the present study was to examine the effects of allopregnanolone in an animal model of stress-induced reinstatement of cocaine seeking by administering the pharmacological stressor, yohimbine. Female and male rats were trained to lever press for i.v. infusions of cocaine for 14 days and were then allowed to extinguish lever pressing for 21 days. Subsequently, rats were tested using a within-subjects reinstatement procedure by administering priming injections of yohimbine (Y), allopregnanolone + yohimbine (A+Y), or saline (S) at the beginning of each session.

2. Methods

2.1. Subjects

Gonadally intact female (n=11) and male (n=8) 90-day-old Wistar rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing 250 – 300 g and 350 – 400 g, respectively, served as subjects in the present study. During testing, the estrous cycle was allowed to randomly vary in female rats so that results would generalize to a range of hormonal conditions. Upon arrival, rats were pair-housed in plastic cages where they had access to ad libitum food and water. Rats were allowed to acclimate for at least 3 days before they were implanted with a chronic indwelling catheter in the jugular vein following methods previously described (Anker et al. 2009). Following surgery rats were placed in operant chambers where they remained for the duration of the study. During this time rats had free access to water, and they were given 16 (females) or 20 (males) g of ground food per day to maintain them at 85% of their free-feeding body weight. Rats were housed in temperature- (24°C) and humidity-controlled holding rooms under a constant light/dark cycle (12/12-h with room lights on at 6:00 am). The experimental protocol (0708A15263) was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC), and the experiment was conducted in accordance with the Principles of Laboratory Animal Care (National Research Council 2003) in laboratory facilities accredited by the American Association for the Accreditation of Laboratory Animal Care.

2.2. Self-administration, extinction, and reinstatement

The self-administration apparatus and procedure during maintenance and extinction were identical to those previously reported by Anker et al. (2009). Briefly, following a 3-day recovery period, rats were trained to lever press for i.v. infusions of cocaine (0.4 mg/kg/infusion) during 2-h sessions (9:00 am to 11:00 am) in stainless-steel octogonally-shaped operant response chambers that contained a drinking spout, recessed food receptacle, 2 stimulus lights, 2 response levers, and a house light. During cocaine self-administration sessions the house light was illuminated, and responses on active lever produced an i.v. infusion of cocaine and activated the stimulus lights located above the lever for the duration of the infusion. A response on the other inactive lever activated the stimulus lights above the lever but did not produce cocaine infusions. Following the acquisition of cocaine self-administration ($\geq$ 20 infusions for 3 consecutive days with a 2:1 active:inactive lever response ratio), rats were allowed to maintain stable cocaine intake for an additional 14 days under a maintenance condition. Subsequently, cocaine solutions were replaced with saline, and rats were allowed to extinguish lever pressing for 21 days, and during that time the stimulus lights, house light, and pump remained active. These stimuli were then unplugged for 3 days before the reinstatement condition to allow rats to extinguish lever pressing for drug-associated cues. During reinstatement, rats received an i.p. injection of S, 2.5 mg/kg Y, or A (15 mg/kg) and Y (A + Y). Injections occurred in the following sequence: S, Y, S, A+Y, S, Y. Yohimbine or S was injected immediately before the beginning of the session, and on the A + Y day A was administered 30 min before the session.

2.3. Drugs

Cocaine HCl was supplied by National Institute of Drug Abuse (Research Triangle Institute, Research Triangle Park, NC). It was dissolved in 0.9 % NaCl at a concentration of 1.6 mg cocaine HCl / 1 ml saline and refrigerated. Allopregnanolone was purchased from Sigma-Aldrich (St. Louis, MO) and was dissolved in peanut oil (20 mg allopregnanolone/ml peanut oil). Yohimbine was injected at the 2.5 mg/kg dose, as this dose had been shown to reliably reinstate cocaine seeking using a similar reinstatement procedure (Feltenstein and See 2006). Allopregnanolone was injected at the 15 mg/kg dose, as this dose effectively attenuated cocaine-primed reinstatement without disrupting food-maintained responding in a previous study (Anker et al. 2009).

2.4. Data analysis

Responses and infusions during maintenance and extinction and responses during reinstatement served as the primary dependent measures. Responses and infusions were averaged across 7-day blocks for the 14-day maintenance and 21-day extinction conditions. Within-group comparisons of active vs. inactive lever responses during the maintenance and extinction phases in female and male groups were analyzed using separate 2-factor repeated-measures ANOVA with block as the repeated measure and lever as the fixed factor. Between-group comparisons of active lever responses and infusions during maintenance and extinction were analyzed using 2-factor repeated measures ANOVA. For reinstatement, responses following S, Y, or A + Y injections were compared in female and male groups using separate single-factor repeated measures ANOVA. Between-group comparisons of responses following priming injections were analyzed using 2-factor repeated-measures ANOVA. After a significant main effect, post-hoc tests were conducted using Fisher's LSD protected t-tests. Statistical analyses were conducted using GB Stat (Dynamic Microsystems, Inc., Silver Spring, MD).

3. Results

3.1. Maintenance and extinction

Females and males responded a similar number of times for i.v. cocaine infusions during the maintenance condition [Mean (±SEM) females = 50.9 (±3.7), males = 54.4 (±8.8)], and the number of responses was stable across the 14 days (Figure 1, left). During the extinction condition, females responded more on the lever previously associated with i.v. cocaine self-administration than males ($F_{1, 56} = 4.64$, $p<0.05$), while both groups decreased responding over the 21 days (Figure 1, right). In addition, both groups responded more on the active lever compared to the inactive lever during the maintenance and extinction conditions.

3.2. Reinstatement

Figure 2 depicts the mean responses on the previously drug-paired lever (active lever) during the reinstatement condition. On the first day of Y administration, both groups responded significantly more than on the preceding day when S was administered (ps<0.5), and females responded more than males (ps <0.01). When A was administered on the second day of Y administration (A + Y), females responded similarly compared to the preceding S day, and responding was lower compared to the first Y day ($p<0.05$). In contrast, males responded more following A + Y when compared to S (ps<0.05), and there were no differences in responding during the first, second, and third day of Y administration in males. Comparison of active vs. inactive responding indicated that females, but not males, responded significantly more on the active lever when Y was administered alone (ps<0.05) (not shown). However, no differences were observed in responding on the two levers on the A+Y day for females.

4. Discussion

In the present study, the effects of allopregnanolone were examined on yohimbine-induced reinstatement of cocaine-seeking behavior in female and male rats. There were no sex differences during the maintenance of cocaine self-administration, however; females were more resistant to extinction of lever pressing following the removal of cocaine. This result supports previous work showing heightened extinction responding in female compared to male rats (Kerstetter et al. 2008; Kippin et al. 2005; Lynch and Carroll 2000; Lynch et al. 2005; Perry et al. 2008), and it suggests that women may have greater difficulty in abstaining from cocaine. Indeed, women cocaine abusers exhibit greater levels of cocaine craving then men (Elman et al. 2001; Kosten and Zhang 2008).

While several preclinical studies have reported yohimbine-induced cocaine seeking in males (Bongiovanni and See 2008; Brown et al. 2009; Dzung Le et al. 2009; Feltenstein and See 2006; Kupferschmidt et al. 2009), this was the first study to extend these results to females. In the present study, female rats were more sensitive than males to the potentiating effects of yohimbine on the reinstatement of cocaine seeking. This finding supports recent clinical work showing that women who abuse cocaine have greater sensitivity to stress during cocaine withdrawal than men (Fox and Sinha 2009). Taken together, these findings suggest that women may be more prone to stress-induced relapse of cocaine abuse.

An additional result was that allopregnanolone attenuated the effects of yohimbine on reinstatement responding in females but not males, suggesting that allopregnanolone's effects on cocaine-seeking behavior may be sex-specific. In a previous study allopregnanolone attenuated cocaine-primed reinstatement of cocaine-seeking behavior in female rats across several priming doses of cocaine, but it had no effect on males (Anker et al. 2009). Similarly, in humans, allopregnanolone's precursor, progesterone, attenuated cocaine-induced positive subjective effects in women (Evans and Foltin 2006; Sofuoglu et al. 2002; Sofuoglu et al.

2004), but this finding did not extend to males (Evans and Foltin 2006). However, only one dose of allopregnanolone and progesterone was tested in these studies, and higher doses may be required to attenuate drug-related measures in males. Taken together, these results suggest the presence of sex-specific treatment effects of progesterone and allopregnanolone on cocaine seeking.

One possible mechanism for allopregnanolone's attenuation of yohimbine-induced cocaine seeking may be related to its effects on the hypothalamic-pituitary-adrenal (HPA) axis. For example, HPA activation is associated with increased cocaine craving and relapse in humans (Sinha et al. 2006; Sinha et al. 2003) and heightened cocaine seeking in animals (Goeders and Clampitt 2002). Furthermore, yohimbine and allopregnanolone have opposite effects on the HPA axis: yohimbine activates the HPA axis (Myers et al. 2005), while allopregnanolone serves as a natural anxiolytic and attenuates HPA function following a stressful event (Patchev et al. 1994). Thus, in the present study, allopregnanolone may have blocked the yohimbine-induced activation of the HPA axis thereby attenuating its potentiation of cocaine-seeking behavior.

In addition to stress, evidence suggests a strong role for allopregnanolone in mediating other aversive aspects of drug abuse. For example, allopregnanolone blocked the development of tolerance and the expression of withdrawal signs after termination of benzodiazepine (Reddy and Kulkarni 1997b) and morphine (Reddy and Kulkarni 1997a) administration in mice. In addition, similar to its effects on cocaine-induced seizures (Kaminski et al. 2003; Leskiewicz et al. 2003), systemic (Luntz-Leybman et al. 1990) or intrahippocampal (Martin-Garcia and Pallares 2005) administration of allopregnanolone resulted in an attenuation of seizures precipitated by large doses of nicotine, purportedly through its mediation of the GABAergic neurotransmitter system (Frye 1995; 2007). Together these results indicate a strong role for allopregnanolone in treating stressful and aversive aspects of drug abuse in women.

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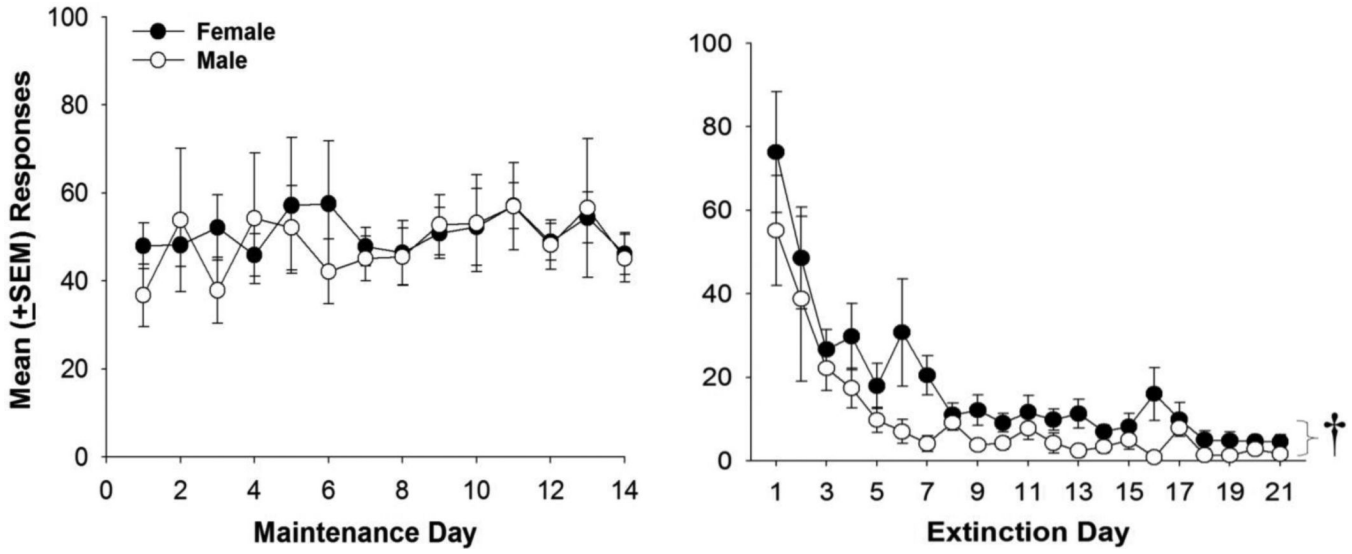

Figure 1.
Mean (+ SEM) cocaine (0.4 mg/kg) infusions earned during maintenance (left) and saline infusions during extinction (right). †= a significant sex difference (females>males) in active-lever responding during the extinction condition ($p < 0.05$).

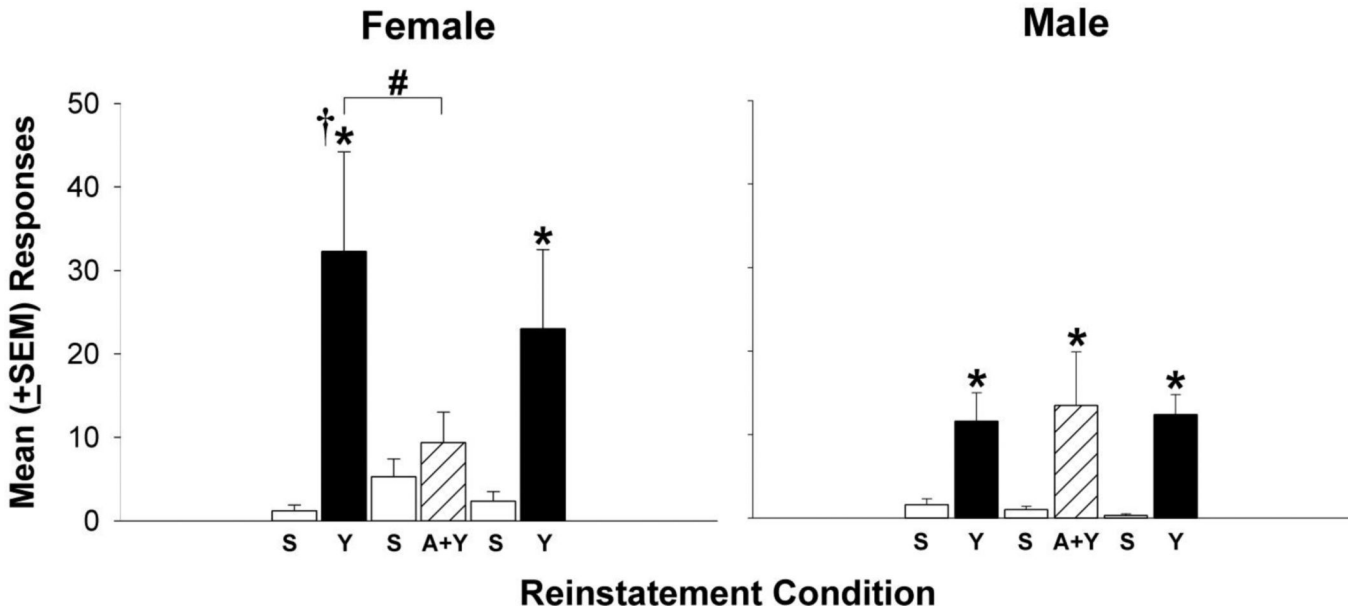

Figure 2.
Mean (± SEM) responses on the previously drug-paired lever following saline (S) or yohimbine (Y) priming injections during the reinstatement procedure. Asterisks indicate significantly greater responding following Y compared to S priming injections ($p < 0.05$). The # symbol indicates a significant difference in Y compared to responding following A+Y in the female group ($p<0.05$). †= a significant sex difference (females>males) in responding following the first Y injection ($p < 0.05$).