Diurnal administration of human growth hormone-releasing factor does not modify sleep and sleep-related growth hormone secretion in normal young men

Philippe Garry¹, Bernard Roussel², Richard Cohen¹, Sylviane Biot-Laporte¹, Abdel Elm Charfi¹, Michel Jouvet³ and Geneviève Sassolas¹

Centre de Médecine Nucléaire¹, Hôpital Neuro-Cardiologique, Lyon, France, Délégation Générale pour l'Armement² - Dret, Paris, and Département de Médecine Expérimentale³, Lyon, France

Abstract. hGRF (iv 50 μg) was administered to 6 normal young adult males at 09.00 and 20.00 h on different days. Nocturnal GH secretion was monitored during polygraphic sleep recordings on both control nights and nights following hGRF administration. Sleep-related GH secretion and sleep parameters were not affected by diurnal hGRF administration.

The close association in adult man between the occurrence of the initial episode of slow-wave sleep (SWS) and the highest peak of plasma GH concentration in a 24-h period led to the concept of sleep-related GH secretion (Takahashi et al. 1968; Sassin et al. 1969), submitted to control mechanisms probably different from those involved in day-time secretion and in pharmacologically induced secretion (Mendelson et al. 1979).

It has been reported that in man administration of GH subsequently can blunt the secretory response to pharmacological stimulation of GH secretion (Hagen et al. 1972) as well as sleep-related GH secretion (Mendelson et al. 1983). This negative feedback appears, then, to be exerted at some common point of the pathways involved in the secretory mechanisms.

Administration of GH in animals (Stern et al. 1975; Drucker-Collin et al. 1975; Stern & Morgane 1977) results in some changes in sleep stages with increased REM sleep and decreased SWS. In man, acute administration of GH (5 U) is followed by a decrease in SWS and an increment in REM sleep (Mendelson et al. 1980; Mendelson 1982), whereas iterative administration has no significant effect on sleep (Mendelson et al. 1983).

Synthetic human growth hormone-releasing factor (hGRF) powerfully stimulates GH release in normal young adult men (Thorner et al. 1983; Rosenthal et al. 1983; Gelato et al. 1984; Sassolas et al. 1984). This study was undertaken in order to determine whether a GH-rise, induced during daytime by hGRF administration, would exert an effect on the sleep-related GH secretion and on sleep stages.

Materials and Methods

Six normal young men (22–26 years of age) taking no medication, within 10% of their ideal body weights, were enrolled for this study. They had given written informed consent. Protocol had been approved by our institutional Ethics Committee.

The subjects were admitted to the Centre de Médecine Nucléaire (Lyon) for two trial periods at intervals of 1 week. The first period included a first control night...
**Results**

Fig. 2 illustrates the GH secretion during the nights and after hGRF injections as well as sleep stages for 1 subject.

**Spontaneous GH secretion during sleep**

On every night studied, all subjects presented the maximum nocturnal GH secretion during the first 3 h of sleep.

Our results from the 12 control nights, determined in 6 subjects, showed significant individual variations in GH secretion during sleep as regards the magnitude of the first nocturnal peak, the sleep-AUC, the 1st cycle-AUC, and the 2nd cycle-AUC (< 0.01 by ANOVA).

**Plasma GH responses to hGRF**

A rise in plasma GH concentrations following every hGRF iv injection was found in all subjects. With respect to the magnitude of the peaks and the AUC of hGRF-induced GH secretion, a high variation was observed between the subjects (< 0.01 by ANOVA).

No significant difference was found as to the time, magnitude or AUC of the peaks (mGRF-AUC and eGRF-AUC) released following hGRF for injections performed at 09.00 h and at 20.00 h.

**Effects of hGRF on nocturnal GH secretion**

The following results were obtained by comparing the GH secretion during control nights and during post-hGRF nights:

1) the total nocturnal GH secretion (20-08-AUC and 23-08-IUC) during the nights following morning injections of hGRF (mGRF-N) and during the
GH secretion during nights and after hGRF injection, and sleep stages for 1 subject. The upper part shows the results obtained during the first trial period; the lower part the results of the second trial period.

first control nights (CN1) did not differ significantly when compared by paired t-test.

2) Conversely, the total GH secretion during the nights following the evening injection of hGRF (eGRF-N) was significantly increased compared with control nights (CN2) during the 20.00 to 08.00 h period ($P = 0.02$). In contrast, during the 23.00 to 08.00 h period no significant difference was observed between GH secretion during eGRF-N and CN2 (paired t-test).

3) Study of GH secretion during sleep (sleep-AUC) during the first 3 h (1st3h-AUC), and during the 1st and 2nd sleep cycles (1st cycle-AUC and 2nd cycle-AUC) revealed no significant difference between the control and the post-hGRF nights (Table 1).

4) Moreover, the first nocturnal peak of GH secretion was not significantly modified by recent hGRF injections either at the time of occurrence or in the latency related to sleep onset (ANOVA).

Table 1.
Nocturnal growth hormone secretion during control nights and nights following mGRF and eGRF.

<table>
<thead>
<tr>
<th></th>
<th>CN1</th>
<th>MGRF-N</th>
<th>CN2</th>
<th>eGRF-N</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak (µg/l)</td>
<td>13.7 ± 5.7</td>
<td>19.4 ± 6.8</td>
<td>9.4 ± 2.0</td>
<td>16.8 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Sleep AUC (µg/l)</td>
<td>16.26 ± 5.34</td>
<td>20.97 ± 5.83</td>
<td>9.63 ± 1.71</td>
<td>16.29 ± 4.35</td>
<td>NS</td>
</tr>
<tr>
<td>1st 3 h AUC (µg/l)</td>
<td>6.17 ± 2.39</td>
<td>10 ± 2.73</td>
<td>4.54 ± 0.74</td>
<td>7.15 ± 1.97</td>
<td>NS</td>
</tr>
<tr>
<td>1st cycle AUC (µg/l)</td>
<td>7.63 ± 2.20</td>
<td>8.02 ± 2.01</td>
<td>6.02 ± 1.92</td>
<td>3.96 ± 1.54</td>
<td>NS</td>
</tr>
<tr>
<td>2nd cycle AUC (µg/l)</td>
<td>4.78 ± 2.70</td>
<td>7.15 ± 3.19</td>
<td>2.12 ± 0.99</td>
<td>7.50 ± 3.65</td>
<td>NS</td>
</tr>
</tbody>
</table>

Peak = mean maximum GH plasma concentration during sleep.
Sleep AUC = mean AUC of GH secretion during sleep.
1st 3 h AUC = mean AUC during the first 3 h of sleep.
1st cycle AUC = mean AUC during first cycle.
2nd cycle AUC = mean AUC during second cycle.
The results are expressed with the standard error of the mean (SEM).
Mean sleep characteristics in the 6 subjects during control nights (CN1 and CN2) and experimental nights after morning and evening iv injection of 50 µg GRF, respectively, (mGRF-N and eGRF-N).

<table>
<thead>
<tr>
<th></th>
<th>CN1</th>
<th>MGRF-N</th>
<th>CN2</th>
<th>eGRF-N</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time (TST)</td>
<td>343.2 ± 32.7</td>
<td>407.2 ± 6.3</td>
<td>347.6 ± 22.4</td>
<td>390.2 ± 21.7</td>
<td>NS</td>
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<tr>
<td>Light sleep (1 + 2)</td>
<td>203.0 ± 21.9</td>
<td>260.7 ± 7.7</td>
<td>216.0 ± 27.3</td>
<td>227.0 ± 18.9</td>
<td>NS</td>
</tr>
<tr>
<td>Delta sleep (3 + 4)</td>
<td>79.8 ± 12.8</td>
<td>49.0 ± 1.9</td>
<td>50.4 ± 12.7</td>
<td>67.2 ± 7.5</td>
<td>NS</td>
</tr>
<tr>
<td>Paradoxical sleep</td>
<td>61.0 ± 18.1</td>
<td>97.6 ± 17.8</td>
<td>61.2 ± 4.7</td>
<td>95.6 ± 10.0</td>
<td>NS</td>
</tr>
<tr>
<td>Wakefulness during sleep</td>
<td>99.9 ± 21.4</td>
<td>52.6 ± 3.2</td>
<td>53.4 ± 11.6</td>
<td>50.0 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Sleep latency</td>
<td>17.4 ± 5.0</td>
<td>11.6 ± 2.0</td>
<td>23.0 ± 10.6</td>
<td>6.8 ± 3.2</td>
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<tr>
<td>Paradoxical sleep latency</td>
<td>196.6 ± 63.4</td>
<td>80.2 ± 10.8</td>
<td>129.0 ± 24.4</td>
<td>100.6 ± 8.5</td>
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<tr>
<td>Mean paradoxical sleep duration</td>
<td>18.0 ± 3.5</td>
<td>20.6 ± 1.4</td>
<td>19.2 ± 2.7</td>
<td>25.8 ± 2.3</td>
<td></td>
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<tr>
<td>Mean cycle duration</td>
<td>141.0 ± 32.6</td>
<td>90.6 ± 7.9</td>
<td>112.2 ± 13.6</td>
<td>98.6 ± 6.3</td>
<td></td>
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</table>

All the results are expressed in minutes ± SEM.

Comparison between spontaneous and induced GH secretion
There were no significant differences between the magnitude and the AUC of GH secretion during the first 3 h of sleep on control nights and the values obtained during the 3 h following hGRF injections (paired t-test (n = 12)). However, a significant correlation (r = 0.81, P < 0.002) was found between the latter parameters.

hGRF injections and sleep parameters
As compared with control nights, a single morning or evening 50 µg iv GRF injection had no effect on the time course and the duration of the different sleep phases (Table 2). Further, the self-assessment of sleep quality did not vary for the GRF nights as compared with the control nights.

Discussion
The results of this study confirm the high degree of variation between individuals both in spontaneous nocturnal GH secretion and in GRF-induced GH secretion, suggesting different secretory abilities among normal subjects belonging to the same age group.

Spontaneous episodic GH secretion during sleep, well defined thanks to an EEG-monitored study, provides a good basis for comparison with induced GH secretion. The positive correlation between the responses to hGRF and the so-called GH sleep-related peaks, as well as the study of individual curves, leads us to suggest that the good respond-
ers are those subjects who secrete most GH (at least during this period of highest secretion), and conversely.

This predictable relationship between spontaneous and induced secretion is in agreement with the results of Shibasaki et al. (1984) showing the age-related impairment in response to hGRF whereas the spontaneous secretion decreases in aging man (Finkelstein et al. 1972). In addition to its possible significance for the aetiological diagnosis of GH deficiency, more thorough study of this relationship in patients suffering from varying degrees of partial GH deficiency could allow a broader understanding of GH secretion. This illustrates the common postulate that meaningfulness can be attributed to the responses to stimulator agents.

Our study also shows that the response to hGRF does not differ significantly when the injection is given in the morning and in the evening. These results are in accordance with those of Takano et al. (1984) in normal subjects of small stature in whom injections were administered in the morning and 2 h after lunch. However, they differ from those obtained by Nathan et al. (1979) showing GH responses to insulin-induced hypoglycaemia to depend on injection time.

Our main aim was to seek a possible effect of hGRF administration on spontaneous GH secretion during sleep. We observed no change in the total nocturnal secretion and, above all, in the characteristics of the first peak as to its chronology and its magnitude in as much as our experimental conditions were very strict: we used the 50 µg dose, which is a dose close to the minimum dose required for maximum effect (Boissel et al., in press), and a...
schedule with times of injections establishing intervals of 15 and 4 h, respectively, between morning and evening injections and the mean time of the sleep-related peak. Moreover, the evening injection increases overall GH secretion during the following 12 h, since GH release induced by GRF was followed by an intact GH secretion during sleep. Such a stable secretion implies that all stages of the pathway leading to GH release during sleep remain unaffected by the sudden previously induced GH rise.

This suggests:

(i) the GH pool susceptible to GRF-stimulated release has been reconstituted.

(ii) the various feedback mechanisms promoted by the increase in GH plasma levels, including the enhancement of somatostatin release and the inhibiting effects of somatostatin and somatomedin-C at the pituitary level (Chihara et al. 1981; Martin et al. 1984; Berelowitz et al. 1981; Brazeau et al. 1982), are no longer active. Thereafter, this allows the somatotropes to be responsive to further stimulation.

(iii) the different mechanisms governing the GH secretion during sleep, probably including hypothalamic hGRF secretion/release as the final step of the pathway, are no longer affected by previous GH release or GRF iv administration.

In contrast, GH administration leads to the disappearance of the pulsatile secretion in the rat (Tannenbaum 1980; Willoughby et al. 1980; Abe et al. 1983), of the sleep-related GH secretion in man (Mendelson et al. 1983), and of the GH response to GRF (Rosenthal et al. 1984) owing to the activation of the different factors involved in the negative control exerted by GH on its own secretion. Plasma GH concentrations subsequent to repeated im GH administrations in the studies of Mendelson et al. (1983) and Rosenthal et al. (1984) are elevated for longer than those subsequent to a single injection of hGRF as employed in our study. It can be supposed that the effects on Sm-C production are different in the different protocols. Indeed, previous studies have shown that GH administration in normal man enhances plasma Sm-C levels 12 or 24 h after injection (Copeland et al. 1980) and that single injections of hGRF had no significant effect on plasma Sm-C concentrations 6, 12, and 24 h after injection (Sassolas et al. 1984).

Other mechanisms undoubtedly play a role.

On the other hand, the results demonstrate that a single 50 µg iv hGRF injection which induces a GH rise has no significant effect on sleep characteristics, even when this injection is realised just 3 h before bed time. This is in agreement with the results of Mendelson et al. (1980) who showed that the sleep parameters in man are not modified after a 2 U im GH injection 15 min before bed time. However, with the same protocol, SWS was markedly decreased and REM sleep markedly increased after a 5 U GH injection. In this case, a tremendous increase in plasma GH concentrations was observed throughout the night, whereas in our experiment the night GH concentrations after hGRF were not different from those in control nights.

The results of our study are not surprising. Indeed, physiologically, GH secretion occurs in bursts, often 3 to 5 h apart, particularly in the adolescent. Our iv GRF injections produced neither greater depletion nor longer inhibition than those attributed to the effects of episodic release of hypothalamic hGRF.

**Acknowledgments**

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**References**


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