Growth hormone-releasing hormone and corticotropin-releasing hormone enhance non-rapid-eye-movement sleep after sleep deprivation

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Schüssler P., A. Yassouridis, M. Uhr, M. Kluge, J. Weikel, F. Holsboer, and A. Steiger. Growth hormone-releasing hormone and corticotropin-releasing hormone enhance non-rapid-eye-movement sleep after sleep deprivation. Am J Physiol Endocrinol Metab 291: E549–E556, 2006; doi:10.1152/ajpendo.00641.2005.—The neuropeptides growth hormone (GH)-releasing hormone (GHRH) and corticotropin-releasing hormone (CRH) regulate sleep and nocturnal hormone secretion in a reciprocal fashion, at least in males. GHRH promotes sleep and GH and inhibits hypothalamic-pituitary-adrenocortical (HPA) hormones. CRH exerts opposing effects. In women, a sexual dimorphism was found because GHRH impairs sleep and stimulates HPA hormones. Sleep deprivation (SD) is the most powerful stimulus for inducing sleep. Studies in rodents show a key role of GHRH in sleep promotion after SD. The effects of GHRH and CRH on sleep-endocrine activity during the recovery night after SD are unknown. We compared sleep EEG, GH, and cortisol secretion between nights before and after 40 h of SD in 48 normal women and men aged 19–67 yr. During the recovery night, GHRH, CRH, or placebo were injected repetitively. After placebo during the recovery night, non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) increased and wakefulness decreased compared with the baseline night. After GHRH, the increase of NREMS and the decrease of wakefulness were more distinct than after placebo. Also, after CRH, NREMS increased higher than after placebo, and a positive correlation was found between age and the baseline-related increase of slow-wave sleep. REMS increased after placebo and after GHRH, but not after CRH. EEG spectral analysis showed increases in the lower frequencies and decreases in the higher frequencies during NREMS after each of the treatments. Cortisol and GH did not differ between baseline and recovery nights after placebo. After GHRH, GH increased and cortisol decreased. Cortisol increased after CRH. No sex differences were found in these changes. Our data suggest that GHRH and CRH augment NREMS promotion after SD. Marked differences appear to exist in peptidergic sleep regulation between spontaneous and recovery sleep.

event history analysis; recovery sleep; cortisol; peptides

Sleep deprivation (SD) is the most powerful method to promote sleep. Recovery sleep following SD is characterized by increases of non-rapid-eye-movement sleep (NREMS), EEG slow-wave activity (SWA), and rapid-eye-movement sleep (REMS) and by decreases of sleep latency and the time spent awake compared with baseline conditions in humans (7) and in laboratory animals (8, 11). NREMS, particularly slow-wave sleep (SWS), and the nocturnal release of growth hormone (GH) are associated strictly, although not absolutely (37, 41, 44). After the selective 5-HT2 receptor antagonist ritanserin (14) and after γ-hydroxybutyrate (42), both SWS and GH increase in normal male subjects. During the recovery night after SD, GH secretion was elevated in some (31), but not all (20), studies. These observations point to common regulators of sleep EEG and sleep-related GH secretion. It is well documented that the hypothalamic neuropeptide GH-releasing hormone (GHRH) stimulates NREMS and GH as well in several species, including humans, at least in male subjects and animals (25, 34). Furthermore, various studies in rodents (25) show that GHRH is involved in the increase of NREMS during recovery sleep after SD. In contrast to GHRH, the key hormone of the hypothalamic-pituitary-adrenocortical (HPA) system, corticotropin-releasing hormone (CRH), promotes wakefulness and impairs sleep (28, 34). Nearly two decades ago, Ehlers and Kupfer (9) proposed the hypothesis that a reciprocal interaction of GHRH and CRH plays a key role in sleep regulation. Today, this theory is well supported.

In more detail, GHRH increases NREMS after central administration to rats and rabbits (10, 21, 46) and after systemic administration to rats (24). Inhibition of GHRH by antagonists (26) and antibodies (27) and by feedback after GH administration (39) decreases NREMS. NREMS is reduced in animal models with reduced GHRH activity as dwarf rats (23) and line 129 mice (22). On the other hand, the very big supermice sleep more than normal mice (19). In young normal male subjects, SWS and GH increase, whereas cortisol is blunted after pulsatile intravenous administration of GHRH during the first few hours of the night (35). The sleep-promoting effect of GHRH is weak in the elderly (15). In a sample of healthy and depressed men with a wide age range, NREMS increased and the hormones of the HPA system, corticotropin (ACTH) and cortisol, decreased (2). In women, however, the effect of GHRH is opposite to that in men, as wakefulness, ACTH, and cortisol increase and NREMS decreases (2, 4).

In the rat, SD results in depletion of hypothalamic GHRH (13). Simultaneously, the GHRH mRNA increases (40, 46). It is thought that this change reflects stimulation of transcription due to the high rate of release (25). The sleep-promoting effect of SD is abolished after GHRH antibodies in rats (27) and after microinjection of GHRH antibodies into the area preoptica of rats (46). In mice with nonfunctional GHRH receptors, the duration of recovery sleep after SD is reduced (22). In the dwarf rat, the increase of SWS after SD is one-half that in control rats (23).

In contrast to the effects of GHRH in males, CRH enhances wakefulness and impairs sleep. After intracerebroventricular administration of CRH, NREMS decreases in rats (10) and rabbits (28). In contrast, NREMS increases after CRH antagonists in rats (29). Similarly, rat strains with reduced CRH...
activity sleep more than the wild-type rats (29). Pulsatile intravenous administration of CRH around sleep onset diminishes SWS, REMS, and GH and stimulates cortisol in young normal male subjects (17). The vulnerability to the sleep-imparing effect of CRH appears to increase during aging. A dose of CRH, which was not effective in young male subjects, impaired sleep in middle-aged men (43). Treatment of patients with depression with the CRH-1 receptor antagonist R121919 for 4 wk prompted decreases of intermittent wakefulness and REM density and an increase of SWS (16).

The effects of GHRH and CRH on sleep EEG and sleep-related hormone secretion during recovery sleep after SD have not yet been investigated. We expected new insights into sleep regulation from addressing this issue. Therefore, we compared sleep EEG and the nocturnal secretion of the major peripheral hormones related to these peptides, GH and cortisol, between nights before and after 40 h of SD in normal women and men of a wide age range. During the recovery night, according to a randomized schedule, one of three treatments, GHRH, CRH, or placebo, was given repetitively. Dosages and time of administration of these substances were the same as in our previous studies on their effects on spontaneous sleep (2, 17, 35). We hypothesized that GHRH augments the sleep-promoting effect of SD, whereas CRH exerts opposite effects.

METHODS

Study Population

The participants were 48 healthy volunteers (24 women and 24 men) with a mean age of 42 ± 2.93 and 37 ± 2.80 yr, respectively (ranging from 19 to 69; male subjects 19–64, female subjects 22–69 yr). Age did not differ between male and female subjects (P = 0.22, t-test for independent samples). A total of 55 subjects had entered the study, but due to technical reasons, seven dropped out and were not included in the analyses. The study was approved by the ethics committee for human experiments at the University of Munich. After the purpose of the study had been explained to the subjects, all of them gave their informed consent according to the tenets of the Declaration of Helsinki. All subjects underwent an extensive physical and psychiatric examination to ensure that they were normal. Sleep disturbances or a personal or family history of psychiatric disorders were ruled out, as well as shift working and a time shift in the last 3 mo before the beginning of the study. They were free of any medication for at least 8 wk. Consumption of alcohol was not allowed throughout the study period starting 1 wk before the first adaptation night. Caffeine was restricted to 200 ml of coffee in the morning. All premenopausal women (n = 17) had regular menstrual cycles and were not taking hormonal contraceptives. They were examined from the 2nd to the 6th day after menstruation. Nine women were postmenopausal.

Experimental Procedure

The study consisted of four consecutive nights: 1) adaptation, 2) baseline, 3) SD, and 4) recovery night. The first of these nights served for adaptation to the laboratory.

During the baseline night and during the recovery night after 40 h of SD, an indwelling intravenous catheter was inserted at 1930 and connected to plastic tubing that ran through a soundproof lock into the adjacent room. This allowed us repeated blood collection without disturbing the study subject. All subjects were in the supine position from 2000. According to our experience from various similar studies (38) and literature (18), cannulation does not affect sleep EEG distinctly. Blood samples were collected to determine plasma levels of GH and cortisol every 30 min between 2000 and 2300 and every 20 min between 2300 and 0700. Specimens collected before 2300 were used only to exclude stress effects after cannulation. In addition, blood was collected between 2300 and 0700 every 10 min for later analysis of renin levels. These results will be reported elsewhere. On the recovery night, between 2200 and 0100 the subjects received hourly bolus injections of either placebo, 4 × 50 μg CRH (CRH-Ferring; Ferring, Kiel, Germany), or 4 × 50 μg GHRH (GHRH-Ferring) according to a randomized schedule. Therefore, care was taken to a homogenous distribution for sex and age within each treatment group.

The subjects were observed on a television screen in an adjacent room. Involuntary napping or microsleep during SD was precluded by keeping the subjects continuously in the company of a study nurse until bedtime of the recovery night. They underwent a standardized activity program (walking, games, sightseeing) without strong physical exercise throughout the study. They had standardized meals at 0800, 1200, and 1800.

Sleep EEG Recordings

Electrodes for polysomnographic recordings (Comlab 32 Digital Sleep Lab, Brainlab V 3.3 Software; Schwarzer, Munich, Germany) were fixed during baseline and recovery night between 2100 and 2200. The subjects were not allowed to sleep until the lights were turned off at 2300. Polysomnographic recordings were obtained from 2300 to 0700 and consisted of two EEGs (C3-A2, C4-A1; time constant 0.3 s, low-pass filtering 70 Hz), vertical and horizontal electrooculograms, and an electromyogram.

Conventional Sleep EEG Analysis

Sleep stages [stage awake, stages 1–4 sleep (stages 3 and 4 sleep are SWS), and REMS] were scored visually in all subjects offline according to conventional criteria (30) by a rater who was unaware of the treatment. Calculations of sleep parameters included sleep period time (SPT; interval from the first episode of stage 2 sleep until final awakening), sleep onset latency (SOL; time between lights off and the first occurrence of stage 2 sleep), and sleep efficiency index (SEI; quotient stages 1 to 4 + REMS/SPT) and the time spent in the different sleep stages with reference to SPT.

Furthermore, the number of transitions between the sleep stages awake, light sleep (LS; stages 1 and 2), SWS, NREMS, and REMS was calculated as described previously (45).

Quantitative Sleep EEG Analysis

The quantitative EEG (qEEG) included all epochs of stable NREMS (stages 2–4). Before qEEG, artifacts and arousals, defined according to criteria of the American Sleep Disorders Association (1), were rejected by visual inspection. The qEEG was performed by the fast Fourier transform routine by use of a rectangular window for consecutive, nonoverlapping 2-s miniepochs, which allowed a frequency resolution of 0.5 Hz. The spectral profiles were calculated as the mean values for the entire sleep and separately for each of the halves and each of the thirds of the night. Furthermore, the spectral power in the δ-frequency range was aggregated through all analyzed NREMS epochs. This approach enabled expression not only of the sleep intensity but also of the accumulated SWA during SPT. The EEG frequency bands were defined as follows: δ (0.5–4 Hz), θ (4.5–8 Hz), α (8.5–12 Hz), β (12.5–15 Hz), γ (15.5–25 Hz), and θ (25.5–30 Hz).

Endocrine Analysis

Plasma cortisol concentrations (intra- and interassay coefficients of variation <7%, RIA Kit J125; ICN Biomedicals, Carson, CA) were measured by radioimmunoassay and GH concentrations (intra- and interassay coefficients of variation <10%. Advantage; Nichols Institute, San Juan Capistrano, CA) were determined by chemiluminescence. Random samples for each hormone were analyzed in duplicate.
According to standard procedures for time series, the remaining specimens were analyzed only once. Nocturnal endocrine activity of cortisol and GH from 2300 to 0700 was analyzed by means of two curve indicators, mean location (ML) and Δ (maximum-minimum) obtained over the profiles of their mean concentrations.

Statistical Analysis

The effect of SD on the considered variables can be best examined in the placebo group, because it was only in this group that subjects underwent the same treatment before and after SD. Therefore, this effect was tested for significance by one-factorial multivariate analyses of variance with “phase” as one within-subjects factor with two levels (baseline vs. recovery night). On the other hand, considering baseline-related values of the sleep EEG and endocrine parameters, we tested the effect of treatment on these normed parameters by one-factorial multivariate analyses of variance, too, where the influential factor was now treatment, a between-subjects factor with three levels (CRH, GHRH, and placebo). Therefore, age and sex were considered according to the questions either as covariates or as influential factors. Next to the analyses of variance, one-sample t-tests were additionally used for testing whether treatment or SD effects were significantly different from baseline or placebo-related changes. As the nominal level for significance, α = 0.05 was accepted. This was corrected according to Bonferroni procedure when and where posteriori tests (like tests of contrasts or of simple effects) had to be performed to keep the type I error less than or equal to 0.05. Represented results in the tables and figures are expressed as means ± SE.

RESULTS

Effects of SD, Age, and Sex on Sleep-Endocrine Activity

Effects of SD on conventional sleep EEG variables. Conventional sleep EEG variables before and after SD for the three treatments are given in Table 1. Analysis of variance revealed a significant phase (sleep deprivation) effect on conventional sleep EEG variables when comparing baseline and recovery night at placebo condition [Wilks multivariate tests of significance (sig); effect of phase on the sleep continuity and architecture parameters: F(9,7) = 8.15, sig of F = 0.006; effect of phase on the transition parameters: F(4,12) = 4.41, sig of F = 0.020]. Apart from stage 2 sleep and the number of transitions, the SD effect was significant on all other investigated parameters (univariate F-tests, P < 0.05).

After SD there were significant increases in SPT (+8% compared with baseline), SEI (+7%), stage 3 (+49%), stage 4 (+109%), SWS (+56%), REMS (+31%), and NREMS (+11%) and in the number of transitions from LS to SWS (+51%), whereas SOL (~37%), stages 1 (~51%) and awake (~35%), and the number of transitions from NREMS to stage awake (~42%) were significantly decreased. These changes indicate a significant improvement in sleep quality after SD.

Additional effects of sex and age on sleep-endocrine activity. When we examined, besides phase, sex also as influential factor we did not find any significant main or interaction effect of sex on the considered sleep EEG parameters. Hence, the observed effects of SD are independent of sex. The sample was then divided into two subsamples according to an age threshold (subjects <40 yr old vs. subjects ≥40 yr old). In considering age as additional influential factor, during the next phase we did not find any effect on the sleep continuity and architecture variables, neither a main nor an interaction effect. However, age revealed a main effect on the transition parameters [Wilks multivariate tests of significance; effect of age on the transitions parameter: F(4,11) = 6.88, sig of F = 0.005]. This effect was significant on the transitions NREMS to REMS and NREMS to stage awake (univariate F-tests, P < 0.05). The younger subjects revealed changes from NREMS to REMS more often than the elder subjects, who showed more frequent changes from NREMS to stage awake.

In the subjects of all treatment groups during the baseline night, we found a significant decrease of SWS with age regardless of sex, but males (r = -0.612) showed a signific-

Table 1. Conventional sleep EEG variables in normal subjects during baseline and recovery night after the treatments GHRH, CRH, and placebo

<table>
<thead>
<tr>
<th>Sleep continuity</th>
<th>GHRH</th>
<th>CRH</th>
<th>Placebo</th>
</tr>
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<tbody>
<tr>
<td>Sleep period time, min</td>
<td>454.47 ± 18.08</td>
<td>475.91 ± 2.22</td>
<td>461.2 ± 18.48</td>
</tr>
<tr>
<td>Sleep onset latency, min</td>
<td>33.69 ± 18.35</td>
<td>17.25 ± 4.24</td>
<td>40.25 ± 11.44</td>
</tr>
<tr>
<td>Sleep efficiency index</td>
<td>0.84 ± 0.30</td>
<td>0.96 ± 0.01</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>Sleep architecture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>70.82 ± 33.99</td>
<td>18.01 ± 10.39</td>
<td>41.97 ± 16.68</td>
</tr>
<tr>
<td>Stage 1</td>
<td>32.38 ± 5.34</td>
<td>15.75 ± 5.49</td>
<td>28.13 ± 6.77</td>
</tr>
<tr>
<td>Stage 2</td>
<td>220.91 ± 24.22</td>
<td>251.25 ± 18.39</td>
<td>227.91 ± 14.78</td>
</tr>
<tr>
<td>Stage 3</td>
<td>28.47 ± 7.91</td>
<td>40.72 ± 10.33</td>
<td>32.32 ± 6.58</td>
</tr>
<tr>
<td>Stage 4</td>
<td>23.38 ± 14.41</td>
<td>51.20 ± 20.51</td>
<td>29.82 ± 12.57</td>
</tr>
<tr>
<td>SWS</td>
<td>51.85 ± 17.75</td>
<td>92.22 ± 25.87</td>
<td>62.13 ± 13.97</td>
</tr>
<tr>
<td>Non-REM (stages 2–4)</td>
<td>272.75 ± 22.31</td>
<td>343.47 ± 18.85</td>
<td>290.03 ± 18.22</td>
</tr>
<tr>
<td>Non-REM</td>
<td>305.13 ± 23.22</td>
<td>359.25 ± 14.74</td>
<td>318.16 ± 16.27</td>
</tr>
<tr>
<td>Stage REM</td>
<td>75.75 ± 12.21</td>
<td>94.26 ± 10.39</td>
<td>84.07 ± 11.78</td>
</tr>
<tr>
<td>Transitions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-REM/REM</td>
<td>9.44 ± 1.46</td>
<td>11.94 ± 0.89</td>
<td>8.44 ± 0.79</td>
</tr>
<tr>
<td>LS/SWS</td>
<td>20.88 ± 3.13</td>
<td>26.00 ± 2.64</td>
<td>20.19 ± 2.03</td>
</tr>
<tr>
<td>Non-REM/awake</td>
<td>21.13 ± 2.80</td>
<td>6.81 ± 1.06</td>
<td>16.13 ± 3.03</td>
</tr>
<tr>
<td>Transition frequency</td>
<td>155.63 ± 10.00</td>
<td>146.19 ± 6.04</td>
<td>135.19 ± 6.39</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 16 in each group. GHRH, growth hormone-releasing hormone; CRH, corticotropin-releasing hormone; REM, rapid eye movement; LS, light sleep; SWS, slow-wave sleep.
In contrast, stage awake increased with age, but here no sex difference was found. Also, after SD, an age-related significant decrease of SWS occurred, although at a higher level (males: \( r = -0.638, P < 0.05 \)).

Effects of SD on quantitative sleep EEG. qEEG variables before and after SD for all three treatments are given in Table 2 and Fig. 2. In the placebo condition, the power densities after SD in the range of 0 to 8 Hz were significantly higher and in the range of 15 to 30 Hz were significantly lower than at baseline (1-sample \( t \)-tests, \( P < 0.05 \)).

Effects of SD on hormones. The baseline concentrations of GH and cortisol are scarcely changed after SD in the placebo condition. No significant differences were found in their curve indicators, ML and \( \Delta \) (Table 3 and Fig. 3), in that condition. Similarly to SD, sex as an additional influential factor did not affect the hormone concentrations neither alone nor in interaction with phase.

Effects of Treatments on Changes in Sleep-Endocrine Activity After SD

Effects of treatments on changes in conventional sleep EEG variables. The baseline-normed sleep EEG parameters of the three treatments, i.e., the quotients between their values during baseline and during recovery nights, were compared. We found a significant treatment effect [Wilks multivariate tests of significance; effect of treatment: \( F(22,64) = 2.04, \) sig of \( F = 0.014 \)]. This effect was significant in stages 1 and awake, REMS, SPT, and SEI. Examination of the differences between the three treatments by tests with contrasts (test of simple effects) showed that, during recovery nights, wakefulness and stage 1 sleep were reduced more distinctly after GHRH than after placebo, whereas SEI was improved more distinctly after both CRH and GHRH than after placebo (data not shown). After CRH, REMS showed the weakest alteration to baseline compared with GHRH and placebo (tests with contrasts, \( P < 0.05 \)). Also, in the transition parameters, a significant treatment effect was found [Wilks multivariate tests of significance; effect of treatment: \( F(12,74) = 1.90, \) sig of \( F = 0.047 \)] and was attributed to the transitions LS to SWS and NREMS to awake. The number of the transitions LS to SWS was significantly higher after GHRH than after CRH or placebo, whereas the number of the transitions NREMS to wake was significantly less (tests with contrasts, \( P < 0.05 \)).

Furthermore, we considered the placebo-normed values of the sleep EEG parameters after the treatments with CRH and GHRH during the recovery night and compared their deviations about significance (see Table 4). By testing the deviation from the reference value 1 with one-sample \( t \)-tests, we found for CRH significant deviations to placebo in SPT, SEI, awake, and NREMS and for GHRH in SEI, awake, NREMS, and in the transition from NREMS to awake. For both treatments, CRH and GHRH, the changes in SEI, awake, and NREMS after SD were significantly higher than after placebo, whereas the changes in the transitions from NREMS to awake after GHRH were significantly less than the corresponding changes after placebo and after CRH. After CRH, fewer transitions between NREMS and REMS were found than after GHRH. Although SWS showed values higher than 1 after GHRH and after CRH, these changes were not significant. In conclusion,

Table 2. Power densities during baseline and recovery nights after GHRH, CRH, and placebo

<table>
<thead>
<tr>
<th>EEG Frequency Bands</th>
<th>GHRH</th>
<th>CRH</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta )</td>
<td>Baseline</td>
<td>Recovery</td>
<td>Baseline</td>
</tr>
<tr>
<td>1,022.60±230.44</td>
<td>1,200.42±269.55</td>
<td>668.61±84.84</td>
<td>831.38±77.55</td>
</tr>
<tr>
<td>( \theta )</td>
<td>143.01±66.39</td>
<td>126.63±32.92</td>
<td>67.64±8.21</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>83.00±43.32</td>
<td>54.63±14.22</td>
<td>44.84±5.64</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>42.50±15.66</td>
<td>26.07±5.44</td>
<td>19.62±1.66</td>
</tr>
<tr>
<td>( \beta )</td>
<td>20.25±8.42</td>
<td>10.75±2.84</td>
<td>8.56±1.00</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>1.50±0.53</td>
<td>0.77±0.18</td>
<td>0.72±0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE in \( \mu V^2 \); \( n = 16 \) in each group.
the increase in NREMS after SD and administration of GHRH or CRH is higher than after placebo. Furthermore, after GHRH and CRH the decrease in awake is more distinct, and after GHRH the transitions from NREMS to awake are less distinct than after placebo and after CRH.

**Effects of sex and age.** After taking into consideration age and sex as covariates, the results of the analysis of covariance were similar to those obtained without these covariates. When the association between age and changes of SWS after SD was examined (expressed in %baseline), we found a significantly positive association only after CRH. This means that after CRH, the SWS change rates are positively correlated with age ($r = 0.579$). The older the subjects were, the stronger the increases in SWS were compared with baseline. GHRH and placebo do not share this effect (see Fig. 4).

**Effects of treatments on changes in qEEG variables.** Multivariate analysis of variance showed no significant effect of treatment on the baseline-normed power densities in the subbands $\delta$, $\theta$, $\alpha$, $\beta$, and $\gamma$ of the range 0–30 Hz between the three treatments (see Fig. 2).

**Effects of treatments on changes in hormone concentrations.** The courses of the GH and cortisol concentrations before and after SD for each treatment group are given in Fig. 3. Testing the effect of treatment on the baseline-normed curve indicators showed significant differences between the treatment groups [Wilks multivariate test of significance; effect of treatment: $F(8,82) = 16.38$, sig of $F < 0.0001$] attributed to both indicators ML and $\Delta$ (univariate $F$-tests, $P < 0.05$).

Comparing the three treatments in the baseline-normed cortisol indicators, we found significantly higher ML and $\Delta$...
DISCUSSION

The major finding of our study is that administration of GHRH and CRH augment the NREMS-promoting effect of SD. In contrast to our previous findings on the effects of GHRH on spontaneous sleep (35), no sexual dimorphism in this augmentation was found. After the combination of CRH and SD, REMS did not differ from baseline, whereas REMS increased after SD combined with placebo and with GHRH as well. Surprisingly, after CRH there was a positive correlation between the increase of SWS and age. SD combined with placebo did not modulate the secretion of cortisol and GH. As expected, administration of GHRH during the recovery night enhanced GH, whereas CRH stimulated cortisol secretion. Furthermore, cortisol was blunted after GHRH regardless of sex.

In detail, in the total sample, NREMS was promoted after SD. As expected (7) during the recovery night after placebo administration, NREMS, SWS, and REMS increased, whereas wakefulness and stage 1 decreased. Furthermore, the transitions from LS (stages 1 and 2) to SWS increased, whereas the transitions from NREMS to awake decreased. Analysis of sex effects showed that these changes are common effects in females and males. When the sample was divided, according to age, into two groups, younger or older than 40 yr, the transitions from NREMS to REMS were more frequent in the younger subjects, and the changes from NREMS to awake were more frequent in the older subjects. On the other hand, changes in sleep architecture and sleep continuity variables after SD were not affected by age. Hence, most of the SD effects on sleep are preserved during aging. This observation resembles our previous finding (20).

Analysis of the effects of the three treatments points to an augmentation of the NREMS-promoting effect of SD by GHRH, because NREMS increased and wakefulness decreased after GHRH more distinctly than after placebo. Furthermore, wakefulness decreased more distinctly, and the number of transitions from NREMS to awake was reduced more distinctly than after placebo or CRH, respectively. As reported in the beginning of this article, it is well established that GHRH promotes spontaneous NREMS in male human subjects and in male rabbits, rats, and mice (25, 34). Furthermore, various preclinical studies (25) point to a key role of GHRH in sleep promotion after SD. The present study suggests that, during the recovery night after SD, exogenous GHRH is capable of enhancing the effects of endogenous NREMS-promoting factors, including probably endogenous GHRH. This observation supports the view that GHRH promotes NREMS, particularly in conditions of high sleep propensity. We showed previously that GHRH increases SWS in young normal male subjects when given intravenously in a pulsatile fashion around sleep onset (35), whereas SWS remains unchanged in young men after administration of GHRH during the early morning, when the amount of SWS is low during baseline conditions (32). Similarly, the sleep-promoting effect of GHRH was weak and did not include an increase of SWS in elderly subjects (15). During the recovery night, GHRH appears to exert an additive effect on NREMS promotion in both females and males. Furthermore, cortisol secretion was blunted after GHRH in both sexes. This is in contrast to our previous finding (2, 4) of a sexual dimorphism of the effects of GHRH on sleep-endocrine activity. Whereas GHRH promotes NREMS and inhibits HPA hormones when given around sleep onset to normal male controls (2, 4, 35) and patients with depression (2, 4), it impairs sleep and stimulates HPA hormones in female normal controls and depressed patients of a wide age range (2, 4). A sleep-impairing effect of GHRH was recently confirmed in young women (36).

Table 4. Placebo-normed changes in the recovery night for the treatments GHRH and CRH

<table>
<thead>
<tr>
<th>Placebo-Related Changes</th>
<th>GHRH</th>
<th>CRH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep period time</td>
<td>0.98±0.01</td>
<td>0.96±0.02*</td>
</tr>
<tr>
<td>Sleep efficiency index</td>
<td>1.04±0.01*</td>
<td>1.03±0.01*</td>
</tr>
<tr>
<td>Awake</td>
<td>0.52±0.15*</td>
<td>0.80±0.05*</td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.83±0.17</td>
<td>1.13±0.16</td>
</tr>
<tr>
<td>Stage 2</td>
<td>1.12±0.04</td>
<td>1.10±0.06</td>
</tr>
<tr>
<td>SWS</td>
<td>1.73±0.15</td>
<td>1.25±0.44</td>
</tr>
<tr>
<td>Non-REM</td>
<td>1.09±0.04*</td>
<td>1.09±0.04*</td>
</tr>
<tr>
<td>REM</td>
<td>1.03±0.08</td>
<td>0.84±0.08</td>
</tr>
<tr>
<td>Non-REM/REM</td>
<td>1.27±0.12</td>
<td>0.92±0.15†</td>
</tr>
<tr>
<td>LS/SWS</td>
<td>1.15±0.12</td>
<td>0.98±0.23</td>
</tr>
<tr>
<td>Non-REM/awake</td>
<td>0.66±0.12*</td>
<td>1.15±0.08†</td>
</tr>
<tr>
<td>No. of transitions</td>
<td>0.92±0.06</td>
<td>1.01±0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant deviations from placebo by 1-sample t-tests; †significant differences between GHRH and CRH by tests with contrast in multivariate ANOVA.
At least in males, CRH is thought to oppose the sleep-endocrine effects of GHRH. A reciprocal interaction of these peptides in sleep regulation was first submitted by Ehlers and Kupfer (9). This hypothesis is supported by studies in rats and rabbits, which show a role of CRH in the maintenance of wakefulness and sleep-impairing effects of this peptide (28, 33). Similarly, SWS, REMS, and GH decrease after CRH administration to young normal male subjects (17). Interestingly, in the present study, in contrast to the influence of CRH on spontaneous sleep, an additive effect of CRH and SD on the increase of NREMS and on the decrease of wakefulness was found. This effect was observed in women and men as well. So far, the effect of CRH on spontaneous sleep in women has not been investigated. A comparison of the sleep-endocrine pattern of young normal women and men showed a lower amount of SWS during the first half of the night, which was thought to be due to elevated CRH levels (3).

The suppression of REMS during spontaneous sleep after CRH (17) resembles the lack of an increase of REMS during the recovery night after CRH administration in the present study. Because REMS suppression is a common effect of acute administration of CRH, ACTH, and cortisol to male subjects, whereas cortisol and CRH exert opposite effects on SWS and GH, (33) it appears likely that the decrease of REMS after CRH is mediated by the peripheral increase of cortisol, whereas the decrease of SWS is a central effect of CRH on spontaneous sleep. In contrast, some, but not all, studies point to a role of CRH in the central nervous system (CNS) in the promotion of REMS (33). This view is further supported, since treatment of patients with depression with the CRH-1-receptor antagonist R 121919 counteracted sleep EEG changes, which are characteristic in affective disorders, because SWS increased and wakefulness and REM density decreased (16). In the present study, after CRH administration, REMS remained unchanged, whereas REMS increased after GHRH and placebo.

It is well established that SWS decreases, whereas intermittent wakefulness increases, during aging (5). This was confirmed during baseline nights in our study. Similarly, a negative correlation was found between age and SWS during the recovery night after placebo, whereas SWS was increased throughout the subjects of different ages. This finding resembles our previous observation (20) that the capacity to promote SWS after SD is preserved during aging. Surprisingly, a distinct effect counteracting the declining sleep propensity during aging appears to occur after CRH, because we found a positive correlation between age and SWS after this peptide. This finding is in contrast to the observation (43) that sleep is disturbed after CRH in middle-aged but not in young men.

Previous studies reported conflicting results on the effects of SD on GH and cortisol secretion. Similar to our previous study (20), these hormones remained unchanged during the placebo condition. As expected, GH was elevated after GHRH, and cortisol increased after CRH. Similar to our previous findings in male subjects (4, 35), cortisol was blunted after the combination of SD and GHRH. In contrast to the increase of HPA hormones after administration of GHRH during spontaneous sleep in women (4), no sex difference was observed in the present study. Cortisol was blunted after GHRH in both female and male subjects.

In all, our results point to distinct differences in the effects of GHRH and CRH on spontaneous and recovery sleep. Whereas the effects of GHRH on sleep EEG and cortisol secretion in male subjects are similar during spontaneous sleep and during recovery sleep, the effects of GHRH in females and the effects of CRH in males on sleep-endocrine activity are different between sleep before and after SD. (The effects of CRH on spontaneous sleep in women are unknown). During spontaneous sleep, both GHRH in women (2, 4) and CRH in men (17) impair sleep and increase cortisol secretion; i.e., GHRH exerts some CRH-like effects in women. In contrast, during recovery sleep, no sex differences in the effects of these peptides were found. GHRH increased NREMS and decreased cortisol in both male and female subjects similarly to its effects on spontaneous sleep in males. Obviously, after SD, the CRH-like influence of GHRH on sleep EEG and hormones in women is converted to the pattern that is found during spontaneous sleep in men. During baseline conditions, GHRH and CRH appear to regulate sleep in a reciprocal fashion, at least in males (9). The NREMS-promoting effect of GHRH during recovery sleep resembles the changes after its central and systemic administration in laboratory animals (25). As discussed in detail elsewhere (38), it appears likely that NREMS changes after intravenous GHRH administration in humans represent central effects that are independent of changes in peripheral hormone secretion. Also, blunting of cortisol may be mediated by suprapituitary mechanisms. The most surprising findings of our study are the augmentation of NREMS and the age-related increase of SWS after CRH during recovery sleep. Similar to the effect of GHRH in women, the effect of CRH on NREMS during recovery sleep is turned to the opposite of its action during spontaneous sleep. Regardless of sex, after SD, CRH appears to exert effects on NREMS that resemble those after GHRH in male subjects during spontaneous sleep. Whereas GH was blunted after CRH during spontaneous sleep in young male controls (17), it remained unchanged during recovery sleep. As expected, cortisol was elevated after CRH. Because cortisol administration increases SWS and GH in young (12) and elderly (6) normal subjects without decreasing wakefulness, it is unlikely that the increase of NREMS and the decrease of wakefulness after CRH were mediated by cortisol. Furthermore, SWS, but not cortisol, showed an age-dependent increase after CRH in the present study. We suggest that central actions independent of peripheral hormone changes mediated the increases of NREMS after GHRH and CRH as well. Preclinical studies on GHRH and CRH activity in the CNS after administration of these peptides during recovery sleep in animals of both sexes are needed to further delineate the exact mechanisms of our findings.

**GRANTS**

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**REFERENCES**


