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DOPAMINE RECEPTORS IN THE RAT PITUITARY AND THE TRANSPLANTABLE PITUITARY TUMOR $MtTF_4$: EFFECT OF CHRONIC TREATMENT WITH OESTRADIOL

J. André[1*], A.M. Marchisio[2], Y. Morel[1] and R. Collu[2]

[1] U.E.R. Lyon-Nord, INSERM U 34, Hôpital Debrousse, 29 Rue Soeur Bouvier 69322 Lyon Cedex 1, France

[2] Neuroendocrine Research Laboratory, Pediatric Research Center, Hôpital Sainte-Justine and Université de Montréal, Montréal, Québec, Canada

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We report that membranes from transplanted pituitary MtF_4 tumor contain ^{3}H-Spiroperidol binding sites. These sites fulfill the criteria for high affinity dopamine receptors similar to those found in normal pituitary. A 15-day estradiol treatment results in a dramatic decrease of these receptors in the tumor and only in a slight decrease in pituitary. These results suggest that estradiol might selectively modulate the action of dopamine in various tissues.

INTRODUCTION

Long term administration of natural or synthetic oestrogens is known to induce pituitary tumors in rats (1, 2). Fischer F344 is a rat strain very sensitive to pituitary tumor induction by diethylstilbestrol (3). One of these tumors, $MtTF_4$, has been maintained by serial transplantation in rats of the same origin. Initially, tumor growth was dependent on diethylstilbestrol treatment of the host, but after few passages the tumor grew both in treated and untreated rats, and was called autonomous (4).

Surprisingly, we have observed that in rats bearing the transplanted $MtTF_4$ tumor, chronic administration of oestradiol enlarged the pituitary but inhibited the growth of the tumor (5). Since both pituitary and tumor contain oestrogen receptors (6, 5), the action of oestradiol on the two tissues might be direct. To explain why oestradiol acts in opposite ways, two hypotheses might be envisaged : either the mechanism of oestradiol action is different in the two tissues or the mechanism of action is the same but its modulation is different.

We have postulated that endogenous dopamine might modulate the action of exogenous oestradiol for the following reasons : 1°) Dopamine inhibits pituitary cell

* To whom inquiries should be addressed.

Abbreviations used : ^{3}H-Spir = ^{3}H-Spiroperidol, E_2 = Estradiol-17β, K_D = equilibrium constant, K_i = inhibitory constant, B_{max} = maximal number of binding sites, Bs = specific binding.

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multiplication in vitro (7). 2°) Bromocriptine – a dopamine agonist – prevents or reduces the enlargement of pituitary induced by oestrogens in vivo (8). 3°) Bromocriptine is used in the treatment of human prolactin adenomas (9). Our working hypothesis is that some differences in dopamine action might be responsible, at least in part, for the opposite action of oestradiol in pituitary and tumor. In the present work we examine : 1°) whether the $MtTF_4$ tumor contains dopamine receptors similar to those found in the pituitary and 2°) the effects of oestrogen treatment on the number and affinity of dopamine receptors in the two tissues.

MATERIAL AND METHODS

Animals and tissues

Adult male Fischer (F344) rats 180-250 g of body weight, exposed to 12 hours dark-12 hours light periods and fed with water and lab chow ad libitum were used. The estrogen treatment was performed with Silastic tubing (602-265 Dow Corning, 1 cm length) filled with 17β-oestradiol and placed subcutaneously in the midline dorsal neck under ether anaesthesia. Empty tubings were placed in control animals.

Hypertrophic anterior pituitaries were collected at autopsy after 15 days or 2 months of estradiol treatment. Normal anterior pituitaries were obtained from control animals bearing the empty Silastic tubing for the same period of time.

$MtTF_4$ tumors resulted from a subcutaneous injection of a suspension of $MtTF_4$ cells as described before (5). Four weeks after the injection, when tumor size was about 2 x 2 cm, animals were treated or not with oestradiol for 15 days as described before. Rats were sacrificed 15 days later by decapitation. Pituitaries and tumors were removed and dropped immediately in liquid nitrogen, then stored at – 70°C until processed. Crude membranes were prepared as previously described (10). Pituitaries were homogenized directly with a teflon glass homogenizer while tumors were previously minced with scissors. Both in control and estradiol treated rats the proteins recovered ranged between 3 and 5.1 % of the pituitary weight and between 1.1 and 2.2 % of tumor weight.

Binding studies

Binding studies were performed as previously described (10) with minor modifications. The membranes freshly prepared or stored for 1 or 2 days at – 70°C were suspended in NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, Tris 50 mM HCl pH 7.4 at 20°C at a final protein concentration of 300-640 µg/ml, as determined by Lowry's method. One ml of the membrane suspension was incubated with 50 µl of increasing concentration of ^{3}H–Spiroperidol (^{3}H–Spir) solubilized in 0.1 % ascorbic acid, in presence or absence of 50 µl of d-Butaclamol 5 µM. For competition studies the incubations were performed with ^{3}H–Spir 1 nM and various drugs at increasing concentrations. The incubation was allowed to proceed for 12 min at 37°C. Membrane-bound ^{3}H-Spir was separated by rapid filtration through Whatman GF/C filters followed by 3 washes with 4 ml of cold incubation buffer. Filters were counted in 5 ml Aquasol in a liquid scintillation counter with a 45 % efficiency as determined by external standardization. Each value is the mean of duplicate determinations. The specific binding was calculated by the difference between the radioactivity bound to the membranes in the absence of d–Butaclamol (total binding) and that retained in its presence (non specific binding). The apparent dissociation constant (K_D) and maximal number of receptor binding sites (Bmax) were determined from Scatchard graphs.

Reagents and drugs

All reagents used were of analytical grade. The following drugs were used : ^{3}H–Spir, specific activity 21 Ci/mmol (New England) ; d– and l–Butaclamol HCl (Ayerst) ; Dopamine (Sigma), Bromocriptine (Sandoz).

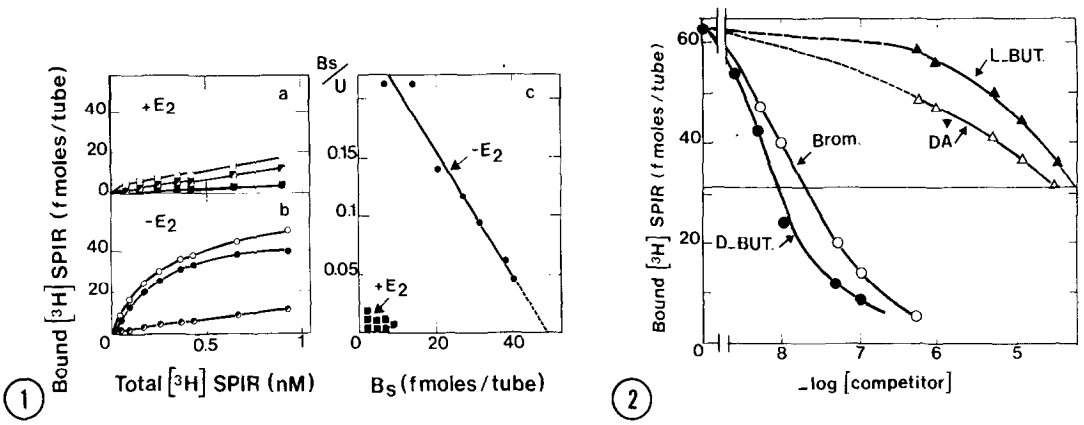

Figure 1. ^{3}H-Spir binding to $MtTF_4$ tumor membranes : Binding curves to membranes prepared from rats treated for 15 days with E_2 (a) or from control rats (b) and Scatchard graphs plotted from a (■) or b (●). Aliquots of each membrane preparation containing 400 mg proteins were incubated with increasing concentrations of ^{3}H-Spir in the presence or absence of 5 µM d-Butaclamol for 12 min. at 37°C. The total binding (○, □) and the non specific binding (◐, ◪) were measured. The specific binding, Bs (●, ■) was the difference between these values. The unbound ^{3}H-Spir (U) was the difference between the total ^{3}H-Spir concentration and Bs.

Figure 2. Specificity of ^{3}H-Spir binding to $MtTF_4$ tumor membranes : Aliquots (470 µg protein) of membranes prepared from a tumor obtained from control rats were incubated for 12 min. at 37°C with ^{3}H-Spir 1 nM and increasing concentrations of non radioactive ligands : d-Butaclamol (d-BUT, ●), Bromocriptine (Brom, ○), Dopamine (DA, △) and l-Butaclamol (l-BUT, ▲). Binding values are the mean of two independent experiments. The competitor concentration required for inhibit [^{3}H]-Spir by 50 % (IC_{50}) was used to calculate the inhibitory constant Ki according to the equation $Ki = IC_{50}/1 + (C)/K_D$. K_D is the equilibrium constant and C the radioactive ligand concentration.

RESULTS

1°) [^{3}H]-Spiroperidol binding to $MtTF_4$ tumor membranes

A typical saturation curve and Scatchard graph of [^{3}H]-Spir binding to crude membranes prepared from $MtTF_4$ obtained from control rats are shown fig. 1a, 1c. The Scatchard graph is linear and reveals a limited number (Bmax = 120 fmoles/mg proteins) of an apparent homogenous class of binding sites. The equilibrium dissociation constant, Kd is 0.18 nM. Similar results were observed in eleven additional experiments using ligand concentrations in the range 0.05 nM-1.2 nM and proteins in the range 270-600 µg. The mean values ± SD for Bmax and Kd were respectively 123 ± 38 fmoles/mg protein and 0.20 ± 0.10 nM. The "dopaminergic" nature of these binding sites was determined by the competition of d-Butaclamol, Bromocriptine, Dopamine and l-Butaclamol for ^{3}H-Spir specific binding (Fig. 2). These drugs had an apparent inhibition constant (Ki) of 1.3 nM, 3.8 nM, 5 µM and 8.3 µM respectively.

After 15 days of treatment with oestradiol the ^{3}H-Spir binding sites were undetectable in 4 experiments (one of which is shown fig. 1) or very low in 2 other experiments. In these experiments the non-specific binding was similar to that measured in tumor membranes obtained from control animals thus the error in determining the specific binding was important. Tentatively in the two last

TABLE I

Volume of membranes from Control rats	Volume of membranes from E_2 treated rats	K_D nM	B_{max} fmoles/mg protein
1	0	0.17	53.7
0.8	0.2	0.15	40.2
0.6	0.4	0.20	30.0
0	1	-	0

Effect of increasing concentrations of $MtTF_4$ tumor membranes prepared from rats treated for 15 days with estradiol on ^{3}H-Spir binding values to $MtTF_4$ tumor membranes prepared from control rats.

The two membrane preparations were adjusted at the same protein concentration (320 µg/ml). ^{3}H-SPIR binding was performed as described in the legend for Fig. 1. K_D and B_{max} were determined from Scatchard graphs.

experiments, the KD values were evaluated at 0.09 and 0.18 nM and Bmax at 9 and 25 fmoles/mg proteins. This low binding could be due to an actual decrease in the number of binding sites but might also have resulted from occupancy of the binding sites, from drug metabolism or from denaturation of the binding sites during preparation or utilisation of the membranes. The following experiment was done to rule out one of these hypotheses. Membranes of tumor obtained from control rats (control membranes) which contained ^{3}H-Spir binding sites were mixed in varying proportions with membranes of tumor obtained from oestrogen-treated rats (treated membranes) which were devoided of any apparent ^{3}H-Spir binding sites. The Bmax and Kd were measured for each mixing. Table 1 shows that the decrease of Bmax paralleled the dilution of control membranes by treated membranes while Kd was not modified. We conclude that, during the incubation period, no diffusible substance which would be able to alter ^{3}H-Spir or its binding sites is extracted from treated membranes.

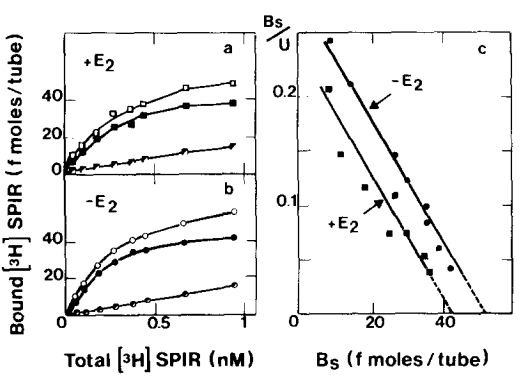

Figure 3. ^{3}H-Spir binding to pituitary membranes : Same legend as in Fig. 1 except the amount of proteins per tube was 640 µg.

Table 2

^{3}H-SPIR binding to pituitary membranes

Treatment	Number of experiments	K_D nM	Bmax fmoles/mg protein
Control	3	0.10 ± 0.04	85.5 ± 11.8
+ E_2 15 days	2	0.12 ± 0.02	62.1 ± 8.0
+ E_2 90 days	3	0.15 ± 0.03	75.1 ± 15.8

Mean ± SD of ^{3}H-SPIR binding to pituitary membranes from control rats and from rats treated with estradiol either for 15 days or 90 days.

2°) [^{3}H]-Spiroperidol binding to pituitary membranes

Fig. 3 shows a typical saturation curve and a Scatchard graph of ^{3}H-Spir binding to pituitary membranes obtained from control rats and from rats treated for 15 days with oestradiol. The two Scatchard graphs are linear in the range 0.05-1 nM of ^{3}H-Spir used and reveal, in both conditions, a limited number of an apparent homogeneous class of binding sites. The Bmax and K_D values determined in these two experiments were 74.5 fmoles/mg prot. and 0.15 nM in control rats and 63.8 fmoles/mg prot. and 0.19 nM in estradiol treated rats. Table 2 shows the mean values ± SD for B_{max} and K_D determined in additional experiments : after estradiol treatment for 15 or 90 days the dissociation constant was not modified while the maximum numbers of binding sites appear slightly decreased after a 15-day treatment.

DISCUSSION

We report here for the first time : 1°) that the transplantable rat pituitary $MtTF_4$ tumor contains ^{3}H-Spir binding sites fulfilling the criteria for high affinity dopamine receptors and 2°) that a 15-day estradiol treatment results in the decrease of the concentration of these receptors in the tumor.

The first point extends the work of Cronin et al (11) indicating that the 7315a tumor, an estrogen-induced rat pituitary tumor, contains ^{3}H-Spir binding sites characterized by the same range of concentration affinity and specificity described here. Such a similarity has to be underlined since, at the opposite, the GH_3D_6 clone derived from a radiation induced pituitary tumor showed no high affinity dopamine receptors (12). Taken together these results suggest that the kind of protocol used for inducing the tumor might be responsible, at least in part, for the presence or absence of dopamine receptors in tumor cells. Moreover our results indicate that the different estradiol action on tumor and pituitary is not due to a difference in the number, affinity or specificity of dopamine receptors at the beginning of the treatment. However, they did not exclude a possible difference at the post receptor level as suggested for the 7315a tumor (13).

The consequences of the estradiol treatment on the concentration of the ^{3}H-Spir binding sites in the $MtTF_4$ tumor are very different from those observed with pituitary or brain. While the decrease of the concentration of these binding sites in the $MtTF_4$ tumor was dramatic this decrease was not obvious in the pituitary (this work). In addition, it has been reported with rats (14) that a treatment with E_2 – 10 µg s.c. twice a day for seven days – did not modify the receptor concentration in the pituitary but increased it in the striatum, nucleus accumbens plus olfactory tubercle and in the frontal cortex. Taken together these results suggest that estradiol might selectively modulate the action of dopamine in various tissues.

The reason(s) for the disappearance of ^{3}H-Spir binding in $MtTF_4$ tumor after estradiol treatment remains to be established. However, the most probable hypothesis is that oestradiol "down regulates" the binding sites in vivo. Work now in progress tries to determine whether this down regulation is due to a decrease of the dopamine receptor bearing cells or to a decrease of dopamine receptors per cell. We rather favor the second possibility for two reasons : 1°) Preliminary observations indicate that tumors from estrogen-treated rats contain a large number of tumoral cells with secretory characteristics (J. Trouillas, unpublished results) but without dopamine receptors ; 2°) After estrogen treatment, the concentration of dopamine receptors in pituitary membranes does not increase (14) while the percentage of mammotroph cells in the pituitary cell population increases.

Whatever the reasons for the dramatic decrease of ^{3}H-Spir binding in the $MtTF_4$ tumor after estradiol treatment, this work demonstrates a clearcut biochemical difference between pituitary and transplantable $MtTF_4$ tumor. It remains to be determined whether the variation of ^{3}H-Spir binding in $MtTF_4$ tumor is the cause or the consequence of the inhibition of tumor growth by oestradiol.

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