Increased Dopamine Receptor Sensitivity After Estrogen Treatment Using the Rat Rotation Model

Abstract. *Estrogen was administered to male rats that had received unilateral injections of 6-hydroxydopamine into the striatum. Following this treatment, their duration of rotation increased in response to amphetamine. Estrogen treatment resulted in a corresponding increase in the number of striatal dopamine receptors. Therefore, both behavioral and biochemical evidence suggests that striatal dopamine function is influenced by peripherally administered estrogens. These results are relevant to the clinical cases of chorea associated with elevated concentrations of estrogen, which occur in pregnancy and during oral contraceptive use.*

The rat rotation model first described by Ungerstedt has been useful in determining the behavioral effects of proposed dopamine (DA) agonists (*1*). The nigrostriatal DA neurons on one side of the brain are destroyed by injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the substantia nigra, the median forebrain bundle, or the striatum (*2*). After supersensitivity develops on the side with the lesion, the systemic injection of direct-acting DA agonists produces rotation in a direction contralateral to the side with the lesion, while indirect-acting DA agonists (which act by promoting release or inhibiting reuptake of DA) produce ipsilateral rotation. This rotation model has been useful for demonstrating that several novel ergot derivatives act as direct DA agonists (*3*). Since estrogen appears to modify DA function (*4*, *5*), we used the rat rotation model to explore the effects of estrogen on extrahypothalamic DA function. We investigated the rotation produced by a direct DA agonist, apomorphine, and an indirect DA agonist, *d*-amphetamine. Also, since the amount of rotation induced by such drugs seems to be correlated with the imbalance in the number of DA receptors measured biochemically (*6*), we tested the effect of estrogen treatment on [^{3}H]spiroperidol binding to DA receptors.

To avoid the adipsia and aphagia often associated with 6-OHDA destruction of the nigrostriatal fibers (*7*), we injected 6-OHDA directly into the striatum (*8*). The uptake of DA was significantly reduced in striatal synaptosomes prepared from the 6-OHDA–injected side of the brain, compared to synaptosomes from the control, uninjected side (Table 1). The uptake of choline was not significantly altered, indicating the relative specificity of the lesion. The number of DA receptors labeled by [^{3}H]spiroperidol increased, demonstrating a postsynaptic supersensitivity after the loss of DA neurons. One week after the unilateral injection of 6-OHDA, the rats rotated contralaterally when they were injected with apomorphine (5 mg/kg) and ipsilaterally when injected with *d*-amphetamine (3 mg/kg).

After the rats were tested for rotation, one-half of them were injected, subcutaneously in the neck, with 17β-estradiol valerate (125 μg) in sesame oil. This single estrogen injection has been reported to have a long-lasting effect, since it increases prolactin levels for 2 weeks (*9*). At 5 to 8 days after estrogen treatment, the rats were again challenged with the same drug (apomorphine or amphetamine) that they received previously, and their rotation was measured. In control rats, the rotation after the second injection increased slightly (about 20 percent) in magnitude, with no change in duration, as compared to the first injection of either drug.

In the rats that were administered estrogen, the duration of the rotation produced by amphetamine significantly increased, compared to the response of control rats treated a second time with amphetamine (Fig. 1). The time at which the peak rotation had declined to half-maximum was significantly increased from 81 ± 5 to 106 ± 8 minutes ($P < .025$). The onset or the peak intensity of the rotation in estrogen-treated rats did not change compared to that in control rats which also received a second injection of amphetamine. The rats treated with estrogen and then a second time with apomorphine did not show alterations in the onset, duration, or peak intensity of rotation, compared to the controls receiving a second apomorphine injection (Fig. 1).

Table 1. Neurochemical changes appearing in the rat striatum on the side injected with 6-OHDA, compared to the control side. Transport and receptor binding methods are given in (*14*) and (*15*), respectively. Number of rats per group is in parentheses.

Parameter	Weeks after injection	Lesion/control (%)
Choline uptake	1	104.1 ± 9.8 (7)
	2	99.7 ± 7.9 (7)
DA uptake	1	34.5 ± 4.4* (8)
	2	34.7 ± 3.6* (7)
Maximum spiroperidol binding	2	120.0 ± 5.4* (4)

*$P < .05$, Student's *t*-test.

The rats treated with 6-OHDA were also studied biochemically after estrogen treatment. The number of DA receptors labeled with [^{3}H]spiroperidol increased in estrogen-treated rats, compared to controls; both the intact and 6-OHDA injected striata showed a similar 20 percent increase in DA receptors (Table 2). Therefore, the total increase on the side of the 6-OHDA lesion after estrogen treatment was about 40 percent; about 20 percent of this increase resulted from the 6-OHDA lesion and 20 percent from the estrogen treatment. The affinity of the striatal DA receptors was not altered after treatment with either 6-OHDA or estrogen (Table 2). Furthermore, the addition of estrogen in vitro did not affect [^{3}H]spiroperidol binding to striatal DA receptors.

These biochemical results in the striatum are consistent with the effects of estrogen on rotation; that is, in estrogen-treated rats, the duration of rotation increases after injection of amphetamine, but apomorphine causes no change in rotation. Since amphetamine requires intact DA neurons for its action, rotation is produced when there is a presynaptic imbalance, such as after 6-OHDA injection. Estrogen treatment increases the number of postsynaptic striatal DA receptors by about 20 percent on both sides of the brain (Table 2). Consequently, the effects of amphetamine on the intact presynaptic side are enhanced by a 21.2 percent increase in receptor sensitivity (Table 2), and this is expressed behaviorally as an increased duration of rotation (Fig. 1). The number of postsynaptic DA receptors on the side of the 6-OHDA lesion has no effect on rotation produced by amphetamine. In contrast, apomorphine produces rotation by acting directly on postsynaptic DA receptors. Rotation produced by apomorphine treatment after 6-OHDA injection is due to the relative increase in the number of DA receptors on the side with the lesion, as compared to the intact side. The net imbalance of the DA receptors in rats injected with 6-OHDA is 22.0 percent, and after estrogen treatment the imbalance in these rats is 19.4 percent (369 compared to 309 fmole per milligram of protein) (Table 2). Therefore, apomorphine produces rotation in rats treated with estrogen with the same peak

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intensity and duration as the second injection of apomorphine produces in control rats because the relative asymmetry of the receptors is the same.

The biochemical data confirm the rotational data, suggesting that the binding of [^{3}H]spiroperidol in striatal tissue is a good indicator for rotation sensitivity (*6*). The effect of estrogen is important, since it increases the number of DA receptor sites labeled by [^{3}H]spiroperidol; it also increases rotation after amphetamine is injected, indicating increased DA receptor sensitivity in vivo. The increases in striatal DA receptors brought about by estrogen and 6-OHDA is apparently additive. This implies that the increase in the number of sites produced by one agent can be increased further by another agent. The summation of the number of sites does not indicate whether or not these agents work through similar sites or mechanisms.

It is possible that the estrogen enhancement of rotation could be caused by a block in the metabolism of amphetamine. In the rat, amphetamine is metabolized primarily by hydroxylation (*10*), and this process could be affected by the estrogen treatment. However, the dose of estrogen administered was small and was given 5 to 8 days before testing with amphetamine. Further, the biochemical results suggest that the increased rotation is correlated with an increased number of DA receptor binding sites.

Our results concur with the diminished DA responses (of stereotypy or DA-sensitive adenylate cyclase activity) measured in ovariectomized guinea pigs or rats, which thus have reduced estrogen concentrations (*4*). These responses could be maintained at normal levels by administering estrogen. We demonstrated previously that estrogen treatment of male rats increased the intensity of stereotypy after injection of either amphetamine or apomorphine, an effect that was also correlated with an increased number of DA receptors in the striatum (*5*). Therefore, small doses of estrogen administered subcutaneously have a profound, long-lasting effect on behavioral and biochemical DA function in the striatum.

The implications of estrogen enhancement of striatal DA response are potentially significant. Estrogen status and treatment may be important to consider in disorders involving a relative decrease in DA function in the striatum, such as parkinsonism, and those disorders involving a relative excess of DA function, such as schizophrenia or Huntington's disease. Although most research has focused on the effect of neurotransmitters on estrogen function in the pituitary and hypothalamus (*11*), there is little clinical or basic evidence linking estrogens to DA function in areas not normally associated with hormonal function, such as the striatum (*12*).

Elevated concentrations of estrogen, present during pregnancy or with oral contraceptive use, have been clinically associated with chorea (*13*). The chorea appears shortly after becoming pregnant or initiating oral contraceptive use, and ceases with the termination of either pregnancy or use of oral contraceptives. This type of chorea is rare and may only occur in individuals with subclinical alterations in the basal ganglia, possibly from a previous episode of rheumatic encephalopathy. Since a relative increase of DA function in the striatum is thought to be associated with choreic disorders, estrogens could act on striatal DA receptors and facilitate chorea development in susceptible women. The present biochemical and behavioral results demonstrate that estrogens increase the number of striatal DA receptors in rats. This may also be true in some humans, and thereby may be one of the causes of this type of chorea. The influence of estrogens on striatal DA function needs to be further explored in disorders involving DA function.

Table 2. Effect of the 6-OHDA-induced lesion and estrogen treatment on [^{3}H]spiroperidol binding to DA receptors in the rat striatum. Abbreviations: K_d, dissociation constant; B_{max}, maximum number of binding sites.

Group	Lesion	K_d (pM)	B_{max} (fmole per mg of protein)	B_{max} increase (%)
Control		13.3 ± 2.8	255 ± 14	
Control	6-OHDA	15.5 ± 1.1	311 ± 9*	22.0
Estrogen		13.2 ± 2.3	309 ± 20*	21.2
Estrogen	6-OHDA	15.2 ± 1.8	369 ± 20†	44.7

*$P < .05$, compared to the control group. †$P < .05$, compared to the control, control and 6-OHDA, or estrogen groups.

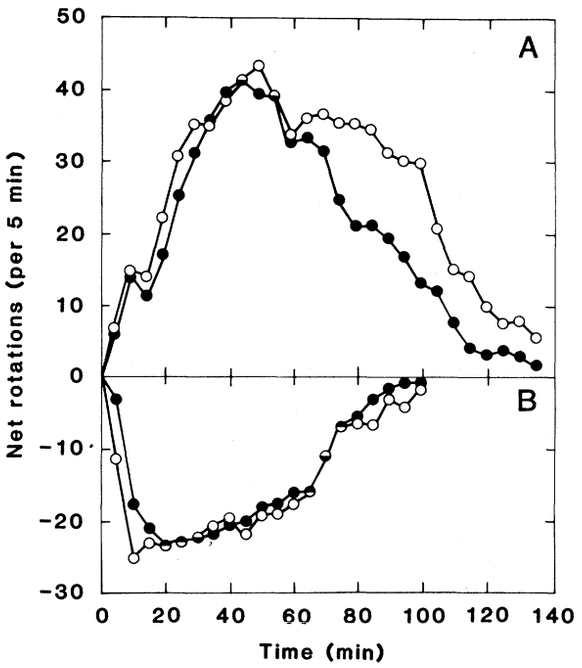

Fig. 1. Rotation of estrogen-treated (○) and control (●) rats in response to injections of (A) *d*-amphetamine sulfate (3 mg/kg, $N = 9$) or (B) apomorphine hydrochloride (5 mg/kg, $N = 6$). The number of rotations per 5-minute period was monitored for each rat at 2 weeks after 6-OHDA injection by using an automated rotometer (*16*). Positive numbers refer to ipsilateral rotation; negative numbers, to contralateral rotation. For the amphetamine-treated rats, the time at which peak rotation had declined to half-maximum was increased by estrogen administration from 81 ± 5 to 106 ± 8 minutes ($P < .025$).

Note added in proof: Removal of the pituitary completely blocked the effect of estrogen on [^{3}H]spiroperidol binding. This suggests that in male rats the effects of estrogen on striatal function are indirect.

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8. Only male rats were used in this study since their endogenous estrogen concentrations are constant and very low. The rats were anesthetized with chloral hydrate and positioned in a stereotaxic apparatus; the coordinates used for injection were: A, 7.9; L, 2.6; and V, 0.4 mm [J. F. R. König and R. A. Klippel, *The Rat Brain, A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem* (Williams & Wilkins, Baltimore, 1963)]. A total of 2 μl of 6-OHDA solution, prepared in saline containing 0.1 percent ascorbate and 20 μg of 6-OHDA, was injected over a 1-minute period.
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14. In all cases, tissue was isolated from the injected striata and the control striata. All results are expressed as a percentage of the control side. The crude synaptosomal fraction was prepared and portions were incubated in 1 ml of a Krebs-Ringer phosphate buffer containing 150 m*M* NaCl, 4.7 m*M* KCl, 1.8 m*M* $CaCl_2$, 1.2 m*M* $MgSO_4$, 17 m*M* sodium phosphate buffer (*p*H 7.4), and 1 mg of glucose per milliliter. Only sodium-dependent transport was measured by subtracting the accumulation in the absence of sodium, using LiCl to replace NaCl, and tris-phosphate to replace sodium phosphate. In the DA transport studies, 0.1 percent ascorbate and 0.1 m*M* nialamide were added to the incubation buffer. The concentration of [^{14}C]choline was 0.42 μ*M*, and [^{14}C]DA was 1.2 μ*M*. The incubation was performed for 5 minutes at 37°C and then terminated by rapid separation over Millipore filters (type HAWP; 0.45 μm in pore size). The tube and filter were rinsed twice rapidly with room temperature saline. Percentages of changes are corrected for protein [O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951)].
15. The receptor binding assays were performed according to standard procedures [J. Z. Fields, T. D. Reisine, H. I. Yamamura, *Brain Res.* **136**, 578 (1977); J. E. Leysen, C. J. E. Niemegeers, J. P. Tollenaere, P. M. Laduron, *Nature (London)* **272**, 168 (1978)]. The fresh striatal tissue was disrupted with a Polytron in 100 m*M* buffer (81 m*M* Na_2PO_4 and 19 m*M* KH_2PO_4, *p*H 7.4) and washed twice. Portions of the tissue suspension were incubated in 10 ml of the buffer containing [^{3}H]spiroperidol (5 to 110 p*M*) for 30 minutes at 37°C. Nonspecific binding was determined in a duplicate set of tubes containing 1 μ*M* (+)-butaclamol and was subtracted from total binding to obtain specific binding. Only specific binding is reported, since nonspecific binding was not affected by any of the treatments. After incubation the bound radioactivity was trapped by rapid filtration over GF/B glass fiber filters, and the tube and filter were rapidly rinsed twice with ice-cold buffer. The maximum binding and dissociation constants were determined from Scatchard plots by least-squares linear regression analyses. The dissociation constants were not affected by the 6-OHDA treatment. All values are corrected for protein concentration.
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Fertilization of Squirrel Monkey and Hamster Ova in the Rabbit Oviduct (Xenogenous Fertilization)

Abstract. *Homologous sperm and ova of either squirrel monkeys or hamsters were placed in the oviducts of pseudopregnant rabbits. Xenogenous fertilization rates of 36 and 60 percent were obtained for squirrel monkey and hamster gametes, respectively.*

The homologous mammalian oviduct provides the optimal environment for fertilization, but the oviduct of a pseudopregnant rabbit is able to support the development of embryos of foreign species (*1*). Additionally, the porcine oviductal environment can support fertilization of bovine ova by bovine sperm (*2*). In a preliminary study, we have reported the potential capacity of the pseudopregnant rabbit oviduct to support fertilization of squirrel monkey (*Saimiri sciureus*) ova (*3*). The present study was designed to test whether the oviduct of a pseudopregnant rabbit could support fertilization of hamster (*Mesocricetus auratus*) and squirrel monkey ova.

We initially transferred hamster oviductal ova (flushed from the excised oviduct with 0.2 ml of medium) in 5 μl of Medium 199 (Gibco) with added newborn calf serum (Agamma) in a ratio of 4:1 (*4*) to the fimbriated end of the rabbit oviduct (surgical transfer) 2 to 9 days after the rabbit received 100 international units (I.U.) of human chorionic gonadotropin (hCG) to induce ovulation and subsequent pseudopregnancy. Sperm from the minced cauda epididymis were added in 0.05 ml of medium to the oviduct. Fertilization was assessed only by the percentage of ova cleaved to the two-cell stage when recovered 30 hours later. Under these conditions, 9 of 66 (or .14) ova were fertilized. In subsequent trials with rabbits of 1, 2, 4, or 10 days of pseudopregnancy and mean inseminated sperm concentration of 2.83×10^8 sperm per milliliter (range, 4.51×10^6 to 8.96×10^8 sperm per milliliter), ova were recovered and examined for evidence of fertilization of either (i) the presence of two pronuclei and two polar bodies or (ii) cleavage of the fertilized ovum.

Squirrel monkey ova were collected by laparoscopically aspirating the developed follicles on the ovary (*5*). Follicular growth was induced with an ovulatory regimen of gonadotropins (*6*), and sperm were collected by electroejaculation (*7*), with mean inseminated sperm concentration of 4.60×10^7 sperm per milliliter (range, 5.00×10^6 to 2.00×10^8 sperm per milliliter). The gametes were then transferred to the rabbit oviduct. The monkey trials were all carried out with rabbit surgery scheduled 24 hours after intravenous injection of 100 I.U. of hCG to the rabbit (1 day of pseudopregnancy). Hamster ova were recovered 28 hours after being deposited by flushing the excised rabbit oviduct with saline through the tubal-uterine junction. Because we suspected slower development on the basis of preliminary trials, we recovered monkey ova by the same procedure 72 hours after they were deposited in the rabbit oviduct. The recovery and xenogenous fertilization rates are shown in Table 1. In all, 16.3 percent of the hamster ova reached the two-cell stage, a value comparable to the preliminary trials.

The overall recovery rates of hamster and squirrel monkey ova were 33 and 55 percent, respectively, and fertilization rates were 60.1 and 36.4 percent. Xenogenously fertilized hamster embryos did not develop beyond the two-cell stage in the pseudopregnant rabbit oviduct, but squirrel monkey embryos developed as far as the eight-cell stage. In one instance a squirrel monkey ovum from a Guyanan-type female (seven acrocentric pairs of chromosomes; point of

Table 1. Xenogenous fertilization of hamster and squirrel monkey ova in oviducts of the pseudopregnant rabbit. Proportions are given in parentheses.

Species	Day of rabbit pseudo-pregnancy	Ovum donor/ rabbit	Embryos recovered	Fertilization
Hamster	1	20/10	108/372 (.29)	59/108 (.55)
Hamster	2	2/1	50/75 (.67)	36/50 (.72)
Hamster	4	2/1	23/58 (.40)	12/23 (.52)
Hamster	10	2/1	7/65 (.11)	6/7 (.9)
Squirrel monkey	1	52/10	22/40 (.55)	8/22 (.36)

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