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Behavioral Effects of Dopamine Agonists and Antagonists: Influence of Estrous Cycle, Ovariectomy, and Estrogen Replacement in Rats

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DÍAZ-VÉLIZ, G., M. S. BENAVIDES, S. BUTRÓN, N. DUSSAUBAT AND S. MORA, S. *Behavioral effects of dopamine agonists and antagonists: Influence of estrous cycle, ovariectomy, and estrogen replacement in rats.* PHARMACOL BIOCHEM BEHAV **62**(1) 21–29, 1999.—The influence of the hormonal condition on the reactivity of central dopamine (DA) receptors was studied in male and in intact and ovariectomized (OVX) female rats. They were injected with selective DA agonists, acting either on D_1 (SKF 38393, 2.5 or 10 mg/kg) or D_2 receptors (PPHT, 31.3 or 125 μg/kg), or with selective DA antagonists, acting either on D_1 (SCH 23390, 6.25 or 25 μg/kg), or D_2 receptors (sulpiride, 10 or 40 mg/kg). The acquisition of an avoidance conditioning response (CAR) and the performance of some spontaneous motor behaviors were tested. Both D_1 and D_2 agonists and antagonists impaired the acquisition of CARs in diestrous, OVX, and male rats. Nevertheless, the effects of these drugs during estrus and in estradiol-primed OVX rats were different according to the drug and the dose injected. Whereas SKF 38393 failed to induce significative changes, PPHT and low doses of SCH 23390 and sulpiride improved the acquisition of CARS in those groups. The effects on conditioning were not accompanied with equivalent changes in spontaneous motor activity. Estradiol level fluctuations that occur in female rats within the estrous cycle or in OVX rats primed with estradiol would be responsive of changes in the response to DA agents. Although the reactivity of central DA systems is differentially affected by the hormonal condition of the rat, the precise mechanism of this modulatory action remains unknown.

Dopamine agonists Dopamine antagonists Estrous cycle Conditioned avoidance responses Estradiol Ovariectomy

SEVERAL studies show that ovarian hormones can modulate behaviors mediated, at least in part, by dopamine (DA) systems (7,31,33). In this respect, we have reported that the acquisition of conditioned avoidance responses in rats is influenced by the estrous cycle (15), the ovariectomy, and the systemic administration of a single dose of estradiol benzoate (EB, 2 μg/rat) (16). Active avoidance conditioning has been related to the activity of central DA systems (9). Moreover, the inhibition of conditioned behavior, without affecting escape responding, is considered as a characteristic action of drugs that block DA transmission (2). Recently, we found that central DA activity can be affected differentially by the hormonal condition of the rat. The systemic administration of amphetamine and apomorphine had contrasting effects on active conditioned avoidance in rats according gender, estrous cycle, ovariectomy, and administration of estrogen (17). These findings imply that the changes in conditioning can be mediated by an interaction of ovarian hormones with DA systems in the CNS.

At least five major DA receptors subtypes have been identified in recent years by using cloning techniques (40,43); but, based on biochemical and pharmacological criteria and func-

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tional significance, all they can be included into the initial classification of D_1 and D_2 dopamine receptors (32,45) in terms of their opposing effects on adenylate cyclase and for which selective agonists or antagonists are available (40). Although the D_3 and D_4 receptors are similar to the D_2 receptor, the D_5 receptor displays structural and pharmacological similarities to the D_1 receptor (40,43). Available evidence indicates that the presynaptic receptors are exclusively D_2 and that the stimulation of these autoreceptors decreases the release and turnover of DA (19). This would explain the paradoxical hypomotility seen with low doses of D_2 agonists. Besides, several studies have shown that expression of D_2 agonist actions to produce the typical D_2 receptor-mediated behaviors requires a certain level of intrinsic D_1 stimulation that may be provided by endogenous DA under normal conditions (46). Following the development of selective D_1 and D_2 agonists and antagonists, extensive efforts have been directed towards evaluating the role of each receptor subtype in DA-mediated behaviors (5,13,44). For example, the selective D_1 agonist SKF 38393 has been reported to induce grooming (35,44) and oral dyskinesia (39). Activation of the D_2 receptor has been implicated in DA-mediated locomotion (3) and stereotypy (4). In some studies, the D_1 antagonist SCH 23390 has been shown to reduce locomotor activity and rearing behavior in rats (25). Although several lines of evidence are consistent with the hypothesis that central DA transmission is involved in learning and memory processes (2,9) the role of DA receptor subtypes in these processes has been less extensively studied (9,10). Neurochemical data have shown that there are sex differences in central DA levels (12), and that these levels also fluctuate across the estrous cycle of the rat (22,47). Furthermore, estrous cycle and estrogen administration affect DA turnover, release, and reuptake (8,14,20,) and influence DA receptors (6,21,23).

The present study was designed to investigate whether the hormonal condition of the rat (gender, estrous cycle, ovariectomy, and EB replacement) might influence the differential involvement of D_1 and D_2 receptors in the expression of avoidance conditioning and some spontaneous motor responses induced by selective DA drugs. We selectively manipulated DA receptor subtypes with systemic administration of the D_1 agonist SKF 38393 (41,42), the D_2 agonist PPHT (27), the D_1 antagonist SCH 23390 (1,28), or the D_2 antagonist sulpiride (29).

METHOD

Animals

Six hundred and forty female and 160 male Sprague–Dawley rats, weighing 180–200 g, were housed six per cage under a 12 L:12 D cycle (lights on 0800 h to 2000 h) with free access to food and water.

Vaginal smears were taken daily from 320 intact female rats to determine the different stages of the estrous cycle. Only rats exhibiting three or more consistent 4-day cycles were utilized. Because a previous report (15) showed great differences in the acquisition of CARs between diestrous and estrous female rats, only these phases were considered for the pharmacological treatments. Additionally, another group of 320 female rats was bilaterally ovariectomized under light ether anesthesia. Fourteen days after surgical removal of the ovaries, ovariectomized (OVX) rats were randomly assigned to two different groups that received either estradiol benzoate (EB, 2 μg/rat) or corn oil vehicle (0.2 ml/rat), injected subcutaneously (SC) into the dorsal region of the neck 48 h prior to the administration of the DA drugs.

A group of 160 male rats was also included in the study to compare their behavioral responses with those exhibited by female rats. Male and OVX rats were handled on several consecutive days before the experiments in the same manner as the intact females, to exclude handling-induced differences between groups.

Drugs

The following drugs were used: SKF 38393 (R(+)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol hydrochloride; Research Biochemicals Inc., Natick, MA); PPHT (N-0434) (±)-2-(*N*-phenyethyl-*N*-propyl)amino-5-hydroxytetralin hydrochloride; Research Biochemicals Inc.); SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride; Research Biochemicals Inc.); and sulpiride (±)-5-(aminosulfonyl)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide; Labomed, Chile). All drugs were dissolved in distilled water containing acetic acid 0.1%, and were administered SC in a volume of 1 ml/kg body weight. Ten rats of each hormonal condition were assigned to each dose of DA drugs: SKF 38393 (2.5 or 10 mg/kg), PPHT (31.3 or 125 μg/kg), SCH 23390 (6.25 or 25 μg/kg) and sulpiride (10 or 40 mg/kg). Control rats received an equivalent volume of solvent. Although separate solvent groups were tested for both doses of each DA drug, they were combined because statistical analysis of the behavioral data revealed no significant differences between them. Experimental procedures were conducted 5 or 30 min after DA agonists and DA antagonists, respectively. Each animal was injected only once to avoid complications associated with multiple injections of DA drugs. All treatments and experimental sessions were conducted between 1000 and 1400 h, by using a fixed design: spontaneous motility measures followed by conditioned avoidance training.

Spontaneous Motor Activity

Each rat was individually placed in a Plexiglas cage (30 × 30 × 30 cm), inside a sound-proof room. The floor of the cage was an activity platform (Lafayette Instrument Co., USA) connected to an electromechanical counter. Spontaneous motor activity was monitored during 30 min and, simultaneously, were recorded the number of times each animal reared, and the time (in seconds) spent in grooming behavior. Each animal was observed continuously via a Sony video camera connected to a VHS tape recorder. Scores were generated from live observations, and video sequences were used for later reanalysis.

Active Avoidance Conditioning

The conditioning experiments were carried out with a two-way shuttle box (Lafayette Instrument Co.) composed of two stainless steel modular testing units, which were equipped with a 18-bar insulated shock grid floor, two 28-V DC lights and a tone generator (Mallory Sonalert 2800 Hz, Lafayette Instrument Co.). Electric shocks were provided to the grid floor by a master shock supply (Lafayette Instrument Co.). Immediately after the spontaneous motor activity test, the rats were individually placed in the shuttle box and were trained in one single session of 50 trials. Each trial consisted of the presentation of a tone that after 5 s was overlapped with a 0.20-mA foot shock until the animal escaped to the op-

posite chamber, with a maximum shock duration of 10 s. A conditioned avoidance response (CAR) was defined as a crossing within the first 5 s (tone alone).

Statistical Analysis

Results were expressed as the means and standard errors. All data were analyzed separately for each drug by using a two-way analysis of variance (ANOVA), followed by a Newman–Keuls multiple comparisons test. In all cases, significance was set at $p < 0.05$.

RESULTS

Conditioned Avoidance Responses (CARs)

Control animals. Under solvent injection, there were no significant differences between intact female rats at diestrus and ovariectomized (OVX) rats in the avoidance performance. However, in estrous female rats and in OVX rats after a single injection of EB the acquisition of CARs was seriously deteriorated ($p < 0.001$ in both cases).

SKF 38393. Figure 1 (top panel) illustrates the effects of SKF 38393 on the active avoidance experiments. The overall ANOVA revealed a significant effect of SKF 38393 treatment, $F(2, 186) = 24.50$, $p < 0.0001$, and hormonal condition, $F(4, 186) = 4.48$, $p < 0.01$. The interaction between these two factors was also significant, $F(8, 186) = 4.67$, $p < 0.001$. Post hoc comparisons indicated that both doses of SKF 38393 significantly impaired the acquisition of CARs in diestrous ($p < 0.01$), OVX ($p < 0.001$), and male rats ($p < 0.05$). However, in estrous rats and in OVX rats with EB administration both doses of SKF 38393 failed to induce significant changes on the acquisition of CARs.

PPHT. Figure 1 (bottom panel) shows the effects of two doses of PPHT on the acquisition of CARs of female and male rats. Although the ANOVA indicated that there were no significant effects of PPHT treatment, $F(2, 186) = 0.12$, $p > 0.05$, a significant hormonal condition effect, $F(4, 186) = 13.18$, $p < 0.0001$, was observed. The significant interaction between PPHT treatment and hormonal condition, $F(8, 135) = 8.46$, $p < 0.0001$, suggests that the effect of PPHT is dependent upon the hormonal condition of the rat. Further analysis revealed that both doses of PPHT significantly enhanced the acquisition of CARs during the estrous stage ($p < 0.05$ and $p < 0.001$ respectively), but only the lower dose improved this behavior in OVX rats treated with EB ($p < 0.05$). In OVX rats both doses of PPHT significantly impaired the response ($p < 0.05$ and $p < 0.001$), whereas only the 125 μg/kg dose impaired this behavior in diestrous rats ($p < 0.01$). Male rats were no reactive to both doses of PPHT.

SCH 23390. This drug modifies the avoidance behavior, $F(2, 186) = 7.35$, $p < 0.001$, and this effect was dependent of the hormonal condition, $F(4, 186) = 35.01$, $p < 0.0001$. A significant dose × hormonal condition interaction was also observed, $F(8, 186) = 8.13$, $p < 0.0001$ (Fig. 2, top panel). Post hoc comparisons revealed that both doses of SCH 23390 produced dose-related decreases in avoidance acquisition at diestrus ($p < 0.005$ and $p < 0.0001$, respectively) and in OVX ($p < 0.0005$ and $p < 0.0001$ respectively), in which the effect of both doses was significantly different from one another ($p < 0.05$ in both cases). In estrous and OVX rats with EB administration the lower dose of SCH 23390 induced a significant improvement in the acquisition, while the higher dose (25 μg/kg) was without effect. In male rats the higher dose decreased the acquisition ($p < 0.001$), while 6.25 μg/kg was without effect.

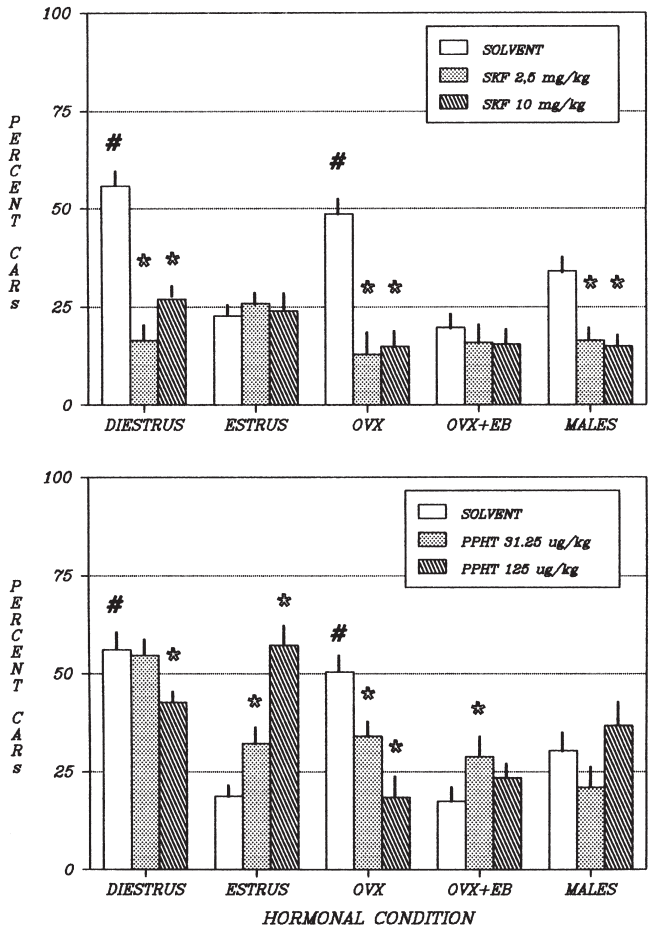

FIG. 1. Influence of hormonal condition (females at diestrus = DI, estrus = E, ovariectomized = OVX, ovariectomized with estradiol replacement = OVX + EB, and male rats) on the effects of DA agonists: SKF 38393 (top panel) and PPHT (bottom panel) on the acquisition of conditioned avoidance responses (CARs). Each bar represents the mean ± SEM of the percentages of CARs for 50 trials. Comparisons were made by using two-way ANOVA followed by post hoc Newman–Keuls test: $^{*}p < 0.05$ compared with its group and $^{\#}p < 0.001$ comparing diestrus with estrus or OVX with OVX + EB rats. The number of rats on solvent group was 20 and on each drug group was 10.

Sulpiride. Figure 2 (bottom panel) illustrates the effect of sulpiride on the avoidance acquisition of female and male rats. Sulpiride induced a significant effect on the acquisition of CARs, $F(2, 186) = 33.33$, $p < 0.0001$, and these effects appeared to vary significantly with the hormonal condition, $F(4, 186) = 120.00$, $p < 0.0001$. A dose × hormonal condition interaction was also significant, $F(8, 186) = 15.08$, $p < 0.0001$. Post hoc comparisons revealed that both doses of sulpiride significantly impaired the acquisition of CARs in diestrous and OVX rats ($p < 0.0001$ in all cases) in a dose-dependent manner. In estrous and in OVX rats with EB administration the lower dose of sulpiride significant improved the acquisition of CARs ($p < 0.001$ in both cases) while the higher dose was without effect. Sulpiride 40 mg/kg decreased the acquisi-

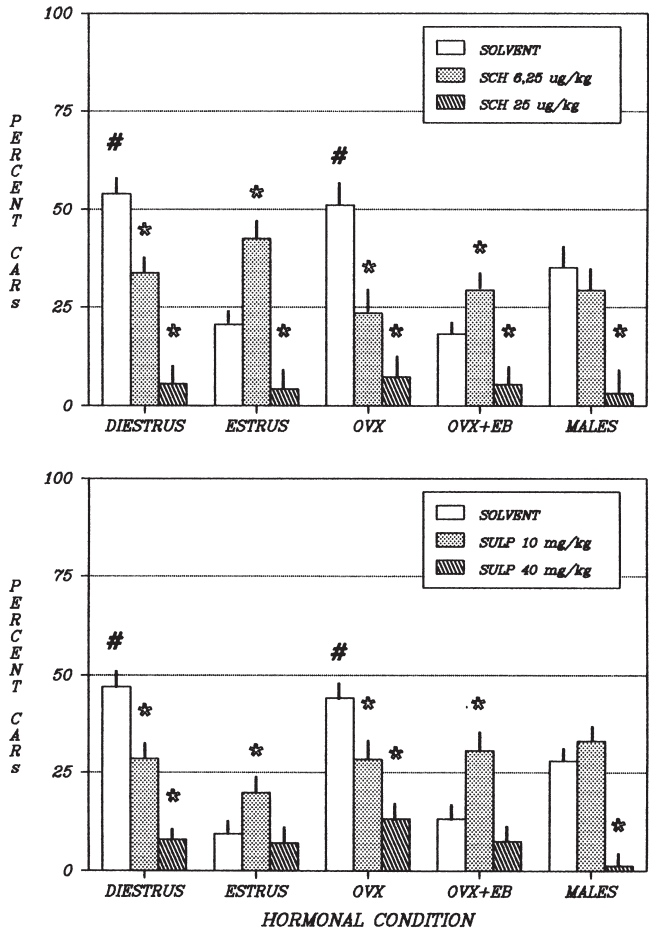

FIG. 2. Influence of hormonal condition (females at diestrus = DI, estrus = E, ovariectomized = OVX, ovariectomized with estradiol replacement = OVX + EB, and male rats) on the effects of DA antagonists: SCH 23390 (top panel) and sulpiride (bottom panel) on the acquisition of conditioned avoidance responses (CARs). Each bar represents the mean ± SEM of the percentages of CARs for 50 trials. Comparisons were made by using two-way ANOVA followed by post hoc Newman–Keuls test: $^{*}p < 0.05$ compared with its group and $^{\#}p < 0.001$ comparing diestrus with estrus or OVX with OVX + EB rats. The number of rats in the group was 20 and on each drug group was 10.

tion ($p < 0.001$) in male rats, while the lower dose was without effect.

Spontaneous Motor Behaviors

Control Animals. Rats injected with solvent exhibited significant changes in motor activity according to their hormonal condition. Motor activity and grooming behavior were significantly lower at estrus than at diestrus ($p < 0.05$). EB replacement to OVX rats produced a reduction in both behaviors ($p < 0.05$). Rearing behavior was not affected by the hormonal condition of the control rat.

SKF 38393. The overall effects of D_1 agonist on motor behaviors are summarized in Table 1. Two-way ANOVA revealed a significant effect of the hormonal condition, $F(4, 186) = 3.10$, $p < 0.05$, and a significant treatment × hormonal condition interaction, $F(8, 186) = 2.45$, $p < 0.05$. Subsequent Newman–Keuls test showed that the higher dose of SKF 38393 increased motility only in rats at estrus ($p < 0.0005$) and in EB-primed OVX rats ($p < 0.005$). Diestrous, OVX, and male rats were not sensitive to this stimulatory effect of SKF 38393. In rearing, behavior was observed only a significant dose × hormonal condition interaction, $F(8, 86) = 3.53$, $p < 0.01$, indicating that the SKF 38393 effect was dependent of the hormonal condition. SKF 38393 (10 mg/kg) increased the number of rears in estrous rats and in OVX rats with EB administration ($p < 0.05$ in both cases). Both doses of SKF 38393 were without effect in diestrous, OVX, and male rats. SKF 38393 induced a highly significant effect on grooming behavior, $F(2, 186) = 53.89$, $p < 0.0001$. The hormonal condition was also significant, $F(4, 186) = 7.07$, $p < 0.001$. However, no significant interaction between these two factors was observed. Both doses of SKF 38393 enhanced the time spent in grooming behavior in all female and male rats ($p < 0.005$ in all comparisons).

TABLE 1

EFFECTS OF SKF 38393 (D_1 AGONIST) ON SPONTANEOUS MOTOR ACTIVITY UNDER DIFFERENT HORMONAL CONDITIONS

Hormonal Condition	*n*	Motor Activity (Counts)	Rearing (No.)	Grooming (s)
Diestrus				
Solvent	20	844.9 ± 64.8	45.8 ± 4.9	459.0 ± 24.1
SKF 2, 5 mg/kg	10	927.9 ± 73.9	45.0 ± 6.0	743.9 ± 58.6*
SKF 10 mg/kg	10	971.8 ± 65.2	54.5 ± 5.2	693.0 ± 56.9*
Estrus				
Solvent	20	638.2 ± 40.9†	54.8 ± 3.8	322.7 ± 44.3†
SKF 2. 5 mg/kg	10	709.8 ± 59.2	56.6 ± 4.8	542.6 ± 53.0*
SKF 10 mg/kg	10	943.0 ± 57.2*	65.3 ± 4.2*	614.3 ± 41.4*
OVX				
Solvent	20	779.5 ± 41.4	33.9 ± 3.2	432.8 ± 43.9
SKF 2, 5 mg/kg	10	746.0 ± 51.8	35.6 ± 3.9	645.1 ± 55.8*
SKF 10 mg/kg	10	837.8 ± 50.9	40.7 ± 4.8	634.6 ± 29.2*
OVX + EB 2 μg				
Solvent	20	545.9 ± 53.6†	42.6 ± 5.2	305.4 ± 33.2†
SKF 2, 5 mg/kg	10	593.9 ± 52.7	44.2 ± 4.5	525.7 ± 53.9*
SKF 10 mg/kg	10	758.8 ± 51.2*	58.9 ± 6.2*	638.9 ± 28.4*
Males				
Solvent	20	694.2 ± 43.9	49.0 ± 5.1	396.8 ± 54.7
SKF 2, 5 mg/kg	10	706.1 ± 58.6	46.3 ± 4.0	729.0 ± 54.4*
SKF 10 mg/kg	10	745.7 ± 61.4	43.6 ± 5.8	623.4 ± 49.7*

Values are expressed as mean ± SEM. Comparisons were made by using two-way ANOVA followed by post hoc Newman-Keuls test: $^{*}p < 0.05$ compared with its solvent group, $^{\dagger}p < 0.05$ comparing diestrus with estrus or OVX with OVX + EB.

PPHT. Table 2 shows the effects of PPHT on spontaneous motor behaviors. Two-way ANOVA revealed highly significant effects of the PPHT treatment on motor activity, $F(2, 186) = 88.87$, $p < 0.0001$, rearing, $F(2, 186) = 62.12$, $p < 0.0001$, and grooming behavior, $F(2, 186) = 79.12$, $p < 0.0001$. Post hoc comparisons indicated that both doses of PPHT significantly decreased the spontaneous motor activity in all the groups treated ($p < 0.005$ and $p < 0.0005$ for each dose). In rearing behavior was also observed a significant interaction PPHT treatment × hormonal condition, $F(8, 186) = 4.33$, $p < 0.05$. Both doses of PPHT produced a significant decrease in the number of rears in diestrous, OVX, and male rats ($p < 0.005$), while only the higher dose of PPHT caused a significant decrease ($p < 0.005$) of this behavior in the other groups.

TABLE 2

EFFECTS OF PPHT (D_2 AGONIST) ON SPONTANEOUS MOTOR ACTIVITY UNDER DIFFERENT HORMONAL CONDITIONS

Hormonal Condition	*n*	Motor Activity (counts)	Rearing (No.)	Grooming (s)
Diestrus				
Solvent	20	787.9 ± 48.6	45.7 ± 4.1	441.4 ± 29.7
PPHT 31.3 μg/kg	10	452.4 ± 57.5*	25.7 ± 3.0*	396.7 ± 34.1
PPHT 125 μg/kg	10	207.0 ± 26.3*	8.7 ± 2.5*	53.6 ± 11.5*
Estrus				
Solvent	20	640.9 ± 37.3†	46.1 ± 4.4	317.6 ± 32.2†
PPHT 31.3 μg/kg	10	422.9 ± 47.1*	37.1 ± 5.0	205.1 ± 27.0*
PPHT 125 μg/kg	10	269.0 ± 23.7*	10.3 ± 1.7*	59.3 ± 10.3*
OVX				
Solvent	20	673.7 ± 39.8	32.4 ± 3.1	379.5 ± 23.9
PPHT 31.3 μg/kg	10	281.0 ± 27.8*	21.1 ± 2.1*	357.0 ± 29.4
PPHT 125 μg/kg	10	215.0 ± 35.9*	8.8 ± 2.1*	112.1 ± 26.9*
OVX + EB 2 μg				
Solvent	20	545.2 ± 39.8†	39.2 ± 4.5	305.6 ± 19.8†
PPHT 31.3 μg/kg	10	375.4 ± 40.6*	34.9 ± 2.3	231.9 ± 12.6*
PPHT 125 μg/kg	10	252.1 ± 47.7*	22.9 ± 3.4*	224.3 ± 11.4*
Males				
Solvent	20	683.9 ± 51.5	49.9 ± 5.0	347.9 ± 43.8
PPHT 31.3 μg/kg	10	286.7 ± 26.4*	16.3 ± 2.5*	295.3 ± 38.9
PPHT 125 μg/kg	10	266.6 ± 49.5*	15.1 ± 3.1*	188.9 ± 34.8*

Values are expressed as mean ± SEM. Comparisons were made by using two-way ANOVA followed by post hoc Newman-Keuls test: *$p < 0.05$ compared with its Solvent group, †$p < 0.05$ comparing diestrus with estrus or OVX with OVX + EB.

In grooming behavior was also observed a significant interaction PPHT treatment × hormonal condition, $F(8, 186) = 4.45$, $p < 0.05$. Post hoc comparisons showed that both doses of PPHT significantly decreased grooming behavior in estrous rats ($p < 0.01$ and $p < 0.0005$ for each dose), and in OVX rats treated with EB ($p < 0.005$ for both doses), but only PPHT 125 μg/kg decrease this behavior in diestrous, OVX, and male rats ($p < 0.01$ in all cases).

SCH 23390. The spontaneous motor responses induced by SCH 23390 are shown in Table 3. SCH 23390 significantly modify motor activity, $F(2, 186) = 3.70$, $p < 0.05$, and rearing behavior, $F(2, 186) = 8.06$, $p < 0.001$. Both doses of SCH 23390 significantly decreased motor activity and rearing behavior in male rats ($p < 0.01$ and $p < 0.0005$ for each dose), whereas the higher dose (25 μg/kg) depressed motor activity and rearing behaviors in all female rats ($p < 0.005$ in all cases). Two-way ANOVA revealed significant effects of the dose, $F(2, 186) = 3.18$, $p < 0.05$, hormonal condition, $F(4, 186) = 4.51$, $p < 0.01$, and interaction treatment × hormonal condition, $F(8, 186) = 4.78$, $p < 0.05$, on grooming behavior. The higher dose of SCH 23390 significantly diminished time spent in grooming behavior in all rats treated ($p < 0.005$ in all female rats and $p < 0.01$ in male rats). The lower dose (6.25 μg/kg) did not induce any significant change in grooming behavior in females at estrus and in OVX rats injected with EB. However, this dose of SCH 23390 significantly increased this behavior in diestrous, OVX, and male rats ($p < 0.05$ in all groups).

Sulpiride. Data from sulpiride effects on spontaneous motor behavior are summarized in Table 4. Two-way ANOVA revealed a significant effects of the dose of sulpiride on motor activity, $F(2, 186) = 6.56$, $p < 0.005$, rearing, $F(2, 186) = 7.92$, $p < 0.001$, and grooming behavior, $F(2, 186) = 16.88$, $p < 0.0001$. Post hoc comparisons showed that the higher dose of sulpiride significantly depressed motor activity and rearing behavior in diestrous, OVX, and male rats ($p < 0.005$ in all cases), while both doses were without effect in estrous rats and in OVX rats injected with EB. Sulpiride 40 mg/kg significantly decreased the time spent in grooming behavior in all female and male groups ($p < 0.05$ in all groups).

DISCUSSION

The present study demonstrates that behavioral responses to selective DA agonists and antagonists are affected by hormonal changes that occur in female rats within the estrous cycle or after ovariectomy and EB replacement, suggesting that the hormonal condition could trigger behavioral changes through the interaction with DA systems in the brain. The findings reported here confirm and extend our previous observation that the impairment in conditioned avoidance responses (CARs) during estrus and in EB-primed OVX rats can be completely reversed by the systemic administration of amphetamine, an indirect DA agonist, and partially by apomorphine, a direct D_1/D_2 agonist (17).

In the present experiments, both D_1 and D_2 selective agonists and antagonists impaired the acquisition of CARs in diestrous, OVX, and male rats, but in estrous and EB-primed OVX rats the effects were different according to the drug and the dose injected. SKF 38390 failed to induce any change in estrous and in OVX rats with EB administration, while PPHT and the lower dose of both DA antagonists improved the ac-

TABLE 3

EFFECTS OF SCH 23390 (D_1 ANTAGONIST) ON SPONTANEOUS MOTOR ACTIVITY UNDER DIFFERENT HORMONAL CONDITIONS

Hormonal Condition	*n*	Motor Activity (Counts)	Rearing (No.)	Grooming (s)
Diestrus				
Solvent	20	778.7 ± 54.2	49.2 ± 5.4	473.6 ± 33.2
SCH 6.25 μg/kg	10	797.9 ± 46.5	37.3 ± 6.8	545.2 ± 21.9*
SCH 25 μg/kg	10	455.1 ± 52.3*	13.2 ± 1.9*	138.3 ± 24.8*
Estrus				
Solvent	20	603.3 ± 47.3†	49.6 ± 6.3	328.8 ± 31.8†
SCH 6.25 μg/kg	10	639.6 ± 43.9	39.0 ± 3.5	288.1 ± 21.6
SCH 25 μg/kg	10	351.4 ± 38.6*	17.2 ± 0.9*	97.0 ± 20.9*
OVX				
Solvent	20	673.0 ± 28.8	31.9 ± 2.8	464.3 ± 33.7
SCH 6.25 μg/kg	10	639.6 ± 53.9	38.6 ± 4.0	548.3 ± 24.2*
SCH 25 μg/kg	10	380.6 ± 40.8*	19.1 ± 3.2*	231.4 ± 17.0*
OVX + EB 2 μg				
Solvent	20	546.6 ± 38.9†	37.7 ± 4.1	335.2 ± 26.2†
SCH 6.25 μg/kg	10	570.6 ± 52.1	31.9 ± 3.7	369.1 ± 38.9
SCH 25 mg/kg	10	322.0 ± 25.5*	11.9 ± 2.5*	208.4 ± 22.5*
Males				
Solvent	20	761.0 ± 45.0	46.0 ± 5.2	401.8 ± 38.4
SCH 6.25 μg/kg	10	574.0 ± 57.8*	27.3 ± 3.2*	563.4 ± 28.3*
SCH 25 μg/kg	10	256.8 ± 46.7*	11.4 ± 2.7*	253.3 ± 37.6*

Values are expressed as mean ± SEM. Comparisons were made by using two-way ANOVA followed by post hoc Newman-Keuls test: *$p < 0.05$ compared with its Solvent group. †$p < 0.05$ comparing diestrus with estrus or OVX with OVX + EB.

quisition in these same groups. As expected, the high dose of SCH 23390 and sulpiride depressed the avoidance conditioning and the spontaneous motor activity in all groups studied. These findings suggest that the role of DA receptors in mediating this behavior is hormone dependent. The stimulant effects of PPHT, SCH 23390, and sulpiride on conditioning behavior were evident only late after the peak plasma concentration of estradiol; that is, during estrus (15) and 48 h after a single injection of EB to OVX rats (16). Nevertheless, the D_2 agonist and the D_1 antagonist completely reversed the impairment of CARs during estrus, while both drugs only antagonized partially the depressant effects of EB in OVX rats, suggesting that these hormonal conditions are not equivalent and that other endogenous agents, in addition to estradiol, may also be necessary to develop the complete stimulant effect of these drugs. The stimulant effect of D_1 antagonist could be the consequence of a facilitatory effect of endogenous DA on D_2 receptors, whereas the stimulant effects of sulpiride could be related to the blockade of D_2 presynaptic receptors. Taken as a whole, these results support the idea that the acquisition improving properties of DA agonists can be mediated by the D_2 receptor. The effect induced by the selective D_2 agonist PPHT was similar to that produced by the nonselective D_1/D_2 agonist apomorphine (17). Our study shows similar effects produced by SKF 38393 and SCH 23390 on avoidance performance. One explanation to the impairment of acquisition of CARs produced by SKF 38393 may be related to its partial efficacy at the D_1 receptor (41) antagonizing endogenous DA from producing its maximal effect. However, this possibility seems unlikely, as there is evidence that both partial SKF 38393 and full agonist SKF 82958 similarly impair responding for conditioned reward (10). In addition, several studies demonstrate that drugs having opposite effects on dopamine release often have similar effects in a variety of behavioral paradigms (26,34). In fact, according to our data, both SKF 38393 and PPHT impaired avoidance in diestrous and OVX rats, but they induced opposite effects in estrous and EB-primed OVX rats, clearly suggesting that the effects of SKF 38393 and PPHT were differentially influenced by the hormonal status of the rat.

Although it is known that conditioned avoidance performance can vary according to the footshock intensity, no significant differences were observed between the footshock thresholds applied to the different groups in our experimental conditions. Therefore, the results could not be explained by changes in pain sensitivity. Nevertheless, we cannot rule out the possibility that changes on avoidance performance could be secondary to influences on emotional or motivational aspects. Female rats at estrus exhibit escape failures, which are not observed at diestrus (38), suggesting that ovarian status could determine changes in the emotional reactivity to the footshock. In this way, rats in diestrus seem to be more responsive to the shock, because they performed faster than estrous rats even during the earlier trials of the 50-trials session.

The higher dose of SKF 38393 stimulated locomotor activity and rearing behavior only in estrous and EB-primed OVX rats, whose exploratory responses were depressed before treatment, while both doses of PPHT reduced motor activity and rearing in all groups studied, regardless of the hormonal condition. The effects of D_1 antagonist on motor behaviors were no distinctively affected by the hormonal condition of the rats. In fact, the higher dose of SCH 23390 decreased mo-

TABLE 4

EFFECTS OF SULPIRIDE (D_2 ANTAGONIST) ON SPONTANEOUS MOTOR ACTIVITY UNDER DIFFERENT HORMONAL CONDITIONS

Hormonal Condition	*n*	Motor Activity (Counts)	Rearing (No.)	Grooming (s)
Diestrus				
Solvent	20	808.7 ± 47.6	39.9 ± 4.4	434.6 ± 32.1
SULP 10 mg/kg	10	749.9 ± 48.0	36.5 ± 5.9	445.1 ± 24.5
SULP 40 mg/kg	10	631.0 ± 22.1*	26.4 ± 2.6*	259.1 ± 32.5*
Estrus				
Solvent	20	674.4 ± 49.5†	46.9 ± 4.1	315.7 ± 35.7†
SULP 10 mg/kg	10	647.4 ± 44.1	38.6 ± 2.5	317.9 ± 26.9
SULP 40 mg/kg	10	670.1 ± 34.1	38.1 ± 3.7	194.5 ± 41.1*
OVX				
Solvent	20	648.6 ± 29.6	37.7 ± 2.3	497.0 ± 39.3
SULP 10 mg/kg	10	729.9 ± 47.0	36.7 ± 2.9	441.9 ± 29.5
SULP 40 mg/kg	10	465.4 ± 54.6*	23.1 ± 5.6*	311.4 ± 39.5*
OVX + EB 2 μg				
Solvent	20	576.6 ± 26.3†	35.8 ± 3.6	395.7 ± 25.4†
SULP 10 mg/kg	10	538.0 ± 34.9	43.9 ± 4.4	451.1 ± 56.7
SULP 40 mg/kg	10	550.0 ± 41.9	38.4 ± 2.7	225.1 ± 51.4*
Males				
Solvent	20	656.1 ± 39.7	20.7 ± 1.9	481.8 ± 56.4
SULP 10 mg/kg	10	728.5 ± 27.6	22.3 ± 1.0	555.6 ± 43.4
SULP 40 mg/kg	10	457.6 ± 45.9*	16.8 ± 0.9*	312.5 ± 42.9*

Values are expressed as mean ± SEM. Comparisons were made by using two-way ANOVA followed by post hoc Newman-Keuls test: *$p < 0.05$ compared with its Solvent group. †$p < 0.05$ comparing diestrus with estrus or OVX with OVX+EB.

tility and rearing behavior in all male and female rats. However, behavioral effects of D_2 antagonist were affected by the hormonal condition of the rat, the higher dose of sulpiride depressed motor activity and rearing behavior in diestrous, OVX, and male rats but not in estrous and EB-primed OVX rats. The hypomotility induced by PPHT could be due to the stimulation of D_2 autoreceptors, which decrease the release, and turnover of DA (19). Some evidence indicate that a certain level of tonic D_1 receptor stimulation is provided by endogenous DA under normal conditions, and that this D_1 receptor tone is necessary for produce the typical D_2 behaviors (46). Then, SCH 23390, by blocking D_1 receptors, which are under tonic stimulation by endogenous DA, could antagonize a postsynaptic D_2 receptor-mediated effect. The improvement in the acquisition of CARs induced by PPHT in intact female rats at estrus and in OVX rats primed with EB cannot be merely explained by the increase in motor activity, considering that this D_2 agonist significantly depressed motor activity in all groups of rats. Moreover, DA antagonists in a dose that was without effect on motility significantly increases acquisition of CARs in rats at estrus and OVX with EB replacement. These data suggest that modulatory influences of gonadal hormones on avoidance response are not necessarily accompanied with equivalent changes in spontaneous motor activity. It is difficult to compare effective doses in different behavioral paradigms (i.e., motor activity and acquisition of CARs) because both classes of behaviors differ not merely in the degree of complexity, but they also reflect changes in DA activity at different sites in the CNS. Moreover, the interaction between DA receptors and gonadal hormones varies in different areas of the brain (6,23,47).

There are reports considering the possibility that DA receptors are involved in the expression of grooming behavior. Some evidence shows that grooming is potentiated by D_1 agents, whereas D_2 agonists had the opposite effect (35,44). Our results are in agreement with this evidence, the selective D_1 agonist SKF 38393 increased the time spent in grooming in all groups studied. On the contrary, the D_2 agonist PPHT inhibited this behavior; however, this effect was more potent in estrous and EB-primed OVX rats. As expected, the high dose of the D_1 antagonist SCH 23390 severely decreased grooming behavior in all experimental groups; nevertheless, the low dose of SCH 23390 enhanced grooming without modify motor activity in male rats and in diestrous and OVX rats, but failed in induce any change in estrous and EB-primed OVX rats. Only the higher dose of the D_2 antagonist sulpiride was able to inhibit grooming behavior in all experimental groups, including those whose exploratory activities were not affected by this drug. It is contradictory that grooming behavior is improved by drugs that are active at D_1 receptors and attenuated by drugs that are active at D_2 receptors, irrespective of whether they are agonists or antagonists. One explanation for these results can be that the expression of this behavior is normally depending on the balance of DA activity at its two different receptors.

In summary, these results suggest a modulating influence of gonadal hormones on behaviors, which are thought to reflect the activity of dopaminergic neurons. Behavioral responses induced by dopaminergic drugs in estrous and EB-primed OVX rats are different, and sometime opposite, to that observed in other hormonal conditions. Although the function of central DA systems is differentially affected by the

hormonal condition of the rat, the precise mechanism of action of this effect remains unknown. The increase in estrogen during the estrous cycle triggers a complex sequence of endocrine events, via genomic mechanisms, which include ovulation as well as the secretion of progesterone and estrogen from the corpus luteum during diestrus. Estrogen administration has been reported to have a direct interaction with dopaminergic systems and affect several nonreproductive behaviors (7,8,12,16,20,22,23,33,47). The effects, sometimes contradictory, seem to depend on procedures employed and, in particular, on the time elapsing between estrogen administration and testing. A short-latency effect occurring less than 1 h after estrogen administration implies a direct neuronal activity, but at longer latencies, hormonal effect implies a more traditional genomic mechanism. Ovarian hormones may act directly, modifying DA receptor sensitivity or influencing other neurotransmitter systems. For example, there is behavioral evidence that central serotonergic activity varies with the hormonal condition of the rat (18), and two drugs used in this study have been reported acting on serotonergic receptors, SKF 38393 with agonist actions at 5-HT_{1C} receptors (11) and SCH 23390 as a potent antagonist of 5-HT_{1C} receptors (11,24). Another mechanism could be secondary to the effects of sex hormones at a hypothalamic level. In fact, fluctuations of estrogen would reflect changes in the activity of DA systems in the hypothalamus, which influence synthesis and release of hypothalamic peptides, like LHRH (33). We have previously reported that the behavioral effects of LHRH could be a consequence of its interaction with dopaminergic systems in the brain (36,37). Finally, the interaction between DA drugs and ovarian hormones may not be a centrally mediated phenomenon, but instead may be due to alteration of the metabolism of drugs by various hormonal conditions (30). Regardless of the site of action, the demonstration of an influence of ovarian hormones on DA drug effects could have important clinical implications.

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