Changes in concentrations of serum prolactin, FSH, oestradiol and progesterone and of the sex skin during the menstrual cycle in the mangabey monkey (Cercocebus atys lunulatus)

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Summary. Daily blood samples were collected from 6 regularly menstruating mangabey monkeys. Because serum LH could not be measured by a radio-immunoassay for human LH, Day 0 was taken as the day of maximum serum oestradiol concentration. The hormone patterns were very similar to those of other cercopithecids and women. However, the peak of serum progesterone was lower in mangabeys than in women. There was no distinct peak of serum oestradiol during the luteal phase of mangabeys but the average levels were higher than during the early follicular phase, a pattern more similar to that in other non-human primates than in women. Serum prolactin rose by about 50%, 48 h after the serum oestradiol peak, then declined during the mid-luteal phase before rising at the end of the cycle. Changes in the sex skin dimensions followed the same pattern as the serum oestradiol concentrations.

Introduction

It has been shown in a previous study that serum prolactin concentrations of mangabey monkeys can be validly measured by an homologous radioimmunoassay for human prolactin (Aidara, Tahiri-Zagret & Robyn, 1981). The purpose of the present study was to investigate whether significant variations in serum levels of prolactin occur during the menstrual cycle in this non-human primate and, if so, to relate them to the variations in serum levels of oestradiol, progesterone and FSH.

Materials and Methods

Animals

Six regularly menstruating mangabeys, with body weights ranging from 5000 to 6270 g were investigated during one complete menstrual cycle. Blood samples were collected daily. The monkeys were transferred from open-air cages (3 x 3 x 2 m) into smaller individual cages (80 x 80 x 60 cm) equipped with a moving panel, thus permitting immobilization for collection of blood samples (Aidara et al., 1981). Merthiolate (1/10 000 w/v, final concentration) was added to the serum samples which were kept at —20°C until hormone assays were performed. For each hormone, all samples from the same animal were tested in the same assay.
Hormone assays

Prolactin. Serum prolactin was assayed as described by Aidara et al. (1981) using the VLS-2 reagents distributed by the National Institute of Arthritis, Metabolism and Digestive Diseases (National Institutes of Health, Maryland, U.S.A.). The results were expressed in micro-units (μU) of the human pituitary prolactin research standard M.R.C. 71/222 (National Institute for Biological Standards and Control, London). The sensitivity of the assay was 10 μU/ml and the within- and between-assay coefficients of variation were 7-7 and 15.5% respectively.

FSH. Serum follicle-stimulating hormone (FSH) was assayed as described by Hodgen et al. (1976). The reagents used are obtained by the NIAMDD, NIH, and have been characterized by Hodgen et al. (1976). The results are expressed in μg equivalents of the rhesus monkey pituitary gonadotrophin reference preparation LER-1909-2. The sensitivity of the assay, defined as the amount detectable from zero, was 0-4 μg/assay tube or 2.0 μg/ml sample. Within the range of values obtained in this study the within-assay variation coefficient was 6.7% and the between-assay variation coefficient was 9.3%.

Progesterone and oestradiol. Serum progesterone and oestradiol were measured by double-antibody radioimmunoassays (Aidara, Courte & Robyn, 1977). The antiserum to progesterone was obtained by immunization of rabbits with progesterone-11-α-terephthalate conjugated to bovine serum albumin (BSA). The antiserum to oestradiol was obtained by immunization of rabbits with oestradiol-7-carboxy-methylxime conjugated to BSA. For the anti-progesterone serum, the cross-reactions (Abraham, 1975) were 43% with 5α-pregnane-3,20-dione, 6.7% with 5β-pregnane-3,20-dione, 4.3% with desoxycorticosterone, 2.8% with pregnenolone, 1-2% with 4-pregnen-20β-ol-3-one and 5α-pregnane-3α-ol-20-one, 0.3% with 17α-hydroxyprogesterone, 0-2% with 4-pregnen-20β-ol-3-one, 0.1% with 5α-pregnane-3-one-20β-ol, 0-08% with testosterone and <0-01% with 5α-pregnane-3α,20β-diol, oestradiol and cortisol.

For the antiserum to oestradiol the cross-reactions were 3.8% with oestradiol, 2.3% with oestrone, 0-5% with 16α-hydroxyoestrone, 0.3% with ethinyloestradiol, 0.3% with 17α-oestradiol and <0-1% with 2-methoxy-oestradiol, 2-methoxy-oestrone, cortisol, desoxycorticosterone, testosterone, progesterone, pregnanediol and pregnenolone.

Serum samples, 1-0 ml for oestradiol and 0-5 ml for progesterone, were extracted with 5 volumes of diethyl ether (E. Merckx A.G., Darmstadt, West Germany). After freezing of the aqueous phase, the organic phase was transferred and dried under a gentle flow of filtered nitrogen. The dry residue was redissolved in 0.5 ml phosphate-buffered (pH 7-0, 0-05 M) saline. Standard solutions and serum extracts (200 μl) were incubated at 4°C for 24 h with the antiserum (500 μl) at 1/20 000 dilution for the progesterone assay and 1/50 000 dilution for the oestradiol assay. The antisera were diluted in phosphate-buffered saline containing 1/1500 serum of non-immunized rabbits. The labelled steroids were [1,2-3H(N)]progesterone and [6,7-3H(N)]-oestradiol, both with a specific activity of 40–60 Ci/mmol and from New England Nuclear, Boston, Massachusetts, U.S.A. After addition of 100 μl 3H-labelled steroid (100 c.p.m. or 230 d.p.m. per μl) in assay buffer, the tubes were incubated again at 4°C for 24 h. Then 200 μl sheep antiserum to rabbit immunoglobulins (Robyn, L’Hermithe, Petrusz & Diczfalusy, 1971) were added for 24 h incubation at 4°C. After addition of 3 ml cold assay buffer, the assay tubes were centrifuged at 3110 g for 30 min. The supernatant was discarded and the immunoprecipitate was dissolved in 1.0 ml Lumagel (Lumac System A.G., Basle). The glass tubes (12 × 66 mm) were counted in plastic vials for 2 min in an Automatic Liquid Scintillation Spectrometer (Packard, Benelux) with special adjustment for the height of the assay tubes (Dixon & Cohen, 1976). Distribution of samples and assay solutions was performed with Hamilton syringes equipped with a Cheney Adaptor (Hamilton Micro-Mesure b.v., The Hague, The Netherlands). Triplicates were used for standards and duplicates for unknowns. Non-specific binding in buffer and in serum extract was 2.5% for both assays. The dilutions of the antisera were selected to give a specific binding of 25% of the total amount of tracer. Procedural losses were assessed by the recovery of
trace amounts of the corresponding \(^3\)H-steroids (10 000 c.p.m. or 23 000 d.p.m.) added to serum aliquots before ether extraction. Recovery after ether extraction of the serum samples ranged from 68 to 80% with a mean value of 74% for progesterone and from 60 to 75% with a mean value of 71% for oestradiol. Assay results were calculated (CDC 1700, mini-computer, San Diego, U.S.A.) after linearization of the standard curve by logit transformation. The sample values were corrected for manipulative losses by using mean recovery values. The slopes of the curves obtained with serum extracts were not significantly different \((P > 0.05)\) from the slopes of the corresponding standard curves. The accuracy of the assays was assessed by adding known amounts of progesterone or oestradiol (80, 160, 320 and 640 pg) to charcoal-treated serum samples. The relationships between the amounts of steroid added and those recovered were linear. For oestradiol the correlation coefficient, the regression coefficient and the y intercept were 0.96, 0.89 and 15 pg respectively. For progesterone, these values were 0.99, 0.92 and 36 pg. The sensitivity of the assay, defined as the smallest amount detectable from 0, was 10 pg/assay tube for both steroids. Within the range of values obtained in this study the within-assay variation coefficient was 12.2% for progesterone and 11.0% for oestradiol. The between-assay variation coefficients were 13.5 and 14.6%, respectively.

**Sex skin**

Diameter, along the upper edge of the ischial callosities, and height of the sex skin area were measured with a ruler. The values reported in this paper correspond to the volume of the sex skin expressed in cm\(^3\).

**Statistical analysis**

Statistical analysis of the data was performed by variance analysis and homogeneity of variances was assessed by the Bartlett test (Snedecor, 1956).

**Results**

Since a valid radioimmunoassay for mangabey LH could not be obtained, the day of the maximal oestradiol value was considered as Day 0. The results are illustrated in Text-fig. 1 and the comparisons for various periods are given in Table 1.

**FSH**

Serum FSH levels were significantly higher during the early follicular phase than during the late follicular phase. The peak value at mid-cycle was not different from that at the end of the luteal phase. Early luteal-phase values were significantly lower than those during the late luteal phase.

**Oestradiol**

Serum levels of oestradiol were low in the early follicular phase but started increasing from Day −10. From Day −4, there was a further and steeper increase which reached a peak value of 647 pg/ml on Day 0. Concentrations then dropped markedly to reach, within 2 days, values similar to those observed during the mid-follicular phase. Concentrations fluctuated around 198 pg/ml between Days +3 and +13.
Text-fig. 1. Mean ± s.e.m. serum concentrations of hormones and sex skin development during the menstrual cycle of 6 mangabey monkeys. The data are related to Day 0 as the time of maximal serum oestradiol values.
Table 1. Significance of the differences in mean serum concentrations of FSH, oestradiol, prolactin and progesterone and in sex skin measurement between various periods of the menstrual cycle, considering the day of the maximal value of oestradiol as Day 0

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Period 1 Days of cycle</th>
<th>Mean</th>
<th>Period 2 Days of cycle</th>
<th>Mean</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH (µg/ml)</td>
<td>-14 to -10</td>
<td>7.5</td>
<td>-9 to -1</td>
<td>6.5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>0 to +1, +14 to +15</td>
<td>8.9</td>
<td>+2 to +13</td>
<td>7.1</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>0 to +1</td>
<td>8.9</td>
<td>+14 to +15</td>
<td>8.9</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>-4 to +1</td>
<td>313</td>
<td>-14 to -5</td>
<td>123</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>-4 to -2</td>
<td>230</td>
<td>-1 to +1</td>
<td>428</td>
<td>P &lt; 0.001</td>
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<tr>
<td></td>
<td>-14 to -5</td>
<td>123</td>
<td>+2 to +14</td>
<td>184</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>+2 to +5, +11 to +14</td>
<td>172</td>
<td>+6 to +10</td>
<td>207</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>+2 to +5</td>
<td>162</td>
<td>+11 to +14</td>
<td>182</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Prolactin (µU/ml)</td>
<td>-14 to +1</td>
<td>407</td>
<td>+2 to +15</td>
<td>552</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>-2 to +1</td>
<td>391</td>
<td>+2 to +5</td>
<td>575</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>+2 to +5, +11 to +14</td>
<td>596</td>
<td>+6 to +10</td>
<td>501</td>
<td>P &lt; 0.05</td>
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<tr>
<td></td>
<td>+2 to +5</td>
<td>575</td>
<td>+11 to +14</td>
<td>618</td>
<td>P &gt; 0.05</td>
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<tr>
<td>Progesterone (ng/ml)</td>
<td>-14 to -2</td>
<td>0.46</td>
<td>-1 to +1</td>
<td>0.80</td>
<td>P &lt; 0.001</td>
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<tr>
<td></td>
<td>+2 to +5, +11 to +14</td>
<td>3.04</td>
<td>+5 to +10</td>
<td>5.06</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>+2 to +5</td>
<td>3.06</td>
<td>+11 to +14</td>
<td>3.01</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Sex skin (cm²)</td>
<td>-10 to -8</td>
<td>28</td>
<td>-7 to -5</td>
<td>39</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>-4 to -2</td>
<td>53</td>
<td>-1 to +1</td>
<td>68</td>
<td>P &lt; 0.05</td>
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<tr>
<td></td>
<td>+2 to +4</td>
<td>38</td>
<td>+5 to +7</td>
<td>27</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* Obtained by variance analysis.

**Sex skin**

The development of the sex skin was closely parallel to the pattern of serum oestrogen concentration. Maximal development of the sex skin occurred 1 day after the oestradiol peak and then subsided very quickly.

**Prolactin**

Concentrations fluctuated at about 400 µU/ml during the follicular phase, and 550 µU/ml during the luteal phase. There was a 50% increase within 48 h after the oestradiol peak.

**Progesterone**

Serum concentrations remained around 0.46 ng/ml during the follicular phase but rose significantly after Day -1. Values remained high until Day +12 and then fell steeply to reach follicular phase values by Day +15.

**Discussion**

The endocrine changes observed in this study during the menstrual cycle of the mangabey monkey, at least for FSH, oestradiol and progesterone, closely resemble those described for the baboon (Goncharov, Aso, Cekan, Pachalia & Diczfalusy, 1976; Kling & Westfahl, 1978), the rhesus monkey (Hotchkiss, Atkinson & Knobil, 1971) and the cynomolgus monkey (Stabenfeldt & Hendrickx, 1972).
The cycle patterns of FSH, oestradiol and progesterone in the mangabey are also similar to those in women. However, the absolute values for the steroids in mangabeys and women are more similar during the follicular phase than during the luteal phase; in the mangabey peak progesterone values during the luteal phase were 5 ng/ml, as compared to more than 10 ng/ml in women (Guerrero et al., 1976; Robyn, Vekemans, Caufriez & L’Hermite, 1976). In women, there is a distinct oestradiol peak during the luteal phase and the levels are higher than during the early follicular phase. In mangabeys, there is no distinct oestradiol peak during the luteal phase.

It has been reported that in the rhesus monkey at mid-cycle (Knobil, 1979) peak values for serum oestradiol and LH concentrations occur simultaneously, while in women the oestradiol peak generally precedes the LH peak by some 24 h. In the mangabey the oestradiol peak coincided with the FSH peak. Unfortunately, immunoreactive LH could not be validly measured by the radioimmunoassay for human LH. The dose–response curve obtained with a mangabey pituitary extract was much flatter than that obtained with the human pituitary LH standard.

The preovulatory peak of oestradiol was higher and of shorter duration than previously described for rhesus monkeys (Hotchkiss et al., 1971), but the values from the 6 animals studied here were arbitrarily grouped around the maximal value of oestradiol at Day 0.

There was a significant change in serum prolactin levels 48 h after the oestradiol peak in the mangabey. A similar increase of serum prolactin concentration occurs at mid-cycle in women (Vekemans, Delvoye, L’Hermite & Robyn, 1977). In vivo and in vitro, oestradiol stimulates not only prolactin release and synthesis but also mitosis in the lactotrophs (Jacobi, Lloyd & Meares, 1977). However, such changes related to oestrogens during the menstrual cycle were not reported for the rhesus monkey or for other cercopithecid monkeys. No significant changes were observed in serum prolactin levels during the menstrual cycle of the chimpanzee (Reyes, Winter, Faiman & Hobson, 1975). A possible explanation of the discrepancy in prolactin between the mangabey and the other non-human primates is that circulating oestradiol levels are higher in the mangabey. Serum prolactin levels fell as progesterone values increased in the mangabey; progesterone is known to antagonize the stimulatory effect of oestrogens on prolactin secretion (Libertum, Kaplan & De Nicola, 1979; Labrie & Veilleux, 1979; March, Marrs, Nakamura & Mishell, 1979).

As stress increases prolactin release in the mangabey (Aidara et al., 1981) and other species, it was expected that, in the experimental conditions as described here, the serum levels of prolactin would be influenced by the stress of the blood collection procedure. However, the basal levels obtained were only slightly higher than those previously reported for mangabeys for which the influence of stress was minimized (Aidara et al., 1981). Furthermore, blood collection was repeated every day at the same time, by the same person and using the same standardized procedure. It is therefore unlikely that the stress involved in these blood collections significantly contributes to the changes in serum prolactin levels observed in this study.

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Aidara, D., Tahiri-Zagret, C. & Robyn, C. (1981) Serum prolactin concentrations in mangabey (Cercocebus atys lunulatus) and patas (Erythrocebus patas)


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