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Nocturnal Secretory Patterns of FSH, GH, LH and TSH

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ABSTRACT

The nocturnal secretory patterns of follicle-stimulating hormone (FSH), growth hormone (GH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH) were studied in 1 female and 4 male volunteers. Immunoreactive FSH, GH, LH and TSH in serum were assayed by a radioimmunosorbent technique. Non-conjugated 11-hydroxycorticosteroids in plasma were also measured in the male volunteers by a fluorimetric method. Blood samples were taken half hourly from 10 p.m. to 8 a.m. Polygraphic monitoring of sleep stages was performed. In all the subjects a marked elevation of serum GH occurred within 60 minutes after the onset of sleep and additional, isolated GH peaks were observed in 3 subjects. The serum concentrations of FSH, LH and TSH also fluctuated, but the variations were small compared with the fluctuations of GH. The serum levels of FSH, LH and TSH respectively were not correlated with the depth or stages of sleep. The observed fluctuations, in the serum levels of all hormones assayed, are larger than could be expected from intra-assay errors and probably represent changes in the secretion rate of the hormones. A lack of concordance between the secretory patterns of FSH, GH, LH and TSH indicates that different mechanisms may be involved in their release.

INTRODUCTION

During recent years several studies have been made on the secretory patterns of hypophyseal hormones. The occurrence of a circadian rhythm, and an episodic secretion of ACTH and cortisol are well established (7, 12). The secretory pattern of the growth hormone (GH), which is characterized by sporadic peaks and a marked elevation of plasma GH at the onset of deep sleep, is also well known (9, 27). However, the results from studies on the plasma levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH) are more divergent. A circadian rhythm of the secretion of FSH (5), LH (26) and TSH (17) have been reported, whereas in other investigations (8, 18) such a rhythm has not been observed. An episodic secretion of FSH and LH has been found in some studies (15, 25), whereas in other investigations the plasma levels have been rather constant (20). Mostly, the plasma levels of only one or two hypophyseal hormones have been determined at the same time. The present investigation was undertaken to ascertain whether there is any correlation between the nocturnal secretory patterns of FSH, GH, LH and TSH or between the secretory patterns of these hormones and different stages of sleep.

MATERIALS AND METHODS

Four healthy male volunteers, in the age range 19–24, and one 24-year-old, healthy female volunteer were studied. None of them took any medicine, tobacco or alcohol during the study. The female (MO) had a normal menstrual cycle and did not take oral contraceptives. Blood samples were obtained from her 9 and 10 days respectively after the onset of the preceding menstrual period. All the subjects reported a normal sleep–waking cycle. All had normal EEG activity during sleep and wakefulness. Three of the subjects (CB, JB and MO) spent 3 nights, and two (HH and BS) spent 4 consecutive nights in a quiet room in the neurophysiological department. Only a weak light was used in the room from 11 p.m. to 7 a.m. The subjects were fasting after 7 p.m. Electroencephalographic, electrooculographic and electromyographic recordings were made continuously from 10 p.m. to 7 a.m. on the last 2 nights. Because of a technical defect, sleep-stage scoring for the female volunteer was possible only for the last night of the study. For the female subject blood sampling took place on the last 2 nights of the study and for the males on the last night. A flexible venous cannula was inserted into an antecubital vein and connected to a 2-foot long plastic tube, so as to permit blood drawing without touching the subject's arm. In 3 cases the system was filled with heparinized isotonic saline between samples, and in 2 cases the cannula was kept patent with a slow drip of isotonic saline. In each case

blood samples were taken half hourly from 10 p.m. to 8 a.m. From the female subject a total volume of approximately 200 ml of blood was removed on each of the 2 nights of the study. Plasma 11-hydroxycorticosteroids were not determined in the female volunteer in order to avoid removal of more blood. A total volume of approximately 300 ml of blood was removed from each male subject. Blood samples for assay of plasma 11-hydroxycorticosteroids were run into heparinized tubes, whereas FSH, GH, LH and TSH were determined in serum. After centrifugation plasma and serum were stored at −20°C until assayed.

Plasma 11-hydroxycorticosteroids were assayed by a fluorimetric method measuring non-conjugated 11-hydroxycorticosteroids (14). During the period of investigation the intra-assay error estimated from duplicate determinations was 4–6% (coefficient of variation).

Immunoreactive FSH, GH, LH and TSH in serum were assayed by a radioimmunosorbent technique (31, 32, 33). FSH in serum was measured by utilizing human pituitary FSH (23) labelled with ^{125}I and guinea-pig anti-human pituitary FSH. The FSH preparation had a biological activity of 14 000 IU (2nd IRP-HMG) per mg. The results were expressed in ng per ml. One ng of FSH was equivalent to 369 ng of LER-907 in the immunoassay.

GH in serum was measured by utilizing human pituitary GH (24) labelled with ^{125}I and rabbit anti-human pituitary GH. The results were expressed in ng per ml using the WHO International Reference Preparations of Growth Hormone, 66/217, as a reference.

LH in serum was measured by utilizing human pituitary LH (23) labelled with ^{125}I and rabbit anti-human pituitary LH. The LH preparation had a biological activity of 14 000 IU (2nd IRP-HMG) per mg. The results were expressed in ng per ml. One ng of LH was equivalent to 83 ng of LER-907 in the immunoassay.

TSH in serum was assayed by utilizing human pituitary TSH (National Pituitary Agency) labelled with ^{125}I and rabbit anti-human pituitary TSH. The activity was expressed in μU per ml using Medical Research Council HTSH Research Standard A as a reference.

In all radioimmunoassays the standards were dissolved in sera with undetectable or low levels of the hormones to be assayed. The samples were assayed in duplicate. The results of the immunoassays were calculated by the use of a logit transformation as proposed by Rodbard et al. (22). The percentage standard error of the mean of the two determinations was between 4 and 6%.

For recordings of EEG, neck muscle EMG and eye movements Ag-AgCl surface electrodes were glued to the skin. Two symmetrical EEG channels were recorded with electrodes in position P_3–O_1 and P_4–O_2.

For recordings of eye movements one electrode was placed at the lateral corner of one eye and another electrode above the same eye. The EMG electrodes were placed symmetrically over the muscles on the back of the neck. The 4 signals were amplified in a standard EEG apparatus (Elema, Sweden) and from there fed to a 4 channel FM tape recorder (PI–6200, USA), where they were stored for later analysis. EEG analysis was made visually and 4 sleep stages were separated. *Stage 1:* Light sleep with low voltage background, sleep spindles and K-complexes. No rapid eye movements. *Stage 2:* Intermediary stage with a fair amount of high voltage slow waves and superimposed spindles. *Stage 3:* Deep sleep dominated by high voltage, slow delta waves. *Rapid eye movement (REM) stage:* Low voltage, fast activity without spindles and with simultaneously occurring rapid eye movements. Absence of EMG activity in the neck muscles.

RESULTS

The results are shown in Figs. 1–3. Onset of sleep occurred between 10 and 11 p.m. in 2 subjects and between midnight and 1 a.m. in 3 subjects. Each person slept well for several hours during the night, but the blood sampling procedure occasionally awakened the subjects. In 2 men (CB and BS) REM sleep constituted about 10% and in 2 men and one woman (JB, HH and MO) REM sleep constituted about 15% of total sleep.

The serum or plasma concentrations of the 5 hormones assayed were, throughout, within the normal range as determined in this laboratory in fertile men and women.

In the 4 male volunteers, in whom plasma 11-hydroxycorticosteroids were determined, maximum levels occurred during the early morning hours. The pattern of plasma 11-hydroxycorticosteroid levels was in all the subjects characterized by sharp increases followed by falls in the plasma level. The lowest levels occurred between 2 and 4 a.m.

It can be seen on the graphs that in all the subjects the serum concentrations of FSH, GH, LH and TSH fluctuated. Each point on the graphs represents the mean of duplicate determinations. The variation between the points in each curve is caused by the error of the mean of the duplicates (intra-sample variation) and a possible true variation between the samples (inter-sample variation). The variance of the results obtained during the period of investigation was calculated for each curve. The difference between this value and the variance of the mean of the duplicate determinations (intra-sample variation) gave an estimate of the inter-sample variance. The median and range of the inter-sample variation, expressed as percentage standard deviation was 12 (4.2–17.3) for FSH, 22.7 (16.4–30.7) for LH, 15.3 (10.0–22.3) for TSH and 95.5 (71.8–130) for GH. Thus, an

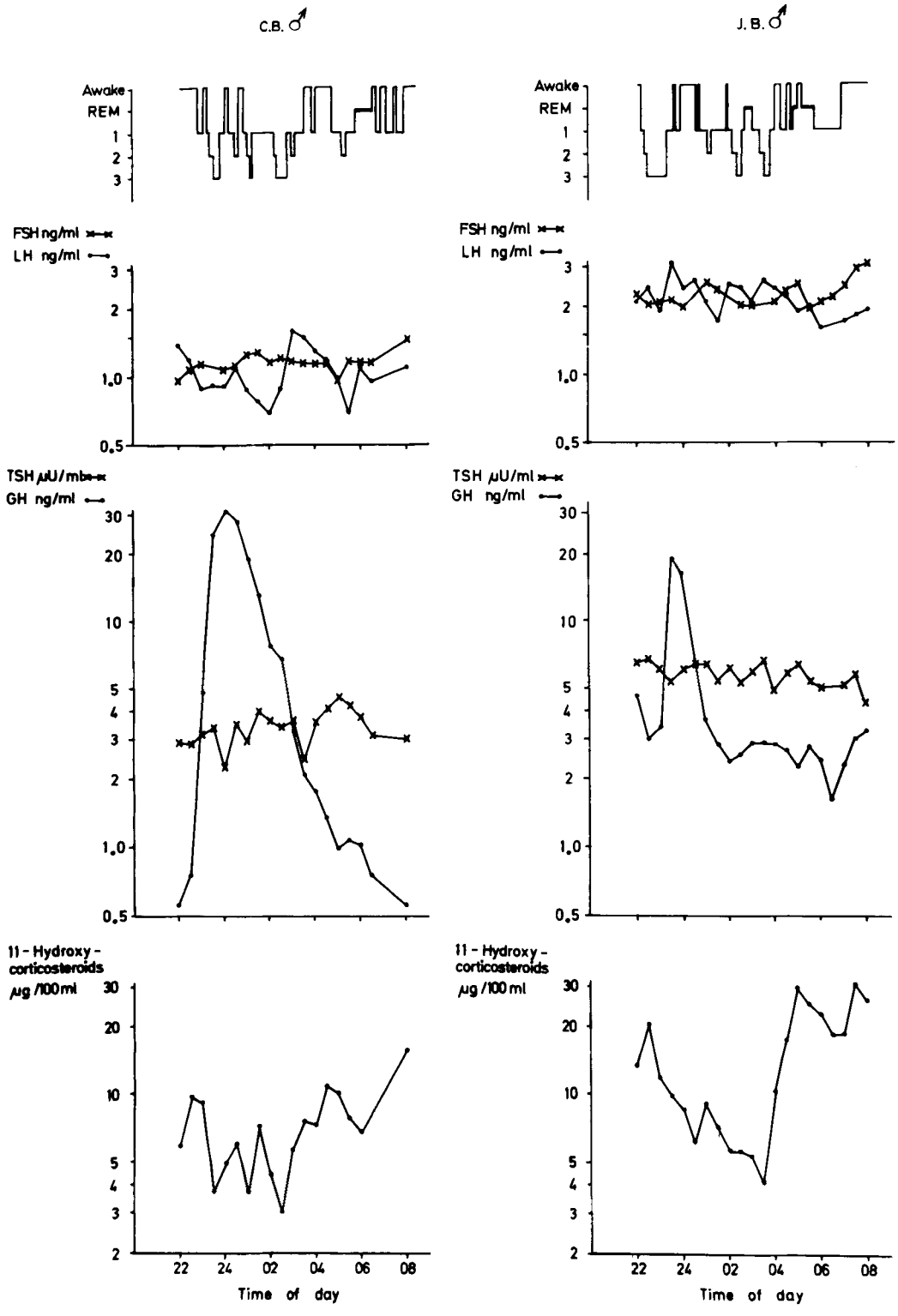

Fig. 1. Patterns of serum FSH, LH, TSH, GH and plasma 11-hydroxycorticosteroid levels and their relationship to sleep stages in male subjects CB and JB. Blood samples taken half hourly from 10 p.m. to 8 a.m.

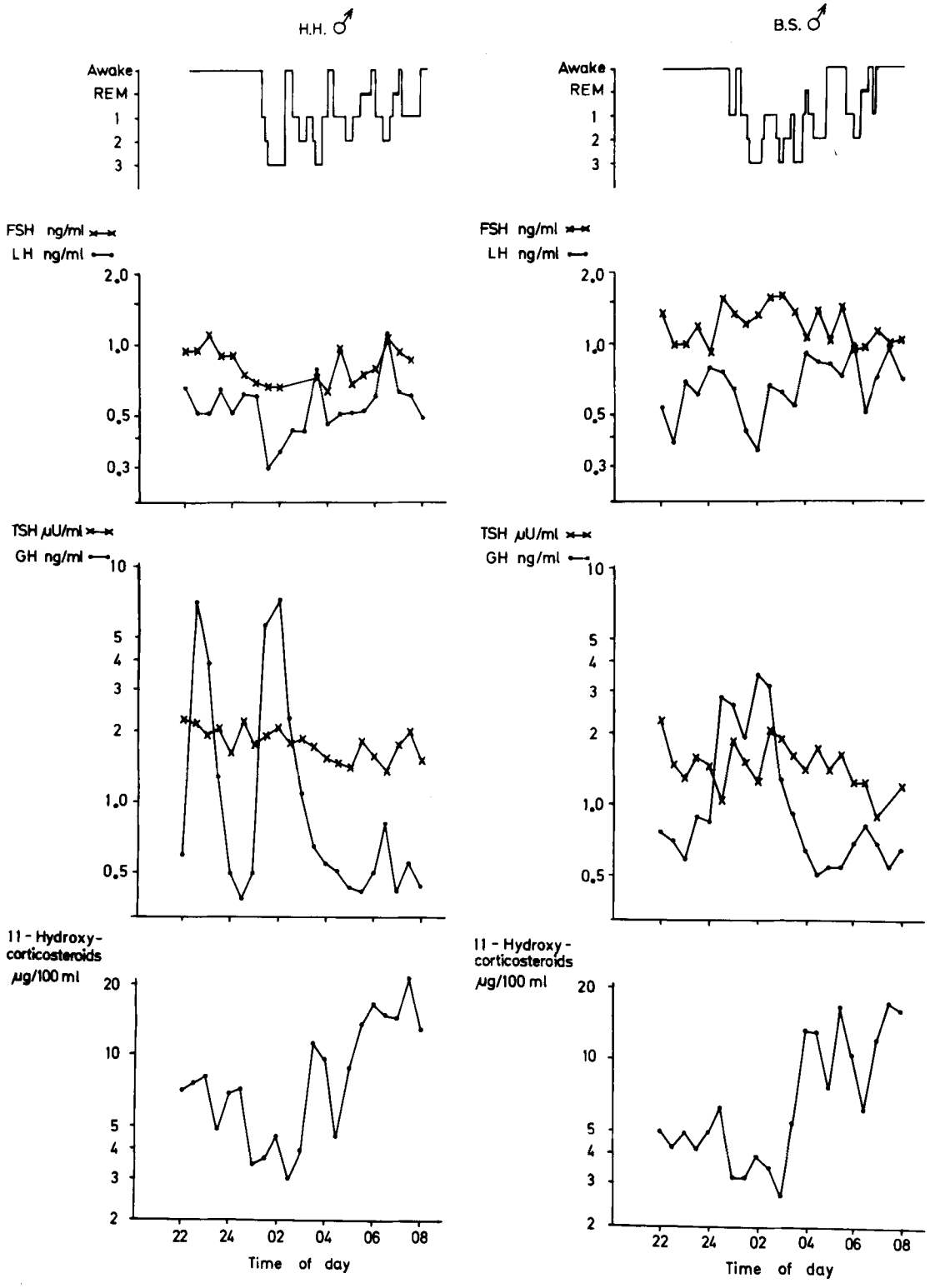

Fig. 2. Patterns of serum FSH, LH, TSH, GH and plasma 11-hydroxycorticosteroid levels and their relationship to sleep stages in male subjects HH and BS. Blood samples taken half hourly from 10 p.m. to 8 a.m.

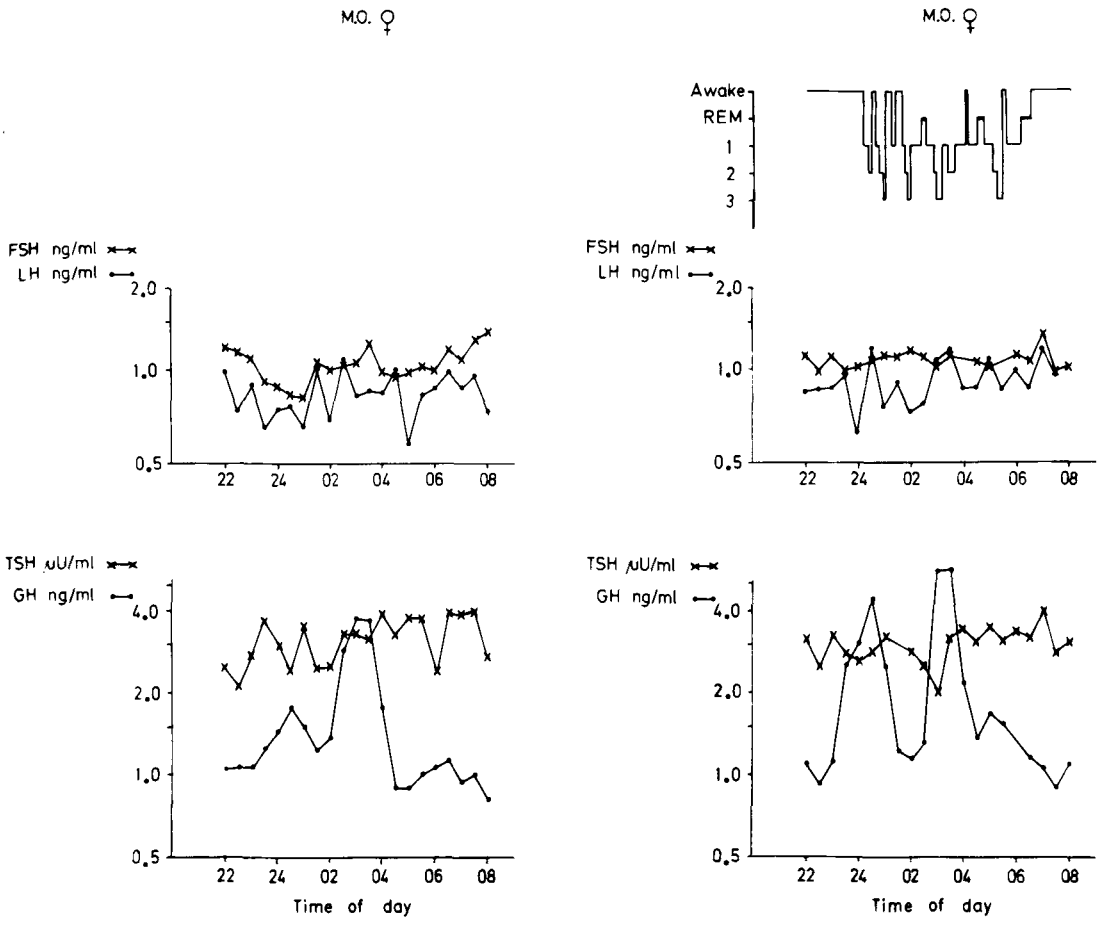

Fig. 3. Patterns of serum FSH, LH, TSH and GH levels in the female subject. Blood samples taken half hourly two consecutive nights, 9 and 10 days respectively after the onset of the preceding menstrual period. Sleep stages recorded the last night.

evident inter-sample variation was found for all four pituitary hormones.

In all the subjects a significant elevation of serum GH concentration occurred within 60 minutes after the onset of sleep. A peak in serum GH concentration preceded the GH peaks associated with the onset of sleep in 2 subjects (HH and BS). In the female subject a second GH peak at 3 a.m. followed the peak which appeared at the onset of sleep.

The variation in the level of LH was greater than those of the FSH and TSH levels. Serum levels of FSH, LH and TSH were not correlated ($p>0.05$) with the depth or stages of sleep. The secretory patterns of FSH, LH and TSH were not constant »between nights» in the female subject studied. Furthermore no relationship was observed between the secretory patterns of FSH, GH, LH and TSH in any of the 5 subjects.

DISCUSSION

Several studies have shown that cortisol is secreted in a series of brief episodes separated by intervals during which there is either no, or only low, secretion. In men and women with a »normal» sleep–waking cycle most of the secretory bursts occur in the early morning hours, with maximum plasma cortisol levels between 4 and 8 a.m. (7, 12, 19, 30). In the present investigation the pattern of plasma 11-hydroxycorticosteroid levels was, in all 4 men in accordance with that de-

scribed previously for plasma cortisol. Thus, all the men had a »normal» pattern of plasma 11-hydroxycorticosteroid levels in spite of a certain amount of stress caused by frequent blood sampling. Krieger et al. (12) found a very good correlation between the times of peaks of plasma corticosteroid levels and those of plasma ACTH levels. The time at which the ACTH peak occurred coincided in most cases with that of the plasma 11-hydroxy-corticosteroid peak. However, there was no apparent proportionality between plasma corticosteroid and plasma ACTH levels.

In all the subjects a marked elevation of serum GH was observed at the onset of sleep. In 3 subjects, 2 male and one female a second GH peak was observed. The peak at 10.30 p.m. in subject HH may be due to the stress of the initial venepunction, that at midnight in subject BS is probably part of the nocturnal peak. The observed secretory pattern of GH is similar to that previously reported (9, 10, 21, 27). Evidently, the peak of serum GH at the onset of sleep is very constant, whereas additional nocturnal peaks are often missing.

Dierschke et al. (4) found, in ovariectomized rhesus monkeys, wide rhythmic oscillations in the plasma LH levels. In intact female monkeys no such fluctuations were observed. Similarly, in castrated male and female rats fairly regularly occurring peaks of LH were found, whereas the serum FSH levels were more stable (6). Yen et al. (34) reported, in women of fertile age, fluctuations in the serum level of LH which were rather regular in frequency and magnitude. However, these parameters varied according to the stage of the menstrual cycle.

In the present study the fluctuations of FSH and LH occurred irregularly, and were of varying magnitude. This finding is in accordance with observations reported in recent investigations in adult men (2, 13, 16, 25) and in one investigation on women of fertile age (15). The fluctuations found for FSH were smaller than for LH which agrees with previous studies (15, 32, 34). There was no correlation between the levels of FSH and LH, and the depth or stages of sleep.

Rubin et al. (25) observed that in adult men the LH values were 14% higher during REM sleep than during other stages of sleep; but release of FSH was not clearly related to the stages of sleep. On the other hand, Boyar et al. (1) did not find, in adult men and prepubertal children, any significant difference between mean LH concentration, regardless of whether the subjects were asleep or awake; and no relation between LH secretory activity and the stages of sleep was observed. In pubertal children, however, the mean, sleep LH concentration was significantly higher than the mean, awake LH concentration (1).

Vanhaelst et al. (28) observed, in 7 of 8 euthyroid subjects, a peak of TSH between 4 and 6 a.m. Moreover, all the subjects exhibited a rhythm of higher frequency, lasting from 1 to 2 hours. The findings of these authors are at variance with those of Webster et al. (29), who demonstrated, in normal subjects and in patients with myxedema, occasional significant variations in serum TSH concentration, but no convincing pattern of circadian rhythm or other mode of variation of TSH secretion. The present study supports the results of Webster et al. (29). Fluctuations of the serum concentrations of TSH were observed, but there was no evidence of a regular periodicity.

The observed fluctuations, in the serum levels of all hormones assayed, are larger than could be expected from intra-assay errors. The fluctuations of the radioimmunoassayed hormones, FSH, GH, LH, TSH, are not synchronized. Therefore it is unlikely that the fluctuations are due to variations of substances in serum, causing nonspecific interference with the assay technique. Kohler et al. (11), and Coble et al. (3) have shown that the metabolic clearance rates of LH and FSH are rather constant, and are not influenced by gonadal function. It is likely that the observed fluctuations in the serum levels of FSH, GH, LH and TSH probably represent changes in the secretion rate of the hormones.

Takahashi et al. (27) did not find any correlation between the secretory patterns of cortisol and GH. Rubin et al. (25) observed no relation between the peaks of FSH and LH in adult men; nor did Krieger et al. (13) detect any relation between the patterns of FSH, GH and LH in adult men. Yen et al. (34) failed to observe any relation between the secretory patterns of FSH and LH in women of fertile age, but in postmenopausal women the fluctuations of FSH and LH usually were coincidental with minor asynchrony. In the study by Midgley & Jaffe (15), in women of fertile age, the changes in the FSH level often paralleled those of LH.

In this study, no correlation was found between

the nocturnal secretory patterns of FSH, GH, LH and TSH.

The mechanisms, regulating the secretion of the different hypophyseal hormones, are still unknown. The lack of concordance between the secretory patterns of FSH, GH, LH and TSH indicates that different mechanisms may be involved in their release. It is not known whether the observed fluctuations in the trophic hormone levels are necessary for the maintenance of a normal function of the target organs. Further similar studies on patients with hypothalamo-hypophyseal diseases may prove valuable for understanding the importance of the observed secretory patterns.

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