Individuality and stability of nocturnal secretion patterns for eight hormones in healthy young men

The concentration of hormones in the bloodstream shows oscillations, reflecting the fact that endocrine physiology is structured over time. In many cases, these oscillations have an ultradian configuration that can be superimposed on a circadian rhythm. Secretion of hormones can be linked to the phases of sleep, as is the case with growth hormone (GH); can depend strongly on the circadian pacemaker, as in the case of cortisol; or be under the influence of both, as seen for thyroid-stimulating hormone (TSH). Thus, the temporal pattern of secretion of several hormones, and the resulting plasma concentration (also influenced by hormone tissue distribution and clearance), depends on impulses from biological clocks and is influenced by endogenous and exogenous masking factors.

The extent of interindividual differences in the phenotypes of temporal patterns of hormone secretion is not well known. In this study, a series of eight hormones were measured over one night, and these measurements were repeated over another night. The study had two goals. The first was to explore the extent of interindividual differences in nocturnal and ultradian rhythms of these hormones. The second was to see how stable the individual patterns of nocturnal hormone secretion could be. Our results indicate that the temporal organization of hormone secretion into the blood is highly individual, and that these intraindividual patterns are conserved over time. This is relevant in view of the changes in secretion of several hormones that have been described in biological psychiatry research.

Methods

Six young males, 20 to 30 years of age, were included in a protocol where repeated blood sampling was carried out over a whole night, from 20:00 to 08:00. They were considered to be healthy on the basis of their physical and psychiatric medical history, and physical examination. Routine laboratory blood tests were performed before inclusion in the study; all values were in the normal range. The protocol had been accepted by the local ethical committee. Early in the evening of the study, a short cannula was inserted into a forearm, and blood was subsequently sampled every 20 minutes. Subjects received a light standardized meal at 18:00. Lights were turned off at 21:30 and on again at 06:30. During the night, the light in the room was kept very dim (less than 50 Lux), and the subjects did not wake up during sampling. This protocol was repeated once in all 6 subjects. There was an interval of at least 7 days between each study night, and each study was performed on the same day of the week for a given subject. Blood was centrifuged, and plasma rapidly frozen. A total of eight hormones were measured using commercially available immunoassays. These assays had intra-assay and interassay coefficients of variation of <5 and <10%, respectively. The hormones measured were cortisol, TSH, prolactin (PRL), luteinizing hormone (LH), GH, melatonin, aldosterone, and testosterone.
Results

Figures 1 and 2 show the nocturnal section of the circadian rhythm for LH and cortisol. The ultradian rhythm of LH was clear, with 1 to 6 nocturnal peaks by visual inspection, depending on the subjects. The amplitude of the secretory peaks varied, but the mean nocturnal value of LH concentration did not depend on the number of peaks. The nocturnal increase in cortisol was absent in one subject (No 3) and the expected nocturnal peak concentrations were only slightly higher than the early evening values in subject No 6. There was quite a lot of variability in the timing of the cortisol surge. It started between 24:00 and 02:00 in two subjects (Nos 1 and 6), and between 02:00 and 04:30 in the other subjects, except for subject No 3 (no increase).

Figure 3 shows the nocturnal plasma concentration of six other hormones in 1 subject (No 5) over 2 nights. The stability of the patterns is apparent, mostly with testosterone and melatonin, while the GH patterns differ. Calculation of intraindividual stability based on area under the concentration versus time curve (AUC)

Figure 1. Nocturnal secretion patterns of luteinizing hormone in six subjects over 2 nights. Closed circles, first night (N1); open circles, second night (N2). Lights on/off indicated by dashed lines.

Figure 2. Nocturnal secretion patterns of cortisol in six subjects over 2 nights. Closed circles, first night (N1); open circles, second night (N2). Lights on/off indicated by dashed lines.

Figure 3. Nocturnal secretion patterns of six hormones in one subject over 2 nights. Closed circles, first night (N1); open circles, second night (N2). Lights on/off indicated by dashed lines.
between individual AUCs from the first and second night (n=6 subjects) showed highest values for LH (Pearson $r=0.98$), and lowest values for cortisol (Pearson $r=0.25$). This correlation on AUC concerns the amount of hormone secreted (assuming a constant hormone clearance). Pearson’s correlations coefficients calculated on hormone concentrations over the 2 nights, within each subject, gives an evaluation of both the amount of hormone and the temporal organization of secretion. Indeed, in this case, the coefficient will be high only if the temporal patterns are similar and occur with no phase shift between the two successive nights of measurement in the same subject. The mean values (n=6 subjects) were lowest for LH (mean Pearson’s $r=0.41$) and highest with melatonin (mean Pearson $r=0.89$).

These data suggest that there can be marked changes in temporal patterns with little changes in the amount of hormone produced during the night (eg, subjects No 1 and 4 for LH, Figure 1) and, conversely, no changes in temporal patterns, but marked changes in nocturnal hormone production (eg, subject No 6 for cortisol, Figure 2).

The study of the nocturnal concentration of several hormones in the plasma showed that the temporal pattern of secretion varied interindividually, and that it was relatively stable over time.

**Stable individual time structure of hormone secretion**

The interindividual differences in nocturnal hormone secretion were of such a magnitude that some of our subjects did not show the pattern of secretion considered to be usual or normal. Indeed, in this small group of six subjects, three showed no nocturnal increase in TSH, one had no nocturnal increase in cortisol and aldosterone, and two had no differences between cortisol concentrations during early-evening and late-night sampling times. The subjects were considered to be physically and mentally healthy, but no standard questionnaire or scale was used to provide a formal confirmation.

Other hormones showed a wide range of secretory patterns, but within the range considered to be normal. This was the case with LH, with nocturnal concentration patterns showing 1 to 6 secretory pulses depending on subject, together with a 3-fold range in mean LH concentration. These interindividual differences in LH secretion in men were stable over time. Several authors have studied the chronobiology of hormones in normal subjects or in patients in protocols where measurements were repeated. Thus, in 10 normal subjects studied over 24 hours on three occasions, the coefficients of variation of the parameters for melatonin, prolactin, LH, and testosterone secretion varied less within subjects than between subjects, indicating that the individual secretion patterns were stable over time, particularly with LH, as also seen in our subjects.

**Coincident secretory pulses of hormones**

Hormones that belong to the same endocrine axis are often secreted with similar temporal patterns, for example cortisol and adrenocorticotropic hormone (ACTH) or β-endorphin, LH and estradiol or progesterone. This coincidence in hormone secretory pulses is to be expected.

However, temporal coincidence is also found between hormones that belong to different axes. Examples are LH and prolactin, testosterone and melatonin, TSH and leptin, LH and leptin in women, and the amino acid L-arginine and insulin.

In addition, more than two hormone secretions can be coupled temporally, as described for cortisol, leptin, LH, and GH. Finally, ultradian coupling can occur between events that belong to different physiological systems. An illustration is the finding by Brandenberger of the coupling between prolactin and electroencephalographic sleep waves. In this particular case, the coupling was so tight that the normalized hormone concentration and delta wave power (expressed as Z-scores) followed almost exactly the same value versus time curve.

**Ultranadian rhythms**

Ultradian and circadian changes in blood hormone concentrations are an indirect marker of the activity of central nervous system pacemakers, but how biological clocks govern ultradian rhythms of hormone secretion is still not well understood. Gonadotrophin-releasing hormone (GnRH, also called luteinizing-releasing hormone or LHRH) stands as an exception and has been well described for several decades. Neurons in the hypothalamus show regular bursts of electrical activity that are accompanied by secretion of GnRH. This hypothalamic ultradian rhythm of GnRH influences the anterior pitu-
itary, and leads to the secretion of LH. Differences in the frequency of LH secretory pulses could be due to individual differences in ultradian biological clocks. Alternatively, not all GnRH pulses lead to LH pulses. Thus, the presence of only a few LH pulses in the peripheral blood, as noted in subject No 4, cannot be taken as an indicator of a slow central ultradian clock function. In contrast, when LH secretory pulses are frequent and regular, one can assume that the period of LH ultradian rhythm corresponds to that of hypothalamic GnRH release. The extent to which this and other ultradian rhythms are independent of the main biological clock located in the suprachiasmatic nucleus remains to be further studied. Indeed, mutations of the circadian clock in the Syrian hamster affect cortisol and LH ultradian rhythms.11

Conclusions

Some biological compounds have a particularly narrow range of normal values. This is for instance the case with plasma electrolytes. In contrast, other biological variables have a wider range of their normal values. This is the case with many hormones. Whether the range of normal values is small or large, one can observe that each individual has his/her own values of the variables, and that these values tend to be temporally stable (when the measurements are repeated at the same time of the day when considering circadian rhythms). In a previous study of daytime hormone concentration in normal subjects, we measured up to a 6-fold range in mean concentration (plasma samples taken on two occasions, between 8:00 and 12:00) for TSH, follicle-stimulating hormone (FSH) and testosterone.12 These interindividual differences were stable over time.

The individuality in mean plasma hormone concentrations and in their temporal pattern of secretion suggests that homeostasis is highly individual, i.e., does not result from a random assemblage of variables within the range of normal values and from general rules of adaptation to the environment. Indeed, each individual has his or her specific configuration of biological variables12 This configuration can be represented as a group of variable values and of their ratios (for example high TSH, medium cortisol, and low testosterone in a given individual). Moreover, individual configurations of variables and of variable value ratios change over time. Rhythm stability over time is a criterion by which biological variables should be evaluated, and this illustrates the complexity of chronobiological studies.1

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