

# The Importance of Luteinizing Hormone in the Control of Inhibin and Progesterone Secretion by the Human Corpus Luteum\*

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**ABSTRACT.** Serum inhibin levels rise markedly during the luteal phase of the human menstrual cycle and are closely correlated with serum progesterone (P) levels, suggesting that the corpus luteum (CL) secretes inhibin. While FSH is the major regulator of inhibin secretion by the granulosa cells, the control of CL inhibin secretion is unclear. We hypothesized that, like P, CL inhibin secretion would be LH dependent. To examine this possibility, normal women were given the GnRH antagonist [Ac-D<sub>2</sub>Nal<sup>1</sup>, D<sub>4</sub>CL Phe<sup>2</sup>, D<sub>2</sub>Pal<sup>3</sup>, Arg<sup>4</sup>, D-Glu<sup>6</sup> (AA), DAla<sup>10</sup>]GnRH (Nal-Glu antagonist) for 3 consecutive days commencing on day 6–8 of the luteal phase. The daily doses were 2.5 (n = 3), 10 (n = 4), and 25 µg/kg (n = 5), sc. Serum LH levels fell 2 h after injection, and the fall was maximal (70–74%) at 6 h; the degree of suppression was not dose dependent. The duration of suppression was dose related, being less than 12 h, between 12, and 24 h, and more than 24 h for the 2.5, 10, and 25 µg/kg doses, respectively. Serum FSH levels declined by 22–43%, but the effect was not dose related. Serum P levels fell by 42–45% 8 h after each dose of antagonist. They returned to baseline 24 h after the 2.5 µg/kg dose, but after both the 10 and 25 µg/kg doses serum P levels continued to fall, and menstrual bleeding commenced within 48–72 h after the first antagonist injection. Serum inhibin levels were not altered relative to normal cycles by the 2.5 µg/kg dose, but fell by 48% and 58%, and 62% and 73% respectively, 48 and 72 h after the 10 and 25 µg/kg doses, respectively. Serum P and inhibin levels correlated closely in all women. To examine the relative roles of FSH and LH in the

control of CL function, Nal-Glu antagonist (25 µg/kg, sc) was administered at 0 and 24 h commencing on day 6–8 of the luteal phase, in combination with either human menopausal gonadotropin (hMG; 150 IU, im, every 12 h) or hCG (1500 IU, im, once), both commencing at 0 h. hMG administration led to a rapid (by 2 h) and marked (3- to 9-fold) rise in serum FSH levels, whereas serum LH remained low, similar to antagonist alone treatment cycles. Serum P levels fell similarly after antagonist with or without hMG administration, as did serum inhibin and estradiol (E<sub>2</sub>), except at 48 h when serum E<sub>2</sub> was significantly higher than during antagonist treatment alone. hCG administration led to a 2- to 8-fold elevation in serum LH-like immunoactivity along with modest increases in serum P (+75%), E<sub>2</sub> (+37%), and inhibin (+60%) levels.

We conclude that 1) the Nal-Glu antagonist is a potent inhibitor of gonadotropin secretion, and the duration of its effect is dose related; 2) under the regimen employed, a threshold dose for the induction of luteolysis exists between 2.5–10 µg/kg; 3) the duration of gonadotropin suppression required to induce luteolysis is between 12–24 h on 3 consecutive days; 4) inhibin and P secretion by the CL are coupled during alterations in gonadotropin levels; 5) coadministration of the Nal-Glu antagonist with hMG failed to maintain CL function; and 6) hCG administration maintained CL P and inhibin secretion after antagonist injection, indicating that these hormones are predominantly under LH control. (*J Clin Endocrinol Metab* 68: 1078, 1989)

**I**NHIBIN is a glycoprotein hormone secreted by the ovary which is believed to be a regulator of FSH secretion (1, 2) and perhaps a paracrine factor within the

ovary (3–5). In women, both granulosa cells and the corpus luteum (CL) appear to be major sources of inhibin. The conclusion that the CL secretes inhibin is based on the finding of elevated serum inhibin levels during the luteal phase of the normal menstrual cycle (6), the secretion of inhibin bioactivity by cultured human granulosa-lutein cells (7), and the localization of inhibin immunoreactivity and bioactivities and mRNA for the inhibin subunits in human CL tissue (8). During the follicular phase, inhibin and estradiol (E<sub>2</sub>) are secreted in parallel, presumably under the influence of FSH (9). The control of CL inhibin

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secretion has not been studied. As progesterone (P) secretion by the CL is known to be at least partially gonadotropin dependent (10–12), and luteal phase serum inhibin and P concentrations are closely correlated (6), we hypothesized that CL inhibin secretion also would be under gonadotropic control.

GnRH antagonists are synthetic peptide analogs of GnRH which inhibit pituitary gonadotropin secretion (13). They have been used to investigate the control of gonadotropin secretion and gonadal function, although their effects in man have not been studied extensively because of their low potency and tendency to release histamine from mast cells. Nonetheless, previous clinical studies with GnRH antagonists have provided valuable insight into the control of ovarian and testicular function (14–17). For example, in women [Ac- $\Delta^3$ Pro<sup>1</sup>,4F-D-Phe<sup>2</sup>,D-Trp<sup>3,6</sup>]GnRH (4F antagonist) was used to demonstrate the gonadotropin dependency of both the developing follicle and the CL (18).

In this study we used a potent new GnRH antagonist [Ac-D<sub>2</sub>Nal<sup>1</sup>,D<sub>4</sub>CL Phe<sup>2</sup>, D<sub>3</sub>Pal<sup>3</sup>,Arg<sup>5</sup>,D<sup>6</sup>Glu<sup>6</sup> (AA), DAla<sup>10</sup>]GnRH (Nal-Glu antagonist) to study the control of CL function in normal women. Initially, the Nal-Glu antagonist was administered to normal women in the midluteal phase to determine 1) the dose-response and time course of action on serum gonadotropin, P, E<sub>2</sub>, and inhibin levels, and 2) whether P and inhibin secretion by the CL were coupled and varied similarly with changes in serum gonadotropin levels. Subsequently, the nature of the gonadotropic support necessary for CL inhibin and P secretion was examined by coadministration of the Nal-Glu antagonist with exogenous gonadotropin preparations.

## Materials and Methods

### Subjects

Fourteen normal young women, aged 22–35 yr, were studied. All had a history of regular menstrual cycles (26–35 days), an ovulatory basal body temperature chart (with luteal phase length of 12–16 days), a midluteal serum P level greater than 25 nmol/L (8 ng/mL), were within 10% of ideal body weight, and were taking no medications. Renal and liver function tests, urinalysis, and complete blood examination were all normal. The women used barrier contraception or abstained from sexual intercourse during the study. This study was approved by the University of Washington Human Subjects Committee, and informed consent was obtained from each woman.

Before the study cycle, skin testing for hypersensitivity to the Nal-Glu antagonist was performed by the intradermal administration in the forearm of 10  $\mu$ g Nal-Glu antagonist in 0.1 mL bacteriostatic water. All women tested had a 2+ or less reaction (wheal >5 mm without pseudopods), and all participated in the study.

### Protocol

**Study 1: GnRH antagonist dose-response study.** During the study cycle, the timing of the midcycle gonadotropin surge (day 0) was determined by urinary LH screening (OvuSTICK, Monoclonal Antibodies, Inc., Mountain View, CA) and was confirmed by serum LH RIA. Blood samples (10 mL) then were drawn daily until the women were admitted to the Clinical Research Center on day 6–8, at which time the first of three once-daily doses of the Nal-Glu antagonist was administered by sc injection into the abdomen. Each woman received the same dose of antagonist on all 3 days. Initially, a dose of 25  $\mu$ g/kg daily was chosen, based on unpublished data in men and animals. This 25  $\mu$ g/kg dose was found to induce luteolysis, and thus, lower doses were used subsequently in order to determine a dose-response relationship. The doses employed were 2.5 (n = 3), 10 (n = 4), and 25  $\mu$ g/kg (n = 5) daily for 3 days. Blood samples (10 mL) were obtained 0, 2, 4, 6, 8, and 12 h after the first dose and immediately before the next two antagonist injections. After the last injection, blood samples were collected daily until day 1–2 of the next menstrual cycle.

**Study 2: Coadministration of GnRH antagonist with human menopausal gonadotropin (hMG) or hCG.** To examine the gonadotropic control of CL function, hMG or hCG was administered along with the dose (25  $\mu$ g/kg) of Nal-Glu antagonist found in study 1 to reliably induce luteolysis. A 48-h protocol was used based on the results in study 1, and the data obtained for the 25  $\mu$ g/kg dose group in study 1 (up to the 48 h time point) were used for comparison with the gonadotropin replacement groups. The eight women in study 2, including six from study 1 in a subsequent cycle, were admitted on day 6–8 of the luteal phase, and the Nal-Glu antagonist (25  $\mu$ g/kg) was administered on 2 consecutive days in combination with either 1) hMG [n = 4; Pergonal, Serono Laboratories, Inc., Braintree, MA; FSH 150 IU/LH (150 IU, im, every 12 h for four doses beginning with the first antagonist dose (0 h)], or 2) hCG [n = 4; Profasi, Serono Laboratories; 1500 IU, im, with the first antagonist dose (0 h)]. Blood samples were obtained at 0, 2, 4, 6, 8, 12, 24, and 48 h.

Serum samples were frozen and stored at –20 C before measurement of FSH, LH, inhibin, P, and E<sub>2</sub> by RIA.

### Nal-Glu Antagonist

The Nal-Glu GnRH antagonist was dissolved in bacteriostatic water plus 4% mannitol and diluted to a concentration of either 1 or 5 mg/mL. The solution was passed through a 0.2- $\mu$ m filter into sterile 5-mL vials, which were frozen and stored at –20 C until use.

### Hormone assays

All samples from an individual woman were analyzed in duplicate in the same assay. Serum FSH and LH were measured by double antibody RIA using reagents supplied by the NIH and LER-907 as the reference preparation. The sensitivity of the LH assay was 3.2  $\mu$ g/L, and the intra- and interassay coefficients of variation (CV) were 5.5% and 8.4%, respectively. The sensitivity of the FSH assay was 25  $\mu$ g/L, and the intra-

and interassay CVs were 7.3% and 9.7%, respectively. The cross-reactivities of  $\alpha$ -subunit in the FSH and LH RIAs, respectively, were 1% and 20–40% (Parlow, A., personal communication).

Serum  $E_2$  was measured by RIA using reagents obtained from ICN Biomedicals, Inc. (RSL Division, Carson, CA). The sensitivity of the assay was 37 pmol/L, and the intra- and interassay CVs were 6.9% and 14.2%, respectively. Serum P was measured by RIA using reagents supplied by Diagnostic Products Corp. (Los Angeles, CA). The cross-reactivity of 17-hydroxyprogesterone was 0.3%; testosterone,  $E_2$ , pregnenolone, and cortisol did not cross-react. The sensitivity of the assay was 14 pmol/L, and the intra- and interassay CVs were 9.5% and 13.2%, respectively.

Serum inhibin was measured in a heterologous double antibody RIA based on purified 31K bovine follicular fluid inhibin (19). The rabbit antiserum (As 1989) was raised against 31K bovine inhibin, and  $^{125}I$ -labeled 31K bovine inhibin was used as tracer. Transforming growth factor- $\beta$ , bovine activin-A, bovine Mullerian inhibitory substance, and free inhibin subunits obtained after reduction and alkylation of 31K bovine inhibin had less than 1% cross-reactivity in the assay. A serum pool obtained from women undergoing ovulation induction was used as the standard and was calibrated in the RIA against a partially purified human follicular fluid preparation described previously (6). The sensitivity of the assay was 100 U/L, and the intraassay CVs in the upper ( $ED_{12}$ ), mid ( $ED_{50}$ ), and lower ( $ED_{88}$ ) regions of the standard curve were 10.7%, 2.8%, and 6.0%, respectively, in 10 assays. The interassay CV was derived from the repeated measurement of multiple dilutions of a quality control serum (obtained from normal women during the midluteal phase) covering the range 90–20% bound to free ratio and was 10.9% in 10 assays.

### Statistics

The results are presented as the percent change from the baseline value obtained immediately before the first antagonist injection and are shown as the mean  $\pm$  SE. The establishment of a baseline value for the interpretation of changes in serum LH and P values during the midluteal phase is difficult because of the relative infrequency and large amplitude of LH pulses at that time. However, in this study, 95% of the single LH values (at 0 h) used in the calculation of percent baseline were less than 12  $\mu$ g/L, suggesting that they were obtained during an interpulse interval. The existence of a significant ( $P < 0.05$ ) difference both with time and between groups was assessed by analysis of variance with repeated measures. The existence of a significant difference between treatment groups at particular time points was assessed by the unpaired  $t$  test.  $P < 0.05$  was considered significant.

## Results

### Study 1: GnRH antagonist dose-response study

The mean serum LH levels fell 2 h after each initial dose of the Nal-Glu antagonist (Fig. 1). The maximal decline (70–74%) occurred 6–8 h after treatment, and

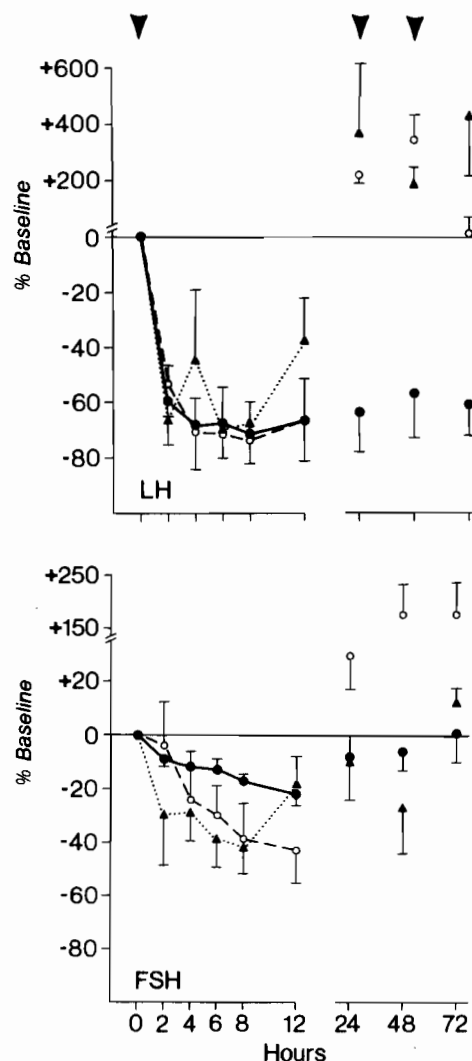


FIG. 1. Mean ( $\pm$ SE) serum LH and FSH levels, expressed as percent change from baseline (zero time), after Nal-Glu antagonist administration in the midluteal phase of the cycle in normal women. The antagonist doses were 2.5 ( $\Delta$ — $\Delta$ ;  $n = 3$ ), 10 ( $\circ$ — $\circ$ ;  $n = 4$ ), and 25  $\mu$ g/kg ( $\bullet$ — $\bullet$ ;  $n = 5$ ), sc, at 0, 24, and 48 h (injections indicated by arrows). Blood samples were obtained between 2 and 12 h after the first injection only; other samples were obtained daily immediately before antagonist injections at 24 and 48 h. Note that data points beyond 12 h have not been joined so as not to suggest that hormone levels did not change during the day after antagonist injections at 24 and 48 h.

serum LH levels during the first 12 h were similar after all three Nal-Glu antagonist doses. The duration of LH suppression was, however, dose related. After the 2.5  $\mu$ g/kg dose, the serum LH level at 12 h was higher than the nadir value, and it was several-fold above baseline 24 h after each of the three injections. After the 10  $\mu$ g/kg dose, the serum LH level was still low at 12 h, but it was several-fold above the baseline levels by 24 h after each dose. The 25  $\mu$ g/kg dose resulted in persistently low serum LH levels throughout the 24-h period. Serum FSH decreased by 22–43%. FSH suppression was not dose

related in either degree or duration (Fig. 1). Serum FSH levels increased (~150% above baseline) 24 h after the second and third 10  $\mu\text{g/kg}$  doses only.

Mean serum P levels fell similarly after each dose of Nal-Glu antagonist from a baseline of  $40.4 \pm 3.2$  nmol/L by 42–45% after 8 h (Fig. 2). By 12 h serum P in the 2.5  $\mu\text{g/kg}$  group had returned toward baseline, and by 24 h serum P levels were similar to those in normal women, after which there was a normal (22%) mid- to late luteal phase decline. The luteal phase length was normal ( $14.8 \pm 0.7$  days), and no abnormal bleeding occurred before the next menses. On the other hand, serum P levels continued to fall after both the 10 and 25  $\mu\text{g/kg}$  doses. The mean serum P levels were  $2.9 \pm 0.3$  and  $2.5 \pm 0.3$  nmol/L at 96 h (48 h after the last antagonist dose) after the 10 and 25  $\mu\text{g/kg}$  doses, respectively. The serum  $E_2$  patterns were very similar to those of P (Fig. 2). These declines in serum P and  $E_2$  were accompanied by the onset of menstrual bleeding within 48–72 h after the first dose of Nal-Glu antagonist in all women, which was normal in flow and duration. In eight of the nine women no further menstrual bleeding occurred until they had normal menses 1 month later. The luteal phase length was shortened to  $10.3 \pm 0.6$  and  $9.6 \pm 0.7$  days by the 10 and 25  $\mu\text{g/kg}$  doses, respectively.

Serum inhibin levels fell more slowly than serum P levels after each dose of antagonist (Fig. 2). After the 2.5  $\mu\text{g/kg}$  dose, serum inhibin levels decreased gradually by 35% in 3 days; the pattern was similar to the mid- to late luteal phase decline of serum inhibin that occurs in normally cycling women (6). The slight initial falls in serum inhibin were similar after all three doses until 48 h, at which time inhibin levels decreased by 50% and 58%, and by 72 h they had decreased by 62% and 73% after the 10 and 25  $\mu\text{g/kg}$  doses, respectively. Serum inhibin and P levels correlated closely in all women throughout the study ( $r = 0.78$ – $0.94$ ;  $P < 0.01$ ).

One woman who received the 25  $\mu\text{g/kg}$  dose had a striking recovery of CL function after Nal-Glu antagonist-induced suppression (Fig. 3). Her serum LH and FSH levels initially fell in a similar fashion to the other women in the group, and her serum P (3.0 nmol/L) and inhibin (<100 U/L) levels decreased profoundly 48 h after the last antagonist dose. Serum LH and FSH levels then rose sharply, followed a day later (72 h after the last antagonist dose) by a rise in serum P and inhibin levels to normal midluteal phase levels (28 nmol/L and 1946 U/L, respectively). A normal late luteal phase profile of declining serum P and inhibin levels and an intercycle gonadotropin rise followed. In contrast, the four other women (three who had received 10  $\mu\text{g/kg}$  and one who had received 25  $\mu\text{g/kg}$ ) studied 72 h after the last antagonist dose had serum P levels below 2.5 nmol/L.

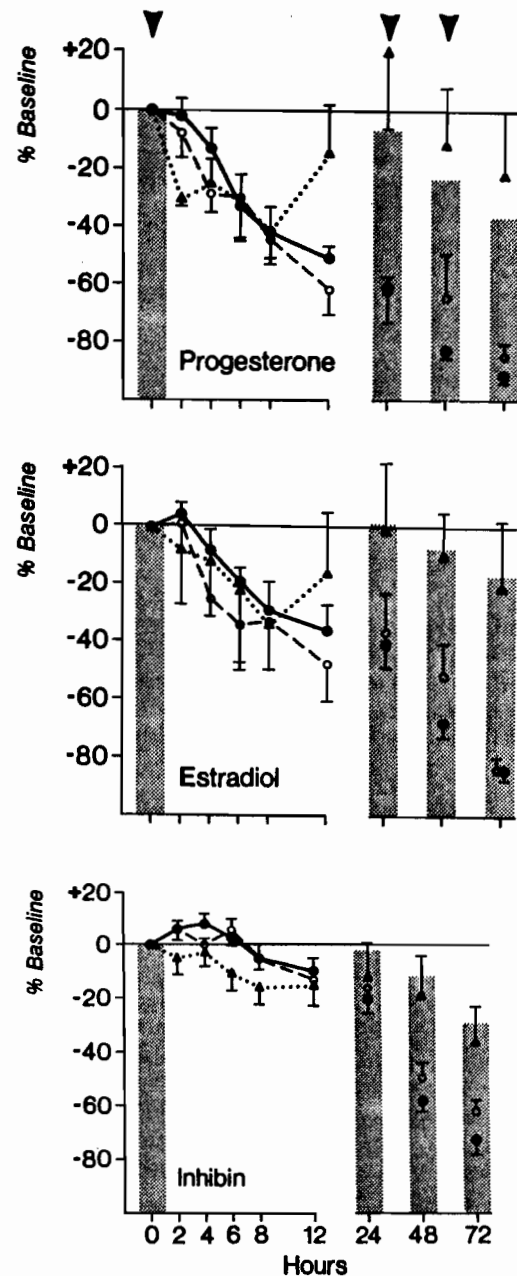


FIG. 2. Mean ( $\pm$ SE) serum P,  $E_2$ , and inhibin levels, expressed as percent change from baseline (zero time), after Nal-Glu antagonist administration in the midluteal phase of the cycle in normal women. The antagonist doses were 2.5 ( $\Delta$ - $\Delta$ ;  $n = 3$ ), 10 ( $\bigcirc$ - $\bigcirc$ ;  $n = 4$ ), and 25  $\mu\text{g/kg}$  ( $\bullet$ - $\bullet$ ;  $n = 5$ ) sc, at 0, 24, and 48 h (injections indicated by arrows). Blood samples were obtained between 2 and 12 h after the first injection only; other samples were obtained daily immediately before antagonist injections at 24 and 48 h. Note that data points beyond 12 h have not been joined so as not to suggest that hormone levels did not change during the day after antagonist injections at 24 and 48 h. The change in each hormone during the menstrual cycles of 30 normal women between days 7 and 10 are shown as histograms to allow comparison with the Nal-Glu antagonist cycles.

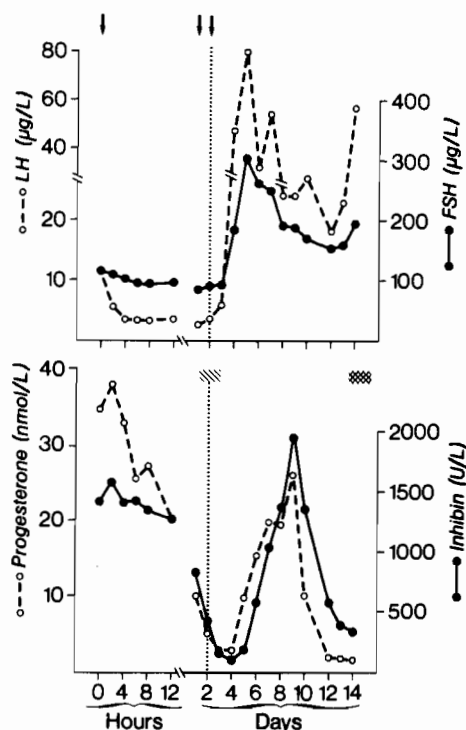


FIG. 3. Serum LH, FSH, inhibin, and P levels during the mid- to late luteal phase in a woman who received 25 µg/kg Nal-Glu antagonist, sc, at 0, 24, and 48 h (indicated by arrows). The timing of the final injection is indicated by the vertical dotted line. The decrease in gonadotropin levels after the first antagonist injection was followed by a marked reduction in P and inhibin levels, along with some light menstrual bleeding (■). 48 h after the last antagonist injection, serum gonadotropin levels increased markedly, followed 24 h later by a rise in serum P and inhibin levels back to normal midluteal phase values. A normal late luteal phase decline in both hormone levels followed by a normal menstrual period then occurred. The overall luteal phase length was 20 days.

#### Study 2: Coadministration of GnRH antagonist with hMG or hCG

Figures 4 and 5 show the changes in serum gonadotropin, P,  $E_2$ , and inhibin levels after two daily injections of 25 µg/kg Nal-Glu antagonist alone or in combination with hMG or hCG. The coadministration of hMG and Nal-Glu antagonist resulted in a rapid (by 2 h) 3- to 9-fold rise in serum FSH (Fig. 4). Serum LH remained low between 2 and 24 h despite hMG administration, and it returned to baseline at 48 h.

Serum P fell rapidly after antagonist injection despite hMG administration, so that the profile was similar to that during antagonist administration alone (Fig. 5). Premature menses occurred within 72 h in two women, while the two other women had low serum P levels with normal luteal phase lengths (data not shown). Serum  $E_2$  also fell initially despite hMG; however, it then increased to be significantly higher ( $P < 0.05$ ) at 48 h in the hMG-treated women compared to that in women who received

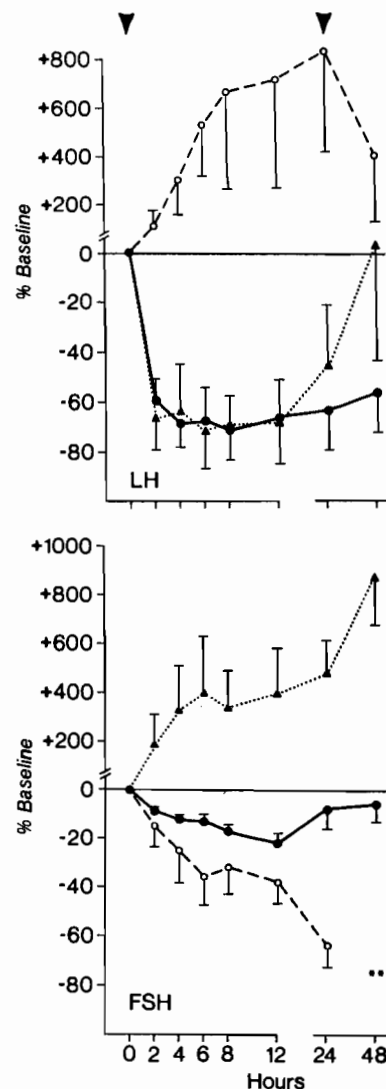


FIG. 4. Mean ( $\pm$ SE) serum LH and FSH levels, expressed as percent change from baseline (zero time), after administration of Nal-Glu antagonist (25 µg/kg, sc) at 0 and 24 h commencing on days 6-8 of the luteal phase in normal women. Nal-Glu antagonist was administered either alone (●—●;  $n = 5$ ) or in combination with either hMG (150 IU, im, every 12 h) at 0, 12, 24, and 36 h (▲—▲;  $n = 4$ ) or hCG (1500 IU, im) at 0 h (○—○;  $n = 4$ ). Blood samples were obtained between 2 and 12 h after the first injection, immediately before the second antagonist injection at 24 h, and at 48 h. \*\*, Note that serum FSH levels were undetectable at 48 h in the hCG treatment group.

antagonist alone (Fig. 5). Serum inhibin levels fell similarly in the antagonist plus hMG group compared with levels in the antagonist alone group (Fig. 5).

The coadministration of hCG with the antagonist resulted in a rapid (by 2 h) 2- to 9-fold increase in serum LH-like immunoactivity (Fig. 4). Serum FSH levels decreased to a significantly ( $P < 0.05$ ) greater degree than during antagonist administration alone and were undetectable at 48 h. Serum P levels increased rapidly to 75% above baseline at 8 h and remained elevated thereafter

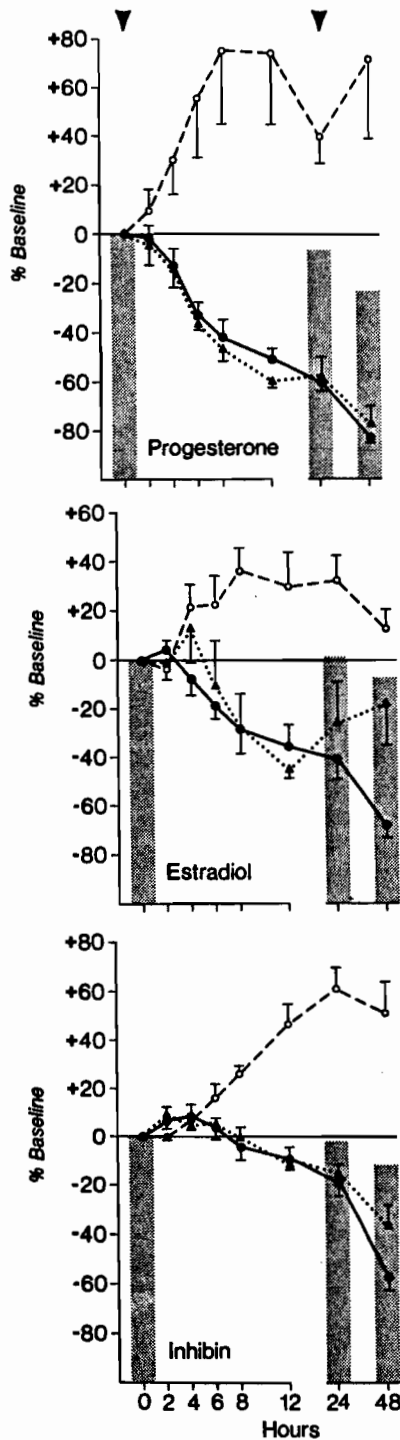


FIG. 5. Mean ( $\pm$ SE) serum P,  $E_2$ , and inhibin levels, expressed as percent change from baseline (zero time), after administration of Nal-Glu antagonist ( $25 \mu\text{g/kg}$ , sc) at 0 and 24 h commencing on days 6–8 of the luteal phase in normal women. Nal-Glu antagonist was administered either alone ( $\bullet$ — $\bullet$ ;  $n = 5$ ) or in combination with either hMG (150 IU, im, every 12 h) at 0, 12, 24, and 36 h ( $\blacktriangle$ — $\blacktriangle$ ;  $n = 4$ ) or hCG (1500 IU, im) at 0 h ( $\circ$ — $\circ$ ;  $n = 4$ ). Blood samples were obtained between 2 and 12 h after the first injection, immediately before the second antagonist injection at 24 h, and at 48 h. The change in each

(Fig. 5). Similarly, serum  $E_2$  levels increased 37% above baseline (Fig. 5). Serum inhibin levels increased by 6 h and were increased to 60% and 52% above baseline at 24 and 48 h, respectively. Luteal phase length was normal in all women (14–16 days).

#### Side-effects

The intradermal skin test was usually associated with local erythema and induration between 8–18 mm. The sc administration of the study doses was often associated with mild local pain lasting 15–30 min. In one woman, the initial  $25 \mu\text{g/kg}$  dose was associated with transient (5-min) tachycardia (120 beats/min), a minor increase in blood pressure (130 mm Hg systolic), and shortness of breath. This reaction was markedly reduced with subsequent injections. No other adverse reactions occurred.

#### Discussion

We have demonstrated that the Nal-Glu antagonist is a potent inhibitor of gonadotropin secretion which, when administered in the midluteal phase, led to a rapid decline in CL function. The consequent decline in inhibin and P secretion by the CL was negated by the coadministration of LH-like bioactivity in the form of hCG, but not by FSH replacement in the form of hMG. The close coupling of serum inhibin and P levels during alterations in the pattern of gonadotropic stimulation indicate that both are under the dominant control of LH.

In the first study we sought to establish the relationship between the dose of antagonist and the degree of suppression of pituitary gonadotropin secretion and CL function. The rapidity (by 2 h) and the maximal degree of LH suppression (70–74% at 6–8 h) were similar among the three doses tested. This suppression was greater than that previously reported using other GnRH antagonists (46–58%) (14–18). The duration of LH suppression was dose related; less than 12 h after the  $2.5 \mu\text{g/kg}$  dose, between 12–24 h after the  $10 \mu\text{g/kg}$  dose, and at least 24 h after the  $25 \mu\text{g/kg}$  dose. This relationship has been noted previously with other antagonists (15, 20). The increased mean levels and wide variance of serum LH apparent 24 h after the  $2.5$  and  $10 \mu\text{g/kg}$  doses suggest that rebound hypersecretion occurred after the antagonist blockade of endogenous GnRH action had abated. Increased endogenous GnRH secretion may have re-

hormone during the menstrual cycles of 30 normal women between days 7 and 10 are shown as *histograms* to allow comparison with the Nal-Glu antagonist and hMG or hCG administration cycles.



sulted from the transiently suppressed sex steroid levels and then acted to enhance LH secretion. The suppression of serum FSH was less than that of LH and was not dose related in either degree or duration. Previous studies with GnRH antagonists have shown less suppression of FSH than LH (14–16, 20), perhaps due to the longer serum half-life of FSH and/or to FSH secretion being less GnRH dependent (21, 22). The apparent rebound increase in FSH 24 h after the 10  $\mu\text{g}/\text{kg}$  dose, like that of LH, may be related to endogenous GnRH action after the antagonist effect had worn off.

The physiological correlate of these changes in gonadotropin levels was the decline in serum P, which fell to a similar degree (42–45%) 8 h after each dose. After the 2.5  $\mu\text{g}/\text{kg}$  dose, P levels began to recover at 12 h and were normal 24 h after each dose. On the other hand, despite restoration of normal to increased serum LH levels 24 h after each 10  $\mu\text{g}/\text{kg}$  dose, serum P levels fell progressively, and these women had premature menstruation and a resultant shortened luteal phase. This pattern was similar to that after the 25  $\mu\text{g}/\text{kg}$  dose. In eight of the nine women who received 10 or 25  $\mu\text{g}/\text{kg}$ , luteolysis was judged to have occurred because of a marked and sustained reduction in P levels, the induction of premature menses of normal duration and flow, and a subsequent normal length menstrual cycle without intermenstrual bleeding.

We conclude that 1) a threshold for the induction of luteolysis in the midluteal phase exists between the 2.5 and 10  $\mu\text{g}/\text{kg}$  doses given daily for 3 consecutive days, and 2) a duration of gonadotropin deprivation between 12 and 24 h daily on 3 consecutive days is necessary for the induction of luteolysis. The latter finding differs somewhat from data in hypothalamic-lesioned female subhuman primates receiving pulsatile GnRH therapy (23). When deprived of GnRH for 3 days in the midluteal phase, serum P levels fell markedly, but were substantially restored, but not normalized, after the reinstitution of pulsatile GnRH. On the other hand, in our study the 10 and 25  $\mu\text{g}/\text{kg}$  doses led to a rapid and sustained fall in serum P, normal menstruation, and a subsequent normal length cycle in eight of nine women. Only one woman demonstrated recovery of CL function after 3 days of gonadotropin withdrawal, in a similar fashion to the subhuman primate (23).

Serum inhibin levels were closely correlated with P levels throughout the dose-response study. Nonetheless, the rapidity of the decline of inhibin after antagonist-induced gonadotropin withdrawal was slower than that of P. This could potentially occur because of a longer serum half-life of inhibin immunoactivity and/or because the cellular machinery for inhibin secretion responds more slowly to changing gonadotropin levels. The gonadotropin dependency and close coupling of CL inhibin and

P secretion also are well demonstrated by the only woman who had recovery of CL function after gonadotropin withdrawal. In this woman the initial fall and secondary rise in gonadotropin levels were associated with parallel changes in serum inhibin and P levels, after a delay of approximately 1 day.

The relative roles of FSH and LH in the control of CL inhibin and P secretion were examined by the coadministration in the midluteal phase of a luteolytic dose of antagonist (25  $\mu\text{g}/\text{kg}$ ) alone or in combination with either hMG or hCG. After hMG administration (300 IU FSH/day), serum FSH levels increased rapidly (by 2 h) and markedly (by 9-fold). On the other hand, serum LH levels remained low between 2 and 24 h and were similar to those after antagonist alone. Serum LH did not increase acutely 2, 4, 6, 8, or 12 h after the first hMG injection. Serum LH did, however, return to baseline at 48 h, suggesting slow accumulation of LH in serum. These data are similar to those obtained during hMG treatment of women with hypogonadotropic hypogonadism, in whom the rise in serum FSH was approximately 5-fold greater than that in LH and the rise in serum LH occurred gradually over several days (24). Thus, the LH present in the hMG dose employed (300 IU LH/day, based on *in vitro* bioassay) led to only a minor increase in serum LH. The predominant replacement of FSH with hMG failed to maintain CL P secretion, although it is possible that a higher dose of hMG may have maintained CL function. Parallel changes in serum  $E_2$  and inhibin occurred during the first 24 h. At the 48 h time point serum  $E_2$  was significantly higher than during treatment with Nal-Glu antagonist alone. This increase in serum  $E_2$  could be due to the development of new follicles occurring during hMG administration. A second possibility is that the FSH stimulus provided by hMG led to increased  $E_2$  secretion by the CL without a parallel increase in P secretion. Evidence has been presented for a large cell subtype in human CL tissue which secretes  $E_2$  in response to FSH (25). A third, less likely, possibility is that the accumulation of LH in serum at 48 h maintained  $E_2$  secretion by the CL without any action on P secretion.

The coadministration of Nal-Glu antagonist with hCG (1500 IU) led to a marked and sustained elevation in serum LH-like immunoactivity. Serum FSH levels fell below those during Nal-Glu antagonist administration alone, perhaps as a result of suppression of gonadotropin secretion by elevated sex steroid and/or inhibin levels. The secretion of inhibin, P, and  $E_2$  were maintained above the baseline for 3 days after hCG treatment. A previous study with the 4F antagonist demonstrated that hCG can negate the effect of the antagonist, indicating that the suppressive action of GnRH antagonists is at the pituitary, rather than the ovarian, level (18). Our

results establish a dominant role for LH in the control of inhibin as well as P secretion.

In conclusion, the Nal-Glu antagonist is a potent inhibitor of gonadotropin secretion which allowed us to study the regulation of CL inhibin and P secretion. During antagonist-induced gonadotropin withdrawal and combined antagonist plus gonadotropin replacement, we found a close coupling of inhibin and P secretion. Both hormones appear to be predominantly under LH control during the midluteal phase.

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