Effects of sexual hormones on ovarian weight in prepuberal female rats

	Treatment day 20, 21, 22		Treatment day 28, 29, 30	
	Body weight (g)	Ovarian weight (mg ovary/100 g body wt.)	Body weight (g)	Ovarian weight (mg ovary/100 g body wt)
Control	40.72 ± 2.23 (11)	39.05 ± 1.72 (11)	63.15 ± 2.52 (14)	24.66 ± 1.05 (15)
Estradiol	42.39 ± 3.78 (10)	29.70 ± 0.71[a] (10)	64.36 ± 2.58 (13)	31.28 ± 1.70[a] (12)
Testosterone	42.53 ± 3.43 (8)	30.77 ± 1.45[b] (8)	62.55 ± 3.75 (9)	25.06 ± 0.71 (9)
Androgenized	46.48 ± 2.17 (6)	17.17 ± 1.42 (6)	67.79 ± 1.49 (13)	19.64 ± 0.96 (13)
Androgenized ± estradiol	47.56 ± 2.73 (5)	11.16 ± 1.59[c] (8)	63.24 ± 2.49 (17)	15.61 ± 1.19[d] (8)
Androgenized + testosterone	45.27 ± 2.40 (7)	10.93 ± 2.17[d] (7)	66.07 ± 2.02 (13)	11.08 ± 1.39[c] (13)

[a] $p < 0.005$ and [b] < 0.001 vs control. [c] $p < 0.01$ and [d] < 0.025 vs androgenized. In parenthesis number of determinations.

in Series I was performed in 28-day-old normal and androgenized rats.

Animals were sacrificed 24 h after the last injection. The ovaries removed and weighed on a precision balance. Results were expressed in mg ovary/100 g body wt. and compared statistically by means of Student's *t*-test.

Results. As can be seen in the Table, estradiol decreased the ovarian weight in 20-day-old normal rats and increased the weight of the gland when administered to 28-day-old rats. In androgenized prepuberal rats, the administration of estradiol resulted in a depression of ovarian weight at 20 and 28 days old. In normal prepuberal rats, testosterone produced an inhibition in the secretion of gonadotrophins at 20 days old, while it did not modify the ovarian weight in 28-day-old rats. On the other hand, the testicular hormone was able to decrease the weight of the ovaries in the androgenized groups at both ages.

Discussion. These results demonstrate that a similar dose of estradiol exerts a negative feed-back effect on gonadotrophin secretion in 20-day-old rats, and a positive action on the secretion of these pituitary hormones in normal 28-day-old rats. These facts indicate that, in the normal rat, the maturation of the different mechanisms implicated in the stimulatory action of estradiol on gonadotrophin secretion takes place between 20 and 28 days of life.

The administration of a single dose of testosterone to female rats soon after birth produces an anovulatory syndrome with persistent estrus, similar to that obtained by electrolytic lesion of the preoptic area of the anterior hypothalamus[3,4].

Much experimental evidence indicates that androgens exert their deleterious action at the level of the suprachiasmatic-preoptic area of the anterior hypothalamus[3-5]. The fact that estradiol decreases the ovarian weight in the androgenized group at 20 and 28 days old indicates that the ovarian hormone does not exert a positive feed-back action on gonadotrophin secretion in such groups, as in normal rats. On this basis it can be postulated that the anterior hypothalamic area is directly involved in the stimulatory effect of estradiol, since androgenized rats, in which such area of the hypothalamus has been altered, did not show the positive effect of the ovarian hormone on gonadotrophin secretion. The fact that neonatally androgenized animals have lost the ability to accumulate ^{3}H-estradiol in the anterior hypothalamus, further supports this hypothesis[6].

The administration of testosterone to 20-day-old normal prepuberal rats decreased the ovarian weight while no effect was observed in 28-day-old rats. On the other hand, the testicular hormone showed in the androgenized group a negative feed-back effect at both ages. These facts appear to indicate that the anterior hypothalamus is also implicated in the increase of threshold of gonadotrophin response to testosterone administration observed in the normal prepuberal rat between 20 and 28 days of life.

Resumen. Los estrógenos ejercen sobre la rata hembra un efecto de retroalimentación negativo sobre la secreción de gonadotrofinas a los 20 días de edad y un efecto positivo a los 28 días de edad. La testosterona inhibió la secreción de gonadotrofinas en ratas de 20 días, pero no tuvo efecto a los 28 días de edad. En los animales androgenizados al nacer ambas hormonas inhiben la secreción de gonadotrofinas a los 20 y a los 28 días de edad.

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Hypophysial Feed-Back in the Hypothalamic Regulation of Luteinizing Hormone Secretion

A previous paper[1] showed the presence of luteinizing hormone releasing factor (LHRF) in plasma from long-term hypophysectomized rats, and that this factor vanished after the median eminence destruction. Several papers demonstrated the same activity in blood of hypophysectomized rats and chickens[2-4]. The fact that hypophysectomy produces a detectable level of LHRF in peripheral blood, suggested that this kind of animal would be useful to clarify the feed-back mechanism in relation to the pituitary luteinizing hormone (LH). Preliminary experiments in 1964 and 1967 (unpublished data) suggested that the level of the circulating LHRF can be lowered by pretreatment of hypophysectomized rats with exogenous LH.

OAAD induced by plasma from hypophysectomized control rats and hypophysectomized rats treated with LH, estradiol and estradiol plus progesterone

Group	No. of experiments	OAAD (%)
Hypophysectomized control rats	6(33) [a]	14.0 ± 0.53 [b]
Hypophysectomized rats + LH standard 5 μg 3 times a day for 10 days	4(20)	2.8 ± 0.51
Hypophysectomized rats + estradiol 5 μg a day for 10 days	4(22)	13.8 ± 0.77
Hypophysectomized rats + 5 μg estradiol and 2 mg progesterone a day for 10 days	5(23)	14.2 ± 0.65
LH standard (0.2 μg/100 g body wt.)	3(18)	11.6 ± 0.23
LH standard (1 μg/100 g body wt.)	5(27)	17.0 ± 0.42
Saline solution (1 ml/100 g body wt.)	6(24)	2.2 ± 0.52

[a] No. of test rats [b] Significant difference: $P < 0.001$

In the present paper we studied the exogenous administration of LH and gonadal steroids to hypophysectomized rats, with the purpose of finding the feed-back site of these hormones in relation with the LH secretion.

Methods. Adult female rats weighing 200–250 g were hypophysectomized according to the technique described by SMITH[5,6] and maintained in group cages at a constant temperature. They were fed with standard rat pellets diet supplemented with raw meat and water-soluble terramicine for animal use, ad libitum.

Three to four months after hypophysectomy, the rats were ovariectomized and divided into 4 groups: 1. Hypophysectomized control group; 2. Hypophysectomized group injected s.c. with 5 μg of LH (ovine NIH-LH-S9), 3 times a day for 10 days; 3. Hypophysectomized rats injected s.c. with estradiol benzoate in oil at the doses of 5 μg a day for 10 days; 4. Hypophysectomized rats injected with 5 μg of estradiol plus 2 mg of progesterone a day for 10 days.

The day after the last injection (experimental day) and 2 h before bleeding, the animals of the second group were injected with 5 μg of LH standard in the jugular vein, and those of groups 3 and 4 were injected through the same way with 10 μg of estradiol and 10 μg of estradiol plus 2 mg of progesterone, respectively.

The animals were bled through the jugular vein. In all the rats serial sections of the base of the brain was made to look for pituitary remnants. The animals which showed pituitary remnants were discarded.

To assay the plasma LH-like activity the ovarian ascorbic acid depletion (OAAD) method of PARLOW[7,8] was used. In each experiment simultaneously 0.2 and/or 1 μg of LH standard per 100 g body wt., and saline solution 1 ml/100 g body wt. were tested. The percentage of OAAD was taken as an index of LH-like activity present in the plasma samples from hypophysectomized rats. The data were statistically analyzed by Student's test and a probability of $P < 0.001$ was accepted as statistically significant.

Results. The results are shown in the Table: a) Plasma LHRF in hypophysectomized rats: immature female rats pretreated with gonadotrophins showed a significant OAAD when injected with plasma from long-term hypophysectomized rats ($P < 0.001$). b) Effects of plasma from hypophysectomized rats treated with LH: it failed to produce OAAD. The effects were similar to those obtained with saline solution. c) Effects of plasma from hypophysectomized rats treated with estradiol or estradiolprogesterone: the administration of gonadal steroids did not inhibit the secretion of LHRF into the circulation of hypophysectomized rats. When plasma from these animals was used, it produced a high OAAD.

In 2 experiments plasma from hypophysectomized rats treated with 5 mg of progesterone in oil solution during 10 days were used. The high dose of progesterone failed to decrease the circulating LHRF.

Discussion. Our experiments clearly indicate that the site for the negative feed-back of gonadal steroids must be the pituitary gland, instead of the median eminence as has been claimed by several authors[9–11]; and this anatomical part of the hypothalamus would be the site for the LH feed-back system. The fact that total hypophysectomy is followed after several weeks by the presence of LHRF in the peripheral blood, supports the hypothesis that the removal of the pituitary gland would release the hypothalamus from its specific signals that are represented by LH for the LHRF. Without the pituitary LH inhibition, the hypothalamus would constantly synthetize and secrete LHRF that, having no target gland (hypophysis) to stimulate, will pass directly into the peripheral circulation where it can be assayed.

Most authors accept that the site for the negative feed-back of gonadal steroids is the median eminence; but our experiments injecting doses of steroids many times higher than the physiological ones, show that without the pituitary gland they have no direct action on the median eminence. On the other hand, the role of the LH in the feed-back mechanism, could be taken into account, according to our experimental design and results.

Resumen. LH exógeno disminuye LHRF circulante no así estradiol ni estradiol-progesterona, concluyéndose que: eminencia media regula LH siendo su nivel plasmático la señal feed-back.

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