SHORT COMMUNICATION

Effects of growth hormone releasing hormone on insulin action and insulin secretion in a hypopituitary patient evaluated by the clamp technique

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The effect of growth hormone releasing hormone (GHRH-44) therapy on insulin action and secretion was evaluated in a hypopituitary patient after one month and one year of treatment. Hepatic and peripheral insulin action was studied with the hyperinsulinenemic-euglycemic clamp in combination with [6,6-\(\text{H}_2\)]glucose tracer infusion. First and second phase insulin secretion was assessed with the hyperglycemic clamp. Prior to GHRH-44 therapy the hypopituitary patient had higher insulin mediated glucose disposal rate and lower basal and stimulated insulin concentrations by more than two standard deviations from the mean of a control group. Following therapy there was no change in basal hepatic glucose production; however, there was evidence of diminished peripheral insulin action. This was manifested by decreased insulin mediated glucose disposal during the hyperinsulinenemic-euglycemic clamp, and increased insulin secretion during the hyperglycemic clamp. We conclude that GHRH-44 therapy in this patient was associated with decreased peripheral insulin action which was compensated for by increased insulin secretion.

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In 1982, the predicted presence of growth hormone releasing hormone (GHRH) was confirmed when it was isolated from two patients with pancreatic islet cell adenomas (1, 2). Subsequently, two major forms of GHRH have been found to be present in the human hypothalamus and are identical to the 1-40 and 1-44 amino acid peptides isolated from the human pancreatic islet cell adenomas (3). Since the discovery of GHRH, rapid development and evaluation of this peptide in human volunteer studies have resulted in the following information. (i) It is estimated that 75% of all children with growth hormone deficiency have GHRH deficiency rather than GH deficiency with intact and responsive pituitary glands (4). (ii) Chronic GHRH administration in growth hormone deficient children has been shown to be an effective and well tolerated therapy for normalizing growth velocity (5). It is classically accepted that GH deficiency is characterized by increased insulin sensitivity, whereas GH excess and/or GH treatment causes insulin resistance (6). The aim of the present study was to evaluate insulin action and insulin secretion by the insulin-glucose clamp technique in hypopituitary patients before and after GHRH-44 therapy as part of a multicenter Hoffman LaRoche, Inc. sponsored study to assess the efficacy and tolerability of GHRH-44 in the therapy of GH deficient children (7). Only one patient was studied due to untimely termination of recruitment. We herein report this case.

Subject and Methods

Subject

The subject at the time of evaluation was a 10.5 year old white female with the diagnosis of idiopathic GH and antidiuretic hormone (ADH) deficiency. Her annual growth rate was 2.8 cm. IGF-1 was low for her age (380 IU/l). Nocturnal GH sampling every 20 min revealed one peak of 4.5 \(\mu\)g/l and a mean of 1.2 ± 0.6 \(\mu\)g/l. During the arginine insulin tolerance test GH peaked at 2.6 \(\mu\)g/l. The rest of her hypothalamic-pituitary endocrine function was intact except for ADH deficiency documented by water deprivation test. An NMR did not reveal any intracranial structural abnormalities. She was started on GHRH-44 therapy (10 \(\mu\)g/kg) subcutaneously twice a day according to the study protocol. After a year of treatment she grew 8.8 cm and after 22 months a total of 16.7 cm.
Methods

The study protocol was approved by the Human Rights Committee of the Children’s Hospital of Pittsburgh. Informed written consent was obtained from the parents and the participants. All studies were performed in the postabsorptive state following a 10 h overnight fast. Before the start of the GHRH-44 therapy and after 1 and 12 months of therapy body composition was assessed and in vivo insulin action and insulin secretion were evaluated by the hyperinsulinemic-euglycemic and hyperglycemic clamp technique (8).

Body composition was assessed by the use of tetrapolar bioelectrical impedance methodology as validated in children with growth disorders (9). Insulin action for suppression of hepatic glucose production and stimulation of glucose disposal was determined by a stepwise sequential hyperinsulinemic-euglycemic clamp in conjunction with isotopic measurement of glucose flux with [6,6-2H2]glucose tracer. The rate of glucose appearance (Ra) and the rate of glucose disappearance (Rd) were quantified following a 10 h overnight fast and during each of the glucose clamp steps by infusing [6,6-2H2]glucose tracer in a primed (30 μmol/kg) constant rate infusion (0.33 μmol·kg⁻¹·min⁻¹) manner (10). [6,6-2H2]glucose (> 98 atom % excess) was purchased from Merck/Isotopes, Quebec, Canada. The tracer was dissolved in normal saline, sterilized by passing through a Millipore filter (pore size 0.22 μm) and tested for pyrogenicity and sterility. After a 2 h baseline period of isotopic equilibration, insulin (Humulin Regular, Lilly) was infused intravenously at 0.6 mU·kg⁻¹·min⁻¹ for 120 min and at 1.2 mU·kg⁻¹ for an additional 90 min. Plasma glucose was clamped at 5.5 mmol/l by concomitant intravenous infusion of variable amounts of glucose as a 10% solution. The rate of glucose infusion was adjusted on the basis of arterialized venous plasma glucose determinations every 5 min at the bedside. During the clamp, blood for determination of plasma glucose enrichment and insulin concentration was drawn every 10 min. First-phase and second-phase insulin secretion was assessed the following day by the hyperglycemic clamp technique for 120 min (8). Plasma glucose was acutely increased to 12.5 mmol/l by a bolus infusion of 25% dextrose and maintained at that level by a variable rate infusion of 10% dextrose solution. Insulin concentration was measured every 2.5 min for 15 min and then every 15 min until the end of the study.

Biochemical measurements

Plasma glucose was measured by the glucose oxidase method using a YSI glucose analyzer (Yellow Springs Instrument Company Inc., Yellow Springs, OH), and insulin by radioimmunoassay. For determining the enrichment of [6,6-2H2]glucose, the plasma samples were deproteinized with zinc sulfate and barium hydroxide. The supernatant was purified through a column of mixed bed of anion and cation ion exchange resins. After eluting with water, the glucose fractions were pooled, dried and converted to pentacetate derivative with acetic anhydride and pyridine. The derivative was analyzed with gas chromatography-electron impact mass spectrometry (10).

Calculations

The rate of endogenous (hepatic) glucose production (HGP) was calculated during the last 30 min of the baseline period according to steady-state isotope dilution principles (10). During the hyperinsulinemic clamp, HGP was calculated by subtracting the glucose infusion rate from Rg. It is recognized that in conditions with high glucose infusion rates, this calculation may give negative values for HGP (11). Negative numbers for HGP were observed during the low and high rate insulin clamp steps. We assumed that in our experiments negative numbers were indicative of complete suppression of HGP, and were calculated as zero. In the basal state, Rb was equal to Rg. During the hyperinsulinemic-euglycemic clamp the Rb was equal to exogenous glucose infusion rate because of complete suppression of HGP. Because the skeletal muscle or the fat free mass (FFM) is the predominant site for insulin mediated glucose disposal (12) Rb was expressed as μmol·min⁻¹·kg⁻¹ fat free mass. First-phase and second-phase insulin secretion were calculated as before (8). The patient was compared to five healthy control subjects (4F/1M), age 11.1±1.8 years, Tanner I pubertal development. The body surface area of the control subjects (1.3±0.1 m²) matched that of the patient (1.2 m²). Two subjects declined the hyperglycemic clamp.

Results

Table 1 summarizes the data of the longitudinal investigation. Following GHRH-44 therapy, both IGF-1 and growth rate increased. In the first year of therapy fat-free mass increased by 5.1 kg, while percentage body fat decreased by 7.3. Prior to GHRH therapy basal plasma glucose concentration in the hypopituitary patient (5.1 mmol/l) was similar to that in the control subjects (5.1±0.2 mmol/l) and did not change with GHRH therapy. Basal insulin concentration was lower in the patient by more than two standard deviations below the control mean (79 vs 129±14 pmol/l); however, it increased to 106 pmol/l after one year of GHRH treatment. There was no difference in basal HGP between the controls and the patient before and after GHRH therapy.

During the hyperinsulinemic-euglycemic clamps steady-state plasma glucose concentration was clamped at 5.5 mmol/l in the patient and (5.6±0.1 mmol/l) in controls, with a coefficient of variation of less than 5.0% in each. There were no differences between the patient and the controls in steady-state plasma insulin concen-
trations during the low (469 vs 417±41 pmol/l) and high (754 vs 731±144 pmol/l) rate insulin clamp steps, and no change following GHRH therapy. HGP was completely suppressed during the low rate insulin infusion in both the controls and the patient with no change following GHRH therapy. R_d was higher in the patient by more than two standard deviations above the control mean (133 vs 72±23 μmol·min⁻¹·kg FFM⁻¹) during the high insulin infusion rate. Following one year of GHRH therapy glucose disposal rates decreased by 20% at low and high insulin infusion rates. During the hyperglycemic clamp plasma glucose was clamped at 12.5 mmol/l in the patient and (11.8 ± 0.5 mmol/l) in controls with a coefficient of variation of less than 7%. Second-phase insulin concentration was lower in the patient by more than two standard deviations below the control mean (708 vs 1,087±108 pmol/l), with no difference in first-phase insulin levels. Following one year of GHRH treatment, second-phase insulin secretion increased by 20% (Table 1).

Discussion

This case study demonstrates that chronic GHRH-44 treatment is associated with the evolution of decreased insulin sensitivity. Prior to GHRH therapy the hypopituitary patient was insulin-sensitive compared with the control subjects. This was manifested in higher insulin-mediated glucose disposal rate and lower basal and stimulated insulin concentrations. Following one year of GHRH therapy, basal insulin concentration increased by 34%, and insulin-mediated glucose disposal rate decreased by 20%. The latter was compensated for by a 20% increase in second-phase insulin secretion.

A number of studies have evaluated the effects of GH administration to normal human subjects (6), but data are scarce in hypopituitary children. In normal subjects there was usually no change in basal glucose concentra-

- **Table 1.** Clinical and biochemical data before and after GHRH-44 therapy.

<table>
<thead>
<tr>
<th></th>
<th>Before GHRH-44</th>
<th>After GHRH-44 (month)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.5</td>
<td>10.6</td>
<td>11.6±1.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.9</td>
<td>20.4</td>
<td>19.5±2.0</td>
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<tr>
<td>FFM (kg)</td>
<td>18.0</td>
<td>19.9</td>
<td>23.1</td>
</tr>
<tr>
<td>% body fat</td>
<td>48.7</td>
<td>45.4</td>
<td>41.4</td>
</tr>
<tr>
<td>Basal HGP (μmol·min⁻¹·kg⁻¹)</td>
<td>15.4</td>
<td>19.9</td>
<td>118±14</td>
</tr>
<tr>
<td>Basal plasma glucose (mmol/l)</td>
<td>5.1</td>
<td>5.1</td>
<td>5.1 ±0.2</td>
</tr>
<tr>
<td>Basal plasma insulin (pmol/l)</td>
<td>79</td>
<td>74</td>
<td>106</td>
</tr>
<tr>
<td>RdL (μmol·min⁻¹·kg FFM⁻¹)</td>
<td>55</td>
<td>59</td>
<td>44</td>
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<tr>
<td>RdH (μmol·min⁻¹·kg FFM⁻¹)</td>
<td>133</td>
<td>118</td>
<td>104</td>
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<tr>
<td>1st phase insulin (pmol/l)</td>
<td>581</td>
<td>568</td>
<td>637</td>
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<tr>
<td>2nd phase insulin (pmol/l)</td>
<td>708</td>
<td>870</td>
<td>850</td>
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</table>

FFM: fat-free mass; HGP: rate of hepatic glucose production; RdL: rate of glucose disposal at low insulin infusion rate (0.6 μU·kg⁻¹·min⁻¹); RdH: rate of glucose disposal at high insulin infusion rate (1.2 μU·kg⁻¹·min⁻¹). Control data are mean ± sd.

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References


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