Augmentation of Pituitary Responsiveness to LH-Releasing Hormone (LH-RH) by Estrogen[1] (35249)

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The concept that the secretory function of the pituitary gonadotrophs is regulated by the hypothalamic neurohumors has been firmly established. However, there has been no clear cut evidence that gonadotropin secretion is only modified by the perfusion rate of the releasing homones nor that the pituitary gland always responds to a given dose of the releasing hormones to the same extent. Several investigators suggested an interaction between the releasing hormones and gonadal steroids (1–3) at the pituitary level in regulating gonadotropin secretion. In our recent studies (4) with highly purified porcine LH-releasing hormone (LH-RH), it was found that in intact diestrous female rats, administration of LH-RH preparation raised serum LH levels significantly, but the magnitude of the maximum rise of the LH levels observed was considerably smaller than that observed at the time of the spontaneous preovulatory surge of LH secretion. In these rats a larger dose of LH-RH did not increase the serum LH to the level observed in the afternoon of proestrus. These findings have led us to assume that the pituitary responsiveness to LH-RH might be modified by some other factors such as gonadal steroids. It is well known that under certain conditions estrogen and progesterone facilitate ovulation (1, 3, 5, 6). Recently Miyake (7) reported that the secretion of estrogen increased rapidly before the preovulatory surge of LH in cycling rats. The present study was undertaken to investigate whether pretreatment of intact rats with estrogen could increase the pituitary responsiveness to exogenous LH-RH.

Materials and Methods. Adult female Sprague-Dawley rats (Cheek Jones, Houston, Texas), weighing about 200 g were used as experimental animals. They were kept at near-constant temperatures (78 ± 1°F) and fed a Purina Chow rat diet supplemented with fresh vegetables, and given water *ad libitum*. Artificial lighting was controlled to give 12 hr of light in each 24 hr (6:00 a.m. to 6:00 p.m.). The animals were kept under these conditions for 7 days after their arrival before examination of vaginal smears was started. Rats which showed regular 5-day cycles were used.

Two-tenth ml of sesame oil or 10 to 20 μg of estradiol benzoate (Progynon, Schering)/0.2 ml of oil was subcutaneously injected into the rats on the first day of diestrus (day 1) at 2:00 or 4:00 p.m. On the following morning or early afternoon, these animals were anesthetized with urethane (175 mg/100 g of body wt, ip). Bood was collected from the jugular vein and then 0.1 μg of a highly purified porcine LH-RH preparation/0.5 ml of saline, or saline alone, was injected into the carotid artery. Twenty min after the injection, the second blood sample was collected. The blood was kept at 4° overnight and the serum was separated by centrifugation.

The LH-RH (AVS 77-3 no. 320-339) was prepared from pig hypothalami as described previously (8). This LH-RH preparation raises serum LH levels in ovariectomized, estrogen–progesterone-pretreated rats when given iv in doses as small as 0.015 μg.

Serum LH was measured by double antibody radioimmunoassay (00-RAT-LH-RIA), as described by Niswender *et al.* (9). A purified ovine LH preparation was used for labelling with ^{125}I. NIH-LH-S-14 was used as the standard. The concentration of LH in rat sera

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TABLE I. Serum LH Levels Before and 20 min After Intracarotid Injection of 0.1 μg of LH-RH or Saline in Diestrous Rats Pretreated with Oil or Estrogen.[a]

	Serum LH level (ng/ml)[b]					
	Oil pretreated			Estrogen pretreated		
Material	Before	After	Δ	Before	After	Δ
				(10 μg of estradiol bz.)		
Saline	1.4 ± 0.89	2.4 ± 1.40	1.0 ± 0.57 (3)	0.3 ± 0.28	0.4 ± 0.29	0.1 ± 0.05 (4)
LH-RH	0.3 ± 0.30	2.5 ± 0.91	2.2 ± 0.70 (5)	0.5 ± 0.22	13.1 ± 2.88[d]	12.6 ± 2.76 (8)
				(20 μg of estradiol bz.)		
Saline	2.6 ± 0.52	2.7 ± 0.16	0.1 ± 0.43 (5)	2.7 ± 1.02	3.1 ± 0.40	0.5 ± 0.75 (4)
LH-RH	2.4 ± 0.32	5.5 ± 0.61[c]	3.1 ± 0.51 (9)	2.0 ± 0.09	10.5 ± 1.56[d]	8.6 ± 1.50 (7)

[a] Number of rats in each group is given in parentheses. 2 × 2 factorial analyses of 4 mean Δ values indicate a significant interaction in both experiments.

[b] Expressed in terms of NIH-LH-S 14.

[c] $p < 0.02$; [d] $p < 0.01$; significant levels in Student's t test as compared with preinjection level.

were read directly on a standard curve constructed with NIH-LH-S-14. Two or three different dilutions were made for each serum sample and the mean value of the LH concentration obtained from these diluted samples was taken as the final value for the concentration of LH.

The difference (Δ) between serum LH levels before and after injection of the samples was calculated for each animal, and the Δ values were subjected to 2 × 2 factorial analysis to determine the interaction between the effects of estrogen and responses to LH-RH (10). To compare the mean LH levels among individual groups, Duncan's new multiple range test (10) was utilized. To compare the mean LH levels before and after injection of LH-RH or saline in each group, the Student's t test was used.

Results. Pretreatment with estradiol benzoate did not change the serum LH levels in diestrous rats determined 18–24 hr later (Table I). Intracarotid injection of saline did not modify the serum LH levels as compared with the preinjection levels both in oil-pretreated and estrogen-pretreated rats. Injection of 0.1 μg of LH-RH resulted in a slight rise (insignificant, $0.05 < p < 0.1$, in one experiment; significant, $p < 0.02$, in another experiment) of serum LH in oil-pretreated animals as compared with the preinjection levels. On the other hand, the same dose of LH-RH induced a greater rise ($p < 0.01$) of serum LH in estrogen-pretreated rats. Factorial analysis of mean Δ values in four treatment groups shows a significant interaction between the effects of LH-RH injection and pretreatment with either 10 or 20 μg of estradiol benzoate. This indicates that estrogen pretreatment enhanced the pituitary response to LH-RH.

Discussion. The results show that in intact diestrous rats pretreated with 10 or 20 μg of estradiol benzoate in oil 18–24 hr previously, the injection of LH-RH induced a greater rise in serum LH than it did in oil-pretreated control animals. The porcine LH-RH preparation used in this experiment was a highly purified preparation which stimulated LH release from the rat pituitary *in vivo* as well as *in vitro* (4, 11). The data accumulated in our laboratories strongly indicates that this LH-RH preparation stimulates LH secretion by acting directly on the pituitary gland (4, 11). Estrogen pretreatment itself did not change the serum LH levels. Therefore, it is reasonable to consider that estrogen pretreatment increased pituitary responsiveness to LH-RH in diestrous rats, whose pituitaries may be somewhat refractory to LH-RH.

Although several investigators (11, 12) postulated that the pituitary responsiveness to LH-RH might change during the estrous

cycle, direct evidence for this hypothesis has been lacking. Antunes-Rodrigues *et al.* (13) investigated the pituitary responsiveness to partially purified sheep LH-RH by measuring plasma LH by bioassay, but found little evidence for such cyclic fluctuations in the pituitary response. Reinvestigation of this problem in rats, using the more sensitive radioimmunoassay, is now in progress in our laboratories. Recently we found in intact ewes that administration of LH-RH induced a greater rise in serum LH levels during an 8 ± 4-hr period on the day of estrus than at any other stage of the estrous cycle (14). On the other hand, a rapid increase of estrogen secretion before the preovulatory LH surge is reported in cycling rats (7). It may be reasonable to assume that increased pituitary responsiveness to LH-RH following increased estrogen secretion as well as increased release of endogenous LH-RH could contribute to the preovulatory surge of LH secretion in cycling rats.

Although estrogen pretreatment augmented the pituitary response to exogenous LH-RH, the magnitude of the rise in serum LH after injection of a relatively large dose of LH-RH (0.1 μg) was considerably smaller than that observed at the spontaneous preovulatory LH surge (15). Intravenous injection of 0.05 μg of this LH-RH preparation into ovariectomized, estrogen- and progesterone-pretreated rats usually increases serum LH levels by 30–90 ng/ml. The dose of estrogen may be too large so that it could also exert inhibition of LH release. The interval after injection of estradiol benzoate might be another factor determining the maximum sensitivity of the pituitary gland. Alternatively, administration of progesterone in combination with estrogen might be necessary. It is also possible that a prolonged infusion of LH-RH, in addition to the possible effects exerted by these steroids, could better mimic the rise of serum LH observed in the afternoon of proestrus in cycling rats. These possibilities, however, remain to be elucidated.

Summary. The effect of pretreatment with estrogen on the LH-RH-induced release of LH was investigated in diestrous rats. Ten or 20 μg of estradiol benzoate in oil were injected into 5-day cycling female rats on day 1 (diestrus). On day 2 (diestrus) 0.1 μg of highly purified porcine LH-releasing hormone (LH-RH) was injected into the carotid artery. Serum LH levels before and 20 min after the injection were determined by radioimmunoassay. In oil-pretreated rats, LH-RH injection induced only a slight rise of serum LH as compared to the preinjection levels. In the estrogen-pretreated rats, the same dose of LH-RH induced a much greater rise in serum LH levels. Estrogen by itself did not affect the level of serum LH. The results suggest that pretreatment with estrogen results in an increased responsiveness of the pituitary to LH-RH in diestrous rats.

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