Temporal Patterns of Integrated Plasma Hormone Levels During Sleep and Wakefulness. I. Thyroid-Stimulating Hormone, Growth Hormone and Cortisol

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ABSTRACT. Plasma levels of TSH, GH and cortisol were determined overnight in 4 healthy adults and over 48 hr in 1 man using a continuous blood sampling technique and polygraphic sleep monitoring. GH levels rose after sleep onset in 3 subjects and correlated with slow wave (Stage 3 & 4) sleep. Correlations between cortisol levels and sleep and wakefulness were found but there was no relationship between changes in TSH levels and sleep stage. While there were negative correlations in time between TSH and cortisol concentrations, consistent with inverse circadian rhythms, there were short-term fluctuations in the levels of both hormones which were positively correlated in the 4 overnight studies. (*J Clin Endocrinol Metab* **37**: 841, 1973)

THE continuous collection of venous blood samples for many hours makes it possible to determine the integrated concentration of plasma constituents over varying periods of time. Using a double lumen catheter (1) and integrated sampling periods of between 2 and 4 hr, it has been shown that there is a circadian rhythm in the plasma concentration of immunoreactive thyroid-stimulating hormone (TSH) in adult men and women (2,3). It was also noted that the elevations in TSH levels occurred simultaneously with rises in growth hormone (GH) levels in some samples taken during day-time sleep. This observation suggested that one component of the mechanism controlling TSH secretion may be the onset of sleep, as it is for GH (4).

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Nicoloff (5) has described an inverse relationship between TSH and cortisol secretion in man; the suppression of GH secretion by pharmacological doses of corticosteroids suggests that a similar inverse relationship might exist between GH and cortisol secretion (6).

In the experiments to be reported in this paper the technique of continuous blood sampling for periods of up to 48 hr has been used to collect 20 or 40-min blood samples for the measurement of integrated plasma levels of TSH, GH and cortisol. The relationships between the plasma levels of these hormones and the different stages of sleep have been examined and an inverse relationship between plasma cortisol and TSH levels has been found.

Materials and Methods

Subjects. Overnight studies were performed on 4 healthy volunteers; 2 men, aged 28 and 30 yr, and 2 women, aged 25 and 35 yr, both of whom were in the early follicular phase of the menstrual cycle. One of the men (FA) was also studied over a 48-hr period 3 months after the overnight study had been performed.

Sleep monitoring. For the overnight studies, subjects slept in a sleep laboratory for 3 con-

secutive nights. On each night sleep was monitored and on the first 2 nights tubing was bandaged onto an arm or connected to a needle inserted subcutaneously in order to accustom the subjects to the environment of the sleep laboratory. On the third night blood was sampled continuously for approximately 11 hr. Electrodes were attached to record continuously a single channel of the EEG (right parietal region to left mastoid process), horizontal eye movements (EOG) and electrical resistance of the skin over the palmar surfaces of the index and middle fingers. All these channels of information enabled the various stages of sleep and wakefulness to be analyzed objectively, using the technique by Johns (7).

In the 48-hr study, one of the men (FA) was studied again by the same method for a continuous period of 48 hr. During the day he ate his usual meals and exercised by walking at fixed times; the remainder of the day was spent sitting and reading.

Continuous blood sampling technique. Venous blood was collected continuously through a double lumen catheter (1) in a forearm vein. A solution of heparin in physiological saline (1,500 U/ml) was infused through the inner lumen of the catheter to prevent clotting. The heparinized blood was withdrawn through the outer lumen by a peristaltic pump at the rate of 0.6 ml/min during the overnight studies, and of 0.3 ml/min during the 48-hr studies. The blood took between 6 and 22 min to travel through the 3–4 m of plastic tubing from the catheter to a fraction collector. In the overnight studies 33, 20-min samples were collected, and in the 48-hr study the sampling period was 40 min, giving a total of 72 samples. Blood was centrifuged immediately after collection of each integrated sample and the plasma stored at −15 C until assayed. The pump, fraction collector and electronic recording equipment were housed in a room adjacent to the sleep laboratory. Two blockages of the blood line occurred which resulted in the loss of one sample in each of the overnight studies in the men.

Assay methods. TSH and GH were measured by double antibody radioimmunoassays previously described (3). Standards used were: GH, 1st IRP (66/127) and TSH, Medical Research Council, Standard A. Cortisol was measured by the competitive protein-binding method of Murphy *et al.* (8), using Fuller's earth to separate free from protein-bound steroid. All samples were assayed in duplicate and those from individual subjects were included in the same assay for TSH and GH; multiple assays using overlapping samples were required for cortisol estimations. Measurement of TSH concentrations was repeated in the samples of the second day of the 48-hr study in order to demonstrate the reproducibility of the TSH assay. The results of all assays, including their errors and sensitivities were calculated by the method of Burger *et al.* (9). Mean intra-assay precision over the range of results obtained expressed as a coefficient of variation was ±5% for TSH and ±8% for GH and cortisol. Mean sensitivities were as follows: GH, 0.3 μU/ml, TSH, 0.2 μU/ml and cortisol 3 ng/ml. The levels of each of these hormones have been found to be stable in blood at room temperature for at least 4 hr. (3).

Statistical analysis. Analyses of variance and covariance were performed as previously described (3). The main sources of variation considered were subject and time in the overnight studies, and day and time in the 48-hr study. In the overnight studies the time effect was defined as the mean of the results over all subjects at each particular sampling period and in the 2 days it was defined as the mean of the results over the 48-hr study at each particular sampling period. Data transformations were performed where appropriate using Tukey's test for nonadditivity (10) as a guide. Because of the missing samples in the sleep studies of each of the men, the number of results of the women were reduced to 32 by discarding the results of the first sample. Initially, the analysis of sleep was made in terms of the usual sleep stages of Rechtschaffen and Kales (11). To test for relationships between the hormone concentrations and sleep, wakefulness was distinguished from REM sleep, slow wave sleep (stages 3 & 4) and nonslow wave sleep (stages 1 & 2), and the proportions of each in the sampling periods were included in the analysis of variance and covariance.

Significant short-term fluctuations were defined as abrupt or progressive rises in hormone

levels followed by progressive declines over at least two consecutive samples where the peak level was significantly different ($p < 0.01$) from both the preceeding and following lowest levels. For this purpose, Student's t tests of the differences between the mean radioactive counts of the appropriate sample duplicates were used as previously described (9).

Results

Sleep. In the overnight studies the subjects went to sleep between 2320 and 0055, and woke spontaneously between 0630 and 0730. In 3 of the subjects the pattern of sleep was similar on each of the 3 nights monitored; however, 1 subject (JA, Fig. 3) was disturbed by pain at the site of the catheter. She was awake for considerable periods during the night, and only had one episode of REM sleep. On each of the 3 nights LG (Fig. 4) had less slow wave sleep than the other subjects. In the 48-hr study FA went to sleep and woke at similar times on both days (Fig. 5) and the distribution of sleep stages was similar on each night.

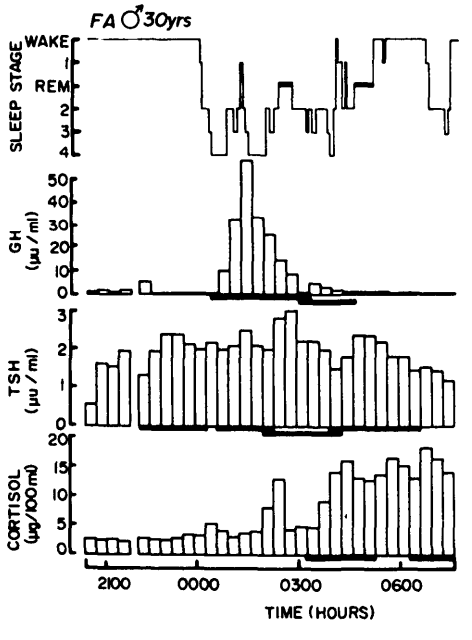

FIG. 1. Integrated concentrations of GH, TSH and cortisol and stages of sleep. The significant short-term fluctuations are underlined. These were defined as abrupt or progressive rises in hormone level followed by progressive declines over at least two consecutive samples where the peak level was significantly different ($p < 0.01$) from both the preceding and following lowest levels.

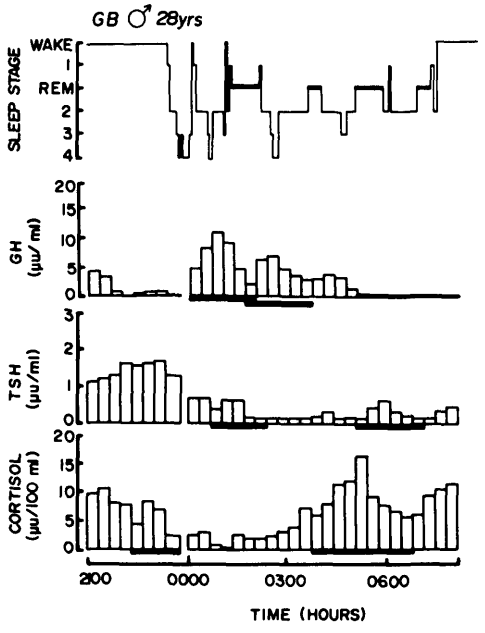

FIG. 2. See legend for Fig. 1.

Growth hormone (Figs. 1–5). In the overnight studies GH levels were elevated during wakefulness in the women, and minor rises also occurred in the men. Some of these increases may have been related to the stress of inserting the catheter and attaching the electrodes. The large increase (50 μU/ml) in the subject LG (Fig. 4) coincided with a period of stress when some air bubbles entered the heparin line. GH levels rose soon after the onset of sleep in 3 subjects, and in 2 (Figs. 2 and 3) additional smaller rises occurred during sleep. Only one minor (6.3 μU/ml) elevation occurred during sleep in LG (Fig. 4) at about 0500. This subject had a very large rise in GH during wakefulness in the evening, and had relatively little stage 4 sleep. In the 48-hr study (Fig. 5) the highest GH levels coincided with the onset of sleep and small elevations occurred later in the night. During the day the levels were below the sensitivity of the assay except for

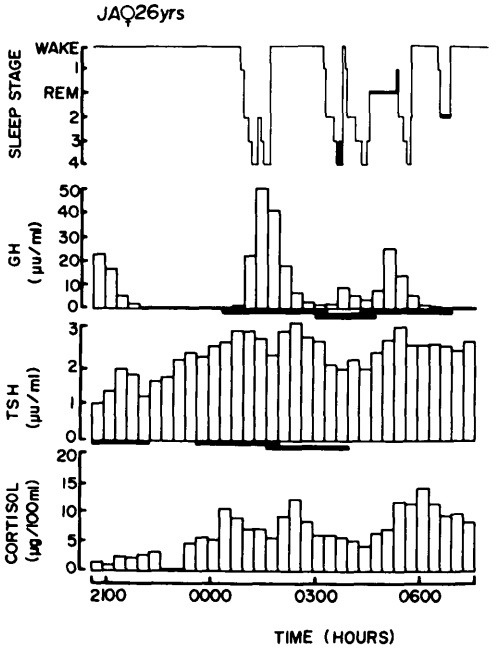

FIG. 3. See legend for Fig. 1.

small rises (3.8 and 1.9 μU/ml) at midday. The patterns were similar on both days and no changes with meals or exercise were observed.

Analysis of variance showed significant

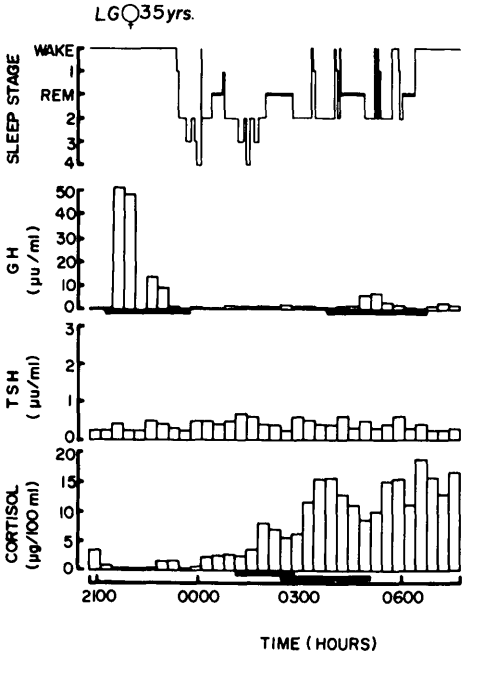

FIG. 4. See legend for Fig. 1.

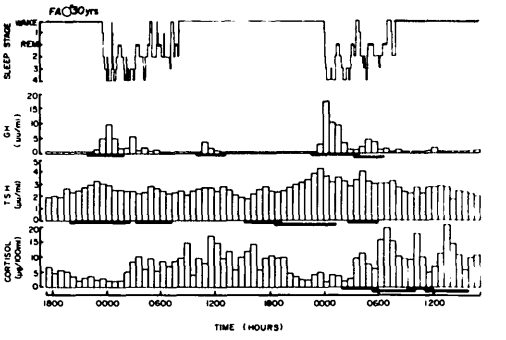

FIG. 5. See legend for Fig. 1.

time effects for the overnight study subjects as a group ($p < 0.05$) and for the 2 days of the 48-hr study ($p < 0.001$). Covariance analysis demonstrated positive correlations between GH levels and slow wave sleep ($p < 0.001$) in both studies, which were due predominantly to between times correlations ($p < 0.001$). However, there was also a between residuals correlation ($p < 0.01$) in the overnight studies, indicating a relationship between GH and slow wave sleep which was independent of time. There were negative correlations with wakefulness ($p < 0.05$ in the overnight studies, $p < 0.001$ in the 48-hr study) again, mainly due to between times correlation.

Thyroid-stimulating hormone. The plasma concentration of TSH varied with time, but not in the same way in all subjects: in the overnight studies both FA (Fig. 1) and JA (Fig. 3) showed a broad peak; in GB (Fig. 2) the highest level occurred in the evening before the onset of sleep and thereafter the levels remained low; in LG (Fig. 4) the levels were low and relatively constant throughout the period of study. Superimposed on these gradual changes there were a number of minor fluctuations. In three subjects significant short-term fluctuations were found which had cycle length of 100–200 min. Although a number of these fluctuations appeared to coincide with or follow periods of REM sleep, they also occurred during wakefulness. In the 48-hr study (Fig. 5) peak levels of TSH occurred between

2300 and 0000 on both days. There were also a number of short-term fluctuations and the levels were generally higher on the second day. The results of the two assays of the TSH levels on the second day of the 48-hr study are shown in Fig. 6. Although the mean levels were different because of inter-assay variability there was good agreement between the patterns. After allowing for the difference in mean levels a test for significance between the residual variations and the assay variations gave an $F_{35,\ 106}$ value of 1.36 (not significant).

In the overnight studies analysis of variance showed that the subjects had significantly different mean TSH levels ($p < 0.001$). There was, however, no significant time effect. In the 48-hr study the mean levels were significantly different on the 2 days ($p < 0.001$) and there was a significant time effect ($p < 0.01$). In the overnight studies there were positive correlations between TSH levels and wakefulness ($p < 0.01$) and slow wave sleep ($p < 0.05$), and negative correlations between TSH levels and nonslow wave sleep ($p < 0.001$) and REM sleep ($p < 0.05$). However, these were mainly due to the large subject differences and no temporal relationships between sleep stage and TSH levels were found. In the 48-hr study there was a negative correlation between TSH and wakefulness in time ($p < 0.01$). There were also positive between times correlations for TSH levels and both slow wave ($p < 0.05$) and non-slow wave sleep ($p < 0.05$). These correlations indicate that in the 48-hr study, TSH levels were lower during the day and higher during the night.

There was a significant relationship between GH and TSH in the overnight studies ($p < 0.05$). However, this was largely due to correlations between subjects. In the 48-hr study there was a positive between times correlation ($p < 0.001$).

Cortisol. In three of the overnight studies (Figs. 1, 2 and 4) and in the 48-hr study

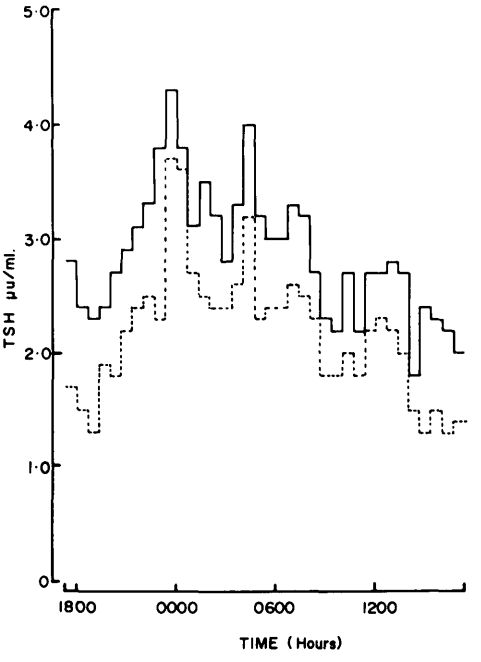

FIG. 6. TSH results from the second day of the 48-hr study from two TSH assays; (——) as in Fig. 5, (- - -) repeat assay.

(Fig. 5) cortisol levels were consistently low (< 5.0 μg/100 ml) between 2100 and 0300, but subsequently increased with a number of peaks in each subject. Several of these peaks appeared to be related to periods of either REM sleep or wakefulness. One subject (JA, Fig. 3) had increased cortisol concentrations when she was trying to fall asleep initially, and again while awake during the night.

There were significant time effects for cortisol levels in both the overnight ($p < 0.001$) and 48-hr studies ($p < 0.01$). In the overnight studies there was an inverse relationship between cortisol levels and wakefulness ($p < 0.05$) indicating that the predominant proportions of the circadian rhythm peaks occurred during sleep. However, there was a positive between residuals correlation between cortisol and wakefulness ($p < 0.001$) which suggests that superimposed on the circadian rhythm, rises in cortisol level occur coincident with periods of wakefulness. There was also a correlation

in time between cortisol levels and REM sleep ($p < 0.05$) but this may only indicate that most REM sleep occurred during the latter part of the night at the same time as the cortisol circadian rhythm peaks. In the 48-hr study there was a negative correlation between cortisol and slow wave sleep ($p < 0.001$) predominantly due to a between times correlation ($p < 0.01$), again probably only indicating that most slow wave sleep occurs during the initial part of the night at the time of the cortisol circadian rhythm nadirs.

In the overnight study there was a negative correlation between GH and cortisol ($p < 0.05$) which was largely due to a between residuals correlation ($p < 0.05$). Thus, there may be an inverse relationship between cortisol and GH secretion unrelated to the circadian rhythm of cortisol. However, no significant correlation between cortisol and GH was found in the 48-hr study.

There was a significant negative correlation in time ($p < 0.01$) between cortisol and TSH in both the overnight and 48-hr studies. This would be consistent with an inverse relationship between the circadian rhythms of cortisol and TSH. However, in the overnight studies there was also a positive between residuals correlation ($p < 0.001$) suggesting that episodes of TSH and cortisol secretion may occur together.

Discussion

The association of elevations in plasma GH levels with slow wave sleep (4) and stress (12) is well known. The absence of a peak after sleep onset in one of the subjects described here (LG, Fig. 4) is reminiscent of findings previously reported in two women (3). Other investigators have also noted that sleep-related GH peaks are less predictable in women (4). However, this subject had a large GH peak in the evening before going to sleep, presumably as a stress response, and this, together with the relatively small amount of slow wave sleep may be related to the lack of a GH peak after sleep onset. In the 48-hr study there was no obvious response of the GH levels to the ingestion of food. This supports the conclusions of a previous study (13) that rises in GH levels are not seen in the late postprandial periods of the usual Western feeding patterns, and that the suggested role of GH as a regulator of intermediary metabolism (14) may not have great significance under these circumstances. Suppression of GH secretion by prolonged administration of corticosteroids has been shown (6) and in the overnight studies there was a negative correlation between GH and cortisol levels. However, the present studies do not permit a statement about the possibility of a physiological inverse relationship between GH and cortisol secretion because no correlation was found in the 48-hr study.

The pattern of changes in cortisol concentrations is similar to that reported by other investigators (15,16). The rises seen around midnight in subject JA (Fig. 3) at the time of episodes of discomfort were probably stress responses. A relationship between cortisol level and REM sleep was found, confirming the observations of Weitzman *et al.* (17). However, this could be explained by the coincidence of the peak cortisol levels and the maximum frequency of REM sleep in the early morning. The correlation between cortisol levels and wakefulness remaining after subject and time effects were removed is more meaningful and indicates episodes of wakefulness during sleep at night were associated with cortisol secretion irrespective of both the time at which they occurred and the cortisol circadian rhythm.

The highest TSH levels in three of the four overnight studies, and on both days of the 48-hr study, occurred between 2200 and 0300. In a previous study (3) using longer integrated sampling intervals over 24 hr in 9 subjects a circadian rhythm of plasma TSH levels was found and the peak levels occurred between 2115 and 0530. There were a number of significant short-term fluc-

tuations in the levels of TSH of 2–3 hr duration. Vanhaelst *et al.* (18) studying the circadian rhythm of TSH levels also noted short-term variations. These fluctuations are probably caused by changes in the rate of secretion of TSH but could result from variations in plasma clearance.

The inverse relationship between TSH and cortisol levels is in accord with the finding of Nicoloff (5). Using thyroidal iodine release as an index of TSH activity, he showed there was an inverse correlation between iodine release and the circadian rhythm of cortisol and suggested that cortisol may be involved in the production of the circadian rhythm of TSH. However, the present study showed the inverse relationship was not exact. In fact there was evidence in the overnight studies that the short-term fluctuations were positively related suggesting that the short episodes of secretion of both hormones may occur together. The overall relationship may simply reflect circadian rhythms for both hormones that are out of phase, the peak TSH levels coinciding with the cortisol nadir, rather than a functionally significant physiological feedback relationship between TSH and cortisol. Studies to determine the effect of infused corticosteroids on TSH levels are in progress.

Although evidence has been presented previously (2,3) that there are both circadian and sleep-related rhythms of TSH secretion, the present data shows that short-term fluctuations occur during sleep and wakefulness, and are not related to any specific sleep stage. However, the possibility of an influence of sleep on TSH secretion has not been finally excluded because no investigation of sleep during the day, or reversal of the pattern of sleep and wakefulness was made.

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