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Rapid conversion of high into low striatal D_2-dopamine receptor agonist binding states after an acute physiological dose of 17β-estradiol

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Ovariectomized female rats injected with 17β-estradiol (100 ng, s.c.) showed, as previously observed, an increase of the dopamine (DA) metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) with no change of DA concentrations in the striatum. This increase was observed as soon as 15 min following the injection while plasma estradiol reached a peak of 78 pg/ml after 5 min and was significantly elevated until 45 min to ultimately return to control values at 60 min. We observed no significant change of the inhibition constants of high- and low-affinity D_2 DA agonist binding sites and of the sum of high + low agonist DAergic agonist binding densities as detected by apomorphine competition of [^{3}H]spiperone binding. By contrast, a significant conversion of high into low agonist affinity binding states was seen at 15 min (38.6% of conversion, $P < 0.05$) and 30 min (40.0% of conversion, $P < 0.01$) after the acute physiological steroid injection. Thus, very small doses of estradiol were able to rapidly increase DA turnover and modulate the striatal agonist affinity states of the D_2 DA receptor. This effect of estradiol is probably non-genomic, presynaptic and may involve a membrane effect at the DA autoreceptor level.

We have previously shown that estradiol (E_2), at a physiological dose, acutely increases dopamine (DA) turnover in the rat brain [9]. This increase was observed 30 min after the steroid injection and coincided with the maximal induction of postural deviation of rats with an unilateral lesion of the entopeduncular nucleus. Furthermore, a small dose of E_2 at a physiological concentration can increase dyskinesia in ovariectomized monkeys with a midbrain electrolytic lesion and this increase was again significant at 30 min following the injection of the steroid [3]. E_2 administered intravenously to ovariectomized rats in doses ranging from 3 to 3000 ng, was shown electrophysiologically to decrease within seconds the spontaneous activity of substantia nigra DA neurons in a dose-dependent manner [6]. More recently, Pasqualini

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et al [16] have shown a rapid and significant decrease of DA receptor density after incubating anterior pituitaries for only 7 min with 10^{-8} M E_2 These results strongly suggest that E_2 can rapidly modulate DAergic transmission at the level of the striatum as well as the anterior pituitary gland The aim of this study was to investigate the effects of an acute physiological dose of 17β-estradiol on the DA agonist binding site of ovariectomized rats in order to elucidate the possible mechanism of the short-term effect of estradiol

One hundred and four adult Sprague-Dawley female rats (Charles River, St-Constant, Que) weighing 300–400 g were housed 2 per cage and maintained at 22–23 C on a 14 10 h light–dark cycle (lights on from 05 00 to 19 00 h) and received rat chow and water ad libitum The rats were bilaterally ovariectomized and used for experiments 14 days later They were injected in the morning (between 8 30 and 10 30 h) with 100 ng (in 0 2 ml, s c) of 17β-estradiol or the vehicle (0 3% gelatin in saline solution) and sacrificed by decapitation 5, 15, 30, 45 and 60 min afterwards for steroid injected rats and 15 min after vehicle injection for control animals The striata were dissected, immediately frozen in dry ice and kept at −70 C until assayed

Trunk blood was collected into heparinized tubes and plasma was separated by centrifugation at 4000 *g* for 10 min and kept at −20°C until assayed for 17β-estradiol and prolactin (PRL) The hormones were measured in duplicate by radioimmunoassays, using rat hormone and rabbit antiserum provided by Dr A Bélanger [4] for E_2 and by double antibody radioimmunoassay using rat prolactin I-3 and rabbit antiserum (anti-rat PRL-S-3) kindly provided by the National Hormones and Pituitary Program Baltimore, U S A Hormone concentrations were measured in individual animals The control, 5, 15 and 30 min groups contained 20 rats while the 45 and 60 min groups included 12 rats From the control, 5, 15 and 30 min groups, 10 half striatum and 6 for the 45 and 60 min groups were used for catecholamine determinations (DA, dihydrophenylacetic acid (DOPAC) and homovanillic and acid (HVA)) while the rest of the tissues were used for the receptor assays

For catecholamine determinations, brain samples were prepared as previously described [9] Namely, striata were homogenized in 1 0 ml of 0 1 M $HClO_4$, at 0–4 C and centrifuged at 10,000 *g* for 20 min to precipitate proteins The supernatants were stored at −70°C in small polyethylene tubes until assayed, while the pellets were dis-

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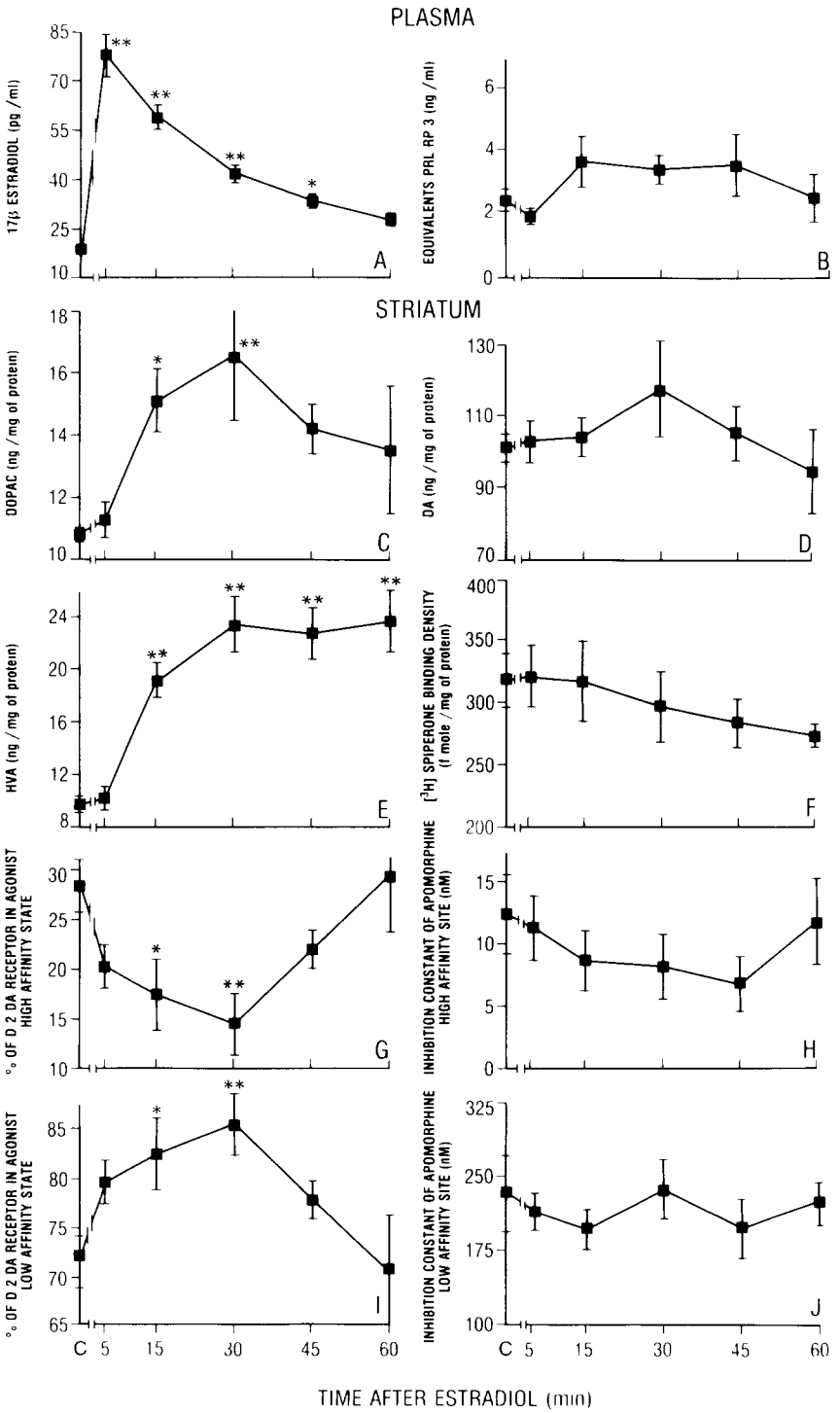
Fig 1 Effect of one injection (100 ng s c) of E_2 on (A) plasma 17β-estradiol concentration (B) Plasma PRL level (C) DOPAC (D) DA and (E) HVA striatal concentrations, (F), sum of high + low DAergic agonist binding sites densities (fmol/mg of protein) of the DA receptor as detected by apomorphine competition for [^{3}H]spiperone (0 2–0 3 nM) binding to ovariectomized female rats, (G) proportion of striatal D_2-receptors observed in the agonist high-affinity state, (H) inhibition constant of the agonist high-affinity state of DA receptor, (I) proportion of striatal D_2-receptors observed in the agonist low-affinity state and (J) inhibition constant of the agonist low affinity state Each time point for hormone concentrations represents the mean ± S E M for 18 20 rats analysed in duplicate for the catecholamine determinations values represent the means ± S E M obtained from 6 to 10 striata measured individually and values of binding experiments represent the means ± S E M of 5 -8 separate determinations $*P<0.05$ and $**P<0.01$ vs control (C)


PLASMA
17β ESTRADIOL (pg /ml)
EQUIVALENTS PRL RP 3 (ng /ml)
A
B
STRIATUM
DOPAC (ng /mg of protein)
DA (ng /mg of protein)
C
D
HVA (ng /mg of protein)
[3H] SPIPERONE BINDING DENSITY (f mole /mg of protein)
E
F
°o OF D 2 DA RECEPTOR IN AGONIST HIGH AFFINITY STATE
INHIBITION CONSTANT OF APOMORPHINE HIGH AFFINITY SITE (nM)
G
H
°o OF D 2 DA RECEPTOR IN AGONIST LOW AFFINITY STATE
INHIBITION CONSTANT OF APOMORPHINE LOW AFFINITY SITE (nM)
I
J
C 5 15 30 45 60
C 5 15 30 45 60
TIME AFTER ESTRADIOL (min)

solved in 1.0 ml of 1 M NaOH for determination of protein content by the method of Lowry et al. [13]. The concentration of DA and its metabolites (DOPAC and HVA) were measured by high-performance liquid chromatography (HPLC) with electrochemical detection according to our previously published procedure [9].

For the [^{3}H]spiperone binding experiments, striata were homogenized in a glass homogenizer in 100 volumes (w/v) of phosphate buffer (81 mM Na_2HPO_4, 19 mM KH_2PO_4, 2 mM $MgCl_2$, pH 7.4) and centrifuged at 40,000 *g* for 15 min. This washing procedure was repeated twice and the final pellet resuspended in 100 volumes of the same cold buffer. The D_2 DAergic agonist binding sites were assayed by competition of [^{3}H]spiperone (Amersham, 80–110 Ci/mmol, 0.2–0.3 nM) binding by apomorphine (10^{-11} to 10^{-4} M) in the presence of 50 nM ketanserin to saturate serotonin (5-HT_2) binding sites. Non-specific binding was estimated using 1.0 μM (+)-butaclamol. Incubation was at 22°C for 1 h and was terminated by rapid filtration through Whatman GF/C glass fiber filters under vacuum followed by 3 rapid rinses (4 ml) with ice cold phosphate buffer. Bound [^{3}H]spiperone was determined by liquid scintillation spectrometry. Dose–response curves and inhibition constants (K_i) were subjected to non-linear least square curve fitting program SCAFIT [14]. Statistical evaluation of K_i and proportions of receptor in the two states as well as hormones and catecholamines data were performed by the Duncan–Kramer multiple-range test [12].

As shown in Fig. 1, one injection of E_2 (100 ng/0.2 ml) to ovariectomized rats led to a significant elevation of this steroid concentration in the plasma as soon as 5 min with a progressive return to control values at 60 min while plasma PRL concentration was not significantly modified. As previously observed [9], DOPAC and HVA concentrations and DA turnover as estimated from the DOPAC/DA and HVA/DA ratios (data not shown) were elevated following the estradiol injection while striatal DA concentrations remained unchanged (Fig. 1).

We observed no significant change of the inhibition constants of high- and low-affinity D_2 DA agonist binding sites after the estradiol injection (Fig. 1). The sum of high + low DAergic agonist binding densities, reflecting total [^{3}H]spiperone binding, remained unchanged following the steroid injection. By contrast a significant conversion of the high into low agonist affinity binding states is observed at 15 min (38.6% of conversion, $P < 0.05$) with a maximal effect at 30 min (40.0% of conversion $P < 0.01$) followed by a return to control values at 45 min after the acute physiological steroid injection.

The present study, as previously reported [9], shows a rapid increase in DA turnover in the striatum of ovariectomized rats following an acute injection of a physiological dose of E_2 at times of increased plasma E_2 concentrations. An injection of 100 ng 17β-estradiol s.c. leads to a rapid surge of 78 pg/ml of plasma E_2 concentration and is similar to the proestrus blood E_2 peak which is in the order of 50 pg/ml [18]. This increase of DA turnover occurs in coincidence with a rapid conversion of the high into the low striatal D_2-receptor agonist binding states.

The chronic effect of E_2 on both post- and presynaptic DA receptors is well documented [19], while short-term effects of E_2 on DA receptors have been less docu-

mented For example, an intravenous injection of estrogen reverses the inhibitory effects of DA applied iontophoretically onto striatal neurons within 2–6 h [1] This indicates that shortly after an acute estrogen treatment, the effects of DA agonists on striatal neurons are suppressed, illustrating a modulation of DA-sensitive events postsynaptic to DA terminals by this hormone Furthermore, in an electrophysiological study Chiodo and Caggiula [7] have shown, after acute and extended exposure to estrogen, alterations in basal firing rate and autoreceptor sensitivity of DA neurons in the substantia nigra When male rats are treated with E_2 and then administered a small dose of apomorphine (APO) thought to affect only DA autoreceptors the hormone significantly attenuates the expected decrease in DOPAC in the striatum [17] This effect is correlated with an attenuation of the motility decrease usually observed after such low doses of APO, suggesting that E_2 may decrease the sensitivity of DA autoreceptors Gordon [11] has found that small doses of estradiol (10 μg kg) reduce the intensity and/or duration of stereotyped behavior elicited 24 h after the hormone administration and that this seems associated with a decrease in the ratio of high- to low-affinity agonist states [5] This effect could underline the estrogen antagonism of DA agonist events In addition, we [8] have observed a significant decrease of [^{3}H]spiperone binding to DA receptors in the anterior pituitary 30 min following a single s c injection of 17β-estradiol (30 ng)

It is well established that DA terminals in the striatum have presynaptic receptors [15] which regulate the release of DA into the synaptic cleft It is likely, in the light of our results where DA turnover and agonist states of DA receptors are altered simultaneously that E_2 treatment could alter the release of this neurotransmitter by a direct action on presynaptic receptors to alter reuptake of DA The present observations are consistent with the electrophysiological [6] and behavioral [3, 9] observations of the acute effects of estradiol Moreover, we have recently [10] observed variations of the proportions of the high/low D_2 DA receptor agonist affinity states in the striatum during the rat estrous cycle suggesting that estrogens are able to modulate DAergic receptors in physiological conditions

Our data show for the first time that an E_2 injection can rapidly and at a physiological dose modulate striatal agonist states of D_2 DA receptor Since the biogenic amino 2 and D_2 DA receptor agonist states changes observed here were rapid short-lasting and closely associated with elevated plasma steroid concentrations it is likely that this is a non-genomic effect of estrogens This steroid action may involve a membrane effect at the DA autoreceptor level, this could alter dendritic release [2], autoreceptor sensitivity and degradation of DA

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