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Influence of Estrogen and Progesterone on behavioral Effects of Apomorphine and Amphetamine

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MICHANEK, A. AND B. J. MEYERSON. *Influence of estrogen and progesterone on behavioral effects of apomorphine and amphetamine.* PHARMAC. BIOCHEM. BEHAV. 16(6) 875–879, 1982.—A study was made of the influence of apomorphine, D- and L-amphetamine on the effects of different hormonal treatments used to induce copulatory behavior in female rats. Female copulatory behavior, (lordosis response), which has been shown to be inhibited by increased DA and 5-HT neuronal activity, was depressed by apomorphine, D- and L-amphetamine in ovariectomized rats treated with estrogen + progesterone. When lordosis behavior was activated by estrogen alone the inhibitory action of the drugs, especially that of apomorphine was considerably less pronounced. However, the effect of D-amphetamine on stereotype activity was not greater after treatment with estrogen + progesterone than after administration of estrogen alone. Estrogen had a slight stimulatory effect on stereotype activity in non-drug-treated rats, while progesterone had an inhibitory effect. Possible mechanisms underlying the observed interactive effect of progesterone and monoamines on lordosis behavior, such as a progesterone-induced alteration of monoaminergic transmission, are discussed.

Sexual behavior Stereotype activity D-Amphetamine L-Amphetamine Apomorphine Estrogen Progesterone

DRUGS altering central monoaminergic activity have been found to influence certain forms of hormone-dependent behavior, such as the copulatory response (lordosis behavior) in the female rat [9, 10, 11]. Neuroanatomic studies have demonstrated a nuclear uptake of estrogen in certain monoaminergic neurons in the brain, indicating a direct hormone-monoamine interaction [5]. The hormone-monoamine relationship can be studied by investigating the influence of hormones on a certain effect of a psychotropic drug. It has been demonstrated that in ovariectomized female rats treated with estradiol benzoate (EB) + progesterone (PROG), the lordosis behavior in response to being mounted by a male is inhibited in a dose-dependent manner by the 5-HT receptor stimulating drug LSD [3]. Inhibition of lordosis by LSD could be increased by raising the dose of PROG, an effect not obtained by increasing the doses of EB [16,17]. This indicates an interaction between PROG and 5-HT mechanisms.

Amphetamine has been shown to inhibit the lordosis response in a dose-dependent fashion, an effect mainly related to dopamine (DA) mechanisms [12,13]. Lordosis was also inhibited by the DA receptor stimulant drug apomorphine [2].

Recent studies indicate that female sex hormones also influence apomorphine- and D-amphetamine-induced stereotype activity [4, 7, 14]. Using lordosis behavior and stereotype activity as behavioral parameters, the effects of different hormonal treatments in combination with drugs acting on DA transmission were investigated.

METHOD

The methods employed were the same as those described in detail in an earlier paper [12].

Female Sprague-Dawley rats (Specific Pathogen Free) weighing 300–350 g, purchased from Anticimex, Sollentuna, Sweden, were used as subjects. They were housed in groups of 5–6 per cage, under a reversed light cycle (the dark period from 0900 hr to 2100 hr), in an air-conditioned room maintained at 21±1°C. Commercial rat food pellets and water were provided ad lib. The rats were ovariectomized two weeks before the first test of lordosis behavior, which was induced by treatment with EB 10 μg/kg 48 hr before PROG 0.4 mg/rat, to assess the response level of the groups. Lordosis tests with drug treatment were started two weeks after the initial test. In the same room as the experimental females, test males were housed. These were of the Wistar strain, sexually experienced, and were kept in individual cages, which also served as test cages.

Copulatory Behavior

Lordosis behavior was activated in two ways: (1) By injection of EB 10 μg/kg given 48 hr before PROG 0.2, 0.4 or 4.0 mg/rat. Testing for the lordosis response was started 4 hr after the latter injection. (2) By injection of EB 10 μg/kg daily for three consecutive days. Testing was started 28 hr after the last EB injection.

All tests were performed between 1300 hr and 1630 hr. Tests for the occurrence of lordosis behavior were per-

formed by placing the female in the cage of a sexually vigorous male. The female was considered positive if she responded with a lordosis reflex to at least two out of six mounts by the male. For each treatment the percentage number of females which showed a positive lordosis response was calculated.

Repetitive Activity and Stereotyped Behavior

Behavior stereotypies are as a role described in the literature as a drug-induced abnormal repetition of certain behavior elements. Further, the behavior as such displayed in this way, seems not relevant for the particular environmental situation. A detailed analysis of the spontaneous behavior of a not drug treated rat shows that also here exists a repetitive activity. The difference between such a repetitive activity and stereotyped behavior is quantitative but also qualtitative. Qualitatively in the sense that the repetitive performance under the non-drug condition is goal-directed. Previous dose-response studies in this laboratory [12,13] have provided evidence for the fact that there exists a continuity between repetitive activity and stereotyped behavior. Low doses of drugs producing stereotyped behavior, such as amphetamine, increases repetitive activity. At higher doses the behaviors displayed become more and more abnormal such as continual licking, gnawing or moving head from side to side. In the following we use the term "stereotype activity" for any repetive activity, including spontaneous behaviors and drug-induced abnormal ones, lasting continually for at least ten seconds.

The rats were placed individually in transparent (Macrolone) cages (41×25×14 cm) and after being allowed to adapt for 15–30 minutes they were tested for the occurrence of stereotype activity. Each rat was observed for a duration of 10 seconds at a time, once a minute for 10 minutes. If the subject performed any activity continually during the 10 seconds observation time it was given one score for that particular behavior. Thus, the maximal possible score for each rat was 10, and the average score for the tested number of rats (N) was calculated. With this operational definition untreated cage-adapted animals reached a score of stereotype activity of 1.6–2.1. The behavior categories recorded were exploratory activities, grooming and gross motor behaviors such as licking, gnawing and head movements. (For a detailed description of behaviors see [12].)

The females were given the following hormonal treatments: (1) EB 10, 20 or 40 μg/kg. Tests were performed 53–54 hr after the injection of EB. (2) PROG 0.1, 0.4 and 1.0 mg/rat. Tests were performed 5–6 hr after the injection of PROG. (3) EB 10 μg/kg given 48 hr before PROG 0.4 mg/rat. Tests were performed 5–6 hr after the injection of PROG. (4) EB 10 μg/kg given daily for three consecutive days. Tests were performed 28–29 hr after the last injection of EB. All tests were carried out between 1400 hr and 1500 hr.

Statistical Methods

Differences between treatments were tested for statistical significance by means of the Chi²-test ($df=1$) for lordosis response and stereotype behavior patterns and by the Mann-Whitney U-test for stereotype activity. Results for pooled groups for each form of treatment are reported in the tables, but comparisons were made between independent groups.

Drugs Injected

Estradiol benzoate and progesterone (NV Organon through Erco Ltd., Stockholm) were dissolved in olive oil, and D- and L-amphetamine (Ulleråker Hospital Pharmacy, Uppsala) and apomorphine HCl (Sandoz, Basel) were dissolved in saline. All compounds were injected subcutaneously, in the dorsal region of the neck.

RESULTS

Lordosis Behavior

Apomorphine. Ten minutes after injection of apomorphine in a dose of 200 μg/kg, given after EB 10 μg/kg + PROG 0.2, 0.4 or 4.0 mg, the lordosis response was inhibited as compared with saline controls (Table 1A). This effect was most pronounced after a PROG dose of 0.4 mg, $\chi^2=13.6$, $p<0.001$. The effect was shortlasting, and after 60 min the response level typical of this hormone treatment was recovered.

The same dose of apomorphine given to rats treated with EB alone was considerably less effective, and there was no significant inhibition of the lordosis response (Table 1B). Thus, apomorphine 200 μg/kg had a significantly higher effect, $\chi^2=7.7$, $p<0.01$, when given to animals treated with EB 10 μg/kg + PROG 0.4 mg/rat than with EB alone. When a higher dose of apomorphine, 500 μg/kg was injected in EB + PROG treated rats (Table 1A), the lordosis response was strongly inhibited 10 min after the injection, $\chi^2=18.2$, $p<0.001$, but had recovered after 60 min. The same effect was observed in females treated with EB alone—the lordosis behavior was inhibited at 10 min, $\chi^2=7.7$, $p<0.01$, and the effect had disappeared after 60 min (Table 1B).

D-Amphetamine. In previous studies on the inhibitory effect of D-amphetamine on lordosis behavior in EB + PROG treated rats the ED_{50} was found to be 2.6 mg/kg [12]. Thirty minutes following injection of D-amphetamine in a dose of 2.5 mg/kg in the present study the lordosis response was significantly, $\chi^2=7.8$, $p<0.01$, inhibited in females treated with these two hormones combined (Table 2A). The same dose of D-amphetamine had a slight, but not statistically significant effect in females treated with EB alone (Table 2B).

L-Amphetamine. Lordosis behavior was depressed in EB + PROG treated animals after administration of L-amphetamine 5 mg/kg, but this effect did not reach statistical significance. The same dose had no inhibitory effect in animals treated with EB alone (Table 2A and B). When the dose of L-amphetamine was increased to 10 mg/kg, the lordosis response was completely abolished after both hormonal treatments (not shown in table; N=18 for both categories).

Stereotype Activity

The influence of increasing doses of EB and of PROG on stereotype activity in non-drug-treated female rats was studied. Stereotype activity was tested after injection of EB in single doses of 10, 20 and 40 μg/kg (Table 3A). Increasing doses of EB resulted in an increased score. After the highest dose of EB the difference from the result following oil treatment reached statistical significance, $U=17$, $p<0.05$. The most prominent effect was on grooming behavior, which was seen in 44% of the females treated with EB 10 μg/kg, while after injection of 40 μg/kg this effect was noted in 89% of the animals.

PROG also influenced the stereotype activity (Table 3A). A dose of 1.0 mg/rat reduced this activity, $U=12$, $p<0.01$, as compared with oil treatment, mainly due to a reduction of

TABLE 1

PERCENTAGE NUMBERS OF OVARIECTOMIZED RATS SHOWING A LORDOSIS RESPONSE AFTER INJECTION OF APOMORPHINE UNDER DIFFERENT HORMONAL CONDITIONS

Hormonal treatment	Drug treatment μg/kg	Percentage with lordosis response Time after drug injection -30	10	60	120	N
A EB 10 μg/kg + PROG 0.2 mg/rat	+ apomorphine 200	63	25*	79	92	(24)
EB 10 μg/kg + PROG 0.2 mg/rat	+ saline 0.3 ml	71	71	83	83	(24)
EB 10 μg/kg + PROG 0.4 mg/rat	+ apomorphine 200	56	11†	78	83	(18)
EB 10 μg/kg + PROG 0.4 mg/rat	+ apomorphine 500	88	8†	83	100	(24)
EB 10 μg/kg + PROG 0.4 mg/rat	+ saline 0.3 ml	61	78	83	89	(18)
EB 10 μg/kg + PROG 4.0 mg/rat	+ apomorphine 200	100	57*	100	100	(23)
EB 10 μg/kg + PROG 4.0 mg/rat	+ saline 0.3 ml	96	100	96	100	(23)
B EB 10 μg/kg × 3	+ apomorphine 200	61	61	78	78	(18)
EB 10 μg/kg × 3	+ apomorphine 500	46	4*	54	100	(24)
EB 10 μg/kg × 3	+ saline 0.3 ml	44	44	50	18	(18)

A. Hormonal treatment: EB 10 μg/kg + progesterone 0.2/0.4/4.0 mg/rat.
B. Hormonal treatment: EB 10 μg/kg × 3.
*$p<0.01$; †$p<0.001$ (comparison with saline).

TABLE 2

PERCENTAGE NUMBER OF OVARIECTOMIZED RATS DISPLAYING LORDOSIS BEHAVIOR AFTER D- AND L-AMPHETAMINE UNDER DIFFERENT HORMONAL CONDITIONS

Treatment	mg/kg	Percentage lordosis response Time after drug injection -30	30	150	N
A D-amphetamine	2.5	44	17*	61	18
L-amphetamine	5.0	78	44	67	18
Saline	0.3 ml	66	69	80	35
B D-amphetamine	2.5	63	36	75	22 (16)
L-amphetamine	5.0	67	59	67	22 (6)
Saline	0.3 ml	54	57	64	28

A. Hormonal treatment: EB 10 μg/kg + progesterone 0.4 mg/rat.
B. Hormonal treatment: EB 10 μg/kg × 3.
*$p<0.01$. Number of animals tested at −30 and 150 min is shown within parentheses.

TABLE 3

STEREOTYPE ACTIVITY

Treatment	Dose	Score (N)
A EB (μg/kg)	10	1.8 (9)
	20	2.3 (13)
	40	2.9* (9)
Oil control (ml)	0.3	1.6 (14)
PROG (mg/rat)	0.1	2.1 (10)
	0.4	1.9 (15)
	1.0	0.8† (9)
Oil control (ml)	0.3	2.1 (15)
B EB (μg/kg) +	10	
PROG (mg/rat)	0.4	2.3 (31)
EB (μg/kg)	10×3	2.7 (26)

A. Stereotype activity after administration of different doses of EB and PROG. The EB or oil control injection was given 53 hr, and the PROG/oil control injection 5 hr before testing.
B. Stereotype activity after administration of EB 10 μg/kg plus PROG, given 53 and 5 hr, respectively, before testing, and of EB 10 μg/kg given on three consecutive days. Tests were performed 29 hr after the last injection.
*$p<0.05$; †$p<0.01$.

rearing activity: this behavior was observed in 80% of the controls versus 11% of the PROG-treated females, $\chi^2=8.2$, $p<0.01$. The occurrence of grooming did not change with increasing doses of PROG.

A comparison was made between the hormonal treatments used in the lordosis tests with respect to stereotype activity (Table 3B). In the group given EB 10 μg/kg × 3, the score was 2.7 and in the group treated with EB 10 μg/kg + PROG 0.4 mg/rat the score was 2.3. This difference was not statistically significant. No striking differences in behaviors were observed between the two groups.

D-Amphetamine in a dose of 2.5 or 5 mg/kg was then added to females treated with EB alone and with EB + PROG, in analogy with the lordosis experiments. Stereotype activity increased dose-dependently in both hormone groups (Fig. 1). PROG did not augment the effect of D-amphetamine. The slightly higher score in the group treated with EB alone was evident, however, after both doses of D-amphetamine.

DISCUSSION

The hormonal condition of an animal can have direct con-

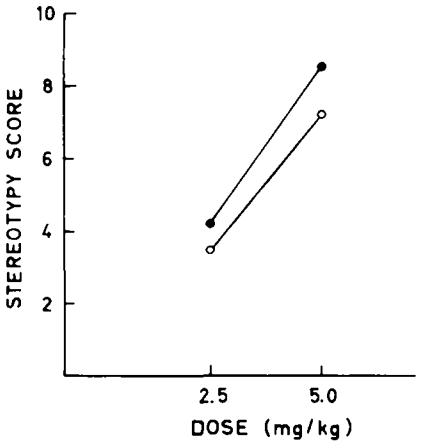

FIG. 1. Stereotype activity in subjects treated with EB 10 μg/kg + PROG 0.4 mg/rat (○) or EB 10 μg/kg × 3 (●) after injection of D-amphetamine 2.5 and 5 mg/kg, given 45 min before testing. N = 10–16.

sequences for the action of drugs influencing monoaminergic transmission. This has previously been demonstrated for LSD, which had a greater lordosis inhibitory action in ovariectomized rats when given after administration of EB + PROG than after EB alone [11,17].

The present study shows that the effects of apomorphine and D- and L- amphetamine also seem to be influenced by hormonal treatment. Inhibition of lordosis by these drugs was greater after treatment with EB and PROG combined than when EB had been given alone, a finding which was most evident for apomorphine and D-amphetamine. When both EB and PROG had been given L-amphetamine 5 mg/kg had some inhibitory effect, though not statistically significant, whereas after treatment with EB alone it caused no inhibition.

Thus, in addition to augmenting the inhibitory effect of LSD on lordosis, PROG has been shown in the present study to have similar influence on the corresponding effects of apomorphine and amphetamine. However, it was found in a previous study that when the dose of PROG was raised, the inhibitory effect of the 5-HT stimulatory drug on lordosis was enhanced [17]. Such a PROG dose related effect was not observed with apomorphine. The lordosis-inhibiting effect of drugs acting on DA transmission seems to be influenced by the presence or absence of a critical dose of PROG.

At present the possibility that PROG interferes with both 5-HT and DA mechanisms, or that one system influences the other, cannot be excluded.

D-Amphetamine-induced stereotype activity, in contrast to lordosis behavior, was not augmented by PROG, as seen from the dose-response relationships (Fig. 1). This rules out the possibility that the effect of PROG may be due to metabolic influence on amphetamine.

In fact, stereotype activity was slightly greater in the animals treated with EB 10 μg/kg × 3 than in those treated with EB 10 μg/kg + PROG. This might reflect an effect of estrogen, which has been shown to increase stereotype activity [7, 8, 14]. Estrogen treatment of male rats results in an increase in the number of striatal DA receptors [6], indicating a direct modifying effect on DA transmission. On the other hand, there was a difference in the stereotype activity score between the two hormonal treatments even before D-amphetamine. Unlike lordosis behavior, stereotype activity is not dependent on hormones for its occurrence, but to a certain extent it seems to be influenced by the action of EB and PROG. Thus, in non-drug-treated animals increasing doses of EB enhanced the stereotype activity, while PROG attenuated it.

An influence of estrogen on DA-dependent diseases is indicated by clinical reports of precipitation of chorea by pregnancy or oral contraceptives [1,18].

The responsiveness of a drug can be altered by different mechanisms, e.g., denervation or chronic treatment with drugs influencing synaptic transmission. Thus, hypersensitivity of receptors can develop following understimulation, and vice versa (see [15] for a review). The basic mechanisms of neuronal actions of PROG are not understood. The hormone can facilitate lordosis behavior in EB-treated rats and, as shown in the present study, enhance the action of lordosis-inhibiting drugs. To explain these effects, it might be hypothesized that PROG in some ways depresses the activity in monoaminergic neurons mediating tonic inhibition of the lordosis response. This action would facilitate the behavior. It is also conceivable that the impaired neuronal activity leads to increased postsynaptic receptor sensitivity, which might influence the effect of drugs acting on monoaminergic transmission. However, it must be emphasized that this is still hypothetical.

To summarize, the results show that in EB-treated rats the effect of D-amphetamine and apomorphine, both of which influence DA pathways, could be increased by adding PROG prior to drug treatment. This effect seemed to be limited to the hormone-dependent copulatory response, as stereotype activity enhanced by D-amphetamine was not augmented in a corresponding way. In addition, it was found that hormonal treatment as such could influence the behavioral repertoire of animals not treated with any drug, as revealed in the stereotype activity test.

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