Effect of oral clonidine, insulin-induced hypoglycemia and exercise on plasma GHRH levels in short-stature children

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Abstract Human growth hormone release is affected by a variety of pharmacological and physiological stimuli. We have studied the effect of oral clonidine, insulin hypoglycemia, and exercise on plasma hGH and GHRH levels in 31 healthy short-stature children. Thirteen underwent an oral clonidine test (0.15 mg/m²), 12 an iv. insulin test (0.1 U/kg), and 6 performed exercise (running for 10 min in a defined route). GHRH-1-44 was extracted from plasma on silica columns and determined by RIA. Although all three stimuli induced a marked increase in plasma hGH levels, only clonidine induced a significant increase in plasma GHRH levels. Maximal increment in GHRH during clonidine was 6.82 ± 1.05 pmol/l (mean ± SEM) as compared with 0.51 ± 0.28 and 0.55 ± 0.62 during hypoglycemia and exercise (p<0.0005 and p<0.005), respectively. An additional 24 subjects received TRH 0.2 mg/kg iv: 8 TRH alone, 8 TRH and insulin, and 8 TRH and clonidine. Only insulin potentiated the TRH-induced TSH response with a peak of 22.0 ± 3.2 vs 16.0 ± 0.8 and 15.3 ± 1.5 mU/l (p<0.025) for TRH alone and TRH and clonidine, respectively. It is suggested that clonidine stimulates hGH secretion mainly through an enhancement of GHRH release, whereas stress stimuli such as hypoglycemia and exercise achieve hGH release by a different mechanism, possibly inhibition of somatostatin.

The isolation and chemical characterization of growth hormone releasing hormone from various tissues (1,2) and the development of sensitive methods for its determination in plasma and hypothalamus (3-5) have made it possible to study the mechanisms by which neuroactive drugs affect growth hormone secretion.

Orally administered clonidine, insulin-induced hypoglycemia, and exercise are widely used hGH stimulation tests (6-8). Clonidine is a central alpha-2-adrenergic stimulatory agent which activates the hypothalamo-hypophysial axis and inhibits ACTH and cortisol secretion (9); hypoglycemia is a stressful stimulus which activates the hypothalamo-hypophysial-adrenal axis and the secretion of ACTH and cortisol, and acute exercise is a physiological stimulus which induces release of the stress hormones resembling the situation during hypoglycemia. Recent studies in the rat provide evidence that clonidine induces GH secretion by activation of hypothalamic GHRH (10,11). On the other hand, there are conflicting results regarding the mechanism by which insulin hypoglycemia stimulates hGH secretion (12-14).

It was the aim of this study to elucidate the mechanism by which these stimuli act to release hGH by measuring their concomitant effect on hGH and GHRH in plasma.

Subjects and Materials

The entire study population comprised 55 healthy short-stature children and adolescents (31 males, 24 females), aged 7-16 years. Height standard deviation score range was from -2 to -4.5 SD. In all the children hGH deficiency could be ruled out by hGH peak values of >0.37 nmol/l. All the procedures were performed within the
frame of developmental evaluation. Explanation was
given to parents and children and informed consent was
obtained. The study was approved by the hospital's Eth¬
ical Committee. Thirty-one subjects (24 males, 7 females)
underwent one of the standard hGH stimulation tests: 12
underwent a standard insulin test (0.1 U/kg iv) and 13 an
oral clonidine test (0.150 mg/m²). Six subjects, 3 receiving
insulin and 3 clonidine, had blood samples withdrawn
every 15 min for 90 min with the aid of a non-thrombo¬
genic catheter and a constant withdrawal pump (Cormed,
New York, USA). In the others blood samples were ob¬
tained through a butterfly needle inserted in the forearm
vein at 0, 30, 60 and 90 min. Six subjects (5 males, 1
female) underwent an exercise test. They were asked to
run along a defined route for 10 min with blood collected
before performance of this exercise, upon its completion,
and 10 min later.

An additional 24 subjects (13 males and 11 females)
were divided into 3 groups of 8 subjects each. The first
group received only TRH 0.2 mg/kg iv, whereas the
second group concomitantly received TRH and insulin
(0.1 U/kg iv) and the third group TRH and clonidine
(0.150 mg/m²) with blood collected at 0, 15, 30, 60 and 90
min.

Blood samples for determination of hGH and TSH
were collected into heparinized tubes, and for determi¬
nation of GHRH, into tubes containing EDTA and
Trasylol® (1000 kIU). Plasma was immediately separated
and frozen at −20°C until extracted and assayed. Blood
glucose was determined by a Beckmann autoanalyzer.

Hormone determinations

Determination of GHRH in plasma was performed ac¬
cording to a method previously described (15). Extraction
of GHRH from plasma was performed on SEP-PAK c-18
columns (Waters, Milford, MA) previously moistened
with acetonitrile and rinsed with H₂O; 2 ml of plasma or
standard solution were pushed slowly through the col¬
umns and washed twice with 10 ml H₂O. The absorbed
GHRH was eluted in 2 ml acetonitrile-acetic acid (80:20
v/v). The eluate was lyophilized and dissolved in 0.5 ml
incubation buffer (PBS 0.05 mol/l, EDTA 0.025 mol/l,
albumin 0.5% and Tween 20 0.5%, pH 6.5). GHRH in the
eluate was assayed by a double-antibody RIA using anti-

Fig. 1.
Effect of oral clonidine (•–•) (0.15 mg/m² po) and 
insulin (0.1 U/kg iv) hypoglycemia (––•–•) on plasma GHRH
levels. * p<0.02 vs basal. Each point represents mean ± SEM.
Table 1.
Individual maximal increment (Δ) of plasma hGH and GHRH over basal levels following oral clonidine, iv insulin or exercise in children.

<table>
<thead>
<tr>
<th>Test</th>
<th>Clonidine</th>
<th>Insulin hypoglycemia*</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.15 mg/m² po)</td>
<td>(0.1 U/kg iv)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hGH  GHRH</td>
<td>hGH  GHRH</td>
<td>hGH</td>
</tr>
<tr>
<td></td>
<td>nmol/l pmol/l</td>
<td>nmol/l pmol/l</td>
<td>pmol/l</td>
</tr>
<tr>
<td>1</td>
<td>0.18 10.4</td>
<td>0.26 -0.08</td>
<td>0.19 2.0</td>
</tr>
<tr>
<td>2</td>
<td>0.23 3.8</td>
<td>0.26 -0.9</td>
<td>0.25 0.4</td>
</tr>
<tr>
<td>3</td>
<td>0.23 6.0</td>
<td>0.28 0.75</td>
<td>0.40 1.6</td>
</tr>
<tr>
<td>4</td>
<td>0.41 12.0</td>
<td>0.37 0.60</td>
<td>0.67 0.8</td>
</tr>
<tr>
<td>5</td>
<td>0.64 10.0</td>
<td>0.45 0.14</td>
<td>0.84 0.8</td>
</tr>
<tr>
<td>6</td>
<td>0.73 4.4</td>
<td>0.60 2.36</td>
<td>0.83 -2.4</td>
</tr>
<tr>
<td>7</td>
<td>0.78 6.4</td>
<td>0.62 -1.16</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.83 9.0</td>
<td>0.66 0.68</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.24 3.0</td>
<td>1.28 0.56</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.42 12.0</td>
<td>1.43 1.20</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.59 6.4</td>
<td>1.47 1.40</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.68 1.1</td>
<td>1.50 0.8</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1.79 4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±SEM</td>
<td>0.9±0.2 6.8±1.1</td>
<td>0.8±0.2 0.5±0.3</td>
<td>0.5±0.1 0.5±0.6</td>
</tr>
</tbody>
</table>

* Mean basal blood glucose was 4.6±0.2 mmol/l (range 4.1-5.0 mmol/l) and following insulin decreased in all subjects to a mean of 2.15±0.4 mmol/l (range 1.8-3.0 mmol/l).

GHRH-1-44 serum and I-125-GHRH (Amersham, Buckinghanshire, UK) and GHRH-1-44 (Sigma, St. Louis, USA) as standard. The specificity of the anti-serum was tested against GHRH-1-40 and found to be 83% on an equimolar basis. No cross-reactivity was found with TRH, CRH, GmRH, SRIF, glucagon, hGH or ACTH. The sensitivity of the assay was 1.2 pmol/l. The intra- and inter-assay variations at 5 pmol/l were 10 and 17%, respectively. Plasma hGH and TSH levels were determined by a standard double-antibody RIA. The sensitivity of the assay for hGH was 0.027 nmol/l and for TSH 0.2 mIU/l. The intra- and inter-assay variations for hGH at 0.037 nmol/l were 7 and 11%, and for TSH at 5 mIU/l 4.5 and 6.5%, respectively. All the levels were expressed in SI units (hGH: 1 μg l = 0.046 nmol/l and GHRH: 1 ng/l = 0.2 pmol/l).

Results

All three stimuli induced a significant increase in plasma hGH; clonidine from 0.21 ± 0.08 nmol/l (mean ± SEM) to a peak of 1.15 ± 0.23 (p<0.01), insulin hypoglycemia from 0.17 ± 0.07 to 0.85 ± 0.14 (p<0.01), and exercise from 0.092 ± 0.023 to 0.58 ± 0.09 (p<0.05). However, only clonidine was effective in inducing a significant increase in GHRH, with a rise from basal levels of 5.62 ± 1.55 to a peak of 12.27 ± 2.5 pmol/l at 60 min (p<0.01). Neither hypoglycemia nor exercise induced a significant rise in plasma GHRH levels (Fig. 1 and 2). The net individual maximal increment in hGH and GHRH is shown in Table 1. The net maximal increment in plasma GHRH induced by clonidine was strikingly greater than that induced by insulin and exercise (6.82 ± 1.05 pmol/l vs 0.51 ± 0.28 and 0.53 ± 0.62, p<0.0005 and p<0.005, respectively).

Although the effect of clonidine on hGH secretion was greater than the effect of hypoglycemia and exercise, the difference did not reach statistical significance. No significant correlation was found between the magnitude of the hGH response and that of GHRH (r = -0.295). The individual variation in GHRH following clonidine or insulin tolerance test is demonstrated by the results obtained in the 6 subjects in whom plasma was withdrawn every
15 min. In the 3 subjects who received clonidine, basal plasma GHRH levels ranged between 4.2 and 7.6 pmol/l and peaked at 45-75 min to 10-16 pmol/l; in all instances the GHRH peak appeared before that of hGH, whereas plasma GHRH levels following insulin fluctuated widely with levels ranging between 5.6-9 pmol/l (Fig. 3).

Administration of TRH induced a significant increase in plasma TSH levels at 15, 30 and 60 min with a peak at 30 min in all the subjects tested. Concomitant administration of clonidine and TRH did not significantly modify this pattern, but administration of insulin and TRH resulted in an anticipation of the peak to 15 min and potentiation of the total response, with a peak level of TSH of 22.0 ± 3.2 mIU/l compared with the mean peak value of 16.0 ± 0.8 (p<0.025) obtained with TRH alone and of 15.3 ± 1.4 mIU/l with TRH + clonidine, respectively (Table 2).

Discussion
The present investigation demonstrates that GHRH secretion is stimulated by oral administration of clonidine, but not by insulin hypoglycemia or exercise despite the fact that all 3 tests significantly stimulated endogenous hGH secretion. These results substantiate previous observations that some hGH provocative stimuli such as L-dopa promote GHRH secretion, whereas others, such as arginine and ornithine, do not (3,16). The results also support the concept that different mechanisms may induce growth hormone stimulation.

Studies in animals also provide evidence that clonidine induces GH secretion by stimulating hypothalamic GHRH release: passive immunization of rats with antiserum to GHRH inhibited the clonidine-induced secretion of GH (17). Long-term treatment with clonidine caused a depletion in hypothalamic GHRH content in the rat (18), and incubation of clonidine with rat hypothalami enhanced the secretion of GHRH (10). Although the above reports strongly suggest a stimulatory role of clonidine on hypothalamic GHRH release, there is no direct evidence regarding its mechanism in man. At variance with our study, Rosskamp & Haverkamp (19) reported no change in plasma GHRH levels following clonidine in short-stature
Table 2.
Effect of insulin hypoglycemia and clonidine on TRH-induced TSH secretion.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRH</td>
<td>8</td>
<td>4.3±0.5</td>
<td>13.4±1.2*</td>
<td>15.8±1.6*</td>
<td>12.5±1.5*</td>
<td>8.1±1.4</td>
</tr>
<tr>
<td>TRH + insulin</td>
<td>8</td>
<td>6.0±1.1</td>
<td>21.9±3.2*#</td>
<td>21.2±2.7*</td>
<td>16.2±3.0*</td>
<td></td>
</tr>
<tr>
<td>TRH + clonidine</td>
<td>8</td>
<td>6.2±0.7</td>
<td>12.9±1.7*</td>
<td>15.5±2.1*</td>
<td>10.8±1.3</td>
<td>8.0±1.7</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
*p<0.001 vs t = 0. # p<0.025 vs TRH and TRH + clonidine.

Fig. 3.
Individual integrated plasma GHRH levels following insulin (0.1 U/kg iv) or clonidine (0.15 mg/m² po) in healthy children.
children. The difference in findings might be related to non-similarity in the methods employed for GHRH determination. The lack of correlation between the increment of hGH and GHRH following clonidine in our study could be due to a contribution of GHRH from peripheral tissues, since GHRH has been found in considerable amounts in the gastrointestinal system (20), and a number of stimuli such as a mixed meal and an oral glucose load were effective in releasing it (21).

The mechanism by which hypoglycemia induces GH secretion is controversial. A few reports suggest the involvement of both somatostatin and GHRH in its effect. In the rat, treatment with antisem to somatostatin reduced the inhibitory effect of stress on GH release (22). In humans, desensitization of GHRH receptors by large amounts of GHRH inhibited the effect of GHRH, but not that of insulin on hGH secretion (23). In middle-aged men hypoglycemia was more effective in releasing hGH than iv administration of GHRH, suggesting a divergence in the mechanisms of the two stimuli (24). Sopwith et al. (12) reported that insulin hypoglycemia led to no change in values of plasma immunoreactive GHRH in young adult subjects. On the other hand, Kashio et al. (13) and Roskamp et al. (14) reported an increase in plasma GHRH immunoreactivity in plasma following both oral glucose load and insulin hypoglycemia. In these two studies, however, the magnitude of the effect of insulin was less marked than the reported effect of L-dopa and that produced by clonidine in our study, supporting the idea that factors other than GHRH are involved in the mechanism of GH stimulation by hypoglycemia, possibly somatostatin inhibition. In favour of such a possibility is our finding that insulin hypoglycemia potentiated the TRH-induced TSH secretion, since this reaction is known to be under the inhibitory influence of somatostatin. Kelijman & Frohman (25) reported other findings, probably due to differences in sampling times.

Although the metabolic work performed during the exercise test carried out in 6 subjects was not standardized, in all 6 it induced a considerable increase in plasma hGH levels without change in plasma GHRH. Acute exercise is regarded as physical stress and like other metabolic and psychological stressors it activates the hypothalamic-hypophyseal-adrenal axis and induces release of CRH, ACTH, cortisol and beta-endorphin (26, 27). It has been suggested that beta-endorphin and the opiate-ergic system play a role in the regulation of GH secretion (28), and studies in the rat provided evidence that the alpha-adrenergic and the opiate-ergic system stimulate GH secretion through activation of GHRH (11). In man, however, pre-treatment with naloxone did not affect the hGH response to exercise, suggesting that the opioid pathway is not involved in this response (29).

The findings of the present study support the concept that clonidine stimulates hGH secretion by an enhancement of GHRH release, whereas stress stimuli achieve this through a different mechanism, possibly by inhibition of somatostatin.

References
11. Miki N, Ono M, Shizume K. Evidence that opiate-ergic


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