# VARIATIONS IN LUTEINIZING HORMONE-RELEASING HORMONE RECEPTORS IN PITUITARY CELLS FROM IMMATURE AND MATURE CYCLING FEMALE RATS

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## 1. Introduction

The pituitary receptors for the hypothalamic decapeptide, luteinizing hormone-releasing hormone (LHRH) have been extensively studied and well characterized [1–4]. Using non-degradable analogs of LHRH [5], it was shown that the pituitary gland contains specific high affinity binding sites for the decapeptide.

Since LHRH exerts its biological function, gonadotropin secretion, via binding to the receptor, it is likely that alteration in the responsiveness of the pituitary to LHRH, may be mediated by changes in the number or in the affinity of the LHRH receptors. It is known that the sensitivity of the pituitary to LHRH stimulation varies with age [6] and sex [7] of the rat and also throughout the estrous cycle [8]. Sex steroids are known to modulate pituitary responsiveness to LHRH: estradiol can facilitate LHRH action in the female rat [9] and is responsible, in part, for the increased sensitivity to LHRH on the day of proestrus. In [10,11], an increase in number of LHRH receptors during proestrus has been reported in a pituitary plasma membrane preparation.

Here we have examined these possibilities by studying LHRH interaction with its receptor in dispersed pituitary cells obtained from immature or adult female rat during different stages of the estrous cycle or from male rats.

## 2. Materials and methods

Wistar-derived rats of the departmental colony were used. They were housed in air-conditioned quarters, illuminated between 05:00 and 19:00 h. Pelleted food (Ralston Purina Co.) and water were offered ad libitum. In experiments which involved the use of cycling rats, only females which exhibited at least 2 normal 4-day cycles, as determined by daily vaginal smears, were used. In all experiments, metestrus female rats were sacrificed at the same time as the experimental animals (proestrus and estrus rats; 12-day-old female rats and male rats) and served as controls. Binding capacity of pituitary cells derived from these experimental groups is expressed relatively to that of metestrus rats.

The method for cell dispersion of the pituitary gland was as in [2]. An LHRH antagonist: DpGlu$^1$, DPhe$^2$, DTrp$^{3,6}$-LHRH was kindly provided by Drs W. Vale and J. Rivier of the Salk Institute (La Jolla CA). The analog was iodinated by the lactoperoxidase method and the specific activity of the labeled peptide was ~1000 $\mu$Ci/$\mu$g. Immediately after dispersion, cells (1 × 10$^6$) were incubated for 90 min at 4°C with the $^{125}$I-antagonist (50–100 pM) and increasing concentration of unlabeled antagonist in 0.4 ml final vol. The reaction was terminated by filtration, under vacuum on Whatman GF/C filters, presoaked in 2% bovine serum albumin (BSA), and washed with 10 ml cold phosphate-buffered saline solution.

## 3. Results

### 3.1. *LH receptor concentration during the estrus cycle*

Pituitary cells derived from female rats were examined for their binding affinity and binding capacity at four stages of the estrus cycle: metestrus, proestrus noon (12:30–13:30 h), evening of proestrus (18:30 h) and estrus. Binding of the LHRH analog to pituitary cells was low (38.2 ± 2.7 fmol/1 × 10$^6$ cells) during

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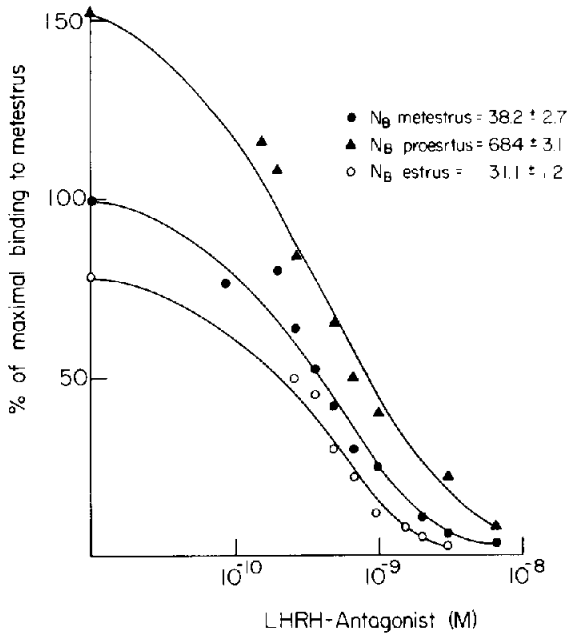


Fig.1. Displacement curves of $^{125}$I-LHRH antagonist by unlabeled antagonist. 1 × $10^6$ Cells derived from (●) metestrus (▲) proestrus and (○) estrus rats, were sacrificed between 12:30–13:30 h and were incubated as in section 2. The maximal binding to metestrus cells was determined as 100%. NB (number of binding sites, mean ± SE) were calculated from the corresponding Scatchard plots. Each point is the mean of duplicate samples. The experiment was repeated 3 times.

metestrus (fig.1) and was increased to 68.4 ± 3.1 fmol/1 × $10^6$ cells at noon of proestrus. A drop in the binding capacity was observed at 18:30 on the day of

Table 1
LHRH receptors concentration in dispersed pituitary cells from rats at different physiological state

| Rats | Number of binding sites[a] (fmol/1 × $10^6$ cells) | *N* |
|---|---|---|
| Metestrus females[b] | 37.1 ± 2.7 | 8 |
| Proestrus females (noon) | 68.4 ± 3.1 | 3 |
| Proestrus females (evening) | 50.4 ± 2.4 | 3 |
| Estrus females | 31.1 ± 1.2 | 3 |
| 12-day-old females | 88.2 ± 4.2 | 2 |
| 2-month-old males | 35.3 ± 2.1 | 2 |

[a] Number of binding sites (mean ± SE) were derived from Scatchard plots

[b] Metestrus females served as controls, and were sacrificed as in section 2

The rats were sacrificed at the same time and dispersed pituitary cells were obtained. Cells were incubated as in section 2

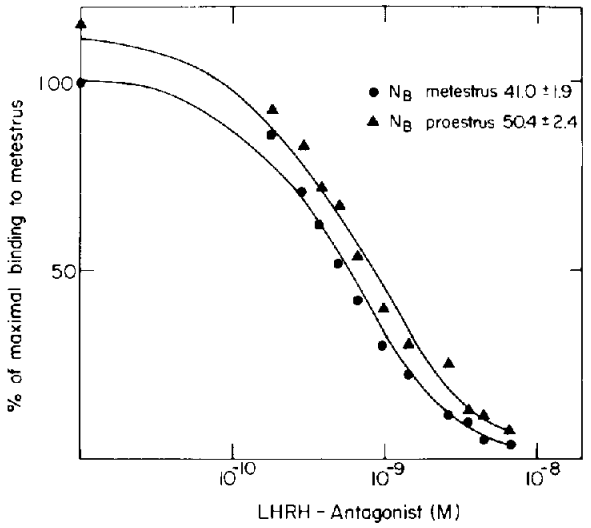


Fig.2. Displacement curves of $^{125}$I-LHRH antagonist by unlabeled antagonist. The cells were derived from (●) metestrus and (▲) procstrus rats, sacrificed at 18:30 h. For details see legend to fig.1.

proestrus (fig.2), after the LH surge which occurs in our colony between 17:00–18:00 h [12]. On the morning of estrus, the number of binding sites was further decreased to value somewhat lower than that of metestrus (31.1 ± 1.2 fmol/1 × $10^6$ cells). Scatchard plots derived from the data presented in fig.1 and 2 have indicated that the dissociation constant ($K_d$) of the LHRH analog did not vary throughout the estrus cycle and was 0.4–0.6 × $10^{-9}$ M.

### 3.2. *LHRH receptor concentration in male rats and 12-day-old female rats*

Binding capacity of the LHRH analog to dispersed pituitary cells derived from 2-month-old male rats was similar to that of metestrus female rats (table 1). A high concentration of LHRH binding sites was found in pituitary cells derived from immature female rats (table 1). This value (88.2 ± 4.2 fmol/1 × $10^6$ cells) exceeds even the relatively high concentration of LHRH receptors observed in pituitary cells of proestrus females.

## 4. Discussion

The pattern of LHRH receptor concentration in the pituitary cells is closely related to the responsiveness of the pituitary gland to LHRH stimulation

as examined in vivo [13] and in vitro [8]. The changes in the pituitary responsiveness to LHRH precede the change in receptor content. On the day of proestrus, doubling of LHRH binding capacity is observed at noon (table 1) and declines soon after the LH-surge, while maximal sensitivity of the pituitary to the neurohormone is delayed to the late afternoon of proestrus, at the time of the LH-surge, and several hours later is still high [13]. Similar results were reported [10,11] when binding of LHRH agonist to pituitary plasma membrane was measured. The several hours delay in the attainment of maximal sensitivity suggests that additional processes are necessary after the induction of LHRH receptors. Gonadal steroids and LHRH itself are probably involved in the regulation of LHRH binding sites. Abolishment of the LH surge on proestrus, by castrating female rats at metestrus [14] suggests a facilitatory effect of estrogen on the sensitivity of the pituitary to LHRH. The increase in plasma estradiol concentration from metestrus to proestrus [15] is well correlated with the increase in pituitary LHRH receptor content. Estradiol can exert its effect on LHRH receptors by a direct action at the level of the pituitary [16] or by altering LHRH release from the hypothalamus which in turn induces or decreases its own receptors. LHRH is known to sensitize the pituitary to its own action [17]. Part of the priming effect could be exerted via induction of LHRH binding sites in the pituitary, namely as an early event at the beginning of the LHRH surge which occurs at the early afternoon of proestrus [18]. However, at the termination of the LHRH surge on the evening of proestrus, a 36% decrease in the pituitary LHRH binding capacity was observed (table 1). This decrease was even more pronounced on the morning of estrus. The loss of LHRH receptors after the LH-surge is probably due to down regulation of the receptors which occurs after the massive increase of LHRH concentration in the pituitary stalk [12,18].

We have demonstrated that pituitaries of 12-day-old female rats are more responsive than those of mature rats to LHRH stimulation [19]. Several factors may determine the increased response of pituitaries derived from immature female rats: positive feedback effect of the elevated plasma estradiol levels, at that age [20], on the pituitary gland and the immaturity of the negative feedback of gonadal steroids on the central nervous system [21]. As suggested for the proestrus rats, it is likely that estrogen is also involved in the induction of LHRH receptors in the pituitary of the immature rats.

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