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Gonadotropin-Releasing Hormone Receptor Binding and Pituitary Responsiveness in Estradiol-Primed Monkeys

Abstract. *Anterior pituitary tissue from ovariectomized cynomolgus macaques* (Macaca fascicularis) *contains a single class of high-affinity receptor for gonadotropin-releasing hormone (GnRH). Exogenous estradiol causes a twofold increase in the tissue concentration of GnRH receptor within 36 hours without affecting receptor affinity. Release of luteinizing hormone in response to exogenous GnRH is initially suppressed by estradiol, but pituitary responsiveness is restored within 36 hours of introduction of estradiol. The pituitary tissue concentration of GnRH receptor is positively correlated with estradiol-induced release of luteinizing hormone only during the phase of potentiated response, an indication that although the augmentation of responsiveness by estradiol may reflect an increased GnRH receptor concentration, the suppression of the luteinizing hormone response by estradiol probably reflects estradiol actions at loci other than the pituitary GnRH receptor.*

In primates, ovulation is the climax of the interplay between ovarian, neural, and adenohypophyseal factors (*1*). At midcycle the schema of functional changes in the hypothalamus and anterior pituitary leading to ovulation is keyed to increased secretion of estradiol (E_2) from the developing Graafian follicle (*1, 2*). Indeed, administration of E_2 to ovariectomized monkeys or to monkeys or women in the early follicular phase of the reproductive cycle induces changes in the hypothalamic-hypophyseal axis that are similar to those obligatory for ovulation in the normal cycle (*1, 3*). Elevation of serum E_2 to midcycle levels (300 to 600 pg/ml), either during the normal cycle or by the introduction of exogenous E_2, augments pituitary responsiveness and induces a marked discharge of gonadotropins (*1, 3, 4*).

The activity of the anterior pituitary gland is regulated by the hypothalamus. Therefore, the impact of E_2 on adenohypophyseal function may reflect E_2 action at either one or both of these loci. However, the recent work of Knobil and coworkers (*2*) suggests that E_2 inputs directed at anterior pituitary loci are sufficient to explain the controlling influence of E_2, provided exogenous gonadotropin-releasing hormone (GnRH) is supplied. Although the cellular and subcellular modifications induced by the estrogenic influence have not been precisely defined, Park *et al.* (*5*) reported that when mice were treated with E_2 in vivo, homogenates of their anterior pituitaries showed increased binding of GnRH. Furthermore, recent reports indicate that the GnRH receptor concentration reaches a maximum in the rodent pituitary during the early afternoon of proestrus (*6, 7*). Perhaps not coincidentally, pituitary responsiveness is also greatest at this time (*8*). In an effort to determine the subcellular changes that underlie E_2 modulation of pituitary responsiveness in primates, we have studied the binding characteristics of the GnRH receptor in anterior pituitary tissue of ovariectomized cynomolgus macaques (*Macaca fascicularis*) at various intervals after the introduction of E_2.

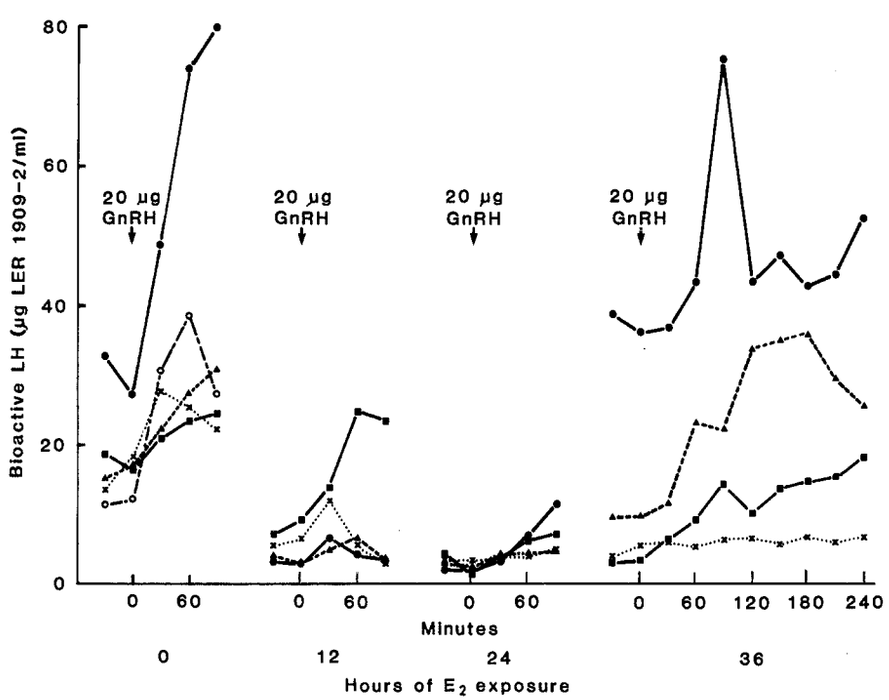

Fig. 1. The effect of hours of exposure to estradiol (E_2) on the magnitude of luteinizing hormone (*LH*) release in response to 20 μg of gonadotropin-releasing hormone (*GnRH*).

Twenty-five monkeys were used in this study, each ovariectomized at least 3 weeks prior to initiation of the experiments. In the first experiment the short-term effect of E_2 on pituitary responsiveness was determined. Serum concentrations of E_2 were increased to midcycle values by subcutaneous insertion of two 3-cm Silastic capsules containing crystalline E_2 (*9*). The concentration of E_2 in serum not subjected to chromatography was determined by radioimmunoassay (*10*). Preimplantation concentrations of serum E_2 (21 ± 8 pg/ml) were increased to 450 ± 30 pg/ml within 6 hours of implantation and were maintained above 400 pg/ml for the duration of E_2 treatment. Animals were challenged with GnRH (20 μg intravenously) at 0, 12, 24, or 36 hours after the insertion of E_2 capsules. Samples of blood were collected at 6-hour intervals until the GnRH challenge and at 30-minute intervals thereafter. The concentrations of luteinizing hormone (LH) in serum were quantified by bioassay with a mouse interstitial cell testosterone (MICT) system (*11*) and the units of LH were expressed in terms of a rhesus macaque standard (LER 1909–2) with a biopotency of 0.003 NIH-S1 units. The magnitude of bioactive LH released in response to challenge with 20 μg of GnRH varied as a function of the length of exposure to E_2 (Fig. 1). Pituitary responsiveness (defined experimentally as the magnitude

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of LH release in excess of basal secretion during the 90-minute postchallenge period) was high prior to introduction of E_2 (T_0 = 49.7 ± 15.5 μg of LH). The anterior pituitary became relatively refractory to GnRH 12 hours (12.6 ± 7.4 μg of LH) and 24 hours (8.0 ± 2.3 μg of LH) after the initiation of E_2 treatment. The pituitary responsiveness after 36 hours (24.6 ± 6.6 μg of LH) was significantly ($P < .05$) greater than that after 24 hours. At hour 36, that is, on the ascending shoulder of the E_2-induced LH surge, the pituitary responsiveness in individual animals was positively correlated (r = .8740) with the basal (T_0) LH concentration.

The effect of exogenous E_2 on GnRH receptor binding characteristics was evaluated in a second experiment. Estradiol was administered to ovariectomized monkeys as described above. Serum concentration of E_2 increased within 6 hours of implantation (462 ± 33 pg/ml) and remained at this level for the remainder of the experiment. In four control monkeys receiving blank capsules, the E_2 level was 23 ± 8 pg/ml. The animals were anesthetized with ketamine and killed at 12, 24, 36, 48, or 72 hours after the introduction of E_2. At each autopsy the calvarium was quickly retraced and the brain and pituitary were excised and placed on ice. Anterior pituitary tissue from each animal was homogenized and prepared for determination of the affinity and amount of GnRH receptor binding (*7*).

The short-term effects of exogenous E_2 on the temporal patterns of serum and tissue concentrations of bioactive LH in ovariectomized monkeys are shown in Fig. 2B. Bioactive LH in serum was depressed within 6 hours of E_2 introduction (T_0 = 43.4 ± 4.9 μg/ml; T_6 = 12.4 ± 3.0 μg/ml) and reached a nadir at hour 24 (T_{24} = 4.3 ± 1.3 μg/ml). The positive feedback effect of E_2 was first expressed at hour 36 (T_{36} = 18.1 ± 5.5 μg/ml), and bioactive LH was maximum 48 to 60 hours after the initiation of E_2 treatment.

Changes in the concentration of bioactive LH in anterior pituitary tissue paralleled the serum LH pattern. Within 12 hours of E_2 introduction, pituitary LH was significantly ($P < .05$) depressed (T_0 = 2.9 ± 0.2 mg per milligram of fresh tissue; T_{12} = 1.9 ± 0.2 mg/mg). The LH concentration increased significantly ($P < .05$) from hour 12 to hour 36 (T_{36} = 4.6 ± 0.5 mg/mg) and declined thereafter, as the E_2-induced surge of serum LH occurred.

Figure 2A shows that although GnRH receptor affinity was stable (affinity constant 7.0 ± 0.5 × $10^8 M^{-1}$, N = 21), the tissue concentration of GnRH receptor [R] varied as a function of length of exposure to E_2. The number of receptors in anterior pituitary tissue (Fig. 2B) increased progressively to a maximum at hour 36 ($[R]_0$ = 4.4 ± 0.6 fmole/mg; $[R]_{36}$ = 9.2 ± 1.9 fmole/mg) and declined thereafter ($[R]_{72}$ = 3.2 ± 0.5 fmole/mg).

The biphasic effect of exogenous E_2 on the secretion of LH in ovariectomized monkeys has been noted repeatedly (*1*). Although the regulatory sites that are sensitive to estrogenic influence have not been precisely defined, present evidence (*2*) indicates that direct action of E_2 at pituitary loci is sufficient to account for the positive feedback effects of E_2.

The initial event in the schema thought to depict activation of LH secretion is the interaction of GnRH with its receptor (*12*). It has been postulated that modulation of LH secretion by E_2 represents direct effects of E_2 on GnRH receptor binding (*13*), although, prior to our study, the short-term effect of E_2 on GnRH receptors had not been rigorously examined (*5, 14*). As reported here, elevation of serum E_2 to midcycle levels has no effect on GnRH receptor binding affinity but does induce a two- to threefold increase in receptor concentration. These data suggest that part of the positive feedback effect of E_2 on LH release in ovariectomized monkeys is mediated subcellularly by increased tissue concentrations of GnRH receptor. Whether the augmented responsiveness of gonadotrophs in an intact primate at midcycle (*4*) is mediated by the same E_2-controlled mechanism will require measurement of GnRH receptor changes during the periovulatory stage of the cycle.

Pituitary responsiveness and the tissue concentration of GnRH receptor were inversely related 12 and 24 hours after

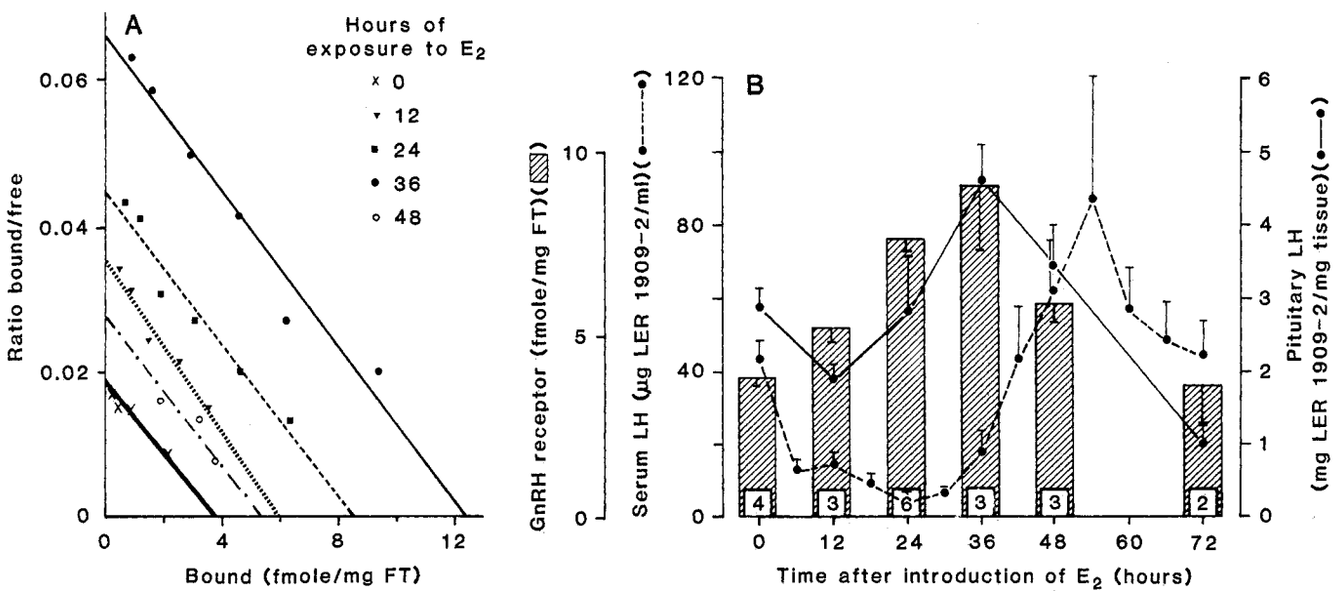

Fig. 2. Effects of hours of exposure to E_2 on four variables in ovariectomized cynomolgus macaques. (A) Binding characteristics of GnRH receptors in anterior pituitary tissue (determined by Scatchard analysis). Animals were killed 12, 24, 36, or 48 hours after insertion of two 3-cm Silastic capsules containing crystalline E_2. Control animals received blank capsules. Abbreviation: *FT*, fresh tissue. (B) Concentration (mean ± standard error) of GnRH receptors after 0 to 72 hours of exposure to E_2. The numbers of animals per group are shown at the base of each histogram bar in (B). The concentrations of bioactive LH in serum (-----) and pituitary tissue (——) after 0 to 72 hours of exposure to E_2 are superimposed on the histogram. The standard errors of the means are plotted below or above the points.

the introduction of E_2. These findings imply that the negative feedback component of the estrogenic influence is mediated at some regulatory loci other than the hormone-receptor complex.

The temporal pattern of responsiveness closely follows a change in the pituitary stores of LH. At any given time the content of LH in gonadotrophs is a function of the relative rates of synthesis, storage, and secretion (*15*). Exogenous E_2 may regulate the rate of one or more of these processes so as to make gonadotrophs refractory or hyperresponsive (*16*).

These findings (*17*) are consistent with the hypothesis that a portion of the estrogenic augmentation of pituitary responsiveness is mediated by an increase in the number of GnRH receptors on the surface of gonadotrophs. However, the negative feedback component of the E_2 effect is not a direct consequence of a corresponding change in GnRH receptor numbers.

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Spontaneous Diabetes in Rats: Destruction of Islets Is Prevented by Immunological Tolerance

Abstract. *Spontaneous diabetes occurring in "BB" rats (derived from a colony of outbred Wistar rats) is the result of destruction of pancreatic islets by infiltrating mononuclear cells (insulitis) and may be a disease very similar to human juvenile onset diabetes. Both diseases probably have an autoimmune etiology. Evidence is presented that islets transplanted to diabetic BB rats are destroyed by the original disease process. Inoculation of bone marrow from normal (nondiabetes-susceptible) rat donors into neonatal BB recipients usually prevents the development of hyperglycemia.*

The spontaneous development of diabetes mellitus was observed in 1977 in several members of a colony of outbred Wistar rats (*1*). Selective breeding of these diabetic "BB" rats in our colony has resulted in an increase in incidence of the disease to about 30 percent. The onset of severe hyperglycemia (3 to 7 mg/ml) in affected animals is sudden and usually occurs between 60 and 180 days of age in previously normal animals and with equal frequency in both sexes. Nonaffected animals remain permanently normoglycemic. Pathophysiologic characteristics of the syndrome include hypoinsulinemia, hyperglucagonemia, and, if insulin therapy is not provided, ketoacidosis and death. Morphologically the pancreas from newly diabetic rats shows a striking mononuclear infiltration (insulitis) with selective β-cell destruction. Both the metabolic and histologic abnormalities closely resemble those of insulin-dependent juvenile onset diabetes of humans. As in the case of the human disease, the etiology is unknown. However, in both BB rats and human diabetics a cell-mediated, organ-specific autoimmune pathogenesis seems possible, based on the characteristic lymphocytic infiltration of the islets. In BB rats an autoimmune mechanism is also implicated by the finding that reversal of the insulitis and return of normoglycemia occurs in 36 to 60 percent of acutely diabetic rats treated with rabbit anti-rat lymphocyte serum (ALS) (*2*).

Since the BB rat may provide an animal model of human insulin-dependent diabetes, it was important to determine whether the pathogenetic process responsible for the disease was also capable of destroying the islets of a transplanted pancreas, an issue of utmost importance in the outcome of this therapy in humans. We previously reported that transplantation of allogeneic islets from Wistar Furth (WF) donors results in long-term correction of the hyperglycemia of BB recipients treated with ALS (*3*). However, when immunosuppression was stopped, hyperglycemia returned within a few days and transplanted islets were noted to be infiltrated by lymphocytes, a histological finding that can be indicative of either rejection or recurrence of the original disease. We have now conducted islet transplantation experiments in diabetic BB rats using procedures designed to exclude the possibly confusing influence of rejection or immunosuppression.

Although BB rats are not genetically uniform, serological lymphocyte typing, mixed lymphocyte culture reactions, and skin graft assays all indicate that inbreeding has significantly minimized the histoincompatibilities in our colony. Thus, not only do all these rats appear to have the same major histocompatibility

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