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LH and Prolactin in Estrogenized Female Rats: Response to LHRH

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Eighty day old female rats postnatally treated with estradiol benzoate (EB) show hyperprolactinemia and increased levels of LH with a positive correlation LH-prolactin, unlike the control animals. Fourteen days after ovariectomy this correlation disappears and the prolactin levels remain higher than control. On the contrary, the increase in LH levels is smaller in the EB group. The administration of LHRH to ovariectomized EB rats produces a decrease in prolactin levels, unobserved in the control group, as well as lower LH levels.

In experimental anovulatory syndromes induced by constant light (1), hypothalamic suprachiasmatic region lesioning (5), anterior deafferentation of basal hypothalamus (8, 10) or steroid injection during early neonatal period (14), plasma prolactin levels were considerably high. Preliminary studies (39) have shown that ovariectomized female rats neonatally treated with estradiol benzoate exhibited a greater prolactin response to estrogen stimulation than control rats and a disappearance of the positive feedback of estrogen to LH. The role that high prolactin levels may play in this anovulatory pattern remains unknown, despite rather extensive investigation (13, 32).

In female rats, LH release is not only determined by the amount of LHRH secreted but also by changes in the pituitary responsiveness due to the action of circulating steroid hormones (2, 4).

In vitro administration of LHRH to the pituitary from intact or gonadectomized rats does not modify prolactin secretion (6). The LHRH dose producing the highest increase of LH in female or male rats *in vivo* has no effect on their prolactin secretion (16, 43). Nevertheless, Fujii *et al.* (19) reported alterations in plasma prolactin concentration after the administration of LHRH in androgen-sterilized female rats and cyclic rats in estrous.

The aim of the present work is to analyze the effect of LRHH on prolactin and LH levels in female rats rendered an-

ovulatory by neonatal estrogen administration.

Materials and Methods

Female Wistar rats, born in our laboratory, housed in a temperature-light (12 h/light/day) controlled environment, were used. On day 5 (day 1 is day of birth), 100 μg of estradiol benzoate (EB) dissolved in 0.1 ml of olive oil were given s.c. to the rats. Control animals received oil injection only. Prior to further experimentation, vaginal smears were taken daily for at least 15 days. Only oil treated rats showing regular 4-5 days cycles were used. All EB treated rats showed cornified vaginal smears during the entire period.

Ovariectomy was performed on 80 day old rats. 100 or 1.000 ng of LHRH (Pevya Lab.) in 0.5 ml of saline, as well as vehicle alone were administered i.p. two weeks after ovariectomy at 10.00 h. Blood samples were taken: *a*) on day 80, before ovariectomy, *b*) two weeks after ovariectomy previous to LHRH or saline administration and *c*) 15 and 45 min after LHRH or saline injection. Blood samples were obtained by jugular puncture under light ether anaesthesia. Samples were collected in heparinized tubes, centrifuged and plasma stored frozen until day of assay.

Plasma concentrations of LH and prolactin were determined by a double antibody radioimmunoassay, with kits supplied by NIAMD. LH and prolactin were labelled with ^{125}I by the chloramine T method of GREENWOOD *et al.* (20). The values are expressed in ng/ml of the reference preparations NIAMD-Rat-LH-RP-1 and NIAMD-Rat-Prolactin-RP-1 respectively. Intraassay and interassay variations were 7 % and 10 % for LH and 9 % and 13 % for prolactin respectively.

Results were analyzed statistically using two-way analysis of variance (35) and Mann Whitney «U» test (34).

Results

LH and prolactin responses to ovariectomy. As shown in table I plasma LH and prolactin levels are similar in diestrus and estrous in oil-treated rats (samples taken at 10.00 h), as previously described (27). Plasma LH concentration in EB-treated rats is higher ($p < 0.01$) than in cyclic rats. Ovariectomy increases ($p < 0.01$) plasma LH levels in both groups. The magnitude of the repsonse to ovariectomy in greater ($p < 0.01$) in control animals than in anovulatory rats.

EB-treated female rats show very high plasma prolactin levels, i.e. about 10 times higher than control females in diestrus and estrous. Plasma levels of this hormone decrease ($p < 0.01$) after ovariectomy. However, prolactin levels are higher in EB treated rats than in controls.

Analysis of variance (mixed model Anova) shows interactions between post-

Table I. *Pre- and postcastration plasma LH and prolactin values in control and estrogenized female rats.*
( ) number of rats; E and D-controls: rats in estrous and diestrous. Results are expressed in ng/ml ($\bar{x} \pm$ s.e.m.).

Group	Basal levels		Post-ovariectomy levels (14 days)	
	LH	PRL	LH	PRL
E-control (12)	19.6 ± 2.3	42.1 ± 9.6	130.9 ± 12.0	26.3 ± 2.8
D-control (8)	24.5 ± 5.1	47.5 ± 11.3		
Sterilized (24)	33.6 ± 2.6	403.0 ± 43.3	60.5 ± 6.1	118.0 ± 15.6

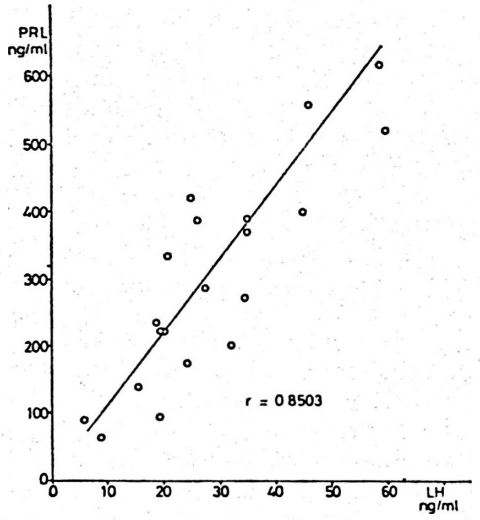

Fig. 1. *Correlation between basal levels of LH and prolactin in EB-treated female rats.*

natal treatment with EB and castration for LH ($p<0.01$) and prolactin ($p<0.01$).

Correlation LH/prolactin. Fig. 1 shows a positive correlation ($r = 0.85$; $p < 0.01$) between basal levels of LH and prolactin in EB-treated female rats. After ovariectomy no correlations are found ($r = -0.37$). In control rats LH and prolactin levels show no correlation either before ($r = 0.25$) or after ($r = 0.33$) ovariectomy.

Effect of LHRH administration. LH levels in control and EB-treated rats are different before LHRH injection. Therefore to allow statistical comparison between the groups, figure 2 shows the temporal patterns of response to LHRH and saline administration, expressed in increment/decrement over the basal levels. The i.p. injection of 100 ng and 1,000 ng of LHRH induces an increase ($p < 0.01$) in plasma LH concentration at 15 and 45 min in both groups. 1,000 ng dose induces a higher increase ($p < 0.01$) than 100 ng dose. EB-treated rats show lower responses to LHRH administration than control animals, without statistically significant differences.

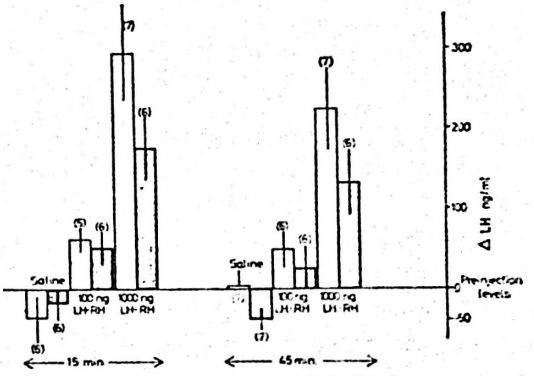

Fig. 2. *Effect of the administration of saline and 100-1000 ng of LHRH on plasma LH levels.*
Open and dark bars show results of control and EB groups respectively.

Saline injection causes a slight decrease in plasma LH levels at 15 min in control rats. This effect is more pronounced in anovulatory animals at 45 min ($p < 0.05$).

No effect is detected in oil-treated rats after LHRH or saline administration on plasma prolactin (fig. 3). EB-treated rats on the contrary respond to 100 and

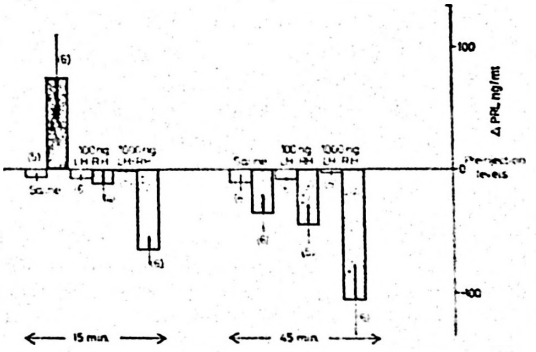

Fig. 3. *Effect of the administration of saline and 100-1000 ng of LHRH on plasma prolactin levels.*
Open and dark bars show results of control and EB groups respectively.

1,000 ng of LHRH injection with a marked decrease in plasma prolactin levels. The lowest concentrations ($p<0.01$) are found for both doses at 45 min. Saline administration to anovulatory rats produces a rise ($p<0.01$) in plasma prolactin levels at 15 min.

Discussion

EB females show higher plasma LH levels than controls. Ovariectomy induces an increase in LH levels in both groups, but higher in controls than in EB-rats (table 1). These results are similar to those found in females postnatally treated with testosterone propionate (TP females) (24, 40).

The lower response to ovariectomy may indicate: *a*) alteration of the sensitivity to negative feedback of estrogens, as pointed out by some authors (37, 39) and not found in TP females (29, 36); *b*) a decrease in the hypophyseal sensitivity to LHRH: in ovariectomized EB females, LHRH produces a lesser increase in LH levels than in controls (fig. 3): the response to LHRH is found higher in TP females than in diestrus females or in males (17) as well as that observed in cyclic females in proestrus (11, 18). On the other hand, the response is less intense after ovariectomy (38). It is possible that the relatively increased levels of estrogens in intact females may improve the hypophyseal response to LHRH, but by eliminating the stimulatory effect of estrogens a lesser hypophyseal sensitivity to LHRH might appear. The decrease in LH levels observed in groups injected exclusively with saline may be due to stress (7, 26).

EB females show high plasma prolactin levels, in accordance with previous results and with data reported in another experimental anovulatory syndromes (see introduction). This hyperprolactinemia has been attributed to a higher production of ovarian estrogens (22, 32, 41) but this hypothesis does not explain the sustained increase of prolactin levels after castration (even 40 days after ovariectomy, unpublished data). It has been suggested that high prolactin plasma levels may produce a stimulatory action over LH secretion (15, 25, 42) or an antigonadotrophic effect (9, 31) acting over a central level (13) through sexual steroids (21, 28). Our data indicate that stimulatory effect of prolactin on LH secretion in EB females is by way of ovarian steroids, because after ovariectomy the positive correlation between LH and prolactin disappears; in other words, LH increases while prolactin decreases.

LHRH injection has no effect on prolactin secretion in control females while in EB females a decrease in plasma prolactin levels is provoked as found also in TP females (19). Possible interrelation between LH and prolactin is indicated by the fact that hyperprolactinemia is found after immunoneutralization of LHRH (12, 23) and the intraventricular injection of prolactin modifies LHRH producing neurons (25).

The increase of prolactin levels after saline in EB females could be due to ether and experimental manipulation stress (3). This effect contradicts results reported by other authors: there is no response to stress with previous high prolactin levels (30) or the response appears as a decrease in those levels (33).

In conclusion our data suggest that in ovariectomized EB females there is less hypophyseal sensitivity to LHRH measured by LH secretion. With regard to prolactin the paradoxical response to stress and the decrease of its levels after LHRH seem to indicate alteration in the mechanisms controlling this hormone secretion.

Resumen

Ratas hembras tratadas postnatalmente con benzoato de estradiol (BE) presentan, a los

80 dias, hiperprolactinemia y niveles elevados de LH respecto a las hembras controles. En el grupo de hembras BE existe una correlación positiva LH-prolactina, ausente en el grupo control. A los 14 días de la ovariectomía desaparece la correlación positiva y los niveles de prolactina siguen siendo superiores en el grupo BE en relación al grupo control, mientras que, por el contrario, la elevación de los niveles de LH es menor en la hembra BE. La administración de LHRH produce descenso de los niveles de prolactina en el grupo BE, no observándose ningún efecto en el grupo control. La elevación de los niveles de LH tras la administración del decapéptido está disminuida en el grupo BE.

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