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MODULATION OF PITUITARY RESPONSE TO HYPOTHALAMIC RELEASING FACTORS

ROBERT B. JAFFE and WILLIAM R. KEYE, JR.

Reproductive Endocrinology Center, Department of Obstetrics and Gynecology, University of California, San Francisco, California, U.S.A.

Sex steroids play a major role in the regulation of pituitary gonadotropin secretion. Two physiologic events during which the effects of changing concentrations of sex steroids on gonadotropin secretion can be determined are (1) the periovulatory phase of the menstrual cycle and (2) the puerperium. This report will present data indicating, first, that estradiol may either inhibit or augment pituitary responsiveness to gonadotropin releasing hormone (GnRH or LRF) in menstruating women. The time course of this phenomenon will be defined. Secondly, the profiles of gonadotropin responses to GnRH in the puerperium will be described.

I. *Studies in the menstrual cycle*

Previous investigations [1, 2] have demonstrated increased pituitary responsiveness to synthetic GnRH late in the follicular phase of the cycle. It has been suggested that this increased responsiveness plays a role in the surge of gonadotropins at midcycle, and may be the result of a postive feedback effect of the increasing concentrations of estradiol-17β which occur in the late follicular phase.

Initially we tested this hypothesis in the following manner [3] (Figs. 1 and 2). Fifteen women, each studied on the second day of their menstrual cycle, were divided into three equal groups. The second day of the menstrual cycle was chosen as endogenous concentrations of estradiol are low at this time. One group of subjects, the control group, did not receive estrogen. The other two received intravenous infusions of estradiol which were designed to achieve circulating concentrations equal to those at midcycle (225 pg/ml) or levels comparable to those in the late follicular phase (150 pg/ml). After a twelve hour infusion with saline alone or saline plus estradiol, each subject received 50 μg GnRH as a rapid intravenous infusion at time zero. As seen in Fig. 1, LH rose to approximately 40 in the control group, 17 in the group with late follicular levels of estradiol, and failed to rise in the group with midcycle levels. The mean maximal increase from baseline values, for individuals in the control group, was significantly greater than either of the two estradiol-treated groups. As seen in Fig. 2, a similar pattern of FSH response was seen in these studies. Again, the average maximal increase from baseline for individuals in the control group was significantly greater than that of either of the two study groups. These data suggested that the increased responsiveness of the pituitary to GnRH at midcyle is not accounted for solely by an increased concentration of estradiol. In addition, the data strongly

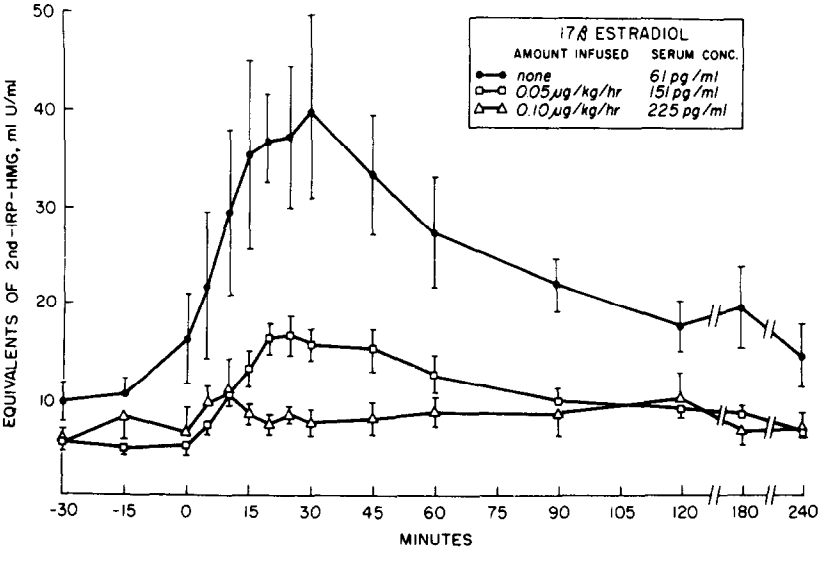

Fig. 1. Serum concentrations of LH (mean ± S.E.) in response to 50 μg GnRH at t = 0 for three groups of women (●——●), (□——□), (△—△—△), that received 17β estradiol in amounts shown in inset. (From Keye W. R. Jr. and Jaffe R. B.: *J. clin. Endocr. Metab.* **38** (1974) 805.

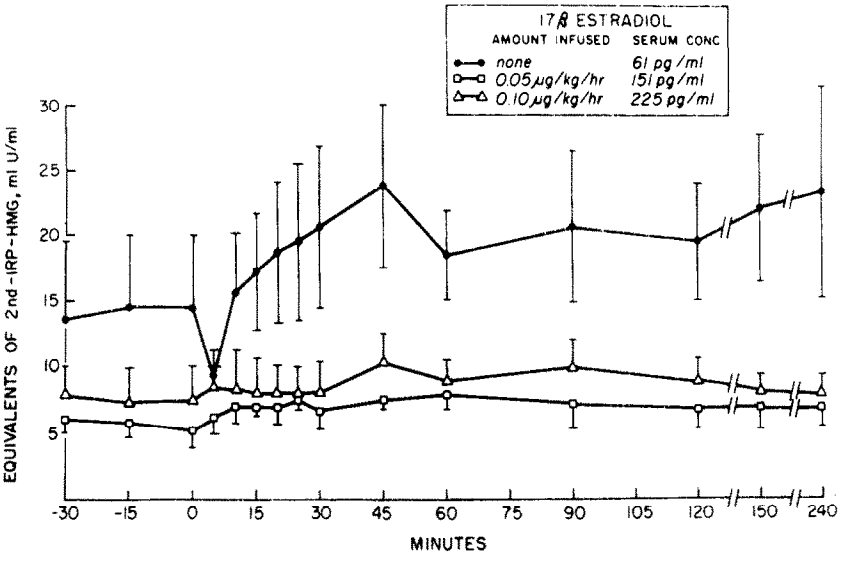

Fig. 2. Serum concentrations of FSH (mean ± S.E.) in response to 50 μg GnRH at t = 0 for three groups of women (●——●), (□——□), III (△—△—△), that received 17β estradiol in amounts shown in inset (From Keye W. R. Jr. and Jaffe R. B.: *J. clin. Endocr. Metab.* **38** (1974) 805.

suggest that one site of action of estradiol is the pituitary, and that under certain conditions this influence is inhibitory.

Nevertheless, we suggested that estradiol may be capable of increasing pituitary responsiveness to GnRH, and that this augmentative effect may be dependent upon the *length* of exposure of the hypothalamic-pituitary system to appropriate amounts of estradiol. Therefore, we designed studies to investigate the effect of the *duration* of estradiol administration upon pituitary response to GnRH in women [4]. If augmentation could be demonstrated with amounts of estradiol which approximate its secretory rate in the late follicular phase, this would suggest that 17β estradiol plays a role in the initiation of the surge of gonadotropins at midcycle by increasing pituitary responsiveness to hypothalamic gonadotropin releasing hormone.

To investigate this possibility, a more protracted regimen of estradiol administration was employed (Fig. 3). Seven women received 2·5 μg/kg estradiol benzoate intramuscularly every 12 h on days 1–6 of the menstrual cycle to approximate the secretory rate of estradiol by the ovary during the late follicular phase. In this study, 20 women, with regular menstrual cycles were divided into 2 groups. One group of subjects, the control group, did not receive estradiol. The treated group received estradiol benzoate on days 1–6 of the menstrual cycle. Subjects in the estradiol-treated group received GnRH 12 h after the last injection of estradiol, on day 7. The mean peak response of 200 in the group treated for 6 days was

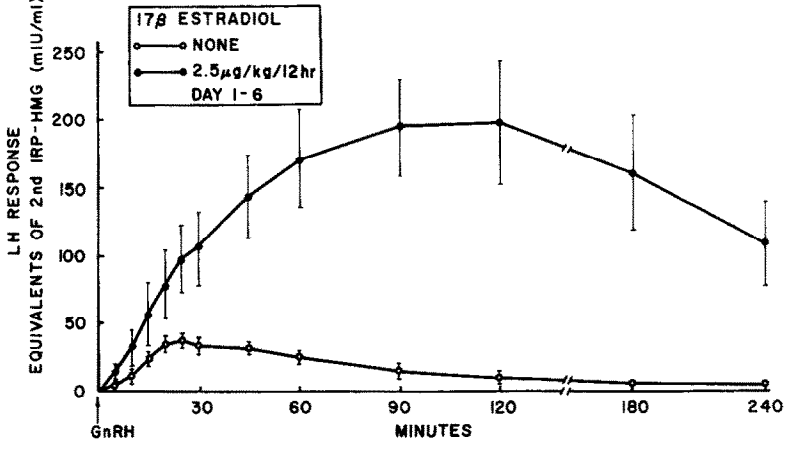

Fig. 3. Net increase of serum LH (mean ± S.E.) in response to 100 μg GnRH at t = 0 in women. GnRH was administered on day 7 (●——●) of the menstrual cycle following treatment with 5 μg/kg/day estradiol benzoate on days 1–6. Data from a control group of women (○——○) that received GnRH on one occasion during the first week of the menstrual cycle, but did not receive exogenous estradiol, also are shown. (From Jaffe R. B. and Keye W. R. Jr.: *J. clin. Endocr. Metab.* **39** (1974) 850.

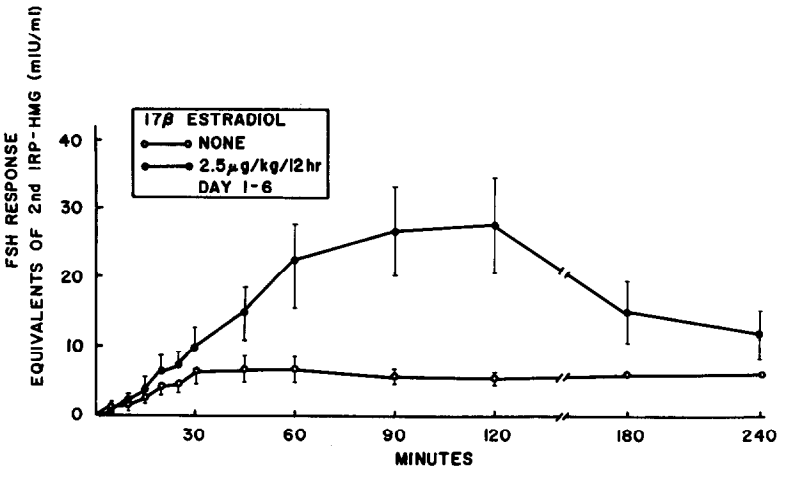

Fig. 4. Net increase of serum FSH (mean ± S.E.) in response to 100 μg GnRH at t = 0 in women. GnRH was administered on day 7 (●——●) of the menstrual cycle following treatment with 5 μg/kg/day estradiol benzoate on days 1–6. Data from a control group of women (○——○) that received GnRH on one occasion during the first week of the menstrual cycle, but did not receive exogenous estradiol, also are shown. (From Jaffe R. B. and Keye W. R. Jr.: *J. clin. Endocr. Metab.* **39** (1974) 850.

significantly greater than that of 37 milli-international units per ml (mIU/ml) in the control, or non-estradiol treated, group. In addition, the peak LH response in the treated group was markedly delayed, occurring at 120 min as compared to 25 min in the control group. Further, the $t_{\frac{1}{2}}$ of LH following the peak response was slower in the estradiol-treated than the control group. The augmentation of FSH responses by estradiol is shown in Fig. 4. The mean maximal response of 35 in the treated group was significantly greater than that in the control group. Similar to LH responses, the peak responses of FSH were delayed, occurring at 120 min or later in the estradiol-treated group in contrast to 45 min in the control group. Thus, it appears that estradiol, acting directly upon the pituitary, or perhaps indirectly by way of an effect upon endogenous GnRH, sensitizes the gland to exogenous GnRH. As a result of this increased sensitivity the gonadotropin response is augmented and prolonged.

One might argue that the increased response to GnRH is simply a consequence of increased stores of LH resulting from an inhibition of LH release during the administration of estradiol. However, LH concentrations did not decrease during or following this dose of estradiol.

While FSH concentrations were not significantly altered during estradiol administration, the mean baseline FSH concentrations on day 7 of 6·0 ± 0·9 was significantly less than that of the control group of 11·9 ± 1·2.

Alternatively, it might be suggested that the augmented response to GnRH is dependent upon a surge of gonadotropins induced by the positive feedback effect of estradiol. That this is not the case is suggested by the data presented in Fig. 5. No surge of

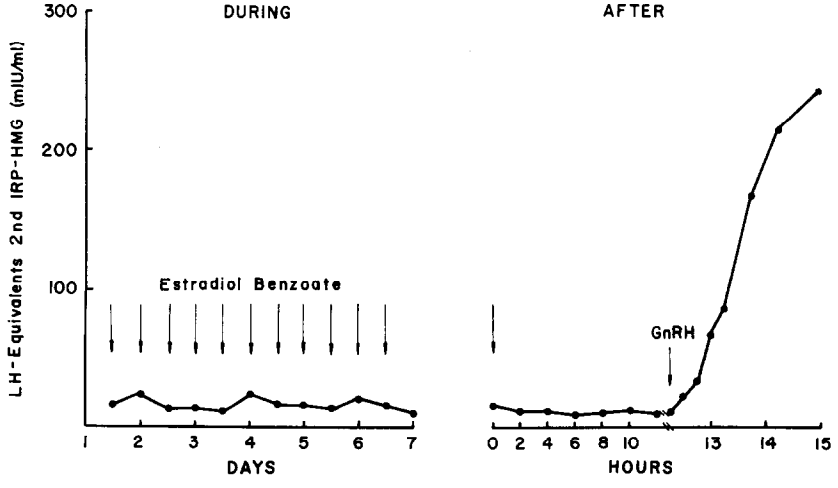

Fig. 5. LH concentrations during (left) and following (right) administration of 5 μg/kg/day estradiol benzoate to a woman on days 1–6 of the menstrual cycle.

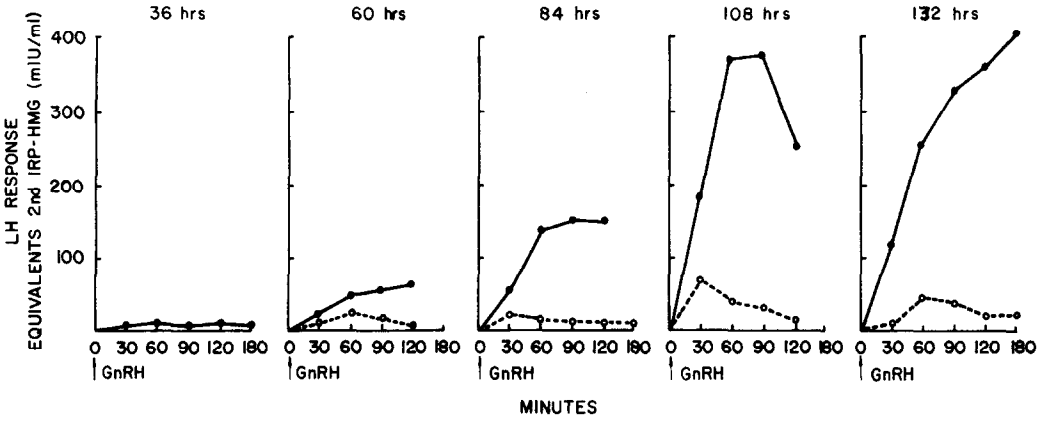

Fig. 6. LH responses to 100 μg GnRH following estradiol benzoate, 2·5 μg/kg/12 h for a total of 3, 5, 7, 9 or 11 injections. GnRH was administered at 36, 60, 84, 108 or 132 h after the first estradiol injection, and 12 h after the last injection. Dashed lines indicate LH data from the antecedent, control, cycles, during which no exogenous estradiol was administered.

LH was observed in sera obtained every 12 h during the administration of estradiol. As shown on the right-hand side of this Figure, no surge was present during the interval between the last estradiol injection and the administration of GnRH, when LH concentrations were determined from sera collected every 2 h.

These studies suggest that the increased response to GnRH is due to an increased sensitivity of the pituitary, and that this effect is not dependent upon the suppression of spontaneous release of LH or upon a surge of LH induced by the estradiol.

Thus, we have demonstrated that the administration of 17β estradiol to women in the early follicular phase of the menstrual cycle can either increase or decrease the sensitivity of the pituitary to GnRH. These observations suggest that the nature of the modulation of gonadotropin responses to GnRH by estradiol is dependent upon the length of exposure of the hypothalamic-pituitary system to estradiol as well as upon the elevated concentrations of this steroid.

The next study was designed to define more precisely the specific relationship between the duration of elevated estradiol concentrations and the alteration in pituitary response to GnRH[5]. (Fig. 6). Five women with regular menstrual cycles were studied. The studies were performed during two consecutive menstrual cycles. The dashed lines illustrate LH data from the first, or control, cycles, and the solid bars indicate LH responses during the second cycle month when subjects received estradiol for varying lengths of time. During the first month, each subject received 100 μg of GnRH i.v. on days 3, 4, 5, 6 or 7 of the menstrual cycle. Each subject was studied again during the first week of the next menstrual cycle. At 8:00 p.m. on the first day of the cycle, each subject received an intra-muscular injection of estradiol benzoate in sesame oil, five μg/kg. Every twelve hours thereafter, subjects received additional injections of estradiol benzoate, 2·5 μg/kg., for a total of 3, 5, 7, 9 or 11 injections. As illustrated, 100 μg GnRH was administered at 36, 60, 84, 108 or 132 h after the first estradiol injection, and 12 h after the last injection. No augmentation of the LH response to GnRH was observed until the estradiol had been given for 3 days prior to the GnRH administration. With increasing duration of exposure to estradiol, there was further

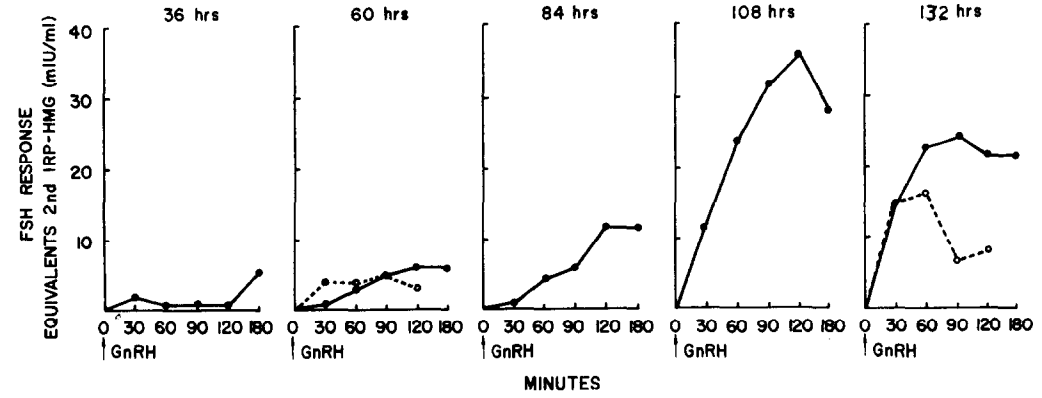

Fig. 7. FSH responses to 100 μg GnRH following estradiol benzoate, 2·5 μg/kg/12 h for a total of 3, 5, 7, 9 or 11 injections. GnRH was administered at 36, 60, 84, 108 or 132 h after the first estradiol injection, and 12 h after the last injection. Dashed lines indicate FSH data from the antecedent, control, cycles, during which no exogenous estradiol was administered.

augmentation of the LH response, reaching a peak between 4 and 5 days.

As shown in Fig. 7, a similar series of responses of FSH was observed. Again, maximal augmentation was seen after 4 to 5 days of estradiol administration. Thus, the duration, as well as concentration, of estradiol is critical in defining the nature of the augmentative effect of estradiol upon pituitary responsiveness to GnRH.

II. *Studies in the puerperium*

The puerperium is a physiologic event during which the influence of a changing hormonal milieu upon the pituitary can be determined. In this study [6], the gonadotropin responses to GnRH were determined in women at weekly intervals begining on the second postpartum day. None of the women were breast feeding, and none had received medication to suppress lactation.

The LH responses are shown in Fig. 8. Each bar represents the maximal increase in LH following the rapid intravenous administration of 100 μg GnRH. These responses are compared with a mean-maximal response in a group of women given GnRH during the first week of the menstrual cycle. This time in the menstrual cycle was chosen for comparison as there was no evidence of ovulation in the postpartum subjects as determined by either basal body temperature or the occurrence of menstruation. Due to the presence of high levels of HCG in sera obtained during the first week following delivery, LH responses were not determined. However, the LH responses during the second, third, fourth and fifth weeks are less than those in the early follicular phase. Following this period of relative unresponsiveness to GnRH, exaggerated responses were seen in all three subjects during the second postpartum month.

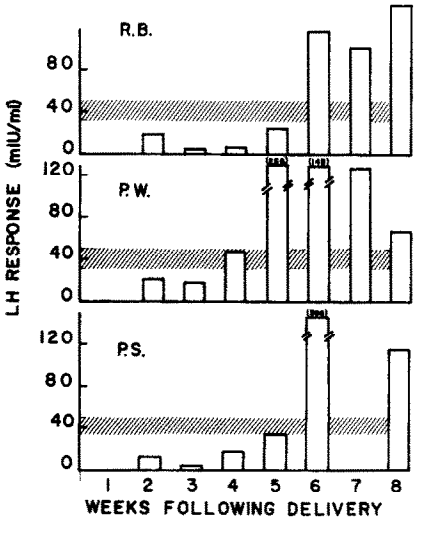

Fig. 8. Serum LH concentrations in response to the weekly administration of 100 μg GnRH during the puerperium. Each vertical bar represents the maximal LH response (maximal LH concentration minus mean baseline concentration). The shaded area is the mean (±S.E.) LH response in the early follicular phase.

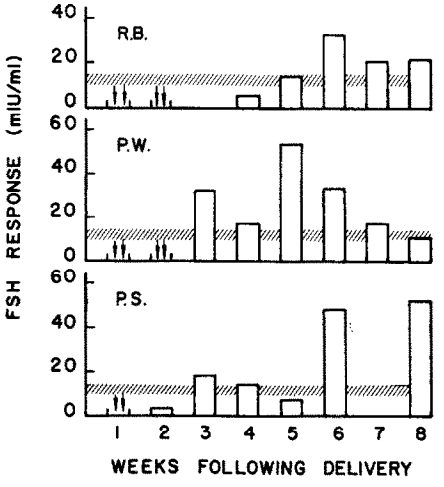

Fig. 9. Serum FSH concentrations in response to the weekly administration of 100 μg GnRH during the puerperium. Each vertical bar represents the maximal FSH response (maximal FSH concentration minus mean baseline concentration). The shaded area is the mean (±S.E.) FSH response in the early follicular phase.

A similar pattern of FSH response to GnRH is shown in Fig. 9. During the first 2–4 weeks following delivery, responses were absent or markedly less than those in the follicular phase. However, there was a shorter period of unresponsiveness of FSH than LH. Responses were equal to those in the follicular phase in two of the three subjects by the third week. Exaggerated FSH responses occurred in all 3 subjects in the last four weeks of study.

These studies demonstrate that the pituitary is relatively unresponsive to GnRH in the first two to four weeks following delivery. In addition, the maximal gonadotropin responses during this second month postpartum were as great as 9 times those of the early follicular phase of the menstrual cycle.

In summary, we have described both the inhibitory and augmentative effects of 17β estradiol on the pituitary response to GnRH in women. These findings suggest that exposure of the hypothalamic-pituitary system to increased concentrations of estradiol for an appropriate length of time in the late follicular phase of the menstrual cycle may play a role in the initiation of the surge of gonadotropins at midcycle by sensitizing the pituitary to GnRH.

In addition, we have described the profile of gonadotropin responses to GnRH following delivery. These findings suggest that anovulation during the first month postpartum is, at least in part, the result of pituitary unresponsiveness to GnRH. However, anovulation which persists beyond the first month is not the result of pituitary unresponsiveness, but may involve hypothalamic and/or ovarian factors.

SUMMARY

These studies were designed to assess the modulating effects of gonadal steroids during the menstrual cycle and puerperium. During the menstrual cycle, women

were treated during the follicular phase with estradiol administered either as infusions for 12 h or intramuscular injections for 6 days. Quantities of estradiol were administered which resulted in circulating levels of estradiol similar to those seen at mid-cycle. Each subject then received a single intravenous bolus of gonadotropin releasing hormone (GnRH or LRF). The short-term infusions of estradiol resulted in marked blunting of pituitary gonadotropin responsiveness to GnRH, while the more prolonged (6 day) injections of estradiol resulted in a marked augmentation of both LH and FSH responsiveness to GnRH. These data suggest that estradiol exerts its effect, at least in part, by direct action on the pituitary gland. The data further suggest that the nature of the modulation of gonadotropin responses to GnRH by estradiol is dependent upon the length of the exposure of the hypothalamic-pituitary system to estradiol as well as upon the elevated concentrations of this steroid. Details of the strength-duration characteristics of the phenomenon of estradiol augmentation of pituitary responsiveness to GnRH also were explored. No augmentation of gonadotropin response was observed until estradiol had been given for 3 days prior to GnRH administration. With increasing duration of exposure to estradiol, there was further augmentation of gonadotropin response reaching a peak between 4 and 5 days.

To study the possible effects of changing hormonal environment upon the pituitary during the puerperium, the gonadotropin responses to GnRH were determined in women at weekly intervals beginning on the second postpartum day. As compared with responses during the follicular phase of the menstrual cycle, LH responses prior to the 5th week postpartum were less than those in the early follicular phase. Following this period of relative unresponsiveness to GnRH, exaggerated responses were seen during the second postpartum month. A similar pattern of FSH response to GnRH was seen in the puerperium. In the first 2–5 weeks following delivery, responses were absent or markedly less than those in the follicular phase. However, there was a shorter period of unresponsiveness of FSH than LH. Responses were equal to those in the follicular phase by the third week. Exaggerated FSH responses occurred in all 3 subjects in the last 4 weeks of study. These studies demonstrate that the pituitary is relatively unresponsive to GnRH in the first 2–4 weeks following delivery. In addition, the maximal gonadotropin responses during the second month postpartum were as great as 9 times those of the early follicular phase of the menstrual cycle. These findings suggest that anovulation during the first postpartum month is, at least in part, the result of pituitary unresponsiveness to GnRH. However, anovulation which persists beyond the first month is not the result of pituitary unresponsiveness, but may involve hypothalamic and/or ovarian factors.

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