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Serum Immunoreactive Inhibin Levels in Polycystic Ovarian Disease (PCOD) and Hypogonadotropic Amenorrhea#

HIDEKI MIZUNUMA, KAZUMICHI ANDOH, MITSUO OBARA,
MASA-AKI YAMAGUCHI, TAKANORI KAMIJO,
YOSHIHISA HASEGAWA AND YOSHITO IBUKI

Department of Obstetrics and Gynecology, Gunma University School of Medicine, Gunma 371, Japan

Abstract. To evaluate the physiological significance of inhibin in various types of amenorrhea, serum immunoreactive (IR)-inhibin levels were measured and compared with those in normal cycling women. Amenorrheic women were as follows: (1) 23 women with PCOD, 11 women with hypogonadotropic amenorrhea (HA, n=23) and 11 women with regular menstrual cycles. Women with HA were further divided into 2 groups according to the presence or absence of withdrawal bleeding (WDB) after progesterone administration. HA with WDB was categorized as HA1, while HA without as HA 2. Serum IR-inhibin levels in women with PCOD were significantly higher than those in HA 2 and normal women at days 2 to 5 from the onset of menstruation and significantly lower than those in normal women in the mid-luteal phase. A significant positive correlation was obtained between IR-inhibin and FSH in HA 2 (r=0.681) and HA 1 (r=0.658), but no significant correlation between these two hormones in PCOD and normal women. These results indicated that basal IR-inhibin levels vary with types of amenorrhea. High IR-inhibin levels in PCOD patients suggest that inhibin plays a part in the discordant gonadotropin secretion in these patients.

Key words: Inhibin, PCOD, Hypogonadotropic amenorrhea.

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INHIBIN is a gonadal glycoprotein hormone consisting of two subunits, which is secreted by the gonad under the influence of FSH and is thought to play an important role in modulating FSH secretion by means of a direct negative feedback on the pituitary gland [1, 2]. With the development of a radioimmunoassay of inhibin with sufficient sensitivity [3, 4], several studies demonstrated changes in serum immunoreactive (IR)-inhibin during normal menstruation [4–6] and stimulated cycles [7, 8], but little has been shown about basal inhibin levels in patients with ovulatory disorders. Many studies thus far reported failed to show an inverse correlation between the two hormones, and it is still controversial whether inhibin is involved in the discordant gonadotropin secretion by selective inhibition of FSH release in women with polycystic ovarian disease (PCOD) [9]. In order to clarify the physiological significance of circulating inhibin in PCOD women, we measured and compared basal inhibin levels in women with amenorrhea due to various causes.

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Correspondence to: Dr. Hideki MIZUNUMA, Department of Obstetrics and Gynecology, Gunma University School of Medicine, 3-39-15 Showamachi, Maebashi, Gunma 371, Japan
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Materials and Methods

Subjects

Forty-six women who visited our clinic complaining of menstrual disorders were enrolled in

this study. The cause of menstrual disorders are polycystic ovarian disease (PCOD, n=23, mean age: 29.8 ± 3.8) and hypothalamic amenorrhea (HA, n=23, mean age: 28.1 ± 7.7). A diagnosis of PCOD was made when there was 1) amenorrhea or chronic anovulation with peripubertal onset of menstrual irregularlities, 2) a high serum LH level in the presence of a normal or low FSH level, 3) multiple cysts on ovaries revealed by pelvic ultrasound, 4) withdrawal bleeding (WDB) after intramuscular administration of 50 mg of progesterone and 5) the concentration of one of the following androgens was high: testosterone (>43.8 ng/dL), androstenedione (>1.45 ng/mL) and free testosterone (>3.7 pg/mL) [10]. Women with HA were put into two groups according to the presence (HA1, n=11) or absence (HA2, n=12) of WDB after progesterone administration. Amenorrhea accompanied with high prolactin was excluded from this study. Eleven infertile but otherwise healthy women with a mean age of 31.7 ± 4.2 years and with regular menstruation and normal mid-luteal progesterone (>10 ng/mL) were taken as a control. The endocrine background of each group is shown in Table 1. Informed consent was obtained from all subjects.

Blood sampling

Blood samples were collected from the cubital vein between 0900 and 1000 h. In the control group, blood samples were taken in the early follicular phase (days 2 to 5), mid-luteal phase and early follicular phase of the next cycle. The blood samples were centrifuged and the plasma frozen and stored at −20°C until assayed.

RIA

Inhibin: Serum IR-inhibin was determined by a double antibody RIA, using the cross-reaction of anti-bovine inhibin antibody (TNDH-1) with human inhibin. Details of this assay have been reported previously [11]. 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, activin or [Tyr30]inhibin-α-(1–30), but had 50% cross binding with bovine inhibin-α monomar [12]. The bovine 32-kDa inhibin was iodinated by the chloramin-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 was used as a standard preparation, and the serum concentration of IR-inhibin was expressed in terms of U/mL. The sensitivity of the assay was 1.5 IU/mL with an ED_{50} of 25 IU/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 with the standard preparation of LER 907, and assay values were expressed as ng/L. Serum E2 was measured by RIA using specific antisera. All samples were assayed in duplicate. The intra- and interassay 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. Serum androstenedione, testosterone and free testosterone were measured with commercially available assay kits (Coat-A-Count Androstenedione, Coat-A-Count-Testosterone and Coat-A-Count

Table 1. Endocrine background of subjects*

Group	N	LH(ng/mL)	FSH(ng/mL)	E2(pg/mL)	Δ4(ng/mL)	T(ng/dL)	free-T(pg/mL)
Control	11	44.5 ± 6.5	512.6 ± 135	62.9 ± 26.7	–	–	–
HA1	11	51.1 ± 22.1	339.6 ± 57.0	64.3 ± 43.5	0.74 ± 0.2	37.9 ± 7.9	1.2 ± 0.4
HA2	12	28.1 ± 16.3	196.3 ± 106[a]	18.6 ± 13.3[b]	–	–	–
PCOD	23	76.0 ± 22.2[a]	376.4 ± 97.4	87.2 ± 41	1.62 ± 0.63	82.2 ± 37.2	5.2 ± 2.5

* Results are the mean ± SD.

[a] Significantly different from control group, $P<0.01$. [b] Significantly different from control group, $P<0.05$.

Free Testosterone, respectively, Japan DPC Corporation, Chiba, Japan).

Statistics

Statistical analysis was performed by Kruskal Wallis analysis followed by Sheffe's nonparametric multiple comparison test, or single factor analysis of variance (ANOV) followed by Student's *t*-test. Correlation coefficients were calculated as the product-moment correlation coefficient and the slope was determined by simple regression analysis. Values were expressed as the mean ± SD unless otherwise indicated. A P value <0.05 was considered significantly different.

Results

Basal IR-inhibin levels

Serum IR-inhibin levels in women with amenorrhea due to various causes were compared to those in 11 normal women during early follicular and mid luteal phases (Fig. 1). The mean IR-inhibin levels of 2 consecutive early follicular phases were 5.82 U/mL and 5.84 U/mL. Both values were significantly lower than those in the mid-luteal phase (31.2 U/mL) and in women with PCOD, but were not statistically different from those of women with HA 1 (6.4 U/mL). The mean IR-inhibin level of PCOD was 10.7 U/mL and was much higher than those of early follicular phases and women with HA 2 (3.2 U/mL).

Correlation between basal FSH and IR-inhibin levels of women with various forms of amenorrhea

In order to investigate the relationship between serum IR-inhibin and FSH during chronic anovulatory conditions, the correlation between two hormones was studied (Fig. 2). There was a significant correlation between basal FSH and IR-inhibin in women with HA (P<0.05, respectively), while no statistical correlations were seen in normal women and women with PCOD.

Discussion

One of the characteristic features of PCOD is high LH with low or normal FSH [13, 14]. This discordant gonadotropin secretion in PCOD women has long been speculated to be produced by selective inhibition of FSH release presumably by increased inhibin [15, 16]. Tanabe *et al.* [17] found high inhibin activity in the follicular fluids (FF) of follicles from PCOD women compared to the levels in the FF of small or atretic follicles of normal women. Our present study supports the results of these previous reports but is not compatible with observations by Buckler *et al.* [9] who found that IR-inhibin concentrations in PCOD women were not significantly different from those in normal women in the early follicular phase. The discrepancy between the two studies is accounted for by the difference in the specificity of the anti-inhibin antibody and by the difference in the experimental design. Our anti-inhibin antibody showed 50% cross-reactivity with bovine inhibin monomar, while that of Buckler *et al.* was reported as showing 144% cross binding with bovine

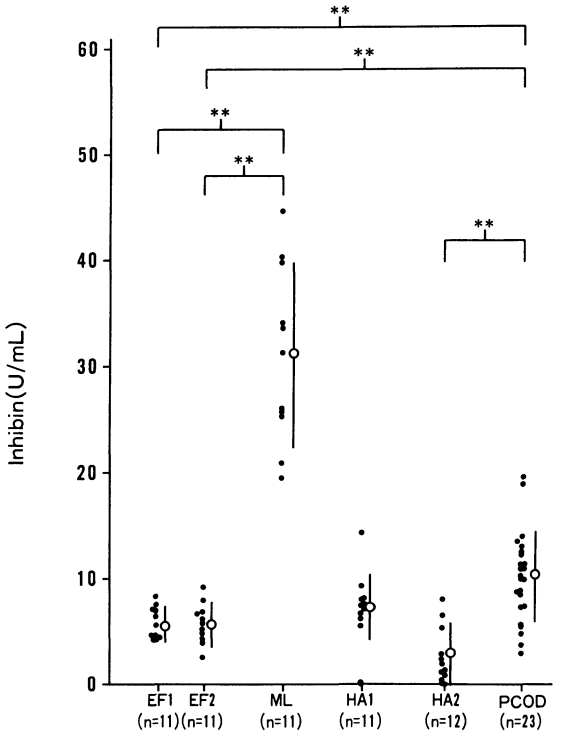

Fig. 1. Serum inhibin levels in normal women, and women with hypothalamic amenorrhea (HA) and PCOD. EF, early follicular phase (days 2 to 5); ML, mid-luteal phase; HA1, hypothalamic amenorrhea (HA) with withdrawal bleeding after progesterone injection. HA2, HA without withdrawal bleeding. ** indicates P<0.01.

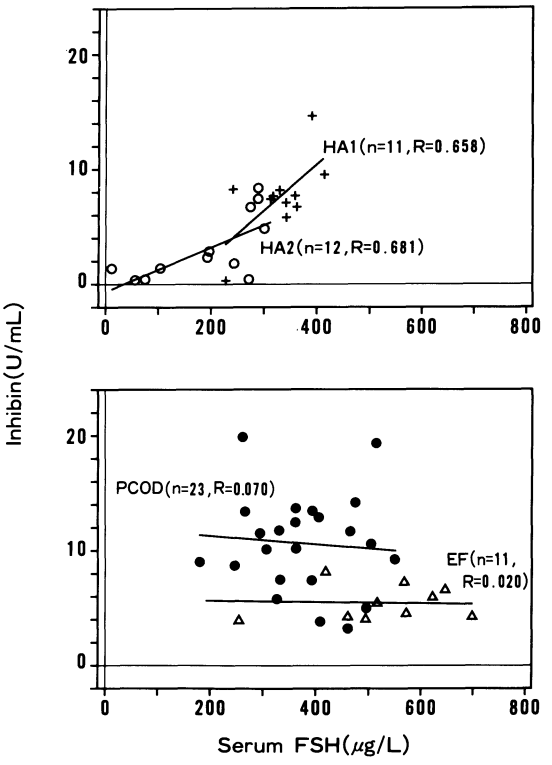

Fig. 2. Correlation between serum inhibin and FSH in women with hypothalamic amenorrhea (HA), PCOD and normal women in the early follicular phase (EF). HA 1 (+) indicates HA with withdrawal bleeding after progesterone injection, and HA 2 (○) without it. There are significant positive correlations between FSH and inhibin in women with HA, but no significant correlation between the two hormones in women with PCOD (●) and normal cycles (△).

inhibin pro-α-subunit [18]. We collected control samples on days 2 to 5 from the onset of menstruation because it had been found that the lowest inhibin levels are detected on day 2 after the onset of menstruation [5], while Buckler *et al.* collected control samples on days −9 to −7 based on the midcycle gonadotropin surge.

Our results indicate that serum IR-inhibin levels in PCOD women are significantly higher than those in normal women in the early stage of the menstrual cycle, but it still remained nuclear whether the inhibin concentration detected in PCOD plays a significant role in selective inhibition of FSH secretion. Immunoneutralization studies revealed that the administration of anti-inhibin antibody causes a significant and selective FSH rise in female rats [19], ewes [20] and heifers [21], suggesting that inhibin plays an important role in suppressing FSH release in non-primate animals. On the other hand, Illingworth *et al.* [22] demonstrated that, despite the significant decrease in serum inhibin after luteectomy, serum FSH was not increased in women. In addition, Fraser *et al.* [23] reported that anti-inhibin serum administration to the stump-tailed macaque in the mid-luteal phase did not have significant effects on serum FSH, but they found a significant increase in serum FSH in the early follicular phase of the following cycle. The report of Fraser *et al.* also indicates that inhibin is not the sole factor in suppressing FSH secretion during the luteal phase [24] but probably plays an important role in the selective inhibition of FSH release, particularly in the luteal-follicular transition phase where circulating progesterone and E2 are quite low [5]. Because PCOD patients have no cycles and their pituitary gland is chronically exposed to slightly increased inhibin, it is likely that inhibin is involved in modulating FSH secretion in women with PCOD.

Most studies thus far reported failed to demonstrate an inverse correlation between inhibin and FSH in normal subjects [11, 25]. This is presumed to be a reciprocal relationship between the two hormones in a negative feedback system [26]. The present study demonstrated a positive correlation between IR-inhibin and FSH in women with hypogonadotropic amenorrhea, indicating that the stimulating effect of FSH on inhibin secretion overcomes the inhibitory effect of inhibin on FSH release in these women. On the other hand, in women with PCOD, the correlation between FSH and inhibin becomes rather negative. Our present study suggests that the reciprocal relationship is probably functioning to keep serum FSH low or normal in women with PCOD, but further studies are necessary to draw a conclusion.

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