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Serum inhibins, estradiol, progesterone and FSH in surgical menopause: a demonstration of ovarian pituitary feedback loop in women

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BACKGROUND: The objective of this study was to confirm the source and study the acute changes and relationship between inhibins and FSH at surgical menopause. METHODS: Regularly cycling women (42–47 years; $n = 10$) undergoing bilateral oophorectomy for non-ovarian pathology were recruited for this study. One blood sample was taken before surgery and after removal of the ovaries, samples were taken every 15 min up to 1 h, hourly up to 6 h, after 12 h and daily during the hospital admission (3 days). RESULTS: There were five women in the follicular phase and five women in the luteal phase of the cycle. For women in both phases, levels of inhibin A, inhibin B, estradiol (E_2) and progesterone decreased after the removal of the ovaries. Serum FSH levels started to rise after 12 h in both follicular and luteal phase women after the surgical menopause. Correlation analysis showed that inhibin A and E_2 were significantly negatively correlated in both phases with FSH concentration. Inhibin B had a negative correlation in the follicular phase and progesterone had a negative correlation in the luteal phase. CONCLUSIONS: This study showed that ovarian inhibin A and B were cleared from the circulation within 12 h of oophorectomy, whereas E_2 and progesterone remain in the circulation for longer. Negative correlation between FSH, inhibin A and inhibin B suggests that inhibins may contribute to the observed early rise in FSH after the surgical menopause.

Key words: FSH/inhibin/menopause/estradiol/progesterone

Introduction

Dimeric inhibins and activins were initially characterized as gonadal glycoprotein hormones regulating pituitary FSH secretion. In addition to the ovary there are reports confirming extragonadal sources (e.g. adrenal, bone marrow, placenta, embryonic tissue, pituitary) of inhibin/activin subunits and follistatin mRNA expression (Meunier *et al*., 1988). In reproductive ageing, circulating concentrations of inhibin A and B decrease with increasing age and disappear after menopause in women. This is evidence that the ovary produces inhibins. Previous studies have shown that, after bilateral oophorectomy, in cycling women levels of FSH rise earlier than LH, suggesting that FSH is more sensitive to ovarian feedback (Barlow *et al*., 1981). Metabolic clearance studies show that secretion rates of FSH and LH are higher during the follicular phase than during the luteal phase of a cycle (Kohler *et al*., 1968; Coble *et al*., 1969). Yen and Tsai reported that immediately after the surgical removal of the ovaries, FSH and LH concentrations rose rapidly in the follicular phase whereas in the luteal phase concentrations rose slowly (Yen and Tsai, 1971). The net increase in serum FSH was higher than that in serum LH after surgery in both phases of the cycle (Yen and Tsai, 1971). In reproductive ageing, there is no clear relationship between increasing FSH and circulating E_2 until the menopause. In this study we investigate the endocrine mechanism involved in the rapid rise in FSH at the menopause induced by surgery. The gradual decline in circulating inhibins in the years preceding the menopause, their absence in post-menopausal women and the reduction of inhibin A and B that is significant after bilateral oophorectomy are indications that ovaries are the predominant source of circulating inhibins in pre-menopausal women (Burger *et al*., 1998; Cobellis *et al*., 2002).

The objectives of this study were: (i) to confirm the source of inhibin A and B in cycling women and their disappearance after the removal of the source; and (ii) to study the endo-

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crine feedback relationship between FSH, inhibins, E_2 and progesterone at surgical menopause.

Materials and methods

Study design

Regularly cycling women between the ages of 42 and 47 years (n = 5, follicular phase; n = 5, luteal phase) with day 1–5 FSH levels <10 mIU/ml were recruited for the study. All women had oophorectomy for non-ovarian pathology and were not on any hormonal therapy prior to surgery. One blood sample was taken before the removal of the ovaries. At surgery the time interval between the removal of one ovary and the removal of the second was ~5 min. The index time for 'time zero' was the removal of the second ovary. Samples were then taken every 15 min from time zero up to 1 h, hourly up to 6 h, after 12 h and daily during their hospital stay (~4 days). On day 3, some women were started on hormone replacement therapy (n = 4). Therefore the time-dependent changes in hormones were studied until 3 days after surgery.

The Barking and Havering Health Authority Research Ethics Committee approved the protocol and written consent was obtained from subjects before enrolling for the study. Serum was separated and stored at –20°C for hormone measurements.

Hormone measurements

Inhibin A

Serum concentrations of dimeric inhibin A were measured in duplicate 50 µl aliquots as described elsewhere (Muttukrishna *et al.*, 1994). The mean intra- and inter-assay coefficients of variation (CV) were 4.3 and 5.1% respectively. Minimum detection limit of the assay for human recombinant inhibin A (National Institute for Biological Standards and Control, Potters Bar, Herts, UK) was 2 pg/ml.

Inhibin B

Serum concentrations of dimeric inhibin B were measured in 50 µl duplicates using an enzyme immunoassay as described elsewhere (Muttukrishna *et al.*, 2000). An in-house standard preparation (partially purified human follicular fluid) was standardized against human recombinant inhibin B (Genentech, San Francisco, CA, USA) and was used as the assay standard. Minimum detection limit of the assay for human recombinant inhibin B was 15 pg/ml. The mean intra- and inter-assay CV were 6.2 and 7.2% respectively.

Gonadotrophins and steroids

Serum concentrations of FSH and LH were measured using Immulite chemiluminescent assay kits (DPC, Glyn Rhonwy, Llanberis, Gwynedd, UK). The detection ranges of FSH and LH were 0.1–170 and 0.7–400 mIU/ml respectively. Progesterone was measured by an in-house radioimmunoassay (following ether extraction and using tritium-labelled tracers) as previously described (Darne *et al.*, 1989). The minimum detection limit of the progesterone assay was 0.1 nmol/l.

Estradiol was measured by a double antibody 125 I sequential radioimmunoassay (DPC). The minimum detection limit of the assay was 4.8 pmol/l (0.0048 nmol/l). The mean intra- and inter- assay CV were <10% for all four assays.

Statistical analysis

Data were log-transformed to obtain a normal distribution. One-way analysis of variance (ANOVA) was carried out to investigate the significance of the time-dependent changes in the parameters studied. Pearson's correlation analysis was carried out to study the relationship between the hormones analysed, using SPSS statistical package (SPSS Inc., USA).

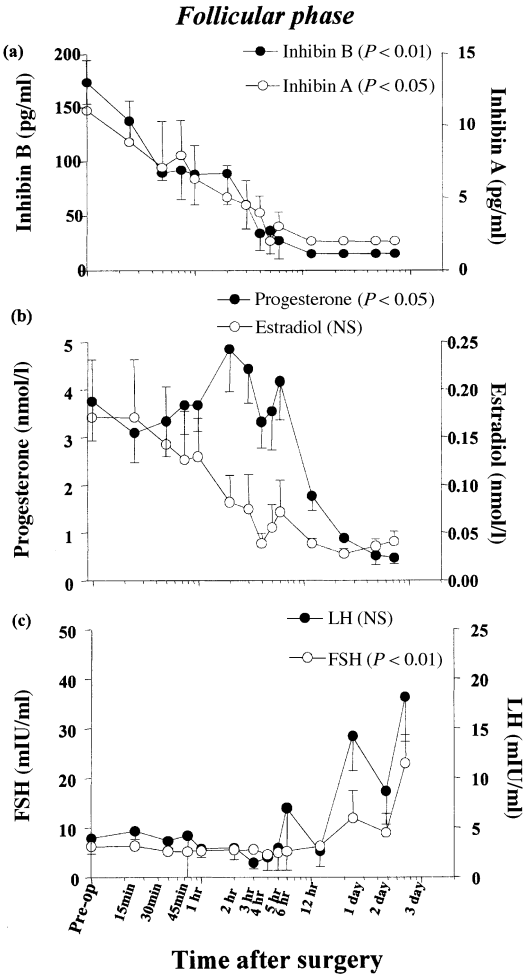

Figure 1. Mean ± SEM serum concentrations of (**a**) inhibin A and inhibin B (**b**) estradiol and progesterone and (**c**) FSH and LH before and after surgical menopause in the follicular phase (n = 5). One-way analysis of variance was carried out to investigate the time-dependent changes in concentration. NS = not significant.

Results

Five of the cycling women were in the follicular phase and five were in the luteal phase. All women had 24–34 day regular cycles with day 1–5 FSH <10 mIU/ml.

Follicular phase

Mean inhibin A and inhibin B levels were 11 ± 4 and 173.5 ± 46 pg/ml respectively in the early follicular phase. Inhibin A and inhibin B levels decreased rapidly and were below the limit of detection after 12 h [inhibin A <2 pg/ml (P < 0.05) and inhibin B 15 pg/ml; ANOVA: P < 0.01] of bilateral oophorectomy in women who had surgery in their early follicular phase (day 3–8; Figure 1a). Mean serum E_2 level was 0.171 ± 0.06 nmol/l in the patients. Estradiol levels fell rapidly within the first 6 h of surgery and then levels were stable after 6 h (0.028–0.042 nmol/l) until the end of the study period (Figure 1b). Mean progesterone level was 3.76 ± 0.84 nmol/l in the early follicular phase patients.

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Serum progesterone levels were not significantly altered for the first 6 h and then levels decreased until the end of the study (0.48 ± 0.1 nmol/l) ($P < 0.001$; Figure 1b).

Mean serum FSH was 6.3 ± 1.6mIU/ml; the levels did not significantly change until 6 h post surgery, after which there was a 2-fold rise between 12 and 24 h followed by a progressive rise throughout the 3 day study period. By the end of the study, mean FSH had risen by ~5 fold ($P < 0.01$, Figures 1c and 3a). There was a trend of rising LH levels after 24 h of surgery (Figures 1c and 3b) that was not significant.

Luteal phase

Mean inhibin A levels were 28.3 ± 7 pg/ml and the mean inhibin B levels were just above the detection limit (18.5 ± 5 pg/ml) in the luteal phase patients (day 16–23). Inhibin A levels fell rapidly within the first 2 h and the levels were below the limit of detection after 12 h ($P < 0.001$). Inhibin B levels fell below the limit of detection (15 pg/ml) within 30 min of bilateral oophorectomy in women who had their surgery in their luteal phase (Figure 2a). The mean serum E_2 and progesterone levels were 0.23 ± 0.06 and 26 ± 4 nmol/l respectively. Estradiol and progesterone levels fell rapidly within the first hour and continued to fall throughout the study period ($P < 0.001$; Figure 2b).

Mean serum FSH was 2.56 ± 0.24 mIU/ml. There was a rise in FSH levels after 6 h and then the levels were similar between 12 and 24 h. Again there was a steady rise after 24 h until 3 days after surgery (~5-fold; $P < 0.001$, Figures 2c and 3a). There was a trend of rising LH levels after 24 h of surgical menopause; the changes in LH concentration were not significantly altered (Figures 2c and 3b).

Relationship between hormones

Follicular phase

Correlation analysis shows that serum inhibin A concentrations were positively correlated with inhibin B ($r = 0.48$, $P < 0.001$) and progesterone ($r = 0.52$; $P < 0.001$), and negatively correlated with FSH ($r = –0.319$, $P < 0.05$). Inhibin B was positively correlated with E_2 ($r = 0.663$, $P < 0.001$) and progesterone ($r = 0.66$, $P < 0.001$) and negatively correlated with FSH ($r = –0.427$, $P < 0.01$). Estradiol was positively correlated with progesterone ($r = 0.41$, $P = 0.001$) and negatively correlated with FSH ($r = –0.53$, $P < 0.01$). Serum progesterone was negatively correlated with FSH ($r = –0.626$, $P < 0.001$) and FSH was positively correlated with LH ($r = 0.64$, $P < 0.001$).

Luteal phase

Correlation analysis shows that serum inhibin A was positively correlated with E_2 ($r = 0.585$; $P < 0.001$) and progesterone ($r = 0.871$, $P < 0.001$), and negatively correlated with FSH ($r = –0.8$, $P < 0.001$) and LH ($r = –0.5$, $P < 0.001$). Estradiol was positively correlated with progesterone ($r = 0.716$, $P < 0.001$) and negatively correlated with FSH ($r = –0.593$, $P < 0.001$). Serum progesterone was negatively correlated with FSH ($r = –0.791$, $P < 0.001$) and FSH was positively correlated with LH ($r = 0.73$, $P < 0.001$).

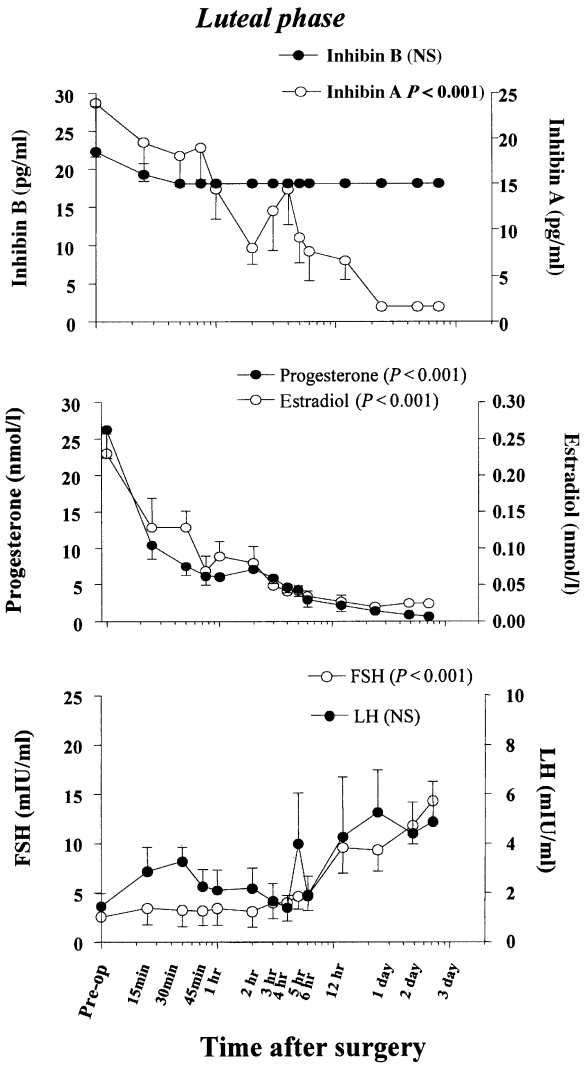

Figure 2. Mean ± SEM serum concentrations of (**a**) inhibin A and inhibin B, (**b**) estradiol progesterone and (**c**) FSH and LH before and after surgical menopause in the luteal phase ($n = 5$). One-way analysis of variance was carried out to investigate the time-dependent changes in concentration. NS = not significant.

Discussion

Several studies have investigated the changes in inhibin A, inhibin B and FSH and have shown that inhibin B is a marker of ovarian follicular reserve and that both inhibins decrease in reproductive ageing (Klein *et al*., 1996; Burger *et al*., 1998; Danforth *et al*., 1998; Santoro *et al*., 1999; Seifer *et al*., 1999). These studies provide evidence that the ovaries produce inhibins.

This is the first study to investigate the dynamic changes in inhibin A and inhibin B after surgical menopause. These data show that inhibin A and B are cleared from circulation within 12 h of bilateral oophorectomy, confirming the ovary as the predominant source for these circulatory proteins in normal cycling women. However, the presence of inhibin/activin subunit proteins in extragonadal tissue has been reported previously (Meunier *et al*., 1988), suggesting that extragonadal inhibins may act locally without making a significant contribution to the systemic circulation. It is clear that soon

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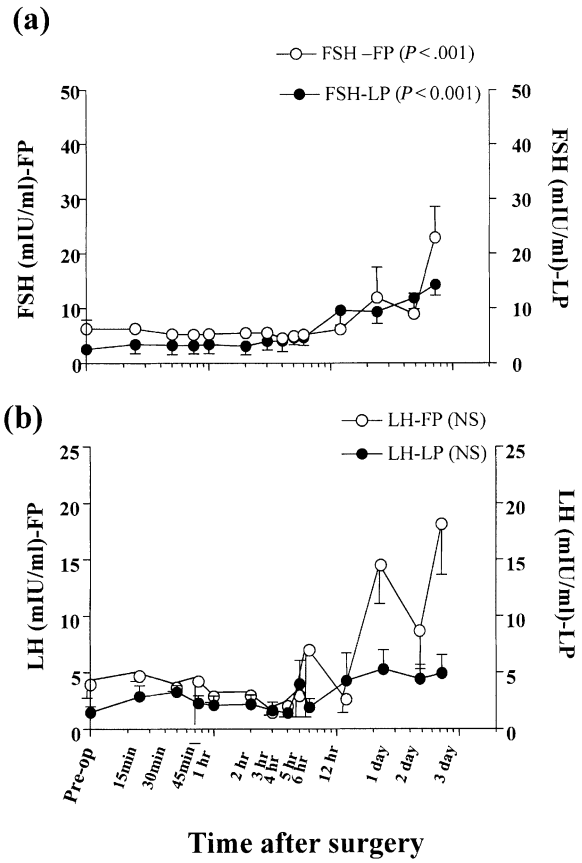

Figure 3. Mean ± SEM serum concentrations of (**a**) FSH and (**b**) LH before and after surgical menopause in the follicular (FP) and luteal (LP) phase (n = 5). One-way analysis of variance was carried out to investigate the time-dependent changes in concentration. NS = not significant.

after the removal of the ovary, inhibin A and inhibin B rapidly decrease in the circulation. Data show that the time taken to reach half-maximal concentration in the circulation (maximal concentration = the concentration before surgery) after surgical castration for inhibin A, inhibin B and E_2 in the follicular phase was 60, 45 and 60 min respectively, whereas in the luteal phase, half-maximal concentrations in the circulation were achieved for inhibin A, E_2 and progesterone after 60, 30 and 15 min respectively post castration, suggesting that E_2 and progesterone were cleared faster in the luteal phase.

We speculate that after the removal of the ovaries and the fall in inhibins, E_2 and progesterone increases the synthesis of FSH followed by increased FSH release. If there was a surge in the release of FSH from a pre-synthesized stored pool, concentrations would have risen with falling concentrations of inhibins and E_2 within the first few hours after surgery. However, although the magnitude of rise of FSH in the follicular phase was similar to the rise in the luteal phase, the absolute concentration of FSH was 2-fold higher in women who had surgical castration in their follicular phase, suggesting that the pituitary gland is more sensitive to negative feedback in the follicular phase. Mean LH levels started to rise within the study period but the rise was not significant, confirming a previous study which showed that FSH is more sensitive to negative feedback than LH (Yen and Tsai, 1971).

The acute changes in inhibin concentrations in this study are consistent with our previous observation in first trimester pregnancy termination (Muttukrishna *et al*., 1997). However, the mechanism(s) by which inhibins are cleared from the circulation is yet to be elucidated. It will be interesting to measure these proteins in urine samples to investigate if they are cleared by the kidney.

We have studied the temporal relationship between FSH rise and fall of inhibins, E_2 and progesterone after menopause. The relationship between E_2 and FSH/LH (but not inhibins) has been studied by other groups in the past (Kohler *et al*., 1968; Coble *et al*., 1969; Yen and Tsai, 1971). This is the first study to investigate the relationship between the acute changes in FSH and inhibins in women at surgical menopause.

The correlation analysis shows a significant negative correlation between inhibin A and FSH in both phases of the cycle, whilst inhibin B levels were negatively correlated with FSH in the follicular phase. This indicates that inhibin B is involved in controlling FSH in the follicular phase whereas inhibin A has a negative feedback role on pituitary FSH suppression in both phases of the cycle. Our observation is consistent with previous studies that have suggested a negative feedback role for inhibin B on FSH in the follicular phase (Groome *et al*., 1996; Klein *et al*., 1996). Inhibin B levels were very low in the luteal phase, as expected. Hence, a lack of a demonstrable relationship between FSH and inhibin B in the luteal phase was not surprising. As in the previous studies, falling E_2 levels were significantly related to the rising FSH in both phases of the cycle. In the luteal phase, falling progesterone levels were also negatively correlated with the FSH rise, suggesting that FSH is regulated by inhibin A, E_2 and progesterone in the luteal phase and by inhibin A and B and E_2 in the follicular phase. However, we are aware that correlation analysis should not be used to evaluate a cause-and-effect relationship. A previous study reported that the effect of oral conjugated estrogens (commencing 1 week after surgery) increased the E_2 concentrations in circulation but did not suppress FSH to pre-menopausal levels until 2.5 mg (achieving circulating E_2 levels above pre-menopausal levels) was administered (Utian *et al*., 1978). Our preliminary observations suggest that concentrations of FSH did not fall significantly 1 day after estrogen replacement therapy in our patients. However, these observations are preliminary and future studies have been planned to investigate the changes in FSH with estrogen replacement in the patients who have had surgical menopause and to analyse the relative contribution of inhibins and E_2 on controlling pituitary FSH.

In summary, this study confirms the ovary as the predominant source for circulating inhibins in normal cycling women. The rise in FSH after surgical menopause is caused by the fall in inhibins, estradiol and progesterone after ovariectomy. The time lag between the fall of the levels of ovarian inhibitors in the circulation and the rise of serum FSH may be due to the time taken for the up-regulation of FSH synthesis before release.

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