0026-895X/91/050659-07$03.00/0

MOLECULAR PHARMACOLOGY, 39:659-665

Dopamine Receptor Reappearance after Irreversible Receptor Blockade: Effect of Chronic Estradiol Treatment of Ovariectomized Rats

DANIEL LÉVESQUE and THÉRÈSE DI PAOLO

School of Pharmacy, Laval University, Quebec, G1K 7P4, and Department of Molecular Endocrinology, CHUL Research Centre, Laval University Medical Centre, Quebec, G1V 4G2, Canada

Received October 22, 1990; Accepted February 25, 1991

SUMMARY

It is well established that estrogen modulates central dopamine functions; however, the mechanism of this interaction is still poorly understood. We have used peripheral *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) administration to induce irreversible blockade of dopamine receptors in ovariectomized female rats, which were pretreated with estradiol (10 μg, twice each day for 2 weeks) or its vehicle, in order to investigate the effect of estradiol on dopamine receptor repopulation kinetics. As previously observed, chronic estradiol treatment increased both striatal D1 and D2 dopamine receptor densities and left affinities unchanged. Anterior pituitary D2 dopamine receptor density remained unchanged. One day after EEDQ administration, a similar decrease (80%) of [^{3}H]SCH 23390 and [^{3}H]spiperone binding to striatum of estradiol- and vehicle-treated animals was observed. Anterior pituitary D2 dopamine receptor specific binding was reduced by about 50% the day after EEDQ. Recovery after EEDQ administration showed that both receptor production rate and degradation rate constants of anterior pituitary D2 and striatal D1 receptors were slowed after chronic estradiol treatment, whereas recovery rates for striatal D2 dopamine receptors were unaffected. EEDQ administration in vehicle-treated rats did not significantly affect plasma prolactin levels, whereas the combination of estradiol pretreatment and EEDQ administration led to increased plasma prolactin levels, compared with estradiol-treated animals that did not receive EEDQ. This suggests that only a fraction of anterior pituitary dopamine receptors are required for a maximal inhibition of prolactin secretion. Estradiol affected both striatal D1 and D2 dopamine receptor densities but only D1 dopamine receptor repopulation kinetics, suggesting that it may act by different mechanisms on each dopamine receptor. Alternatively, estradiol may affect dopamine receptor interaction. Thus, the present study raises the possibility that a biochemical D1/D2 receptor interaction affects dopamine receptor biosynthesis, turnover, and/or gene expression and that estradiol may influence this dopamine receptor interaction in the striatum.

It is now well established that steroid gonadal hormones, particularly estradiol, affect mammalian central nervous system DA receptors (1). For example, it has been shown that chronic estradiol treatment leads to an increased density of rat striatal D1 (2, 3) and D2 (4–8) DA receptors. The modulation by estrogen of rat anterior pituitary D2 DA receptors is more complex and has not yet led to a consensus. This discrepancy may come from differences in the doses and times of exposure to estrogen (9, 10). Estrogens and their catecholestrogen metabolites have no or very low affinity for DA receptors, as measured *in vitro* with binding of dopaminergic ligands (10, 11). Anterior pituitary cells, including lactotrophs, contain high estradiol receptor levels (12), as measured with autoradiographic techniques using [^{3}H]estradiol, whereas this technique fails to show such receptors in the striatum (13). Therefore, an estradiol receptor-mediated action on DA receptor biosynthesis and gene expression could be expected in the anterior pituitary but seems unlikely in the striatum. All these studies demonstrate that chronic administration of estrogen can affect central nervous system DA receptors, but the mechanism underlying this interaction is still poorly understood.

EEDQ produces, *in vivo*, a profound and irreversible inactivation of DA receptors in rats (14). It can also irreversibly alkylate other monoaminergic (15), cholinergic (16), and serotonergic (17) receptors. Peripheral administration of EEDQ markedly reduces striatal D1 and D2 DA receptor binding, without affecting either the catalytic subunit of adenylate cyclase or guanosine nucleotide binding to regulatory protein components (18, 19). Because of its characteristics, EEDQ has been proven useful in the study of DA receptor turnover and occupancy (20–22). However, because EEDQ extensively decreases DA receptor levels, thus disrupting the equilibrium of DA transmission, it is possible that the exact physiological receptor turnover is not measured, because EEDQ may affect the normal homeostatsis of DA receptor kinetics. Nevertheless, EEDQ has been shown to be a good tool to investigate the

This work was supported by the Medical Research Council of Canada.

ABBREVIATIONS: DA, dopamine; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; OVX, ovariectomized; PRL, prolactin.

mechanism involved in the DA receptor changes observed after denervation (23) or neuroleptic (24) or reserpine (25) treatments.

The receptor steady state level, which is generally measured as the receptor maximal density (B_{max}), is the product of the rates of receptor production and degradation. Therefore, alterations in the rates of receptor production and/or degradation may be responsible for changes in steady state levels of receptors. In this paper, we have studied DA receptor recovery at various time intervals after peripheral EEDQ administration in rats chronically treated with 17β-estradiol or its vehicle, in order to evaluate the possible effect of estrogens on DA receptor kinetic parameters.

Materials and Methods

Adult Sprague-Dawley female rats were purchased from Charles River Canada Inc. (St-Constant, Québec). Female rats weighing 250–350 g were bilaterally OVX under ether anesthesia, housed two/cage, and maintained at 22–23° on a 14/10-hr light-dark cycle (lights on from 5:00 a.m. to 7:00 p.m.). They received rat chow and water *ad libitum*. The day after ovariectomy, animals received injections of 10 μg of 17β-estradiol (Sigma) in 0.2 ml, subcutaneously, twice each day for 2 weeks, while a second group received injections of the vehicle (0.3% gelatin in saline solution). Estradiol was initially dissolved in a minimum of ethanol and then diluted to the appropriate concentration in the vehicle. The final volume of ethanol never exceeded 0.01% of the volume injected (0.2 ml), which was negligible. EEDQ (Aldrich Chemical Co.), freshly dissolved in ethanol/water (1:1, v/v), was injected intraperitoneally at a dose of 10 mg/kg, on a 0.5 ml/kg basis, after chronic estradiol or vehicle treatment. Two groups of rats, chronically treated with estradiol or the vehicle, received the ethanol/water (1:1) mixture as controls. All animals were sacrificed by decapitation in the morning (between 9:00 and 11:00 a.m.) 24 hr (day 1), 48 hr (day 2), 96 hr (day 4), or 168 hr (day 7) after EEDQ injection. Only rats demonstrating a good response to EEDQ (intense catalepsy with marked decrease of DA receptor density after 24 hr were included in the study. Estradiol was also injected during recovery times after EEDQ, to allow constant exposure to the hormone. The effect of chronic estradiol treatment for 1–4 weeks on striatal D2 DA receptors was previously shown to be similar (7, 10). Control groups (vehicle- and estradiol-treated animals that did not receive EEDQ) were sacrificed at the same time as the last recovery time group (day 7). Trunk blood was collected into glass tubes and serum was separated, after coagulation, by centrifugation at 4000 × *g* for 10 min and kept at −20° until assayed for PRL in duplicate by double-antibody radioimmunoassay, using rat PRL-I-5 and rabbit antisera (anti-rat PRL-S-9) kindly provided by National Hormone and Pituitary Program (Baltimore, MD).

Striata were immediately dissected, frozen in dry ice, and kept at −70° until assayed. They were homogenized (at 4°) in a glass-Teflon homogenizer, in 100 volumes (w/v) of Tris buffer containing 15 mM Tris·HCl and 1 mM $MgCl_2$ (pH 7.4), and centrifuged (at 4°) at 40,000 × *g* for 20 min. This washing procedure was repeated twice, and the final pellet was resuspended in 100 volumes of a Tris buffer containing 15 mM Tris·HCl, 1 mM, $MgCl_2$, 2 mM $CaCl_2$, 5 mM KCl, 12.5 μM nialamide, 0.1 mM EDTA, 0.1% ascorbic acid, and 120 mM NaCl (pH 7.4). D1 DA receptors were labeled using [*N-methyl*-^{3}H]SCH 23390 (Amersham), as previously described (3). Namely, we used six to eight concentrations (0.05–0.8 nM) of [*N-methyl*-^{3}H]SCH 23390 (specific activity, 80–95 Ci/mmol) with tissue preparations containing approximatively 100 μg of protein, as determined by the method of Lowry *et al.* (26), in a final volume of 2.0 ml. Nonspecific binding was estimated using 1 μM unlabeled SCH 23390 (Schering Co.). D2 DA receptors were labeled with [^{3}H]spiperone (Amersham), as described previously (5). We used six to eight concentrations (0.025–0.5 nM) of [^{3}H]spiperone (specific activity, 60–80 Ci/mmol), with 50 nM ketanserin to block [^{3}H]spiperone binding to the serotonin (5-HT_2) receptor. Nonspecific binding was estimated using 1 μM (+)-butaclamol (RBI).

Anterior pituitary tissues were immediately separated from the intermediate and posterior lobes, rapidly frozen in dry ice, and kept at −70° until assayed. Anterior pituitary tissues (individuals for estradiol-treated and pools of two or three pituitaries for vehicle-treated animals) were homogenized (at 4°), in 100 volumes of Tris buffer containing 25 mM Tris·HCl, 2 mM $MgCl_2$, and 0.25 M sucrose (pH 7.4), and centrifuged (4°) at 100 × *g* for 10 min. The pellet of this centrifugation was discarded, and the supernatant was centrifuged at 40,000 × *g* for 20 min. The second pellet was resuspended in 100 volumes of the same buffer without sucrose, homogenized, and centrifuged at 40,000 × *g* for 20 min. The final pellet was resuspended in 50 volumes of 25 mM Tris·HCl, 2 mM $MgCl_2$, 0.1% ascorbic acid, 0.1 mM EDTA, 12.5 μM nialamide buffer (pH 7.4). Anterior pituitary D2 DA receptors were measured in duplicate with [^{3}H]spiperone (0.12 nM) with the tissue preparation (approximately 200 μg of protein) in a final volume of 0.5 ml.

Incubation at 22° for 1 hr was terminated by rapid filtration through Whatman GF/C glass fiber filters under vacuum, followed by three rapid rinses (4 ml each) with the appropriate ice-cold buffer. Bound [*N-methyl*-^{3}H]SCH 23390 and [^{3}H]spiperone were measured by liquid scintillation counting, at a counting efficiency of about 56%. Striatal tissue was analyzed by saturation isotherm experiments, whereas specific anterior pituitary binding was obtained by subtraction of nonspecific from total binding at the ligand concentration used. For striatal DA receptor binding, Scatchard plots were constructed from saturation data, and least squares linear regression analyses were performed to calculate the equilibrium dissociation constant (K_d) and B_{max}.

Results

Saturation analysis of [^{3}H]spiperone and [^{3}H]SCH 23390 binding to striatal tissue showed a marked decline (about 80%) after EEDQ (10 mg/kg) administration (Tables 1 and 2). Binding affinities were essentially unaffected by chronic estradiol or by EEDQ administration, except for day 1 after EEDQ, when affinity of striatal D2 DA receptors was decreased (Table 2), and for day 7 after EEDQ for striatal D1 DA receptors, when affinities were increased (Table 1). Seven days after EEDQ, 90% of striatal D1 receptors had recovered in vehicle-treated animals, whereas only 68% of striatal D1 receptors from estradiol-treated group had reappeared (Table 1). Similar striatal D2 DA receptors recoveries in the vehicle- and estradiol-treated groups were seen at the various recovery intervals studied after EEDQ administration (Table 2). Anterior pituitary D2 receptors reached almost 100% recuperation after 4 days in vehicle-treated rats, whereas estradiol-treated animals reached this level of recuperation after 7 days (Table 3). Chronic administration of estradiol to OVX female rats increased the B_{max} of striatal D1 and D2 DA receptors and left affinity unchanged (Tables 1 and 2), as we (3, 5) and others (2, 6–8) have previously observed.

Specific [^{3}H]spiperone binding to anterior pituitary D2 DA receptors showed a 50% decrease 1 day after EEDQ injection in both vehicle-treated and estradiol-treated groups (Table 3). Plasma PRL levels were not significantly affected by EEDQ administration in rats chronically treated with vehicle, in spite of a 50% loss of specific D2 DA receptor binding sites. In chronically estradiol-treated rats, PRL levels were elevated, as expected, but EEDQ administration induced significantly higher levels 24 hr after EEDQ injection (Table 4). Estradiol also induced hyperplasia of pituitary tissue, resulting in a significant increase of pituitary weight, compared with vehicle-treated groups (control, 12.6 ± 0.2 mg; estradiol, 21.1 ± 0.9 mg; $p < 0.01$), concomitantly with an increase of protein content

TABLE 1

Recovery of striatal D1 DA receptors after EEDQ administration in chronically vehicle- and estradiol-treated OVX female rats

OVX Sprague-Dawley female rats were treated with either vehicle (0.3% gelatin in saline solution) or estradiol (10 μg, twice each day) for 2 weeks before EEDQ administration (10 mg/kg, intraperitoneally). They were sacrificed by decapitation, and striata were rapidly dissected. B_{max} and K_d values were determined from Scatchard analysis of [^{3}H]SCH 23390 (six to eight concentrations done in duplicate, ranging from 0.05 to 0.8 nM) saturation data, as described in Materials and Methods. Nonspecific binding was estimated in the presence of 1 μM SCH 23390. Values represent the means ± standard errors from five to seven animals assayed individually.

Time after EEDQ	Vehicle-treated rats			Estradiol-treated rats		
	B_{max}		K_d	B_{max}		K_d
days	*fmol/mg of protein*	*% of control*	*nM*	*fmol/mg of protein*	*% of control*	*nM*
Control	805 ± 35	100	0.197 ± 0.012	982 ± 21[a]	100	0.182 ± 0.006
1	142 ± 16	17.6	0.184 ± 0.008	179 ± 18	18.2	0.206 ± 0.009
2	285 ± 20	35.4	0.206 ± 0.009	306 ± 8	31.2	0.184 ± 0.012
4	482 ± 28	59.9	0.202 ± 0.009	464 ± 27	47.3[b]	0.195 ± 0.004
7	729 ± 32	90.6	0.162 ± 0.014[c]	671 ± 34	68.3[d]	0.160 ± 0.003

[a] $p = 0.0014$, versus respective vehicle-treated group, according to an unpaired student t test.
[b] $p < 0.02$, versus respective vehicle-treated group, according to an unpaired student t test.
[c] $p < 0.01$, versus control vehicle-treated group, according to an analysis of variance Fisher's protected least significant difference test.
[d] $p = 0.0005$, versus respective vehicle-treated group, according to an unpaired student t test.

TABLE 2

Recovery of striatal D2 DA receptors after EEDQ administration in chronically vehicle- and estradiol-treated OVX female rats

OVX Sprague-Dawley female rats were treated with either vehicle (0.3% gelatin in saline solution) or estradiol (10 μg, twice each day) for 2 weeks before EEDQ administration (10 mg/kg, intraperitoneally). They were sacrificed by decapitation, and striata were rapidly dissected. B_{max} and K_d values were determined from Scatchard analysis of [^{3}H]spiperone (six to eight concentrations done in duplicate, ranging from 0.025 to 0.5 nM) saturation data (in the presence of 50 nM ketenserin to preclude binding to serotonin receptors), as described in Materials and Methods. Nonspecific binding was estimated in the presence of 1 μM (+)-butaclamol. Values represent the means ± standard errors from five to seven animals assayed individually.

Time after EEDQ	Vehicle-treated rats			Estradiol-treated rats		
	B_{max}		K_d	B_{max}		K_d
days	*fmol/mg of protein*	*% of control*	*pM*	*fmol/mg of protein*	*% of control*	*pM*
Control	245 ± 7	100	29.2 ± 1.7	274 ± 10[a]	100	26.8 ± 1.9
1	44 ± 4	18.0	42.6 ± 5.2[b]	46 ± 5	16.8	39.2 ± 3.7[c]
2	87 ± 4	35.5	31.0 ± 2.6	85 ± 5	31.0	28.2 ± 1.6
4	114 ± 7	46.5	28.2 ± 1.8	130 ± 6	47.4	28.0 ± 2.3
7	171 ± 12	69.8	29.9 ± 1.8	196 ± 13	71.5	23.5 ± 1.7

[a] $p = 0.0004$, versus vehicle-treated group, according to an unpaired Student t test.
[b] $p < 0.01$, versus control vehicle-treated group, [c] $p < 0.05$, versus control estradiol-treated group, according to an analysis of variance Fisher's protected least significant difference test.

TABLE 3

Recovery of anterior pituitary D2 DA receptors after EEDQ administration in chronically vehicle- and estradiol-treated OVX female rats

OVX Sprague-Dawley female rats were treated with either vehicle (0.3% gelatin in saline solution) or estradiol (10 μg, twice each day) for 2 weeks before EEDQ administration (10 mg/kg, intraperitoneally). Anterior pituitaries were obtained from the same animals used for striatal dopamine receptor binding. Anterior pituitary D2 DA receptors were measured with a concentration of 0.12 nM [^{3}H]spiperone, and specific binding was obtained by subtraction from total [^{3}H]spiperone binding of nonspecific binding, estimated with 1 μM (+)-butaclamol. Values represent the means ± standard errors of three to five separate determinations done in duplicate from pooled anterior pituitaries (one to three) of respective groups.

Time after EEDQ	Specific binding			
	Vehicle-treated rats		Estradiol-treated rats	
days	*fmol/mg of protein*	*% of control*	*fmol/mg of protein*	*% of control*
Control	46.0 ± 3.7	100	56.0 ± 2.8	100
1	22.8 ± 0.4	49.6	30.0 ± 2.9	53.6
2	39.5 ± 4.2	85.9	34.3 ± 5.8	61.3
4	44.8 ± 1.9	97.0	42.0 ± 3.5	75.0[a]
7	49.3 ± 4.8	107.1[b]	52.3 ± 4.6	93.4

[a] $p = 0.03$ versus respective vehicle-treated group, according to an unpaired Student t test.
[b] This time point was not included in the repopulation curve, because 100% recovery was already reached at the earlier time recovery interval.

TABLE 4

Effect of chronic estradiol or vehicle treatments on OVX female rat plasma PRL levels after EEDQ administration

Plasma PRL concentrations were measured in duplicate by double-antibody radioimmunoassay, using rat PRL-I-5 and rabbit antisera (anti-rat PRL-S-9) kindly provided by National Hormone and Pituitary Program (Baltimore, MD). Values represent the means ± standard errors of 10 to 15 separate determinations.

Time after EEDQ	Plasma PRL	
	Vehicle-treated rats	Estradiol-treated rats
days	*ng/ml*	
Control	2.1 ± 0.5	54.4 ± 20.0[a]
1	3.7 ± 0.9	134.2 ± 25.3[b,c]
2	2.5 ± 0.6	43.2 ± 11.0[a]
4	1.9 ± 0.4	68.9 ± 17.6[b]
7	2.1 ± 0.6	58.6 ± 15.5[b]

[a] $p < 0.05$, [b] $p < 0.01$ versus respective vehicle-treated group; [c] $p < 0.01$, versus other estradiol-treated times after EEDQ, according to an analysis of variance Fisher's protected least significant difference test.

(control, 0.862 ± 0.010 mg/ml; estradiol, 1.045 ± 0.023 mg/ml; $p < 0.01$), with no significant differences in recovery time intervals after EEDQ administration.

According to the work of Mauger *et al.* (27), if receptor production rate is constant (zero-order) and receptor degradation is dependent on receptor concentration (first-order), then repopulation kinetics after irreversible blockade of receptors may be described by the equation

$$[R_t] = (r/k)\ (1 - e^{-kt}) \quad (1)$$

where $[R_t]$ is the receptor concentration at time t, r is the receptor production rate, and k is the rate constant for receptor degradation. When t tends to infinity, R_t approaches r/k, which is equal to $[R_{ss}]$, the concentration of receptor at steady state (B_{max}); therefore,

$$[R_{ss}] = r/k \quad (2)$$

Logarithmic transformation of eq. 1 (28) gives

$$\ln([R_{ss}]/[R_{ss}] - [R_t]) = kt \quad (3)$$

Plots of our receptor recovery data according to eq. 3 are shown in Figs. 1–3. The experimental repopulation curves showed that repopulation was always a monoexponential process. Slopes of the plots, which are equal to the rate constant of receptor degradation, k, as well as other kinetic parameters derived from the recovery data (receptor repopulation half-time and receptor production rate) are shown in the legends to their respective figures. Anterior pituitary D2 DA receptors showed the fastest degradation rate constant, whereas striatal D1 DA receptors had the highest receptor production rate. Rats chronically treated with estradiol had a significantly lower receptor degradation rate and receptor production rate of striatal D1 and anterior pituitary D2 DA receptors, compared with vehicle-treated rats, whereas striatal D2 DA receptor recovery parameters were not significantly affected by chronic estradiol treatment (Figs. 1–3). Estradiol treatment doubled and tripled the half-time for receptor repopulation of striatal D1 and anterior pituitary D2 receptors, respectively, whereas the half-time for receptor repopulation of striatal D2 receptors remained unchanged (see legends to Figs. 1–3).

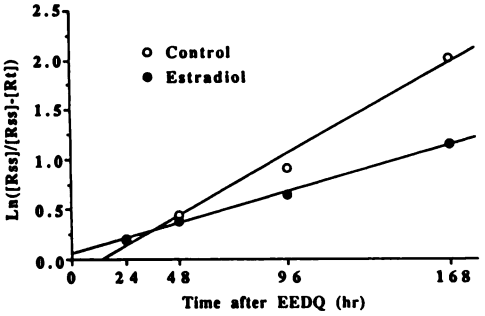

Fig. 1. Semilogarithmic plot of time course of striatal D1 DA receptor recovery in vehicle- (0.3% gelatin in saline solution) and 17β-estradiol-treated (10 μg, twice each day) OVX female rats after EEDQ administration (10 mg/kg). $[R_{ss}]$, respective steady state receptor levels (B_{max}) of rats (see Table 1) that did not receive EEDQ (they received the EEDQ vehicle, ethanol/water, 1:1 mixture, on a 0.5 ml/kg basis). $[R_t]$, receptor concentration at various time intervals after EEDQ blockade. The slope values, which represent the receptor degradation rate constant k, are equal to 0.0127 hr^{-1} (95% confidence interval from 0.0080 to 0.0170 hr^{-1}) and 0.0065 hr^{-1} (95% confidence interval from 0.0060 and 0.0070 hr^{-1}) for vehicle- and estradiol-treated rats, respectively. Corresponding receptor production rates, r, are 10.2 and 6.4 fmol/mg of protein/hr and half-times of receptor repopulation, $t_{1/2}$ are 54.6 and 106.6 hr (obtained by substitution of k into the equation $t_{1/2} = 0.693/k$). Statistical comparison between slopes of respective treated groups was performed according the method described by Neter and Wasserman (43), in which β_3 terms of multiple regression curve equations are tested for zero equality. This analysis showed that the slopes for vehicle- and estradiol-treated groups were not parallel ($p = 0.004$), indicating distinct k values.

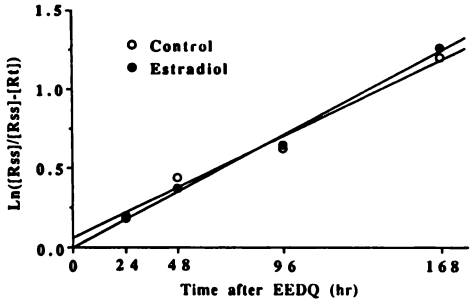

Fig. 2. Semilogarithmic plot of time course of striatal D2 DA receptor recovery in vehicle- (0.3% gelatin in saline solution) and 17β-estradiol-treated (10 μg, twice each day) OVX female rats after EEDQ administration (10 mg/kg). $[R_{ss}]$, respective steady state receptor levels (B_{max}) of rats (see Table 2) that did not receive EEDQ (they received the EEDQ vehicle, ethanol/water, 1:1 mixture, on a 0.5 ml/kg basis). $[R_t]$, receptor concentration at various time intervals after EEDQ blockade. The slope values, which represent the receptor degradation rate constant k, are equal to 0.0066 hr^{-1} (95% confidence interval from 0.0040 to 0.0090 hr^{-1}) and 0.0073 hr^{-1} (95% confidence interval from 0.0050 and 0.0090 hr^{-1}) for vehicle- and estradiol-treated rats, respectively. Corresponding receptor production rates, r, are 1.6 and 2.0 fmol/mg of protein/hr and half-times of receptor repopulation, $t_{1/2}$, are 105.0 and 94.9 hr (obtained by substitution of k into the equation $t_{1/2} = 0.693/k$). Statistical comparison between slopes of respective treated groups was performed according the method described by Neter and Wasserman (43), in which β_3 terms of multiple regression curve equations are tested for zero equality. This analysis showed that the slopes for vehicle- and estradiol-treated groups were parallel ($p = 0.4193$), indicating that the k values were not different.

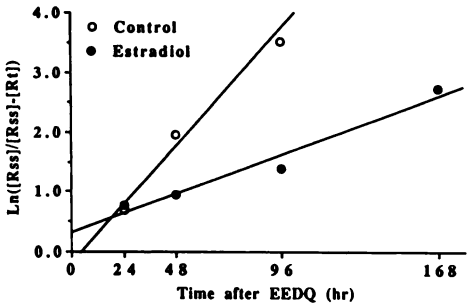

Fig. 3. Semilogarithmic plot of time course of anterior pituitary D2 DA receptor recovery in vehicle- (0.3% gelatin in saline solution) and 17β-estradiol-treated (10 μg, twice each day) OVX female rats after EEDQ administration (10 mg/kg). $[R_{ss}]$, respective densities of receptors, measured with 0.12 nM [^{3}H]spiperone binding (see Table 3), from rats that did not receive EEDQ (they received the EEDQ vehicle, ethanol/water, 1:1 mixture, on a 0.5 ml/kg basis). $[R_t]$, receptor concentration at various time intervals after EEDQ blockade. The slopes values, which represent the receptor degradation rate constant k, are equal to 0.0382 hr^{-1} (95% confidence interval from 0.0270 to 0.0650 hr^{-1}) and 0.0136 hr^{-1} (95% confidence interval from 0.0060 to 0.0220 hr^{-1}) for vehicle- and estradiol-treated rats, respectively. Corresponding receptor production rates, r, were obtained in a separate experiment. In this experiment, B_{max} values were obtained from Scatchard analysis of [^{3}H]spiperone binding (0.05–0.5 nM) to DA receptors of vehicle- (0.3% gelatin in saline solution) and 17β-estradiol-treated OVX female rats (30 rats/group). Nonspecific binding was estimated with 1 μM (+)-butaclamol. Values obtained are: control, B_{max} = 82 ± 14 fmol/mg of protein, K_d = 79 ± 19 pM; estradiol, B_{max} = 73 ± 5 fmol/mg of protein, K_d = 63 ± 2 pM); they represent means ± standard errors of three to five separate determinations done in duplicate with pooled (four to eight) anterior pituitaries. With these B_{max} values, calculated receptor production rates are 3.1 and 1.1 fmol/mg of protein/hr, respectively. Half-times of receptor repopulation, $t_{1/2}$ are 18.1 and 50.9 hr, respectively (obtained by substitution of k into the equation $t_{1/2} = 0.693/k$). Statistical comparison between slopes of respective treated groups was performed according the method described by Neter and Wasserman (43), in which β_3 terms of multiple regression curve equations are tested for zero equality. This analysis showed that the slopes of vehicle- and estradiol-treated groups were not parallel ($p = 0.015$), indicating distinct k values.

Discussion

We observed that chronic estradiol treatment increased both D1 and D2 DA receptor densities in the striatum, whereas affinities of both DA receptor binding sites remained unchanged, as previously reported (2–8). In terms of numbers of new DA receptor molecules induced by estradiol in the striatum, the effect of this steroid is more important for D1 DA receptors, where this hormone has induced almost as many new D1 DA

receptors as there are D2 receptors in the striatum (see Tables 1 and 2). Anterior pituitary D2 DA receptor density is modulated in a more complex fashion by estradiol; there is an increase after chronic estradiol at low concentrations (1 ng), whereas with higher concentrations no effect is observed, and at doses higher than 1 μg a decrease is measured (29). In the present experiments, we did not observe significant changes of D2 DA receptor-specific binding (Table 3), whereas the B_{max} tended to be lower (see legend to Fig. 3). This may be due to a shift in the dose response, with the rats used in the present experiment being less responsive to estradiol than those reported in our previous study. This could also be the case for the effect of estradiol on striatal D2 receptor density, which is somewhat smaller than previously reported (4–8).

Administration of EEDQ in vehicle-treated animals did not significantly affect plasma PRL levels, in spite of a loss of about 50% of DA receptors in the anterior pituitary. Twenty-four hours after EEDQ administration, estradiol-treated rats showed a significant increase of plasma PRL levels, compared with estradiol-treated rats not treated with EEDQ. Estradiol has an antidopaminergic activity on anterior pituitary PRL secretion (30). This antidopaminergic effect was proposed to result from an action on D2 DA receptor-mediated inhibition of cyclic AMP formation by promotion of a functional uncoupling of D2 DA receptors from the adenylate cyclase effector system (31). EEDQ administration in the present experiments seemed to potentiate the antidopaminergic effect of chronic estradiol on PRL secretion. This indicates that the DA receptor-blocking action of EEDQ in combination with the effector moiety-blocking activity of estradiol could be more potent in blocking DA actions on PRL secretion than are either of them individually. This also suggests the presence of spare DA receptors for inhibition of PRL secretion in the anterior pituitary, as previously suggested by Shin (32) for cultured pituitary cells after phenoxybenzamine pretreatment. Moreover, chronic bromocriptine treatment, which reduces estradiol-induced pituitary tumor [^{3}H]spiperone binding by about 75%, is able to decrease plasma PRL secretion (33).

The rate constant for synthesis, r, describes a multistep process that includes both the synthesis and insertion of receptor into the membrane. Similarly, the degradation rate constant, k, describes multiple processes, including the internalization and degradation of receptors. *In vivo* EEDQ administration affects specifically the membrane receptor recognition site, leaving adenylate cyclase moieties and GTP binding to regulatory components intact (18, 19). Moreover, cycloheximide administration before EEDQ inhibits the receptor recovery rates, indicating that the process of receptor repopulation is dependent on newly synthesized receptor proteins (20). The response to a decrease in the level of neurotransmission, which ultimately leads to an increase in the steady state level of receptor, may result from an increase in transcription of the gene(s) responsible for the synthesis of new receptor proteins for that particular neurotransmitter or result from a decrease in the degradation rate constant of this receptor. Alternatively, changes in processes responsible for receptor membrane internalization or receptor membrane insertion could also be altered.

After EEDQ administration, recovery of anterior pituitary D2 DA receptors was slowed by chronic estradiol pretreatment; both receptor production and degradation rates were decreased by about 3-fold, whereas half-time repopulation was about tripled (see legend to Fig. 3). The altered state of the anterior pituitary DA receptor induced by chronic estradiol (31) may reduce DA neurotransmission and ultimately affect receptor turnover kinetic parameters. DA receptors that are uncoupled from their effector by estradiol (31) may interfere with receptor membrane internalization and insertion processes. Moreover, the presence of estradiol receptors in the anterior pituitary may lead to a genomic action of this steroid by an interaction with regulatory components of the D2 DA receptor gene or with the coupling effector system (GTP-binding protein) gene(s), as previously suggested (34, 35). If that is the case, estradiol may decrease *de novo* DA receptor biosynthesis, which is reflected by the slower repopulation kinetics observed here, after EEDQ administration.

EEDQ administration produced a marked decrease of both striatal D1 and D2 DA receptor densities (Tables 1 and 2), which is consistent with previous reports (24, 25, 36, 37). We also observed a decrease of striatal D2 DA receptor affinities 24 hr after EEDQ. This was not reported consistently previously; for example, Leff *et al.* (20) have shown such effects, whereas Pich *et al.* (24) did not. The reason for this discrepancy is unclear and may result from experimental difficulties in measuring very low concentrations of DA receptors at this recovery time interval. Indeed, we did not observe a similar effect on binding affinities of D1 receptors, which have a higher receptor density at this time. On the other hand, increased affinity of D1 DA receptor binding on day 7 after EEDQ may result from a greater efficacy of newly synthesized receptor molecules. The recovery kinetic parameters of striatal D1 and D2 receptors measured here are in agreement with those previously reported in a similar paradigm (20, 24, 25, 37). Striatal D1 DA receptor production rate, measured in the present study, was about 6 times higher than for striatal D2 receptors, and a 2-fold higher receptor degradation rate constant for D1 receptors, compared with D2 receptors, was observed. These rate constants would lead to a steady state level of striatal D1 DA receptors about 3-fold higher than the D2 receptor steady state level. We observed here, in striatal homogenates, a 3-fold higher density of D1 receptors, compared with D2 receptors. This is also in good agreement with autoradiographic analysis of DA receptor densities in the caudate putamen, which indicates a 3- to 4-fold higher density of D1 compared with D2 receptors (38, 39). Therefore, our results suggest that recovery kinetic parameters, as measured with irreversible blocking agents like EEDQ, are a good reflection of apparent observed steady state levels of the respective DA receptors.

To the best of our knowledge, this is the first report of recovery kinetic data after EEDQ for anterior pituitary D2 receptors. We observed that D2 receptors in the striatum and in the anterior pituitary have distinct recovery parameters; the receptor degradation rate constant for anterior pituitary D2 receptors is 6 times higher and the receptor production rate is 2 times higher, whereas the half-time repopulation is 6 times lower than that for striatal D2 receptors (see legends to Figs. 2 and 3). This is reflected by the higher level of D2 receptors measured 24 hr after EEDQ in anterior pituitary (50%), compared with the level of striatal D2 receptors (20%) observed at this time, which results from a faster receptor recovery in the former structure. This indicates that, in spite of the fact that both structures have pharmacologically and biochemically similar D2 receptors, they are differently processed in their respective cell membrane.

Chronic neuroleptic treatment has been shown to slow the

recovery rates (24), whereas chronic reserpine treatment or lesion of the ascending DA nigrostriatal pathway by 6-hydroxydopamine increases the striatal D2 receptor production rate (23). All these treatments reduce striatal dopaminergic transmission and lead to the development of D2 DA postsynaptic receptor supersensitivity. Chronic neuroleptic treatment produces a postsynaptic blockade, whereas a depleting agent like reserpine or nigral 6-hydroxydopamine lesion induces impairment of DA release. The decrease of receptor degradation rate constant observed by Pich *et al.* (24) is more important than the decrease of receptor production rate. Thus, it can be proposed that the up-regulation of striatal D2 receptors after chronic neuroleptic treatment may preferentially result from receptor membrane stabilization caused by a more constant occupancy of the DA receptor during the neuroleptic treatment (which may interfere with receptor internalization), instead of an increase in receptor production. It is interesting to note that the pattern of action of estradiol on striatal D1 DA receptor kinetic parameters is similar to what is observed with neuroleptics.

Estradiol can affect both pre- and postsynaptic elements of DA neurons (1). However, estrogen treatment produces an increased density of striatal D2 DA receptors even after denervation of the nigrostriatal DA pathway, which eliminates the presynaptic elements (40), whereas estrogen cannot increase spiperone binding after kainic acid lesion of the striatum, which destroys mainly the postsynaptic DA receptor located on intrinsic neurons (41). Thus, increased striatal D2 dopamine receptor density after chronic estradiol treatment cannot be viewed as a compensatory effect of estradiol-induced presynaptic dysfunction and is not the result of a direct interaction with the DA receptor, as mentioned earlier (10, 11).

The effect of estradiol on DA receptor recovery in the striatum may be more complex than the effect on pituitary gland D2 DA receptors, because of the existence of D1/D2 DA receptors interactions in the brain. Indeed, Cameron and Crocker (42) demonstrated that stimulation of D1 DA receptors facilitates D2 receptor recovery after EEDQ administration, suggesting that D2 receptor turnover may be influenced by D1 receptors. Therefore, estradiol may act on striatal D2 DA receptor repopulation processes after EEDQ, but this effect could be compensated for by perturbation of striatal D1 receptor functions.

Nevertheless, our results raise the possibility that DA receptor biosynthesis and/or gene expression can be influenced by heterologous alteration of DA receptor functions induced by chronic estradiol. Thus, a functional interaction between DA receptors at these levels may exist and can be affected by chronic estradiol treatment. We cannot rule out the possibility that an action of estradiol on other transmitters may have contributed to the effects of estradiol on DA receptors observed here; more experiments on DA receptor gene regulatory elements and on DA receptor post-transcriptional events are needed to confirm this assertion.

References

1. Van Hartesveldt, C., and J. N. Joyce. Effects of estrogen on the basal ganglia. *Neurosci. Biobehav. Rev.* **10**:1–14 (1986).
2. Hruska, R. E., and M. W. Nowak. Estrogen treatment increases the density of D_1 dopamine receptors in the rat striatum. *Brain Res.* **442**:349–350 (1988).
3. Lévesque, D., and T. Di Paolo. Chronic estradiol treatment increases ovariectomized rat striatal D-1 dopamine receptors. *Life Sci.* **45**:1813–1820 (1989).
4. Di Paolo, T., R. Carmichael, F. Labrie, and J.-P. Raynaud. Effects of estrogens on the characteristics of [^{3}H]spiperone and [^{3}H]RU 24213 binding in rat anterior pituitary gland and brain. *Mol. Cell. Endocrinol.* **16**:99–112 (1979).
5. Di Paolo, T., P. Poyet, and F. Labrie. Effect of prolactin and estradiol on rat striatal dopamine receptors. *Life Sci.* **31**:2921–2929 (1982).
6. Hruska, R. E., L. M. Ludman, and E. K. Silbergeld. Characterization of the striatal dopamine receptor supersensitivity produced by estrogen treatment of male rats. *Neuropharmacology* **19**:923–926 (1980).
7. Hruska, R. E. Elevation of striatal dopamine receptors by estrogen: dose and time studies. *J. Neurochem.* **47**:1908–1915 (1986).
8. Gordon, J. H., and K. O. Perry. Pre- and postsynaptic neurochemical alterations following estrogen-induced striatal dopamine hypo- and hypersensitivity. *Brain Res. Bull.* **10**:425–428 (1983).
9. Clopton, J. K., and J. H. Gordon. Separate molecular mechanisms for estrogen-induced up- and down-regulation of striatal dopamine receptors, in *Dopaminergic Systems and Their Regulation* (G. N. Woodruff, J. A. Poat, and P. J. Roberts, eds.). McMillan Press, London, 293–302 (1986).
10. Di Paolo, T., and N. Barden. Effect of oestrogen and prolactin on dopamine receptors: further characterization, in *Dopaminergic Systems and Their Regulation* (G. N. Woodruff, J. A. Poat, and P. J. Roberts, eds.). McMillan Press, London, 323–337 (1986).
11. Hiemke, C., M. Banger, R. Ghraf, and R. Knuppen. Do catecholestrogens interact with the *in vitro* binding of radioligands to catecholamine receptors? *J. Steroid Biochem.* **19**:1469–1474 (1983).
12. Stumpf, W. E., and M. Sar. Autoradiographic techniques for localizing steroid hormones. *Methods Enzymol.* **36**:135–156 (1975).
13. Stumpf, W. E., M. Sar, and D. A. Keefer. Atlas of estrogen target cells in the rat brain, in *Anatomical Neuroendocrinology, International Conference on Neurobiology of CNS-Hormone Interactions.* Karger, Basel, 104–119 (1975).
14. Hamblin, M. W., and I. Creese. Behavioral and radioligand evidence for irreversible dopamine receptor blockade by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. *Life Sci.* **32**:2247–2255 (1983).
15. Belleau, B., R. Martel, G. Lacasse, M. Ménard, N. Weinberg, and Y. G. Perron. *N*-Carboxylic acid esters of 1,2- and 1,4-dihydroquinoline: a new class of irreversible inactivators of the catecholamine *alpha* receptors and potent central system depressants. *J. Am. Chem. Soc.* **90**:823–824 (1968).
16. Chang, K. J., J. F. Moran, and D. J. Triggle. Mechanism of cholinergic antagonism by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). *Pharmacol. Res. Commun.* **2**:63–66 (1970).
17. Battaglia, G., B. Andrew, P. L. Newton, and I. Creese. *In vivo* and *in vitro* irreversible blockade of cortical S2 serotonin receptors by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline: a technique for investigating S2 serotonin receptor recovery. *J. Neurochem.* **46**:589–593 (1986).
18. Hess, E. J., G. Battaglia, A. B. Norman, and I. Creese. Differential modification of striatal D_1 dopamine receptors and effector moieties by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline *in vitro* and *in vivo*. *Mol. Pharmacol.* **31**:50–57 (1986).
19. Cameron, D. L., and A. D. Crocker. Alkylation of striatal dopamine receptors abolishes stereotyped behavior but has no effect on dopamine stimulated adenylate cyclase activity. *Neurosci. Lett.* **90**:165–171 (1988).
20. Leff, S. E., R. Gariano, and I. Creese. Dopamine receptor turnover rates in the rat striatum are age-dependent. *Proc. Natl. Acad. Sci. USA* **81**:3910–3914 (1984).
21. Fukuchi, I., N. Fujita, M. Nakahiro, K. Saito, and H. Yoshida. D-2 dopamine receptors synthesis and turnover in rat striatum. *Eur. J. Pharmacol.* **127**:291–294 (1986).
22. Nowak, G., J. Arnt, and J. Hyttel. EEDQ, a tool for *ex vivo* measurement of occupancy of D-1 and D-2 dopamine receptors. *Eur. J. Pharmacol.* **153**:309–311 (1988).
23. Neve, K. A., S. Loeschen, and J. F. Marshall. Denervation accelerates the reappearance of neostriatal D-2 receptors after irreversible receptor blockade. *Brain Res.* **329**:225–231 (1985).
24. Pich, E. M., F. Benfenati, C. Farabegoli, K. Fuxe, E. Meller, M. Arosson, M. Goldstein, and L. F. Agnati. Chronic haloperidol affects striatal D_2-dopamine receptor reappearance after irreversible receptor blockade. *Brain Res.* **435**:147–152 (1987).
25. Norman, A. B., G. Battaglia, and I. Creese. Differential recovery rates of rat D_2 dopamine receptors as a function of aging and chronic reserpine treatment following irreversible modification: a key to receptor regulatory mechanisms. *J. Neurosci.* **7**:1484–1491 (1987).
26. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
27. Mauger, J.-P., F. Sladeczek, and J. Bockaert. Characteristics and metabolism of α_1 adrenergic receptors in a nonfusing muscle cell line. *J. Biol. Chem.* **257**:875–879 (1982).
28. Sladeczek, F., and J. Bockaert. Turnover *in vivo* of *alpha*$_1$-adrenergic receptors in rat submaxillary glands. *Mol. Pharmacol.* **23**:282–288 (1983).
29. Di Paolo, T., and P. Falardeau. Modulation of brain and pituitary dopamine receptors by estrogens and prolactin. *Prog. Neuro-Psychopharmacol. Biol Psychiatry* **9**:473–480 (1985).
30. Raymond, V., M. Beaulieu, F. Labrie, and J. Boissier. Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. *Science (Washington D. C.)* **200**:1173–1175 (1978).
31. Munemura, M., A. Takashi, and D. R. Sibley. Chronic estrogen treatment promotes a functional uncoupling of the D_2 dopamine receptor in rat anterior pituitary gland. *Endocrinology* **124**:346–355 (1989).

32. Shin, S. H. Dopamine-induced inhibition of prolactin release from cultured adenohypophysial cells: spare receptors for dopamine. *Life Sci.* **22**:67–74 (1977).
33. Di Paolo, T., and P. Falardeau. Dopamine receptors in rat pituitary and estradiol-induced pituitary tumors: effect of chronic treatment with bromocriptine. *Biochem. Biophys. Res. Commun.* **123**:312–316 (1984).
34. Enjalbert, A., P. Bertrand, J. Bockaert, S. Drouva, and C. Kordon. Multiple coupling of neurohormone receptors with cyclic AMP and inositol phosphate production in anterior pituitary cells. *Biochimie (Paris)* **69**:271–279 (1987).
35. Maus, M., P. Bertrand, S. Drouva, R. Rasolonjanahary, C. Kordon, J. Glowinski, J. Prémont, and A. Enjalbert. Differential modulation of D_1 and D_2 dopamine-sensitive adenylate cyclase by 17β-estradiol in cultured striatal neurons and anterior pituitary cells. *J. Neurochem.* **52**:410–418 (1989).
36. Battaglia, G., A. B. Norman, E. J. Hess, and I. Creese. Functional recovery of D_1 dopamine receptor-mediated stimulation of rat striatal adenylate cyclase activity following irreversible receptor modification by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ): evidence for spare receptors. *Neurosci. Lett.* **69**:290–295 (1986).
37. Fuxe, K., L. F. Agnati, E. M. Pich, E. Miller, and M. Goldstein. Evidence for a fast receptor turnover of D_1 dopamine receptor in various forebrain regions of the rat. *Neurosci. Lett.* **81**:183–187 (1987).
38. Boyson, S. J., P. McGonigle, and P. B. Molinoff. Quantitative autoradiographic localization of the D_1 and D_2 subtypes of dopamine receptors in rat brain. *J. Neurosci.* **6**:3177–3188 (1986).
39. Savasta, M., A. Dubois, J. Benavidès, and B. Scatton. Different plasticity changes in D_1 and D_2 receptors in rat striatal subregions following impairment of dopaminergic transmission. *Neurosci. Lett.* **85**:119–124 (1988).
40. Hruska, R. E., and E. K. Silbergeld. Increased dopamine receptor sensitivity after estrogen treatment using the rat rotation model. *Science (Washington D. C.)* **208**:1466–1468 (1980).
41. Hruska, R. E., and K. T. Pitman. Distribution and localization of estrogen-sensitive dopamine receptors in the rat brain. *J. Neurochem.* **39**:1418–1423 (1982).
42. Cameron, D. L., and A. D. Crocker. Stimulation of D-1 dopamine receptors facilitates D-2 dopamine receptor recovery after irreversible receptor blockade. *Neuropharmacology* **27**:447–450 (1988).
43. Neter, J., and W. Wasserman. Indicator variables, in *Applied Linear Statistical Models* (R. D. Irwin, ed.). R. D. Irwin, Inc., Homewood, IL, 301–308 (1974).

Send reprint requests to: Dr. Thérèse Di Paolo, Department of Molecular Endocrinology, CHUL Research Centre, Laval University Medical Centre, 2705, Laurier Boulevard, Ste-Foy, Québec, Canada, G1V 4G2.