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RELATIONSHIP BETWEEN ADENOHYPOPHYSEAL AND STEROID HORMONES AND VARIATIONS IN SERUM AND URINARY MELATONIN LEVELS DURING THE OVARIAN CYCLE, PERIMENOPAUSE AND MENOPAUSE IN HEALTHY WOMEN

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Summary—Morning levels of serum melatonin, FSH, LH, prolactin (PRL), progesterone and estradiol were studied by RIA during the ovarian cycle, perimenopause and menopause in 79 healthy women. FSH and LH levels showed a slight nonsignificant increase from the fertile period to perimenopause, exhibiting a significantly greater increase during menopause. PRL, progesterone and estradiol showed parallel changes, reaching lower levels during menopause. Serum melatonin levels decreased with age, attaining minimum levels in menopause. FSH and estradiol were significantly correlated with melatonin in the follicular phase, while in the luteal phase a negative correlation was found between melatonin, progesterone and estradiol. No significant correlations were noted between serum hormone levels during the perimenopausal period. In menopause, as during the follicular phase, melatonin and FSH were negatively correlated. As expected, a significant positive correlation was found between morning serum levels of melatonin and nocturnal urinary excretion of this indoleamine in all groups studied.

INTRODUCTION

The pineal gland, as a neuroendocrine organ, may markedly influence the endocrine system through its hormone melatonin [1, 2]. Changes in gonadotropin secretion in response to melatonin have been frequently studied, principally in animals in the natural environment [3].

On the other hand, increasing evidence indicates that the pineal gland is affected by endogenous hormonal signals. Some of the better studied hormonal regulatory factors of pineal secretory activity are gonadal steroids and gonadotropins [4, 5]. Although most of the data published to date center upon castration and/or steroid treatment [3, 4], several studies have appeared on the effects of various developmental and sexual factors on the synthesis and secretion of pineal melatonin. In this sense, increases in urinary melatonin excretion during menstrual bleeding [6] and a decrease in morning serum melatonin at mid-ovarian cycle and in the luteal phase in women [7–9] have been reported. Similarly, a decrease in 5-methoxytryptophol, another methoxyindole, was found during the luteal phase as compared to the follicular phase [10]. Recently, it has been reported that metabolic changes in gonadotropins and melatonin from the fertile period to menopause can be influential in the control of circulating levels of these hormones in women [11]. Other authors, however, have been unable to detect variations in either serum melatonin levels or urinary excretion of melatonin metabolites during the ovarian cycle in women [12, 13], and there is as yet no information on melatonin changes during the menopausal period.

In the present paper, we report the changes in several endocrine parameters of reproductive activity (i.e. FSH, LH, prolactin, progesterone and estradiol), and day-time values of melatonin in women from the reproductive period to menopause. Our aim was to determine whether a relationship exists between the pineal gland and the hypothalamic–hypophyseal–gonadal axis in women.

MATERIALS AND METHODS

Subjects and samples

Three groups of women in different reproductive stages were studied. Informed consent was obtained from all subjects, and they were classified as follows:

A—Fertile stage: A group of 20 healthy women, aged 25–30, with regular menstrual cyclicity (28–30 days), and not undergoing anovulatory treatment. Midfollicular and midluteal phases were examined separately.

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B—Perimenopausal period: A group of 19 healthy women, aged 38–40, with ovarian cycle alterations (prolongation of ovarian cycles and some anovulatory episodes). This period, which begins several years before menopause, is also characterized by endocrine changes (increase in FSH, decrease in estradiol and progesterone), which, in all probability, are due to waning ovarian follicular activity and its eventual cessation [14].

C—Menopausal period: A group of 40 healthy women, aged 46–51, in whom regular menses had disappeared at least 3–4 yr earlier.

Women in Groups B and C received no hormonal treatment for at least 1 yr prior to the study.

20 ml of blood were taken by venipuncture between 0800 and 0900 h. Sera were separated by centrifugation and stored at −20°C until assay. Night/day fractions of urine were collected, and HCl was added as a preservative. Total urinary volume was recorded, and aliquots were frozen at −20°C until assay.

Analyses

Hormone levels in serum and urine were determined by RIA using commercial kits (CEA–SORIN). FSH, LH, prolactin and progesterone were estimated directly in unextracted serum. FSH MRC 78/549 and LH MRC 68/40 were used as the standard for the FSH and LH RIAs, respectively. Standard PRL, supplied in the kit, was calibrated against the WHO International Standard, IRP 75/504. Estradiol determinations were carried out following serum extraction by ether. Briefly, diethylether (3 ml) was added to 1 ml serum and mixed by rotatory shaking for 30 min (25–30 rpm). The tubes were then centrifuged at 3000 *g* for 5 min. The aqueous phase was discarded and the organic phase was evaporated to dryness under an air stream. The residue was dissolved in 0.04 M phosphate buffer, pH 7.4, for RIA.

FSH and LH determinations in urine were performed in 10 ml samples previously concentrated (10:1) by polyethylene-glycol (a 40 kDa polymer) dialysis. Briefly, urine samples were placed in 1 × 10 cm dialysis bags which were then sealed and placed in a 1 cm layer of polyethylene-glycol at 0–4°C for 2 h. This time was previously shown to be sufficient to concentrate the urine samples about 10 times. Aliquots of concentrated samples were assayed by RIA using the same procedure as in serum.

Quality controls of the RIAs were performed. Sensitivity, i.e. the minimum amount of hormone different from zero, was FSH: 1 mIU/ml; LH: 1 mIU/ml; prolactin: 0.8 ng/ml; progesterone: 0.06 ng/ml; estradiol: 4.5 pg/ml. Intra- and interassay coefficients of variation for the hormone RIAs were as follows: FSH: 1.9, 1.9%; LH: 2.4, 3.8%; prolactin: 2.8, 6.6%; progesterone: 4.6, 6.9%; estradiol: 8.0, 7.9%.

Melatonin in serum and urine was measured by a commercial RIA kit (WHB, Box 19018, S-161, Bromma, Sweden) following extraction. Briefly, 0.05 M phosphate buffer at pH 7.5 (2 ml) was added to 1 ml serum or urine, and the mixture was extracted with 5 ml dichloromethane. The aqueous phase was discarded and sodium hydroxide (1 ml, 0.1 M) was added. After centrifugation, the organic phase was filtered in Pasteur pipettes through glass fiber, and then evaporated to dryness under an air stream. The residue was dissolved in 0.5 ml phosphosaline buffer (0.05 M phosphate, pH 7.5, 0.14 M NaCl, containing 0.1% gelatin).

200 μl of this extract or of a standard melatonin solution were mixed with 100 μl of antiserum solution, and 100 μl [^{3}H]melatonin (New England Nuclear Co., Boston, Mass) diluted with phosphosaline buffer containing 0.1% gelatin, yielding 1800 cpm/100 μl, were added. The mixture was shaken in a vortex and incubated for 1 h at 4°C. After centrifugation, the precipitate was dissolved in 200 μl 0.1 N NaOH, and 300 μl distilled water were added. Scintillation fluid (Biofluor, New England Nuclear Co.) was used for liquid scintillation counting. The intra- and interassay coefficients of variation were 11.3 and 16.3%, respectively. The recovery of added melatonin was 84.4%.

Gas chromatography was used to determine urinary excretion of adrenal and ovarian steroid metabolites. Aliquots of 24 h urine collections were used for this purpose, as previously described [15].

Student's *t*-test (when variances were similar) or Welch's test (when variances were different) were employed for the comparison of variables between groups. When appropriate, an analysis of variance followed by Schaffe's test were performed. Linear regression was used to determine the relationships between the variables studied. The data are presented as means ± SE.

RESULTS

Serum FSH and LH levels, which did not differ between the follicular and the luteal phases, showed a slight non-significant rise during the perimenopausal period and increased significantly in the menopausal period (Fig. 1, left axis). Progesterone, prolactin and estradiol levels were highest in the luteal phase and decreased during the perimenopausal and menopausal periods. As shown in Fig. 1 (right axis), morning serum melatonin concentration changed as a function of the stage of the estrous cycle, decreasing significantly during the luteal phase. Serum melatonin levels also decreased in peri- and menopausal periods, but this decrease was significant in comparison to the levels found during the ovarian cycle only in menopause (Fig. 1, right axis).

The correlations between individual melatonin levels and other hormones in the different reproductive stages are depicted in Figs 2–5. Figure 2 shows a significant negative correlation between melatonin

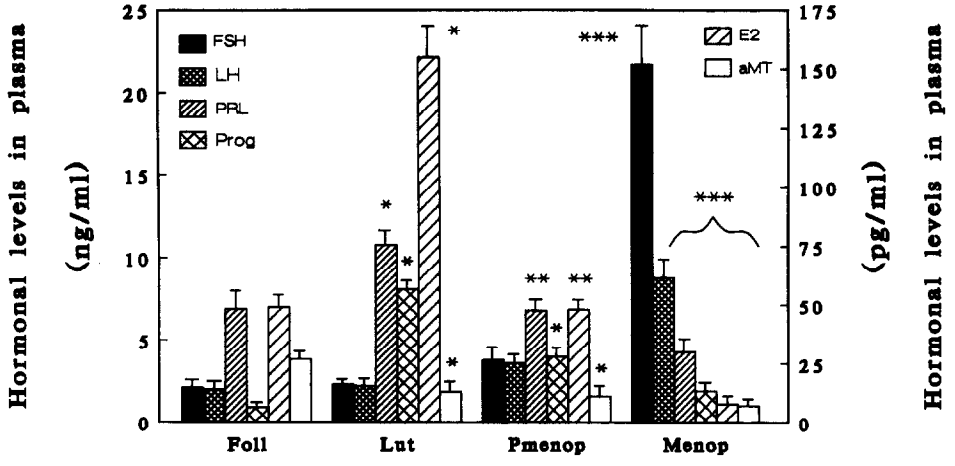

Fig. 1. Serum levels of FSH, LH, prolactin (PRL), and Progesterone (Prog) (left), estradiol (E_2) and melatonin (aMT) (right), during the ovarian cycle, perimenopause and menopause. *$P < 0.001$ vs follicular phase. **$P < 0.001$ vs luteal phase. ***$P < 0.001$ vs fertile and perimenopausal periods.

and FSH ($P < 0.001$) and melatonin and estradiol ($P < 0.05$) in the follicular phase of the cycle. During the luteal phase, however, significant negative correlations existed between melatonin and the ovarian steroids estradiol and progesterone, but not with FSH (Fig. 3, $P < 0.001$). There was no significant correlation between melatonin and the other hormones during the perimenopausal period. In menopause, however, a significant negative correlation was found between melatonin and FSH (Fig. 4, $P < 0.001$), as also occurred during the follicular phase.

Regardless of the reproductive stage, morning serum melatonin levels showed a significant positive correlation with 12 h-night urinary melatonin excretion (Fig. 5, $P < 0.001$).

Urinary excretion of the following steroid metabolites were within the expected normal values for a given reproductive period: androsterone, etiocholanolone, 11-keto-etiocholanolone, 11-β-hydroxy-androsterone, pregnanediol and pregnanetriol (data not shown).

DISCUSSION

The foregoing data indicate that morning serum melatonin concentration significantly decreases during the luteal phase in cycling women. The levels found in peri- and menopausal periods were lower than in the fertile period, although differences were significant only in the menopausal period. These results are in agreement with earlier findings [16, 17], which reported an age-dependent reduction in serum melatonin concentration.

Our results also support earlier studies [6–9] which showed lower melatonin levels in serum samples taken at morning hours in the luteal phase, as well as those [18] which demonstrated a night-time maximum of serum melatonin in women sampled during the night at midluteal phase.

Variations in serum melatonin levels during the ovarian cycle can be interpreted as evidence of a modulating effect of the gonadotropins and steroid hormones on pineal melatonin synthesis [19–21]. Indeed, in a number of experimental situations both effects are apparent in rats [22], and estradiol

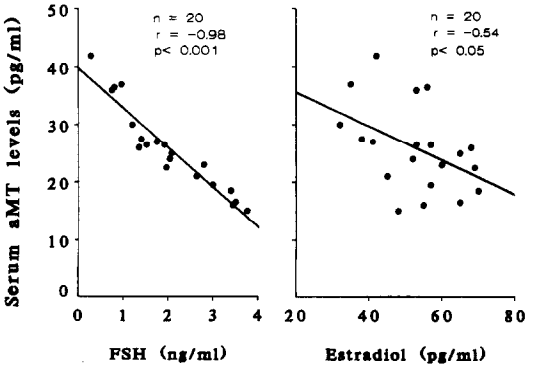

Fig. 2. Correlations between day-time serum melatonin levels and FSH (left) and estradiol (right) during the follicular phase of the ovarian cycle.

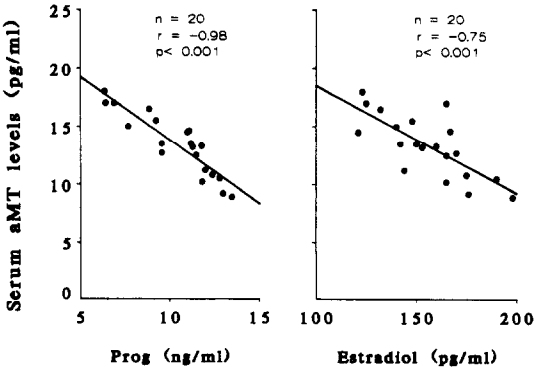

Fig. 3. Correlations between day-time serum melatonin levels and progesterone (left) and estradiol (right) during the luteal phase of ovarian cycle.

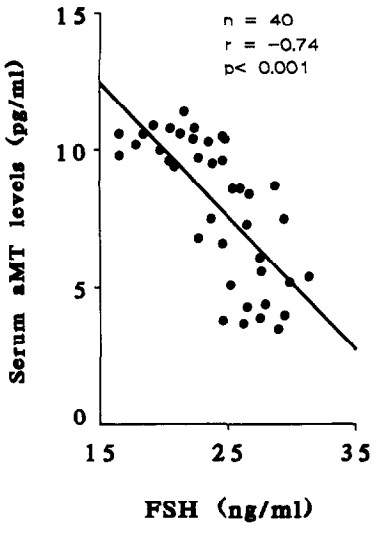

Fig. 4. Correlations between day-time serum melatonin levels and FSH during the menopausal period.

and progesterone receptors have been detected in the pineal gland of a number of mammalian species [23, 24]. Our results effectively show that progesterone and estradiol are negatively correlated with melatonin during the luteal phase. Therefore, the decrease in melatonin at this time may reflect a direct negative feedback effect of both steroids on melatonin synthesis through pineal progesterone and estradiol receptors [23, 24]. It could be argued that the depressed levels of aMT at this time may aid the ovulatory process by removing an inhibitory influence [25].

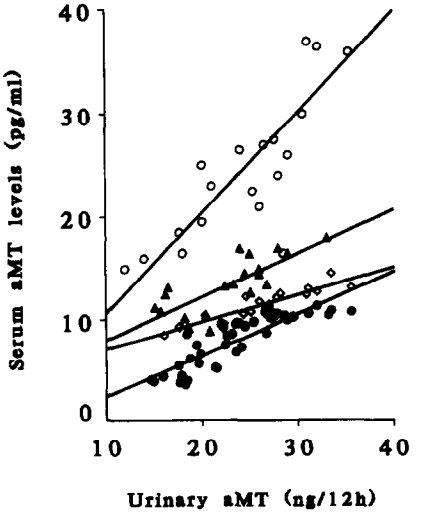

Fig. 5. Correlations between day-time serum melatonin levels and 12 h-night urinary melatonin excretion in the reproductive stages studied. (Follicular phase: ○——○; $P > 0.001$, $r = +0.89$. Luteal phase: ▲——▲; $P > 0.001$, $r = +0.73$. Perimenopause: ◇——◇; $P > 0.001$, $r = +0.92$. Menopause: ●——●; $P > 0.001$, $r = +0.89$).

In the follicular phase, serum melatonin, while showing no correlation with serum progesterone or estradiol, was found to be significantly correlated with serum FSH concentration. Although the effect of FSH on melatonin synthesis has not been explored in detail in animals, data have come to light suggesting that, at least in rats, gonadotropins can affect pineal physiology [22]. Indeed, the decrease in melatonin menopausal levels can be traced to an increase in LH secretion in this period [26]. Peri- and menopausal women, in addition to showing the expected changes in serum estradiol, progesterone, prolactin and gonadotropin levels [27], also showed a decrease in melatonin levels, which was significant in the latter group.

During the menopausal period, however, only serum FSH was found to be significantly correlated with melatonin, neither estradiol nor progesterone being correlated with pineal hormone. It is interesting to note that the increase in progesterone and estradiol seems to exert an inhibitory role on melatonin secretion, since these hormones are most influential during the luteal phase, the latter also acting to a lesser extent during the follicular phase. By contrast, when levels of these steroids fall (i.e. follicular phase or menopausal period), this inhibitory role seems to be exerted by FSH. Although these two distinct inhibitory effects are not yet explainable, it is possible that two different negative feedback mechanisms may operate in the sexual hormone control of melatonin secretion. Indeed, FSH effects may be exerted mainly through the superior cervical ganglion, since gonadotropins can act on this structure [28]; while progesterone and estradiol receptors are known to exist in the pineal gland [23, 24]. Hence, the presence of higher levels of progesterone and estradiol could lead to a more significant negative influence on melatonin synthesis by acting directly upon the pinealocyte, whereas FSH acts by modulating pineal innervation [19, 21, 29]. During the perimenopausal period, no significant correlations were found between any of the hormones measured, perhaps because the changes that take place in this period are so profound as to disguise any possible relationship.

In the present study we compared the amount of melatonin excreted in urine with serum melatonin levels. Significant correlation was noted in all groups between morning serum melatonin and 12 h-night urinary melatonin excretion. Urine melatonin excretion reflects the total hormone secreted over a known period of time. Considering the day/night rhythmicity of melatonin secretion [30], and the considerable individual variations in rhythmicity, determinations in urine could represent a meaningful, noninvasive approach to study the influence of different factors on melatonin secretion. However, in contrast to circulating melatonin levels, minor changes in urinary melatonin excretion were observed between the mid-luteal and mid-follicular phase in cycling women, and between cycling and postmenopausal women. Taking

into account that most urinary excretion of melatonin takes place at night [31], changes in melatonin clearance rates throughout the different periods of reproductive activity could be cited as a plausible explanation for the fact that serum melatonin levels fall off from the fertile to the menopausal periods, whereas urinary excretion does not change. Previous data showed reduced urinary excretion of 6-hydroxymelatonin (the major urinary melatonin metabolite) with age, indicating that increases in clearance could not account for the decline in plasma melatonin [17]. However, by measuring urinary melatonin excretion directly, we recently reported that melatonin clearance rates, like gonadotropin clearance rates, change in the different periods of reproductive activity; these changes can be important in modifying serum levels of these hormones [11].

In summary, our results support the view that not only environmental light but also hormone-related events are operational in the control of melatonin synthesis in humans.

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