0021-972X/96/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 81, No. 4
Printed in U.S.A.

# Measurement of Dimeric Inhibin B throughout the Human Menstrual Cycle*

NIGEL P. GROOME, PETER J. ILLINGWORTH, MARTIN O'BRIEN, ROGER PAI, FAYE E. RODGER, JENNIE P. MATHER, AND ALAN S. McNEILLY

*School of Biological and Molecular Sciences, Oxford Brookes University (N.P.G.), Oxford, UK; Medical Research Council Reproductive Biology Unit, Center for Reproductive Biology (P.J.I., F.E.R., A.S.M.), Edinburgh, UK; and Genentech, Inc. (R.P., J.P.M.), South San Francisco, California 94080*

**ABSTRACT**

This report describes the development of a specific and sensitive assay for inhibin B and its application to the measurement of inhibin B concentrations in plasma during the human menstrual cycle. A monoclonal antibody raised against a synthetic peptide from the βB-subunit was combined with an antibody to an inhibin α-subunit sequence in a double antibody enzyme-linked immunosorbent assay format. The validated assay had a limit of detection of 10 pg/mL and 0.5% cross-reactivity with inhibin A.

Using this immunoassay, we found that the plasma concentration of inhibin B rose rapidly in the early follicular phase to a peak of 85.2 ± 9.6 pg/mL on the day after the intercycle FSH rise, then fell progressively during the remainder of the follicular phase. Two days after the midcycle LH peak, there was a short lived peak in the inhibin B concentration (133.6 ± 31.2 pg/mL), which then fell to a low concentration (<20 pg/mL) for the remainder of the luteal phase. In contrast, the inhibin A concentration was low in the early follicular phase, rose at ovulation, and was maximal during the midluteal phase. The concentration of inhibin B in individual follicular fluid samples was 20- to 200-fold higher than the concentration of inhibin A and was highest in follicular fluid samples from the early follicular phase.

Inhibin B appears to be the predominant form of inhibin in the preovulatory follicle. The different patterns of circulating inhibin B and inhibin A concentrations observed during the human menstrual cycle suggest that these forms may have different physiological roles. (*J Clin Endocrinol Metab* **81:** 1401–1405, 1996)

THE NONSTEROIDAL hormone, inhibin, is defined by the property of suppression of gonadotropin secretion (1). The role of this hormone in the follicular phase of the female menstrual cycle, however, has been controversial. This is largely because previous measurements of inhibin have shown no change in concentration during the early follicular phase, with maximum levels occurring after ovulation (2, 3). Inhibin, however, exists in a wide variety of forms; the bioactive molecules consist of an α-subunit disulfide-linked to either a βA-subunit (inhibin A) or a βB-subunit (inhibin B) (1). Early techniques for inhibin immunoassay had extensive cross-reaction with potentially inactive precursor forms (4). More recently, double antibody enzyme-linked immunosorbent assays (ELISAs) have been developed that are specific for dimeric inhibin (2, 5, 6). Only one assay has had sufficient sensitivity to measure plasma concentrations throughout the female menstrual cycle (2). However, it has been unclear whether this assay format could distinguish between inhibin A and inhibin B. Indeed, the demonstration of specificity for the A and B forms of inhibin has been hampered until recently by the lack of a suitable inhibin B standard.

We report here that our previously described assay for dimeric inhibin (2) does not cross-react with inhibin B and is, thus, specific for inhibin A forms. The development is reported here of the first highly sensitive assay format with specificity for inhibin B, and this is used to study changes in the circulating concentration of inhibin B during the normal female menstrual cycle.

Received August 8, 1995. Revision received November 29, 1995. Accepted December 26, 1995.

Address all correspondence and requests for reprints to: Prof. N. P. Groome, School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, United Kingdom OX3 0BP.

* This work was supported by a project grant from the United Kingdom Medical Research Council.

## Materials and Methods

### *Subjects*

Daily plasma samples were collected for at least 5 weeks from six women (aged 25–39 yr) with regular menstrual cycles of between 26–31 days in duration. The period of sampling was extended to include one complete menstrual cycle as well as one luteal-follicular transition for each woman. Eight other women with regular menstrual cycles who were undergoing laparotomy for benign gynecological conditions at different stages of the physiological follicular phase underwent aspiration of the largest visible ovarian follicle at the time of surgery. All subjects studied were healthy, and none had received any form of hormonal therapy within the preceding 3 months. All samples were stored at −20 C. The plasma samples were assayed for dimeric inhibin (inhibin A), inhibin B, estradiol, progesterone, LH, and FSH. The follicular fluid aspirates were assayed for inhibin A, inhibin B, estradiol, and testosterone.

Informed consent was obtained from all subjects. All studies were approved by the Lothian ethics of medical research committee, reproductive medicine subcommittee.

### *Inhibin B assay*

*Monoclonal antibodies.* Preparation of the R1 monoclonal antibody reacting with the human inhibin α-subunit and preparation and use of its F(ab) fragment coupled to alkaline phosphatase have been previously described for use in our inhibin A assay (2, 7, 8). A new monoclonal antibody to the inhibin βB-subunit was made by immunizing HPG hypogonadal mice with a synthetic peptide from near the C-terminal of the mature βB-subunit (9). The synthetic peptide IPTKLSTMSMLYFDDEYNI VKRDVPNMIVEECG, made by standard Fmoc chemistry (10), was coupled to tuberculin and used to immunize BCG-primed mice, as

described previously for a similar peptide from the βA-subunit (8). The mice were immunized sc three times at monthly intervals with conjugate containing 50 μg peptide in Freund's incomplete adjuvant. Finally, the mice were boosted iv with the same amount of antigen in saline. Four days later, the spleen was removed, and the splenocytes were fused to SP2/0 myeloma cells using polyethylene glycol and standard protocols (11). The fusion supernatants were screened using microplates coated with purified porcine activin B and standard ELISA procedures, as previously described (7, 8). Clone C5, secreting an IgG2a antibody, was selected on the basis of its strong ELISA reaction. The hybridoma was grown to produce ascites fluid in nude mice, and the IgG was purified by protein chromatography by standard methods (12).

*Inhibin B standard.* Recombinant human inhibin B was used as the standard. The material was prepared at Genentech (South San Francisco, CA) by coexpression of the human inhibin α and βB genes in Chinese hamster ovary cells. Conditioned medium was collected, and inhibin B was purified using several steps. These steps include anion exchange chromatography on diethylaminoethyl-Sepharose, hydrophobic interaction chromatography on phenyl-Toyopearl and phenyl-Sepharose CL-4B, and gel filtration chromatography on Sephacryl S-100. This procedure produces highly purified inhibin B (>95%), and the protein concentration of the purified material was determined by either amino acid analysis or absorbance at 280 nm using an extinction coefficient of 1.5. The identity of the final material was confirmed by N-terminal sequencing. Amino acid sequencing revealed only two sequences: STPLMSWPWSPSALR (170 pmol), corresponding to the expected intact amino-terminal of the α-subunit, and GLEXDGRTNLXXRQQ (215 pmol), corresponding to the expected amino-terminal of the βB-subunit. The slight excess of the latter may be accounted for by the presence of trace amounts of contaminating activin dimer seen on the silver-stained gel (estimated to be <5% of protein, but with two sequences per dimer) or by the presence of N-terminally truncated forms of the inhibin dimer. However, if these were present, they were not detected by sequencing and, therefore, did not constitute a major contaminant. Truncation of the N-terminus, which has been observed previously during purification and storage of inhibin A, does not affect the bioactivity of inhibin A (our unpublished results), but would eliminate the epitope detected by the R1 monoclonal antibody in both our inhibin A and inhibin B ELISAs.

*ELISA procedure for inhibin B.* The monoclonal antibody C5 was biotinylated by standard procedures (12) and reacted with commercially available streptavidin-coated microplates (Polymer Laboratories, Church Stretton, UK). To each well of the dry streptavidin plates, 50 μL 5 μg/mL C5 IgG in 1% (wt/vol) BSA were added. The plates were then incubated overnight at room temperature, shaken dry, and blocked by the addition of 100 μL 1% (wt/vol) BSA in 25 mmol/L Tris-HCl buffer, pH 7.5, containing 0.1% (wt/vol) sodium azide. Coated plates were stored in this form in a moist box at 4 C and washed immediately before use with ELISA wash solution [0.05% (wt/vol) Tween 20 and 0.15 mol/L NaCl in 0.05 mmol/L Tris-HCl (pH 7.5)].

Recombinant inhibin B standards were diluted in postmenopausal human serum. Fifty microliters of 6% (wt/wt) aqueous SDS (Sigma Chemical Co., Poole, UK) were added to 100-μL portions of standards or patients' plasma samples in Eppendorf tubes. The tubes were heated at 100 C for 3 min and cooled, followed by the addition of 100 μL assay diluent. The assay diluent was 5% (wt/vol) Triton X-100 (Sigma), 10% (wt/vol) BSA (A3294, Sigma), 5% mouse serum (Serotec, Kidlington, UK), and 0.15 mol/L NaCl in 25 mmol/L Tris-HCl buffer, pH 7.5. To each tube were added 50 μL 6% (wt/vol) hydrogen peroxide (BDH, Poole, UK), and the samples were allowed to stand for 30 min at room temperature. This step oxidizes methionine residues in the epitope for the C5 monoclonal and improves immunoreactivity. A similar procedure was previously described for our inhibin A assay (2, 13).

A C5 monoclonal antibody-coated microplate, prepared as described above, was washed and shaken dry, and 100 μL of each treated sample were added in duplicate to the plate. The plate was covered and incubated overnight on a shaker at room temperature. The following day, the plate was washed, and 50 μL F(ab) R1 alkaline phosphatase conjugate were added in assay diluent. The optimal concentration of this was determined for each batch of conjugate. The plate was shaken at room temperature for 1 h and thoroughly washed before the addition of 50 μL alkaline phosphate substrate (BRL, Paisley, UK) before being covered and shaken for 2 h at room temperature. After this time, 50 μL amplifier (BRL) were added to each well, and color was allowed to develop for 10–20 min. The reactions were then stopped by the addition of 50 μL 0.4 mol/L HCl to each well, and the plate was read at 490 nm on an automated ELISA plate reader (Biotek Instruments, Winooski, VT). Curve fitting and dose interpolation were performed by software associated with the reader.

*Assay performance.* The assay detection limit was less than 10 pg/mL. Activin A, activin B, follistatin, and purified human pro-αC had less than 0.1% cross-reaction, whereas recombinant inhibin A had 0.5% cross-reaction in the inhibin B ELISA. Within- and between-plate coefficients of variation (CVs) were less than 10%. Recovery of inhibin B spiked into female plasma samples was quantitative (mean, 115%; n = 5). Serially diluted patient samples gave dose responses parallel to that of the recombinant standard (Fig. 1).

### *Other assays*

Inhibin A assays were carried out as previously described (2). Activin A, activin B, follistatin, and inhibin B all had less than 0.1% cross-reaction, thus indicating that this assay is specific for inhibin A. The assay sensitivity was 2 pg/mL.

The concentrations of FSH and LH were measured by previously described RIAs (14), with assay sensitivities of 0.9 and 0.8 IU/L, respectively, and within-assay variabilities of 5.0% and 4.6%, respectively. Testosterone was measured by a previously described RIA (15). Progesterone was assayed in duplicate by direct RIA with a sensitivity of 0.6 nmol/L, an interassay CV of 15.0%, and a within-assay CV of 6.4%. Estradiol was assayed by RIA after extraction by diethyl ether (14). The assay sensitivity was 25 pmol/L, and the inter- and intraassay CVs were 15.8% and 4%, respectively.

All data on plasma hormone concentrations are expressed as the mean ± SEM.

## Results

The mean concentrations of inhibin A, inhibin B, LH, FSH, estradiol, and progesterone through a normal menstrual cycle are shown in Fig. 2 aligned relative to the day of the midcycle plasma LH peak. Each of the six subjects undergoing daily sampling was judged to have ovulated during the study cycle, as demonstrated by a midcycle LH peak and serial changes in the concentrations of estradiol and progesterone. To demonstrate changes relative to the luteal-follicular transition, the mean concentrations of inhibin A, inhibin B, FSH, and estradiol were aligned relative to the day of the early follicular phase FSH peak and are shown in Fig. 3.

The plasma concentration of inhibin A remained low (<10 pg/mL) during the early follicular phase (Fig. 2) and only

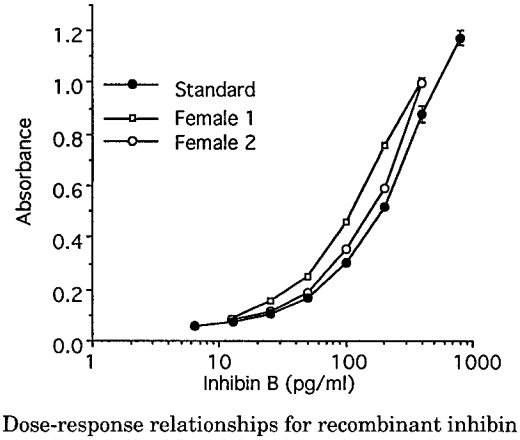


FIG. 1. Dose-response relationships for recombinant inhibin B standard alongside serially diluted female serum samples.

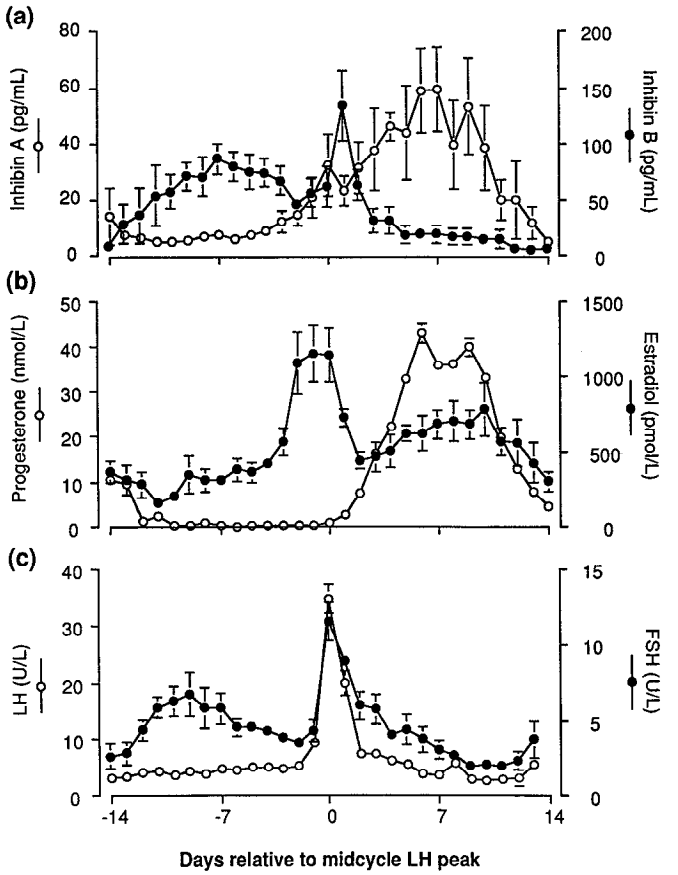


FIG. 2. Plasma concentrations of a) inhibin A and inhibin B, b) estradiol and progesterone and c) LH and FSH, during the female menstrual cycle. Data were aligned with respect to the day of the midcycle LH peak. Mean concentrations are shown ± SE.

rose in the late follicular phase to reach a peak concentration (32.3 ± 11.0 pg/mL) on the day of the LH peak. After the LH peak, the concentration fell briefly before rising to reach a maximum concentration (59.5 ± 15.0 pg/mL) during the midluteal phase. The inhibin A concentration fell synchronously with the drop in progesterone during the late luteal phase.

The pattern of change in the plasma concentration of inhibin B was very different (Fig. 2). The plasma concentration was high in the early follicular phase (86.8 ± 13.8 pg/mL) and fell in the late follicular phase during the days before ovulation. There was no increase in inhibin B at the time of the midcycle LH peak, but 2 days later, there was a short lived peak in the hormone concentration (133.6 ± 31.2 pg/mL) before the concentration dropped to a low level (<20 pg/mL) by the middle of the luteal phase (Fig. 2). The plasma inhibin B concentration subsequently remained low during the luteal phase and did not change during luteal involution.

During the luteal-follicular transition (Fig. 3), the inhibin B concentration rose rapidly from a very low concentration (<10 pg/mL) to reach a peak of 85.2 ± 9.6 pg/mL 4 days after the peak concentration of FSH was reached. In contrast, the inhibin A concentration fell synchronously with estradiol and progesterone (data not shown) during the late luteal phase to remain at a concentration less than 10 pg/mL during

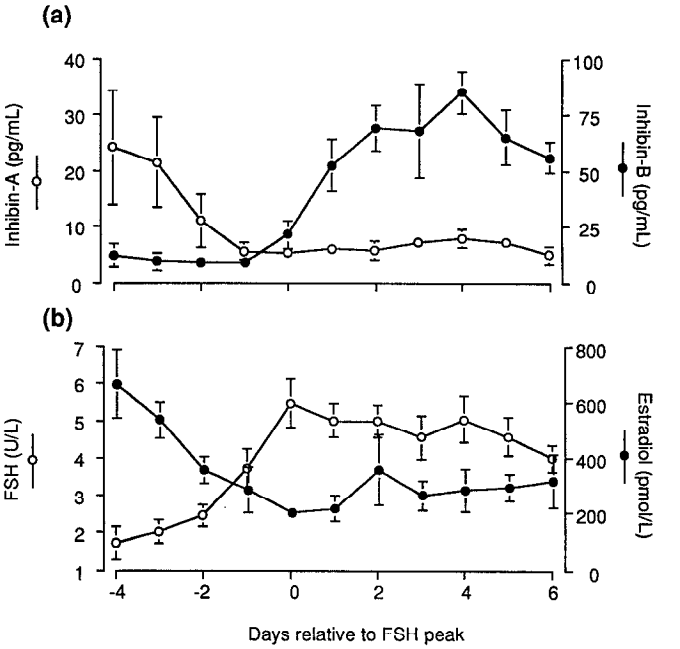


FIG. 3. Plasma concentrations of a) inhibin A and inhibin B, and b) estradiol and FSH during the luteal-follicular transition. Data were aligned with respect to the day of the intercycle FSH peak. Mean concentrations are shown ± SE.

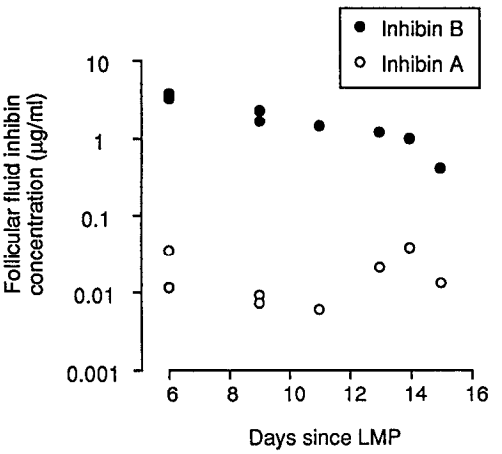


FIG. 4. Individual concentrations of inhibin A (○) and inhibin B (●) in the largest visible ovarian follicles relative to the number of days since menstruation.

the early follicular phase rise in FSH. During the early follicular phase, there was only a small rise in the mean estradiol concentration from a nadir of 201.3 ± 18.0 pmol/L to a concentration of 318.7 ± 95.1 pmol/L by day 6 after the early cycle FSH peak.

The concentrations of inhibin A and inhibin B for each of the individual follicles studied are shown in Fig. 4. The inhibin B concentration in each follicular fluid sample was from 20- to 100-fold higher than the corresponding follicular fluid inhibin A concentration. The inhibin B concentration was relatively higher in the early follicular phase follicles and fell as the fluid was aspirated from progressively more mature follicles. In contrast, the follicular fluid inhibin A concentrations tended to be higher in the more mature follicles.

The estradiol concentration in the follicular fluid samples ranged from 481-1860 ng/mL, whereas the testosterone concentration ranged from 22–145 ng/mL. The ratio of estradiol/testosterone was greater than 5:1 in all follicles studied. Although the estradiol concentrations tended to be higher in the more mature follicles, no consistent trends were observed.

## Discussion

This is the first time that inhibin B has been successfully assayed in normal women throughout the menstrual cycle. The demonstration that our previously described assay for dimeric inhibin (2) has less than 0.1% cross-reaction with recombinant inhibin B indicates that this assay is specific for inhibin A. The plasma concentration profile of dimeric inhibin A during the female menstrual cycle measured with this assay (2) is broadly similar to that of inhibin measurements obtained by other less specific assays (16, 17).

The different pattern of inhibin B concentration during the menstrual cycle, however, was quite unexpected. The timing of the early rise in the plasma inhibin B concentration suggests that it is secreted by antral follicles in response to FSH. However, it is unclear whether the inhibin B originates from one selected dominant follicle or from all the antral follicles present. The low concentration seen during the late luteal phase, when there are no follicles greater diameter than 4 mm (18, 19), suggests that despite expression of messenger ribonucleic acid (mRNA) for the βB-subunit (20, 21), these small follicles do not secrete intact inhibin B. Secretion of dimeric inhibin B, therefore, may be dependent upon a FSH-induced increase in the level of α-subunit expression to reach the relative excess of α-subunit mRNA required for dimeric inhibin synthesis (22).

Inhibin B secretion from the antral follicle(s) may be short lived. The concentration of inhibin B in both follicular fluid and plasma falls approaching ovulation, and βB mRNA expression is markedly reduced in the mature preovulatory follicle (20, 21, 23). The source of the peak in plasma inhibin B concentration occurring on the day after ovulation is, therefore, unclear. This peak is clearly later than the periovulatory peaks of inhibin A (2) and pro-αC-related immunoreactivity (24), and the corpus luteum does not express mRNA for the βB-subunit (20, 21, 23). The peak may represent release from the ruptured follicle into the peritoneal cavity rather than *de novo* secretion. The low inhibin B concentration in the midluteal phase makes it unlikely that the corpus luteum secretes inhibin B. This is supported by the observation that stimulation of the corpus luteum with hCG (leading to an increase in pro-αC-RI and inhibin A concentrations) has no effect on the inhibin B concentration (25). All of these observations are in direct contrast to the pattern for inhibin A, where both the plasma concentration and expression of the βA-subunit (20, 23) are higher in the luteal phase than the follicular phase.

The discrepancy between the pattern of inhibin B concentrations reported here and previous reports of inhibin concentration during the female menstrual cycle may be due to a number of factors. Previous inhibin assays have exhibited a high degree of cross-reaction with α-subunit inhibin precursor forms (4). We recently reported high circulating concentrations of pro-αC-RI during the early follicular phase (24). In addition, conformational differences imparted by the presence of different β-subunits appear to cause large differences in the immunoreactivity detected with various α-subunit-directed immunoassays. In the Monash assay, for example, inhibin B had only 12.3% the cross-reactivity of inhibin A (26). This limited cross-reactivity combined with the limited sensitivity of the Monash assay (detection limit, 360 pg/mL) may explain why the rise in inhibin B we report in the early follicular phase was not detectable by the Monash assay. In contrast, the Genentech CK/CK assay (5) has an 80% cross-reactivity with inhibin B (26) and detects almost equal amounts of inhibins across the menstrual cycle (6).

The absolute values for inhibin B described here should be regarded as provisional. Genentech recombinant 32-kDa inhibin B is the only well characterized human inhibin B standard available. However, human inhibin B, like inhibin A, occurs in several size variants with different degrees of α-subunit glycosylation (26, 27). Different inhibin A assays are known to have varying cross-reactivities with the different inhibin A forms (26). The exact forms detected in this assay and the extent of their cross-reactivity are, therefore, unknown.

The rapid rise in inhibin B concentration after the intercycle FSH rise represents the first observation of a major change in the plasma concentration of an ovarian product at this stage of the menstrual cycle. The timing raises the possibility of a role for inhibin B in limiting the duration of the intercycle FSH rise. Estradiol is currently thought to be the critical factor in the regulation of FSH during the early follicular phase and the preceding fall in estradiol (with or without inhibin A) associated with luteolysis is thought to initiate the intercycle FSH rise (28). Inhibition of estradiol action by the administration of either antiestradiol antibodies in primates (29) or estrogen antagonists in women (30) has been shown to raise FSH secretion, leading to an increase in the number of follicles selected. Conversely, the administration of moderate amounts of exogenous estradiol has been shown to exert a suppressive effect on FSH secretion (31). However, although it is clear from this that estradiol can have an important effect on FSH secretion, the plasma concentration of estradiol exhibits only a limited rise during the very early follicular phase.

It is not clear whether these circulating concentrations of inhibin B are sufficient to fulfill an endocrine role in FSH suppression. It has recently been shown that human inhibin B has little apparent effect compared to that of inhibin A in the sheep pituitary cell bioassay (26), although this may be due to species differences. We recently demonstrated a clear inverse correlation between similar plasma levels of inhibin B and the corresponding FSH concentration in both fertile and infertile men (32).

These data show that inhibin B is the predominant form of inhibin present within follicular fluid. Previous studies have demonstrated critical paracrine roles for inhibin A and activin A in the local regulation of ovarian function (33). Due to the lack of suitable preparations of inhibin B for *in vitro* studies, it remains unknown whether inhibin B and activin B have the same paracrine effects as inhibin A and activin A.

In summary, the inhibin B ELISA offers a potentially in-

valuable tool for elucidation of the control of follicular development as well as potential clinical applications in investigating disorders of female fertility. The possibility suggested by these data, that inhibin B exerts a critical physiological role in the follicular phase, requires further investigation.

## Acknowledgments

The authors thank Dr. H. Sugino for the gift of porcine activin B, Mr. Len Bagnall for assistance with the animal work, and Mr. Ian Swanston and Ms. Vivian Grant for expert technical assistance. Dr. H. Charlton generously donated the hypogonadal mice used.

## References

1. **Burger HG, Igarashi M.** 1988 Inhibin: definition and nomenclature, including related substances. J Clin Endocrinol Metab. 66:885–886.
2. **Groome NP, Illingworth PJ, O'Brien M, et al.** 1994 Detection of dimeric inhibin throughout the human menstrual cycle by two-site enzyme immunoassay. Clin Endocrinol (Oxf). 40:717–723.
3. **Robertson DM, Tsonis CG, McLachlan RI, et al.** 1988 Comparison of inhibin immunological and *in vitro* biological activities in human serum. J Clin Endocrinol Metab. 67:438–443.
4. **Schneyer AL, Mason AJ, Burton LE, Zeigner JR, Crowley WF.** 1990 Immunoreactive inhibin α-subunit in human serum: implications for radioimmunoassay. J Clin Endocrinol Metab. 70:1208–1212.
5. **Baly DL, Allison DE, Krummen LA, et al.** 1993 Development of a specific and sensitive two-site enzyme-linked immunoadsorbent assay for measurement of inhibin-A in serum. Endocrinology. 132:2099–2108.
6. **Woodruff TK, Krummen LK, Baly D, et al.** 1994 The measurement of the inhibin family of hormones in human serum. In: Burger HG, Findlay JK, Robertson DM, de Kretser D, Petraglia F, eds. Inhibin and inhibin-related proteins. Rome: Ares-Serono Symposia; 55–68.
7. **Groome NP, Hancock J, Betteridge A, Craven R.** 1990 Monoclonal and polyclonal antibodies reactive with the 1–32 amino terminal peptide of 32K human inhibin. Hybridoma. 9:31–41.
8. **Groome NP, Lawrence M.** 1991 Preparation of monoclonal antibodies reacting with beta-A subunit of human ovarian inhibin. Hybridoma. 10:309–316.
9. **Mason AJ, Hayflick JS, Ling N, et al.** 1985 Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor-B. Nature. 318:659–663.
10. **Atherton E, Sheppard RC.** 1989 Solid phase peptide synthesis. a practical approach. Oxford: IRL Press.
11. **Galfre G, Milstein C.** 1981 Preparation of monoclonal antibodies: strategies and procedures. In: Langone JJ, Vunakis HV, eds. Methods in enzymology. New York: Academic Press; 73:3–36.
12. **Harlow E, Lane D.** 1988 Antibodies—a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory.
13. **Knight PG, Muttukrishna S, Groome NP.** 1996 Development and application of a two-site enzyme immunoassay for the determination of "total" activin-A concentrations in serum and follicular fluid. J Endocrinol. 148:267–279.
14. **Backstrom CT, McNeilly AS, Leask R, Baird DT.** 1982 Pulsatile secretion of LH, FSH, prolactin, oestradiol and progesterone during the human menstrual cycle. Clin Endocrinol (Oxf). 7:29–42.
15. **Corker DS, Davidson DW.** 1978 Radioimmunoassay of testosterone in various biological fluids without chromatography. J Steroid Biochem. 9:373–374.
16. **McLachlan RI, Robertson DM, Healy DL, Burger HG, de Kretser DM.** 1987 Circulating immunoreactive inhibin levels during the normal menstrual cycle. J Clin Endocrinol Metab. 65:954–961.
17. **Roseff SJ, Bangah ML, Kettel LM, et al.** 1989 Dynamic changes in circulating inhibin levels during the luteal-follicular transition of the human menstrual cycle. J Clin Endocrinol Metab. 69:1033–1039.
18. **Govan ADT.** 1970 The human ovary in early pregnancy. J Endocrinol. 40: 421–429.
19. **McNatty KP, Hillier SG, Van Den Boogaard AMJ, Trimbos-Kemper TCM, Reichert LEJ, Van Hall EV.** 1983 Follicular development during the luteal phase of the human menstrual cycle. J Clin Endocrinol Metab. 56:1022–1031.
20. **Roberts VJ, Barth S, Elroeiy A, Yen SSC.** 1993 Expression of inhibin/activin subunits and follistatin messenger ribonucleic-acids and proteins in ovarian follicles and the corpus-luteum during the human menstrual-cycle. J Clin Endocrinol Metab. 77:1402–1410.
21. **Fraser HM, Lunn SF, Whitelaw PF, Hillier SG.** 1995 Induced luteal regression: differential effects on follicular and luteal inhibin/activin subunit mRNAs in the marmoset monkey. J Endocrinol. 144:201–208.
22. **Mason AJ, Schwall RH, Renz M, Rhee LM, Nikolics K, Seeburg PH.** 1987 Human inhibin and activin: structure and recombinant expression in mammalian cells. In: Burger HG, de Kretser DM, Findlay JK, Igarashi M, eds. Inhibin: non-steroidal regulation and recombinant expression in mammalian cells. New York: Raven Press; 89–103.
23. **Schwall RH, Mason AJ, Wilcox JN, Bassett SG, Zeleznik AJ.** 1990 Localization of inhibin/activin subunit mRNAs within the primate ovary. Mol Endocrinol. 4:75–79.
24. **Groome NP, Illingworth P, O'Brien M, Priddle J, Weaver K, McNeilly AS.** 1995 Quantification of inhibin forms containing pro-αC in human serum by a new ultrasensitive ELISA. J Clin Endocrinol Metab. 80:2926–2932.
25. **Illingworth PJ, Groome NP, Duncan WC, et al.** 1995 Measurement of circulating inhibin forms during the establishment of pregnancy. J Clin Endocrinol Metab. 81:1471–1475.
26. **Robertson D, Burger H, Sullivan J, et al.** 1995 Biological and immunological characterization of inhibin forms in human plasma. J Clin Endocrinol Metab. In press.
27. **Robertson DM, Sullivan J, Watson M, Cahir N.** 1995 Inhibin forms in human plasma. J Endocrinol. 144:261–269.
28. **Baird DT, Baker G, McNatty KP, Neal P.** 1975 Relationship between the secretion of the corpus luteum and the length of the follicular phase of the ovarian cycle. J Reprod Fertil. 45:611–619.
29. **Zeleznik AJ, Hutchison JS, Schuler HM.** 1985 Interference with the gonadotropin-suppressing actions of estradiol in macaques overrides the selection of a single preovulatory follicle. Endocrinology. 117:991–999.
30. **Vaitukaites JL, Bermudez JA, Cargille M, Ipsett B, Ross T.** 1971 New evidence for an anti-estrogenic action of clomiphene citrate in women. J Clin Endocrinol Metab. 32:503–508.
31. **Zeleznik AJ.** 1981 Premature elevation of systemic estradiol reduces serum levels of follicle-stimulating hormone and lengthens the follicular phase of the menstrual cycle in rhesus monkeys. Endocrinology. 109:352–355.
32. **Illingworth PJ, Groome NP, Byrd W, Rainey WE, Mather J, Bremner WJ.** 1995 Inhibin-B: a potential candidate for the principal bioactive inhibin form in plasma in men. J Clin Endocrinol Metab. In press.
33. **Hillier SG.** 1991 Regulatory functions for inhibin and activin in human ovaries. J Endocrinol. 131:171–175.