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ROLE OF ESTROGENS ON STRIATAL DOPAMINERGIC ACTIVITY

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Summary—Studies were undertaken to evaluate the effects of estradiol and prolactin on striatal dopamine receptor activity. Dopamine receptors were quantified in partially purified striatal membranes by equilibrium binding using [^{3}H]spiroperidol. When we investigated whether the D-2 dopamine receptor activity changes during the estrous cycle, the results suggest an increase in dopamine receptor density in diestrous, without modifications in the affinity. The finding that in ovariectomized rats the dopamine receptor binding parameters remained unchanged, suggests that gonadal steroids are not essential in the mechanism of action of this receptor. Results of activity of D-2 dopamine receptors showing that hyperprolactinemia fails to increase the number of these receptors do not support the hypothesis that circulating prolactin regulates the activity of these striatal dopamine receptors. Administration of estradiol benzoate (250 μg/kg per day) to hyperprolactinemic rats, by s.c. injection, significantly decreased both the density and the affinity of the striatal dopamine receptors. The present data indicate that, although prolactin does not seem to modify the activity of striatal dopamine receptors, it could modulate the estrogen-induced hypersensitivity of these receptors.

INTRODUCTION

It is now well established that estrogens can affect the nigrostriatal dopaminergic system. In the female rat, striatal dopaminergic content [1], as well as *in vitro* amphetamine-stimulated dopamine release [2] vary as a function of the estrous cycle. Moreover, *in vitro* dopamine release of ovariectomized female rats is markedly altered following exogenous estrogen and progesterone manipulations [2]. Chronic estrogen treatment increases the density of striatal dopamine receptors in the rat [3]. On the other hand, administration of low doses of estrogen for a brief period of time has been shown to result in striatal dopamine receptor hyposensitivity 24 h after the last dose [4], while an hypersensitive state is observed after 48 h or longer. This biphasic response may be mediated by separate molecular mechanisms, and it has been suggested that this striatal dopamine receptor hypersensitivity may be mediated, at least in part, by the catecholestrogens [5].

The effects of estrogens on the central nervous system have been proposed to be mediated via the modulation of pituitary prolactin release [6]. Dopamine receptor concentrations are increased following prolactin treatment [3, 4], and this hormone can also stimulate dopamine release from perifused striata of male rats when infused *in vitro* [7]. However, other reports do not agree with this conclusion. Some investigators have found that chronic estradiol treatment of hypophysectomized rats lead to increased dopamine receptor density [3]. Others observed that hyperprolactinemia failed to increase striatal receptors in male and female mice [8] or in female rats grafted with prolactin secreting adenomas [9].

It has been suggested that the central effects of estrogens on the dopamine system are independent of their effects on the pituitary [10]. Thus, prolactin and estradiol may have similar but independent actions on striatal dopamine receptors.

In order to clarify the effects of prolactin and estradiol on striatal dopamine receptors, the following series of experiments were carried out.

EXPERIMENTAL

Animals

Female Wistar rats were kept under controlled conditions of light (12 h light/12 h darkness) and temperature. Sanders (Madrid) rat chow and water were available *ad libitum*.

Studies during the estrous cycle

Estrous cycles were monitored by daily vaginal smears, and only those animals exhibiting 3 or more consecutive 4-day cycles were used in this study. Groups of 6 rats, 76 days old, were killed in 3 different phases of the estrous cycle (diestrous, proestrous and estrous).

Ovariectomy

A group of 6 female rats, 30 days old, were ovariectomized, under i.p. tribromoethanol (0.25 g/kg body wt) anesthesia. Rats of the same age were sham-operated to be used as controls. The two groups of rats were decapitated 46 days after the operation.

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Induction of hypeprolactinemia

At the age of 30 days, groups of 6 rats were submitted to a medial laparotomy under i.p. tribromoethanol (0.25 g/kg body wt) anesthesia. After exposing the right kidney, a deep pocket was opened between the capsule and the renal parenchyma at the inferior kidney pole. A litter-mate donor was decapitated, its pituitary gland quickly removed, the neural lobe discarded and the pars distalis grafted in the kidney pocket under the capsule [11]. As a control, rats of the same age were sham-operated by just opening the kidney pocket. To study striatal dopamine receptors in this situation, a group of 6 hyperprolactinemic rats and a control group were sacrificed 46 days after the induction of hyperprolactinemia.

Estrogen administration

Twenty-four days after the induction of hyperprolactinemia two groups of 6 rats received for 21 days a s.c. injection (0.2 ml) of either estradiol benzoate (250 μg/kg body wt) or the oil vehicle. Rats were sacrificed 24 h after the last injection.

Striatal membrane preparation

After respective treatments, all animals were killed by decapitation between 0830 and 0930 h. The corpus striatum was dissected as defined by a coronal cut through the optic chiasm and dorsal and lateral cuts within the perimeter of the corpus callosum to a depth of approximately 2 mm.

Striata were homogenized in 50 vol. ice-cold 50 mM Tris–HCl buffer (pH 7.4). Homogenization was performed in a glass homogenizer with a glass pestle at 1500 r.p.m. (0–4°C) for 20 s. The crude homogenate was centrifuged at 25,000 **g** for 10 min and the pellet was washed twice with the same buffer.

Binding experiments

The binding experiments were performed according to Creese and Snyder[12] with modifications. Triplicate 200-μl aliquots of the membrane preparations (150–200 μg protein, as determined by the Lowry method [13]) were incubated with seven different concentrations of [^{3}H]spiroperidol [31 Ci/mmol, New England Nuclear) at 37°C for 15 min. The reactions were terminated by filtering the incubation medium through Whatman glass filters (GF/F). The filters were washed 3 times with 5 ml of homogenization medium. Filter bound radioactivity was determined by liquid scintillation counting (Biofluor New England Nuclear). The difference between the amounts of [^{3}H]spiroperidol bound to striatal membranes in the absence and in the presence of (+)butaclamol (10^{-6} M) was designated as specific binding of [^{3}H]spiroperidol to D-2 dopamine receptors.

A Scatchard analysis of the data using linear regression was performed to determinate the equilibrium dissociation constant (K_d) and site numbers (B_{max}).

Statistical analysis

Differences between group means were evaluated using analysis of variance followed by *t*-tests.

RESULTS

[^{3}H]Spiroperidol binding characteristics during the estrous cycle

The binding of [^{3}H]spiroperidol to partially purified rat striatal plasma membranes was quantified during the estrous cycle. Receptor affinity remained unchanged all along the cycle. The number of dopamine binding sites (B_{max}) may fluctuate during the estrous cycle as shown in Fig. 1. The

Fig. 1. Characteristics of affinity and density of striatal dopamine receptors in three different phases of the estrous cycle (diestrous, proestrous and estrous). Each value represents the mean ±SEM of six determinations.

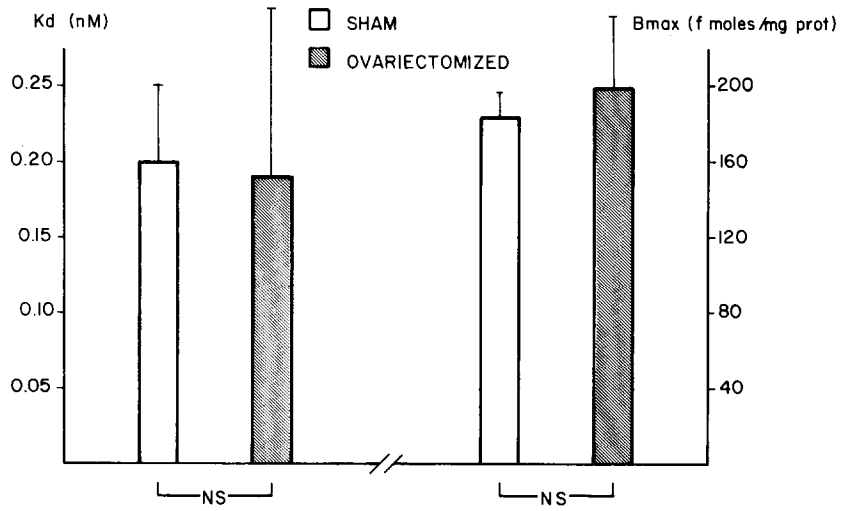

Fig. 2. Effects of ovariectomy on the affinity and density of striatal dopamine receptors. Female rats, ovariectomized at 30 days of life, were killed 46 days after this operation. Each value represents the mean ± SEM of six determinations.

maximal binding capacity was observed in diestrous (152 ± 7 fmol/mg protein) while it was lower in proestrous (134 ± 7 fmol/mg protein) and estrous (137 ± 8 fmol/mg protein).

The increase in the striatal dopamine binding sites is significant in diestrous vs proestrous ($P < 0.05$) but not vs estrous. These results suggest that the hormonal state in the different phases of the estrous cycle may influence the number of D-2 receptors striatum.

Effects of ovariectomy on the activity of striatal dopamine receptors

Affinity and density of striatal dopamine receptors were determined in ovariectomized rats in order to test the possible effect on these receptors of the absence of gonadal steroids. As illustrated in Fig. 2, ovariectomy failed to alter either the affinity or the density of striatal dopamine receptors. They were identical in ovariectomized rats ($B_{max} = 199 \pm 38$ fmol/protein; $K_d = 0.19 \pm 0.11$ mM) and in sham-operated controls ($B_{max} = 184 \pm 12$ fmol/mg protein; $K_d = 0.20 \pm 0.05$ nM).

The absence in ovariectomized rats of variations in the affinity or in the density of striatal dopamine receptors, suggest that gonadal steroids are not essential for the action of this receptor, even though they can participate in the modulation of its activity.

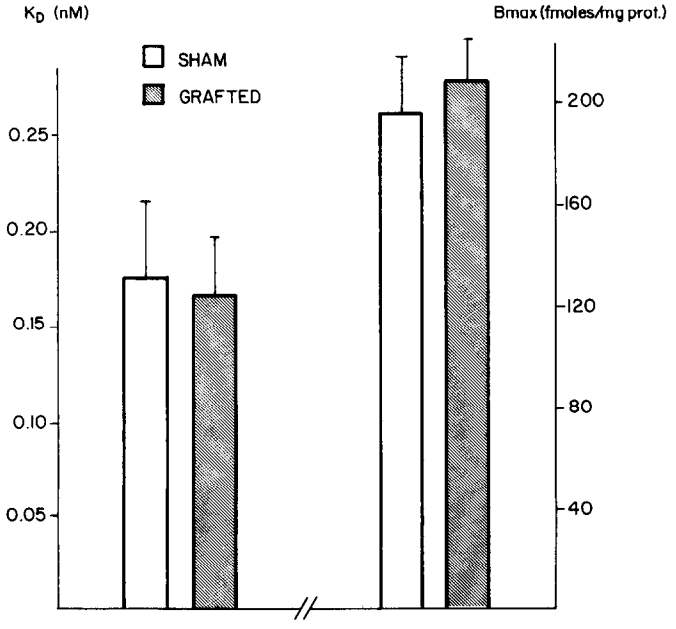

Fig. 3. Characteristics of affinity and density of striatal dopamine receptors of hyperprolactinemic rats. This hormonal state was induced at 30 days of life and the rats were killed 46 days after this operation. Each value represents the mean ± SEM of six determinations.

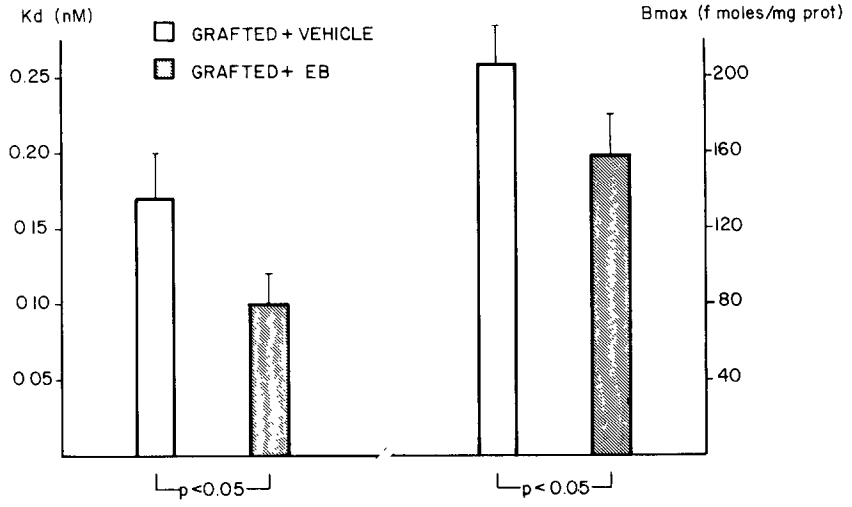

Fig. 4. Effects of estradiol benzoate on the characteristics of affinity and density of striatal dopamine receptors in hyperprolactinemic rats, 24 days after the induction of hyperprolactinemia, animals received during 21 days a s.c. injection (0.2 ml) of either estradiol benzoate (250 μg/kg body wt) or the oil vehicle. Rats were sacrificed 24 h after the last injection. Each value represents the mean ±SEM of six determinations.

Effects of prolactin on the activity of striatal dopamine receptors

Another study was carried out with hyperprolactinemic rats to determine whether elevation of prolactin levels in plasma was able to affect dopamine receptors in striatum. As indicated in Fig. 3, differences were not found in the affinity nor in the number of striatal dopamine receptors between hyperprolactinemic rats ($B_{max} = 210 \pm 16$ fmol/mg protein; $K_d = 0.16 \pm 0.03$ nM) and sham-operated controls ($B_{max} = 197 \pm 22$ fmol/mg protein; $K_d = 0.17 \pm 0.04$ nM). Thus, chronic hyperprolactinemia failed to increase the concentration of striatal dopamine receptors, although, as has been previously reported [11], the concentration of prolactin in plasma is several-fold higher in the hyperprolactinemic than in control animals. Therefore, it seems likely that prolactin has no effect on the activity of these dopamine receptors.

Administration of estradiol to hyperprolactinemic rats decreases the striatal dopamine receptor sensitivity

As shown in Fig. 4, administration of estradiol (250 μg/kg body wt, s.c.) to hyperprolactinemic rats for 21 days, caused a significant decrease in the binding capacity ($P < 0.05$), as well as in the affinity ($P < 0.05$) of striatal dopamine receptors ($B_{max} = 159 \pm 17$ fmol/mg protein; $K_d = 0.10 \pm 0.02$ nM), when compared with sham-operated controls ($B_{max} = 207 \pm 20$ fmol/mg protein; $K_d = 0.17 \pm 0.02$ nM). Thus, the permanently elevated levels of prolactin in plasma were able to counteract the reported capacity of estrogens to induce hypersensitivity of the striatal dopamine receptor [3].

DISCUSSION

The results of the study of striatal dopaminergic binding sites during the estrous cycle in the rat indicate an increase in dopamine receptor density in diestrous, without modifications in the affinity. These results are similar to those described in rat anterior pituitary, although they are not conclusive because it is necessary to increase the number of determinations with each day of the cycle.

It has been reported in the rat anterior pituitary [14] that the number of these receptors was constantly high from diestrous to proestrous as long as serum prolactin remained low and that in proestrous there was a rapid and marked decrease in receptor number which was coincident in time with the preovulatory prolactin surge. Subsequently, [^{3}H]spiroperidol binding gradually increased, while prolactin returned to basal levels. It is possible that striatal and anterior pituitary dopamine receptors may be regulated by ovarian steroids and/or prolactin. However, it is quite intriguing that the decrease in the number of these receptors occurs after estrogens in the blood have reached their highest levels [14].

Progesterone, which is known to reverse the stimulatory effect of estradiol on prolactin secretion, could participate in the increase of receptor number observed in diestrous. A few reports have indicated that this steroid can modify the activity of the nigrostriatal dopaminergic system [15, 16]. Nevertheless, the possibility that slight increases in serum prolactin may also participate in the decrease of dopamine receptor number cannot be excluded. A major decrease in D-2 receptor levels was observed

in hypothalamus during sexual development between 19 and 30 days of age [17]. This decrease is coincident in time with the previously reported increases in plasma levels of prolactin and free estradiol.

The absence in ovariectomized rats of variations in the affinity or in the density of striatal dopamine receptors suggests that the gonadal steroids are not essential for the action of this receptor, even though they can participate in the modulation of its activity.

Differences were not found in the affinity or number of striatal dopamine receptors between hyperprolactinemic and control rats. These data do not support the hypothesis that circulating prolactin regulates the activity of this striatal dopamine receptor [3, 6]. They are consistent with results previously reported showing that hyperprolactinemia failed to increase both the number of D-2 dopamine receptors [15, 16, 18] and dopaminergic activity [19] in the nigostriatal dopaminergic neurons.

The administration of estradiol to hyperprolactinemic rats decreased the striatal dopamine receptor sensitivity. In the literature, the effects of estrogens and prolactin on tuberoinfundibular dopamine neurons terminating in the median eminence and on the incertohypothalamic dopaminergic neurons, are well documented [20, 21]. However, the initial proposition that the effect of estrogen on the nigrostriatal system is modulated by pituitary prolactin [6] has been contested by other reports [3, 10]. In fact, it has been suggested that estradiol and prolactin may have similar but independent actions on striatal dopamine receptors [10].

It has been shown that doses and timing of estradiol treatment may influence the up- or down-regulation of striatal dopamine receptors [10]. This biphasic response may be mediated by separate molecular mechanisms [4]. The situation of hypersensitivity may be mediated, at least in part, by 2-hydroxy-estradiol [5]. The catecholestrogens may be involved in the expression of some of the actions of the estrogens in the central nervous system, as they have been shown to decrease the *in vivo* turnover rate of catecholamines in the anterior part of the medio-basal hypothalamus [22] and to inhibit tyrosine hydroxylase activity in the corpus striatum [23].

Recently, it has been reported that very small doses of estradiol were able to increase dopamine turnover with no changes of dopamine concentrations in the striatum and nucleus accumbens [24]. This effect was seen 30 min after the steroid injection and it is probably non-genomic and presynaptic. Diffusion or active transport of this steroid across the cell membrane could generate ion conductance changes and thereby alter the spontaneous activity of these cells in a way similar to that observed in the hypothalamus. Thus, a membrane-linked effect of estrogens could alter dentritic release, autoreceptor sensitivity and degradation of dopamine. These modifications may affect the activity of the enzyme adenylate cyclase. A cAMP-dependent protein kinase has been involved in the activation of soluble tyrosine hydroxylase from rat striatum [25]. This activation, coupled with presynaptic mechanisms, may then result in maximal stimulation of dopamine metabolism, as reflected by increased dopamine levels in the corpus striatum, high spontaneous release of dopamine and high responsivity of the dopaminergic terminal to amphetamine-stimulated dopamine release during a facilitatory phase [26].

In vitro treatment of secretory endometrial membranes with 17-β-estradiol stimulates 3–4-fold the activity of adenylate cyclase [27]. Cyclic nucleotides have been also implicated in short-term regulation of hormone binding in target tissue, probably by controlling the phosphorylation state of the receptor [28]. If this peripheric mechanism exists in the central nervous system, it may be responsible, at least in part, for the effects of estradiol on the nigrostriatal system.

In rabbits, the existence of binding sites for prolactin in substantia nigra and, to a lesser extent, in striatum, has been described [29]. Prolactin is also synthesized in hypothalamus [30]; the cell bodies in the medial basal hypothalamus have fibers projecting to the rest of brain. Thus, it seems clear that prolactin has to be considered as a neural as well as a pituitary product. This raises the possibility that this hypothalamic product, acting as a neuromodulator, may participate in managing the different possible responses to sex steroids, in addition to the classical neurotransmitter system.

It has been reported [32] that both the level and the cycling pattern of cerebrospinal fluid prolactin production were maintained unchanged, even though hypophysectomy had completely obliterated its presence in the plasma, and also that a sustained and intense hyperprolactinemia indeed affects the cerebrospinal fluid prolactin level. The mechanism of action of prolactin on striatal dopaminergic receptors may be similar to that described for other peptides [31] and could be mediated by adenylate cyclase. It has been described above that this enzyme may be also regulated by estrogens. Therefore prolactin and estrogens could play a modulating role on dopamine receptor activity via regulation of adenylate cyclase.

The results indicate that the hyperprolactinemic situation antagonizes the action of estradiol on striatal dopamine receptor sensitivity. This modulatory effect of prolactin may be similar to the previously described one for hypophysectomized rats treated with haloperidol [33]. It has been reported that the administration of prolactin to hypophysectomized rats could partially antagonize the development of the neuroleptic-induced dopamine receptor hypersensitivity. A possible explanation for this effect could be that prolactin may increase

dopamine release in the striatum [7]. This release could regulate the number of dopamine receptors and decrease their density and/or affinity ir diverse physiological or pathological conditions. Two of these situations could be the development of dopamine receptor hypersensitivity induced by the administration of haloperidol or by the elevation of estrogen levels in plasma.

In summary, estrogens participate in the regulation of the activity of striatal dopamine receptors. This action is not essential for the actuation of D-2 dopamine receptors. Prolactin does not seem to modify the activity of these receptors, but it may modulate, at least under some circumstances, the estrogen-induced striatal dopamine receptor hypersensitivity.

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