GH response to GHRH, insulin, clonidine and arginine after GHRH pretreatment in children

Sergio Bernasconi, Cecilia Volta, Antonella Cozzini, Mariangela Ziveri, Lucia Ghizzoni, Costantino Panza and Ezio Ghigo

Departments of Pediatrics and Endocrinology, Universities of Parma and Turin, Italy

To determine whether differences in the neuroendocrine control of GH are present between children and adult subjects, the GH response to GHRH (1 µg/kg) (group 1), insulin-induced hypoglycemia (0.1 U/kg iv) (group 2), clonidine (150 µg/m² po) (group 3) and iv arginine (0.5 g/kg in 30 min) (group 4) after GHRH pretreatment (1 µg/kg) was studied in 26 short-statured normal children (mean age 10.2 years). The results were compared with historical data in adults. No differences were present among mean peak GH levels after the first and second stimuli in groups 1, 2 and 3, while in group 4 the GH response to arginine administration was lower than that obtained after the initial GHRH (0.43 ± 0.04 vs 0.9 ± 0.13 nmol/l). Moreover, comparing the GH peak values following the second stimulus, it appears that the greatest GH responses were elicited by GHRH (1.31 ± 0.23 nmol/l) and clonidine (1.11 ± 0.22 nmol/l), while the lowest was elicited by arginine (0.43 ± 0.04 nmol/l). In adults, sequential GHRH administration leads to inhibition of the response of the somatotropes, probably mediated by an increase in hypothalamic somatostatin. Our results confirm that after GHRH pre-stimulation GHRII elicits a significant GH response suggesting that activation of the somatostatinergic tone is less effective in children. This hypothesis also explains the low GH response to arginine which acts selectively through somatostatin inhibition.

Sergio Bernasconi, Clinica Pediatrica, Via Gramsci 14, 43100 Parma, Italy

In the last few years, several authors have demonstrated that the plasma GH response of adult subjects to acute GHRH stimulation is abolished by a previous GHRH injection (1, 2). It has been speculated that an increase in the somatostatinergic tone following the first GHRH challenge might be responsible for the pituitary lack of response to the second stimulus.

This hypothesis has been further confirmed by studies in which it was shown that pyridostigmine, a cholinesterase inhibitor capable of reducing the somatostatinergic tone (3, 4), is effective in reinstating the GH responsiveness to repeated GHRH administration (5). The same mechanism has been advocated to explain the enhanced GH levels after GHRH pretreatment obtained by arginine, another somatostatin inhibiting agent (6), when injected either alone (7) or in combination with the second GHRH bolus (8).

On the basis of this evidence, it has been suggested that stimuli eliciting a GH increase after GHRH pretreatment might act through mechanisms different from GHRH release and most likely involving somatostatin inhibition, as happens after insulin-induced hypoglycemia (1) and, according to some authors (9), after clonidine.

On the other hand, children show quite different GH responses to different stimuli. In fact, a persistent somatotrope responsiveness to repeated GHRH stimulation was demonstrated in this age group, suggesting that a discrete autoregulation of the somatostatin-mediated GH secretion might be present in children (10).

The aim of the present study was to investigate the GH response to some commonly used somatotropin stimuli such as insulin-induced hypoglycemia, clonidine and arginine after GHRH pretreatment. This was in order to clarify their mechanisms of action and to establish whether differences in the neuroendocrine control of GH secretion are present between children and adult subjects.

Materials and methods

Twenty-six children (13 males – age range 5.9–14.9 years; and 13 females – age range 7 – 12.2 years) (mean age 10.2 ± 3.1 years) referred to our department for evaluation of their short stature, were studied. Sixteen of them were prepubertal and 10 in Tanner stage II or III of pubertal development; none of them was affected by endocrine or chronic disease and their short stature was considered idiopathic or constitutional.

The study was approved by the Ethics Committees of the departments and informed consent was obtained from parents and children.
At 09.00 h, after an overnight fasting, an iv line was inserted into an antecubital vein of each subject and blood samples for the measurement of serum GH concentration were obtained 30 min before and 0, 15, 30, 45, 60, 90 and 120 min after the iv bolus injection of 1 µg/kg of GHRH 1–29 (Sanofi, Paris).

Two hours after the first GHRH administration, seven patients received a second 1 µg/kg iv bolus of GHRH (group 1), seven received 0.1 IU/kg of insulin iv (Actrapid HM Novo) (group 2), and six children 0.15 mg/m² of clonidine (Catapresan® Boehringer) were administered po (group 3). The remaining six patients underwent a 30' iv infusion of 0.5 g/kg of arginine (1-arginine Damor, Naples) (group 4). Blood samples were collected at 135, 150, 165, 180, 210 and, after arginine and clonidine only, 240 min after injection of the first GHRH bolus.

The clinical features of the subjects of each group are reported in Table 1.

### Table 1. Main clinical features of the subjects of the four different groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (N = 7)</th>
<th>Group 2 (N = 7)</th>
<th>Group 3 (N = 6)</th>
<th>Group 4 (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± SD Range</td>
<td>Mean ± SD Range</td>
<td>Mean ± SD Range</td>
<td>Mean ± SD Range</td>
</tr>
<tr>
<td></td>
<td>10 ± 2.9 7–13</td>
<td>10.5 ± 2.3 8–14.1</td>
<td>9.6 ± 2.6 5.9–12.9</td>
<td>10.6 ± 1.8 8.1–12.1</td>
</tr>
<tr>
<td>Sex</td>
<td>M 4 3 3</td>
<td>F 3 4 3</td>
<td>M 5 4 4</td>
<td>F 3 2 3</td>
</tr>
<tr>
<td>Tanner</td>
<td>I 5 4 4</td>
<td>II–III 2 3 2</td>
<td>I 5 4 4</td>
<td>II–III 2 3 2</td>
</tr>
</tbody>
</table>

Plasma glucose levels measured using the hexokinase enzymatic method reached a nadir equal to or less than 50% of the baseline value after insulin administration.

Serum GH concentrations were measured using the Nichols Institute Diagnostic human GH immunoradiometric assay kit (San Juan Capistrano, California). All samples from each subject were measured in duplicate in the same assay. Sensitivity of the assay was 0.09 nmol/l. The mean intra-assay coefficients of variation were 3.3, 4.0 and 4.5% at 0.23, 0.69 and 2.3 nmol/l respectively.

The GH secretory responses were expressed as absolute values of peak levels and area under the curve (AUC µg·1⁻¹·h⁻¹) calculated with trapezoidal integration.

Because of the skewed distribution of the data, the results were log-transformed and analyzed by the paired two-tailed Student t-test. Multiple comparisons among independent groups of data were performed using analysis of variance followed by the Bonferroni correction. Correlations were evaluated using linear regression analysis.

### Results

Table 2 gives the GH blood concentrations before and after injection of the GHRH pretreatment and before and after the second stimulus (GHRH, insulin, clonidine and arginine) in the groups considered. Mean baseline GH levels were similar among all four groups of subjects tested. In all of them the first GHRH administration elicited a significant GH elevation and the magnitude of the increase was similar in all subjects. Blood GH concentrations returned to baseline levels after 120 min.

### Table 2. GH values expressed as mean ± SEM, median and range of baseline, peak and area parameters after GHRH and the second stimulus in the groups considered (a = p < 0.05 vs baseline GH levels; b = p < 0.05 vs GH peak levels after arginine stimulation).

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>After GHRH</th>
<th>Area</th>
<th>After 2nd stimulus</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/l</td>
<td>Peak nmol/l</td>
<td>nmol·1⁻¹·h⁻¹</td>
<td>nmol/l</td>
<td>Peak nmol/l</td>
</tr>
<tr>
<td>1</td>
<td>0.06±0.02</td>
<td>0.74±0.12a</td>
<td>34.04±7.57</td>
<td>0.08±0.02</td>
<td>1.31±0.23a</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.04</td>
<td>0.6</td>
<td>28.6</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.01–0.2</td>
<td>0.4–1.3</td>
<td>18.1–74.1</td>
<td>0.02–0.2</td>
</tr>
<tr>
<td>2</td>
<td>0.37±0.15</td>
<td>1.53±0.28a</td>
<td>92.37±25.2</td>
<td>0.22±0.07</td>
<td>0.89±0.19a</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.09</td>
<td>1.37</td>
<td>81.4</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.01–1.16</td>
<td>0.6–2.3</td>
<td>26.1–183</td>
<td>0.04–0.6</td>
</tr>
<tr>
<td>3</td>
<td>0.33±0.1</td>
<td>1.77±0.22a</td>
<td>86.85±16.6</td>
<td>0.12±0.04</td>
<td>1.11±0.22a</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.29</td>
<td>1.82</td>
<td>65.3</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.01–0.7</td>
<td>0.9–2.3</td>
<td>59.8–149.2</td>
<td>0.04–0.3</td>
</tr>
<tr>
<td>4</td>
<td>0.23±0.06</td>
<td>0.9±0.11b</td>
<td>44.25±7.65</td>
<td>0.2±0.1</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.26</td>
<td>1.1</td>
<td>41.9</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.1–0.4</td>
<td>0.5–1.3</td>
<td>17.1–74.6</td>
<td>0.02–0.8</td>
</tr>
</tbody>
</table>
Injection of a second GHRH (group 1) and insulin (group 2) bolus, as well as oral clonidine administration (group 3), led to an additional significant increase in GH plasma levels. In each of these three groups, GH peak levels and area under the curve were similar to those obtained by the preceding GHRH challenge. In contrast, following arginine infusion, no significant GH increase could be detected and peak values were significantly lower ($t = 3.5; p < 0.05$) than those obtained in the same subjects after the GHRH pretreatment.

No correlation was found between GH peak levels and area under the curve obtained in response to GHRH pretreatment and those elicited by each of the following stimuli.

Comparing the GH responses to different challenges (inter-group analysis) (Fig. 1), the GH response to arginine, expressed as mean peak values and area under the curve appears to be lower than that elicited by the other stimuli. However, the difference reached statistical significance only when compared with the GH levels obtained after GHRH and clonidine administration (peaks: $t = 4.1; p < 0.001$ and $t = 3.4; p < 0.005$ respectively; area under the curve: $t = 3.8; p < 0.001$ and $t = 3.6; p < 0.005$ respectively).

No correlation was found between GH responses to the second stimulus and age, sex and pubertal development.

Discussion

The results of the present study confirm (10) that in children GHRH pretreatment does not affect the ability of the pituitary to respond to a second GHRH stimulation. The same is true for insulin hypoglycemia and clonidine, but not for arginine, which failed to induce a GH response comparable to that obtained after GHRH administration.

In part at variance with the present findings are the results of studies performed in adult subjects in whom GHRH administration was found to inhibit the GH response to the second GHRH bolus (2). This phenomenon has been explained by an increase in the somatostatinergic tone secondary to GHRH administration, which antagonizes the action of the following GHRH challenge, but not that of the stimuli acting through inhibition of SRIH release such as arginine and pyridostigmine (5).

Based on this hypothesis, the lack of inhibition of GH response to the releasing hormone after GHRH pretreatment in children might be accounted for by the different negative somatostatinergic feedback in this age group (10).

Arginine is generally thought to stimulate GH release through mechanisms selectively SRIH-mediated (6–8, 11, 12). In the present study this amino acid fails, following GHRH pretreatment, to induce a consistent GH augmentation, further supporting this hypothesis. At variance with our observations, in adults pretreated with GHRH the amino acid induces a clear-cut GH rise (7). This discrepancy suggests that the GHRH challenge in children does not trigger an increase in somatostatinergic tone, as happens in adult subjects.

Conflicting results have been reported on the mechanism by which clonidine stimulates GH secretion. In fact the GH response to clonidine has been shown to be inhibited by previous GHRH administration by Valcavi et al. (13), but not by others (9). The children we studied exhibited a GH response to clonidine administration following GHRH pretreatment, similar to that obtained after the second GHRH administration and higher than that achieved by arginine. These results further support the hypothesis of a GHRH-mediated mechanism of action of clonidine in agreement with previous in vitro (9) and in vivo (14) studies. However, an inhibiting effect on SRIH release by alpha-2 adrenergic agents has also recently been suggested (15).
The mechanisms involved in the stimulation of GH release by insulin hypoglycemia have also been widely investigated, but not clearly understood. Partial inhibition of the GH response to hypoglycemia by alpha-adrenergic blockade with phentolamine tends to indicate a stimulatory effect of catecholamines on GH secretion through a discharge of GHRH from the hypothalamus (16). However, since the alpha-adrenergic blockade was not complete, the effect of hypoglycemia on GH secretion cannot be entirely mediated by catecholamines. The possibility that the GH release induced by hypoglycemia might partially be due to SRIF inhibition has to be taken into account. The fact that in adults insulin-induced GH release is not inhibited by GHRH pretreatment further supports this hypothesis (1). Furthermore, it might be hypothesized that in a life-threatening situation such as hypoglycemia, both mechanisms can activate the release of counter-regulatory hormones such as GH. Involvement of the somatostatinerergic together with the GHRH mediated pathways in the GH release induced by hypoglycemia in children is also indicated by the results of the present investigation. In fact, the GH responses to insulin stimulation are intermediate between those obtained after GHRH and clonidine on the one hand and arginine on the other.

Finally, GH responses to the different stimuli seem to be independent of sex and stage of pubertal development. It should be pointed out, however, that our patients were either prepubertal or at the beginning of pubertal development. Further studies are needed to establish whether sex steroids may be responsible for the different GH behavior after challenge with different substances, as suggested by studies in animals (17).

In conclusion, our data demonstrate that the regulation of GH secretion in children is partially different from that in adults. In particular, based on the results obtained in adults, the different GH responses after GHRH, clonidine, insulin-induced hypoglycemia and mainly arginine suggest a different modulation of the somatostatinergic tone.

References

Received April 15th, 1991
Accepted September 2nd, 1991