Biochemical Pharmacology, Vol. 45, No. 3, pp. 723–733, 1993.
Printed in Great Britain.

0006-2952/93 $6.00+0.00

MODULATION BY ESTRADIOL AND PROGESTERONE OF THE GTP EFFECT ON STRIATAL D-2 DOPAMINE RECEPTORS

DANIEL LÉVESQUE and THÉRÈSE DI PAOLO*

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

(*Received* 24 *March* 1992; *accepted* 8 *October* 1992)

Abstract—Agonist binding properties of rat striatal D-2 dopamine (DA) receptors were investigated after *in vivo* or *in vitro* estradiol or progesterone exposures in order to elucidate the mechanism of action of steroid hormones on DA receptors. Chronic estradiol treatment of ovariectomized rats (10 μg, twice each day, for 2 weeks) increased lateral striatum total receptor density and left unchanged the proportion and affinity of the agonist high- and low-affinity states of this receptor in the striatum. In addition, when GTP was added in DA competition for [^{3}H]spiperone binding experiments, D-2 receptors in the medial part of the striatum from estrogen-treated animals were more sensitive to GTP than those in the lateral part, whereas GTP had equal activity in both parts of the striatum in vehicle-treated rats. With apomorphine, but not with DA competition for [^{3}H]spiperone binding, addition of estradiol (1 nM) to striatal homogenates of intact male rats prevented the expected shift of the high- to the low-affinity state of D-2 receptors, normally induced by GTP (100 μM) under these conditions. This effect of estradiol was not observed in the presence of 4 mM $MgCl_2$, while *in vitro* progesterone (100 nM) had no effect in either the absence or presence of $MgCl_2$. In addition, *in vivo* chronic progesterone treatment of ovariectomized rats left striatal [^{3}H]spiperone density and affinity unchanged. Moreover, 1 nM estradiol increased the IC_{50} of GTP for inhibition of [^{3}H]*N*-propylnorapomorphine binding to the high-affinity state of striatal D-2 receptors. This effect was also observed but decreased by 2-fold in the presence of $MgCl_2$. Our data suggest that estradiol *in vivo* and *in vitro* interferes with the effect of GTP on striatal D-2 DA receptors.

Dopamine (DA)† D-2 receptors, both in anterior pituitary and striatum, exist in high- and low-affinity states for DA and dopaminergic agonists [1, 2]. Coupling of the high-affinity state of the receptor to an inhibitory guanine nucleotide regulatory protein (G-protein or G_i) that mediates the association of the receptor with adenylate cyclase activity is well known [3]. Guanine nucleotides have been shown to convert the agonist high-affinity state of the receptor into its low-affinity form in both anterior pituitary and striatum [4, 5].

17β-Estradiol (E_2) is well known to be involved in reproductive physiology and behaviors [6–8], and much evidence suggests that this hormone is also involved in the modulation of DA neurotransmission in basal ganglia [9]. These studies show that E_2 has multiple actions upon DA systems. For example, chronic administration of pharmacological doses of E_2 increases the density of D-2 receptors in the striatum of rats of both sexes [10–14], as measured by antagonist binding, whereas smaller doses of E_2 or fluctuations of hormone levels as occur during the rat estrous cycle leave total D-2 receptor density unchanged [15, 16]. In addition, an injection of E_2 or progesterone (P), at a physiological dose, acutely increases DA and/or its metabolite levels in the rat striatum [17, 18]. This short-term effect of E_2 is associated with behavioral activation and a shift of the high-affinity state of striatal D-2 receptors to the low affinity state [17, 19]. Moreover, proportions of the high- and low-affinity D-2 agonist states were shown to fluctuate during the rat estrous cycle or after ovariectomy [16].

The increase in D-2 receptor density in the striatum caused by chronic estradiol treatment was shown previously by us and other investigators to be the same in ovariectomized (OVX) female rats [10, 11, 14, 15, 20] and intact male rats [12, 13]. In our laboratory, we have used mostly OVX female rats in our chronic steroid treatments. Since this work directly follows our previous studies, for a better comparison with past results we again used OVX rats. Indeed, in the present study, we confirmed our past observation that the estradiol effect was restricted to an increase of D-2 receptor density in the lateral striatum and left these receptors in the medial striatum unchanged. In the present studies we further characterized the different responses of

* Corresponding author: Dr Thérèse Di Paolo, Department of Molecular Endocrinology, CHUL Research Centre, Laval University Medical Centre, 2705, Laurier Boulevard, Ste-Foy, Québec, Canada G1V 4G2. Tel. (418) 654-2296; FAX (418) 654-2761.

† Abbreviations: APO, (−)-apomorphine; B_{max}, maximal receptor density; DA, dopamine; E_2, 17β-estradiol; Gpp(NH)p, guanosine 5′-[β-γ-imido]triphosphate; K_d, equilibrium dissociation constant; K_{high}, high-affinity inhibition constant; K_{low}, low-affinity inhibition constant; NPA, *N*-propylnorapomorphine; OVX, ovariectomized; P, progesterone; PLG, L-propyl-L-leucyl glycinamide; R_{high}, density of receptor in its high-affinity state; and R_t, total receptor density.

the lateral versus the medial striatum by investigating the agonist site of this receptor as well as its coupling with G-proteins with the effect of GTP. No publication as of yet has described in the striatum the *in vitro* effect of steroids on the agonist sites of D-2 receptors and their conversion by GTP. Nevertheless, preliminary data reported in an abstract showed that E_2 regulates *in vitro* the DA receptor interaction with G-proteins in the striatum of male rats [21]. To further explore these preliminary data and because we did not have any background information such as for our *in vivo* experiments, we chose to use intact males as a model for the *in vitro* studies in this manuscript. The choice of male rats for the present *in vitro* studies was also a simpler model to start with because in female rats the D-2 agonist sites fluctuate during the estrous cycle and are different in OVX rats [16].

In the present study, agonist binding properties of D-2 receptors after *in vivo* or *in vitro* exposure to steroid gonadal hormones were examined in the rat striatum to further investigate the possible mechanism of action of these hormones on D-2 receptors and on guanine nucleotide coupling to this receptor.

MATERIALS AND METHODS

Materials

Hormones (E_2 and P) were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.) [^{3}H]-Spiperone (80–110 Ci/mmol) was obtained from Amersham Int. (Canada) and [^{3}H]*N*-propylnor-apomorphine ([^{3}H]NPA, 55–70 Ci/mmol) from New England Nuclear (Dupont NEN, Canada). GTP was purchased from Boehringer Mannhein Canada Ltd. and (+)-butaclamol, (−)-apomorphine (APO) and ketanserin were obtained from Research Biochemical Inc. (RBI, U.S.A.).

Animals

Adult Sprague–Dawley rats were purchased from Charles River Canada Inc., St-Constant, Québec. Ovariectomized female rats (70 rats, each weighing 250–350 g) were used in the *in vivo* chronic steroid treatment experiments shown, whereas in the *in vitro* studies on the effects of steroids on DA receptors the striata of intact male rats (300 rats each weighing 200–250 g) were used. Female rats were bilaterally ovariectomized under ether anesthesia, housed two per 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 lib*. The day after ovariectomy, female rats were injected subcutaneously with 10 μg of E_2 (thirty) in 0.2 mL or 1 mg of P (five) in 0.2 mL twice each day, for 2 weeks, while another group of rats (thirty five) received injections of the vehicle (0.3% gelatin in saline solution), as control. Rats were killed by decapitation the morning after their last injection.

Tissue preparation

Brains were quickly removed immediately after decapitation of the rats. Striata from vehicle- and E_2-treated female rats were dissected in their lateral and medial parts. Total striata were dissected for intact male rats and in the experiment with chronic P treatment of female rats.

The lateral/medial dissection was performed as reported previously [20], namely a coronal section of the brain was made to cut the forebrain from the rest of the brain. This coronal brain section exposed the striatum which was then cut in half by eye to separate lateral from medial striatum. The lateral/medial cut was made before removing the striatum from the coronal forebrain block in order to have a good evaluation of the middle of the striatum and hence a more precise dissection. Then both lateral parts were removed from the forebrain block and pooled; pools of both medial parts were also done.

Striata were immediately frozen in dry ice and kept at −70° until assayed. Tissue homogenates were obtained according to previously published procedures [10, 11]. Namely, striata were homogenized (at 4°) in a glass–teflon homogenizer in 100 vol. (w/v) of Tris buffer containing 15 mM Tris–HCl and 4 mM $MgCl_2$, when stated (tissue preparation buffer, pH 7.4), and centrifuged (at 4°) at 40,000 *g* for 20 min. This washing procedure was repeated twice and the final pellet resuspended in 100 vol. of a Tris buffer containing 15 mM Tris–HCl, 4 mM $MgCl_2$ (when stated), 2 mM $CaCl_2$, 5 mM KCl, 12.5 μM nialamide, 0.1 mM EDTA, 0.1% ascorbic acid and 120 mM NaCl (incubation buffer, pH 7.4).

Receptor binding assays

Agonist competition experiments. The D-2 agonist high- and low affinity states were determined by measuring the competition of [^{3}H]spiperone (0.1 to 0.2 nM) binding by APO (10^{-12} to 10^{-4} M), as previously described [19], and by DA (10^{-11} to 10^{-3} M) in the presence of 50 nM ketanserin to block serotonin (5-HT_2) binding sites in a final volume of 2.0 mL (incubation buffer with or without 4 mM $MgCl_2$). Nonspecific binding was estimated using 1.0 μM (+)-butaclamol. Binding experiments were performed in duplicate. E_2 (1 and 10 nM final concentration, for DA competition and 1 nM for APO competition) or P (100 nM for APO competition) was added directly into the incubation buffer in the presence of absence (control) of 100 μM GTP for the *in vitro* studies. Agonist binding properties of D-2 receptors from striatal homogenates of vehicle- and E_2-treated animals were assessed by measuring DA competition of [^{3}H]spiperone binding in the absence or presence (100 μM) of GTP.

GTP competition experiments. The IC_{50} of GTP for inhibition of [^{3}H]NPA specific binding was evaluated by determining the competition of [^{3}H]-NPA binding (0.25 nM) with increasing concentrations of GTP (10^{-8} to 10^{-3} M) in a final volume of 2.0 mL, with the incubation buffer described in the tissue preparation section. The concentration of [^{3}H]NPA used in these experiments selectively labels the high-affinity component of D-2 receptors [22]. These experiments were done in duplicate and were performed in the absence or presence of $MgCl_2$ (4 mM) in the buffers. E_2 (0.1, and 1.0 and 10 nM, final concentration) was also included in some of these experiments. Specific binding to the high-affinity component of D-2 receptors in the striatum was estimated by subtracting nonspecific binding

Table 1. Effect of chronic 17β-estradiol treatment of OVX rats on agonist states of D-2 receptors and their conversion with GTP in subregions of the striatum

Striatum	K_{high} (nM)	K_{low} (μM)	R_{high} (%)	R_t (fmol/mg protein)
Control				
Lateral	18.1 ± 2.6	0.90 ± 0.12	64.5 ± 2.4	215 ± 7
Lateral + GTP	39.4 ± 6.7*	2.43 ± 0.22†	44.6 ± 3.1*	
Medial	13.0 ± 1.2	0.61 ± 0.04	61.8 ± 1.3	213 ± 8
Medial + GTP	43.5 ± 8.3*	2.05 ± 0.19*	38.7 ± 2.4*	
Estradiol treated				
Lateral	10.9 ± 1.3	0.60 ± 0.09	61.0 ± 3.2	242 ± 11‡
Lateral + GTP	34.2 ± 3.5*	2.02 ± 0.16*	43.1 ± 2.3*	
Medial	14.5 ± 1.2	0.77 ± 0.08	66.5 ± 1.6	211 ± 8
Medial + GTP	17.3 ± 2.4	1.72 ± 0.05*	34.3 ± 2.2*§	

Chronic treatment consisted of subcutaneous injections of E_2 (10 μg, twice each day, for 2 weeks) to OVX female rats. High- and low-affinity states were assessed by determining DA competition for [^{3}H]spiperone binding in the absence or presence of GTP (100 μM). Note that these experiments were done with buffers containing $MgCl_2$. Values are means ± SEM obtained from six independent determinations done in duplicate from pools of 2–3 rats each for a total of 60 rats for this experiment.

† Significantly different ($P < 0.01$ and †$P < 0.05$) vs respective lateral or medial striatum without GTP (Duncan–Kramer test).

‡ Significantly different ($P < 0.05$) vs control lateral group without GTP (Duncan–Kramer test).

§ $P = 0.01$ for the effect of GTP on the lateral vs the medical part of the striatum in E_2-treated animals (two-way analysis of variance test).

measured in the presence of 1.0 μM (+)-butaclamol. The GTP-resistant D-2 receptor population was evaluated using the residual level of receptors labeled with [^{3}H]NPA in the presence of 1 mM GTP.

Incubation at 22° for 30 min was terminated by rapid filtration through Whatman GF/C glass filters under vacuum followed by three rapid rinses (4 mL each) with the appropriate ice-cold buffer using a cell harvester (Brandel). This short incubation time was used to avoid degradation of GTP. Equilibrium of tritiated ligands binding to DA receptors was reached under these conditions. Bound [^{3}H]-spiperone and [^{3}H]NPA were measured by liquid scintillation (Formula-963, NEN) spectrometry at a counting efficiency of about 56% (Beckman LS3801).

Data analysis

Dose–response curves of APO and DA competitions for [^{3}H]spiperone binding were subjected to the non-linear least square curve fitting program LIGAND [23]. Statistical analysis of high- and low-affinity inhibition constants (K_{high} and K_{low}, respectively) for agonist binding sites, proportions (in percent) of the receptors in the high-affinity state (R_{high}), total receptor density (R_t) of [^{3}H]spiperone binding and the IC_{50} of GTP were performed by the Duncan–Kramer multiple range test [24], whereas the interaction between effects was assessed with a two-way analysis of variance test. Protein contents were determined by the method of Lowry *et al.* [25]. All values are presented as the means ± SEM for the number of determinations (N) indicated.

RESULTS

In vivo *exposure of OVX female rats to hormones*

Chronic E_2 treatment of OVX rats increased the total density of [^{3}H]spiperone binding to D-2 receptors only in the lateral part of the striatum (Table 1), as observed previously [20]. Chronic treatment with E_2 did not affect the agonist binding characteristics [affinities or proportions (R) of high- and low-affinity sites] of D-2 receptors as measured with DA (Fig. 1 showing a representative example and Table 1 including means of six such experiments) or APO (three determinations using a total of 25 rats, data not shown) competition for [^{3}H]spiperone binding. Addition of GTP in the competition experiments produced a partial shift of the high-affinity to the low-affinity state of D-2 receptors and increased both high- and low-affinity inhibition constants (K_{high} and K_{low}, respectively) of DA in lateral and medial striatum of vehicle-treated animals. In general, similar effects of GTP were observed in the striatum of control and estradiol-treated rats except for the medial striatum where the high-affinity inhibition constant remained unchanged after GTP in steroid-treated rats. In addition, D-2 receptors in the medial part of the striatum of estradiol-treated rats were more sensitive to the action of GTP, with a greater conversion of the high-affinity state to the low-affinity state of the receptor ($P = 0.01$, two-way analysis of variance test), than were D-2 receptors in the lateral part of the striatum (Table 1 and Fig. 1). GTP had equal activity in both parts of the striatum in vehicle-treated animals (Table 1 and Fig. 1).

Chronic P treatment (2 mg/day) of OVX rats did not affect maximal receptor density (B_{max}) or the dissociation constant (K_d) of striatal D-2 receptors in the striatum as measured with [^{3}H]spiperone binding saturation isotherm experiments (control: $K_d = 25 \pm 8$ pM, $B_{max} = 320 \pm 15$ fmol/mg protein,

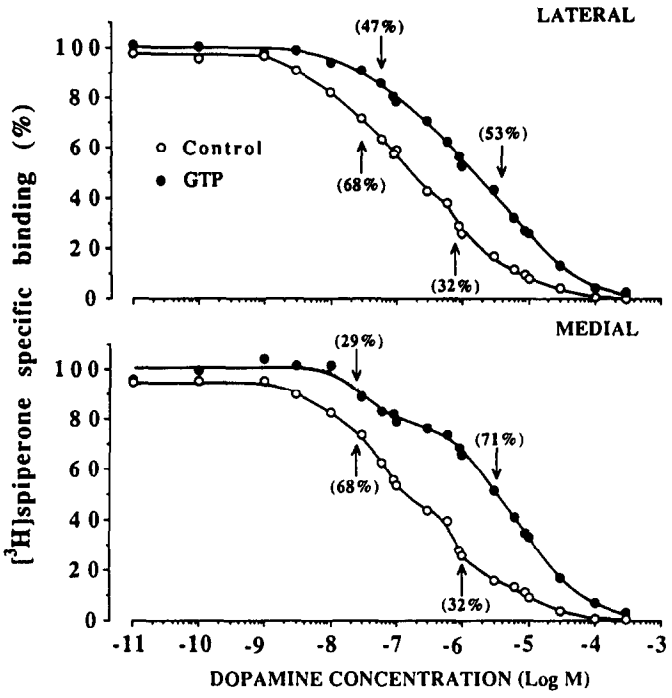

Fig. 1. Representative examples showing the effect of GTP (100 μM) on agonist binding characteristics of D-2 receptors in lateral and medial striatum of OVX female rats (using a pool of 2–3 rats for each curve) chronically treated with E_2 (10 μg, twice each day, for 2 weeks). This was determined by measuring the effect of GTP on DA competition for [^{3}H]spiperone specific binding in buffers containing $MgCl_2$. Arrows represent high- and low-affinity inhibition constants ± SE from the mathematical fit (control lateral = 15 ± 3 and 780 ± 137 nM; GTP lateral = 38 ± 8 and 2030 ± 195 nM; control medial = 14 ± 3 and 898 ± 170 nM; GTP medial = 12 ± 4 and 1653 ± 126 nM); the respective percentages of receptors in the high- or low-affinity state are shown in parentheses. Note the greater GTP-induced high-affinity state conversion for the medial D-2 receptors compared to receptor conversion in the lateral part of the striatum of E_2-treated animals. One hundred percent values (± SEM) were 253 ± 14, 256 ± 10, 181 ± 12 and 201 ± 7 fmol/mg protein for control lateral, GTP lateral, control medial and GTP-medial, respectively.

N = 5; P: K_d = 27 ± 13 pM, B_{max} = 305 ± 24 fmol/mg protein, N = 5), whereas a similar treatment with E_2 increases the density of striatal D-2 receptors [10, 11, 20].

In vitro *exposure of male striata to hormones*

Agonist competition experiments. The actions of gonadal hormones (E_2 and P) on agonist binding properties of striatal D-2 receptors were studied *in vitro* by adding the hormones to the incubation buffer of the receptor assay. No effect of hormones alone, at the concentrations used here, were observed on agonist binding characteristics of striatal D-2 receptors (control: K_{high} = 0.84 ± 0.22 nM, K_{low} = 30.6 ± 7.9 nM, R_{high} = 31.8 ± 2.9%; P: K_{high} = 0.83 ± 0.10 nM, K_{low} = 33.6 ± 1.0 nM, R_{high} = 28.0 ± 1.2%; E_2: K_{high} = 0.73 ± 0.10 nM, K_{low} = 23.4 ± 1.1 nM, R_{high} = 26.0 ± 2.5% using a total of fifty rats) with APO competition in the absence of $MgCl_2$. There was no difference between responses of lateral and medial striatal D-2 receptors with *in vitro* exposure to E_2 (data not shown).

Figures 2 (one representative example) and 3 (means of 5–7 such experiments) illustrate the *in vitro* effect of GTP alone or in combination with E_2 (1 nM) or P (100 nM) on agonist properties of striatal D-2 receptors in the absence or presence of 4 mM $MgCl_2$, as measured by APO competition for [^{3}H]-spiperone binding. The E_2 and P concentrations used were chosen to reflect physiological levels of these hormones as measured in rat striatum during the estrous cycle [26], and these respective steroid hormone levels have been shown to affect striatal DA activity [17–19].

GTP induced the expected shift of the high-affinity to the low-affinity state of D-2 receptors in the striatum as assessed with APO or DA (Figs. 3 and 4). Addition of Mg^{2+} induced a higher proportion of the high-affinity receptor population with both agonists (Figs. 3 and 4), but the extent of the shift in both assay conditions (with or without $MgCl_2$) was not significantly different (P = 0.2, two-way analysis of variance test), despite the fact that there are more receptors in the high-affinity state susceptible to being converted. The high- and low-affinity inhibition constants of APO were increased with the addition of GTP only in the presence of Mg^{2+} whereas inhibition constants of DA were

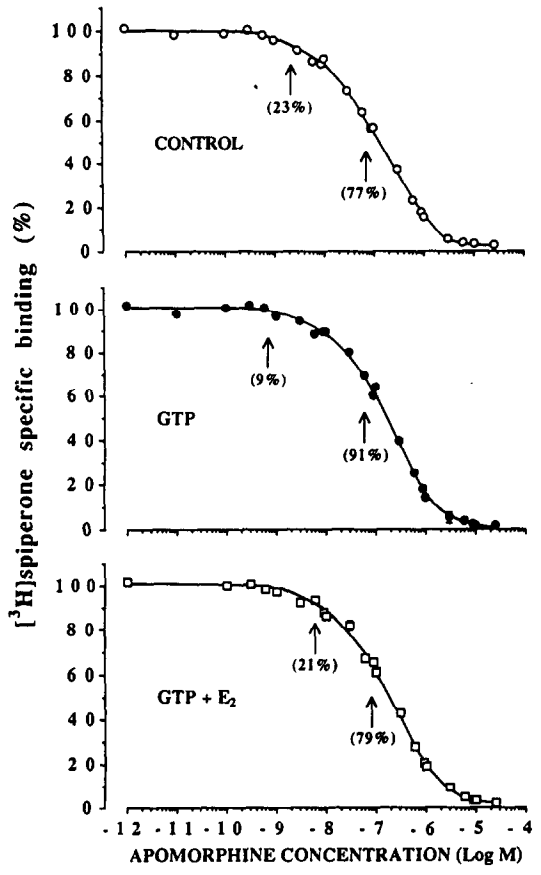

Fig. 2. Representative examples showing the effects of *in vitro* addition of GTP (100 μM) or GTP (100 μM) + E_2 (1 nM) on the agonist binding characteristics of D-2 receptors as measured by determining APO competition for [^{3}H]spiperone specific binding in buffers without $MgCl_2$ in striatum homogenates of intact male rats (using a pool of two rats for each curve). Arrows represent high- and low-affinity inhibition constants ± SE from the mathematical fit (control = 1.8 ± 1.3 and 46 ± 6 nM; GTP = 0.4 ± 0.9 and 38 ± 4 nM; GTP + E_2 = 2.3 ± 1.5 and 58 ± 6 nM); the percentages of receptors in the high- or low-affinity state are shown in parentheses. Note that the addition of E_2 into the incubation buffer prevented the GTP-induced high-affinity state conversion. One hundred percent values (± SEM) were 273 ± 41, 270 ± 40 and 288 ± 46 fmol/mg protein for control, GTP and GTP + E_2 groups, respectively.

increased by GTP independently of the presence of $MgCl_2$ (Figs. 3 and 4).

Addition of E_2 prevented the increase of the low-affinity inhibition constant of APO induced by GTP in the presence of Mg^{2+} (Fig. 3). Mg^{2+} and GTP did not generally affect [^{3}H]spiperone binding to D-2 receptors (Figs. 3 and 4). Most interestingly, addition of E_2 simultaneously with GTP prevented the expected conversion of the high-affinity state to the low-affinity state of the receptor normally induced by GTP with APO competition for [^{3}H]spiperone binding (Fig. 3). The combination of E_2 with GTP was ineffective on APO competition in the presence of $MgCl_2$ as well as the combination of P + GTP in the absence or presence of $MgCl_2$ (Fig. 3). E_2 remained without effect upon the action of GTP on DA competition with either concentration of E_2 used and in both assay conditions (Fig. 4).

GTP competition experiments. Figure 5 (one representative example) and 6 (means of 5–7 such experiments) illustrate the effect of various concentrations of E_2 on the IC_{50} of GTP for the inhibition of [^{3}H]NPA binding to the high-affinity component of striatal D-2 receptors. Addition of 1 nM (final concentration) E_2 into the buffer of competition experiments induced an increase of the IC_{50} of GTP to displace [^{3}H]NPA binding, while a lower (0.1 nM) or a higher (10 nM) concentration of this hormone was without effect (Figs. 5 and 6). The addition of $MgCl_2$ reduced this effect by 2-fold but the effect remained significant compared to respective control values (Fig. 6). Mg^{2+} also increased the total receptor density of the high-affinity state of D-2 receptors labeled with the agonist [^{3}H]NPA and increased the proportion of the receptor population which remained resistant to the action of GTP (GTP-resistant population), when compared to the receptor level observed with 1 mM GTP in the absence of this bivalent cation (Fig. 7). However, E_2 at the three concentrations studied (0.1, 1.0 and 10 nM) did not influence the effect of Mg^{2+} (Fig. 7).

DISCUSSION

The main finding of this study is that E_2 interacts with the GTP effect to modulate D-2 receptor agonist binding characteristics. Indeed, chronic *in vivo* exposure to this hormone induced a different effect of GTP between D-2 receptors in the lateral and those in the medial part of the rat striatum. Moreover, an *in vitro* exposure to a low dose (of the same range as the levels observed during the rat estrous cycle [26]) of this hormone affected agonist binding characteristics of D-2 receptors in striatal homogenates. E_2 prevented the expected shift of the high- to the low-affinity state of D-2 receptors normally induced by GTP with APO competition and increased the IC_{50} of GTP for inhibition of [^{3}H] NPA binding.

In E_2-treated animals, GTP induced more conversion in the medial striatum than in the lateral striatum, while in control animals both portions of the striatum responded similarly to GTP. Moreover, this nucleotide did not increase the K_{high} of D-2 receptors in the medial striatum of estrogen-treated rats, as it did in the lateral striatum of estrogen-treated animals, or in control rats. This combined effect may be responsible for the overall absence of effect of E_2 on the total receptor density of D-2 receptors in the medial striatum, since these receptors became more sensitive to the effect of GTP and remained in a high-affinity state, while E_2 interfered with DA neurotransmission in the lateral striatum causing an increase of receptor density in this part of the striatum after chronic exposure to this hormone. In comparison, Hall and Sällemark [27] have observed that repeated treatment with neuroleptics decreases the affinity of DA for the D-2 low-affinity state of the receptor and increases the

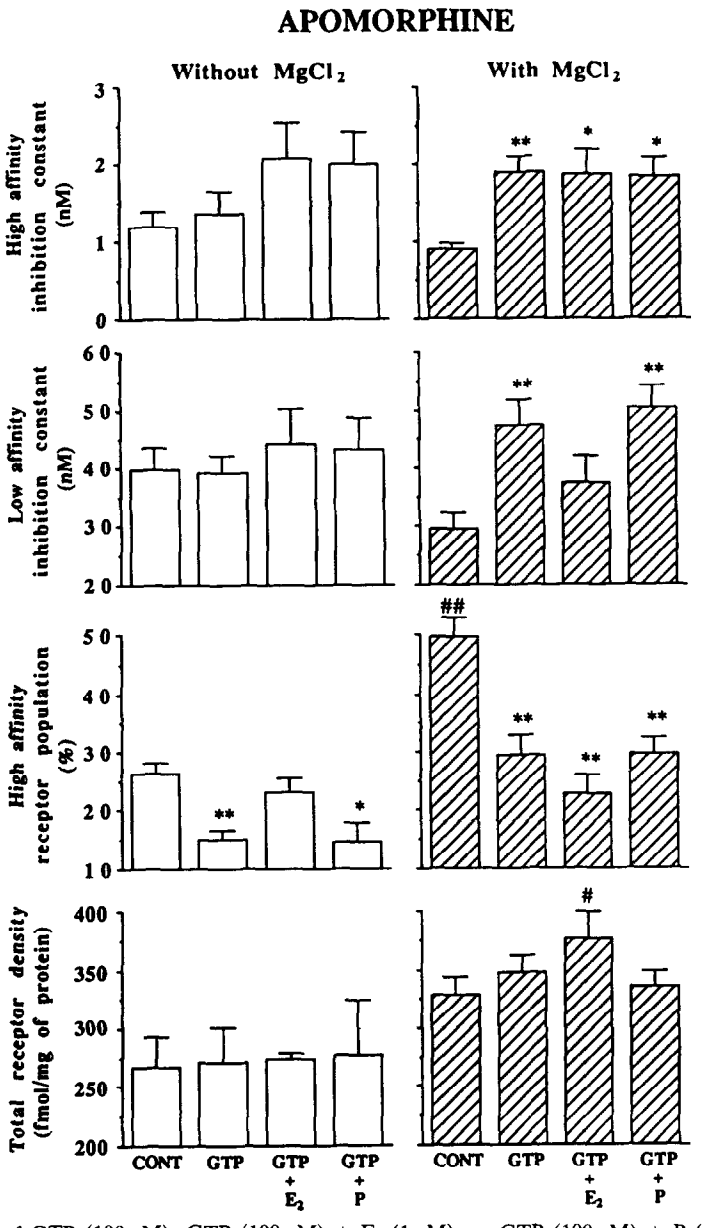

Fig. 3. Effects of GTP (100 μM), GTP (100 μM) + E_2 (1 nM), or GTP (100 μM) + P (100 nM) on agonist binding characteristics of D-2 receptors in striatal homogenates from intact male rats, as measured by determining APO competition for [^{3}H]spiperone specific binding in buffers without $MgCl_2$ or with 4 mM $MgCl_2$. High- and low-affinity inhibition constants and high-affinity receptor population represent agonist characteristics of APO, while the total receptor density represents the amount of D-2 receptors labeled with the antagonist [^{3}H]spiperone (100% specific binding on competition curves). Values are means ± SEM obtained from 5–7 separate determinations done in duplicate using a total of about 100 rats. For each determination the striata from 10–12 rats were pooled for preparation of a striatal tissue homogenate. This homogenate was then divided for each assay condition for one *in vitro* steroid study. This enabled comparison of the effects of GTP, Mg^{2+} and steroids in the same homogenate. Note that experiments with and without Mg^{2+} were done simultaneously. Key (*) $P < 0.05$ and (**) $P < 0.01$ vs respective control; (#) $P < 0.05$ and (##) $P < 0.01$ vs respective group without $MgCl_2$ according to a Duncan–Kramer test.

percentage of the high-affinity state of the D-2 receptors in the rat striatum. These effects are both suggested to compensate for the continuous blockade of D-2 receptors by the neuroleptic treatment [27]. In contrast, MacKenzie and Zigmond [28] observed no effect of 6-hydroxydopamine or chronic haloperidol treatment on the high- and low-affinity states of the D-2 receptors.

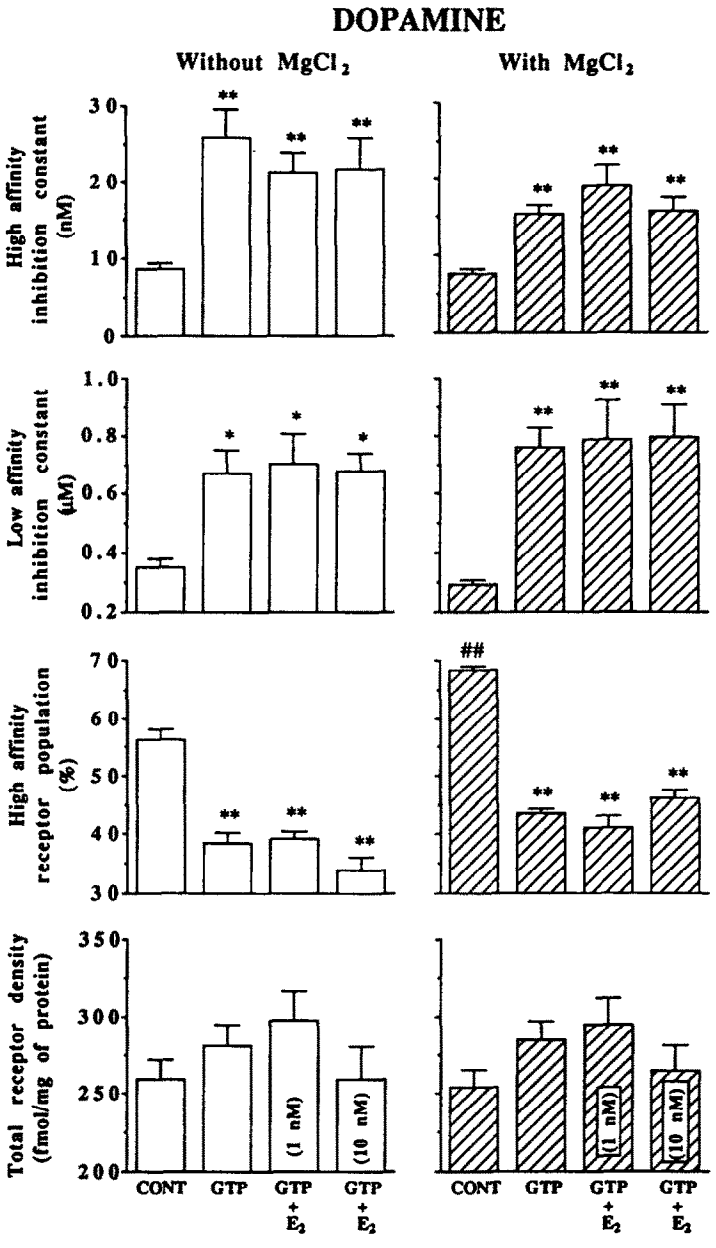

Fig. 4. Effect of GTP (100 μM) or GTP (100 μM) + E_2 (1 and 10 nM) on agonist binding characteristics of D-2 receptors in striatal homogenates from intact male rats, as measured with DA competition for [^{3}H]spiperone specific binding in buffers without $MgCl_2$ or with 4 mM $MgCl_2$. High- and low-affinity inhibition constants and high-affinity receptor population represent agonist characteristics of DA, while the total receptor density represents the amount of D-2 receptors labeled with the antagonist [^{3}H]-spiperone (100% specific binding on competition curves). Values are means ± SEM obtained from 5–7 separate determinations done in duplicate using a total of about 100 rats (see details in legend to Fig. 3). Key: (*) $P < 0.05$ and (**) $P < 0.01$ vs respective control; (##) $P < 0.01$ vs respective group without $MgCl_2$ according to a Duncan–Kramer test.

The heterogeneity of the action of E_2 in the striatum may result from the presence of two distinct D-2 receptor populations in respect to the action of GTP, which may come from differences in G-protein coupling mechanisms between lateral and medial D-2 receptors in the striatum. Regional variations of the effect of E_2 in the striatum could also result from the presence of different subpopulations of DA receptors (D-2_A, D-2_B, D-3 or D-4, for example) [29, 30], which bind [^{3}H]spiperone, but may react differently to E_2.

The proportions of the high-affinity agonist state of D-2 receptors and the GTP-induced partial conversion observed here (20–30% of conversion)

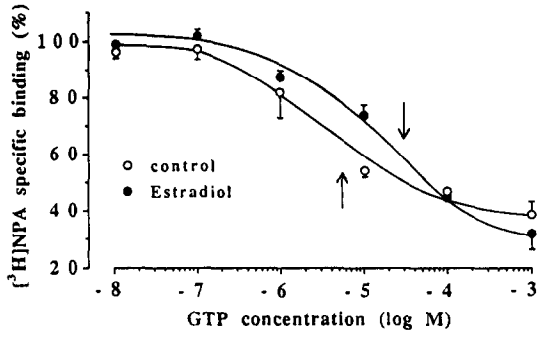

Fig. 5. Representative example showing the effect of *in vitro* addition in the incubation buffer of E_2 (1 nM) on the IC_{50} of GTP for the inhibition of specific [^{3}H]NPA (0.25 nM) binding to the high-affinity state of D-2 receptors in striatal homogenates from intact male rats. A pool of two rats was used for each curve. This experiment was performed in a buffer without $MgCl_2$. Arrows represent respective IC_{50} (± SEM) values from the mathematical fit for control (2.8 ± 0.9 μM) and E_2-treated (21.4 ± 12.5 μM) homogenates obtained by computer analysis of the data. Each data point represents duplicate values of one representative experiment using a homogenate of the striatum of two rats for each curve. One hundred percent values (± SEM) were 123 ± 4 and 119 ± 5 fmol/mg protein for the control and the E_2-treated group, respectively.

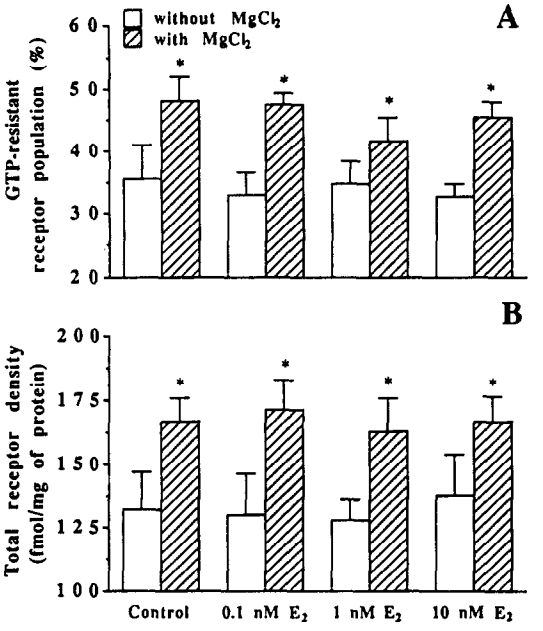

Fig. 7. Effect of various concentrations of E_2 on GTP-resistant D-2 high affinity receptor population (A) as measured by determining the specific binding of [^{3}H]NPA (0.25 nM) in the presence of 1 mM GTP in male rats striatal homogenates, and on total receptor density (B) of [^{3}H]-NPA (0.25 nM) specific binding (in the absence of GTP). Experiments were performed in the absence or presence of 4 mM $MgCl_2$ in buffers. Values are means ± SEM obtained from 5–7 separate determinations done in duplicate and are from the same group of rats described in the legend to Fig. 6. Key: (*) $P < 0.05$ vs respective condition without $MgCl_2$ according to a Duncan–Kramer multiple range test.

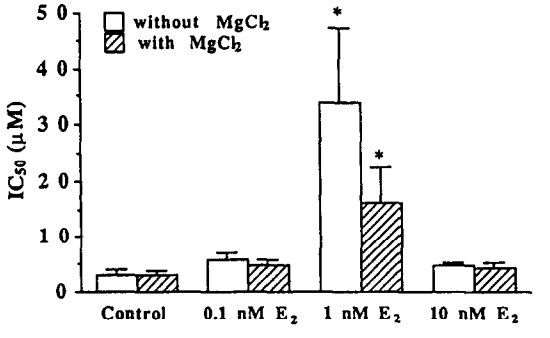

Fig. 6. Effect of various concentrations of E_2 on the IC_{50} of GTP for the inhibition of [^{3}H]NPA (0.25 nM) specific binding to the high-affinity site of D-2 receptors in striatal homogenates from intact male rats. Experiments were performed in the absence or the presence of 4 mM $MgCl_2$ in buffers. Values are means ± SEM obtained from 5–7 separate determinations done in duplicate using a total of fifty rats. For each determination the striata from eight rats were pooled for preparation of a striatal tissue homogenate (without $MgCl_2$). Another similar pool of striatal homogenate was prepared with $MgCl_2$. These homogenates were then divided for each essay condition (addition of various E_2 concentrations). This enabled comparison of the effect of E_2 at various concentrations in the same homogenate. Key: (*) $P < 0.05$ vs respective control according to a Duncan–Kramer multiple range test.

are similar to those of previous reports under similar experimental conditions [5, 27, 31–35]. Inhibition of binding of [^{3}H]spiperone by antagonists and agonists, even in the presence of GTP, has been reported to be associated with Hill coefficients of less than one [33]. In addition, a 3- to 4-fold increase in the IC_{50} of agonists competing for [^{3}H]spiperone binding was observed after GTP with no increase [36] or only a slight increase in their Hill coefficients (from 0.5 to 0.6 [3]). The IC_{50} for GTP (about 3 μM for the control group) reported here is similar to that of previous reports (5 μM [36]). We observed as have others [3, 33, 36] both partial conversion of the high- to the low-affinity state of the receptor and only minor increases of Hill coefficients with a shift to the right of the agonist competition curves (DA and APO) for [^{3}H]spiperone binding. This was quantified as increased inhibition constants of both high- and low-affinity states of the receptor. The partial conversion by GTP of the high-affinity state of D-2 receptors in the striatum may be because of the contribution from D-2 receptors uncoupled with a G-protein in the striatum [4, 37]. It is also possible that under our experimental conditions the amount of available G-proteins was not sufficient to promote a total conversion of D-2 receptors in high affinity

[38]. GTP-induced shifts in affinity as observed here would not be predicted by the ternary complex model, suggesting that this model is insufficient to fully accommodate experimental observations.

Recently, we have shown in bovine anterior pituitary and in 7315a tumors grown in female Buffalo rats that *in vitro* E_2 (1 and 10 nM), such as observed in the present manuscript, opposes the effect of GTP to shift the D-2 receptor from the high to the low agonist state [39]. The 7315a tumors secrete large amounts of prolactin and this interferes with the estrous cycle; hence, these female rats do not cycle normally. These data suggest that the similar opposing effect of E_2 to the GTP shift is a general finding for D-2 receptors in the pituitary (normal bovine, tumoral) and the striatum (male rats, this manuscript). The choice of intact male rats as a model in the present manuscript may prove to be a useful and more general model. Nevertheless, female rats will have to be tested in future experiments for the *in vitro* effect of E_2 in the striatum to get a general idea of the effect of E_2 *in vitro*.

E_2 added *in vitro* influenced the effect of GTP on APO competition for [^{3}H]spiperone binding curves in a Mg^{2+}-dependent manner, whereas the effect of GTP on DA competition for this tritiated antagonist was not affected by E_2 and was not Mg^{2+} dependent (in its effect on inhibition constants). Mg^{2+} and other divalent cations have been shown to enhance binding of dopaminergic agonist ligands and to increase the potency of agonists at D-2 sites labeled by butyrophenone [31], while leaving binding of antagonists relatively unaffected [32]. The shifts to the right of competition curves induced by GTP may be influenced by divalent cations since this effect is Mg^{2+} dependent with APO competition for binding. The fact that this was not observed with DA competition for binding may be because of differences between agonists and their interactions with Mg^{2+} and/or other ions, which may depend on the chemical structure of the ligand [40], or may also be related to differences between the full and the partial agonist nature of the competing agent. The absence of activity of E_2 with DA competition may also be related to differences between the full and partial agonist nature of the competing agent. This suggests that the effect of E_2 on GTP activity in APO competition of [^{3}H]spiperone binding could involve a Mg^{2+}/GTP associated site on G-proteins.

The effect of E_2 on G-proteins could explain the effects on both adenylate cyclase and D-2 receptors reported previously [41, 42]. Maus *et al.* [43] demonstrated that E_2 pretreatment of embryogenic striatal neurons in primary culture increases ADP-ribosylation of pertussis toxin sensitive G-proteins ($G\alpha_{0,i}$) without modification of the amount of respective G-protein subunits. Testosterone mimics this effect, while P remains ineffective [43]. Moreover, E_2 does not influence the effect of GTP on D-1 receptor agonist binding, indicating some specificity to the effect of E_2 on certain G-protein subunits [21]. The interaction of E_2 on the GTP-shift of D-2 receptor agonist binding can ultimately affect agonistic binding properties at the D-2 receptor level probably by acting upon the ternary complex association/dissociation processes of this complex composed of D-2 receptor, G-protein coupling system and adenylate cyclase enzyme or, as suggested by Maus *et al.* [43], E_2 may stabilize the trimeric form of the G-protein complex.

The *in vitro* effect of E_2 observed here likely involves membrane components associated with the coupling system of D-2 receptors in the striatum. The effect of E_2 on striatal membranes may be through changes in fluidity of the membrane. Indeed, increasing the membrane cholesterol/phospholipid ratio inhibits the activity of DA-stimulated adenylate cyclase [44]. Similarly, E_2 could modify this ratio substituting for cholesterol to affect adenylate cyclase activity. This enzyme activity can also be influenced by membrane fluidizing agents [45] and GTP action on the loss of the high-affinity state of D-2 receptors can be enhanced by ascorbate-induced lipid peroxidation [46]. Phospholipid methylation is a rapid process which can affect membrane fluidity by modifying the asymmetry of phospholipid distribution between the two lipid bilayers of cell membranes [47]. Some methyltransferases are Mg^{2+} dependent, and phospholipid methylation affects binding characteristics and the effect of GTP on β-adrenergic receptors [47]. In addition, the activity of methyltransferases was shown to vary during the estrous cycle in the rat pituitary [48]. Thus, our results suggest that circulating hormone levels of E_2 may influence agonistic binding characteristics as well as adenylate cyclase activity by a complex interaction at the level of G-protein in modifying membrane fluidity, which may modify interactions in ternary complex association/dissociation processes. *In vivo* or *in vitro* exposures to P did not affect D-2 receptors in the present experiments. We have observed, however, an increase of DA metabolism or release after an acute injection of a small dose of this hormone [18], and Dluzen and Ramirez [49] observed a direct action of this hormone on perfused striatal tissue DA release. This suggests that the mechanism of action of P on central DA systems may not be the same as for E_2.

L-Propyl-L-leucyl glycinamide (PLG), a hypothalamic tripeptide, has also been shown to modulate agonist binding characteristics to striatal D-2 receptors [50]. PLG-treated striatal membranes have an increase in the affinity of agonist sites and an increase in the ratio of high- to low-affinity forms of the receptor [50]. PLG also increases the IC_{50} of guanosine 5'-[β-γ-imido]triphosphate (Gpp(NH)p)-induced inhibition of [^{3}H]NPA binding [50], and only a specific concentration of PLG (1 μM) will induce this effect, whereas lower or higher concentrations are ineffective [50], as observed here with E_2. The similarities between the E_2 and PLG activity upon DA receptors suggest that they may have a common mechanism of action on the association/dissociation processes of the ternary complex.

In summary, E_2 can influence the effect of GTP on striatal D-2 receptors *in vivo* and *in vitro*, suggesting that fluctuations of E_2 concentrations, as occur during the rat estrous cycle, may affect striatal membrane integrity and may be an important factor

in synaptic plasticity of neuronal cells in the rat striatum.

Acknowledgements—This work was supported by a grant to T. D. P. and by a Studentship to D. L. from the MRC of Canada.

REFERENCES

1. Sibley DR, De Léan A and Creese I, Anterior pituitary dopamine receptors. Demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. *J Biol Chem* **257**: 6351–6361, 1982.
2. Hamblin MW, Leff SE and Creese I, Interactions of agonists with D-2 dopamine receptors: Evidence for a single receptor population existing in multiple agonist affinity-states in rat striatal membranes. *Biochem Pharmacol* **33**: 877–887, 1984.
3. Zahnizer NR and Molinoff PB, Effect of guanine nucleotides on striatal dopamine receptors. *Nature* **275**: 453–455, 1978.
4. Creese I, Usdin T and Snyder SH, Guanine nucleotides distinguish between two dopamine receptors. *Nature* **278**: 577–578, 1979.
5. Wreggett KA and Seeman P, Agonist high- and low-affinity states of the D_2-dopamine receptor in calf brain. Partial conversion by guanine nucleotide. *Mol Pharmacol* **25**: 10–17, 1984.
6. De Paolo LV and Barraclough CA, Interaction of estradiol and progesterone on pituitary gonadotrophin secretion: Possible sites and mechanisms of action. *Biol Reprod* **20**: 1173–1185, 1979.
7. Pfaff DW, *Estrogens and Brain Function: Neural Analysis of Hormone-Controlled Mammalian Reproductive Behavior*. Springer, New York, 1980.
8. McEwen BS, Jones KJ and Pfaff DW, Hormonal control of sexual behavior in the female rat: Molecular, cellular and neurochemical studies. *Biol Reprod* **36**: 37–45, 1987.
9. Van Hartesveldt C and Joyce JN, Effects of estrogen on the basal ganglia. *Neurosci Biobehav Rev* **10**: 1–14, 1986.
10. Di Paolo T, Poyet P, Labrie F and Raynaud JP, Effect of prolactin and estradiol on striatal dopamine receptors. *Life Sci* **31**: 2921–2929, 1982.
11. Di Paolo T, Poyet P and Labrie F, Prolactin and estradiol increase striatal dopamine receptor density in intact, castrated and hypophysectomized rats. *Prog Neuropsychopharmacol Biol Psychiatry* **6**: 377–384, 1982.
12. Hruska RE and Silbergeld EK, Increased dopamine receptor sensitivity after estrogen treatment using the rat rotation model. *Science* **209**: 1466–1468, 1980.
13. Hruska RE, Ludman LM and Silbergeld EK, Characterization of the striatal dopamine receptor supersensitivity produced by estrogen treatment of male rats. *Neuropharmacology* **19**: 923–926, 1980.
14. Gordon JH, Perry KO, Pre- and postsynaptic neurochemical alterations following estrogen-induced striatal dopamine hypo- and hypersensitivity. *Brain Res Bull* **10**: 425–428,1983.
15. Di Paolo T and Falardeau P, Modulation of brain and pituitary dopamine receptors by estrogens and prolactin. *Prog Neuropsychopharmacol Biol Psychiatry* **9**: 473–480, 1985.
16. Di Paolo T, Falardeau P and Morissette M, Striatal dopamine agonist binding sites fluctuate during the rat estrous cycle. *Life Sci* **43**: 665–672, 1988.
17. Di Paolo T, Rouillard C and Bédard P, 17β-Estradiol at a physiological dose acutely increases DA turnover in rat brain. *Eur J Pharmacol* **117**: 197–203, 1985.
18. Di Paolo T, Lévesque D and Daigle M, A physiological dose of progesterone affects rat striatum biogenic amine metabolism. *Eur J Pharmacol* **125**: 11–16, 1986.
19. Lévesque D and Di Paolo T, Rapid conversion of high into low striatal D_2-dopamine receptor agonist binding states after an acute physiological dose of 17β-estradiol. *Neurosci Lett* **88**: 113–118, 1988.
20. Falardeau P and Di Paolo T, Regional effect of estradiol on rat caudate-putamen dopamine receptors: Lateral–medial differences. *Neurosci Lett* **74**: 43–48, 1987.
21. Hruska RE, 17β-Estradiol regulation of DA receptor interactions with G-proteins. *Soc Neurosci Abstr* **14**: 454, 1988.
22. Titeler M and Seeman P, Selective labeling of different dopamine receptors by a new agonist ^{3}H-ligand: ^{3}H-*N*-propylnorapomorphine. *Eur J Pharmacol* **56**: 291–292, 1979.
23. Munson PJ and Rodbard D, LIGAND, a versatile computerized approach for characterization of ligand-binding systems. *Anal Biochem* **107**: 220–239, 1980.
24. Kramer CY, Extension of multiple range tests for group means with unequal number of replications. *Biometrics* **12**: 307–310, 1956.
25. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
26. Morissette M, Garcia-Segura L-M, Bélanger A and Di Paolo T, Membrane effects of steroid hormones in the rat striatum: Changes during the estrous cycle. *Posters Neurosci* **1**: 95–98, 1991.
27. Hall H and Sällemark M, Effects of chronic neuroleptic treatment on agonist affinity states of the dopamine-D_2 receptor in the rain brain. *Pharmacol Toxicol* **60**: 359–363, 1987.
28. MacKenzie RG and Zigmond MJ, High- and low-affinity states of striatal D_2 receptors are not affected by 6-hydroxydopamine or chronic haloperidol treatment. *J Neurochem* **43**: 1310–1318, 1984.
29. Sokoloff P, Giros B, Martres M-P, Bouthenet M-L and Schwartz J-C, Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature* **347**: 146–151, 1990.
30. Van Tol HHM, Bunzow JR, Guan H-C, Sunahara RK, Seeman P, Niznik HB and Civelli O, Cloning of the gene for a human dopamine D_4 receptor with high affinity for antipsychotic clozapine. *Nature* **350**: 610–614, 1991.
31. Creese I, Sibley DR, Hamblin MW and Leff SE, The classification of dopamine receptors: Relationship to radioligand binding. *Annu Rev Neurosci* **6**: 43–71, 1983.
32. De Vries DJ and Beart PM, Role of assay conditions in determining agonist potency at D_2 dopamine receptors in striatal homogenates. *Mol Brain Res* **1**: 29–35, 1986.
33. Huff RM and Molinoff PB, Quantitative determination of dopamine receptor subtypes not linked to activation of adenylate cyclase in rat striatum. *Proc Natl Acad Sci USA* **79**: 7561–7565, 1982.
34. Grigoriadis D and Seeman P, Complete conversion of brain D_2 dopamine receptors from high- to low-affinity state for dopamine agonists, using sodium ions and guanine nucleotide. *J Neurochem* **44**: 1925–1935, 1985.
35. De Vries DJ and Beart PM, Magnesium ions reveal nanomolar potency of dopamine at [^{3}H]spiperone labelled D-2 receptors in rat corpus striatum. *Eur J Pharmacol* **109**: 417–419, 1985.
36. Creese I, Usdin TB and Snyder SH, Dopamine receptor binding regulated by guanine nucleotides. *Mol Pharmacol* **16**: 69–76, 1979.
37. De Keyser J, Walraevens H, De Backer J-P, Edinber G and Vauquelin G, D_2 dopamine receptors in human brain: Heterogeneity based on differences in guanine nucleotide effect on agonist binding, and their presence

on corticostriatal nerve terminals. *Brain Res* **484**: 36–42, 1989.

38. Wreggett KA and De Léan A, The ternary complex model. Its properties and application to ligand interactions with the D_2-dopamine receptor of the anterior pituitary gland. *Mol Pharmacol* **26**: 214–227, 1984.
39. Abolfathi Z and Di Paolo T, Modulation of dopamine receptor agonist binding sites by cations and estradiol in intact pituitary and 7315a tumors. *Biochem Pharmacol* **42**: 2163–2169, 1991.
40. Urwyler S, Affinity shifts induced by cations do not reliably predict the agonistic or antagonistic nature of ligands of brain dopamine receptors. *J Neurochem* **49**: 1415–1420, 1987.
41. Maus M, Bertrand P, Drouva S, Rasolonjanahary R, Kordon C, Glowinski J, Prémont J and Enjalbert A, Differential modulation of D1 and D2 dopamine-sensitive adenylate cyclases by 17β-estradiol in cultured striatal neurons and anterior pituitary cells. *J Neurochem* **52**: 410–418, 1989.
42. Munemura M, Agui T and Sibley DR, Chronic estrogen treatment promotes a functional uncoupling of the D_2 dopamine receptor in the rat anterior pituitary gland. *Endocrinology* **124**: 346–355, 1989.
43. Maus M, Homberger V, Bockaert J, Glowinski J and Prémont J, Pretreatment of mouse striatal neurons in primary culture with 17β-estradiol enhances the pertussis toxin-catalyzed ADP-ribosylation of $G\alpha_{o,i}$ protein subunits. *J Neurochem* **55**: 1244–1251, 1990.
44. Maguire PA and Druse MJ, The influence of cholesterol on synaptic fluidity, dopamine D_1 binding and dopamine-stimulated adenylate cyclase. *Brain Res Bull* **23**: 69–74, 1989.
45. Rimon G, Hanski E, Braun S and Levitski A, Mode of coupling between hormone receptors and adenylate cyclase elucidated by modulation of membrane fluidity. *Nature* **276**: 394–396, 1978.
46. Andorn AC, Bacon BR, Nguyen-Hunh AT, Parlato SJ and Stitts JA, Guanyl nucleotide interactions with dopaminergic binding sites labeled by [^{3}H]spiroperidol in human caudate and putamen: Guanyl nucleotides enhance ascorbate-induced lipid peroxidation and cause an apparent loss of high affinity binding sites. *Mol Pharmacol* **33**: 155–162, 1988.
47. Hirata F and Axelrod J, Phospholipid methylation and biological signal transmission. *Science* **209**: 1082–1090, 1980.
48. Drouva SV, Rerat E, Leblanc P, Laplante E and Kordon C, Variations of phospholipid methyltransferase(s) activity in the rat pituitary: Estrous cycle and sex differences. *Endocrinology* **121**: 569–574, 1987.
49. Dluzen DE and Ramirez VD, Progesterone effects upon dopamine from the corpus striatum of female rats. II. Evidence for a membrane site of action and the role of albumin. *Brain Res* **476**: 338–344, 1989.
50. Srivastava LK, Bajwa SB, Johnson RL and Mishra RK, Interaction of L-prolyl-L-leucyl glycinamide with dopamine D_2 receptor: Evidence for modulation of agonist affinity states in bovine striatal membranes. *J Neurochem* **50**: 960–968, 1988.