Sleep enhances nocturnal plasma ghrelin levels in healthy subjects

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Ghrelin is a 28-amino acid polypeptide that has been discovered as an endogenous ligand for the growth hormone secretagogue receptor (11). Ghrelin is synthesized mainly in stomach endocrine cells but has also been identified in other tissues, including the hypothalamus (7, 10, 12). Ghrelin stimulates growth hormone secretion, increases food intake and appetite, and promotes body weight gain (17, 37, 38). Recent findings suggest that ghrelin might be involved in the regulation of sleep in humans by promoting slow-wave sleep (SWS) (35).

Plasma ghrelin levels increase during fasting and decline within 1 h after food intake (4). Furthermore, ghrelin levels have been observed to increase during the night in sleeping subjects and to decrease in the morning some hours before awakening and, thus, before breakfast (4).

This time course in sleeping subjects suggests the existence of a mechanism regulating ghrelin production that is not dependent on food intake. Possible candidates are the circadian rhythms regulating numerous endocrine networks, such as, for example, the secretion of cortisol or leptin (5, 13), which, in turn, are related to insulin and blood glucose levels (27). Alternatively, sleep might directly influence ghrelin production, as it has been demonstrated, for example, for the secretion of growth hormone by the pituitary (28).

To dissect diurnal variations from sleep-related influences on ghrelin production, we performed a study on the 24-h pattern of ghrelin secretion in healthy males. During two experimental sessions from 0900 to 1000 the following morning, the subjects were allowed to sleep or were sleep deprived in balanced order. Nocturnal ghrelin secretion was compared between the two conditions using a within-subjects design.

MATERIALS AND METHODS

Experimental subjects. We investigated 10 healthy, nonobese male volunteers [age 28 ± 3.1 yr, body mass index 24.0 ± 2.9 kg/m² (range from 20.5 to 29.5 kg/m²)] under both sleep and sleep deprivation conditions. Written informed consent was obtained from all participants before inclusion, and the study was approved by an independent ethics committee. The subjects underwent detailed medical history, evaluation of sleep habits, physical examination, laboratory investigations, electroencephalogram (EEG), and electrocardiogram to exclude acute or chronic diseases. Subjects with a personal or family history of psychiatric disorders, a history or symptoms of any sleep disorder, use of any medication, or alcohol or substance abuse or dependence were not included. Furthermore, we excluded subjects with a history of recent irregular sleep-wake schedules, e.g., shift work or intercontinental flights within the previous 4 wk. To control for regular sleep-wake behavior, subjects kept a sleep log the week before both experimental sessions.

Experimental procedure. The study was performed using a balanced, randomized, within-subject design. Every subject participated in two 24-h experiments. Once, the subjects were totally sleep deprived, and the other time they were allowed to sleep from 2300 to 0700. The time interval between the two sessions was ≥2 wk. To adjust to the experimental settings, the subjects spent the night before each session (2300 to 0700) in the sleep laboratory. At 0800, electrodes for EEG, electromyogram, and electrooculogram recordings were placed according to standardized criteria (20). The subjects remained in a semirecumbent position and received standardized meals at four different time points. The subjects received a standardized diet from our hospital kitchen with 1,800 kcal/24 h containing ~30% fat, 60% carbohydrates, and 10% protein. The subjects were not allowed to eat additional food. Measurements were started at 0900. Subjects stayed in bed for 25 h, until 1000 the next morning. Artificial light intensity was always <200 lux. Lights were turned off, and the subjects were allowed to sleep between 2300 and 0700 in the sleep condition only. During sleep deprivation, another person stayed continuously in the same room with the subject to prevent lapses into sleep. Standardized meals were served at 0800, 1200, 1900, and again.
at 0800 the next morning. Water was freely available during the whole experimental session.

Blood sampling. Blood samples were taken hourly via an intravenous catheter placed into an antecubital vein, which was kept patent by continuous 0.9% saline infusion containing 400 IU of Na-heparin/l at a rate of 30 ml/h. To minimize disturbances of sleep continuity, blood sampling was done through the wall via a long line from a room adjacent to the sleep laboratory. Blood was collected in tubes, which were stored on ice and contained Na-EDTA (1 mg/ml blood) and aprotinin (300 kallikrein inhibitor units/ml blood). Immediately after withdrawal, blood was centrifuged at 2,600 g for 7 min at 4°C, and plasma was aliquoted and frozen to −20°C until the various assays were performed.

Sleep recordings. To ensure that sleep deprivation was successful, polygraphic recordings were obtained throughout 24 h. All records were visually scored in 30-s epochs with standardized criteria (20) by the same experienced person blind to the experimental condition. During sleep deprivation, none of the subjects displayed rapid eye movement (REM) sleep or non-REM sleep stages 2–4. The sleep parameters from the sleep phase during the sleep condition are shown in Table 1. Sleep quality and distribution of the different sleep stages were comparable to data from other studies on healthy male volunteers who underwent sleep recordings with intermittent blood sampling (15, 19, 26) (Table 1).

Determination of ghrelin, human growth hormone, and cortisol plasma levels. Total plasma ghrelin levels were determined by a radioimmunoassay (Phoenix Pharmaceuticals, Mountain View, CA). The limit of detection was 80 pg/ml. The intra- and interassay coefficients of variation were <13%. Human growth hormone (hGH) plasma levels were determined using radioimmunoassays purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA). The limit of detection was 1.5 ng/ml, and the intra- and interassay coefficients of variation were <7%. Plasma cortisol levels were determined by means of a radioimmunoassay from ICN Biomedicals (Carson, CA). The limit of detection was 0.2 ng/ml, and the intra- and interassay coefficients of variation were <7%.

Statistics. To compare nocturnal hormone plasma levels between the sleep condition and the sleep deprivation condition, ANOVA for repeated measures was performed with time and condition (sleep vs. sleep deprivation) as within-subject factors. To avoid too many a posteriori tests, which would imply a strong reduction of the level of significance, only five time points between 2300 and 0700 were included (2300, 0100, 0300, 0500, and 0700) in the ANOVA. For each hormone, the averages of the measured plasma concentrations before the sleep period were considered as baseline values, and conditions were tested about significance in them by a separate ANOVA. If, for some hormone, baseline plasma concentrations differed significantly between the two conditions, then all corresponding values in the sleep period had to be normed with the baseline values before the ANOVA application. Additionally, ghrelin levels were compared between conditions by a recently developed methodology for the comparison of the stability of hormone concentration curves (36). Finally, the peak level of hGH between 2300 and 0300 was determined and associated with the ghrelin concentrations between 2300 and 0300 and their area under the curve in this interval as well by using Pearson’s correlation coefficient. The level of statistical significance was set to 0.05. One subject had to be excluded from statistical analysis, because no blood samples were available between 2300 and 0500 during the sleep condition due to technical reasons. In the figures, data are depicted as means ± SE; in the table, data are given as means ± SD.

RESULTS

Condition did not reveal a significant effect on the baseline values of any of the considered hormones. In the sleep condition, ghrelin levels rose sharply around midnight and then slowly declined until the next morning. In contrast, when subjects were sleep deprived, ghrelin levels increased steadily up to a plateau in the early morning hours and declined just after breakfast (Fig. 1). ANOVA for repeated measures revealed a significant difference between the conditions with respect to nocturnal (2300 and 0700) ghrelin secretion [F(4;32) = 6.65, P < 0.01]. This was confirmed by a Z-score of −2.31 (P < 0.05) in the comparison of the hormone curves between sleep and sleep deprivation with the aforementioned stability testing (36).

ANOVA also revealed a significant difference in nocturnal hGH secretion between the conditions [F(4;32) = 8.1, P < 0.01], confirming the well-established hGH peak around sleep onset and its strong attenuation by sleep deprivation (Fig. 1). The peak hGH level during night sleep was positively correlated to ghrelin secretion during the first 4 h of sleep (r = 0.705, P < 0.05; Fig. 2). A similar but nonsignificant (NS) relationship (r = 0.433, NS) was obvious when hGH and ghrelin levels were suppressed during sleep deprivation. During the day, both ghrelin and hGH plasma levels increased before meals and declined thereafter (Fig. 1).

Cortisol levels across the 24-h experimental period showed the well-known circadian variability, with a maximum in the morning and a nadir during the night. ANOVA for repeated measures revealed no significant differences between the two conditions [F(4;32) = 2.3, NS].

DISCUSSION

In the present study, we investigated the influence of nocturnal sleep compared with sleep deprivation on ghrelin plasma levels in healthy volunteers. We confirmed the finding of Cummings et al. (4) that in sleeping subjects ghrelin levels rise during the early part of the night and decline in the morning before breakfast. We discovered, however, that this increase is blunted by sleep deprivation. When subjects stayed awake, ghrelin levels rose slowly and steadily to reach a plateau in the early morning and did not decrease until breakfast. This relative increase of ~25% during nocturnal wakefulness was similar to the effects of a 12-h fast observed during the day (1).
The suppressive effect of sleep deprivation on ghrelin levels suggests that the bell-shaped time course observed during sleep might not be due to an influence of circadian rhythms but rather might be related to sleep itself. Hence, nocturnal ghrelin secretion shows similarities to hGH production, which is promoted by sleep as well, and particularly by sleep onset (3). In addition, peak hGH levels correlated positively to ghrelin secretion during the first hours of sleep (Fig. 2), further supporting the idea of a tight interaction.

Sleep-related growth hormone secretion is probably caused by an increased production of hypothalamic growth hormone-releasing hormone (GHRH) acting on the pituitary (28). Ghrelin, in contrast, is produced mainly by the stomach and other splanchnic organs (1, 14). Small quantities might also be produced in the hypothalamus (for review see Ref. 9), but these are very unlikely to contribute to systemic levels (34). Hence, it is almost certain that the increased ghrelin levels that we found in sleeping subjects are due to peripheral production. Yet it is unclear at present how the brain might act on ghrelin-producing cells. Despite the positive correlation between ghrelin and hGH secretion, a causative role of hGH is unlikely, because a number of studies demonstrated that hGH administration does not affect ghrelin levels (2, 8, 16, 30). Studies investigating the interaction between ghrelin production and feeding suggest an influence of vagal cholinergic efferents on ghrelin production and transmission of the ghrelin signal to the brain through vagal afferents (29, 30). These studies indicate a negative influence of the vagus on ghrelin secretion. Therefore, the increased parasympathetic tone occurring during sleep (30) is unlikely to explain increased ghrelin secretion, because the opposite would rather be expected. Hence, although the present results clearly demonstrate increased ghrelin levels during sleep, the question remains to be solved how the sleeping brain stimulates ghrelin secretion. At first glance, it is not easy to understand why the production of an appetite-stimulating hormone should be increased during sleep. However, the interaction among metabolic, endocrine, and sleep-regulating networks is complex, and ghrelin has numerous functions. Ghrelin has recently been shown to increase SWS, and this effect is probably dependent on the GHRH receptor (33, 35). Therefore, ghrelin might represent a peripheral feed-forward signal to support SWS initiation and/or maintenance during the first hours of night sleep in concert with central SWS-promoting actions of GHRH (18). Similarly, ghrelin being a potent growth hormone secretagogue (11) might reinforce the central GHRH

Fig. 1. Plasma levels of ghrelin, human growth hormone (hGH), and cortisol in 9 healthy subjects during sleep (○) and sleep deprivation (□). Arrows indicate food intake; black bar indicates the time subjects were allowed to sleep or were kept awake, respectively.

Fig. 2. Scatter plot of hGH peak vs. area under the curve (AUC) of ghrelin secretion between 2300 and 0300 during sleep in 9 healthy subjects. Pearson’s correlation coefficient revealed a significant positive correlation ($r = 0.71, P < 0.01$).
signal for enhanced hGH production. This idea is supported by the correlation between hGH and ghrelin production shown in Fig. 2. Studies on the interactions between sleep and neuroendocrine factors date back to the late 1960s (e.g., Ref. 31), but it was only very recently that a particularly intriguing link between sleep and the regulation of appetite became evident. Orexins, hypothalamic peptides that were initially thought mainly to regulate food intake but also to have potent arousing properties (21), play a crucial role in the pathophysiology of narcolepsy. Narcolepsy is characterized by excessive daytime sleepiness, cataplexy (a sudden, short-lasting loss of muscle tone triggered by emotions), and an acquired complete loss of hypothalamic orexin production in the hypothalamus in most patients (21). In addition to sleep-related symptoms, patients display various endocrine and metabolic abnormalities, such as obesity and hypoleptinemia (23–25). Like ghrelin plasma levels, orexin cerebrospinal fluid levels are increased at night (6), and ghrelin-induced food intake is mediated by orexinergic pathways (22). Therefore, it might be of great interest to investigate ghrelin under baseline conditions, in association with sleep and sleep deprivation and in association with meals in narcoleptic patients. To conclude, ghrelin production is enhanced during night sleep in healthy humans and correlates with the increased release of hGH occurring in parallel. Although the underlying mechanisms remain to be elucidated, the present study further supports the idea that ghrelin is a novel, important player within the sleep-neuroendocrine interplay.

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REFERENCES


