Persistent pregnanediol glucuronide secretion after gonadotrophin suppression indicates adrenal source of progesterone in premature ovarian failure

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Materials and methods
The study protocol was approved by the New Jersey Medical School Institutional Review Board. Four women with premature ovarian failure meeting the following criteria were recruited and consented for the study: (i) onset of ovarian failure after menarche but prior to age 35; (ii) 46 XX karyotype in at least 50 cells with no evidence of mosaicism; (iii) no prior history of ablative ovarian surgery; (iv) no hormonal therapy for at least one month before beginning the study; (v) at least 90% normal weight for height; (vi) amenorrhoea for at least 6 months prior to the study or no menses without oestrogen

Introduction
Premature ovarian failure is a syndrome which typically occurs prior to the age of 40, and is characterized by amenorrhoea, elevated gonadotrophin values, and oestrogen deficiency (de Moraes-Ruehsen and Jones, 1967). This diagnosis accounts for ~12% of secondary amenorrhoea (Hull et al., 1979) and is a major cause of infertility. The majority of these women have no identifiable aetiological factor (Aiman and Smentek, 1985). However, abnormal karyotypes, autoimmunity, surgery, chemotherapy, and radiation treatment are known to be causative. Occasional reversibility of this syndrome can occur as evidenced by increased sex steroid secretion and even conception (Szlachter et al., 1979; Rebar et al., 1982; Santoro and Schmidt, 1990; Nelson et al., 1992). A 2–3 fold higher urinary pregnanediol glucuronide excretion has previously been reported in women with premature ovarian failure compared with age-appropriate menopausal women (Brown et al., 1993). These women collected daily urine samples for 1 year. Comparison of their mean urinary pregnanediol glucuronide with each other and with eumenorrhoeic controls (data over a complete menstrual cycle) demonstrated significantly higher pregnanediol glucuronide concentrations in premature ovarian failure and eumenorrhoeic women relative to age-appropriate menopausal women.

Precursors of urinary pregnanediol glucuronide are circulating progesterone and pregnenolone (Tait et al., 1962; Arcos et al., 1964; Lasley et al., 1985). These are both in relatively low concentrations in premature ovarian failure and age-appropriate menopausal women. Their source may be either ovarian or adrenal. An ovarian source of this increased pregnanediol glucuronide would suggest the presence of a productively ‘younger’ ovary in women with premature ovarian failure, despite the premature loss of ovarian follicles. An adrenal source would lend support to the phenomenon of adrenopause, an age-related decline in androgen secretion (Abraham et al., 1973; Vermeulen, 1976; Vermeulen and Verdonck, 1979; Crilly et al., 1979).

The purpose of this study was to test our hypothesis that the major glandular source of the circulating progesterone in women with premature ovarian failure was ovarian. To remove the bulk of ovarian stimulation by pituitary down-regulation, monthly depot leuprolide acetate was administered to women with premature ovarian failure for 3 months. Urinary pregnanediol glucuronide, follicle stimulating hormone (FSH), and luteinizing hormone (LH) concentrations were measured pretreatment and compared with post-leuprolide acetate administration and compared with post-leuprolide acetate concentrations.
Table I. Median values of luteinizing hormone (LH), follicle stimulating hormone (FSH) and pregnanediol glucuronide concentrations prior to leuprolide acetate administration

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>LH range (mIU/mg creatinine)</th>
<th>FSH range (mIU/mg creatinine)</th>
<th>Pregnanediol glucuronide range (µg/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.9–31.1/19.0</td>
<td>73.8–132.7/96.9</td>
<td>0.7–1.3/1.0</td>
</tr>
<tr>
<td>2</td>
<td>1.8–8.7/4.6</td>
<td>5.9–60.2/27.1</td>
<td>0.9–2.0/1.3</td>
</tr>
<tr>
<td>3</td>
<td>3.7–30.8/14.6</td>
<td>17.5–146.3/66.5</td>
<td>0.2–1.2/0.5</td>
</tr>
<tr>
<td>4</td>
<td>1.3–21.1/12.5</td>
<td>11.9–84.5/54.6</td>
<td>0.4–9.0/0.7</td>
</tr>
</tbody>
</table>

and/or progestin therapy; (vi) no underlying diseases affecting gonadotrophin or sex steroid secretion, clearance, or excretion; and (viii) normal thyroid stimulating hormone (TSH) and prolactin concentrations.

To establish a baseline value for gonadotrophins and pregnanediol glucuronide, daily first morning voided urine samples were collected for at least 30 days. Leuprolide acetate (7.5 mg) was then administered monthly for 3 months with patients instructed to continue collecting the urine specimens. Although pituitary suppression is usually achieved within 8–10 days after the first leuprolide acetate dose, to assure complete pituitary down-regulation, the initial 2 weeks of the data were not used. Collection interruption was no more than 4 consecutive days for any patient. The urine was collected in glycerol coated test tubes yielding a final glycerol concentration of 7%. This procedure protects the gonadotrophin immunoreactivity during storage and the glucuronide has no effect on the assay (Livesey et al., 1983). Specimens were frozen at –20°C until time of assay. Duplicate measurements of LH and FSH were obtained by a solid phase two-site double immunosorbent assay (ELISA) in duplicate using antisera provided by Dr Bill Lasley (Davis, CA, USA). The inter- and intra-assay coefficients of variation for LH and FSH were 17.1 and 20.6% respectively. The intra-assay coefficients of variation for LH and FSH were 9.2 and 8.2% respectively. This assay system has previously been validated for use in urine (Saketos et al., 1994).

Pregnanediol glucuronide was measured by an enzyme-linked immunosorbent assay (ELISA) in duplicate using antisera provided by Dr Bill Lasley (Davis, CA, USA). The inter- and intra-assay coefficients of variation were 15.3 and 7.0% respectively. It should be noted that values for coefficients of variation refer to the entire working ranges of the assays used. Direct calorimetric reaction (Taussky, 1954) was used to assay creatinine. All results are normalized for creatinine and corrected for the glycerol volume.

Results

The data were not normally distributed. Hence, non-parametric statistical tests using the Wilcoxon rank sum test were performed for data analysis. The pre-leuprolide acetate data are presented in Table I and the post-leuprolide acetate data are presented in Table II. All range values describe the lowest and the highest values during the collection period.

Pre-leuprolide acetate data demonstrated considerable daily variation in gonadotrophin concentrations. This is shown by the relatively large range of LH values, from a maximum of 27.1 mIU/mg creatinine (subject 3) to a minimum of 6.9 mIU/mg creatinine (subject 2). Similarly, considerable variation in FSH range existed, from a maximum of 128.8 mIU/mg creatinine (subject 3) to a minimum of 54.3 mIU/mg creatinine (subject 2). The range for pregnanediol glucuronide values did not demonstrate significant variation; the maximum and minimum range being 1.1 (subject 2) and 0.6 (subject 1) µg/mg creatinine respectively.

After pituitary down-regulation, all gonadotrophin ranges decreased significantly to a maximum LH range of 2.5 mIU/mg creatinine (subject 3) and FSH range of 56.3 mIU/mg creatinine (subject 1). The maximum pregnanediol glucuronide range was 1.5 µg/mg creatinine (subject 2). Interestingly, the overall variability also declined somewhat in all four patients. The median LH values for all four women post-leuprolide acetate are lower than pre-leuprolide acetate. This was statistically significant (P < 0.0001) using the Wilcoxon rank sum test. Suppression of FSH concentrations also occurred post-leuprolide acetate (P < 0.0001). However, there was no detectable decline in median pregnanediol glucuronide values post-leuprolide acetate (P = 0.43).

Discussion

Premature ovarian failure is a major cause of secondary amenorrhoea and infertility. Previous data strongly suggest that the hormonal milieu in premature ovarian failure is dissimilar to age-appropriate menopausal women (Brown et al., 1993). This detailed study was undertaken to elucidate the source of the increased urinary pregnanediol glucuronide concentrations in premature ovarian failure relative to age-appropriate menopausal women. A significant decrease in pregnanediol glucuronide after pituitary down-regulation in premature ovarian failure would have implied an ovarian source. This finding would suggest a fundamental difference between the ovaries of women with premature ovarian failure and their age-appropriate menopausal counterparts.

The administration of leuprolide acetate 7.5 mg depot was highly successful in down-regulating the pituitary in premature ovarian failure, thereby lowering urinary LH concentrations to the limits of detection in the assay system used (0.1 mIU/mg creatinine). Lesser but significant suppression of FSH was also noted. This suppressive effect resulted in considerable reduction of the previous highly variable daily urinary gonadotrophin values. Median pregnanediol glucuronide concentrations for all four women, on the other hand, were statistically no different despite a 10–100-fold LH suppression. This lack of alteration in pregnanediol glucuronide concentrations, after removing the bulk of ovarian stimulating factors, strongly suggests an adrenal source for the enhanced pregnanediol glucuronide excretion.

Table II. Median values of luteinizing hormone (LH), follicle stimulating hormone (FSH) and pregnanediol glucuronide concentrations following leuprolide acetate administration

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>LH range (mIU/mg creatinine)</th>
<th>FSH range (mIU/mg creatinine)</th>
<th>Pregnanediol glucuronide range (µg/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1–2.0/0.4</td>
<td>4.2–60.5/10.6</td>
<td>0.5–1.5/0.9</td>
</tr>
<tr>
<td>2</td>
<td>0.1–1.2/1.0</td>
<td>0.4–3.5/1.5</td>
<td>0.6–2.1/1.2</td>
</tr>
<tr>
<td>3</td>
<td>0.1–2.6/2.0</td>
<td>0.2–44.7/8.1</td>
<td>0.2–1.0/0.4</td>
</tr>
<tr>
<td>4</td>
<td>0.1–0.7/0.1</td>
<td>0.8–24.2/4.4</td>
<td>0.3–1.0/0.6</td>
</tr>
</tbody>
</table>

*P < 0.0001 for median values compared with pre-leuprolide acetate values.
The adrenal gland secretes most of the circulating progesterone after menopause. Declining pregnanediol glucuronide concentrations after age-appropriate menopause may reflect a gland reaching senescence. This reinforces the concept of adrenopause described by previous investigators (Abraham et al., 1973; Vermeulen, 1976; Crilly et al., 1979; Vermeulen and Verdonck, 1979) as an age-related decline in adrenal function that may occur rapidly during the early menopausal years. Interestingly, a similar age-related decline in adrenal function exists in men (Vermeulen and Verdonck, 1976).

Several investigators have recently demonstrated increased glucocorticoid secretion with advancing age (Peskind et al., 1995; Raskind et al., 1995; Van Cauter et al., 1996). This suggests a shift in the steroid biosynthesis pathway. Additionally, progesterone is known to have anti-glucocorticoid activity in vitro (Chrousos et al., 1983) and possibly in vivo (Grünfeld et al., 1985). The data for the latter effect is less clear due to the metabolic conversion of progesterone to glucocorticoids. Declining progesterone and/or increased metabolic conversion rate to glucocorticoids in age-appropriate menopause may lead to a lack of glucocorticoid suppression and possibly allow cortisol concentrations to increase. More direct experiments to elucidate the nature of progesterone metabolism and its specific actions in vivo are necessary.

References


Adrenal source of progesterone in premature ovarian failure


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