Pituitary Gonadotropin-Releasing Hormone Receptors During the Rat Estrous Cycle

Abstract. *The binding of [6-alanine]gonadotropin-releasing hormone to pituitary plasma membranes increased threefold between metestrus and early proestrus in female rats. Receptor numbers fell rapidly on the afternoon of proestrus coincident with the preovulatory gonadotropin surge. The numbers of receptors for gonadotropin-releasing hormone were positively correlated with concentrations of estradiol in serum; this pattern may be a necessary component of increased pituitary sensitivity to gonadotropin-releasing hormone observed during proestrus.*

Previous studies of binding of gonadotropin-releasing hormone (GnRH) to receptors in the pituitary have been hampered by the large amount of low-affinity binding observed when radioiodinated GnRH is used as ligand (*1*). We have recently shown that the use of nondegradable agonist GnRH analogs as ligand allows the measurement of high-affinity GnRH receptors on anterior pituitary plasma membranes (*2*). This method has allowed us to determine the pattern of pituitary GnRH receptors throughout the rat estrous cycle and to correlate receptor changes with the shifting hormonal milieu.

Between late estrus and the early afternoon of proestrus in the rat, circulating pituitary gonadotropin concentrations are low (*3, 4*). A marked increase in both luteinizing hormone (LH) and follicle-stimulating hormone (FSH), the proestrous gonadotropin surge, begins at around 1600 on the day of proestrus. Gonadotropin release in response to exogenous GnRH is increased during the cycle to a maximum during proestrus (*5*), a time when hypothalamic GnRH secretion is also increased (*6*). Thus, the preovulatory gonadotropin surge appears to result both from increased GnRH secretion and increased pituitary sensitivity to GnRH.

The mechanisms of altered pituitary sensitivity to GnRH are not fully understood, but could be related to fluctuations in number or affinity of GnRH receptors present on pituitary gonadotrophs. The present study was designed to measure GnRH binding to pituitary receptors during the rat estrous cycle and to integrate observed changes with current hypotheses of gonadotropin control.

Female Sprague-Dawley rats were housed under conditions previously described (*4*). Animals demonstrating regular 4-day estrous cycles were caged individually at least 12 hours prior to decapitation. Every 4 hours beginning at midnight, and also at 0600 and 1800, rats were decapitated by guillotine in an adjacent room. During dark periods, all decapitations were performed under red light. Within 2 minutes, trunk blood was collected, and the pituitary was removed. Anterior pituitaries were rinsed in sucrose (*7*), quick-frozen, and stored at −70°C until assay. Serum LH, FSH, estradiol, and progesterone concentrations were measured by radioimmunoassay (*8*).

Competition curves generated during initial studies indicated that the affinity of ^{125}I-labeled [D-Ala^6]des-Gly^{10}-GnRH ethylamide (D-Ala^6 analog) binding was unchanged on different days of the cycle either at 0600 (Fig. 1) or at 1800 (not shown). The association constant was between $4.0 \times 10^9 M^{-1}$ and $6.0 \times 10^9 M^{-1}$ at all times studied [Scatchard analysis (*9*)]. Since the receptor affinity remained constant, the number of binding sites could be conveniently estimated by saturation analysis, which was the method used for the rest of the studies (*10*).

Binding of D-Ala^6 analog was low (376 to 432 fmole per milligram of membrane protein) during the first 12 hours of metestrus, but progressively increased to 1070 ± 60 [mean ± standard error of the mean (S.E.)] fmole/mg by 0800 during diestrus (a threefold increase: Fig. 2). Binding capacity remained elevated through noon on the day of proestrus. Between 1200 and 1600, there was a rapid decrease from 856 ± 84 to 452 ± 17 fmole/mg. Subsequently, D-Ala^6 analog binding capacity increased briefly at midnight between proestrus and estrus, before gradually declining during estrus to values similar to those of early metestrus. The observed changes in GnRH receptor number correspond to previously described changes in gonadotroph sensitivity to GnRH (*5*) and to pituitary gonadotropin content (*11*).

The mechanisms involved in altering the number of GnRH receptors are unknown, but several possibilities can be considered. First, the increase in the

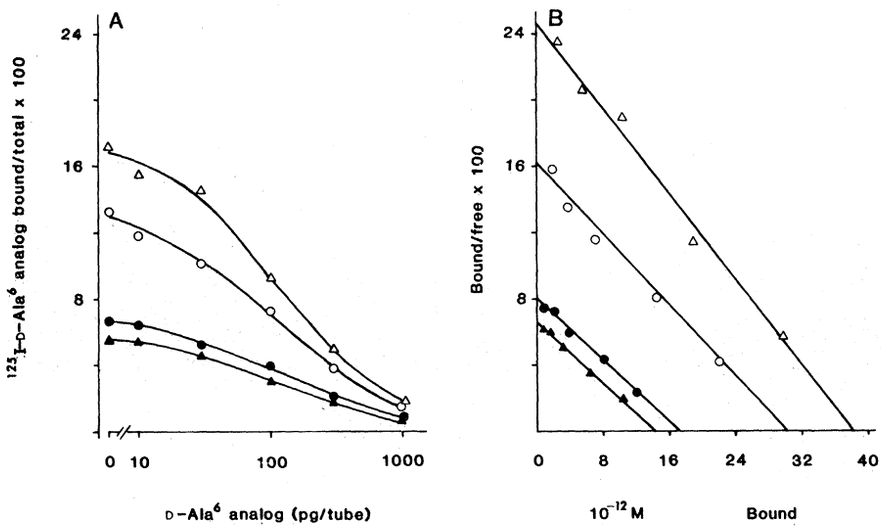

Fig. 1. Competition curves of (A) ^{125}I-labeled D-Ala^6 analog binding to rat pituitary membrane preparations by unlabeled D-Ala^6 analog and (B) Scatchard plots of the curves. After being thawed, two pituitary glands were hand-homogenized in 2 ml of sucrose (*7*). The homogenate was centrifuged at 300*g* for 5 minutes, and the supernatant was centrifuged at 10,800*g* for 20 minutes. The 10,800*g* pellet (crude membrane preparation) was washed three times in buffer (10 m*M* tris-HCl, *p*H 7.7) prior to use in the binding assay. Between 13 and 24 μg of membrane protein were incubated with 15 pg of ^{125}I-labeled D-Ala^6 analog (specific activity, 900 μCi/μg) with or without unlabeled D-Ala^6 analog. The reaction was carried out in a total volume of 500 μl (assay buffer 10 m*M* tris-HCl with 1 m*M* dithiothreitol, and 0.5 percent bovine serum albumin (BSA), *p*H 7.7) in polypropylene tubes (Falcon Plastics) for 60 minutes at 4°C. After centrifugation at 27,000*g* for 15 minutes, the pellet was washed and counted. The data shown are means of duplicate incubations of membranes prepared from rats decapitated at 0600; values have been corrected for protein content (each curve equivalent to 20 μg of membrane protein). Symbols indicate the day of the cycle: (▲) metestrus, (○) diestrus, (△) proestrus, and (●) estrus. Similar curves (data not shown) were obtained with membranes from rats decapitated at 1800 on the same days of the cycle. Previous studies have indicated that 60 percent of the ^{125}I-labeled D-Ala^6 analog is bound in the presence of excess receptor preparation. Therefore, 60 percent of the total counts added (the active fraction) was used to calculate the affinity constants and binding capacities.

number of GnRH receptors parallels the slowly increasing serum estradiol concentration from metestrus to diestrus (Fig. 2C). Serum estradiol concentration is positively correlated with GnRH binding capacity during this period, and indeed throughout the entire estrous cycle [$r = .5204$, $P < .001$, $N = 106$ (*12*)]. Increased concentrations of estradiol have a facilitatory effect on gonadotropin release in response to GnRH (*13*), an action that may be mediated by an increase in the number of GnRH receptors. Estradiol increases the number of thyrotropin-releasing hormone binding sites on pituitary membranes (*14*), and this suggests that the steroid may directly affect pituitary membrane receptors. However, from metestrus to diestrus, serum estradiol increased at a slower rate than GnRH binding capacity, an indication that other factors may be involved in determining the number of GnRH receptors. Serum progesterone, which is increased during metestrus, decreases during early diestrus as the corpora lutea regress. Progesterone may initiate the increase in GnRH receptors during diestrus, and receptor number may be maintained subsequently by the increasing estradiol concentrations secreted from developing ovarian follicles. If estradiol is important in increasing GnRH binding, then this effect clearly precedes the positive feedback of estradiol on LH release, which is exerted between 2300 on the day of diestrus and 0300 on the day of proestrus (*15*), and is important in triggering the preovulatory gonadotropin surge (*11*). Exogenous GnRH can evoke LH and FSH surges on the afternoon of diestrus (*5*), demonstrating that the GnRH receptors present at this time are functional. However, during a normal cycle, the positive feedback action of estradiol (*15*) and increased endogenous GnRH secretion (*6*) will not trigger a gonadotropin surge until the afternoon of proestrus.

Whether estradiol alone or the sequence of progesterone followed by estradiol is responsible for the GnRH receptor pattern from metestrus through noon on the day of proestrus, there is a marked disruption by 1600 of proestrus. Despite the presence of increased progesterone and estradiol, GnRH binding capacity is halved. The stimulatory effect of these steroids may be reasserted in the brief increase in receptor numbers observed late on the day of proestrus, but both receptors and steroid hormones have declined by 0400 on the day of estrus and remain low until the afternoon of metestrus. The overall patterns of steroid hormones and receptors suggest that either progesterone or estradiol, or both, exert a positive effect on GnRH binding capacity.

Alternatively, GnRH could be altering its own receptor. Injections of low doses of GnRH increase receptor number without changing receptor affinity in intact male rats (*16*). Thus, the increase in pituitary GnRH receptors through late metestrus and diestrus might be related to increasing endogenous GnRH secretion, an effect that could be augmented by the changing steroid milieu. Although endogenous GnRH secretion appears to be minimal and constant during that time (*6*), other membrane receptors increase when circulating concentrations of tropic hormones are low (*17*). Endogenous GnRH secretion may also be involved in the abrupt decrease in GnRH binding capacity during proestrus, as pituitary stalk blood levels increase five- to sixfold at the time of the gonadotropin surge (*6*). Thus, marked increase in GnRH secre-

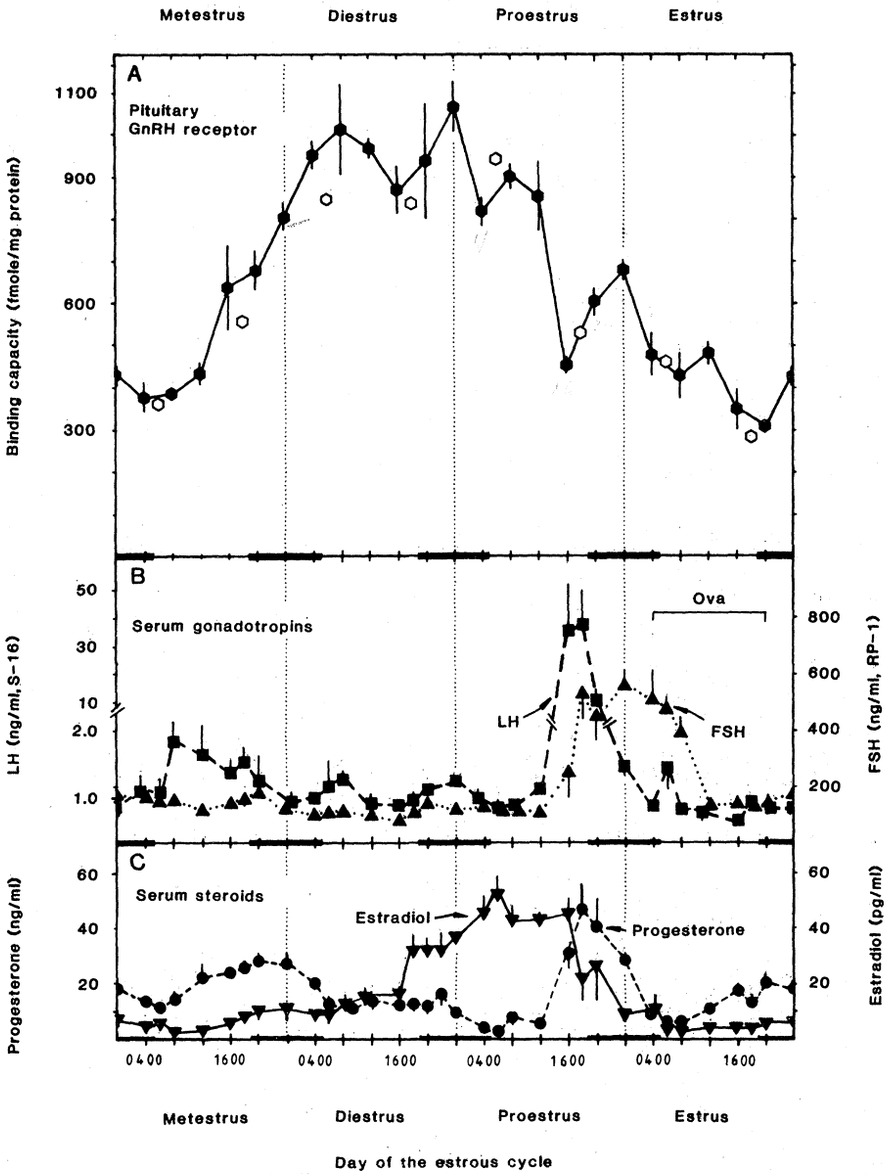

Fig. 2. (A) The binding capacity of D-Ala^6 analog to pituitary plasma membrane preparations from rats decapitated at 4-hour intervals during the estrous cycle. Membrane protein (15 to 20 μg) was incubated with a near-saturating dose (600 pg per tube) of D-Ala^6 analog (200 pg of ^{125}I-labeled D-Ala^6 analog plus 400 pg of D-Ala^6 analog per assay tube). Other incubation conditions are described in Fig. 1. Means ± S.E. for three to five triplicate determinations are shown at each time point. Open symbols indicate the binding capacity calculated by Scatchard analysis of competition curves using membranes from rats decapitated at 0600 and 1800. (B) Serum LH (■) measured in units of NIH LH S-16 and serum FSH (▲) measured in units of NIH FSH RP-1. Note the scale break and scale change for LH values. Means ± S.E. are plotted, with $N = 5$ at each time point. Limit of detectability was 0.4 ng/ml for the LH assay and 58.5 ng/ml for the FSH assay. Presence of ova in the oviducts is indicated on estrus. Black bars on the time scale indicate periods of darkness, and the light vertical dotted lines divide the days of the cycle. (C) Serum estradiol (▼) and progesterone (●) are represented; $N = 5$ for each time point.

tion could down-regulate the GnRH receptor, an effect that has been observed with large doses of other peptide hormones and their receptors (*17*). That low concentrations of GnRH may increase the number of GnRH receptors, whereas high concentrations may reduce receptor number, is not without precedent (*18*). Alternatively, the rapid decrease in observed receptor numbers during proestrus may be due to occupation of the receptors by endogenous GnRH. However this appears unlikely, as the washing procedures performed during membrane preparation take approximately 2 hours, well in excess of the time required for GnRH to dissociate from its receptor in vitro (*19*). Further, we have been unable to demonstrate decreased receptor binding capacity following exogenous GnRH administration (*20*). These results suggest that occupation of the receptor by endogenous GnRH cannot account for the observed reduction in the number of receptors on the afternoon of proestrus.

Another explanation for the abrupt decrease in the number of receptors during proestrus is that LH or FSH could reduce the number of available GnRH receptors. In general, the number of GnRH receptors appears to be inversely related to gonadotropin concentrations throughout the estrous cycle (Fig. 2). It has been shown that LH inhibits its own secretion (*21*), a short-loop feedback effect that could occur via a reduction in GnRH receptor. The striking divergence of FSH from LH that occurs late during proestrus corresponds to an increased number of GnRH receptors. This secondary FSH surge, previously observed in rats (*3, 4*) may be related to increased GnRH binding capacity, to increased GnRH secretion between midnight and 0200 on estrus (*6*), or to the postulated reduction of peptide hormone feedback from the ovary (*22*).

We have demonstrated that the pituitary GnRH receptor affinity is constant, even though the numbers of receptors fluctuate throughout the estrous cycle of the rat. The increase in GnRH binding during metestrus and diestrus appears to be related to increasing serum estradiol concentrations, though progesterone and endogenous GnRH may also be involved in initiating increased receptor numbers. Receptors for GnRH in the pituitary are maximal by 0400 on diestrus, yet increased hypothalamic secretion of GnRH will not occur until the afternoon of proestrus. The pituitary is clearly prepared for the proestrous GnRH surge at least 30 hours in advance. The positive feedback action of estradiol that stimulates the LH and FSH surges is not evident until 24 hours after GnRH binding is maximal. Separation in time of these two events suggests that the trigger mechanism for estradiol positive feedback must be exerted at higher centers (*23*). The mechanisms controlling the availability of GnRH receptors await further study. It remains to be demonstrated that exogenously administered progesterone, followed by estradiol to replicate endogenous steroid hormone profiles, can cause alterations in pituitary GnRH receptor binding corresponding to those observed during the estrous cycle.

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