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Physiology

Decreased release of gonadotropin-releasing hormone during the preovulatory midcycle luteinizing hormone surge in normal women

(menstrual cycle/neuroendocrine/follicle-stimulating hormone/gonadotropin-releasing hormone antagonist)

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ABSTRACT **To investigate the contribution of hypothalamic gonadotropin-releasing hormone (GnRH) secretion to the midcycle gonadotropin surge in the human, the response of luteinizing hormone (LH) to competitive GnRH receptor blockade achieved by administration of a range of doses of a pure GnRH antagonist was used to provide a semiquantitative estimate of endogenous GnRH secretion. The LH response to 5, 15, 50, and 150 μg/kg s.c. of the NAL-GLU GnRH antagonist ([Ac-D-2Nal[1],D-4ClPhe[2],D-Pal[3],Arg[5],D-4-*p*-methoxybenzoyl-2-aminobutyric acid[6],D-Ala[10]]GnRH, where 2Nal is 2-naphthylalanine, 4ClPhe is 4-chlorophenylalanine, and 3Pal is 3-pyridylalanine) was measured in normal women in the early and late follicular phases of the menstrual cycle, at the time of the midcycle LH surge and in the early luteal phase. LH decreased in a dose–response fashion after administration of the GnRH antagonist in all cycle phases ($P < 0.0001$). When this suppression was expressed as maximum percent inhibition, there was no difference in response during the early and late follicular and early luteal phases. However, at the midcycle surge, there was a leftward shift of the dose–response curve with significantly greater suppression of LH at the lower antagonist doses in comparison to the other cycle phases ($P < 0.005$), but no difference at the highest dose. Thus, we draw the following conclusions. (*i*) There is a consistently greater degree of LH inhibition by GnRH antagonism at the midcycle surge at submaximal degrees of GnRH receptor blockade than at other phases of the menstrual cycle in normal women. (*ii*) This leftward shift of the dose–response relationship to GnRH receptor blockade suggests that the overall amount of GnRH secreted at the midcycle surge is less than at other cycle stages. (*iii*) These data confirm the importance of pituitary augmentation of the GnRH signal at the time of the midcycle gonadotropin surge in the human.**

The human menstrual cycle requires a tightly integrated series of neuroendocrine and peripheral hormonal signals involving the hypothalamus, pituitary, and ovaries for normal folliculogenesis, ovulation, and maintenance of the corpus luteum. Despite an increasing understanding of the neuroendocrine mechanisms governing normal reproductive cycles, the precise mechanisms responsible for the dramatic increase in luteinizing hormone (LH) secretion at midcycle (the preovulatory gonadotropin surge) remain unclear, particularly in the human. Studies in women with congenital gonadotropin-releasing hormone (GnRH) deficiency and rhesus monkeys with induced GnRH deficiency unequivocally demonstrate that normal LH surges can be generated with no change in the amplitude or frequency of GnRH replacement from that required for follicular development (1–3), indicating that, in these species, an increment in GnRH release is not required for this critical event. What is less certain is the nature of actual changes in hypothalamic GnRH secretion at the time of the gonadotropin surge in normal women.

Direct measurements of GnRH in pituitary portal blood indicate that a surge of GnRH secretion is generally associated with spontaneous and sex steroid-induced gonadotropin surges in sheep (4–6). GnRH levels also appear to increase at the time of the proestrus surge in rats (7) and rhesus monkeys (8, 9). These direct techniques of accessing information regarding the hypothalamic component of the preovulatory surge are clearly not feasible in the human and measurements of GnRH in the peripheral circulation do not accurately reflect hypothalamic GnRH secretion if at all (10). Therefore, an understanding of the physiology of GnRH secretion and pituitary responsiveness to this releasing hormone in the human can only be achieved by combining a number of indirect approaches. Monitoring of pulsatile LH secretion can provide insight into the frequency of GnRH secretory episodes (11) but does not permit estimates to be made of the amount of GnRH secreted. The dose of exogenous pulsatile GnRH required to mimic normal menstrual cycles and a normal midcycle surge in GnRH-deficient women (3) has been determined empirically, by comparison with "target" or reference ranges derived from studies in normal women, but does not provide information about potential changes in the actual quantity of endogenous GnRH secreted under various physiologic conditions in the intact human.

A competitive receptor antagonist can be used to assess the relative amount of an unmeasurable endogenous ligand by determining the susceptibility of a marker of its action to specific blockade. This principle derives from quantitative theories of drug action (12) and has proven extremely useful in determination of opioid tone using the opiate receptor blocker, naloxone (13, 14). We have applied an analogous approach to the assessment of the overall amount of GnRH secreted by using a GnRH antagonist that competitively blocks the GnRH receptor and LH secretion as the marker of GnRH action. The underlying assumption is that the amount of GnRH present at a given time is directly proportional to the dose of GnRH antagonist required to inhibit LH secretion. The NAL-GLU GnRH antagonist ([Ac-D-2Nal[1],D-4ClPhe[2],D-Pal[3],Arg[5],D-4-*p*-methoxybenzoyl-2-aminobutyric acid[6],D-Ala[10]]GnRH, where 2Nal is 2-naphthylalanine, 4ClPhe is 4-chlorophenylalanine, and 3Pal is 3-pyridylalanine) (15) was used as a probe to determine whether an increase in the

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Abbreviations: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; NAL-GLU, [Ac-D-2Nal[1],D-4ClPhe[2],D-Pal[3],Arg[5],D-4-*p*-methoxybenzoyl-2-aminobutyric acid[6],D-Ala[10]]GnRH, where 2Nal is 2-naphthylalanine, 4ClPhe is 4-chlorophenylalanine, and 3Pal is 3-pyridylalanine; P, progesterone; FSH, follicle-stimulating hormone; E_2, estradiol.

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overall amount of GnRH secreted from the hypothalamus is seen at the time of the midcycle surge in the human. Full dose–response curves of LH inhibition over a range of antagonist doses in the early follicular phase (16) served as a reference for comparison with studies at three additional stages of the menstrual cycle, the late follicular phase, the midcycle surge, and the early luteal phase. A greater ease of inhibition of LH at the midcycle surge suggests that the overall amount of GnRH released at this critical time may in fact be less than in other cycle phases.

METHODS

Experimental Protocol. Studies were performed in euthyroid normoprolactinemic women aged 18–40 years with proven ovulatory cycles who had either undergone a tubal ligation or consented to the careful use of barrier contraception during the month of study. All subjects had a negative urinary human chorionic gonadotrophin measurement on the day of the study. The studies were approved by the Subcommittee on Human Studies of the Massachusetts General Hospital and each subject signed a statement of informed consent.

Previous studies had been performed in the early follicular phase alone with the NAL-GLU GnRH antagonist at doses of 15, 50, and 150 μg/kg (16). Additional studies were performed at a dose of 5 μg/kg in the early follicular phase (5 or 6 women studied at each dose), at all four doses in the late follicular phase (6–11 women studied at each dose) and the midcycle surge (3–6 women studied at each dose), and at the three highest doses in the early luteal phase (4 or 5 women studied at each dose). Cycle phases were determined prospectively by previous menstrual cycle history, transvaginal ultrasound examination, and basal body temperature charts and confirmed retrospectively by comparison of hormonal characteristics on the day of the study with the previously published series of 64 normal women studied in this laboratory (17). Women studied in the early follicular phase were admitted between days 2 and 5 from the onset of menses, and those in the late follicular phase group were studied >9 days from the onset of menses and had a mean baseline LH value within 2 SEMs of late-follicular-phase LH values (17). In the midcycle surge group, the baseline LH was >2 SEMs above the late-follicular-phase values and progesterone (P) was <6 nmol/liter. Women in the early luteal phase were studied between 16 and 22 days from the onset of menses and had a P level of ≥9.5 nmol/liter. Subjects were admitted to the Clinical Research Unit of the Massachusetts General Hospital on the designated cycle day and had an intravenous cannula inserted for blood sampling. Blood was sampled at 10-min intervals for the first 12 hr of the study and hourly for a further 12 hr. A single subcutaneous dose of the NAL-GLU GnRH antagonist was administered after the first 4 hr of blood sampling. All samples were assayed for LH, and follicle-stimulating hormone (FSH) was measured in hourly samples. Estradiol (E_2) and P were measured at the beginning of each study. Studies were not performed later in the luteal phase due to the long interpulse interval of LH secretion at this stage of the cycle relative to the short pretreatment baseline and the errors that this might introduce in the assessment of percent inhibition.

Assays. LH, FSH, E_2, and P were measured by RIA, as described (18, 19). All samples from an individual subject's study were measured in duplicate in the same assay. The intraassay coefficient of variation for LH was 6.1 ± 0.2% and the lower limit of detection for LH and FSH was 0.8 international unit/liter. Gonadotropin values are expressed in units/liter as equivalents of the Second International Reference Preparation of human menopausal gonadotropins.

Data Analysis. Pretreatment values of E_2, P, LH, and FSH for each subject were compared to published data (17) to assure that these hormonal characteristics were appropriate to the cycle stage assigned. To determine the significance of gonadotropin decreases after the antagonist, the studies were divided into the baseline (pretreatment) 4-hr period and three subsequent postantagonist 4-hr periods. Values were logarithmically transformed before analysis and each value was subtracted from the baseline. Thus, differences can be interpreted as percent change from baseline. A repeated measures analysis of convariance was performed on these data with logarithmic dose as a continuous covariate and cycle stage as a factor.

The maximum amount of suppression of LH and FSH at each dose can most easily be examined by calculation of percent inhibition as [(mean pretreatment baseline − nadir)/mean pretreatment baseline] × 100. The nadir was calculated by using a moving average (six points for the 10-min LH values and three points for the hourly FSH values). This approach permits comparisons to be made of the response of a given hormone between cycle phases when baseline hormone levels are different and of the response of different hormones (LH and FSH). Two-way analysis of variance was used to determine the effect of dose and cycle phase on percent inhibition. Results are expressed as the mean ± SEM, and unless specified, a P value of <0.05 was considered to be statistically significant.

RESULTS

Pretreatment levels of E_2, P, LH, and FSH prior to antagonist administration were compatible with normal values (17) and confirmed the assignment of all studies to the appropriate cycle phase (Table 1).

LH Response to GnRH Receptor Blockade. GnRH receptor blockade resulted in a decrease in LH in response to all doses of the antagonist in all cycle phases ($P < 0.0001$) with an additional effect of both dose ($P < 0.0001$) and cycle phase ($P < 0.0007$). In the early and late follicular and early luteal phases, LH remained suppressed for at least 20 hr after antagonist administration at the highest two doses but returned to pretreatment levels after the lower two doses within this period of observation (Fig. 1). At both 50 μg/kg and 150 μg/kg, occasional values after antagonist administration in the early luteal phase studies were suppressed to assay sensitivity that did not occur at any other cycle phase. In the midcycle surge studies, LH did not return to pretreatment levels by 20 hr after antagonist administration at any dose of the GnRH antagonist (Fig. 1). This difference in degree and time course of suppression and recovery was particularly apparent when individual values were expressed as percent of mean pretreatment levels (Fig. 2). The pattern and duration of LH suppression were not different in studies in the early and late follicular or early luteal phases; however, at the midcycle surge, a greater and more prolonged suppression of LH was evident at antagonist doses of 5 and 15 μg/kg in comparison to all other cycle phases, with no significant difference at the higher two doses.

Percent Inhibition. Expression of data as percent inhibition from the pretreatment baseline permits comparisons to be

Table 1. Hormonal values: Pretreatment baseline

Phase	E_2, pmol/liter	P, nmol/liter	LH, IU/liter	FSH, IU/liter
EFP	93 ± 6	2.4 ± 0.3†	9.2 ± 1‡	9.1 ± 1‡
LFP	299 ± 29*	2.0 ± 0.1†	15.0 ± 1*‡	8.4 ± 1‡
MCS	337 ± 37*	2.7 ± 0.3†	49.4 ± 6*	15.5 ± 2*
ELP	207 ± 28*	28.2 ± 3.2	14.1 ± 3*‡	7.0 ± 1*‡

EFP, early follicular phase; LFP, late follicular phase; MCS, midcycle surge; ELP, early luteal phase; IU, international unit(s). Data are the mean ± SEM. *, $P < 0.005$ vs. EFP; †, $P < 0.0001$ vs. ELP; ‡, $P < 0.0005$ vs. MCS.

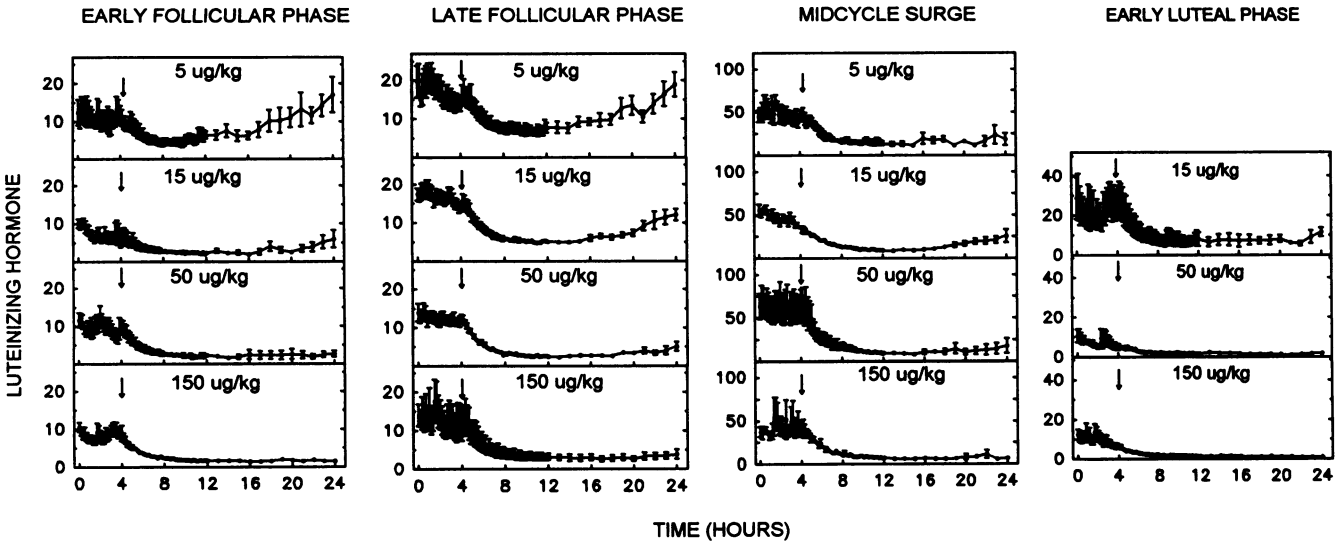

FIG. 1. LH before and after administration of the NAL-GLU GnRH antagonist at doses of 5, 15, 50, and 150 μg/kg (ug/kg) as indicated, in normal women in the early follicular phase, late follicular phase, midcycle surge, and early luteal phase. The arrow indicates the timing of antagonist administration. Note the difference in scale for the midcycle surge and the early follicular phase. Data for the higher three doses in the early follicular phase are from Hall *et al.* (16). LH is presented as international units/liter (mean ± SEM).

made of the dose–response curves of LH in response to GnRH receptor blockade between cycle phases with each subject's study contributing a single value (Fig. 3). Two-way analysis of variance revealed an overall effect of dose ($P < 0.0001$) and cycle phase. The effect of cycle phase was greater with inclusion of all four doses in the early and late follicular phase and the midcycle ($P < 0.005$) but was also present when data from the three highest doses were analyzed for studies in all four cycle phases ($P < 0.05$). The effect of cycle phase was due entirely to the greater inhibition of LH in studies at the midcycle surge in comparison to all other cycle phases at the doses of 5, 15, and 50 μg/kg with no difference in maximum percent inhibition of LH at 150 μg/kg. Given the lower limit of detection of the assay and the differing pretreatment LH levels, there is some variability in the maximum percent suppression that would be possible in the different cycle phases, being approximately 91, 94, 98, and 94% in the early and midfollicular phases, the midcycle surge, and the early luteal phase, respectively. Although the maximum percent inhibition achieved at the higher GnRH antagonist doses approached the maximum inhibition possible, in no instance was full inhibition achieved, making comparison of percent inhibition meaningful across cycle phases.

FSH Response to GnRH Receptor Blockade. FSH decreased in response to all doses of the GnRH antagonist during all stages of the cycle ($P < 0.001$) (Fig. 4). The decrease in FSH was delayed in comparison with LH, reflecting the longer half-life of FSH with changes not always reaching significance within the first 4 hr after antagonist administration. In addition, FSH did not return to baseline by the completion of the study (20 hr after antagonist administration) at any dose in any cycle phase. There was an overall effect of cycle phase ($P < 0.0001$) but not dose on the FSH response to GnRH receptor blockade. This effect was greater at the midcycle

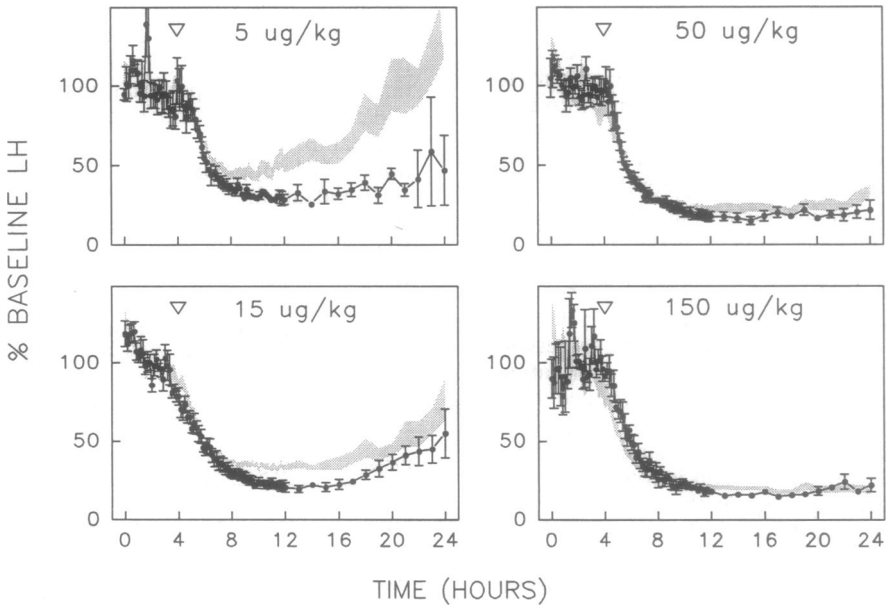

FIG. 2. LH expressed as a percent of baseline (mean ± SEM) in studies in the midcycle surge (●) in relation to the combined data from the early follicular phase, late follicular phase, and early luteal phase (shaded area). The triangle indicates the timing of antagonist administration. ug/kg, μg/kg.

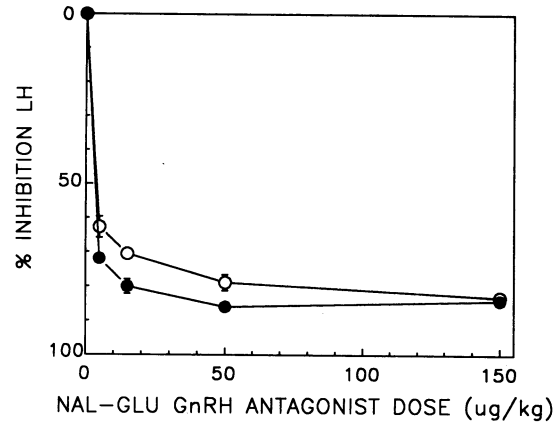

FIG. 3. Maximum percent inhibition of LH in response to the NAL-GLU GnRH antagonist at doses of 5, 15, 50, and 150 μg/kg. Data for the early follicular phase, late follicular phase, and early luteal phase have been combined (○) as there was no difference between the dose–response curves for these cycle phases. There was a greater inhibition of LH at submaximal doses at the midcycle surge (●). Where not obvious, the SEM is included in the symbol. ug/kg, μg/kg.

surge than at the late follicular phase ($P < 0.0001$) and greater for these cycle phases than for the early follicular phase ($P < 0.0001$) and early luteal phase ($P < 0.0001$ and $P < 0.02$ vs. the midcycle surge and late follicular phase, respectively).

DISCUSSION

The principles that govern competitive inhibition of the interaction of ligands with receptors (12) predict that a semiquantitative estimate of endogenous GnRH secretion can be derived from the effect of a competitive GnRH antagonist on LH secretion. At a given GnRH antagonist dose that results in a submaximal degree of LH inhibition, the amount of endogenous GnRH secreted will be inversely proportional to the degree of inhibition of LH. We have demonstrated a leftward shift in the response of LH to increasing degrees of GnRH receptor blockade at the time of the midcycle surge in comparison to other phases of the menstrual cycle in normal women. These data suggest that in normal women the overall amount of GnRH released at the midcycle surge is less than at other cycle stages. This approach is made possible by the fact that GnRH is the only known independent secretogogue for LH, that GnRH and its antagonist bind to a single receptor type, and that there has been no demonstration of changes in GnRH receptor affinity over a wide range of physiologic and pharmacologic conditions (21). The general approach is analogous to the use of naloxone, an opiate receptor blocker, to determine endogenous endorphin "tone" (13, 14). The use of this tool for discriminating between different physiologic states requires a precision in measurement that is demonstrated in these studies. Expression of the data in relation to the pretreatment baseline allows comparisons to be made between cycle phases and is only justified because the absolute nadir reached is not at the lower limit of detection of the assay. In the current study, it was possible to define the GnRH antagonist dose that produced the maximum degree of GnRH receptor blockade as there is no difference in suppression of LH at the higher two doses of the GnRH antagonist in any cycle phase although the duration of this effect was greater at the highest dose. In addition the NAL-GLU GnRH antagonist doses of 5 and 15 μg/kg are clearly submaximal doses. Thus, the relationships of endogenous ligand, receptor, and receptor blocker in these studies are such that a competitive effect should be seen when it is present.

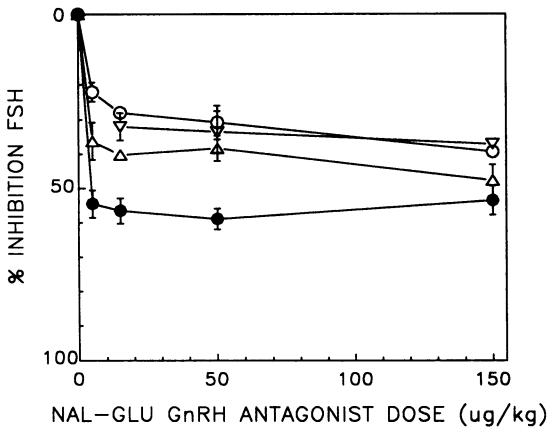

FIG. 4. Maximum percent inhibition of FSH after GnRH antagonist administration at the doses indicated in the early follicular phase (○), late follicular phase (△), midcycle surge (●), and early luteal phase (▽). Where not obvious, the SEM is included in the symbol. ug/kg, μg/kg.

We have used the LH response to a GnRH antagonist to quantify the amount of GnRH secreted in different cycle phases relative to the early follicular phase. By expressing the data in relation to pretreatment baseline LH levels, we have controlled for differences in LH responsivity to GnRH in different cycle phases. Both increases in GnRH receptor number and post-receptor amplification have been proposed to account for the augmented LH response to GnRH at the midcycle (22, 23). The effect of either change would be expected to make it more difficult to block the LH response to GnRH with an antagonist. The increased inhibition of LH at the midcycle in the current studies could be explained by a decrease in receptor number coincident with the peak of LH as demonstrated in the rat (24), perhaps as a result of desensitization. However, there is evidence that this decrease in receptor number at the peak of LH secretion is likely to be an artifact of receptor quantitation (25), supported by the recent demonstration that a decrease in GnRH receptor mRNA in the rat occurs only after the LH peak (26). Factors that dampen the LH signal in response to GnRH and operate at a post-receptor level or through an independent receptor could also explain the results of the current studies. Numerous putative factors have been proposed (27), but to our knowledge, data are currently not available to evaluate these possibilities. The consistency of response to GnRH receptor blockade among the early and late follicular and the early luteal phases in the current study is particularly notable and bespeaks a certain constancy of these factors that influence GnRH responsivity over a wide range of sex steroid environments. In a previous study, a single subcutaneous dose of a less potent antagonist resulted in inhibition of LH that was not different in the early or mid-follicular phase or the midluteal phase, also in agreement with our findings (28).

The increased sensitivity of LH to GnRH receptor blockade at the midcycle suggests that the overall amount of GnRH secreted at this time in normal women may, in fact, be decreased. This finding is supported by earlier observations in rhesus monkeys that GnRH antisera do not block sex steroid-induced gonadotropin surges (29) and that a gonadotropin surge can be generated in response to estrogen in the absence of GnRH for up to 24 hr (30). However, recent studies in rhesus monkeys indicate that GnRH levels are increased in both spontaneous and steroid-induced gonadotropin surges (8, 9). Our findings in normal women are, therefore, not in agreement with direct measurement of GnRH in monkeys, sheep, and rats that have demonstrated variable increases in the amount of GnRH during gonadotropin surges (4–9).

In the human there is ample evidence that the pituitary plays a key role in the positive feedback that generates the midcycle gonadotropin surge. A marked increase in pituitary responsiveness to a fixed dose of exogenous GnRH has been demonstrated at the midcycle surge in normal women (31). The mechanisms responsible for this increase in pituitary sensitivity to GnRH at the midcycle have not been completely elucidated but may be due to an increase in receptor number or to post-receptor effects on the gonadotrope (23). Both estrogen (21, 22) and inhibin (32) have been shown to increase receptor number in lower animals. In addition, studies in the rat suggest that neuropeptide Y (7, 33) and galanin (34) may play a role in generation of the midcycle surge through a direct pituitary effect that is unlikely to be mediated via the GnRH receptor. In GnRH-deficient women receiving exogenous GnRH, an LH surge is consistently generated with no further change in the frequency or dose of exogenous GnRH from that required for maturation of a single dominant follicle (2, 3). However, some degree of GnRH stimulation of the pituitary is undoubtedly essential for generation of the midcycle surge, as demonstrated by the finding that GnRH antagonists block gonadotropin surges in women (35).

A greater degree of FSH suppression was observed after GnRH antagonist administration at the midcycle surge than in other cycle phases and in the late follicular phase compared with the early follicular and early luteal phases. We have shown that LH and FSH are differentially regulated by GnRH and that the contribution of GnRH to total FSH secretion is considerably less than for LH (16, 20). In addition to its control by GnRH, FSH secretion is very sensitive to the negative feedback effects of E_2 at the pituitary (36), is negatively regulated by inhibin (37), and is positively regulated by activin (38, 39). The results of the current studies suggest that the contribution of GnRH to FSH secretion relative to other controlling factors is greater at the midcycle than in the early follicular phase and that this effect is initiated in the late follicular phase but abolished after ovulation.

In conclusion, we have shown that the inhibitory effect of GnRH antagonism on LH and FSH secretion is precise and remarkably consistent. The different sex steroid environments of the early and late follicular and early luteal phases do not influence the LH response to GnRH receptor blockade, suggesting that the amount of GnRH secreted during these particular cycle phases is unlikely to be grossly different. At the midcycle surge, however, in contrast to what has been demonstrated in a number of other species, the overall amount of GnRH secreted in the human may well be decreased. Although these conclusions remain to be confirmed by direct observations, the foregoing studies add an important dimension to the body of information that addresses the hypothalamic and pituitary mechanisms responsible for this central event in the human menstrual cycle.

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