Progesterone Prevents Sleep Disturbances and Modulates GH, TSH, and Melatonin Secretion in Postmenopausal Women

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Context: A number of neuroactive progesterone metabolites produce sedative-like effects. However, the effects of progesterone administration on sleep are not well characterized.

Objective: To investigate the effects of a 3-wk progesterone administration on sleep architecture and multiple hormonal profiles.

Subjects: Eight healthy postmenopausal women, 48–74 yr old, without sleep complaints or vasomotor symptoms. None was on hormone replacement therapy. They did not take any medication for $\geq$2 months.

Design: Randomized, double-blind, placebo-controlled study. For 3 wk, subjects took daily at 2300 h a capsule of either 300 mg of progesterone or placebo. Sleep was polygraphically recorded during the last two nights, and blood samples were obtained at 15-min intervals for 24 h.

Results: During the first night (no blood sampling), sleep was similar in both conditions. Under placebo, blood sampling procedure was associated with marked sleep disturbances, which were considerably reduced under progesterone treatment: mean duration of wake after sleep onset was 53% lower, slow-wave sleep duration almost 50% higher, and total slow-wave activity (reflecting duration and intensity of deep sleep) almost 45% higher under progesterone than under placebo ($P \leq 0.05$). Nocturnal GH secretion was increased, and evening and nocturnal TSH levels were decreased under progesterone ($P \leq 0.05$).

Conclusions: Progesterone had no effect on undisturbed sleep but restored normal sleep when sleep was disturbed (while currently available hypnotics tend to inhibit deep sleep), acting as a "physiologic" regulator rather than as a hypnotic drug. Use of progesterone might provide novel therapeutic strategies for the treatment of sleep disturbances, in particular in aging where sleep is fragmented and of lower quality. **(*J Clin Endocrinol Metab* 96: E614–E623, 2011)**

We have previously shown that in normally cycling women, endogenous progesterone could modulate the secretion of hormones primarily or partially regulated by the sleep-wake cycle (GH, prolactin, TSH), though no effects on sleep could be evidenced (1). Very few studies have objectively characterized the effects of progesterone administration on sleep (2, 3), although preclinical studies have shown that certain neuroactive progesterone metabolites produce sedative-like effects (4). The purpose of the present study was to simultaneously inves-

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Abbreviations: DHEAS, Dehydroeipandrosterone sulfate; FT4, free thyroxine; REM, rapid eye movement; SWA, slow-wave activity; SWS, slow-wave sleep; WASO, wake after sleep onset.

tigate, in healthy postmenopausal women, the effects of a 3-wk progesterone administration both on sleep architecture and on multiple hormonal profiles. The protocol allowed us to explore the effects of the treatment both on normal sleep and on sleep disturbed by an iv catheter.

Materials and Methods

Subjects

Eight postmenopausal women, aged 48–74 yr (mean 57.4 yr), were selected after a careful clinical and biological evaluation. Investigations were performed 2–17 yr (mean 8.0 yr) after natural menopause (seven subjects) or bilateral ovariectomy (one subject). Mean age at menopause was 49.4 yr (range, 41–57 yr). Three subjects had never received hormone replacement therapy, and five subjects were off such treatment for 2–6 months (mean 4.4 months) at the time of enrollment. Their body weight was in the normal range for all (mean ± SEM of body mass index 22.1 ± 0.8 kg/m^2). In all subjects, estradiol plasma levels were <10 ng/liter. FSH plasma levels averaged 90 IU/liter (range, 66–136 IU/liter). Smokers, shift workers, subjects who had traveled across time zones during the last 2 months, individuals with personal history of drug abuse or with personal or family history of psychiatric, neurological, endocrine, or metabolic disorders, and subjects with current vasomotor symptoms, dieting, or intensive physical exercise were excluded from the study. Each volunteer was examined by one of the authors (A.C.) and had to answer a battery of specific questions concerning her sleep habits. To be included in the study, volunteers had to comply with the following requirements: regular sleep schedules (bedtimes from 2200–2400 h to 0600–0800 h), no difficulty to fall asleep, no complaints of awakenings during the sleep period, no snoring, no periodic limb movements, and no daytime fatigue and sleepiness. Written informed consent was obtained from all volunteers.

Experimental protocol

The protocol, approved by the institutional review board, was designed as a randomized, double-blind, placebo-controlled study.

Before the beginning of the investigation, subjects spent two consecutive nights in the sleep laboratory to habituate to the sleep laboratory environment and recording procedures. On both nights, electrodes for polygraphic sleep recordings were placed around 2230 h. Polygraphic sleep recordings were obtained during the second night. Thereafter, all subjects participated in random order in two studies (referred to as placebo and progesterone), separated from each other by at least 1 month. The placebo study preceded the progesterone study in four subjects and followed the progesterone study in the other four volunteers.

Each study included an initial 20-d ambulatory period. During this period, the subjects were asked to maintain regular sleep-wake cycles (bedtime 2300–0700 h in darkness) and meal schedules (0800 h, 1230 h, 1900 h). They took daily, at 2300 h, a capsule of either 300 mg of progesterone (nonmicronized preparation) or placebo. At the end of the ambulatory period, they were admitted at about 1600 h to the sleep laboratory for 44 h. During hospitalization, subjects were maintained in seated/upright position in normal indoor light conditions (± 300 lux), and no naps were allowed during the scheduled wake periods (0700–2300 h). Meals were served at 0800 h, 1230 h, and 1900 h. At 2300 h, they received their usual capsule of either 300 mg of progesterone or placebo. During both scheduled sleep periods (2300–0700 h), subjects were kept recumbent in total darkness and sleep was polygraphically recorded. On the morning after the first night, a sterile heparin-lock catheter was inserted iv in the forearm and blood samples were collected for 24 h at 15-min intervals. The iv line was kept permeable by a slow drip of heparinized saline. During the sleep period, the indwelling catheter was connected to plastic tubing extending to an adjacent room and blood samples were collected remotely, as previously described (5). The total amount of blood withdrawn was less than 300 ml for each inpatient study. The subjects were discharged at about 1200 h after the second night.

Hormonal assays

Each blood sample was centrifuged at 4 C. Plasma samples were frozen at −20 C. For each hormone, all samples from a given 24-h study were analyzed in the same assay run. Circulating levels of LH, FSH, melatonin, ACTH, cortisol, TSH, prolactin, and GH were measured at 15-min intervals. Progesterone, androstanediol glucuronide, testosterone, estradiol, and estrone levels were measured at 4-h intervals from 1100 h till 0700 h the following morning, with additional measurements at 2200 h, 0100 h, and 0200 h. Dehydroeipandrosterone sulfate (DHEAS) and free thyroxine (FT4) were measured at 4-h intervals during 24 h. IGF-I was measured on two to three plasma samples between 0930 h and 1100 h.

LH, FSH, ACTH, cortisol, TSH, prolactin, GH, DHEAS, and FT4 were assayed by a chemiluminescent enzyme immunometric assay (Immulite, Siemens Healthcare Diagnostics, Deerfield, IL). Melatonin was measured by RIA, using a rabbit antiserum (Stockgrand, University of Surrey, UK) (6). IGF-I was assayed by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA) after acid-ethanol extraction. The following hormones were assayed using various commercially available RIA kits: progesterone and testosterone, Coat-a-count, Siemens Healthcare Diagnostics; androstanediol glucuronide and estrone, Diagnostic Systems Laboratories, Webster, TX; estradiol, DiaSorin, Saluggia, Italy. Lower limits of sensitivity and intraassay and interassay coefficients of variation are shown in the Supplemental Data Table (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org/).

Analysis of hormonal profiles

When measured at 15-min intervals, plasma hormonal levels were averaged over the entire 24-h period, over the scheduled wake period (0700–2300 h, referred to as daytime), and over the scheduled sleep period (2300–0700 h, referred to as nighttime). Individual circadian variations of plasma cortisol and prolactin profiles were quantified by a best-fit curve using a robust locally weighted regression procedure (7) with a regression window of 4 h. For each cortisol and prolactin profile, the acrophase and the nadir were defined as, respectively, the maximum and minimum of the best-fit curve. The amplitude was defined as 50% of the difference between the acrophase value and the nadir value. The quiescent period of cortisol secretion was defined as starting (ending) when concentrations lower (higher) than 30 μg/liter were observed for at least three consecutive samples. The end of the quiescent period corresponds to the onset of the nocturnal cortisol circadian rise toward the morning maximum. The onset and the offset of the melatonin circadian rise were defined, respectively, as the timing of the first plasma level exceeding the mean + 3 SD of baseline levels recorded over the 1300–1600 h period not followed by a return to lower concentra-

tions before the nocturnal peak, and the timing of the last value occurring after the peak exceeding the mean + 3 SD of those baseline values. The onset of the TSH circadian rise was defined as the timing of the first plasma level exceeding the mean + 3 SD of baseline levels recorded over the 1300–1700 h period, not followed by a return to lower concentrations before the nocturnal peak. The timings of melatonin, cortisol, and TSH onsets are currently considered as reliable markers of the circadian phase (8).

Significant hormonal pulses of individual hormonal profiles were identified and characterized using the computer algorithm ULTRA (9). The threshold for significance of a pulse was set at twice the intraassay coefficient of variation in the relevant concentration range. A modification of the algorithm ULTRA was used to estimate the amount of GH secretion by a deconvolution procedure using a one-compartment model for GH clearance and subject-adjusted half-lives (13–21 min in the present study). A volume of distribution of 7% of body weight was used in these calculations. The total amount of GH secreted over a given time interval was calculated by summing the amounts secreted in each of the significant pulses occurring during that time interval. If a pulse overlapped two periods, the amount of GH secreted was divided proportionally.

Sleep analysis

Polygraphic sleep recordings were visually scored at 30-sec intervals, using standardized criteria (10) by the same experienced scorer who was blind to the clinical condition of the subject. Sleep onset and morning awakening were defined as, respectively, the times of the first and last 30-sec intervals scored II, III, IV, or rapid eye movement (REM). The sleep period was defined as the time interval separating sleep onset and final awakening. Total sleep time was defined as the sleep period minus the total duration of wake after sleep onset (WASO). Sleep latency was defined as the time interval from lights off until sleep onset. Sleep efficiency was calculated as the total sleep time, expressed as percentage of the time allocated to sleep. Slow-wave sleep (SWS) was defined as stages III+IV.

A spectral analysis was performed on the central electroencephalogram lead (PRANA, PhiTools, Strasbourg, France) (11). Muscular, ocular, and movement artifacts were eliminated before spectral analysis. δ activity was calculated as the absolute spectral power in the frequency band 0.5–4 Hz. Mean δ power was calculated per 30-sec epoch. δ power in non-REM sleep quantifies slow-wave activity (SWA) (*i.e.*, the intensity of SWS).

Statistical tests

Nonparametric tests (Friedman test, Wilcoxon signed rank test, Spearman rank test) were used for statistical calculations (StatView SE+ software for Macintosh computers, Abacus Concepts Inc., Berkeley, CA). Detection of outliers was performed by the Grubbs test (12, 13). All group values are expressed as the mean ± SEM.

Results

Steroids (Table 1 and Fig. 1)

Under placebo, 24-h progesterone levels averaged 0.15 ± 0.04 μg/liter. Under oral nonmicronized progesterone treatment, 24-h levels averaged 8.1 ± 1.2 μg/liter. Minimal values (3.6 ± 0.3 μg/liter) were observed at 2200–2300 h, just before progesterone administration. Thereafter, levels increased rapidly to reach maximum values averaging 20.3 ± 5.6 μg/liter (range 8.3–59.0 μg/liter) at 0100–0200 h (*i.e.*, 2–3 h after drug ingestion).

TABLE 1. Progesterone, androstanediol glucuronide, testosterone, estradiol, estrone, DHEAS, LH, and FSH variables (mean ± SEM, $n = 8$)

	Placebo treatment	Progesterone treatment	*P*
Progesterone			
24-h levels (μg/liter)	0.15 ± 0.04	8.1 ± 1.2	**0.01**
Androstanediol glucuronide			
24-h levels (μg/liter)	1.32 ± 0.34	3.58 ± 0.79	**0.01**
Testosterone			
24-h levels (μg/liter)	0.12 ± 0.01	0.13 ± 0.01	0.12
Estradiol			
24-h levels (ng/liter)	6.33 ± 0.74	6.62 ± 0.69	0.78
Estrone			
24-h levels (ng/liter)	11.39 ± 1.30	11.83 ± 1.23	0.58
DHEAS			
24-h levels (μg/liter)	547 ± 131	626 ± 197	0.35
LH			
24-h levels (IU/liter)	25.9 ± 2.9	24.8 ± 3.3	0.16
0700–2300 h levels (IU/liter)	26.5 ± 2.9	25.6 ± 3.4	0.21
2300–0700 h levels (IU/liter)	24.6 ± 2.9	23.3 ± 3.2	0.26
Pulse frequency (nb/24 h)	14 ± 1	14 ± 2	0.48
Pulse amplitude (IU/liter)	7.9 ± 0.9	8.5 ± 1.9	0.21
Pulse duration (min)	91 ± 6	102 ± 15	0.23
FSH			
24-h levels (IU/liter)	79 ± 8	75 ± 7	**0.02**
0700–2300 h levels (IU/liter)	82 ± 8	78 ± 8	**0.03**
2300–0700 h levels (IU/liter)	73 ± 7	67 ± 7	***0.07***
Pulse frequency (nb/h)	11 ± 1	11 ± 1	1.00
Pulse amplitude (IU/liter)	18 ± 2	16 ± 2	0.48
Pulse duration (min)	114 ± 11	108 ± 11	0.57

P values denote the results of Wilcoxon tests. Bold values denote statistically significant *P* level (≤0.05). Italic-bold value denotes a statistical trend ($0.05 < P < 0.10$).

Under placebo, androstanediol glucuronide levels were stable over the 24-h span. Under progesterone, levels were increased ($P = 0.01$), and 24-h profiles paralleled simultaneous progesterone patterns, with maximum values at 0100–0200 h. At 0200 h, individual differences in androstanediol glucuronide values between progesterone and placebo conditions correlated positively with simultaneous differences in progesterone values ($r_s = 0.86$, $P = 0.02$).

Testosterone profiles exhibited similar diurnal variations in both conditions, with similar low nocturnal levels

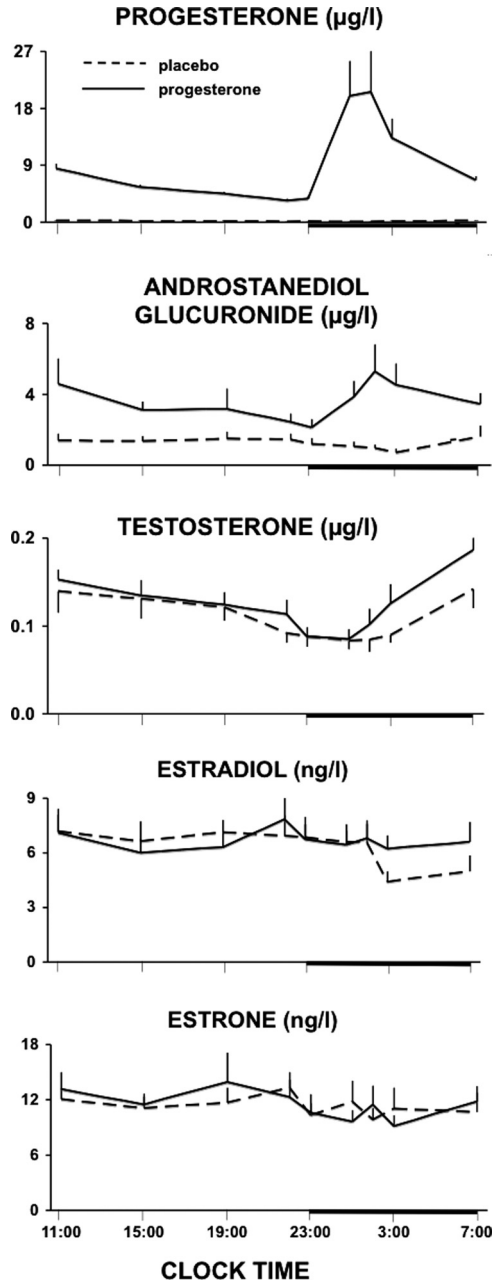

FIG. 1. Mean (+ SEM, n = 8) 24-h profiles of plasma progesterone, androstanediol glucuronide, testosterone, estradiol, and estrone under placebo (*dotted line*) and progesterone (*solid line*) treatment. *Black bars* indicate scheduled sleep periods. The drug was given at 2300 h.

and high morning levels. Estradiol and estrone levels were low, stable, and similar in both conditions. Individual DHEAS levels were stable and similar in both conditions but varied considerably among individuals. No correlation was detected between progesterone and testosterone, DHEAS, or estrogens.

Gonadotropins (Table 1 and Fig. 2)

Classic postmenopausal gonadotropins profiles were observed in both conditions. Mean 24-h LH levels, pulse frequency, duration, and amplitude were similar in both conditions. Individual values under progesterone correlated positively with corresponding values under placebo for pulse frequency ($r_s = 0.98$, $P = 0.009$), duration ($r_s = 0.95$, $P = 0.01$), and amplitude ($r_s = 0.86$, $P = 0.02$). Mean 24-h FSH levels were slightly but significantly lower under progesterone than under placebo ($P = 0.02$). FSH pulse characteristics were similar in both conditions.

Melatonin (Table 2 and Fig. 2)

Melatonin profiles were obtained in six subjects. In both conditions, classic profiles with stable, low daytime values, an evening circadian rise, and a return to low values in the morning were observed. The 24-h levels and the timings of onset and offset of the circadian rise in both conditions were not significantly different from each other. However, over the 2300–0500 h period, melatonin levels were decreased by more than 40%, compared with placebo ($P = 0.03$).

ACTH and cortisol (Table 2 and Fig. 2)

In both conditions, typical ACTH and cortisol profiles were observed, with a quiescent period of minimal secretion centered around midnight, followed by an abrupt rise during the second part of the night to reach an early morning maximum, and declining levels during daytime. All variables characterizing ACTH and cortisol profiles were similar in both conditions. Individual pulse amplitude values under progesterone correlated positively with corresponding values under placebo for ACTH ($r_s = 0.81$, $P = 0.05$) and cortisol ($r_s = 0.89$, $P = 0.02$).

TSH and FT4 (Table 3 and Fig. 2)

In both conditions, TSH concentrations followed the expected pattern, with relatively stable daytime levels, followed by an early evening circadian rise, a nocturnal decrease, and a transient rebound after final morning awakening. The timings of the onset of the circadian rise and of the peak were similar in both conditions. However, TSH concentrations were 25–30% lower under progesterone than under placebo over the 1500–2300 h and 2300–0700 h periods ($P \leq 0.05$), resulting in a mean 24% decrease over the 24-h period ($P = 0.07$). In contrast, FT4 levels were similar in both conditions.

Prolactin (Table 3 and Fig. 2)

Prolactin profiles were obtained in seven subjects. In both conditions, classic profiles, with a major nocturnal elevation, were observed. All variables characterizing prolactin profiles were similar in both conditions.

GH and IGF-I (Table 3 and Fig. 2)

Daytime GH secretion was similar in both conditions. In contrast, nighttime GH secretion was 50% higher un-

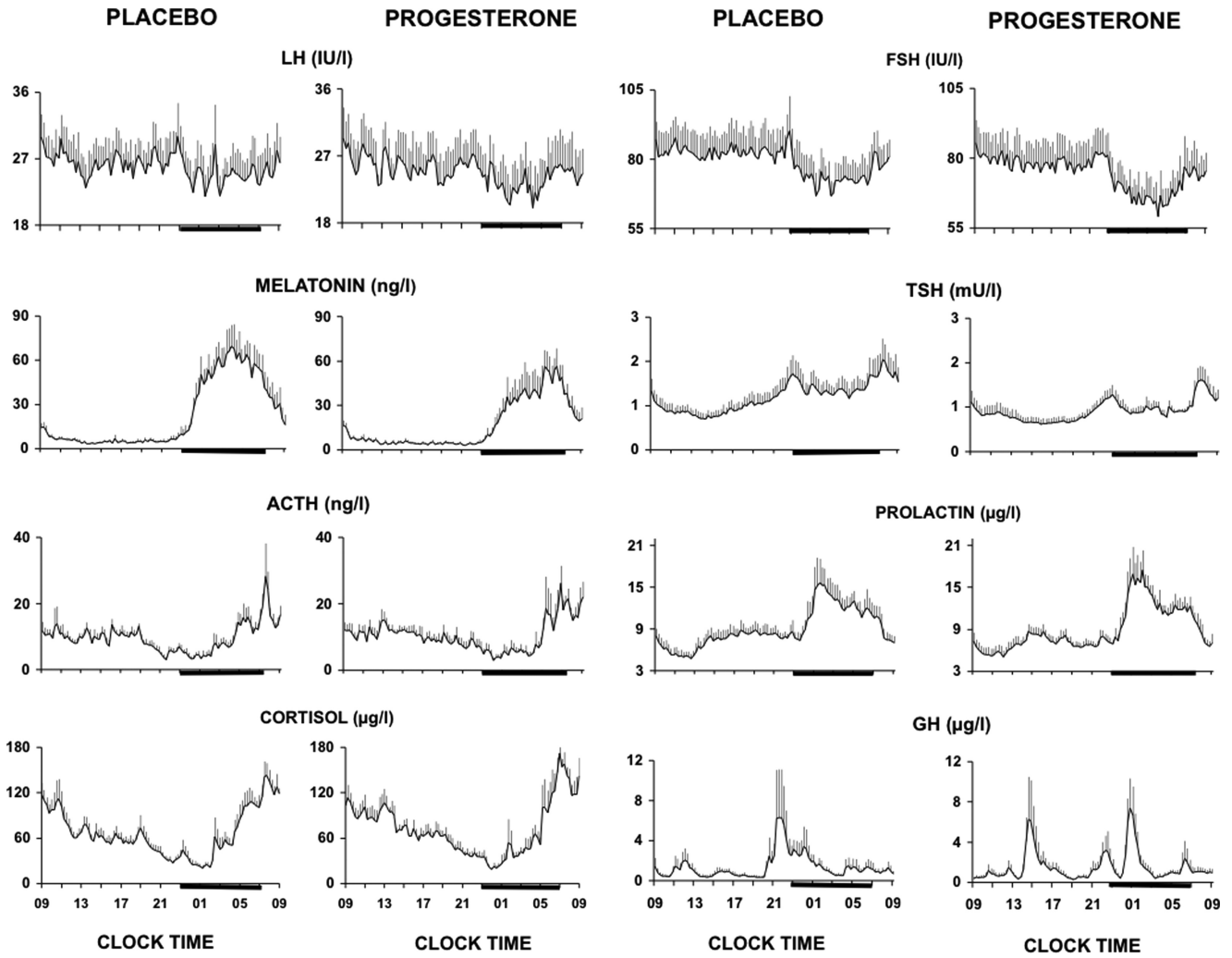

FIG. 2. Mean (+ SEM; n = 8, except for melatonin: n = 6, and for prolactin: n = 7) 24-h profiles of plasma LH, FSH, melatonin, ACTH, cortisol, TSH, prolactin, and GH under placebo and progesterone treatment. *Black bars* indicate scheduled sleep periods.

der progesterone than under placebo ($P = 0.05$). A trend for an increase in IGF-I values was detectable ($P = 0.09$).

Sleep (Fig. 3)

During night 1 (without catheter), sleep duration (averaging nearly 7 h) and architecture were in the normal range for this population of postmenopausal women (11, 14). There were no differences between placebo and progesterone treatment ($P \geq 0.33$).

The effects on sleep of the blood sampling procedure (which included the presence of an iv catheter) were quite different under placebo and under progesterone. Under placebo, all sleep variables, except stage I, sleep latency, and REM latency, were considerably disturbed during night 2, compared with night 1 ($P \leq 0.02$): mean WASO duration was increased by more than 250% (from 43 ± 9 min to 152 ± 35 min), and dramatic decreases were observed in total sleep time (almost 30%), sleep efficiency (35%), the duration of stage II (more than 30%), SWS duration (almost 45%), and total SWA (almost 40%). The decrease in REM sleep averaged almost 40% (Friedman test, not significant). In contrast, only minor alterations were observed under progesterone treatment: total sleep time ($P = 0.05$), sleep efficiency ($P = 0.07$), and stage II ($P = 0.04$) decreased by about 20%. Other sleep variables were not significantly altered.

During night 2 (with catheter), disturbances in sleep duration and quality observed under placebo were considerably reduced under progesterone treatment. Indeed, under progesterone compared with placebo, mean WASO duration was more than 50% lower ($P = 0.05$), total sleep time was 20% higher ($P = 0.04$), sleep efficiency 15% higher ($P = 0.04$), and total SWA more than 40% higher ($P = 0.04$). SWS was similar under placebo (69 ± 16 min) and progesterone (84 ± 10 min), but this apparent similarity was attributable to an outlier ($P < 0.01$): SWS duration under placebo was 175 min in this outlier, *vs.* values ranging from 36 to 86 min in the other seven subjects. If this outlier was excluded from the analysis, SWS averaged 53 ± 7 min and 79 ± 10 min

TABLE 2. Melatonin, ACTH, and cortisol variables (mean ± SEM, n = 8, except for melatonin, n = 6)

	Placebo treatment	Progesterone treatment	*P*
Melatonin			
24-h levels (ng/liter)	22.5 ± 3.7	17.0 ± 3.3	0.17
Onset circadian rise (clock time ± min)	23.10 ± 21	23.53 ± 22	0.14
Offset circadian rise (clock time ± min)	11.20 ± 32	10.30 ± 29	0.34
2300–0500 h levels (ng/liter)	46.9 ± 9.7	27.6 ± 8.6	**0.03**
ACTH			
24-h levels (ng/liter)	9.8 ± 1.2	10.4 ± 1.3	0.61
0700–2300 h levels (ng/liter)	10.9 ± 1.2	11.6 ± 1.1	0.53
2300–0700 h levels (ng/liter)	8.2 ± 1.4	8.3 ± 1.8	0.46
Pulse frequency (nb/24 h)	21 ± 1	22 ± 1	0.28
Pulse duration (min)	59 ± 2	56 ± 4	0.27
Pulse amplitude (ng/liter)	7.4 ± 0.5	7.0 ± 0.8	0.31
Cortisol			
24-h levels (μg/liter)	67 ± 8	72 ± 8	0.12
0700–2300 h levels (μg/liter)	72 ± 8	81 ± 8	0.94
2300–0700 h levels (μg/liter)	57 ± 9	59 ± 11	0.89
Circadian amplitude (% 24-h mean)	88 ± 9	81 ± 9	0.50
Acrophase value (μg/liter)	135 ± 13	138 ± 17	0.58
Acrophase timing (clock time ± min)	08.15 ± 23	08.38 ± 31	0.59
Nadir value (μg/liter)	23 ± 5	23 ± 5	0.67
Nadir timing (clock time ± min)	01.15 ± 24	01.11 ± 26	0.85
Duration quiescent period (min)	291 ± 43	253 ± 47	0.50
Onset circadian rise (clock time ± min)	02.49 ± 46	02.23 ± 45	0.67
Pulse frequency (nb/24 h)	15 ± 1	13 ± 1	0.14
Pulse duration (min)	87 ± 7	96 ± 5	0.26
Pulse amplitude (μg/liter)	49 ± 14	47 ± 5	0.67

P values denote the results of Wilcoxon tests. Bold value denotes statistically significant *P* level (≤0.05).

TABLE 3. TSH, FT4, prolactin, GH and IGF-I variables (mean ± SEM, n = 8, except for prolactin, n = 7)

	Placebo treatment	Progesterone treatment	*P*
TSH			
24-h levels (mU/liter)	1.19 ± 0.22	0.91 ± 0.13	***0.07***
0700–1500 h levels (mU/liter)	1.10 ± 0.22	0.97 ± 0.19	0.58
1500–2300 h levels (mU/liter)	1.07 ± 0.21	0.80 ± 0.12	**0.04**
2300–0700 h levels (mU/liter)	1.39 ± 0.24	0.98 ± 0.18	**0.05**
Onset circadian rise (clock time ± min)	20.38 ± 23	20.00 ± 24	0.23
Peak (clock time ± min)	22.51 ± 9	22.41 ± 4	0.27
FT4 (ng/liter)			
24-h levels	10.1 ± 0.4	10.3 ± 0.4	0.53
Prolactin			
24-h levels (μg/liter)	9.0 ± 1.0	8.9 ± 0.7	0.83
0700–2300 h levels (μg/liter)	7.5 ± 1.0	7.1 ± 0.7	0.27
2300–0700 h levels (μg/liter)	12.0 ± 1.5	12.5 ± 1.4	0.60
Circadian amplitude (% 24-h mean)	106 ± 17	119 ± 14	0.50
Acrophase value (μg/liter)	14.6 ± 2.0	15.6 ± 1.8	0.50
Acrophase timing (clock time ± min)	03.19 ± 41	02.56 ± 37	0.34
Nadir value (μg/liter)	5.1 ± 0.8	5.0 ± 0.9	0.53
Nadir timing (clock time ± min)	11.47 ± 15	11.09 ± 34	0.60
GH			
24-h secretion (μg)	344 ± 105	393 ± 118	0.26
0700–2300 h secretion (μg)	241 ± 89	239 ± 91	0.99
2300–0700 h secretion (μg)	103 ± 25	154 ± 44	**0.05**
IGF-I			
Morning levels (μg/liter)	90 ± 20	103 ± 23	***0.09***

P values denote the results of Wilcoxon tests. Bold values denote statistically significant *P* level (≤0.05). Italic-bold values denote statistical trends ($0.05 < P < 0.10$).

under placebo and progesterone, respectively ($P = 0.04$). There were no differences in sleep latency, stage I, stage II, REM sleep, and REM latency. Possibly because of the modest sample size, no relation was evidenced between sleep variables and progesterone or other hormonal values.

Discussion

This study, performed under well-controlled experimental conditions in a group of eight healthy postmenopausal women, is the first investigation where the effects of a semi-chronic progesterone treatment on sleep architecture and on multiple 24-h hormonal profiles were simultaneously analyzed. Despite the modest sample size, the present results provide valuable indications about progesterone action on the neuroendocrine system.

None of the volunteers complained of sleep problems or of vasomotor symptoms. To be habituated to the study procedure, they were hospitalized in the sleep laboratory for two consecutive nights several weeks before the beginning of the investigation.

During the laboratory session, sleep duration and architecture during night 1 (without catheter) were normal (11, 14) and progesterone administration had no effect.

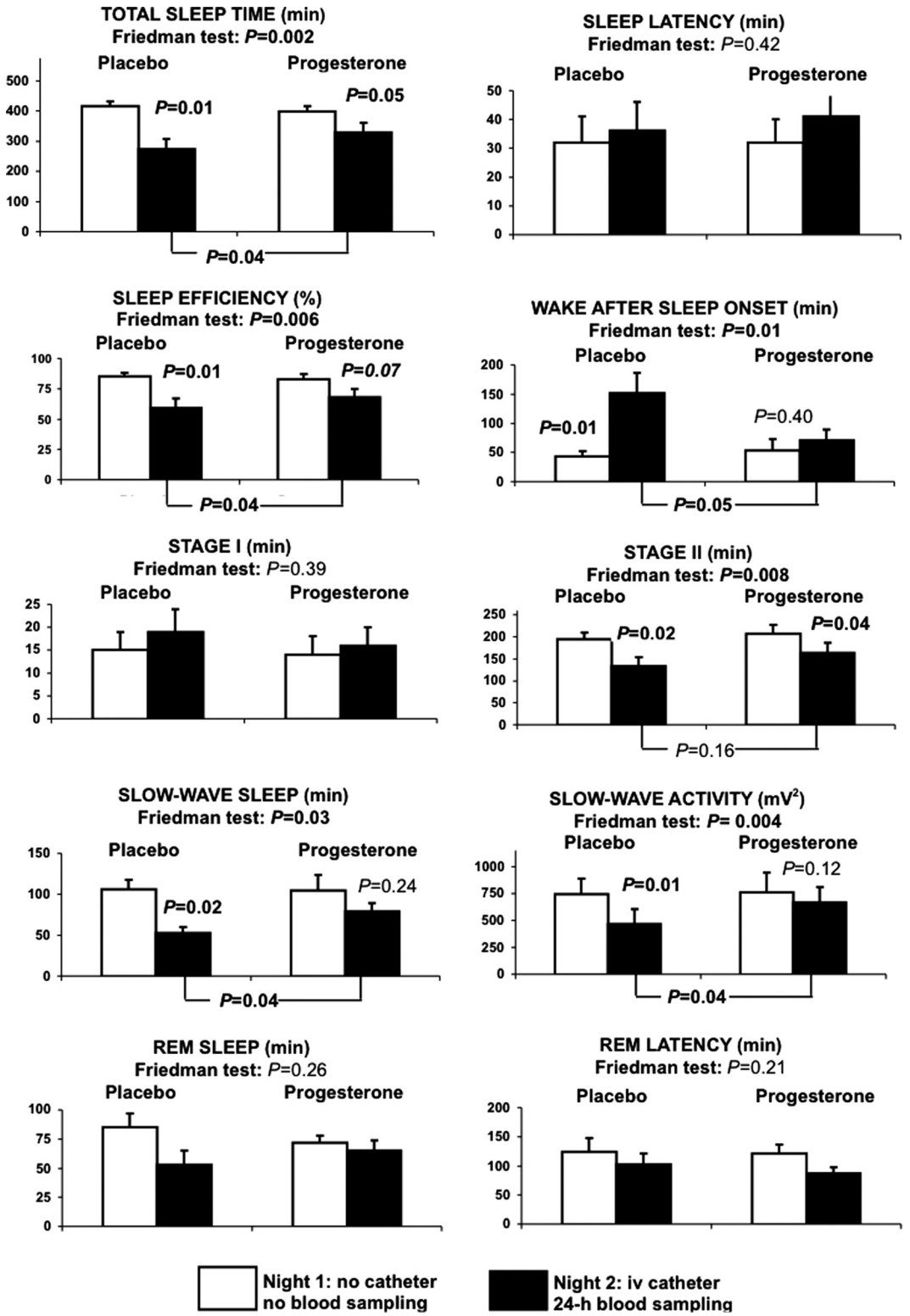

FIG. 3. Mean (+ SEM; n = 8) values of sleep variables during night 1 (no iv catheter, no blood sampling; *blank columns*) and during night 2 (iv catheter, blood sampling; *filled columns*) under placebo and under progesterone treatment. One outlier was not included in SWS values (see text). One outlier ($P < 0.01$) was not included in REM latency values: in this subject, the sleep latency during night 2 under placebo was 426 min, *vs.* values ranging from 39 to 169 min in the other seven subjects. The result of the Friedman test was identical when the outlier was included in the analysis. Except when otherwise stated, *P* values denote the results of Wilcoxon tests. *Bold values* denote statistically significant *P* level (≤0.05). *Italic-bold value* denotes a statistical trend ($0.05 < P < 0.10$). Wilcoxon tests were not performed for sleep latency, REM sleep, and REM latency because the Friedman test was not significant. Comparisons between placebo and progesterone during night 1 ($P \geq 0.33$) are not indicated.

Consistent with the findings previously reported in healthy older women (11, 15), sleep profiles under placebo were markedly disrupted during night 2 by the indwelling iv catheter and the blood sampling procedure. In contrast, those sleep alterations were largely prevented under progesterone. Thus, progesterone administration had no effect on normal sleep, but dramatically improved environment-related sleep perturbations: most importantly, both sleep duration and sleep quality (in particular increases in SWS and SWA) were improved under progesterone.

Very few studies have investigated the effects on sleep patterns of progesterone administration. They have yielded inconsistent results, probably because the protocol design varied widely from one study to another, in particular in view of the fact that progesterone could exert opposite effects at low or high concentrations (16). In the absence of nocturnal blood sampling, prolonged administration of progesterone alone (3) or of progesterone combined with estrogen (17) to healthy postmenopausal women without sleep disorders reduced wakefulness, but no other significant effect on sleep architecture was detected. In young men submitted to concomitant nocturnal blood sampling, a single dose of progesterone was reported to induce an increase in non-REM sleep and a possible decrease in δ power (2). In contrast, the present study was performed at the end of a 3-week treatment, when minimal progesterone levels, observed immediately before evening administration of the drug, were more than 20 times higher than corresponding levels under placebo.

Typical postmenopausal LH, FSH, estradiol, estrone, testosterone, DHEAS, and androstanediol glucuronide profiles were observed under placebo. The diurnal profile of testosterone was consistent with an adrenal origin of this hormone in postmenopausal women, as previously suggested (18).

Progesterone administration had no effect on LH but was associated with a slight but significant decrease in FSH levels, consistent with a negative feedback action at the hypothalamo-pituitary level (19, 20). Under placebo, progesterone and androstanediol glucuronide levels were not related to each other. However, after progesterone administration, the acute nocturnal elevation in progesterone concentrations was associated with a proportional and parallel increase in androstanediol glucuronide, while testosterone, DHEAS, estradiol, and estrone profiles were not altered. This indicates a possible conversion of progesterone to androstanediol via 5α-reduced metabolites of 17-hydroxyprogesterone, a pathway bypassing testosterone and dihydrotestosterone previously described in rat and rabbit testes and ovaries (21–24). Because liver is a major site of androstanediol glucuronide production (25), we hypothesize that this pathway could be operative in the liver when high progesterone concentrations are obtained in the hepatic portal system after oral administration of the drug. These results also cast doubt upon the reliability of the androstanediol glucuronide levels as a specific marker of androgenicity.

Diurnal profiles of ACTH and cortisol, two hormones primarily regulated by the circadian clock, were essentially similar in both conditions, and the timings of the onset/offset of the melatonin circadian rise were not affected by progesterone administration. These findings are consistent with those observed in younger cycling women in the luteal phase compared with the follicular phase (1). However, in the present study, progesterone treatment was associated with a transient inhibition of melatonin levels temporally coincident with the peak in progesterone circulating concentrations, suggesting a pharmacological effect of the drug, as evidenced in *in vitro* studies (26).

Temporal TSH patterns were similar in both conditions, but progesterone treatment was associated with lower TSH concentrations. In contrast, FT4 values remained unchanged, suggesting that lower concentrations of TSH were required to maintain adequate thyroxin levels. These data are consistent with our previous findings in luteal phase compared with follicular phase (1). On the other hand, at variance with the data obtained in younger women in luteal phase (1), no effect of progesterone on prolactin secretion was observed in the present study, possibly because of the low estradiol levels (27).

Consistent with the well-known dramatic age-related decline in GH levels (28), GH secretion in our postmenopausal women was markedly decreased compared with younger women (1). However, as in younger women (1)—though, as expected, to a lesser degree— elevated progesterone levels were associated with increased GH secretion. Interestingly, enhanced GH secretion was observed during nighttime, when progesterone levels were highest, suggesting a pharmacological action of the drug on GH release.

In summary, progesterone administration was associated with a reduction in sleep disturbances, increased GH secretion, and decreased TSH levels. All those effects could be mediated by the γ-aminobutyric acid (GABA) system, via neuroactive progesterone metabolites (including pregnanolone, allopregnanolone, and possibly androstanediol) (29–34).

Thus, the present results indicate that progesterone may restore normal sleep in postmenopausal women when sleep is disturbed by environmental conditions. Progesterone would not act as a conventional hypnotic (*i.e.*, it would not induce artificial sleepiness), but it would rather act as a "physiologic" regulator. Moreover, it would im-

prove sleep quality, while currently available hypnotics (benzodiazepines and Z-drugs) tend to inhibit, rather than enhance, SWA (35). These observations cannot be transposed to progesterone analogs, referred to as progestins, because they may act on different receptors and therefore have different— or even opposite— effects (17, 36–38).

In conclusion, although they need to be confirmed by larger studies performed in various experimental conditions, including postmenopausal women complaining about sleep problems, the present findings suggest that use of progesterone (or of specifically designed progesterone agonists) might provide novel therapeutic strategies for the treatment of sleep disturbances, in particular in the elderly.

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