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ESTRADIOL AND GUANINE NUCLEOTIDE MODULATION OF DOPAMINE RECEPTOR AGONIST AND ANTAGONIST BINDING SITES IN 7315a PITUITARY TUMORS

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Abstract—The agonist high- and low-affinity states of the dopamine (DA) receptor were investigated with apomorphine competition for [^{3}H]spiperone binding to DA receptors in 7315a tumors grown in intact female rats, while the antagonist site was investigated with saturation of [^{3}H]spiperone binding. Such as for the intact pituitary, the antagonist binding site density in 7315a tumors was not affected by NaCl and/or Gpp(NH)p, and its binding affinity was increased in the presence of NaCl. The DA receptor in 7315a tumors existed in high- and low-affinity agonist states, and the two apomorphine sites had similar affinities in tumoral and intact tissue. However, the proportion of the high affinity state was slightly lower in the 7315a tumor compared to intact tissue. Tumor (7315a) growth in ovariectomized rats was slower than in intact animal; chronic 17β-estradiol treatment inhibited growth of these tumors. Prolactin (PRL) concentration and density of DA receptors were higher in tumors grown in ovariectomized than in intact female rats, whereas both decreased after 23 days of 17β-estradiol treatment. Estradiol treatment decreased the affinity of the high- and the low-apomorphine binding sites, whereas their proportions remained unchanged. Thus, changes of DA receptors and 7315a tumor growth seem to be related; however, their relationship is complex.

Dopamine (DA) receptors in the normal anterior pituitary have been shown to exist in a high-affinity state that is considered to be functional and which mediates the inhibition of prolactin (PRL) release and of a low-affinity state for DA and DA agonists [1]. Guanine nucleotides have been shown to modulate the conversion of the high-affinity agonist state to the low, in normal pituitary [2]. PRL secreting 7315a tumor DA receptors are apparently normal although DA agonists do not inhibit PRL secretion [3]. Chronic luteinizing hormone releasing hormone (LHRH) agonist treatment decreases 7315a tumor growth and plasma PRL levels while the density of D2 DA receptors increases [4]. Agonist affinity for [^{3}H]spiperone-labeled sites in 7315a tumors was found to be reduced in the presence of guanine nucleotides, although high- and low-affinity sites were not separated [3]. The aim of the present study was to investigate in detail the antagonist as well as the high- and low-agonist affinity states of the DA receptor in 7315a tumors and its regulation by estradiol and guanine nucleotides.

MATERIALS AND METHODS

Adult female Buffalo rats weighing 175–200 g were 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*. Rats were inoculated s.c. with a suspension of 7315a tumors under the back skin. The 7315a tumor suspension was prepared as follows. Tumor tissue was taken immediately after decapitation of two rats bearing these tumors. Tumor tissue from different parts of the whole tumor was taken, the volume was estimated, and the tissue was suspended in the same volume of a PBS buffer, pH 7.2 (2.7 mM KCl, 1.5 mM KH_2PO_4, 0.5 mM $MgCl_2 \cdot 6H_2O$, 138 mM NaCl, 8 mM $Na_2HPO_4 \cdot 7H_2O$). This mixture was then gently passed with a glass piston through a metal mesh to obtain a homogenous suspension. All rats in a specific experiment were inoculated with 0.5 ml of the same suspension. In the first experiment, tumors were left to grow for 3 weeks in seven intact rats. In the second experiment, twenty-four rats were inoculated under ether anesthesia and separated in four groups as follows: (1) the rats of one group were ovariectomized (OVX) at the same time as tumors were implanted and were injected twice daily (b.i.d.) with 0.9% gelatin saline solution; (2) rats of a second group were injected for 16 days with 17β-estradiol (E_2, 20 μg/0.2 ml in 0.9% gelatin saline solution b.i.d.) and then for 1 week with the vehicle alone (called 17β-E_2 DC); (3) rats of a third group were injected for 23 days with E_2 (20 μg/0.2 ml in 0.9% gelatin saline solution b.i.d.) (called 17β-E_2); and (4) control rats were injected b.i.d. with the vehicle. The rats were decapitated in the morning of day 24 after tumor implantation. Tumors were quickly removed, immediately frozen, and kept at −90° until assayed. The PRL concentration in tumors was measured in homogenized tumors (diluted 1/10) in PBS buffer. PRL was measured in duplicate by double-antibody radioimmunoassay as previously described [4]. The second experiment was repeated twice; in one of these experiments, trunk blood was

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collected into heparinized tubes at the time of decapitation of the animals for measurement of plasma PRL and E_2 by specific radioimmunoassays as previously described [4, 5]. Tumor growth was measured with microcalipers, and volume was calculated according to the formula length × width × thickness × 0.5236 as previously reported [6].

Tumor tissue preparation for DA receptor assay was done at 0–4°, essentially as before [4]. Namely, tumor tissue was weighed, minced with a scalpel, and homogenized using a glass–Teflon homogenizer in 6 vol./wet weight of tissue; the buffer contained 0.25 M sucrose, 15 mM Tris–HCl, 100 μM EDTA, 12.5 μM nialamide, 0.01% ascorbic acid, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$ and 120 mM NaCl, pH 7.45, at 0–4°. In the first experiment, for the control and Gpp(NH)p assays, NaCl was excluded from the buffer. This homogenate was centrifuged at 100 *g* for 10 min; the pellet of this centrifugation was discarded and the supernatant fraction was centrifuged at 40,000 *g* for 20 min. The pellet was resuspended in 6 vol./wet weight of cold buffer without sucrose and centrifuged for 20 min at 40,000 *g*. The final pellet was resuspended in buffer (20 vol./wet weight), preincubated at 37° for 15 min, and then kept on ice until binding assay.

DA receptor agonist affinity states were investigated by competition of [^{3}H]spiperone (0.2 to 0.3 nM) binding by (−)apomorphine (25–26 concentrations, 10^{-12} to 10^{-4} M), whereas the antagonist binding site was investigated by saturation with increasing concentrations of [^{3}H]spiperone (7–8 concentrations, 0.025 to 0.3 nM); 2 μM (+)butaclamol was used to estimate non-specific binding. [^{3}H]Spiperone (Amersham, 88 Ci/mmol) was incubated in triplicate for 75 min at 25° in a total volume of 2.0 ml. Incubation was initiated by the addition of tumor membrane suspension (0.2 ml containing an average of 0.2 mg protein) to tubes containing 0.05 ml [^{3}H]spiperone and 0.05 ml (+)butaclamol or apomorphine or 0.1% ascorbic acid and 1.7 ml of buffer without sucrose (or 1.5 ml of buffer plus 0.2 ml of guanosine 5′-[β-γ-imino]triphosphate (Gpp(NH)p) dissolved in buffer). At the end of incubation, samples were diluted with 2 ml of cold washing buffer and rapidly filtered through Whatman (GF/C) glass fibre filters under reduced pressure using a cell harvester (Brandel). Following two 10-sec washes (4–6 ml each), the filters were placed into vials with 10 ml of scintillation fluid (Formula-963, from NEN Research Products, Boston, MA, U.S.A.). Radioactivity was monitored in a Beckman LS3801 instrument with an efficiency of 66%. Protein concentration was determined by the method of Lowry *et al.* [7].

Competition binding curves were subjected to a non-linear least squares curve-fitting (program LIGAND) [8] generalized model for complex ligand–receptor systems. Data were fit successively to one population and two populations of binding sites. A two-site model was obtained as appropriate only when a statistically significant improvement of the fit to the data was obtained over a one-site model, based on the assumption that deviations of the data from that expected for a one-site bimolecular reaction (law of mass action) resulted from different affinities of the ligand to more than one class of binding site present. Scatchard plots were constructed from saturation data, and least square linear regression analysis was performed to calculate the dissociation constant (K_D) and the maximum number of binding sites (B_{max}). Statistical evaluation of K_D, B_{max} and K_i values, as well as proportions of receptor in the two states, were performed by the Duncan–Kramer multiple range test.

RESULTS

In the first experiment, 7315a tumors used were grown in seven intact female rats and weighed 33.1 ± 7.0 g at sacrifice. The density (B_{max}) of [^{3}H]spiperone binding sites in 7315a tumors was not affected by sodium ions or Gpp(NH)p alone or in combination (Table 1 and Fig. 1). The affinity (K_D) of [^{3}H]spiperone binding sites was increased in the presence of NaCl whereas Gpp(NH)p alone or in combination with NaCl had no effect on affinity.

The DA receptor existed in a high and a low agonist affinity state in this tumor as assessed with competition for [^{3}H]spiperone binding by apomorphine (Table 2 and Fig. 2). Sodium ions increased the affinity of the low-affinity site of apomorphine, whereas the high-affinity site was not influenced by this cation. Gpp(NH)p alone decreased the affinity of high- and low-affinity apomorphine sites, whereas complete conversion into the low-affinity site was observed in combination with NaCl. The proportions of high to low affinity apomorphine sites remained unchanged in the presence of NaCl alone. With Gpp(NH)p alone either

Table 1. Effects of sodium (120 mM NaCl) and guanine nucleotide (100 μM Gpp(NH)p) on the equilibrium dissociation constant (K_D) and density (B_{max}) of [^{3}H]spiperone binding sites in 7315a tumors grown in intact female rats

Group	K_D(nM)	B_{max} (fmol/mg protein)
Control (N = 5)	0.058 ± 0.006	36 ± 2
NaCl (N = 5)	0.040 ± 0.004*	41 ± 2
Gpp(NH)p (N = 3)	0.061 ± 0.009	30 ± 5
NaCl + Gpp(NH)p (N = 3)	0.058 ± 0.004	38 ± 6

Values are means ± SEM obtained from N separate determinations.
* $P < 0.05$ vs control.

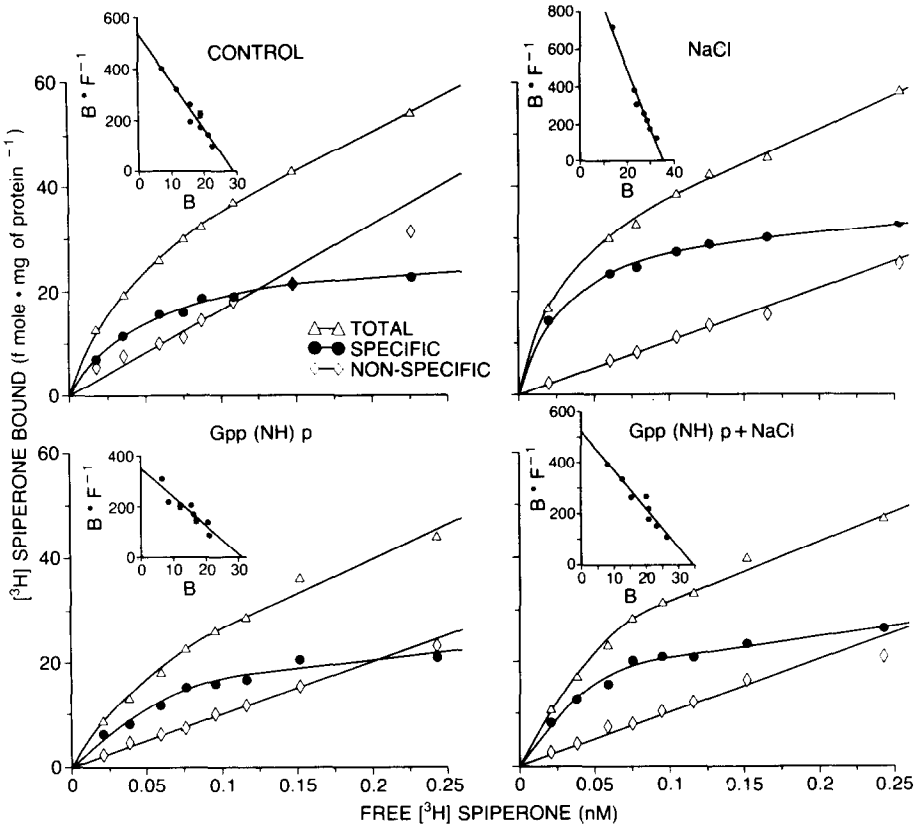

Fig. 1. Representative saturation experiments showing the effects of NaCl (120 mM) and Gpp(NH)p (100 μM) on [^{3}H]spiperone binding to DA receptors in 7315a tumors. The K_D values were 0.058 ± 0.008, 0.028 ± 0.002, 0.078 ± 0.012 and 0.065 ± 0.006 nM for the control, NaCl, Gpp(NH)p, and NaCl + Gpp(NH)p experiments respectively. Scatchard plots showing bound/free ($B \cdot F^{-1}$) data in fmol·(mg protein)$^{-1}$·nM^{-1} as a function of bound (B) ligand in fmol·(mg protein)$^{-1}$ are shown in insert.

complete conversion or no conversion of high into low apomorphine site was obtained depending on the tumor used, whereas complete conversion was observed invariably with the combination NaCl + Gpp(NH)p.

In the second series of experiments, we observed that the growth of 7315a tumors was greater in intact compared to OVX rats, while, surprisingly, chronic E_2 treatment at pharmacological doses decreased tumor growth both when E_2 treatment was discontinued for 1 week or pursued (Fig. 3). Tumor PRL content was increased in OVX compared to intact

Table 2. Effects of sodium (120 mM NaCl) and guanine nucleotide (100 μM Gpp(NH)p) on the inhibition constant (K_i) and proportion (%) of DA receptors in the agonist high- and low-affinity states as detected by apomorphine competition for [^{3}H]spiperone (0.2 to 0.3 nM) binding to 7315a tumors grown in intact female rats

D2 Receptor state	Control (N = 6)		NaCl (N = 7)		Gpp(NH)p (N = 4, 2)		NaCl + Gpp(NH)p (N = 6)	
	K_i (nM)	%	K_i (nM)	%	K_i (nM)	%	K_i (nM)	%
High	1.39 ± 0.61	35 ± 5	2.98 ± 1.61	39 ± 4	31 ± 8*	44 ± 1	—	0
Low	810 ± 237	65 ± 5	288 ± 49†	61 ± 4	1604 ± 222*	56 ± 1	247 ± 19†	100
High					—	0		
Low					323, 205	100		

Values are means ± SEM obtained from N separate determinations.
* $P < 0.01$ vs control.
† $P < 0.05$ vs control.

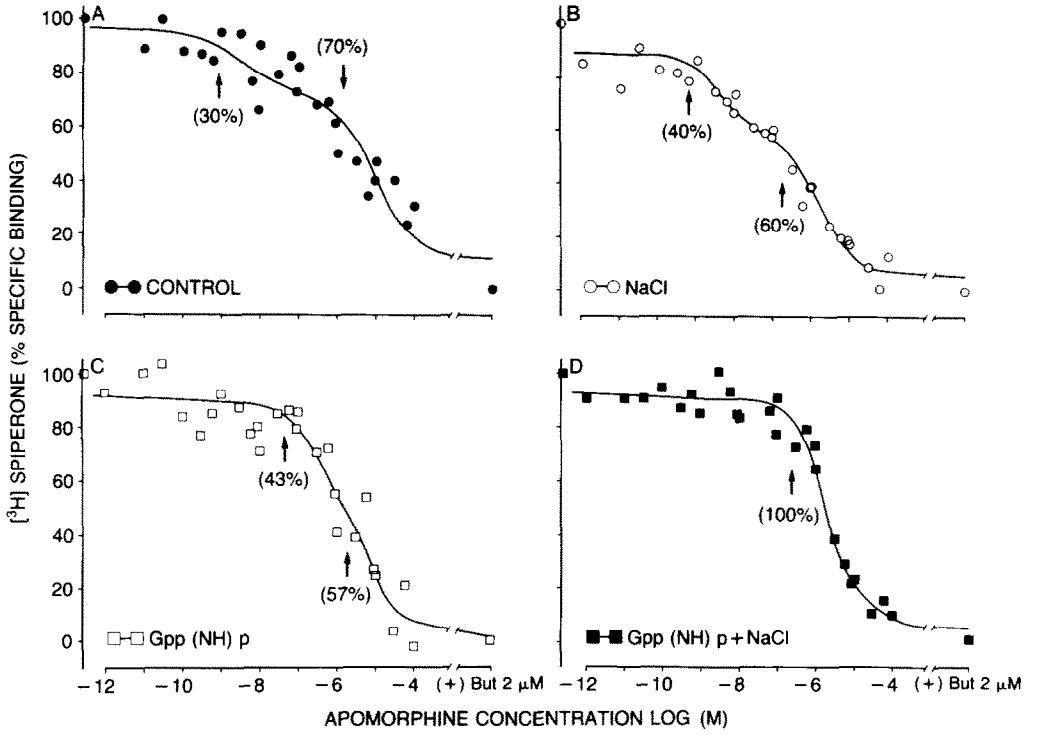

Fig. 2. Representative competition curves of apomorphine (M in molar concentrations) for [^{3}H]spiperone binding to DA receptors in 7315a tumors showing the effects of NaCl (120 mM) and Gpp(NH)p (100 μM). The K_{high} and K_{low} values for control, NaCl and Gpp(NH)p curves were, respectively, 1.09 ± 1.60, 1112 ± 601 nM; 0.556 ± 0.352, 215 ± 76 nM; and 46.6 ± 52.4, 1641 ± 1515 nM. The K_{low} for NaCl + Gpp(NH)p curve was 387 ± 56 nM. For each curve (control, NaCl, Gpp(NH)p, and Gpp(NH)p + NaCl) [^{3}H]spiperone binding (0.28, 0.26, 0.28, and 0.27 nM respectively) with tumor membranes (at protein concentrations of 1.02, 0.89, 0.83 and 0.90 mg/ml respectively) gave 2010, 2280, 1540 and 2560 dpm/tube total and 1150, 1010, 751 and 1310 dpm non-specific binding or 20, 38, 38 and 36 fmol/mg of protein specific binding.

rats, while 23 days of E_2 treatment decreased its PRL content (Table 3). Plasma PRL concentration (y) was positively correlated with tumor weight (x)($y = 35.79x + 83.45$; $N = 36$, $R = 0.84$, $P < 0.001$) or volume ($y = 44.51x + 256.59$; $N = 36$, $R = 0.52$, $P < 0.001$). By contrast, no significant correlation was found between plasma PRL concentration and PRL tumor tissue concentration ($R = 0.05$, $P > 0.1$). Nor was any correlation with tumor PRL content found if plasma PRL concentrations were expressed per gram of tumor tissue ($R = -0.25$, $P > 0.1$). Plasma E_2 concentration was observed to be low in intact (22.4 ± 0.3 pg/ml), in OVX (20.9 ± 1.0 pg/ml) as well as in rats in the 17β-E_2 DC group (22.6 ± 2.3 pg/ml) and elevated compared to the other groups ($P < 0.01$) in the 17β-E_2 group (45.8 ± 7.6 pg/ml). Uterus weight was lower in OVX (136.5 ± 5.5 mg, $P < 0.01$) compared to intact (406.9 ± 13.2 mg) rats, was unchanged for rats in the 17β-E_2 DC group (430.8 ± 22.2 mg), and increased in rats in the 17β-E_2 group (613.5 ± 60.4 mg, $P < 0.01$ vs intact).

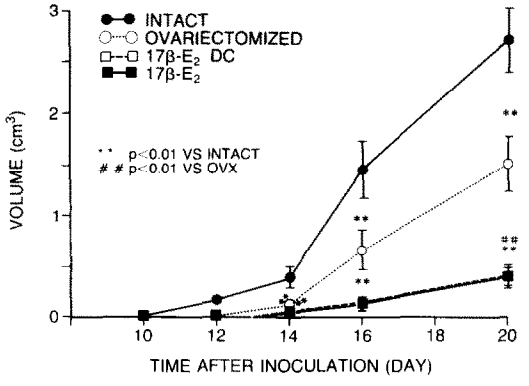

Fig. 3. Effect of ovariectomy or 17β-estradiol treatment of intact rats pursued (17β-E_2) or discontinued for 1 week (17β-E_2 DC) until sacrifice on *in vivo* 7315a tumor growth. Treatment was started the day after tumor inoculation.

In addition, plasma PRL levels expressed in ng/ml of plasma per gram of tumor were elevated in the 17β-E_2 group (162.3 ± 17.3, $P < 0.001$) compared to animals in the 17β-E_2 DC group (78.0 ± 8.4) as well to intact (82.5 ± 8.2) or OVX (55.2 ± 8.5) rats.

The affinity and density of [^{3}H]spiperone binding was decreased in tumors of E_2-treated rats, whereas tumors of OVX rats had a higher density of receptors with no change of affinity compared to tumors carried by intact rats (Table 3 and Fig. 4).

The high and low apomorphine affinities and pro-

Table 3. Effect of ovariectomy or 17β-estradiol treatment of intact rats on 7315a tumor PRL content as well as [^{3}H]spiperone affinity and density of binding to DA receptors

Group	K_D(nM)	B_{max} (fmol/mg protein)	Prolactin (ng/mg tissue)
Ovariectomized	0.043 ± 0.005	74 ± 4*	7.00 ± 0.25†
Intact	0.040 ± 0.004	42 ± 3	5.68 ± 0.54
17β-Estradiol (DC)	0.112 ± 0.022*‡	29 ± 3*‡	5.42 ± 0.19§
17β-Estradiol	0.185 ± 0.038*‡	24 ± 4*‡	3.29 ± 0.59*‡

Values are the mean ± SEM of five to eight separate determinations.
* $P < 0.01$ vs intact.
† $P < 0.05$ vs intact.
‡ $P < 0.01$ vs ovariectomized.
§ $P < 0.05$ vs ovariectomized.

portions were essentially the same in tumors from intact or OVX rats (Table 4 and Fig. 5). By contrast, E_2 treatment, either discontinued or continued until decapitation of the animals, decreased the affinity of the high and low apomorphine sites, while their proportions remained unchanged (Table 4 and Fig. 5). The sum ($R_{high} + R_{low}$) of the densities of the high- and low-affinity apomorphine sites showed a similar hormonal influence as observed for the B_{max} obtained with [^{3}H]spiperone saturation binding experiments.

DISCUSSION

It has been well documented that 7315a tumors contain high-affinity [^{3}H]spiperone binding to DA receptors [3, 4], whereas the high and low agonist states of the DA receptors have not been studied. For the first time, we report that in 7315a tumors, such as in intact pituitary tissue, the DA receptors exist in high- and low-affinity states for DA agonists. In addition, the high-affinity site for apomorphine in 7315a tumors observed here is in excellent agreement with the IC_{50} for inhibition of PRL by rat anterior pituitary cells in culture (3 nM) [1]. The apomorphine inhibition constants for the 7315a tumor DA receptors are also in good agreement with those observed in porcine pituitaries although they are slightly higher [1]. The proportion of [^{3}H]spiperone binding in the high-affinity state, as recognized by apomorphine in the porcine anterior pituitary, is 52% [1], 45% in rat anterior pituitary tissue [9] and is somewhat higher than our observation in 7315a tumors. In 7315a tumors, the lower proportion of

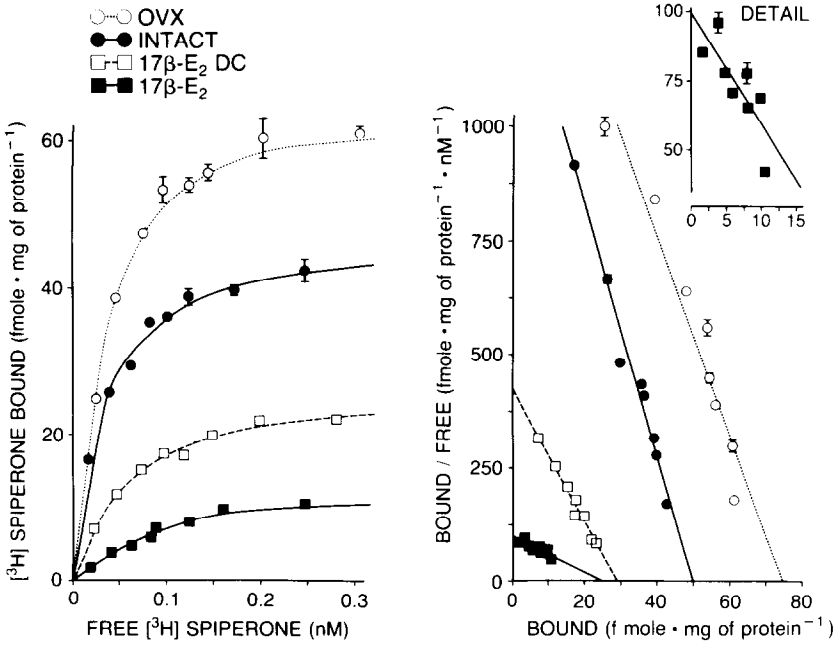

Fig. 4. Representative saturation experiments of [^{3}H]spiperone binding to the DA receptor in 7315a tumors, showing the effect of ovariectomy or E_2 treatment. The K_D values for OVX, intact, 17β-E_2 DC and 17β-E_2 groups were, respectively, 0.051 ± 0.006, 0.036 ± 0.003, 0.080 ± 0.027 and 0.200 ± 0.045 nM. The 17β-E_2 Scatchard plot is also included in an expanded form ("DETAIL").

Table 4. Effect of ovariectomy or 17β-estradiol treatment of intact rats on the inhibition constant (K_{high}, K_{low}), proportion (R_{high}, R_{low}) (%) and density ($R_{high} + R_{low}$) of DA receptors in the agonist high- and low-affinity states as detected by apomorphine competition for [^{3}H]spiperone (0.2 to 0.3 nM) binding to rat 7315a tumors at 25°

Group	K_{high} (nM)	R_{high} (%)	K_{low} (nM)	R_{low} (%)	$R_{high} + R_{low}$ (fmol/mg protein)
Ovariectomized	0.794 ± 0.344	37 ± 3	425 ± 214	63 ± 3	66.1 ± 1.8*
Intact	0.579 ± 0.131	39 ± 4	261 ± 50	61 ± 4	40.7 ± 2.1
17β-Estradiol (DC)	16.7 ± 5.3*	40 ± 4	2951 ± 850*	60 ± 4	25.7 ± 3.3*†
17β-Estradiol	5.9 ± 2.6	30 ± 6	1740 ± 461‡	70 ± 6	20.7 ± 2.6*†

Values are the mean ± SEM of five independent determinations.
* $P < 0.01$ vs intact.
† $P < 0.01$ vs ovariectomized.
‡ $P < 0.05$ vs intact.

the high-affinity DA agonist state, which has been considered as functional in the inhibition of PRL secretion, could partly explain the refractory response to DA of this tumor; alternatively, these differences may only reflect different assay conditions. At 25° in 7315a tumors, such as in the intact pituitary at 20° [2], complete conversion of the high- into the low-affinity state was obtained only in the presence of both NaCl and Gpp(NH)p, whereas with Gpp(NH)p alone, it was sometimes obtained, but not always. Interconversion of high- to low-affinity state is temperature dependent in intact pituitary tissue [2], and this may also explain the incomplete conversion with Gpp(NH)p alone in 7315a tumors. Such as for the intact pituitary, the antagonist [^{3}H]spiperone binding site density in 7315a

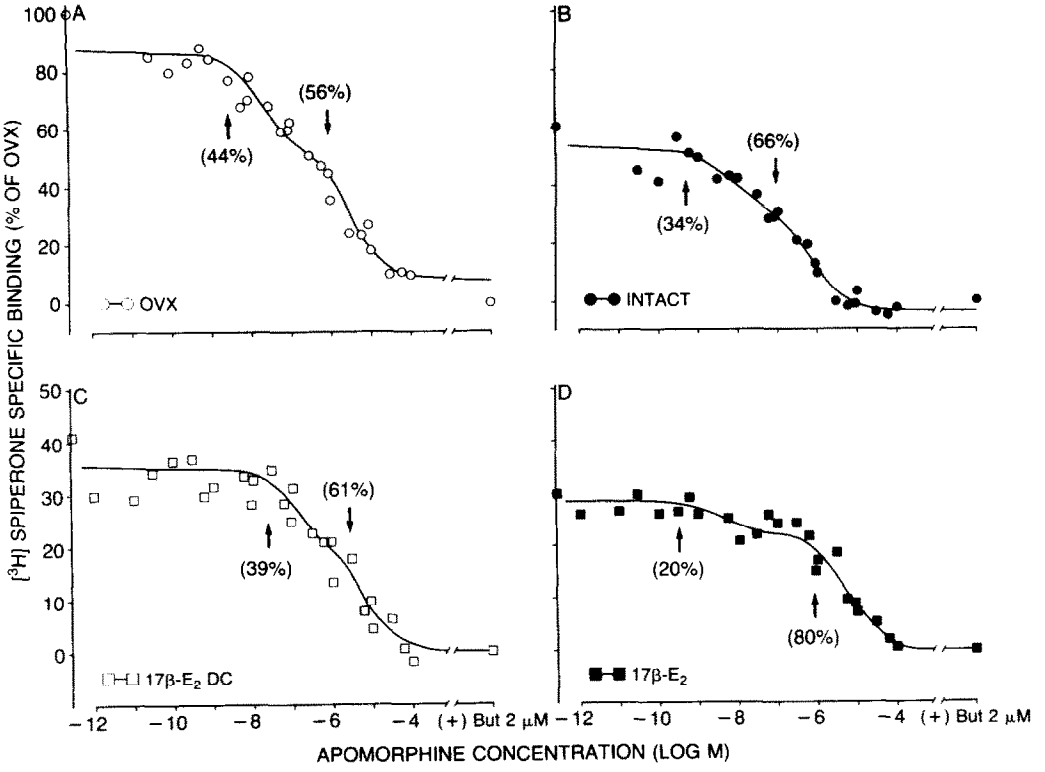

Fig. 5. Representative competition curves of apomorphine (M in molar concentrations) for [^{3}H]spiperone binding to DA receptors in 7315a tumors showing the effect of ovariectomy or E_2 treatment. The K_{high} and K_{low} values from OVX, intact, 17β-E_2 DC and 17β-E_2 groups were, respectively, 3.66 ± 2.40, 757 ± 264 nM; 0.778 ± 0.767, 75.1 ± 26.7 nM; 33.5 ± 38.6, 2145 ± 1455 nM; and 0.394 ± 0.605, 1415 ± 337 nM. Percent specific binding was relative to the OVX curve. For each curve (OVX, intact, 17β-E_2 DC, and 17β-E_2), [^{3}H]spiperone binding (0.28, 0.23, 0.22 and 0.22 nM respectively) with tumor membranes (at protein concentrations of 1.09, 1.20, 0.91 and 0.89 mg/ml respectively) gave 3600, 2510, 1420 and 1190 dpm/tube total and 962, 1010, 679 and 700 dpm non-specific binding or 66, 41, 26 and 21 fmol/mg of protein specific binding.

tumors was not affected by NaCl and/or Gpp(NH)p. [^{3}H]Spiperone affinity was increased only in the presence of NaCl in 7315a tumors, whereas this has been observed with NaCl both with or without Gpp(NH)p in intact pituitary tissue [2].

As previously reported [10], we observed that 7315a tumors, like the PRL-secreting MtTW15 tumors [11], grow at a slower rate in OVX compared to intact female rats. Surprisingly, for MtTW15 [11] and MtTF4 tumors [12], and as was shown by Lamberts *et al.* [10] for 7315a tumors, estrogen treatment inhibited the growth of 7315a tumors.

A higher density of DA receptors was observed in 7315a tumors from OVX compared to those from intact rats, whereas [^{3}H]spiperone affinity and apomorphine high and low affinities and proportions remained unchanged. This was associated with a higher PRL tumor content and smaller tumor volume and growth. It may be that, among other factors, the increased density of DA receptors enables some inhibition of PRL secretion and thus accumulation of PRL tumor content or that the synthetic rate of PRL is affected by these receptor changes. The observation that ovariectomy resulted in increased DA receptor density and a slower rate of tumor growth was also observed in MtTW15 tumors [13] although PRL concentrations were not measured. In addition and in agreement with these findings, we [4] have reported previously that treatment with an agonist of LHRH slows 7315a and MtTW15 tumor growth and is associated with higher tumor DA receptor density.

E_2 treatment, either discontinued for 1 week or pursued until sacrifice of the animals, led to smaller 7315a tumor volume as well as to a decrease in [^{3}H]spiperone binding affinity and density. The high and low apomorphine sites were also decreased in affinity, whereas the proportion of high to low remained unchanged. Similar results for the antagonist DA site have been observed for the MtTW15 [13] and $MtTF_4$ [14] tumors, whereas the agonist site was not investigated previously. However, the decrease by E_2 of the density of DA receptors was not reversed after 1 week of withdrawal as observed after diethylstilbestrol withdrawal from MtTW15 tumor-bearing rats [13]. The experimental conditions were somewhat different, and it may be that a longer period of time is required to reverse the effects of E_2 in our experimental paradigm. This is associated with unchanged PRL content in tumors treated for 16 days with E_2 and left untreated for 1 week, whereas PRL tumor content was decreased in tumors of rats treated with E_2 (23 days) until sacrifice. This may be explained by the fact that DA receptors of a lower density and also of lower affinity inhibit less adequately PRL release and thus less PRL is accumulated in the tumors. We might also suggest, as did Lamberts *et al.* [10], that treatment with pharmacological doses of E_2 leads to a down-regulation of the activity of the estrogen receptors on this pituitary tumor and that this is in some way related to tumor growth and changes in DA receptors. Responsiveness of the system is probably more complex than a simple inverse relationship between E_2 levels and DA receptor density. In this respect, we have reported for intact pituitary a biphasic effect of chronic E_2 treatment on DA receptors [15] with an increase of receptors at small doses of this steroid and a decrease at high doses. A biphasic effect of E_2 may also be involved in the regulation of 7315a tumor DA receptors. It may also be possible that chronic E_2 treatment alters the protein content of the tumor and membrane composition and that our results of DA receptor density in semi-purified membranes expressed in femtomoles per milligram of protein is not the best reflection of the number of receptors per tumor cell.

In summary, our results suggest that 7315a tumors have normal high and low DA agonist affinity states and regulation by sodium ion and guanine nucleotides. By contrast, 7315a tumor growth and DA receptors, like MtTW15 and MtTF4 tumors, are uniquely regulated by estrogen and differently from intact pituitary tissue [13]. A relationship between 7315a tumor growth and DA receptor density and affinity was observed, and its further characterization may give valuable information.

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REFERENCES

1. S. R. George, M. Watanabe, T. Di Paolo, P. Falardeau, F. Labrie and P. Seeman, *Endocrinology* **117**, 690 (1985).
2. M. Watanabe, S. R. George and P. Seeman, *Biochem. Pharmac.* **34**, 2459 (1985).
3. M. J. Cronin, C. A. Valdenegro, S. N. Perkins and R. M. MacLeod, *Endocrinology* **109**, 2160 (1981).
4. T. Di Paolo and P. Falardeau, *Eur. J. Pharmac.* **102**, 383 (1984).
5. A. Bélanger, S. Caron and V. Picard, *J. Steroid Biochem.* **13**, 185 (1980).
6. P. Janik, P. Briand and N. R. Hartmann, *Cancer Res.* **35**, 3698 (1975).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. P. Munson, A. Léan and D. Rodbard, *User's Guide to LIGAND*. Endocrinology and Reproduction Research Branch, National Institute for Children's Health and Human Development, NIH, U.S.A. (1980).
9. T. Di Paolo and P. Falardeau, *Life Sci.* **41**, 1149 (1987).
10. S. W. J. Lamberts, I. Nagy, P. Uitterlinden and R. M. MacLeod, *Endocrinology* **110**, 1141 (1982).
11. R. V. Lloyd, T. D. Landefeld, I. Maslar and L. A. Frohman, *Am. J. Path.* **118**, 379 (1985).
12. Y. Morel, V. Albaladejo, J. Bouvier and J. André, *Cancer Res.* **42**, 1492 (1982).
13. R. V. Lloyd and K. L. Fields, *Molec. cell. Endocr.* **44**, 133 (1986).
14. V. Albaladejo, R. Collu and J. André, *Endocrinology* **114**, 2344 (1984).
15. T. Di Paolo and P. Falardeau, *Prog. Neuro-Psychopharmac. biol. Psychiat.* **9**, 665 (1985).