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Ovarian hormone replacement affects cocaine-induced behaviors in ovariectomized female rats

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Abstract

To determine whether cocaine-induced behavioral alterations are modulated by ovarian hormones, ovariectomized rats were randomly assigned to one of two drug treatment conditions: "binge" cocaine (three 15-mg/kg intraperitoneal (ip) injections, 1 h apart) or saline administration; and four hormone pretreatment sub-groups: vehicle control, estrogen, progesterone, or estrogen + progesterone. Cocaine-treated animals displayed more locomotor activity than saline-treated animals and locomotor activity was higher after the third injection than after the first two injections. When analyzed according to hormone group, the administration of estrogen + progestrone suppressed cocaine-induced locomotion after the first injection; this effect was significant when compared to estrogen-pretreated animals. While in each condition cocaine-treated animals displayed significantly higher stereotypic activity than saline-treated animals, in the estrogen + progesterone replacement group, there was more activity after the second injection of cocaine than after the first. Interestingly, animals in the estrogen + progesterone group had significantly lower plasma levels of the cocaine metabolite, benzoylecgonine, than animals in the progesterone or estrogen groups. These results extend our earlier findings in the intact female rat, which suggest an interaction between the endocrine environment, cocaine metabolism, and cocaine-induced behaviors. These effects may underlie reported sex and estrous cycle differences in cocaine-induced behavioral activity.

Keywords: "Binge" pattern cocaine; Female; Ovariectomized; Estrogen; Progesterone; Stereotypy; Locomotion; Fischer

1. Introduction

Cocaine is one of the most widely abused drugs in Western countries. Based on the 1998 National Household Survey on Drug Abuse, an estimated 1.75 million Americans used cocaine in a month's time; 36% of those users were female. Recent studies suggest that male and female humans and animals respond differently to different psychomotor stimulants [4,11,21,22]. For example, Lukas et al. [13] reported significant sex differences in response to acute cocaine administration in humans, with male participants achieving a faster and higher peak of plasma cocaine levels than females. Furthermore, men reported having experienced more episodes of euphoria than women [13]. However, Mendelson et al. [14] reported no gender or menstrual cycle differences in cocaine peak plasma cocaine levels. Rodents show behavioral sex differences in response to psychostimulants. Female rats display more intense behavioral responses to drugs of abuse than males [20], and have markedly enhanced stereotypic behaviors to their first cocaine dose and significantly higher locomotor activity than males [4]. Females also show less toxicity to cocaine than male rats [4]. Cocaine-induced stereotypic and locomotive behaviors have been shown to vary across the estrous cycle of the rat [19]. The fluctuating steroid hormone levels during the estrous cycle may play an important role in modulating the behavioral effects of cocaine. Furthermore, there may be an interaction between ovarian hormones and the behavioral effects of cocaine. These hormonal influences may contribute to the sex differences in the neurobiological effects of cocaine.

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It is well established that estrogen and progesterone function in the brain to regulate neuronal activity and influence behavior. These hormones alter a variety of reproductive [1] and non-reproductive behaviors [18] possibly through their actions on the dopamine, serotonin, and opioid systems [7–9,12,17,23]. Due the modulating effects of estrogen and progesterone on the CNS, these hormones may be an integral part of the cascade of events that are involved in cocaine's actions in the CNS. However, it is not clear what role(s) each of these hormones play individually or in combination in cocaine-induced subjective and physiological alterations. The present study was conducted to understand how estrogen and progesterone affect cocaine-induced stereotypic and locomotor activities. We hypothesize that due to the profound effect of ovarian hormones in the CNS, these steroids may affect cocaine-induced alterations in behavior. The aim of this study is to test this postulate. Results from this study may help to explain previous observations of gender and estrous cycle differences in response to cocaine [4,11,19].

2. Methods

2.1. Animals

Two cohorts (each with 24 animals) of ovariectomized (OVX) female Fischer rats purchased from Charles River were individually housed in standard cages, in a stress-minimized facility with free access to food and water, and maintained on a 12-h light/dark cycle with lights on at 10:30 a.m. EST. All NIH Guidelines for the Care and Use of Laboratory Animals were followed.

2.2. Drug and hormone treatment

All chemicals were purchased from Sigma (St. Louis, MO). Two weeks after ovariectomy, rats were randomly assigned to either cocaine- or saline-treatment groups, and then, further divided into one of four hormone pretreatment conditions: vehicle control (vehicle), estrogen, progesterone, or estrogen + progesterone ($n = 6$ animals per group). Forty-eight hours prior to the start of drug treatment, animals in the estrogen and estrogen + progesterone groups received subcutaneous injections of estrogen benzoate (50 μg) dissolved in sesame oil, and control groups (vehicle and progesterone) received vehicle injections. Four hours prior to cocaine treatment (44 h post-estrogen treatment) animals in the progesterone and estrogen + progestrone groups received subcutaneous injections of progesterone (500 μg) dissolved in sesame oil. Vehicle and estrogen animals received injections of sesame oil. This administration paradigm and the doses of steroids used have been shown to induce lordosis behavior in ovariectomized rats [16].

Four hours after progesterone or vehicle administration (11:00 a.m. EST), animals received the first of three interperitoneal (ip) injections, administered 1 h apart, of 0.9% saline (1 ml/kg) or cocaine (15 mg/kg dissolved in 0.9% saline at a concentration of 15 mg/ml). This "binge" dosing schedule was chosen to mimic the manner in which cocaine is often self-administered by humans both in terms of temporal pattern and in relation to circadian rhythm [3]. Throughout the study all injections were administered in each rat's home cage.

2.3. Locomotor activity

The spontaneous locomotor activity of each animal was monitored electronically. The monitor consists of a frame in which the standard cage is placed; three channels of digital information record interruptions of red light beams that traverse the cage [26]. The sum of counts on all three channels for each animal in 6-min time bins was used as a measure of spontaneous locomotor activity. With this technique, there is no change in the animal's environment during behavioral recording and it permits the simultaneous videotaping for later stereotypy scoring.

2.4. Stereotypic behaviors

Each animal was videotaped in its home cage for 25 s at 15, 30, and 45 min after the first two injections of cocaine or saline (no filming was done after the third injection in order to prepare to sacrifice the animals 30 min after the last injection of cocaine). The videotapes were analyzed for behavioral stereotypy by a trained observer blind to each animal's treatment condition. The rating for cocaine-induced stereotypic behavior was based upon a modification [6] of the Creese and Iversen scale [5] (see Table 1). A score of 10 was never observed during the course of this experiment.

2.5. Plasma levels of cocaine metabolite

Thirty minutes after the third injection, animals were sacrificed by decapitation, following brief (10–15 s)

Table 1
Rating scale from Daunais and McGinty [6]

Score	Behavior
1	asleep, inactive
2	alert, actively grooming
3	increased sniffing in one location
4	intermittent rearing and sniffing
5	increased locomotion and sniffing
6	intense sniffing in one location
7	continuous pivoting and sniffing
8	continuous rearing and sniffing
9	maintained rearing and sniffing for >25 s
10	splayed hind limbs

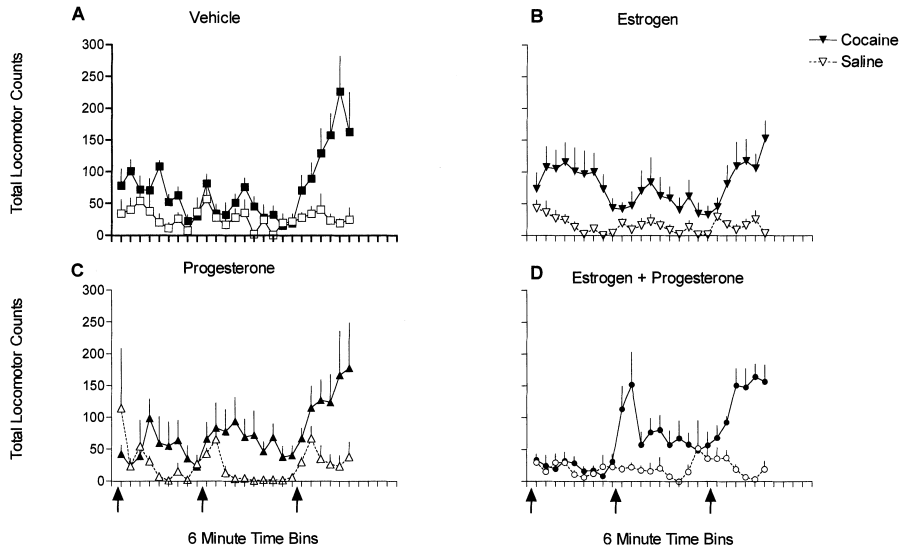

Fig. 1. Locomotor behavior of cocaine- and saline-treated animals in each of the four hormone pretreatment groups. Mean (±S.E.M.) total counts of locomotor behavior in the home cage after administration of cocaine or saline to OVX Fischer rats pretreated with one of the hormone regimens: vehicle, estrogen, progesterone, or estrogen + progesterone.

exposure to CO_2. This procedure is in compliance with NIH Guidelines for the Care and Use of Laboratory Animals and AVMA standards. Trunk blood was collected, allowed to clot, and then centrifuged at 3000 rpm for 15 min at 4°C. Plasma was collected and stored at −40°C until radioimmunoassay for the cocaine metabolite benzoylecgonine. Internal standards containing known amounts were run to correct for extraction losses. Samples (diluted 1:100; 25 μl) and standards (0 to 5400 ng/ml) were analyzed with Count-A-Coat Cocaine Metabolite radioimmunoassay kit from Diagnostic Products (CA). The intra-assay coefficient of variation was less than 3%. Results for assays were determined using a log–logit computer program.

2.6. Data analysis

2.6.1. Stereotypic behaviors

Since the distribution of the cumulative scores of stereotypy does not depart from normality, we examined the effects of hormone treatment on cumulative scores of stereotypic behavior using one- and two-way repeated measures ANOVAs. Due to differences in the scores of behavioral stereotypy between hormone treatment groups

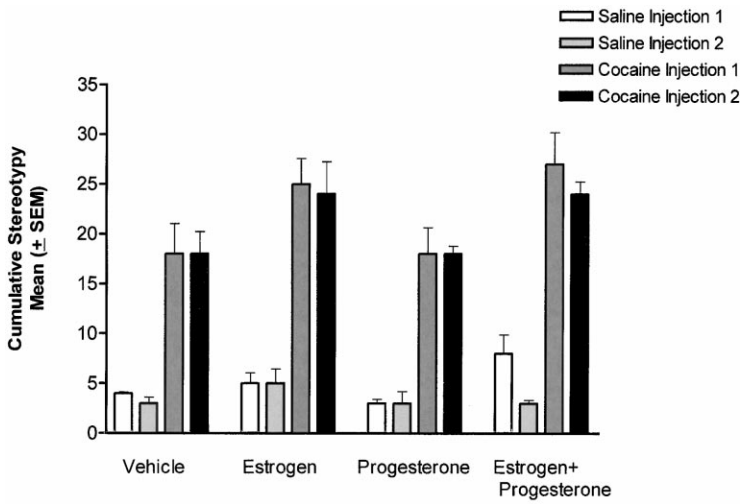

Fig. 2. Cocaine-induced difference scores of stereotypic behavior. Cumulative behavior scores after injection one (gray bars) and two (black bars) with data expressed as difference scores (each cocaine-treated animal's score minus the mean of saline-treated animal's in the same hormonal condition). Values shown are mean ± S.E.M.

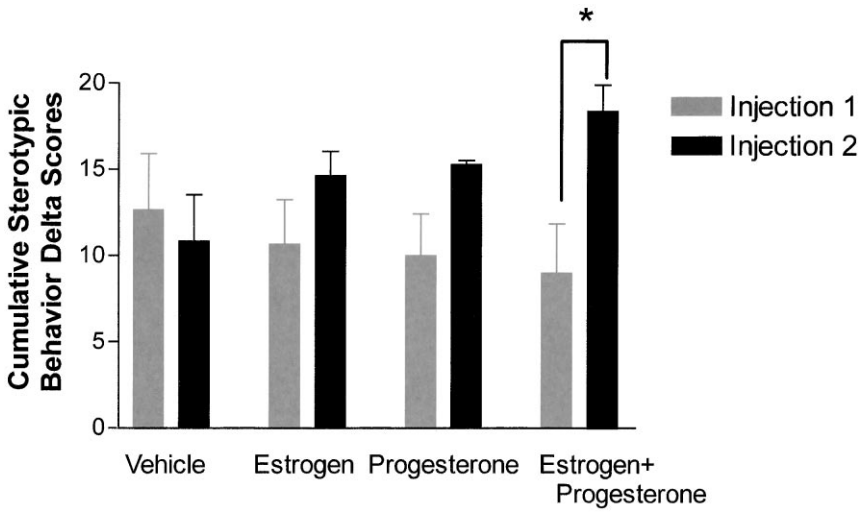

Fig. 3. Behavioral stereotypy of cocaine- and saline-treated female rats in each of the four hormone-treated conditions. Mean (±S.E.M.) scores of stereotypic behavior in the home cage after administration of cocaine or saline to OVX Fischer rats pretreated with one of the hormone regimens: vehicle, estrogen, progesterone, or estrogen + progesterone.

in the saline baseline condition, examination of the effect of cocaine was made using difference scores: the mean saline score of the same hormone treatment group was subtracted from each cocaine-treated animal's score.

2.6.2. Locomotor activity

To examine the response to "binge" pattern cocaine administration for different hormone conditions, a three-way ANOVA of total locomotor counts was used: "condition" (saline vs. cocaine) × "hormone treatment" (vehicle, estrogen, progesterone or estrogen + progesterone) × "injec-injection" (first 30 min of behavior after each injection) with repeated measures on the last factor, followed by Newman–Keuls post-hoc tests.

3. Results

3.1. Locomotor activity

Overall, cocaine treatment significantly increased locomotor activity [$f(1,40) = 29.61$, $P < .000005$]. In addition, there was a significant increase in locomotor activity of the cocaine-treated animals over time [$f(2,80) = 13.924$, $P < .00001$]. Interestingly, cocaine-treated animals had significantly higher locomotor activity after the third injection compared to the second and third injections ($p < .0002$ and $P < .005$, respectively, Fig. 1). When data for cocaine-treated animals were analyzed by hormone group, estrogen + progesterone pretreatment appeared to suppress cocaine-induced locomotor activity after the first injection. Post-hoc tests revealed that this effect was significant when compared to estrogen-pretreated animals (Newman–Keuls, $P < .05$).

3.2. Stereotypic behaviors

After the first injection, saline-treated animals in the estrogen-pretreatment group had higher stereotypic activity scores than those in the other three hormone groups [$f(3,20) = 4.81$, $P < .02$; Fig. 2]. Due to these differences in the saline baseline data, the scores of stereotypy from each cocaine-treated animal were expressed as the difference from saline-treated control mean (delta values; Fig. 3). A significant "hormone" × "injection" interaction was found [$f(3,17) = 3.261$, $P < .05$]; the estrogen + progesterone group displayed significantly more stereotypic behavior after the second cocaine injection, but not after the first ($p < .02$).

3.3. Benzoylecgonine levels

Pretreatment of ovariectomized rats with estrogen + progesterone resulted in differences in cocaine-induced behaviors. Interestingly, this hormone pretreatment group also differed from the other groups in plasma levels of the cocaine metabolite, benzoylecgonine (Table 2). Benzoylecgonine plasma levels differed significantly across the different hormone groups [$f(3,18) = 4.589$, $P < .05$]. Animals

Table 2
The mean (±S.E.M.) plasma levels of benzoylecgonine for all cocaine-treated rats

Hormone-treatment group	*n*	Benzoylecgonine level (ng/ml)
Vehicle control	6	400 ± 81
Estrogen	6	582 ± 61
Progesterone	6	644 ± 79
Estrogen + progesterone	5	349 ± 53 *

* Differs from all other hormone groups, $P < .05$.

in the estrogen + progesterone group had significantly lower levels of cocaine metabolite than animals in the estrogen- or progesterone-pretreatment groups (Newman–Keuls, $P < .05$ and $P < .02$, respectively).

4. Discussion

Cocaine increases both stereotypic and locomotor behaviors in ovariectomized rats pretreated with different steroid replacement treatments. This finding confirms and extends previous research in intact male and female rats [19,26]. Male Fischer rats, treated with chronic "binge" pattern cocaine administration for 14 days displayed increases in spontaneous locomotor activity for approximately 30 min following each injection [26]. Interestingly, Quiñones-Jenab et al. [19] reported that the locomotor activity of cycling female rats in response to acute "binge" pattern cocaine administration was similar to the pattern of the male response. However, the overall activity for females was higher than that for males, suggesting that females may be more "sensitive" to cocaine than males [19]. In the present study, ovariectomized rats had a different pattern of locomotor activity in response to cocaine than that reported for male and intact female rats; after the third cocaine injection, locomotor activity was higher than after the first two injections. With the exception of the ovariectomized control rats, the locomotor response of ovariectomized female rats to acute "binge" pattern cocaine administration and estrogen, progesterone, or estrogen + progesterone pretreatment appears to be similar to that of intact females. Overall, the level of locomotor activity for OVX females in this study was higher than that previously induced by acute cocaine administration in male rats.

No differences in cocaine-induced activity after estrogen or progesterone pretreatment were observed. Interestingly, cocaine-induced locomotion after the first injection appeared to be suppressed in the estrogen + progesterone replacement group. However, after the second and third injections locomotor behavior levels for estrogen + progesterone-pretreated animals reached levels comparable to the other three groups' (control, estrogen, or progesterone). In lordosis behavior, the interaction between estrogen and progesterone is bimodal. Progesterone can first act in synergy with estrogen facilitating reproductive behavior and than act to inhibit sexual receptivity [15]. This temporal relationship is important in the control of lordotic behaviors. The regulation of cocaine-induced behaviors and female reproductive behaviors may overlap in similar CNS mechanisms; progesterone may exert similar modulation on either the motor components of cocaine-induced behavior or rewarding aspects of the drug stimulation. The possible temporal relationship between estrogen and progesterone in the control of cocaine-induced locomotor activity remains to be elucidated.

Cocaine administration has been shown to increase stereotypic behaviors in both intact male and female Fischer rats [19,25]. We have previously demonstrated estrous cycle effects on cocaine-induced stereotypic behaviors after acute "binge" cocaine administration [19] finding that after the second injection of cocaine, rats during estrus (when both estrogen and progesterone are present) had higher stereotypic scores than those in the other phases of the cycle. In the present study, there were higher levels of stereotypic behaviors in the estrogen + progesterone group in the saline baseline condition. This effect may be related to the increase in motor activity associated with the facilitation of reproductive behaviors by ovarian hormones. When stereotypic behavior scores for cocaine-treated animals were expressed as difference from saline control groups, there were increases in scores of stereotypy in response to the second injection of cocaine when compared to the first injection in the estrogen + progesterone group. This finding suggests an interaction between hormones and cocaine-induced stereotypic behaviors and further, suggests a possible temporal interaction between estrogen and progesterone in the modulation of cocaine-induced behaviors.

Estrous cycle [19] and gender differences [2] in benzoylecgonine levels have been observed. It has been postulated that ovarian hormones modulate cocaine metabolism. Although it has been reported that cocaine can be spontaneously hydrolyzed to benzoylecgonine in solution [10], when levels of benzoylecgonine were measured in a cocaine–saline solution (pH 7.4, 25°C, 15 mg/ml) as used in our study, no detectable levels of benzoylecgonine were found (data not shown). The cocaine methyl esterase and ethyl transferase activities in the liver are significantly greater in male than in female rats [23]. It is possible that steroid regulation of these enzymes may underlie gender [2] and estrous cycle [19] differences in pharmacokinetics of cocaine metabolism [24]. In the present study, plasma levels of benzoylecgonine were affected by estrogen + progesterone administration. Thus, it is possible that these esterase mechanisms of degradation may be sensitive to estrogen and progesterone concentrations in the plasma, as is the case of the hepatic P450-related enzymes. Interestingly, the estrogen + progesterone pretreatment group also differed from the other groups in cocaine-induced behaviors. Thus, differences in cocaine metabolism may contribute to the observed differences in cocaine-induced behaviors.

Based on these observations, we hypothesize that cocaine-induced behavioral alterations are affected by the animal's endocrine profile. Because gonadal hormones have profound effects on brain functioning, the female's hormonal state at the time of cocaine administration may influence the effects of cocaine on brain functions involved in cocaine-induced behavior. This may be the basis of gender and estrous cycle differences in response to cocaine.

These results confirm our earlier findings in intact female rats, which suggest an interaction between the endocrine

environment and cocaine-induced behaviors. The present study extends our previous findings because estrogen and progesterone were directly manipulated, thus adding to the body of evidence showing an interaction between the endocrine profile of the female at time of drug administration and effects of the drug on the CNS.

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References

[1] Arnold AP, Breedlove SM. Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. Horm Behav 1985;19:469–98.
[2] Bowman B, Vaughan SR, Walker DQ, Davis SL, Little PJ, Scheffler NMTBF, Kuhn CM. Effects of sex and gonadectomy on cocaine metabolism in the rat. J Pharmacol Exp Ther 1999;290:1316–23.
[3] Branch AD, Unterwald EM, Lee SE, Kreek MJ. Quantitation of preproenkephalin mRNA levels in brain regions from male Fischer rats following chronic cocaine treatment using a recently developed solution hybridization procedure. Mol Brain Res 1996;14:231–8.
[4] Craft RM, Stratmann JA. Discriminative stimulus effects of cocaine in female versus male rats. Drug Alcohol Depend 1996;42:27–37.
[5] Creese I, Iversen D. The role of forebrain dopamine system in amphetamine induced stereotypic behaviors in the rat. Psychopharmacology 1974;39:345–57.
[6] Daunais JB, McGinty JF. Cocaine binges differentially alter striatal preprodynorphin and zif/268 mRNAs. Mol Brain Res 1995;29:201–10.
[7] Di Paolo T, Carmichael R, Labrie F, Raymond JP. Effects of estrogen on the characteristics of {3-H} spiroperidol and [3-H]RU24213 binding in rat anterior pituitary gland and brain. Mol Cell Endocrinol 1979;16:99–112.
[8] Di Paolo T, Poyet P, Labrie F. Effects of prolactin and estradiol on rat striatal dopamine receptors. Life Sci 1982;31:2921–9.
[9] Funabashi T, Brooks PJ, Pfaff DW. Preproenkephalin regulation during the estrous cycle of the female rat. Mol Brain Res.
[10] Isenschmid DS, Levine BS, Caplan YH. A comprehensive study of the stability of cocaine and its metabolites. J Anal Toxicol 1989;13:250–6.
[11] Kuhn C, Francis MS. Gender differences in cocaine-induced HPA axis activation. Neuropsychopharmacology 1997;16:399–407.
[12] Lauber AH, Romano GJ, Mobbs CV, Howells RD, Pfaff DW. Estradiol induction of proenkephalin messenger RNA in hypothalamus: dose-response and relation to reproductive behavior in the female rat. Mol Brain Res 1990;8:47–54.
[13] Lukas SE, Sholar MB, Fortin M, Wines J, Mendelson JH. Sex differences in plasma cocaine levels and subjective effects after acute cocaine administration in human volunteers. Psychopharmacology 1996;125:346–56.
[14] Mendelson JH, Mello NK, Sholar JW, Seigel AJ, Kaufman MJ, Levin JM, Renshaw PF, Cohen BM. Cocaine pharmacokinetics in men and in women during the follicular and luteal phases of the menstrual cycle. Neuropsychopharmacology 1999;2:294–303.
[15] Morin LP. Progesterone: inhibition of rodent sexual behavior. Physiol Behav 1976;18:701–15.
[16] Pfaff DW, Schwartz-Giblin S. Cellular mechanism of female reproductive behavior. In: Knobil E, Neill J, editors. The physiology of reproduction. New York: Raven Press, 1995. pp. 1487–568.
[17] Pfaus J, Pfaff DW. μ-, δ-, and κ-Opioid receptor agonists selectively modulate sexual behaviors in the female rat: differential dependence on progesterone. Horm Behav 1992;26:457–73.
[18] Priest CA, Pfaff DW. Actions of sex steroids on behaviours beyond reproductive reflexes. Non-reproductive actions of sex steroids. Chichester: Wiley, 1995. pp. 74–89.
[19] Quiñones-Jenab V, Ho A, Schlussman SD, Franck J, Kreek MJ. Estrous cycle differences in cocaine-induced stereotypic and locomotor behaviors in Fischer rats. Behav Brain Res 1999;101:15–20.
[20] Roberts DCS, Bennett SAL, Vickers GJ. The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. Psychopharmacology 1989;98:408–11.
[21] Robinson TE, Becker JB, Presty SK. Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex differences. Brain Res 1982;253:231–41.
[22] Robinson TE, Camp DM, Jacknow DS, Becker JB. Sex differences and estrous cycle dependent variation in rotational behavior elicited by electrical stimulation of the mesostriatal dopamine system. Behav Brain Res 1982;6:273–87.
[23] Romano GJ, Mobbs CV, Pfaff DW. Estrogen regulation of proenkephalin gene expression in the ventromedial hypothalamus of the rat: temporal qualities and synergism with progesterone. Mol Brain Res 1989;5:57–8.
[24] Sharma A, Plessinger MA, Miller RK, Woods JR. Progesterone antagonist mifepristone (RU 486) decreases cardiotoxicity of cocaine. PSEBM 1993;202:279–87.
[25] Spangler R, Zhou Y, Schlussman SD, Ho A, Kreek MJ. Behavioral stereotypies induced by 'binge' cocaine administration are independent of drug-induced increased in corticosterone levels. Behav Brain Res 1997;86:201–4.
[26] Unterwald EM, Ho A, Rubenfeld JM, Kreek MJ. Time course of the development of behavioral sensitization and dopamine receptor upregulation during binge cocaine administration. J Pharmacol Exp Ther 1994;270:1387–96.