Evidence against a role for the growth hormone-releasing peptide axis in human slow-wave sleep regulation

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Center for the Study of Biological Rhythms (CERB), Department of Endocrinology and Laboratory of Experimental Medicine, and Sleep Laboratory, Department of Psychiatry, Hôpital Erasme, Université Libre de Bruxelles, B-1070 Brussels, Belgium; Department of Medicine, University of Virginia, Charlottesville, Virginia 22908; and Department of Medicine, University of Chicago, Chicago, Illinois 60637

Moreno-Reyes, Rodrigo, Myriam Kerkhofs, Mireille L’Hermite-Balériaux, Michael O. Thorner, Eve Van Cauter, and Georges Copinschi. Evidence against a role for the growth hormone-releasing peptide axis in human slow-wave sleep regulation. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E779–E784, 1998—A complex interrelationship exists between sleep and somatotropic activity. In humans, intravenous injections of growth hormone-releasing hormone (GHRH) given during sleep consistently stimulate slow-wave (SW) sleep, particularly when given in the latter part of the night. In the present study, the possible somnogenic effects induced under similar conditions by GH-releasing peptide (GHRP) were investigated in seven young healthy men. Bolus intravenous injections of GHRP-2 (1 µg/kg body wt) or saline, in randomized order, were given after 60 s of the third rapid-eye-movement period. All GHRP injections were immediately followed by transient prolactin elevations and by GH pulses of a magnitude within or around the upper limit of the physiological range. Except for a nonsignificant tendency to increased amounts of wakefulness during the 1st h after the injection, no effects of GHRP-2 administration on sleep were detected. There was in particular no enhancement of SW sleep. Thus, in contrast to GHRH, late-night single injections of GHRP-2 at a dosage resulting in similar GH elevations have no stimulatory effects on SW sleep. The present data provide evidence against the involvement of the GHRP axis in human SW sleep regulation.

Conversely, there is also good evidence to indicate that somatotropic activity is involved in the quality and the maintenance of sleep (20). In particular, the role of GHRH has been evidenced in humans as well as in laboratory animals. In rodents, intracerebral as well as systemic injections of GHRH stimulate non-rapid-eye-movement (non-REM) sleep, even in hypophysectomized animals (11, 25–27), and inhibition of endogenous GHRH decreases both non-REM sleep and GH secretion (29, 30). In humans, no effects of GHRH on sleep quality were found when the peptide was injected during daytime or before sleep onset (13, 21) or when it was given as an infusion (19, 23). In contrast, studies with intravenous GHRH injections given during sleep have been consistent in demonstrating robust stimulatory effects on SW sleep (18, 23, 35). A detailed study performed in normal young men indicated that the effects of the peptide on sleep quality depend on the timing of administration. Single intravenous bolus injections of GHRH at a dosage eliciting GH responses of a magnitude similar to those of spontaneous sleep-onset GH pulses induced a modest increase in REM sleep without a change in SW sleep when injected early during sleep, i.e., at a time when SW sleep is predominant over REM sleep. In contrast, injection of a similar dosage of GHRH in the latter part of the night, i.e., at a time when REM sleep is predominant, was followed by an almost 10-fold increase in SW sleep, without change in REM sleep (18). In rats, there is evidence that the SW sleep-enhancing effects of GHRH are exerted centrally (27), whereas the REM sleep-enhancing effects would appear to be mediated by GH (26, 27). There are no comparable data in humans.

Numerous studies have documented a complex interrelationship between sleep and somatotropic activity in animals as well as in humans. In animals, elevated growth hormone (GH) circulating levels are found during sleep (17, 22, 24). In normal young men, a consistent temporal association between slow-wave (SW) sleep (SW: stages III and IV) and GH nocturnal secretion has been demonstrated. On a daily basis, the major GH secretory episode is a sleep onset-associated pulse occurring during the first phase of SW sleep (15, 36, 39). A close relationship between the amount of GH secreted during sleep and the duration of SW stages has been evidenced in a pulse-by-pulse analysis of GH secretory profiles (39). Pharmacological stimulation of deep sleep by ritanserin or by γ-hydroxybutyrate also increases GH secretion in close temporal and quantitative relationship (14, 40). A recent study using a GH-releasing hormone (GHRH) antagonist has indicated that sleep-related GH secretion appears to be primarily mediated by GHRH release (31).
have a stimulatory effect on SW sleep when given during the latter part of the night at a time when SW sleep is not naturally abundant. The present study was therefore designed to investigate the effects on sleep quality of a late-night intravenous bolus injection of GHRP-2 (a more potent GH secretagogue than GHRP-6) at a dosage resulting in elevations of GH within the physiological range.

MATERIALS AND METHODS

Subjects. Seven healthy men, 24–30 yr old, were selected after a careful physical, psychiatric, and biological evaluation. All subjects were of normal weight (body mass index 23–25 kg/m²). Night and/or shift workers, subjects having crossed time zones or having taken any drug during the previous 6 wk, smokers, and subjects with sleep complaints or with a personal or family history of psychiatric, neurological, metabolic, or endocrine disorder were excluded. The protocol was approved by the Institutional Review Board, and written informed consent was given by all subjects after they had received a complete explanation of the aims and means of the study. All experiments were performed in the Sleep Laboratory of the Department of Psychiatry, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium.

Protocol. One week before the beginning of the investigation, the subjects spent two consecutive nights in the sleep unit to become habituated to laboratory conditions and experimental procedures. During the first night, electrodes for polygraphic sleep recording were attached around 2245, but no recordings were performed. Sleep was polygraphically recorded during the second night. This pretest polygraphic recording served to exclude subjects with abnormal sleep patterns. Thereafter, the subjects had to comply with a standardized schedule of meal times (breakfast: 0800–0900; lunch: 1200–1300; dinner: 1900–2000) and bedtimes (from 2230–2300 until 0700–0900).

On day 1, the subjects were admitted to the sleep laboratory at 2100 for four consecutive nights. Throughout the entire study, above mentioned mealtimes were strictly enforced, except on days 2 and 4, when a snack was served at 1700 and only water was thereafter allowed until the following morning. Every day, the subjects were assigned to bed at 2230 and lights were turned off at 2300. The subjects remained recumbent in total darkness at least until 0700 and had to get up before 0900. Daytime naps and recumbency, snacks between meals, and alcoholic beverages were prohibited. On day 2 and on day 4, a heparin-lock catheter was inserted into an antecubital vein at 1400, and 1-ml blood samples for GH and prolactin determinations were drawn at 15-min intervals for 25 consecutive hours starting at 1500.

Table 1. Characteristics of sleep during nights with saline or GHRP injections

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>GHRP</th>
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<tbody>
<tr>
<td>Sleep period time, min</td>
<td>531 ± 9</td>
<td>508 ± 17</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>80 ± 2</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>Sleep onset latency, min</td>
<td>17 ± 5</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>REM latency, min</td>
<td>83 ± 12</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>Stage duration, min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wake</td>
<td>81 ± 12</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>I</td>
<td>49 ± 8</td>
<td>50 ± 6</td>
</tr>
<tr>
<td>II</td>
<td>229 ± 17</td>
<td>232 ± 13</td>
</tr>
<tr>
<td>III</td>
<td>49 ± 7</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>IV</td>
<td>22 ± 7</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>SW</td>
<td>72 ± 14</td>
<td>74 ± 37</td>
</tr>
<tr>
<td>REM</td>
<td>100 ± 7</td>
<td>102 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SE. GHRP, growth hormone-releasing peptide; REM, rapid-eye-movement stage; SW, slow-wave stage.
The intravenous line was kept patent with a slow drip of heparinized saline. During sleep hours, the intravenous line was connected to plastic tubing that extended to an adjoining room, as previously described (10), to perform blood sampling and intravenous injections without disturbing the subject's sleep. During both nights with blood sampling, polygraphic sleep recordings were obtained and bolus intravenous injections of GHRP-2 (1 µg/kg body wt) or saline, in randomized order, were given via the catheter after 60 s of the third REM period (i.e., between 0323 and 0509) as determined by extemporaneous examination of the sleep recordings.

Hormonal assays. All samples from the same individual were analyzed in the same assay. Plasma GH and prolactin levels were measured by commercially available immunoradiometric assays (Medgenix, Fleurus, Belgium). The GH assay had a lower limit of sensitivity of 0.10 µg/l, an intra-assay variation coefficient of 4%, and an extra-assay variation coefficient of 11%. The prolactin assay had a lower limit of sensitivity of 0.25 µg/l, an intra-assay variation coefficient of 7%, and an extra-assay variation coefficient of 13%.

Sleep recording and analysis. Polygraphic sleep recordings were scored at 20-s intervals in wake, I, II, III, IV and REM stages by use of standardized criteria (32). Sleep onset and morning awakening were defined as, respectively, the times of the first and last 20-s intervals scored II, III, IV, or REM. The sleep period was defined as the time interval between sleep onset and final morning awakening. Sleep efficiency was calculated as the total sleep period minus the time spent awake, expressed as percentage of the total recording time. SW sleep was defined as the total duration of stages III and IV.

To analyze possible somnogenic effects of GHRP, the total amounts of each sleep stage recorded during the 1st, 2nd, and 3rd h after GHRP were calculated and compared with the amounts recorded during corresponding hours in the placebo study. Similar calculations were performed to consider successive 90-min time intervals over this 3-h period.

Statistical methods. Significant pulses of GH secretion were identified using a modification of the computer algorithm ULTRA (37, 38). The threshold for significance of a pulse was set at two times the intra-assay coefficient of variation in the relevant range of concentration. For each significant pulse, the amount of GH secreted was estimated by deconvolution based on a one-compartment model for GH clearance and variable individual half-lives, as previously described (39).

Statistical tests. All data are expressed as means ± SE except when otherwise stated. Paired comparisons were performed using the nonparametric Wilcoxon test. All statistical calculations were performed using the Statview SE+ software for Macintosh computers (Abacus Concepts, Berkeley, CA).

RESULTS

Mean GH and prolactin profiles in GHRP and saline experiments are shown in Fig. 1. Spontaneous sleep-onset GH pulses were observed in all individual profiles. Their amplitude was similar in presaline and pre-GHRP experiments. The amount of GH secreted in the sleep-onset pulse ranged between 59 and 1,344 µg, averaging 483 µg. No significant GH response was observed after saline administration, whereas all GHRP injections were immediately followed by GH pulses. The magnitude of these pulses varied widely across individuals. In one subject (subject 3), the amount of GH secreted (2,080 µg) exceeded markedly the upper limit of spontaneous sleep-onset pulses. In the other six subjects, the GH secretion in response to GHRP injection ranged between 210 and 1,206 µg, averaging 818 µg, i.e., within the range of the spontaneous sleep-onset pulses.
The classical 24-h profile of plasma prolactin levels was observed in all experiments. In addition, a transient significant elevation was observed within the 1st h after GHRP injection (P < 0.01 vs. saline).

Sleep parameters are shown in Table 1 and in Fig. 2. Sleep period time, sleep efficiency, and total durations of wake, I, II, III, IV, SW, and REM stages were similar during nights with GHRP administration and nights with saline injection. Except for a nonsignificant tendency (P < 0.08) to increased amounts of wake stage during the 1st h after the injection, no effects of GHRP administration on sleep were detected by the hourly analysis and by the 90-min analysis of sleep stages performed during the first 3 h postinjection. Similar results were obtained if subject 3, who exhibited a GH response to GHRP well beyond the upper limit of spontaneous sleep-onset pulses, was excluded from the analysis.

DISCUSSION

Transient stimulations of GH and prolactin secretion observed in the present study after single intravenous injections of GHRP-2 are consistent with the results of previous studies (3). In all subjects but one, the magnitude of the GHRP-induced GH secretory pulse was within the range of spontaneous sleep-onset GH pulses.

Our data indicate that single injections of GHRP-2 in the latter part of a normal night, i.e., at a time when sleep is shallow and fragmented, had no effect on sleep in young healthy men. In particular, there was no enhancement of SW sleep and no decrease in the duration of awakenings. This is in contrast to the effects elicited in similar conditions by GHRH injections (18). In both studies, the GH secretagogue was given as a single intravenous bolus injection after 60 s of the third REM period at a dosage resulting in elevations of GH secretion within or around the upper limit of the physiological range. Because GHRH and GHRP bind to different receptors in the central nervous system and stimulate GH release by different mechanisms (2), the present results support the view that SW sleep-promoting effects of physiological doses of GHRH are mediated by increased GH secretion but result from a direct action on brain centers, consistent with the concept that stimulation of GH release and of SW sleep by GHRH represents two independent processes, involving GHRH neurons in two different hypothalamic areas (4, 20, 28) and possibly different GHRH receptor systems.

GHRPs are GH secretagogues that act as somatostatin antagonists via specific receptors localized both in the pituitary and in the arcuate ventromedial and infundibular hypothalamus (6, 16, 33). Inconsistent data have been reported concerning the action of somatostatin on sleep. In the rodent, REM sleep was inhibited by immunoneutralization of endogenous somatostatin (9) and enhanced by intracerebroventricular administration of exogenous somatostatin (8), and non-REM sleep was inhibited by subcutaneous injections of a long-acting somatostatin analog (1). In the human, repeated intravenous injections of somatostatin did not influence sleep quality in normal young subjects (35), but REM sleep was decreased by somatostatin in the elderly (34).

GHRP-6 and GHRP-2 appear to stimulate GH secretion through different receptors in sheep but not in rats (5, 41). No comparable data are available in humans. There are no published studies of the effects of GHRP-2 on sleep. As far as GHRP-6 is concerned, a modest enhancement of stage II, without any increase in SW sleep, has been reported (12) in a study involving injections of 50 mg of GHRP-6 administered hourly from 2200 to 0100, i.e., from 1 h before until 2 h after bedtime, resulting in an important and sustained elevation of GH concentrations. The absence of stimulation of SW sleep could, however, have represented a ceiling effect, because during the early part of the night, SW stages are already abundant. The present results, obtained with GHRP-2, are consistent with the previous findings reported with GHRP-6 in failing to demonstrate a stimulation of SW sleep. They do not exclude, however, the possibility that these two related peptides involve different mechanisms of action. Additional studies on larger subject populations with both GHRP-2 and GHRP-6 are needed to further clarify the respective effects of these compounds on sleep regulation. The evidence available at the present time indicates that, in contrast to GHRH, single injections of GHRP-2 at a dosage resulting in similar GH elevations have no stimulatory effects on SW sleep, even when given at a time when SW sleep is not predominant.

Recently, we have shown that 7-day oral treatment with MK-677, a functional agonist of GHRH acting via the GHRP receptor, is associated with an increase in stage IV in normal young men (7). This intriguing finding is difficult to interpret in the context of the present study, because plasma GH levels were not elevated at the time of the sleep study (although acutely MK-677 is a powerful GH secretagogue), but plasma insulin-like growth factor 1 levels were markedly increased. Multiple complex mechanisms could be involved in the effects of MK-677 on sleep, with dubious relevance to the effects of direct, acute stimulation of the GHRP axis as examined in the present study.

In conclusion, the present study supports the concept that the well-documented relationship between somatotropic activity and human SW sleep regulation is primarily dependent on GHRH.

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