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Effects of Ovariectomy and Estrogen Replacement on Intrastriatal Dopamine-Elicited Postural Deviation[1]

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SMITH, R. L. AND C. VAN HARTESVELDT. *Effects of ovariectomy and estrogen replacement on intrastriatal dopamine-elicited postural deviation.* PHARMACOL BIOCHEM BEHAV 22(5) 689–693, 1985.—The effects of ovariectomy, sham ovariectomy, and estradiol benzoate replacement on unilateral intrastriatal dopamine-induced postural deviation were studied in rats. Animals were tested prior to surgery, and at both two and seven days after surgery. Relative to the pre-surgery test, ovariectomized rats greatly increased this behavioral response two days after surgery while sham ovariectomy resulted in no significant change. Estradiol benzoate treatment in ovariectomized animals not only prevented this increase but significantly suppressed it at both two and seven days after surgery relative to pre-surgery levels. Thus, removal of endogenous estrogen in female rats resulted in an acute increase in a striatal DA-mediated behavior which could be prevented by hormone replacement. These results are consistent with the hypothesis that estrogen suppresses some striatal DA-mediated behaviors.

Female rats Striatum Dopamine Estradiol benzoate Postural deviation Ovariectomy

SUBSTANTIAL evidence has now accumulated showing that estrogen affects the behavioral consequences of drugs thought to act on the nigrostriatal dopamine (DA) system. For example, catalepsy induced by dopamine antagonists reduces the functional activity of DA in the striatum [6], and catalepsy is enhanced by estrogen [4, 7, 9]. On the other hand, stereotyped behavior induced by DA agonists acting at least in part on the striatum [14] is enhanced by removal of endogenous estrogen [10, 11, 13, 28] and antagonized by estrogen, if a small dose of estradiol benzoate is used [10]. However, the conclusions which can be drawn from these studies are limited by the facts that the drugs are given systemically, and may have multiple sites of action; and that hormones are known to alter the rates of metabolism of many drugs (e.g., [8]), thus changing the intensity and duration of their behavioral effects.

In order to specify the site of drug action and eliminate the problem of hormonal effects on drug metabolism, we have injected DA directly into the striatum and measured its effects on postural deviation. Previous work from this laboratory has shown that intrastriatal DA-induced postural deviation in female rats is suppressed in intact animals when plasma levels of endogenous estrogen are high, on the morning of estrus [17], and within 1/2 hour of administration of exogenous estrogen [15]. In the present experiment we have chosen to examine the effect of ovariectomy on intrastriatal DA-induced postural deviation. If estrogen suppresses the action of striatal DA, then removal of the primary source of endogenous estrogen by ovariectomy should enhance it, increasing postural deviation. If ovariectomy results in an increase in DA-related behavior due to removal of estrogen, then replacement therapy with estradiol benzoate should cancel the increase.

METHOD

Subjects

Thirty-two adult female Long-Evans hooded rats weighing 200–250 g were individually housed with food and water ad lib. They were maintained on a 12:12 hour light:dark cycle throughout the experiment, with lights on at 0800 hours. The regularity of the animal's estrous cycle was assessed by daily vaginal smears, taken between 0900–1000 hours. The smears were obtained by inserting a thin metal loop, lubricated with water, into the vagina. The vaginal specimen was placed on a slide, stained with toluidine blue and categorized with the aid of a light microscope. Only those females showing at least two consecutive four-day estrous cycles were used for behavioral testing.

Stereotaxic Surgery

Each animal was bilaterally implanted with 21 ga stainless steel guide cannulae positioned in the dorsal striatum. A 27 ga stainless steel stylet was inserted into each cannula to prevent prolonged exposure to air. The coordinates for the implantation were taken from Pellegrino, Pellegrino and Cushman [21]: +2.0 to +3.0 mm with respect to bregma;

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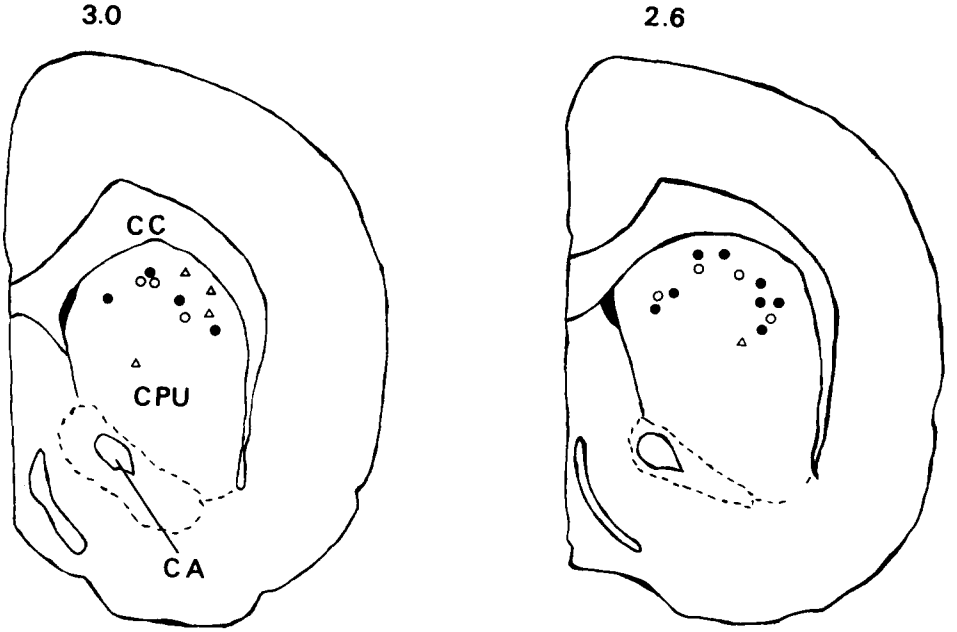

FIG. 1. Locations of cannula tips in the dorsal striatum (caudate-putamen) in diagrams derived from Pellegrino *et al.* [21]. Open circles indicate sham ovariectomized rats; closed circles, ovariectomized rats; and open triangles, ovariectomized rats treated with estradiol benzoate. CC=corpus callosum; CPU=caudate-putamen; CA=anterior commissure.

2.0–4.0 mm lateral to bregma; and 3.5–5.0 mm below the surface of the brain. After cannula implantation the animals were given a one week recovery period.

Drug and Hormone Administration

Unilateral intrastriatal infusions were made with a 27 ga injection cannula which was inserted into the guide cannula. The infusion consisted of 25 μg of DA dissolved in 0.25 μl phosphate buffer solution to a pH of 7.4, or 0.25 μl of the vehicle control. The phosphate buffer was a 7.0 mM sodium phosphate/140 mM sodium phosphate dibasic solution. The vehicle control consisted of a 0.13 mM sucrose solution in the phosphate buffer, acidified with acetic acid to a pH of 7.4. The infusion was made at a constant rate over a 30 sec period, after which the injection cannula was left in place for an additional 30 sec.

Estradiol benzoate was dissolved in peanut oil to a concentration of 20 μl/ml.

Behavior

After the intrastriatal injection was made, the animals were placed in a circular Plexiglas chamber and continuously observed for 30 min. The time spent in ipsilateral and contralateral postural deviation, with respect to the side of injection, was recorded every 5 min. The behavioral score was determined by subtracting the total time spent in ipsilateral deviation from the total time spent in contralateral deviation. Behavioral testing occurred between 1400–1600 hours. Animals not showing contralateral deviation were excluded from the study.

Experimental Design

The animals were randomly distributed in three treatment groups. All animals were behaviorally tested for postural deviation in response to intrastriatal injection of DA prior to implementation of the treatments. Behavioral testing before surgery occurred only during estrus or the first day of diestrus in the female rat's estrous cycle. The treatment groups were as follows:

(1) OVX group (n=12): animals were bilaterally ovariectomized under light ether anesthesia on the evening of the pretest. Half of the animals were tested with DA, and the other half with the drug vehicle.

(2) Sham-OVX group (n=7): these animals were anesthetized with ether, given a midline incision, their ovaries displaced and replaced, and the incision sutured on the evening of the pretest to DA.

(3) OVX-EB group (n=5): animals were bilaterally ovariectomized on the evening of the pretest, but received 2 μg of estradiol benzoate (EB) twice daily, starting immediately after the ovariectomy.

All three groups were tested again 2 and 7 days after ovariectomy or sham ovariectomy.

Statistical Analysis

An analysis of variance and covariance with repeated measures was performed on the behavioral scores of the three treatment groups over the three test days. One-way analyses of variance were carried out for each of the day and treatment groups. Post-hoc comparisons were made using the Newman-Keuls test.

Histology

The rats were sacrificed under pentobarbital anesthesia and perfused with saline and a 10% buffered formalin solution. The brains were removed from the skull and frozen, and 30 μ sections were cut, mounted on slides and stained with cresyl violet. The localization of cannula tip placement

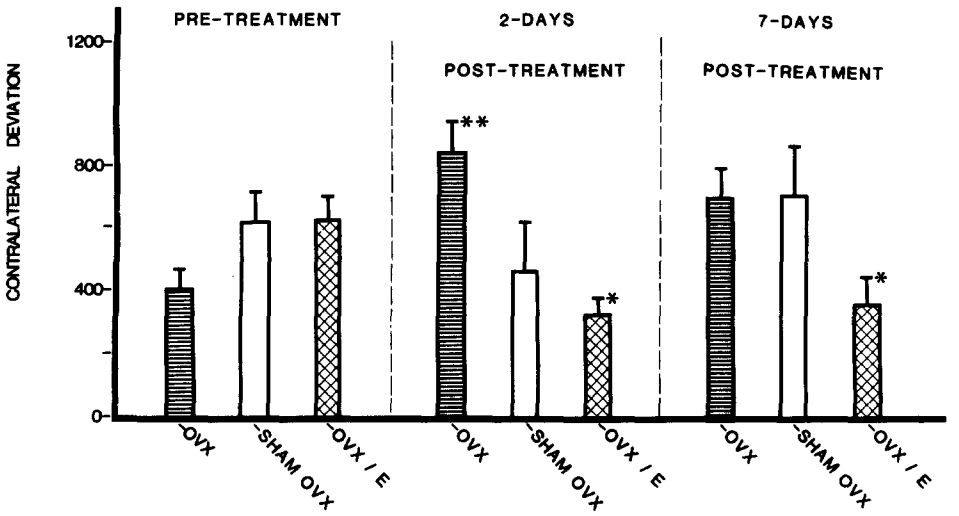

FIG. 2. Behavioral responses to unilateral injection of 25 μg DA in a volume of 0.25 μl into the dorsal part of the anterior striatum. The ordinate represents the average difference score for postural deviation expressed in 0.01 min; positive scores represent predominantly contralateral deviation. The graph shows the mean difference score ±S.D. for the total observation period. Animals of each group were tested before surgery (pre-treatment) and two and seven days after surgery (post-treatment). OVX, ovariectomized (n=12); SHAM-OVX, sham ovariectomized (n=7); OVX/E, ovariectomized and treated with estradiol benzoate (n=5). *Significantly different from pre-OVX scores at 2 days, $p<0.01$; at 7 days, $p<0.05$. **Significantly different from any other group, $p<0.01$.

was determined according to the rat brain atlas of Pellegrino *et al.* [21].

RESULTS

Cannula tips were located in the dorsal striatum (Fig. 1). There were no differences in cannula placements among groups.

The overall analysis of variance and covariance indicated that there were no significant differences among the Groups prior to surgery, and thus a covariate analysis was not performed. The overall ANOVA showed neither significant Group nor Day main effects, but revealed a significant interaction, $F(4,36)=4.64$, $p<0.0004$. One-way ANOVA's were run by Day and by Group. The scores of OVX females changed significantly across days, $F(2,20)=8.73$, $p<0.002$. DA-induced postural deviation was greater at two days after OVX relative to pre-OVX scores ($p<0.01$). At seven days after OVX, behavioral scores for the OVX Group were not significantly different from either the pre-OVX or the two day post-OVX scores. Females tested with the vehicle did not show contralateral postural deviation, and OVX did not change their scores. The behavioral scores of sham-OVX females did not change significantly across days. Estradiol benzoate treatment of OVX females significantly changed behavioral scores across days, $F(2,8)=9.50$, $p<0.008$; reducing scores at two days after OVX relative to pre-OVX ($p<0.01$) and at seven days after OVX relative to pre-OVX ($p<0.05$).

ANOVA's were also carried out for each day across Groups. At two days after OVX, there were significant Group differences, $F(2,18)=10.03$, $p<0.002$. DA-induced postural deviation was greater in OVX females than in either sham-OVX or EB-treated OVX females ($p<0.01$). There were no significant differences across Groups at seven days after OVX. The results for all groups are shown in Fig. 2.

DISCUSSION

The results of the present study are consistent with the hypothesis that estrogen suppresses the striatal DA mechanisms underlying postural deviation. Previous work has shown that postural deviation elicited by intrastriatal DA is suppressed either when the endogenous plasma level of estrogen is high, as on the morning of proestrus [17], or within 24 hours of administration of a small dose of estradiol benzoate [15]. In the present experiment, removal of the primary source of endogenous estrogen by ovariectomy led to an increase in DA-elicited postural deviation by two days after surgery. The OVX-induced increase appeared to last for seven days after surgery, but further experiments must be done to confirm that OVX leads to a chronic increase in DA-elicited postural deviation. Estradiol benzoate cancelled this increase, suggesting that the loss of estrogen was the cause of the behavioral change.

The results of the present experiment are consistent with a large body of literature relating to the effects of estrogen on DA-mediated behavior. For example, Bedard and associates have found that three weeks after OVX, apomorphine-induced turning in a rat with a unilateral entopeduncular lesion is increased [2], and that estradiol benzoate can reduce this behavior [3]. In this model, it is assumed that the entopeduncular lesion disrupts a primary striopallidal outflow, allowing assessment of the effects of drugs and hormones on the intact side. Apomorphine-induced stereotyped behavior, also thought to be mediated at least in part by striatal DA, is also affected by estrogen. Ovariectomy leads to an increase in APO-induced stereotyped behavior [10, 11, 13, 28] which gradually becomes even greater with time after surgery [11]. However, no change in stereotyped behavior in rats after OVX is found when high doses of apomorphine and amphetamine are used [1,22]. Estradiol benzoate decreases

APO-induced stereotyped behavior in OVX female rats when given in small doses and when behavioral measurements are made within about 24 hours [12, 22, 23]. It should be noted that opposite results are found when large doses of estradiol benzoate are used and behavioral measurements are made 48 or more hours after hormone treatment [5, 10, 15, 26].

There may also be important species differences in the effects of estrogen on dopamine-related behaviors. In mice as in rats, ovariectomy leads to increased behavioral responses including stereotyped behavior in response to L-DOPA [27] and increased apomorphine-induced stereotyped behavior, which can be reversed by administration of estradiol benzoate [19]. However, guinea pigs respond in the opposite manner both to ovariectomy and to estrogen replacement; ovariectomy leads to a decrease in apomorphine-induced stereotyped behavior, and chronic treatment with estradiol valerate results in an increase [18,20].

The effects of ovariectomy on other behaviors related to striatal DA are not the same as on intrastriatal DA-elicited postural deviation, apomorphine-induced circling in entopeduncular-lesioned animals, or apomorphine-induced stereotyped behavior. For example, electrical stimulation of the nigrostriatal bundle leads to contralateral turning behavior through release of DA in the striatum, and, according to the authors, possibly the nucleus accumbens [24,25]. Ovariectomy of female rats led to a gradual decrease in electrical stimulation-induced rotation which became significantly lower beginning at day 10 after surgery. These results appear to be the mirror image of those obtained for the behaviors above. It is possible that ovariectomy reduces rotation elicited by electrical stimulation of the nigrostriatal tract by a presynaptic action on these neurons, while it increases behaviors such as apomorphine-induced stereotypy via postsynaptic effects in the striatum. However, since turning induced by electrical stimulation may involve mesolimbic DA as well as nigrostriatal DA, it is important to consider the effects of OVX and estradiol replacement on this system. Ovariectomy blocks amphetamine-induced locomotor activity [26], which is thought to be mediated by mesolimbic dopaminergic neurons. With increased time after OVX, locomotion elicited by amphetamine injected into the nucleus accumbens decreases and is restored by estradiol benzoate [15]. The effect of ovariectomy on rotation elicited by electrical stimulation more closely resembles the effect of ovariectomy on behaviors known to be mediated by the mesolimbic DA system than those mediated by the nigrostriatal DA system. Thus, the results of hormone manipulations on electrical stimulation-induced rotation may represent a combination of very different effects on nigrostriatal and mesolimbic DA systems.

Comparing the effects of estrogen and its removal across behaviors, species, DA systems, dose levels, and times after hormone injection has led to confusion concerning the nature of its effects. However, when the effects of estrogen are studied on postural deviation elicited by intrastriatal dopamine, estrogen clearly has a suppressant effect.

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