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Melatonin enhances cortisol levels in aged but not young women

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In spite of animal data showing an effect of melatonin in the regulation of the hypothalamus–pituitary–adrenal (HPA) axis, no effect of melatonin on cortisol has been evidenced in young men. Gender and aging are believed to influence the regulation of the HPA axis, and may thus modulate the melatonin effect on cortisol. In this study we investigated whether an effect of melatonin on cortisol can be observed in women of different age. Six young women in early follicular phase (22–32 years; EFW) and eight aged women in postmenopause (54–62 years; PMW) were studied. At 08.00 h on two consecutive days each woman received, randomly and in double-blind fashion, a pill of placebo or melatonin (100 mg). Serum levels of melatonin and cortisol were evaluated at 20-min intervals for 48 h. In comparison to EFW, PMW showed an earlier onset of nocturnal melatonin ($p < 0.05$) and cortisol rise ($p < 0.01$) and higher cortisol levels at lunch ($p < 0.05$) and early evening ($p < 0.01$). Melatonin administration did not modify serum cortisol levels in EFW but elicited a marked increase of daytime cortisol levels in PMW ($p < 0.02$). The present data reveal that in aged PMW the cortisol levels are enhanced at selected circadian times and are stimulated by melatonin.

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Melatonin is the main hormone of the pineal gland, produced at night following an adrenergic stimulus at pinealocytes (1). In animals, a role for melatonin in the regulation of the hypothalamo–pituitary–adrenal (HPA) axis has been suggested by evidence showing an inhibitory or stimulatory effect of melatonin (2–5), exerted at the adrenal (6–8) or/and in the brain. Indeed, in the brain melatonin influences neurotransmitters regulating CRF secretion (9–14) and may impair HPA axis negative feedbacks, reducing the affinity of hippocampus adrenocortical steroid receptors (15).

In humans the data are limited to studies showing that in young men the pharmacological (16, 17) or physiological (18) levels of melatonin do not modify cortisol. Because the activity of the HPA axis is influenced by such biological factors as gender (7, 18–21) and aging (22–26), in this study we investigated in contrast to men, whether melatonin may influence cortisol in women of both young and old age.

Materials and methods

Young women (22–32 years) in the early follicular phase (days 3–6 of the menstrual cycle) (EFW) and aged women (54–62 years) in postmenopause (PMW) gave informed consent to the present study, which was approved by our local Ethical Committee. All subjects were free from medication and entrained to a normal sleep–wake cycle. The body mass index of each subject was within 15% of the ideal. Subjects were admitted at the Clinical Research Center the evening before testing; the investigation was initiated at 08.00 h on the following day. To exclude circadian variations, possibly associated with modifications of day length, all studies were performed in the same period of the year. During the experiments the subjects were in bed rest, with the light intensity measured by a photometer (United Dector) at eye level < 10 lux between 23.00 and 07.00 h and > 1000 lux between 07.00 and 17.00 h. Furthermore, in order to define the onset of the nocturnal melatonin rise, which is considered a reliable index of the circadian rhythm phase, light intensity was kept at < 100 lux between 17.00 and 23.00 h (28). Standardized meals were given at 07.00, 12.00 and 17.00 h. Sleep was only allowed between 23.00 and 07.00 h.

A gelatine capsule containing either placebo or melatonin (100 mg) was administered orally in double-blind fashion at 08.00 h on two consecutive days. The melatonin dose was chosen in accordance to that used previously in men (17, 29).

Blood samples were collected at 20-min intervals through a polyethylene catheter placed in an antecubital vein of the non-dominant arm. After separation the serum was stored at $-25°C$ until assayed for melatonin and cortisol by radioimmunoassays (RIAs). Melatonin, expressed as picomoles per liter (pmol/l), was measured without extraction in each serum sample by RIA, using a sheep polyclonal antibody (Guildhay

Antisera, Guildford, UK) (30, 31). The assay, previously validated by gas chromatography, has intra- and interassay coefficients of variation (CVs) of 5% and 8%, respectively, and a sensitivity of 28 pmol/l. Cortisol, expressed as nanomoles per liter (nmol/l), was measured without extraction in each serum sample by RIA (32). The assay has intra- and interassay CVs of 5.8% and 6.5%, respectively, and a sensitivity of 20 nmol/l. Serum levels of FSH, estrone (E_1), estradiol (E_2), androstenedione and testosterone, and plasma levels of ACTH were determined by RIAs (32, 33) in samples collected at 09.00, 13.00, 17.00, 22.00, 02.00 and 06.00 h.

The onset of the nocturnal melatonin rise was identified as a hormone increase two standard deviations above mean daytime values (10.00–17.00 h). The acrophase (time of maximal circadian value) of the nocturnal melatonin rise was determined by visual inspection of the serum profile, after smoothing with a moving average (34). The first nocturnal cortisol peak was defined by a hormone increase two standard deviations above the preceding circadian nadir (at least four samples). Single cosinor analysis was used to evaluate the mesor (mean cosine value), acrophase (time of maximal cosine value) and amplitude (difference between mesor and acrophase) of the circadian cortisol rhythm (31).

Statistical analysis of the results was performed by the Mann–Whitney and Wilcoxon tests for unpaired and paired data, respectively.

Results

Circulating levels of FSH, E_1, E_2, androstenedione and testosterone are reported in Table 1.

In PMW, ACTH levels, evaluated as the mean of the six samples analyzed, were elevated slightly but not significantly (6.5 ± 1.2 vs 4.9 ± 0.4 pmol/l). Similarly, mean 24-h cortisol and nocturnal melatonin levels were not significantly different from those of EFW (Table 2). However, in PMW, lunchtime cortisol levels were higher both as peak values (405.5 ± 29.0 vs 288.8 ± 56.5 nmol/l; $p < 0.05$) (Fig. 1) and as the mean of the values observed between 11.00 and 14.00 h (Table 2). The nocturnal rise of cortisol ($p < 0.01$) and melatonin ($p < 0.05$) and their circadian rhythm acrophases ($p < 0.025$) were phase advanced in PMW (Fig. 2 and Table 2). Furthermore, the time lag between the onset of the nocturnal melatonin rise and the first nocturnal cortisol peak was significantly reduced in PMW (182.5 ± 39.3 vs 310.0 ± 27.3 min; $p < 0.05$) (Fig. 3). As a consequence

Table 1. Mean (± SEM) circulating levels of FSH, estrone (E_1), estradiol (E_2), androstenedione (A) and testosterone (T), calculated by the mean of six samples (09.00, 13.00, 17.00, 22.00, 02.00 and 06.00 h) collected on the placebo day, in six early follicular phase women (EFW) and in eight postmenopausal women (PMW).

	EFW	PMW
FSH (IU/l)	11.3 ± 1.4**	166.1 ± 11.5
E1 (pmol/l)	35.6 ± 6.7*	20.0 ± 3.5
E2 (pmol/l)	37.0 ± 5.3**	14.0 ± 3.8
A (pmol/l)	665.5 ± 5.8**	227.5 ± 29.8
T (pmol/l)	230.7 ± 19.0*	145.2 ± 37.0

*$p < 0.05$ and **$p < 0.01$ vs PMW.

Table 2. Mean (± SEM) parameters of melatonin and cortisol secretion in six early follicular phase women (EFW) and eight postmenopausal women (PMW).

	EFW	PMW
Melatonin (pmol/l)		
24-h levels	131.8 ± 31.7	99.2 ± 16.7
Nocturnal levels	239.8 ± 57.5	179.3 ± 25.5
Maximum peak levels	406.6 ± 109.2	307.6 ± 51.4
Nocturnal onset (h)	2123 ± 0.32*	1952 ± 0.27
Acrophase (h)	0225 ± 0.27**	0023 ± 0.28
Cortisol (nmol/l)		
24-h mean	202.2 ± 13.8	225.5 ± 12.3
Lunch (11.00–14.00 h)	195.0 ± 27.5*	250.4 ± 11.6
Early night (20.00–01.00 h)	67.2 ± 12.2***	214.6 ± 54.7
Late night (01.00–08.00 h)	309.7 ± 30.9	329.9 ± 15.7
Circadian amplitude	141.0 ± 14.9	116.7 ± 17.4
Circadian acrophase (h)	0816 ± 0.27**	0644 ± 0.21
First nocturnal peak (h)	0053 ± 0.32***	2207 ± 0.28

*$p < 0.05$, **$p < 0.025$ and ***$p < 0.01$ vs PMW.

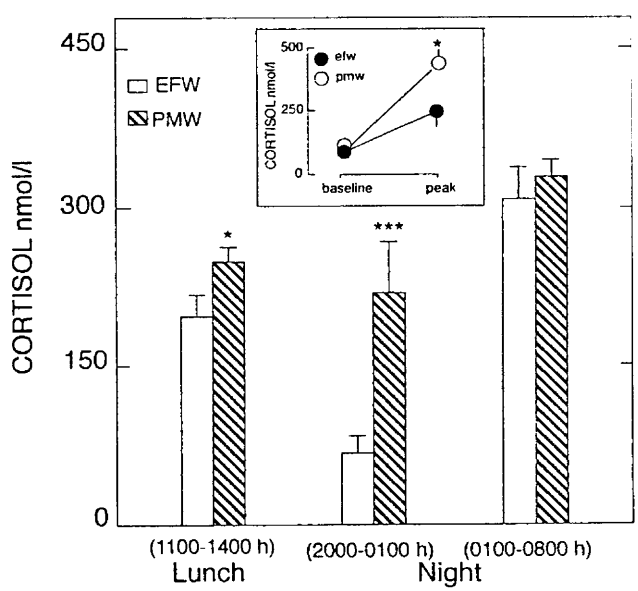

Fig. 1. Transverse mean (± SEM) of cortisol levels at lunch (11.00–14.00 h), early night (20.00–01.00 h) and late night (01.00–08.00 h) in six early follicular phase women (EFW) and eight postmenopausal women (PMW). The included graph shows the mean (± SEM) cortisol increase at lunch, from baseline (10.40–11.20 h) to maximal levels, in the two groups of subjects. *$p < 0.05$ and ***$p < 0.01$ vs PMW.

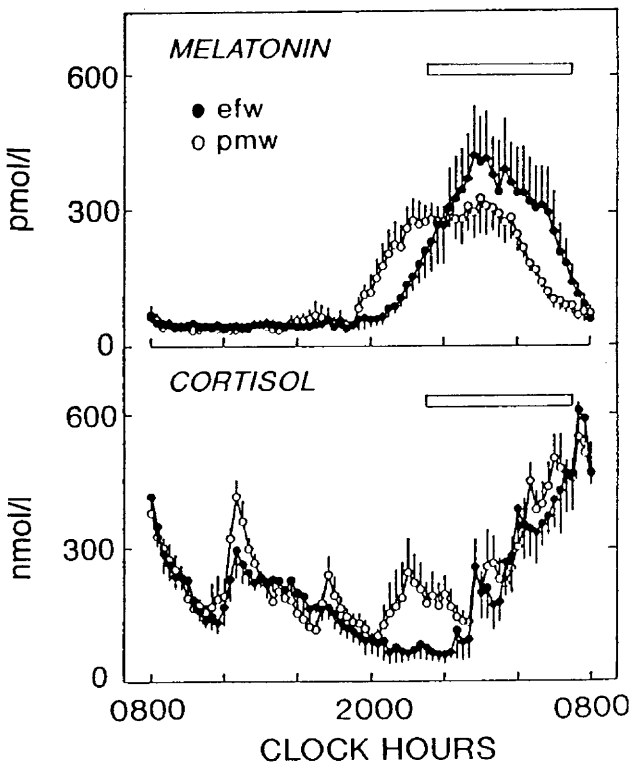

Fig. 2. Mean (± SEM) circadian melatonin (top panel) and cortisol (bottom panel) rhythms in six early follicular phase women (EFW) and eight postmenopausal women (PMW). Open bars indicate sleep time. Earlier onsets of nocturnal melatonin ($p < 0.05$) and cortisol rises ($p < 0.01$) are observed in PMW.

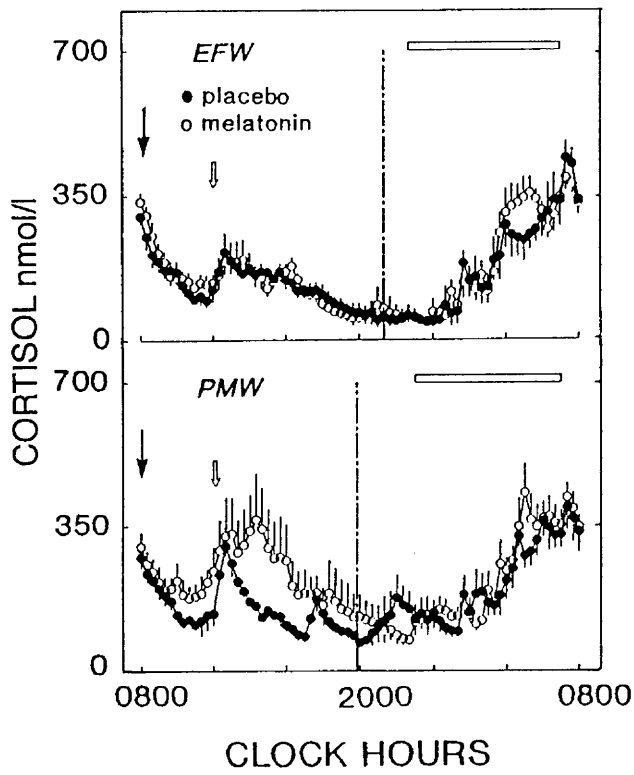

Fig. 3. Mean (± SEM) 24-h circadian rhythm of cortisol observed after the administration of placebo (closed circles) or 100 mg of melatonin (open circles) at 08.00 h in six early follicular phase women (EFW) and eight postmenopausal women (PMW). Closed arrows indicate time of placebo/melatonin administration, and open arrows indicate time of lunch. Open bars indicate sleep time and vertical broken lines indicate the onset of the nocturnal melatonin rise, as evaluated on the placebo day. Exogenous melatonin does not modify cortisol levels in EFW, whereas it enhances daytime cortisol levels in PMW (08.00–20.00 h; $p < 0.02$).

of the advanced rise, early evening (20.00–01.00 h) cortisol levels were significantly higher in PMW ($p < 0.01$) (Fig. 1).

Following its administration in both EFW and PMW, circulating melatonin increased to pharmacological levels within 20 min (49389 ± 5850 and 44128 ± 8560 pmol/l, respectively), peaked after 100 min and then progressively declined to reach a physiological level at 0540 ± 0.45 h in EFW and at 0500 ± 0.55 h in PMW. In comparison to placebo, in EFW the melatonin did not modify either daytime ACTH levels (mean of samples collected at 09.00, 13.00 and 17.00 h) (5.1 ± 0.5 vs 4.9 ± 0.4 pmol/l) or daytime (08.00–20.00 h) cortisol levels (195.4 ± 14.9 vs 188.8 ± 14.5 nmol/l). By contrast, in PMW the melatonin enhanced daytime ACTH (9.3 ± 2.8 vs 6.1 ± 1.2 pmol/l; $p < 0.02$) and cortisol (311.3 ± 65.2 vs 187.4 ± 15.9 nmol/l; $p < 0.02$) levels (Fig. 3). Melatonin did not modify night-time ACTH levels (mean levels of samples collected at 22.00, 02.00 and 06.00 h) in either EFW (4.8 ± 0.3 vs 5.0 ± 0.5 pmol/l) or PMW (6.4 ± 1.0 vs 6.1 ± 1.0 pmol/l). Similarly, melatonin did not modify night-time cortisol levels in either EFW or PMW (Fig. 3).

Discussion

Herein, by the analysis of the circadian melatonin and cortisol rhythms, we confirm previously reported age-associated phase advances of endogenous circadian rhythms (35–38). Although, this age-related phase advance is believed to be induced by acquired modifications in the activity of endogenous circadian pacemakers (37), shifts of daytime customary activities, usually associated with the aging process (37), may also act as contributing factors.

In association with this phase advance, cortisol levels of PMW were elevated at selected circadian times. The observed concomitant elevation of ACTH did not reach the statistical significance, probably because of the infrequency of the determinations. Animal studies have shown that aging is associated with a progressive loss of hippocampus adrenocortical steroid receptors, leading to a progressive impairment of the HPA axis negative feedbacks and to an elevation of circulating cortisol (23–27). In both animals and humans these

age-associated alterations of the HPA axis are more pronounced in females (7, 19–22). Thus, it is conceivable that the HPA axis of PMW is at a critical level of regulation, and that this impairment is revealed at selected circadian times, such as the time of lunch or the early night hours. The possibility that, in PMW, the mild and time-selective hypercorticolism influences metabolic processes, bone turnover, cardiocirculatory mechanisms and immunological responses (23, 27), contributing to the pathogenesis of metabolic and cardiovascular alterations (27, 39) cannot be disregarded.

In PMW, melatonin may represent a factor capable of impairing the regulation of the HPA axis. Indeed, while pharmacological levels of melatonin were not capable of modifying cortisol levels in young EFW, they induced an elevation of ACTH and cortisol in PMW. Whether this stimulatory effect can be achieved with melatonin levels in the physiological range cannot be ascertained, and could only be clarified by a dose–response study. Melatonin exerts dose-dependent effects on rat suprachiasmatic nuclei activity (40), but data on the body temperature of women have indicated that its effect is not dose related but is, rather, a threshold phenomenon, with just detectable circulating melatonin levels that are as effective as those in the pharmacological range (31, 41, 42). Regardless of whether such a phenomenon is applicable to the regulation of the HPA axis, present data may have important physiological implications. Indeed, the possibility that in PMW endogenous melatonin represents a stimulus for cortisol is suggested by the analysis of the temporal relation existing between the endogenous rise of melatonin and that of cortisol. The time lag between the two is greatly reduced in PMW, which indicates that the physiological nocturnal rise of melatonin may stimulate the onset of the nocturnal cortisol peak.

The mechanisms through which melatonin enhances cortisol in PMW is unclear. Melatonin, by reducing the affinity of residual hippocampus adrenocortical steroid receptors (15), may further imbalance HPA axis negative feedbacks, and favour an increase of cortisol. Additional mechanisms of action may derive by influences on hypothalamic neurotransmitters regulating CRF secretion (9–14) or/and on the circadian pacemaker regulating cortisol secretion, whose aging-associated weakened activity (37) could be modified more easily by melatonin (10, 34). Although these central effects of melatonin are supported by the elevation of ACTH, concomitant with that of cortisol, the possibility that melatonin magnifies the cortisol response to ACTH by an action at the adrenal levels (6–8) cannot be dismissed completely.

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