Changes in Serum Immunoreactive Inhibin during Ovulation Induction in Women with Amenorrhea#

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Abstract. Changes in serum immunoreactive (IR)-inhibin were measured by RIA in two studies, in order to elucidate, firstly whether the pattern of IR-inhibin secretion is similar to that of estradiol (E2), and secondly, whether inhibin suppresses endogenous FSH release. Study 1: Purified urinary FSH (pFSH) or human menopausal gonadotropin (hMG) were daily injected intramuscularly into women with hypogonadotropic amenorrhea at 12 to 14 week intervals. PFSH and hMG stimulated IR-inhibin release in a similar fashion in the ovulatory cycles, but the increase in estradiol (E2) during pFSH administration was delayed and lower than that during the hMG cycles. This suggests that E2 and IR-inhibin are secreted independently from the granulosa cells. Study 2: Ovulation induction was performed in 18 cycles of 9 women with polycystic ovarian disease (PCOD) by the step-down administration of pFSH. The serum FSH concentration in cycles with premature LH release increased even after the dose of pFSH was reduced, and were significantly higher than those of cycles without premature LH release. It was also found that the serum IR-inhibin concentration in cycles with the premature LH release was 2 to 4 times as high as in cycles without premature LH release. This suggests that IR-inhibin does not suppress endogenous FSH release associated with premature LH release.

Key words: Inhibin, Hypogonadotropic amenorrhea, Ovarian hyperstimulation syndrome

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INHIBIN and estradiol (E2) are gonadal hormones secreted by the ovary under the influence of FSH and are thought to play important roles in female reproduction [1, 2]. Both hormones are produced and released by the granulosa cells during the menstrual cycle [3–6]. In fact a parallel increase in the two hormones in response to exogenous gonadotropin has been demonstrated in normal women [7–9] and in amenorrheic women with polycystic ovarian disease (PCOD) [10]. These reports indicate that measurement of inhibin can be a useful marker for evaluating granulosa cell function [6] or predicting the clinical outcome of the treatment [7–9], but there is still a lack of knowledge of inhibin secretion during ovulation induction. In this study, we attempted to clarify firstly whether the pattern of IR-inhibin secretion is similar to that of estradiol (E2), and secondly whether inhibin elicits an acute effect on FSH secretion from the pituitary gland, particularly during the course of ovarian hyperstimulation. Ovarian hyperstimulation syndrome is one of the most serious problems in gonadotropin therapy, and endogenous FSH release during ovulation induction has been shown to be involved in the etiology of this syndrome in women with PCOD [11].
Materials and Methods

Subjects

Study 1. Three women with hypogonadotropic amenorrhea were participated in this study: the first woman with secondary amenorrhea with an unidentified cause (case 1) and the remaining two with primary amenorrhea (cases 2 and 3). They were outpatients who visited our clinic complaining of infertility. Before the experiments, the purpose of the study was explained and informed consent was obtained from all the subjects. The endocrine profile of the subjects is shown in Table 1.

Study 2. Eighteen treated cycles of 9 women with polycystic ovarian disease were analyzed in this study. A diagnosis of PCOD was made on 1) the basis of the presence of amenorrhea or chronic anovulation, 2) a high serum LH concentration in the presence of normal or low FSH, 3) the presence of multiple cysts on ovaries revealed by pelvic ultrasound and 4) the presence of withdrawal bleeding after intramuscular administration of 50 mg progesterone. Women with hyperprolactinemia were not included in this study.

Ovulation induction

Study 1. Ovulation induction was attempted with hMG (Pergonal, LH:FSH=1:1; Teikoku Zohki, Tokyo, Japan) and pFSH (Fertinorm P; Serono Japan Co., Tokyo, Japan) in two women with primary amenorrhea and a woman with secondary hypogonadotropic amenorrhea. Initially all the women received an intramuscular injection of 150 IU of pFSH for 8 consecutive days, then 225 IU for >1 week until human chorionic gonadotropin (hCG, Pregnyl; Organon Co., Tokyo, Japan) injection. In order to separate as much as possible the effects of pFSH and hMG, hMG was administered 12 to 14 weeks after the administration of pFSH. The treatment protocol for hMG administration was the same method as for the pFSH cycle.

Study 2. Ovulation induction was done with pFSH in the women with PCOD. PFSH was administered daily by the step down method [12]. This regimen started with a daily dose of 225 IU of pFSH for the first two days, then the daily dose was reduced to 75 IU from the third day. In both studies, follicular development was monitored by pelvic ultrasound, and 5000 IU of hCG was injected when the maximal follicular diameter reached >18 mm. Subsequently, hCG was injected to support luteal function, twice ever 2 to 3 days, unless the ovarian diameter exceeded 60 mm.

RIA

Inhibin: Serum immunoreactive (IR)-inhibin was determined by a double antibody RIA, by means of the cross-reaction of antibovine inhibin antibody (TNDH-1) with human inhibin. Details of this assay were reported previously [13]. Briefly, the antibody was raised in a castrated male rabbit against partially purified bovine follicular fluid inhibin prepared by immunoaffinity chromatography. The antibody did not cross-react significantly with human FSH, LH, transforming growth factor-β, FSH activin or [Tyr30]inhibin-α-(1-30). The bovine 32-
kDa inhibin was iodinated by the chloramine-T method and used as a tracer after purification with Affigel-10 (Bio-Rad, Richmond, CA) coupled with a monoclonal antibody to bovine 32-kDa inhibin. Recombinant human inhibin B synthesized at our laboratory was used as a standard preparation, and the serum concentration of inhibin was expressed in terms of U/mL. The sensitivity of the assay was 1.5 U/mL with an ED50 of 25 U/mL. The intra- and inter-assay coefficients of variation were 7.0% and 5.4%, respectively.

Other hormones: Serum LH and FSH were measured by a double antibody RIA kindly supplied by NIDDK with the standard preparation of LER 907, and assay values were expressed in terms of ng/mL. The sensitivity of LH assay was 10 ng/mL with an ED50 of 160 ng/mL. The sensitivity of FSH assay was 40 ng/mL with an ED50 of 640 ng/mL. Serum E2 was measured by RIA with specific antisera kindly supplied by Dr. G. D. Niswender. All samples were assayed in duplicate. The intra- and inter-assay coefficients of variation were 6.5 and 3.8% for LH, 9.0 and 9.8% for FSH, and 16.2 and 5.4% for E2, respectively.

Statistics

Comparison of means for the two groups was done by Mann-Whitney non-parametric analysis.

Results

Study 1

The serum FSH concentration increased similarly both pFSH and hMG injection. Although hMG contains the same amount of FSH and LH, there were no remarkable differences between pFSH and hMG cycles in the serum LH concentration (Fig. 1).

Ovulation was successfully achieved in cases 1 (secondary amenorrhea) and 2 (primary amenorrhea) by both hMG and pFSH administration. The rise in serum inhibin in cases 1 and 2 was remarkable and the magnitude of the increase was similar in both hMG and pFSH cycles. Serum E2, however, showed a remarkable rise, but it was delayed and was lower during pFSH administration than that seen in hMG cycles, suggesting that LH plays an important role in E2 secretion, but not on IR-

inhbin secretion from the granulosa cells (Fig. 2).

In case 3 (primary amenorrhea), follicular development was not detected during pFSH administration, but serum IR-inhibin showed a slight but significant increase, while serum E2 showed no change. During hMG therapy, a single dominant follicular development was detected by pelvic ultrasonography and serum IR-inhibin increased greatly, but the increase in E2 was small (Fig. 2).

Study 2

Figure 3 showed changes in the serum FSH, LH, E2 and IR-inhibin concentration for 7 days before hCG administration in women with PCOD. Cycles were grouped into 2 according to the presence of the premature LH release. The premature LH release was defined as an LH release that occurred during pFSH administration and exceeded the pre-

Fig. 1. Changes in serum LH and FSH during ovulation induction in women with hypogonadotropic amenorrhea with pFSH and hMG. Initially every woman received 150 IU of pFSH for 8 days, then 225 IU for >1 week until hCG injection. After 12 to 14 weeks from pFSH administration, hMG was given in the same manner.
treatment LH value for at least 2 consecutive days before hCG administration. Serum FSH increased greatly after the initiation of the pFSH injection, but the serum FSH concentration in the cycles with premature LH release (group A, n=5) remained high even though the dose of pFSH was reduced, whereas the concentration in cycles without premature LH release (group B, n=13) decreased after reducing the dose of pFSH and were significantly lower that those in group A.

Both inhibin and E2 showed a remarkable increase after the initiation of pFSH administration, but the degree of increase in E2 and IR-inhibin in group A was much greater than that in group B. The mean ovarian diameter during the luteal phase of group A was 9.5 ± 1.1 cm and was much greater than that of group B (5.1 ± 0.3 cm, P<0.01).

Discussion

Inhibin and E2 are produced and secreted by the granulosa cells [14] and are demonstrated to show a parallel increase in normal menstrual cycles [3–6] or during ovulation induction. There is still a clinical concern, whether inhibin and E2 has the greater importance to evaluate granulosa cell function and to predict the clinical outcome of the treatment. To determine this, we carried out two independent studies with an attempt to clarify, firstly whether inhibin is secreted in parallel with E2, and secondly whether increased IR-inhibin suppresses endogenous FSH release.

It is accepted that LH and FSH are necessary for E2 production by the theca/granulosa cells [14], but little has been shown in in vivo studies to indicate whether LH is involved in inhibin secretion from the granulosa cells. Most previous reports demonstrated a parallel increase in IR-inhibin and

![Fig. 2. Changes in serum IR-inhibin and estradiol (E2) during ovulation induction in women with hypogonadotropic amenorrhea.](image)

![Fig. 3. Changes in serum LH, FSH, E2 and IR-inhibin during ovulation induction in women with PCOD. PFSH was administered by the step-down method. Cycles were grouped into 2, according to the presence of the premature LH release. Serum FSH levels of cycles without the premature LH release increased for the first 2 days, but decreased after the dose of pFSH was reduced. Open circles indicate cycles with the premature LH release (group A), while closed circles without the premature LH release (group B). *, P<0.05; **, P<0.01.](image)
E2 during ovulation induction in normal women [7-9] or women with polycystic ovarian syndrome [10], and indeed a significant correlation between the two hormones was shown in women undergoing ovulation induction by pFSH [15]. However these findings were obtained under conditions where the effect of endogenous LH was present. To investigate whether LH is necessary for inhibin secretion during the follicular phase in women, we administered pFSH and hMG to the same patients with hypogonadotropic amenorrhea. Although the study was conducted with a small sample, our results indicated that LH is less involved in IR-inhibin secretion and that E2 and IR-inhibin are secreted independently from the granulosa cells. The transient but different pattern of IR-inhibin and E2 has been pointed out by McLachlan et al. [16] in normal cycling women, and our results support this.

As shown in Fig. 1, there were no significant differences between pFSH and hMG cycles in serum LH levels, but the low LH levels during hMG therapy can be accounted for by the short half-life of LH [17]. It has already been shown that intramuscularly administered LH is eliminated from the circulation within 4 hours after the injection [18].

We have demonstrated that a high serum FSH concentration in the late stage of gonadotropin therapy is primarily important for producing multiple follicular development, which is mandatory for ovarian hyperstimulation syndrome. In addition, we have shown that endogenous gonadotropin secretion is involved in increased FSH and LH at that stage [11]. Before this experiment we had presumed that endogenous FSH release would be caused by low inhibin secretion, but as shown in Fig. 3, serum IR-inhibin as well as E2 was rather high, and the intervention of inhibin in endogenous FSH release is less involved. The exact mechanisms of endogenous gonadotropin release is still unknown.

Thus our present study raised the question of an acute effect of inhibin on the suppression of FSH secretion. It is well established in non-primate animals that inhibin elicits acute suppression of FSH secretion [1, 2], but in primates including man, it is still unknown. Fraser et al. [19] reported that anti-inhibin serum administration to the stump-tailed macaque in the mid-luteal phase did not have a great effect on serum FSH, but they found a significant increase in serum FSH in the early follicular phase of the following cycle. Illingworth et al. [20] showed that despite a significant decrease in serum inhibin after luteectomy, serum FSH was not increased in women. These reports suggest that changes in serum inhibin do not cause an immediate effect on serum FSH, and that inhibin is not a sole factor in suppressing FSH secretion. The results of our present study agree with these findings and suggest that FSH release can be induced even if circulating inhibin is superphysiologically increased. Because the antibody we used in this study cross reacts with α subunits as well as intact inhibin, further studies are necessary to draw final conclusion.

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References


