GLP-1-induced alterations in the glucose-stimulated insulin secretory dose-response curve

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1The Clinical Research Unit for Gastrointestinal Endocrinology, Department of Internal Medicine, Philipps University, 35033 Marburg, Germany; 2Department of Internal Medicine, Washington University, St. Louis, Missouri 63110; and 3Department of Gastroenterology, Inselspital, University of Bern, CH-3010 Bern, Switzerland

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Brandt, Andreas, Martin Katschinski, Rudolf Arnold, Kenneth S. Polonsky, Burkhard Goke, and Maria M. Byrne. GLP-1-induced alterations in the glucose-stimulated insulin secretory dose-response curve. Am J Physiol Endocrinol Metab 281: E242–E247, 2001.—The present study was undertaken to establish in normal volunteers the alterations in β-cell responsiveness to glucose associated with a constant infusion of glucagon-like peptide-1 (GLP-1) or a pretreatment infusion for 60 min. A high-dose graded glucose infusion protocol was used to explore the dose-response relationship between glucose and insulin secretion. Studies were performed in 10 normal volunteers, and insulin secretion rates (ISR) were calculated by deconvolution of peripheral C-peptide levels by use of a two-compartmental model that utilized mean kinetic parameters. During the saline study, from 5 to 15 mM glucose, the relationship between glucose and ISR was linear. Constant GLP-1 infusion (0.4 pmol·kg⁻¹·min⁻¹) shifted the dose-response curve to the left, with an increase in the slope of this curve from 5 to 9 mM glucose from 71.0 ± 12.4 pmol·min⁻¹·mM⁻¹ during the saline study to 241.7 ± 36.6 pmol·min⁻¹·mM⁻¹ during the constant GLP-1 infusion (P < 0.0001). GLP-1 consistently stimulated a >200% increase in ISR at each 1 mM glucose interval, maintaining plasma glucose at <10 mM (P < 0.0007). Pretreatment with GLP-1 for 60 min resulted in no significant priming of the β-cell response to glucose (P = 0.2). Insulin clearance rates were similar in all three studies at corresponding insulin levels. These studies demonstrate that physiological levels of GLP-1 stimulate glucose-induced insulin secretion in a linear manner, with a consistent increase in ISR at each 1 mM glucose interval, and that they have no independent effect on insulin clearance and no priming effect on subsequent insulin secretory response to glucose.

IN NORMAL SUBJECTS, insulin secretion is far more enhanced in response to an oral than an intravenous glucose load, resulting in similar plasma glucose concentrations (9, 30). This hyperinsulinemia occurring after oral glucose results from a combination of increased insulin secretion and diminished insulin clearance (29). The enhanced insulin response is mediated by the gastrointestinal hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which act as physiological incretins in humans. GLP-1 is a natural enteric peptide that results from posttranslational processing of the glucagon precursor proglucagon in the intestinal L-cells (6). It is secreted in response to a mixed meal and has been shown to potentiously stimulate glucose-induced insulin secretion in animals (1, 2, 19, 21) and in humans (15, 20, 23, 28). It thereby plays a physiological role in the control of postprandial glucose levels (8). However, the precise in vivo alterations in the glucose-insulin secretion dose-response relationships in response to GLP-1 in healthy human subjects and its effects on insulin clearance have not been described.

In addition to its effect as an incretin, in vitro studies have proposed that GLP-1 regulates β-cell function by maintaining at least a portion of the β-cell population in a glucose-competent state by making resistant islets glucose sensitive (17, 18). Pretreatment of isolated islets (18) or the isolated perfused pancreas (11) with GLP-1 in vitro has been shown to enhance the insulin secretory response to a subsequent stimulation with glucose or other secretagogues.

The present study was therefore undertaken to establish the in vivo glucose dependency of the action of GLP-1 in normal healthy volunteers and the effect of GLP-1 on insulin clearance, and to establish whether a priming effect of GLP-1 on subsequent insulin secretory response to glucose exists. The protocol first involved characterizing the dose-response relationship between glucose and insulin secretion rates (ISR) during a high-dose graded glucose infusion and then examined the alterations that occurred after pretreatment with GLP-1 or with a constant infusion of GLP-1, resulting in postprandial physiological levels. The advantage of this protocol is that it yields ISR at each 1

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mM glucose interval, and therefore ISR can be compared at corresponding glucose levels in the same individuals by use of factors that may alter the sensitivity of the pancreatic β-cells to glucose. This protocol also allows changes in insulin clearance to be defined.

MATERIALS AND METHODS

Subjects

Studies were performed in 10 healthy male Caucasian volunteers aged 22–27 yr (mean ± SE, 25.2 ± 0.6). Subjects were within 10% of ideal body weight (body mass index, 23.5 ± 0.5 kg/m²). None of the participants had a personal or family history of diabetes. Subjects had no other medical illnesses and were not receiving any medications. All subjects were placed on a weight maintenance diet consisting of ≥200 g carbohydrate/day for 2 wk before each study. All studies were performed in the Clinical Research Center of the University of Marburg. The protocol was approved by the ethics committee, and all subjects gave written informed consent.

Experimental Protocol

Each subject was studied on three occasions separated by intervals of ≥1 wk. The studies were performed in random order in each subject. All studies were performed after a 12-h overnight fast beginning at 0800, unless otherwise stated, with subjects in the recumbent position. Indwelling catheters were placed in an antecubital vein for the administration of glucose, saline, or GLP-1 as needed, and in a retrograde fashion in a contralateral dorsal hand vein for blood sampling. The hand was warmed to 40°C throughout the studies using the “heated-hand” technique to ensure arterialization of the venous sample.

High-Dose Graded Intravenous Glucose Infusion Protocol

This protocol was designed to explore the dose-response relationship between glucose and ISR and to study the effects of GLP-1 on this relationship. The protocol is similar to that previously validated (4, 5) but utilizes higher glucose infusion rates. Baseline samples were drawn every 10 min for 30 min to define baseline glucose, insulin, C-peptide, and glucagon. A stepped-up infusion of glucose (20% dextrose) was then started at +30 min at a rate of 1 mg·kg⁻¹·min⁻¹, followed by infusions of 4, 8, 16, and 24 mg·kg⁻¹·min⁻¹. Each infusion rate was administered for a period of 40 min. Samples were drawn at 10, 20, 30, and 40 min into each 40-min period for the measurement of glucose, insulin, and C-peptide. Blood samples were drawn at 90, 120, and 210 min for the measurement of GLP-1. This protocol was performed once with saline infusion, once with infusion of GLP-1 at a constant rate of 0.4 pmol·kg⁻¹·min⁻¹ for 200 min beginning at time point 30 min (i.e., the point of glucose infusion), and once after a 60-min infusion of GLP-1 at 0.4 pmol·kg⁻¹·min⁻¹ from −60 to 0 min (with glucose infusion starting at +30 min). Synthetic human GLP-1(7–36) amide was synthesized by Saxon Biochemicals (Hannover, Germany), with a peptide content of 88.08% and a peptide purity of >99%. The infusion rate of the peptide was calculated from the net peptide content (88%) rather than the total weight. GLP-1(7–36) amide was dissolved in 1% human serum albumin (Bayer, guaranteed to be free of hepatitis-B surface antigen and human immunodeficiency virus antibodies), filtered through 0.2-μm nitrocellulose filters, and then stored at −70°C in individual glass ampules under sterile conditions until the day of the experiment. HPLC after sterile filtration showed a single peak for GLP-1(7–36) amide. Samples were tested for pyrogens, bacteria, and endotoxins.

Assays

Plasma glucose levels were measured by the Yellow Springs Instrument glucose oxidase technique (Schlag, Bergisch-Gladbach, Germany). The coefficient of variation of this method is <2%. Plasma insulin was measured by the Abbott IMx Microparticle Enzyme Immunoassay, which shows cross-reactivity with proinsulin of <0.005%. The average intra-assay coefficient of variation was 5%. Plasma C-peptide was measured as previously described (10). The lower limit of sensitivity of the assay is 20 pmol/l, and the intra-assay coefficient of variation averaged 6%. Glucagon was measured by using a commercially available radioimmunoassay kit (Biermann, Bad Nauheim, Germany), and the intra-assay coefficient of variation averaged 8%. IR-GLP-1 was measured using the specific polyclonal antibody GA 1178 (Affinity Research, Nottingham, UK) (16). It has 100% reactivity with GLP-1(1–36) amide and the truncated GLP-1(7–36) amide. Immunoreactive GLP-1-like material was extracted from plasma samples on C₁₈ cartridges with use of acetonitrile for elution of the samples. The detection limit of the assay was 2 fmol/tube. The antiserum did not cross-react with GIP, pancreatic glucagon, glicentin, oxyntomodulin, or GLP-2. Intra- and interassay coefficients of variation were 3.4 and 10.4%, respectively.

Data Analysis

Determination of ISR. Standard kinetic parameters for C-peptide clearance adjusted for age, sex, and body surface area were used (32) to derive, in each 10-min interval between blood sampling, the ISR from the plasma C-peptide concentrations by deconvolution, as previously described (7, 26).

Relationship between glucose and ISR, and glucose and insulin. Baseline glucose, insulin, C-peptide, and ISR were calculated as means of the values in the −30, −20, −10, and 0 min samples. ISR, insulin, and glucose concentrations used in the analysis represented the average of the values between 10 and 40 min during each glucose infusion period. Mean ISR and mean insulin for each glucose infusion period were then plotted against the corresponding mean glucose level to define the dose-response relationship between glucose and these variables. Mean ISR and mean insulin were determined for 1 mM glucose concentration intervals by calculating the area under the curve for each interval with the trapezoidal rule. This area was then divided by 1 mM to obtain the correct units. The local absolute slope of the glucose-ISR dose-response curve at a given glucose interval was defined as the increment in ISR from that interval to the next, divided by the glucose interval. This definition yields the units of picomoles per minute times concentration in mM. Insulin clearance was calculated by dividing the mean ISR by the mean plasma insulin at each period of glucose infusion (30) adjusted for body surface area. Mean insulin clearance at each glucose infusion rate was plotted against the corresponding mean insulin level to compare insulin clearance at corresponding insulin levels.

Statistical Analysis

All results are expressed as means ± SE. Data analysis was performed using the Statistical Analysis System (SAS version 6 edition for personal computers; SAS Institute, Cary, NC).
Cary, NC). The significance of intraindividual differences induced by GLP-1 infusion was determined using paired t-tests. Two-way analysis of variance for repeated measures was used to assess whether there were significant differences between study conditions in glucose, ISR, insulin, and insulin clearance. Differences were considered to be significant at \( P < 0.05 \).

RESULTS

Mean Glucose, Insulin, and Glucagon Levels During the 60-Min Pretreatment Infusion of GLP-1

Mean glucose, insulin, and glucagon profiles are shown in Fig. 1. Plasma glucose levels were reduced from 4.59 ± 0.12 mM at −60 min to 4.01 ± 0.10 mM at 0 min (\( P < 0.0002 \)), with minimum values of 3.9 ± 0.1 mM at 35 min. This was secondary to increased plasma insulin levels after 10 min of GLP-1 infusion from 32.6 ± 3.2 to 56.6 ± 6.5 pmol/l (\( P < 0.003 \)), and decreased plasma glucagon from 85.6 ± 8.9 pg/ml at −60 min compared with 70.5 ± 5.4 at 0 min (\( P < 0.02 \)).

Plasma GLP-1 Levels

Mean GLP-1 levels during the constant GLP-1 infusion from 30 to 240 min were 16.3 ± 1.6 vs. 1.1 ± 0.2 pmol/l during saline infusion vs. 1.2 ± 0.2 pmol/l after pretreatment with GLP-1; \( P < 0.0002 \). Pretreatment with GLP-1 yielded levels of 16.4 ± 0.9 pmol/l from −60 to 0, which declined to 2.2 ± 0.4 pmol/l at time point +30 min.

Fig. 1. Mean glucose (A), insulin (B), and glucagon (C) values during a 60-min infusion of glucagon-like peptide-1 (GLP-1).

Responses in Glucose, Insulin, and ISR during a High-Dose Graded Intravenous Glucose Infusion with Saline, after 60-Min Pretreatment with GLP-1 and During Constant Infusion with GLP-1

Mean glucose and ISR profiles are shown in Fig. 2. Mean levels of plasma glucose, insulin, and ISR for each period of glucose infusion are shown in Table 1. To allow the ISRs at the same plasma glucose levels to be compared during the three studies, the average amount of insulin secreted at each glucose infusion rate was plotted against the corresponding glucose level. The resulting glucose-ISR dose-response relationships are shown in Fig. 3. The curve obtained during saline infusion is linear in the glucose range from 5 to 15 mM. Pretreatment with GLP-1 resulted in no alteration of the dose-response curve. Constant GLP-1 infusion shifted the dose-response curve upward and to the left. The mean slope of the glucose-ISR dose-response curve from 5 to 9 mM glucose in the respective studies was 71.0 ± 12.4 vs. 82.2 ± 11.5 vs. 241.7 ± 36.6 pmol·min⁻¹·mM⁻¹; \( P < 0.0001 \). This represented an increase of 228 ± 28% in ISR from 5 to 9 mM glucose compared with the saline study (\( P < 0.0002 \)). A >200% increase in ISR during the constant GLP-1 infusion is maintained at each 1 mM glucose interval (\( P < 0.0007 \)).

Fig. 2. Mean profiles of glucose (A) and insulin secretion rates (ISR, B) during a high-dose graded intravenous glucose infusion with saline (C), with constant infusion with GLP-1 (D), and after 60 min of pretreatment with GLP-1 (E). Time +30 min corresponds to the start of glucose infusion. Arrows indicate points at which the glucose infusion rate was increased.
study at glucose infusion rates of 16 and 24 mg·kg⁻¹·min⁻¹. Clearance significantly decreased during the saline insulin clearance rates (Fig. 4) revealed that insulin during the constant GLP-1 infusion. Calculation of insulin was greater than the increase in ISR, implying 80% compared with the saline study. The increase in pmol/l;

<table>
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<th>GINF, mg·kg⁻¹·min⁻¹</th>
<th>Saline</th>
<th>GLP-1 Pretreatment</th>
<th>Constant GLP-1</th>
<th>P Value</th>
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<tr>
<td>Mean glucose, mM</td>
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<tr>
<td>0</td>
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<td>4.39 ± 0.08</td>
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<td>6.62 ± 0.19</td>
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<td>5.36 ± 0.18* &lt;0.001</td>
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<td>15.26 ± 1.30</td>
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<tr>
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<td>637.2 ± 129</td>
<td>1,623.8 ± 376.7*&lt; 0.03</td>
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<td>Mean ISR, pmol/min</td>
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<td>189.0 ± 25.7*&lt; 0.02</td>
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<tr>
<td>4</td>
<td>242.6 ± 35.5</td>
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<td>452.4 ± 56.8*&lt; 0.008</td>
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<td>566.8 ± 54.8 = 0.3</td>
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<td>16</td>
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<td>737.2 ± 100</td>
<td>1,027.7 ± 119.4 = 0.1</td>
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<td>943.5 ± 123.3</td>
<td>1,547 ± 207.2*&lt; 0.03</td>
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</table>

Values are means ± SE. ISR, insulin secretion rates; GINF, glucose infusion rates; GLP-1, glucagon-like peptide-1. *Significant difference vs. saline and GLP-1 pretreatment.

Average insulin levels from 5 to 9 mM glucose were increased from 72.9 ± 7.4 to 77.1 ± 7.8 to 511.7 ± 121.7 pmol/l;  P < 0.0002, representing an increase of 539 ± 80% compared with the saline study. The increase in insulin was greater than the increase in ISR, implying that there was a change in the clearance rate of insulin during the constant GLP-1 infusion. Calculation of insulin clearance rates (Fig. 4) revealed that insulin clearance significantly decreased during the saline study at glucose infusion rates of 16 and 24 mg·kg⁻¹·min⁻¹ (  P < 0.001) and was significantly further reduced during the last three periods of glucose infusion during the constant GLP-1 infusion (  P < 0.0005). Interesstingly, when insulin clearance was compared at corresponding insulin levels, there was no significant difference between the studies. For saline vs. constant GLP-1 infusion, clearance at insulin levels from 0 to 250 pmol/l was 1.64 ± 0.14 vs. 1.82 ± 0.11 l·min⁻¹·m⁻²;  P = 0.15, at insulin levels of 250–500 pmol/l, 1.14 ± 0.05 vs. 1.06 ± 0.05 l·min⁻¹·m⁻²;  P = 0.3, and from 500 to 750 pmol/l, 0.87 ± 0.05 vs. 0.90 ± 0.05 l·min⁻¹·m⁻²;  P = 0.7.

**DISCUSSION**

The present study was undertaken to explore the dose-response relationships between glucose and insulin secretion in normal human volunteers during a high-dose glucose infusion and to study the alterations resulting from constant GLP-1 infusion and pretreatment with GLP-1. We infused GLP-1 at a dose of 0.4 pmol·kg⁻¹·min⁻¹, as this dose yields plasma levels in the upper range of postprandial physiological levels, which have previously been shown to potently increase the insulin response to intravenous glucose (3, 20). The high-dose graded glucose infusion resulted in changes in plasma glucose from 5 to 15 mM. Use of the two-compartment mathematical model with standard kinetic parameters for C-peptide enabled ISR to be derived from peripheral C-peptide concentrations by deconvolution and thereby enabled changes in insulin clearance to be detected. The ability of the β-cell to respond to glucose over a wide glucose range was examined in the same individual in response to factors known to alter the sensitivity of the pancreatic β-cells to glucose.

The high-dose graded glucose infusion resulted in a dose-response curve that was linear in the glucose concentration range from 5 to 15 mM, with no evidence of tapering off of insulin secretion as higher glucose concentrations are achieved. Short-term constant GLP-1 infusion resulted in a shift of the dose-response curve to the left, with an increase in slope from of 71.0 ± 12.4 to 241.7 ± 36.6 pmol·min⁻¹·m⁻² from 5 to 9 mM glucose. At each 1 mM glucose interval, there

![Fig. 3. Relationship between average glucose and ISR during a high-dose graded intravenous glucose infusion with saline (○), during constant GLP-1 infusion (▲), and after 60 min of pretreatment with GLP-1 (●). Lowest glucose levels and ISR were measured under basal conditions, and subsequent levels were obtained during glucose infusion rates of 1, 4, 8, 16, and 24 mg·kg⁻¹·min⁻¹.](http://ajpendo.physiology.org/)

![Fig. 4. Average insulin clearance at each glucose infusion (GINF) rate of 0, 1, 4, 8, 16, and 24 mg·kg⁻¹·min⁻¹ during saline (open bars), constant GLP-1 infusion (solid bars), and pretreatment with GLP-1 (cross-hatched bars).](http://ajpendo.physiology.org/)

Fig. 4. Average insulin clearance at each glucose infusion (GINF) rate of 0, 1, 4, 8, 16, and 24 mg·kg⁻¹·min⁻¹ during saline (open bars), constant GLP-1 infusion (solid bars), and pretreatment with GLP-1 (cross-hatched bars).
was consistently a >200% increase in ISR, with no evidence of β-cell exhaustion.

In vitro studies have shown that GLP-1 has a priming effect on the β-cell response to glucose (11, 18) and constitutes biphasic insulin release in human fetal pancreatic β-cells (24). These priming effects of GLP-1 may reflect the prolonged action of cytosolic second messengers and/or slow dissociation of GLP-1 from its receptor, or they may be due to increased GLUT-2 expression and glucokinase mRNA (33). In our study, pretreatment with GLP-1 resulted in no shift of the glucose insulin secretion dose-response curve. These results suggest that GLP-1-induced priming of β-cells, demonstrated in in vitro studies (11, 18), cannot be detected in humans, at least at physiological levels. It is well known that glucose sensitivity of individual β-cells is diminished compared with β-cells of whole intact islets. Examination of single cells may well yield different results from those obtained in a human, where glucose competence may already exist, if it is envisioned as a metabolic state in which the glucose signaling system is fully primed and ready to go. In a previous study (27), no priming effects of GLP-1 were seen in type 2 diabetic subjects in whom fasting glucose levels were normalized during an overnight infusion of GLP-1 (1.2 pmol·kg⁻¹·min⁻¹) but returned to diabetic levels within 30 min of discontinuing the GLP-1 infusion. In line with these findings, “glucose competence” has been shown to be preserved in mouse pancreatic β-cells after disruption of the GLP-1 receptor gene (13).

The results presented here demonstrate that, when insulin levels are matched, GLP-1 did not have an independent effect on insulin clearance beyond simply stimulating ISR to a greater level. Insulin clearance saturates as the plasma insulin concentration increases (12, 22). Stimulation of endogenous insulin secretion is associated with a reduction in insulin clearance at peripheral insulin concentrations in the high physiological range of 60–70 μU/ml (366–488 pmol/l) (25, 29, 31). This corresponds to the reduction in insulin clearance seen here during and after the 8 mg·kg⁻¹·min⁻¹ infusion period. Reduced insulin clearance is presumably due to alterations in insulin receptor number and/or affinity, in view of the importance of the insulin receptor in mediating insulin clearance (14).

In conclusion, this study demonstrated the in vivo glucose dependency of the action of postprandial physiological levels of GLP-1 in healthy subjects over the glucose range of 5–10 mM. ISR were consistently increased at each 1 mM glucose interval. GLP-1 had no independent effect on insulin clearance beyond stimulating ISR to a greater level, and there was no priming effect of GLP-1 on subsequent insulin secretory response to glucose. These results enhance our knowledge of the regulation of insulin secretion by GLP-1 in humans.

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