Plasma levels of growth hormone-releasing hormone and somatostatin in response to a mixed meal and during sleep in children

R. Rosskamp, M. Becker, F. Haverkamp, B. Thomas, S. Brühl, J. Klumpp and N. Liappis

Department of Paediatrics, University of Bonn, Bonn, FRG

Abstract. Following a mixed meal, plasma levels of GHRH, GH, SRIH and insulin were measured in 7 prepubertal children with constitutional delay of growth and adolescence (CDGA) and in 3 children with proven GH-deficiency which responded to GHRH-injection. In children with CDGA, plasma levels of GHRH increased between 60 and 120 min (10.1 ± 1.2 ng/l vs 25.5 ± 4.4 ng/l; P < 0.01). Although no GH increase occurred in patients with GH-deficiency, their plasma GHRH increases were comparable to those in CDGA children. No time relationship was present between circulating GHRH and GH, SRIH, or insulin, nor was there any correlation between their integrated hormone response areas. Sleep-induced plasma GHRH, GH and SRIH values were determined in 10 prepubertal children with CDGA. Spontaneous variations of plasma GHRH and GH values occurred with no temporal or quantitative relationship. SRIH values did not change during nocturnal sleep. In one child with GH-deficiency, comparable GHRH plasma fluctuations occurred, although GH values were all below 1 μg/l. Our results support the concept that circulating GHRH does not only represent hypothalamic GHRH, but derives mainly from extrahypothalamic sources, possibly from the gastrointestinal tract.

GHRH was primarily isolated from human pancreatic tumours (Guillemin et al. 1982; Rivier et al. 1982). Its presence in the human hypothalamus could be confirmed by purification and sequencing from this same source (Ling et al. 1984). By using immunostaining techniques, GHRH could also be detected in non-neural tissues such as the gut and pancreas (Christofides et al. 1984; Shibasaki et al. 1984). GHRH is also detectable in the peripheral plasma (Chihara et al. 1986; Donnadieu et al. 1985), but its origin and function are still controversial. Since GHRH$_{1-40}$ injection has been found to stimulate insulin and SRIH in a dose- and glucose-dependent fashion (Hermansen et al. 1986), we investigated whether there is a relationship between peripheral GHRH concentrations and these two endocrine pancreatic hormones following the physiological stimulus of a mixed meal.

The neurosecretion of pituitary GH is under hypothalamic control of GHRH and SRIH. Pharmacological doses of GHRH stimulate whereas those of SRIH inhibit pituitary GH secretion. Therefore we wanted to determine whether such an opposite effect on GH secretion is present at a peripheral level. For this purpose we measured the circulating concentrations of GHRH and SRIH during nocturnal sleep when GH secretion is augmented (Illig et al. 1971).

Patients and Methods

Seventeen patients (5.9–13.7 years) with short stature were studied. Informed consent was obtained from the parents. The patients were initially referred to our endocrine unit to exclude GH-deficiency. All patients were in a prepubertal state according to the classifica-
tion of Tanner and accordingly had prepubertal values of oestradiol and testosterone. Their skeletal age was found to be delayed by more than 2 years and their height was more than 2 SD below the mean value for the corresponding age. A potential GH deficiency could be ruled out in these children with GH peak values of > 10 µg/l on the basis of either an arginine infusion or an insulin hypoglycaemia test. In almost all patients, a family history of delayed puberty was confirmed. The final diagnosis in all these patients revealed constitutional delay of growth and adolescence (CDGA). Three additional patients (10.8–14.3 years) with probable hypothalamic GHRH deficiency were studied: They had peak plasma GH levels below 4 µg/l following both insulin hypoglycaemia and arginine tests, and after a GHRH1–44 injection (Bissendorf Peptide, Wedemark, FRG) of 1 µg/kg body weight their peak plasma GH levels ranged between 18.8 µg/l and 24.7 µg/l. In two of these patients extirpation of a suprasellar craniopharyngioma had been performed, whereas in the case of the third patient a hypothalamic spongioblastoma had been removed.

In order to investigate spontaneous GH secretion during a 12-h interval (data not shown), a venous catheter was placed in 7 children with CDGA and in 3 children with hypothalamic GHRH deficiency at 08.00 h. A test meal (500–600 kcal; 50% carbohydrates, 35% protein, and 15% fat) was given at 12.00 h. Blood samples for investigation of GHRH, SRIH, GH and insulin were drawn before (0) and 30, 60, 90, 120, 150 and 180 min after the ingestion of the meal.

The spontaneous GH secretion during nocturnal sleep was measured in 10 children with CDGA and in 1 child with hypothalamic GHRH deficiency according to the method of Bierich et al. (1985). Sleep-induced GH secretion was determined during the first 5.5 h of nocturnal sleep. The first plasma samples were taken 30 min after the patient had fallen asleep in order to investigate GHRH, SRIH and GH levels.

For the determination of plasma GHRH and SRIH concentrations, aliquots were added to chilled tubes containing 1.2 mg EDTA/ml and 1000 KIU/ml aprotinin. The samples were centrifuged within 20 min and stored at −40°C until assayed.

Plasma GH and insulin were measured with standard commercially available radioimmunoassay kits (Serono, Freiburg, FRG). The intra- and inter-assay coefficients of variation were below 7.8% and 11.6%, respectively.

Plasma SRIH was measured in unextracted plasma, as reported previously (Rosskamp et al. 1987a). The sensitivity of this assay is 6 ng/l. At a level of 16 ng/l, the intra-assay coefficient of variation was determined to be 8.5%, whereas the corresponding inter-assay coefficient of variation was 14%. Plasma GHRH concentrations were measured by RIA according to a recently described method (Frohman & Downs 1986). The plasma (1.5–4 ml) was extracted using C18 Sep-Pak cartridges.

Waters, Milford, USA and the eluate was lyophilized subsequently. 125I-GHRH-44 was used as tracer (Amersham Laboratories, Buckinghamshire, England) together with a highly specific rabbit antiserum raised against synthetic GHRH1–44 (Amersham Laboratories; final dilution 1:25 000). This antiserum cross-reacts 100% with GHRH1–44 on an equimolar basis. The recovery of synthetic GHRH1–44 (Bachem, Basel, Switzerland) from pooled human plasma averaged 87.5%. The plasma GHRH values reported here were not corrected for recovery. The sensitivity for standard GHRH1–44 was 4 pg/assay tube. The intra-assay coefficient of variation at 8 ng/l was 11% (N = 10) and the inter-assay coefficient of variation was 14% (N = 10).

Hormone secretory responses were determined by measuring the area under the curve within 180 min following the test meal and for 5.5 h during nocturnal sleep applying the trapezoidal method. Statistics were performed by means of the following non-parametric tests: the Mann-Whitney U-test to compare different groups, the Wilcoxon test to compare results within the same group, and the Spearman rank correlation coefficient. P < 0.05 was considered statistically significant.

Values are expressed as mean ± SEM.

Results

Hormone responses following the mixed meal

Fig. 1 shows the mean responses of plasma GHRH, GH, SRIH and insulin following the ingestion of the mixed meal.

Circulating GHRH levels were measurable in all children with baseline levels ranging from 6.9 ng/l to 14.9 ng/l. In response to the mixed meal in children with CDGA, mean plasma GHRH levels increased significantly at 90, 120 and 180 min (P < 0.05). Peak values were found between 60 min and 120 min with values ranging from 14.0 ng/l to 38.8 ng/l. These peak levels were significantly higher than baseline levels (10.1 ± 1.2 ng/l vs 25.5 ± 4.6 ng/l; P < 0.01). The GHRH increase in 3 children with hypothalamic GHRH-deficiency was comparable following the test meal (Fig. 1), even though the increase occurred somewhat delayed (peak values between 120 and 150 min ranging from 16.6 ng/l to 23.8 ng/l). There was no difference between the GHRH response areas of these 3 children or of those with CDGA (Table 1).

For the CDGA children, plasma GH levels showed wide variations with peak values occurring between 30 and 150 min, suggesting spontaneous GH fluctuations rather than any effect...
Mean ± SEM plasma levels of GHRH, GH, SRIH, and insulin following a mixed meal in 7 prepubertal children with constitutional delay of growth and adolescence (CDGA) (— —) and 3 children with hypothalamic GHRH-deficiency (——); *P < 0.05 vs patients with GHRH-deficiency.

Mean ± SEM integrated response areas of plasma GHRH, GH, SRIH, and insulin following a mixed meal in 7 children with constitutional delay of growth and adolescence (CDGA) and 3 children with hypothalamic GHRH-deficiency.

<table>
<thead>
<tr>
<th>Hormone response area</th>
<th>CDGA</th>
<th>Hypothalamic GHRH-deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHRH (ng x 1⁻¹ x 3 h⁻¹)</td>
<td>2542 ± 347</td>
<td>1845 ± 448</td>
</tr>
<tr>
<td>GH (µg x 1⁻¹ x 3 h⁻¹)</td>
<td>491 ± 96</td>
<td>121 ± 54*</td>
</tr>
<tr>
<td>SRIH (ng x 1⁻¹ x 3 h⁻¹)</td>
<td>2790 ± 283</td>
<td>2554 ± 440</td>
</tr>
<tr>
<td>Insulin (mU x 1⁻¹ x 3 h⁻¹)</td>
<td>5657 ± 440</td>
<td>6586 ± 990</td>
</tr>
</tbody>
</table>

* P < 0.05 vs CDGA.
Sleep-induced GH, GHRH and SRIH concentrations

Fig. 2 shows the individual plasma GH and GHRH concentrations in 10 children with CDGA during nocturnal sleep. The lowest GHRH values peaked from a mean value of 5.2 ± 0.1 ng/l (range from 4.5 ng/l to 5.8 ng/l) to mean maximum values of 26.5 ± 3.3 ng/l ($P < 0.01$) ranging from 16.5 ng/l to 49.5 ng/l. Spontaneous plasma GH pulses occurred in all subjects, with a mean peak value of 16.3 ± 1.7 µg/l. No time relationship between GHRH and GH peaks was observed except for subject No. 4. Subject No. 5 showed the highest GHRH peak value (49.5 ng/l), which was not followed by any GH increment. The patient with hypothalamic GHRH deficiency exhibited comparable GHRH increments (peak value 24.8 ng/l GHRH) although his GH values were all below 1 µg/l.

The SRIH concentrations did not change during nocturnal sleep (data not shown).

No correlation was present between the integrated areas under the curve during the 5.5 h of GHRH, GH and SRIH measurements.

Discussion

Our observation of a significant increase in plasma GHRH concentrations in children following a mixed meal is in accordance with the findings of a recent study on adults (Sopwith et al. 1985). The GHRH response patterns are similar in the two studies, yet the magnitude of the GHRH response is slightly higher among the adult patients. In both studies, the RIA used for the determination of GHRH did not discriminate between the 1–40
and 1-44 peptides. Therefore, significant fluctuations of one peptide could well be masked by larger amounts of the other non-fluctuating peptide. However, a recent chromatographic study of GHRH revealed evidence that all molecular forms of GHRH, except GHRH1-37, increase in peripheral plasma following a mixed meal (Penny et al. 1986). Since the molecular forms GHRH1-40 and GHRH1-44 are also present in human hypothalamic tissue (Ling et al. 1984), it is difficult to draw conclusions as to the sources of circulating GHRH following a mixed meal. However, in the present study, three children with suspected hypothalamic GHRH-deficiency exhibited a similar plasma GHRH increase when compared with the response in short normal children. This result confirms a similar study in adults (Sopewith et al. 1986). In addition, normal basal concentrations of plasma GHRH have been detected in patients with hypothalamic lesions (Kashio et al. 1987). These results support the view that both the basal and the meal-stimulated GHRH concentrations originate — at least in part — from gastrointestinal sources.

Little is known about the physiological role of circulating GHRH. In rats, a dose-related plasma gastrin release following human GHRH1-44 has been demonstrated (Accary et al. 1986). In response to GHRH1-40 injection using the isolated perfused canine pancreas, a significant increase in SRIH and insulin occurred (Hermansen et al. 1986). Recently, we have demonstrated a significant negative linear correlation between GHRH and SRIH increments following an insulin-induced hypoglycaemia (Rosskamp et al. 1987b). In the present study we found no correlation between the increments of plasma GHRH and SRIH or insulin in response to a mixed meal. However, this fact does not exclude the possibility that circulating GHRH is connected with gastrointestinal hormone secretion, since GHRH might chiefly act locally in the gastrointestinal tract, having a paracrine or an autocrine effect.

In normal subjects, plasma GHRH has been reported to increase both after L-dopa stimulation (Chihara et al. 1986; Donnadieu et al. 1985) and insulin-induced hypoglycaemia (Kashio et al. 1987; Rosskamp et al. 1987b). Patients with a probable hypothalamic defect do not exhibit such a GHRH increase following these tests (Chihara et al. 1986; Kashio et al. 1987). These findings favour a central origin of circulating GHRH. However, pharmacological tests of GH secretion do not represent the exact status of GH secretion (Bercu et al. 1986). Our more physiological investigation of sleep-induced GH secretion reveals no correlation between the spontaneous pulses of plasma GHRH and the spontaneous GH bursts.

Furthermore, we could demonstrate spontaneous plasma GHRH pulses in a child with suspected hypothalamic GHRH-deficiency. This provides further evidence for the hypothesis that nocturnal GHRH plasma levels originate mainly extrahypothalamically, possibly from gastrointestinal sources. It is well known for other gastrointestinal hormones that they reach remarkably high levels in the late evening and at night, peaking some hours after the last meal (Jorde & Burhol 1985). The physiological relevance of these diurnal patterns is unclear, however. In light of these findings the previously reported dramatic increases of basal GHRH values during puberty and their significant positive linear correlation with basal plasma somatomedin-C values (Argente et al. 1986) seems difficult to explain. These findings emphasize the central role of circulating GHRH in pubertal development which is known to be accompanied by an increasing GH secretion (Finkelstein et al. 1972). Since our studies were limited to prepubertal children, we cannot assess with certainty the potential role of circulating GHRH in the increase of GH secretion during puberty.

In conclusion, we have demonstrated a significant plasma GHRH increase following a mixed meal both in children with CDGA as well as in children with suspected hypothalamic GHRH-deficiency. During nocturnal sleep, a spontaneous GHRH secretion occurs, which shows no correlation to the GH bursts. Our findings support the concept that circulating GHRH does not merely represent hypothalamic GHRH, but might also be involved in the regulation of gastrointestinal functions. The exact role of circulating GHRH in this regard needs to be elucidated further.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (Grant Ro 717/1-1). We wish to thank Ms D. Fischer and Ms G. Bargon for their assistance in preparation of the manuscript and Ms A. Starke and Ms A. Möllenbeck-Madsen for their excellent technical assistance.
References


Kashio Y, Chihara K & Kita T et al. (1987): Effect of oral glucose administration on plasma growth hormone-releasing hormone (GHRH)-like immunoreactivity levels in normal subjects and patients with idiopathic GH deficiency: evidence that GHRH is released not only from the hypothalamus but also from extrahypothalamic tissue. J Clin Endocrinol Metab 64: 92–97.


Received June 18th, 1987.
Accepted September 10th, 1987.

Dr R. Rosskamp,
Universitäts-Kinderklinik,
Adenauerallee 119,
D-5300 Bonn I, FRG.