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Effects of Estradiol on Circulating Levels of Prolactin in Female Rats Bearing Ectopic Pituitaries

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The existence of local mechanisms controlling the prolactin (PRL) release from anterior pituitaries (AP) grafted to an ectopic location has been recently described. To study if these mechanisms are affected by estrogens, pituitary-grafted (GRAFT) and sham-operated (SHAM) rats were injected with a single dose of estradiol benzoate (EB), their plasma PRL levels as well as their hypothalamic and AP contents of norepinephrine (NE) and dopamine (DA) being analyzed. Administration of EB to GRAFT animals produced a small increase in their previously high plasma PRL levels, with both an increased NE and a decreased DA content in the ectopic AP. Since NE enhances the PRL release from ectopic AP and DA partially inhibits this secretion these changes may explain such a small increase in PRL levels. However, an additional increase in the decreased PRL release from the *in situ* AP of these animals cannot be discarded since EB produced also a decrease of the DA content in this tissue with an unaltered hypothalamic content. Finally, administration of this steroid to SHAM animals produced an important increase in plasma PRL levels. Since this increase was correlative to a decrease in DA and NE hypothalamic contents and unaltered AP contents. EB may be suppose to be able to reduce the DA synthesis in the tuberoinfundibular neurons, while the changes in noradrenergic inputs could be more related to the feedback effects of estrogens on the gonadotrophin release.

Key words: Prolactin, Estradiol, Dopamine, Norepinephrine, Ectopic pituitary, Hyperprolactinemia.

Grafting of one or more anterior pituitaries under the kidney capsule of rodents has been currently used to study the physiological effects of chronically increased prolactin (PRL) levels (1, 4, 8). The anterior pituitary located in an ectopic site is able to increase its secretory pattern of PRL, showing an increased number of lactotroph cells and a high degree of vascularization (22). This occurs presumably as a consequence of the absence of a normal dopaminergic inhibitory influence from the hypothalamus (16). Moreover, this tissue does not present relevant immunological rejection symptoms, surviv-

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ing for many months after the grafting (22).

Nevertheless, it has been recently proposed that this apparently uncontrolled PRL secretion from ectopic pituitaries could be modulated by several local mechanisms in which some biogenic amines could be involved (9-11, 20). The most important seems to be norepinephrine (NE) which exerts a stimulatory effect on PRL secretion, while dopamine (DA) inhibits only partially this secretion (12, 13). The origin of both amines present in ectopic pituitaries could be the *in situ* synthesis (11) and the uptake from the systemic circulation (14).

On the other hand, it is well established that estrogens increase PRL release in both male and female rats (15, 34), and moreover, they induce hyperplasia of lactotrophs with adenoma formation when they are administered in a prolonged treatment (29, 32). Their action would be produced at hypothalamic level by modifying the activity of tuberoinfundibular dopaminergic (TIDA) neurons (5, 21, 28, 33), as well as at pituitary level by altering the PRL synthesis and release from lactotrophs (2, 3, 7, 18, 25-27).

The present work has been designed to study the acute effect (1-day) of estrogens on the PRL release in rats with previously increased plasma levels of this hormone and, specially, if these steroids are able to alter the local catecholaminergic mechanisms modulating the ectopic PRL release in these animals. Measurements of peripheral PRL levels and hypothalamic and anterior pituitary contents of DA and NE in pituitary-grafted and control animals have been used to evaluate this possibility.

Materials and Methods

Animals. — Female rats of the Wistar strain were housed from the birth under controlled photoperiod (12 h light/12 h darkness; light on at 8 h a.m.) and temperature (23 ± 1° C) and with constant access to standard food (Sanders, Madrid, Spain) and water. At the age of 30 days, animals were implanted with an anterior pituitary gland under the right kidney capsule or were sham-operated (1). Litter-mate males were used as pituitary donors. Surgery was performed under i.p. tribromoethanol (0.25 g/kg body weight) anesthesia. Six weeks after the grafting or sham-operation, animals were submitted to a single s.c. injection of estradiol benzoate (0.2 mg/kg body weight) or sesame oil. At this time, control animals exhibited a normal cyclic pattern (60 % diestrous; 14 % proestrous and 26 % estrous), whereas pituitary-grafted rats showed a nearly constant diestrous condition (93 % diestrous and 7 % estrous). Treatments were performed with both control and pituitary-grafted animals showing a diestrous smear. Injections were carried out at 10 h a. m. Animals were killed by decapitation 24 hours later. Trunk blood was collected in heparinized tubes, immediately centrifuged (1,500 g) during 5 min at 4° C and the plasma separated and stored frozen at −70° C until analyzed. The hypothalamus and the *in situ* anterior pituitary were removed according to GLOWINSKI and IVERSEN (17). The ectopic pituitary was also removed in the pituitary-grafted rats. Tissues were weighed and homogenized in 50-100 vol of 0.1 N perchloric acid with 0.05 % EGTA. Homogenates were centrifuged (1,500 g) during 5 min at 4° C and the supernatant stored frozen at −70° C analyzed.

Prolactin determinations. — Plasma PRL levels were measured by a specific double antibody RIA system using materials kindly supplied by the NIH (Bethesda. Md. USA). The assay was previously validated in our laboratory. Plasma PRL levels were expressed as ng/ml of rat-PRL-RPI with a sensitivity

Table I. *Plasma prolactin (PRL) levels of pituitary-grafted (GRAFT) and sham-operated (SHAM) animals submitted to a single dose of estradiol benzoate (EB) or vehicle.*

Details in the text. Values are means ± SEM of six determinations. Statistical differences were obtained by analysis of variance. Values with a different superscript are statistically different ($p < 0.05$).

Animals	PRL (ng/ml)
SHAM + oil	24.3 ± 2.1[a]
SHAM + EB	90.0 ± 6.5[c]
GRAFT + oil	53.2 ± 8.4[b]
GRAFT + EB	78.8 ± 8.2[c]

of 0.4 ng/ml, an intraassay variation of 6 % and an interassay variation of 9 %.

Dopamine and norepinephrine determinations. — DA and NE contents were analyzed by a radioenzymatic assay according to DA PRADA and ZURCHER (6), previously validated in our laboratory. Intraassay variation was 8 %, interassay variation was 12 % and sensitivity was 0.1 ng/mg of protein. Results were expressed as ng of amine *per* mg of protein measured by the LOWRY method (24).

Statistics. — Data were assessed by analysis of variance for multiple group comparisons and by Student's t-test to compare only two groups.

Results

Grafting of an additional anterior pituitary gland under the kidney capsule produced a significant increase in plasma PRL levels of recipient animals (table I). This was accompanied by an increase in the DA content of the hypothalamus and the *in situ* anterior pituitary and unaltered NE contents (table II). Moreover, the ectopic anterior pituitary showed the presence of significant amounts of both amines (table III).

Administration of estradiol benzoate to pituitary-grafted animals led to a small increase in plasma PRL levels (table I) with a decreased DA content in the *in situ* anterior pituitary and unaltered hypothalamic contents of DA and NE (table II). Besides, administration of this steroid was followed by an increase in NE content and a decrease in DA content of the ectopic anterior pituitary (table III).

Finally, the administration of estradiol benzoate to control animals produced the highest plasma PRL levels (table I). This

Table II. *Dopamine and norepinephrine contents (ng/mg prot.) in hypothalamus and* in situ *anterior pituitary of pituitary-grafted (GRAFT) and sham-operated (SHAM) animals submitted to a single dose of estradiol benzoate (EB) or vehicle.*

Details in the text. Values are means ± SEM of six determinations. Statistical differences were obtained by analysis of variance. Values with a different superscript are statistically different ($p < 0.05$). ND = not detectable (< 0.1 ng/mg protein).

Determinations	SHAM		GRAFT	
	+ oil	+ EB	+ oil	+ EB
Hypothalamus				
Dopamine	7.4 ± 2.1[b]	3.0 ± 1.2[a]	16.2 ± 1.9[c]	13.9 ± 1.5[c]
Norepinephrine	12.9 ± 2.3[b]	5.3 ± 1.6[a]	13.9 ± 1.1[b]	15.6 ± 2.7[b]
Anterior pituitary				
Dopamine	1.0 ± 0.3[a]	1.4 ± 0.5[ab]	4.2 ± 0.8[c]	2.2 ± 0.5[b]
Norepinephrine	ND	ND	ND	ND

Table III. *Ectopic pituitary contents (ng/mg prot.) of dopamine and norepinephrine of pituitary-grafted (GRAFT) animals submitted to a single dose of estradiol benzoate (EB) or vehicle.* Details in the text. Values are means ± SEM of six determinations. Statistical differences were obtained by Student's t-test.

Determinations	GRAFT + oil	GRAFT + EB
Dopamine	2.6 ± 0.2	0.9 ± 0.1*
Norepinephrine	6.8 ± 2.7	14.3 ± 1.8*

* $p < 0.05$.

increase was concomitant with a decrease in the hypothalamic content of DA, while the *in situ* anterior pituitary content of this amine was not modified (table II). Moreover, NE content in the hypothalamus was decreased after estradiol administration (table II).

Discussion

Administration of estrogens in a single and pharmacological dose was followed by a significant increase in plasma PRL levels of both pituitary-grafted and control rats. However, the increase observed in the animals with previously increased PRL levels was lesser than the observed in the animals with normal PRL levels. A similar small increase in plasma PRL levels was also observed in patients with prolactinomas treated with estradiol (23), and, moreover, according to our previous studies the PRL response to estradiol in pituitary-grafted rats was less evident and with absence of pulsatility as compared to the high and in a pulsatile manner response found in control rats (30). In addition, PRL levels after ovariectomy were less reduced in pituitary-grafted rats than in controls (30). This could be supporting the possible existence of a double action of estradiol in dependence of PRL levels.

This small increase in plasma PRL levels induced by estradiol in pituitary-grafted animals could be apparently related with a direct effect of this steroid at pituitary level, acting directly on the PRL-producing cells. In this respect, the high DA content observed in the *in situ* anterior pituitary after the grafting had partially been reduced after administration of estradiol, while the activity of TIDA neurons was not apparently modified by the steroid since the hypothalamic content of DA remained unaltered. These findings confirm previous observations of our group in which it was suggested that the small PRL response to estradiol in pituitary-grafted animals (31) could be due to a direct effect of this steroid on the pituitary cells. Since a pulsatile response to estradiol, was absent in these animals supposed that the possibility of an effect on TIDA neurons could be discarded (30).

Nevertheless, the administration of estradiol to pituitary-grafted animals was also followed by changes in the endogenous amounts of DA and NE in the ectopic pituitary. Since NE seems to enhance the PRL secretion from ectopic pituitaries and DA slightly inhibits this ectopic secretion (12, 13), and considering that much of the PRL present in the circulation of these animals was originated from this tissue (1), it is likely that the estrogen-induced increase in the peripheral PRL levels of pituitary-grafted rats can be most clearly explained on the basis of estrogen-induced changes in the local catecholaminergic mechanisms controlling ectopic PRL release.

The positive effect of estradiol on circulating PRL levels in pituitary-grafted rats was different from the effect of this steroid in control animals in which administration of estradiol produced a very high increase of peripheral levels of this hormone. This increase was concomitant with a presumably decreased hypothalamic synthesis of DA in the TIDA neurons induced by estradiol, which is supported by previous reports (5, 30).

Finally, we have observed a decreased NE content in the hypothalamus after estradiol administration to control animals. This decrease could probably be indicating the existence of an altered hypothalamic activity of this amine induced by estradiol. This could affect the PRL release since it has been previously reported that NE stimulates the release of this hormone from the *in situ* anterior pituitary acting at hypothalamic level (12). However, it is most likely that the estradiol-induced changes in the hypothalamic activity of this amine may be rather related to the feedback effects of this steroid on the hypothalamic control of gonadotrophin secretion, which are well documented (19).

To sum up, estradiol-induced increase in the peripheral PRL levels of pituitary-grafted animals can be explained on the basis of changes in the local noradrenergic and dopaminergic influences acting at ectopic pituitary level. This increase was small and contrast with the high increase induced by estradiol in plasma PRL levels of control animals probably due to a decreased DA synthesis in the TIDA neurons.

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Resumen

Con el objetivo de estudiar si los mecanismos locales que controlan la liberación de prolactina (PRL) por adenohipófisis (ADHF) implantadas en una localización ectópica se afectan por los estrógenos, se han medido los niveles plasmáticos de PRL y los contenidos hipotalámicos y adenohipofisarios de dopamina (DA) y norepinefrina (NE) después de la administración de una única dosis de benzoato de estradiol (BE) a ratas con implante adenohipofisario (EXP) o con operación simulada (CT). La administración de BE a animales EXP produce un pequeño incremento en los ya elevados niveles plasmáticos de PRL de estos animales, así como incrementa el contenido de NE y disminuye el de DA en la ADHF ectópica. Estos últimos cambios pueden explicar el pequeño incremento en los niveles plasmáticos de PRL producidos por el BE, ya que previamente se ha demostrado que la liberación de PRL por la ADHF ectópica es estimulada por NE y parcialmente inhibida por DA. Sin embargo, no se puede descartar que adicionalmente el BE produzca una recuperación de la baja liberación de PRL por la ADHF *in situ*, ya que el esteroide produce también una disminución del contenido de DA en este tejido sin alterar su contenido hipotalámico. Finalmente, la administración de BE a animales CT produce una importante elevación en los niveles plasmáticos de DA y NE, sin alterar sus contenidos en la ADHF. Esto induce a suponer que el BE puede reducir la síntesis de DA en las neuronas tuberoinfundibulares, mientras que los cambios en la actividad noradrenérgica hipotalámica podrían estar relacionados más bien con los efectos *fedback* de los estrógenos sobre la secreción de gonadotropinas.

Palabras clave: Prolactina, Estradiol, Dopamina, Norepinefrina, Adenohipófisis ectópica, Hiperprolactinemia.

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