The separate and combined impact of the intestinal hormones, GIP, GLP-1, and GLP-2, on glucagon secretion in type 2 diabetes

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Lund A, Vilsbøll T, Bagger JI, Holst JJ, Knop FK. The separate and combined impact of the intestinal hormones, GIP, GLP-1, and GLP-2, on glucagon secretion in type 2 diabetes. Am J Physiol Endocrinol Metab 300: E1038–E1046, 2011. First published March 8, 2011; doi:10.1152/ajpendo.00665.2010.—Type 2 diabetes mellitus (T2DM) is associated with reduced suppression of glucagon during oral glucose tolerance test (OGTT), whereas isoglycemic intravenous glucose infusion (IIGI) results in normal glucagon suppression in these patients. We examined the role of the intestinal hormones glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2) in this discrepancy. Glucagon responses were measured during a 3-h 50-g OGTT (day A) and an IIGI (day B) in 10 patients with T2DM [age (mean ± SE), 51 ± 3 yr; body mass index, 33 ± 2 kg/m2; HbA1c, 6.5 ± 0.2%]. During four additional IIGIs, GIP (day C), GLP-1 (day D), GLP-2 (day E) and a combination of the three (day F) were infused intravenously. Isoglycemia during all six study days was obtained. As expected, no suppression of glucagon occurred during the initial phase of the OGTT, whereas significantly (P < 0.05) lower plasma levels of glucagon during the first 30 min of the IIGI (day B) were observed. The glucagon response during the IIGI + GIP + GLP-1 + GLP-2 infusion (day F) equaled the inappropriate glucagon response during OGTT (P = not significant). The separate GIP infusion (day C) elicited significant hypersecretion of glucagon, whereas GLP-1 infusion (day D) resulted in enhancement of glucagon suppression during IIGI. IIGI + GLP-2 infusion (day E) resulted in a glucagon response in the midrange between the glucagon responses to OGTT and IIGI. Our results indicate that the intestinal hormones, GIP, GLP-1, and GLP-2, may play a role in the inappropriate glucagon response to orally ingested glucose in T2DM with, especially, GIP, acting to increase glucagon secretion.

Type 2 diabetes mellitus (T2DM) is characterized not only by defects in pancreatic β-cell function and insulin action but also by dysfunction of the pancreatic α-cell (3). In contrast to subjects with normal glucose tolerance [Supplemental Fig. 1 (Supplemental data for this article may be found on the American Journal of Physiology: Endocrinology and Metabolism website.)], patients with T2DM exhibit elevated plasma glucagon levels in the fasting state, and glucagon concentrations fail to decrease appropriately or even, paradoxically, increase in response to oral glucose tolerance test (OGTT) or meal test (20, 21, 36, 41, 43, 44). This hyperglucagonemia contributes, through a stimulatory effect on hepatic glucose production, to the fasting and postprandial hyperglycemia observed in patients with T2DM (1, 9, 44). Postprandial hyperglucagonemia of T2DM has been proposed to arise as a consequence of insufficient secretion of insulin (known to inhibit glucagon secretion) combined with reduced glucose sensitivity of α-cells (the secretion of which is normally inhibited by elevated glucose levels). However, we and others (20, 21, 23, 30) have found that patients with T2DM, in contrast to their inappropriate glucagon response to OGTT, are able to suppress glucagon secretion following isoglycemic intravenous glucose infusion (IIGI) mimicking the plasma glucose profile obtained during OGTT, very much like normal glucose-tolerant subjects (Supplemental Fig. 1). This difference in glucagon responses to oral vs. intravenous glucose administration in patients with T2DM is currently unexplained. Theoretically, it could be attributed to the release of glucagonotropic/glucagonostatic peptides secreted from the gastrointestinal tract, as previously proposed (20, 27). The incretin hormone, glucagon-like peptide-1 (GLP-1), secreted from entodcrine L cells in the intestinal mucosa in response to feeding, is known to inhibit glucagon secretion (6, 34), whereas the other incretin hormone, glucose-dependent insulinotropic poly-peptide (GIP), has been shown to stimulate glucagon secretion (2, 11, 28, 31, 48). Also, glucagon-like peptide-2 (GLP-2), which is cosecreted with GLP-1, has glucagonostotic properties (8, 29). We speculated that, during OGTT in healthy subjects, the glucagonostatic effects of GLP-1 and insulin outweigh the glucagonostotic effects of GIP and GLP-2, resulting in normal glucagon suppression. In contrast, we hypothesized that this glucagonotropic/glucagonostatic equilibrium of the gastrointestinal hormones is disturbed in patients with T2DM, explaining the difference in glucagon responses to OGTT and IIGI. In the present study, we investigated whether a response similar to the inappropriate glucagon response following OGTT in patients with T2DM could be elicited by infusions of the gastrointestinal hormones GIP, GLP-1, and GLP-2 during IIGI (which normally suppresses glucagon secretion in T2DM patients).

Research Design and Methods

The study was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (registration no. H-A-2008–025) and registered with ClinicalTrials.gov (ID: NCT00716170). The study was performed in accordance with the Helsinki Declaration II, and written informed consent was obtained from all participants before inclusion.

Subjects. Ten patients (4 males) with T2DM [age (mean ± SE), 51 ± 3 yr; body mass index, 33.2 ± 1.6 kg/m²; fasting plasma glucose (FPG), 9.3 ± 0.6; HbA1c, 6.5 ± 0.2%; duration of diabetes, 4.8 ± 1.2 yr] were studied. The participants were all diagnosed with T2DM according to the World Health Organization criteria at least six
months before study inclusion. Nine patients were treated with metformin alone and one with metformin and sulfonylurea in combination. None of the patients was treated with other drugs likely to affect the responses of glucose, insulin, C-peptide, or any of the intestinal hormones (GIP, GLP-1, or GLP-2). All participants were negative for both islet cell autoantibodies and glutamate decarboxylase-65 autoantibodies and were well treated with regard to plasma lipids and blood pressure. None of the patients had impaired renal function (normal plasma creatinine and no microalbuminuria), proliferative retinopathy or impaired liver function, or suffered from diabetic neuropathy.

Methods. All participants were studied on six different occasions (days A, B, C, D, E, and F) preceded by a screening visit. The experiments were separated by at least 24 h and were performed over a 6-wk period. Before each occasion, the patients had not taken their oral antidiabetic therapy for a period of no less than a week. On all occasions, the patients were studied in the morning after an overnight (10 h) fast, including liquids, medication, and use of tobacco.

On the first experimental day (day A), a 3-h 50-g OGTT was performed. A cannula was inserted in a cubital vein for collection of arterialized blood samples. The forearm was placed in a heating box (50°C) throughout the experiment. The participants ingested 50 g of water-free glucose dissolved in 300 ml water over the first 3 min of the experiment. Blood samples were drawn 20, 10, and 0 min before and 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, and 180 min after initiation of ingestion of glucose. Blood was distributed in chilled tubes (on ice) containing EDTA, aprotinin (500 kIU/ml blood; Trasylol; Bayer, Leverkusen, Germany), and a specific dipeptidyl peptidase 4 inhibitor (valine-pyrrolide, final concentration: 0.01 mM; a gift from Novo Nordisk, Bagsværd, Denmark) for analysis of GIP, GLP-1, GLP-2, and glucagon. For analysis of insulin and C-peptide, blood was distributed in plain tubes for coagulation (20 min at room temperature). All samples were centrifuged for 20 min at 1,200 g and 4°C. Plasma samples for GIP, GLP-1, GLP-2, and glucagon analyses were stored at −80°C until analysis. For bedside measurement of plasma glucose, blood was distributed in fluoride tubes and centrifuged immediately at 7,400 g for 2 min at room temperature.

Days B, C, D, E, and F were performed following day A in randomized order. On each occasion, an IIGI (20% wt/vol), aiming at duplicating the plasma glucose profile attained during day A, was performed as previously described (20). In addition to the adjustable
intravenous glucose infusion, intravenous infusions of saline (day B), GIP (day C), GLP-1 (day D), GLP-2 (day E), or GIP + GLP-1 + GLP-2 (day F) were carried out. The infusion rates of the hormones were varied with the intention to reach high physiological concentrations (see below). Cannulas were inserted in cubital veins in both arms, one for collection of arterialized blood samples (50°C) and one for glucose and hormone infusion. A solution containing GIP, GLP-1, GLP-2, GIP + GLP-1 + GLP-2, or saline mixed with 10 ml of 5% human albumin was prepared each morning before the experiment. The amount of hormone in the infused was calculated according to individual patient weights so that the infusion speed was fixed and patient blinding intact. At time 0 min, a continuous infusion of either GIP (4.0 pmol·kg⁻¹·min⁻¹), GLP-1 (0.6 pmol·kg⁻¹·min⁻¹), GLP-2 (1.0 pmol·kg⁻¹·min⁻¹), GIP + GLP-1 + GLP-2 (in the aforementioned concentrations), or saline was initiated. At time 20 min, the infusion rate was halved, and, at time 50 min, the infusion was stopped. Blood was sampled as on day A.

**Peptides.** Synthetic GIP synthesized by Pegasus Pharma (Hannover, Germany) was received as a gift from Dr. M. Nauck, Diabetestzentrum Bad Lauterberg, Germany. The peptide was dissolved in sterilized water containing 2% human serum albumin and subjected to sterile filtration. Appropriate amounts of peptide for each experimental subject were dispensed into capped vials and stored frozen (−20°C) under sterile conditions until the day of the experiment. The purity and correctness of structure were confirmed by mass sequence and HPLC analysis. Synthetic GLP-1(7–36) amide (PolyPeptide Laboratories, Linhamn, Sweden) was dissolved in sterilized water containing 2% human serum albumin and subjected to sterile filtration. The GLP-1 peptide solution was demonstrated to be >97% pure and identical to the natural human peptide by HPLC, mass, and sequence analysis. Synthetic human GLP-2, corresponding to human proglucagon-(126–158), synthesized by PolyPeptide Laboratories (Wolfenbüttel, Germany), was received as a gift from Dr. P. B. Jeppesen, Department of Medical Gastroenterology, Rigshospitalet, Denmark (13). The peptide, provided as the acetate salt, was mixed with 0.9% NaCl, and 0.5% ammonia was added until the peptide dissolved (pH ~8–9); then pH was neutralized using 0.1 M acetic acid. The preparation was subjected to sterile filtration, after which peptide content was confirmed by amino acid analysis. Next, the preparation was dispensed into capped vials and heat sterilized for 20 min at 100°C. The preparation was checked for sterility and kept at −20°C until use. The purity and correctness of structure were confirmed by mass sequence and HPLC analysis.

**Analyses.** Plasma glucose concentrations were measured by the glucose oxidase method, using a glucose analyzer (Yellow Springs Instrument model 2300 STAT plus analyzer; YSI, Yellow Springs, OH). Serum insulin and C-peptide concentrations were measured using a two-sided assay, Electro Chemi Luminescens Immuno Assay (D-68298 Roche/Hitachi Modular analytics; Roche Diagnostic, Mannheim, Germany). Total GIP, total GLP-1, intact GLP-2, and glucagon were measured by RIAs as previously described (7, 10, 25, 37a). Glucagon was measured with an assay directed against the COOH-terminal of the glucagon molecule (antibody code no. 4305–8) and, therefore, measures glucagon of mainly pancreatic origin. The assay does not cross-react with GIP, GLP-1, or GLP-2.

**Statistical analyses and calculations.** Baseline, peak, area under curve (AUC), and incretin effect values are expressed as means ± SE. For analyses of differences of means between days, repeated-measure ANOVA (rmANOVA) after Greenhouse-Geisser (G-G) or Huynh-Feldt (H-F) was performed, depending on the sphericity (ε) assumption (G-G was used if ε was lower than 0.75, and H-F was used if ε was higher than 0.75; significance level P < 0.05) (16). As post hoc analyses, Bonferroni corrections were used. Differences between time courses were compared using two-factor rmANOVA values were calculated using the trapezoid rule and are presented as the total area if nothing else is stated. Insulin secretion rate (ISR) values were calculated by deconvolution of measured C-peptide

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**Table 1. GIP, GLP-1, and GLP-2**

<table>
<thead>
<tr>
<th>Table 1. GIP, GLP-1, and GLP-2</th>
<th>Mean baseline, pM</th>
<th>AUC (0-30 min)</th>
<th>iAUC (0-30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day A</strong></td>
<td><strong>Day B</strong></td>
<td><strong>Day C</strong></td>
<td><strong>Day D</strong></td>
</tr>
<tr>
<td>GIP</td>
<td>12.1 ± 1.4</td>
<td>10.0 ± 1.3</td>
<td>5.20 ± 0.9</td>
</tr>
<tr>
<td>GLP-1</td>
<td>5.20 ± 0.9</td>
<td>8.70 ± 1.7</td>
<td>2.98 ± 0.3</td>
</tr>
<tr>
<td>GLP-2</td>
<td>8.70 ± 1.7</td>
<td>11.1 ± 0.8</td>
<td>16.0 ± 2.0</td>
</tr>
<tr>
<td><strong>Day E</strong></td>
<td><strong>Day F</strong></td>
<td><strong>Day A</strong></td>
<td><strong>Day B</strong></td>
</tr>
<tr>
<td>GIP</td>
<td>14.3 ± 1.5</td>
<td>12.5 ± 1.5</td>
<td>10.7 ± 2.2</td>
</tr>
<tr>
<td>GLP-1</td>
<td>12.5 ± 1.5</td>
<td>14.3 ± 1.5</td>
<td>13.9 ± 1.4</td>
</tr>
<tr>
<td>GLP-2</td>
<td>14.3 ± 1.5</td>
<td>12.5 ± 1.5</td>
<td>13.9 ± 1.4</td>
</tr>
</tbody>
</table>

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**Statistical analysis of responses.** Insulin secretion rate (ISR) values were calculated using the trapezoid rule and are presented as the total area if nothing else is stated. Insulin secretion rate (ISR) values were calculated by deconvolution of measured C-peptide
concentrations and application of population-based parameters for C-peptide kinetics (18, 19, 46). ISR is expressed as picomoles insulin secreted per minute per kilogram body weight. The incretin effect was calculated by relating the difference in β-cell secretory responses (AUC for insulin, C-peptide, and ISR) between stimulation with OGTT and IIGI to the response after OGTT, which was taken as 100% [incretin effect (%) = 100% × (AUCOGTT – AUCIIGI)/AUCOGTT]. The "incretin effect" of each of the hormone infusions was calculated for the hormone infusion days (days C, D, E, and F) in the same manner as mentioned above by relating the difference in β-cell secretory responses between stimulation with IIGI + hormone(s) and IIGI + saline to the response after IIGI + hormone(s).

RESULTS

Glucose. Plasma glucose excursions are displayed in Fig. 1. Fasting plasma glucose concentrations were similar on all experimental days [ranging from 8.9 ± 0.6 to 9.4 ± 0.7 mM; P = not significant (NS)], and isoglycemia during all study days was obtained with no significant differences in AUCs between the days (day A, 2.564 ± 133; day B, 2.564 ± 130; day C, 2.702 ± 169; day D, 2.568 ± 125; day E, 2.597 ± 142; day F, 2.569 ± 136 pM × 3 h; P = NS). The amount of intravenous glucose needed to copy the plasma glucose profile obtained on day A (using 50 g glucose) was similar on all test days (day B, 49.0 ± 1.3 g; day C, 48.4 ± 2.4 g; day D, 51.4 ± 2.5 g; day E, 46.1 ± 2.0 g; day F, 50.3 ± 1.0 g; P = NS).

GIP. Time courses for plasma concentrations of total GIP are shown in Fig. 2. Baseline values were similar on all study days (Table 1). During days A (OGTT), C (IIGI + GIP), and F (IIGI + GIP + GLP-1 + GLP-2), significant GIP responses were observed (with a greater response during day C compared with day A; Table 1), whereas no significant changes (flat lines) occurred during days B (IIGI + saline), D (IIGI + GLP-1), and E (IIGI + GLP-2) (Table 1).

GLP-1. Time courses for plasma concentrations of total GLP-1 are shown in Fig. 2. Baseline values were similar on all study days (Table 1). During days A (OGTT), D (IIGI + GLP-1), and F (IIGI + GIP + GLP-1 + GLP-2), significant GLP-1 responses occurred (with greater responses during days D and F compared with day A; Table 1), whereas no significant changes (flat lines) occurred during days B (IIGI + saline), C (IIGI + GIP), and E (IIGI + GLP-2) (Table 1).

GLP-2. Time courses for plasma concentrations of intact GLP-2 are shown in Fig. 2. As indicated in Table 1, mean baseline values on the six study days ranged from 9 to 18 pM (P < 0.05). During days A (OGTT), E (IIGI + GLP-2), and F (IIGI + GIP + GLP-1 + GLP-2), significant GLP-2 responses occurred (with greater responses during days E and F compared with day A; Table 1), whereas no significant changes (flat lines) occurred during days B (IIGI + saline), C (IIGI + GIP), and D (IIGI + GLP-1) (Table 1).

Insulin, C-peptide, ISR, and incretin effect. Time courses for serum insulin and C-peptide concentrations and ISR values are shown in Fig. 3. As indicated in Table 2, similar fasting values of insulin and C-peptide were observed on all experimental days (P = NS). No significant differences in total serum insulin responses were observed between the experimental days (Table 2). However, significantly greater initial (0–30 min) serum insulin responses occurred during days C (IIGI + GIP), D (IIGI + GLP-1), and F (IIGI + GIP + GLP-1 + GLP-2) compared with days B (IIGI) and E (IIGI + GLP-2) (Table 2). Incretin effect values calculated on the basis of the three integrated β-cell secretory responses (insulin, C-peptide, and GLP-2) (Table 1) during OGTT and IIGI (left) and during IIGI with concomitant infusions of saline, GIP, GLP-1, GLP-2, or a combination of the three hormones (right) in patients with type 2 diabetes. Data are mean values ± SE.
Table 2. β-Cell responses and incretin effects during 50-g OGTT (Table 2). Day A (IIGI) and OGTT.

<table>
<thead>
<tr>
<th>Day</th>
<th>β-Cell secretion</th>
<th>Insulin, mean ± SD</th>
<th>C-peptide, mean ± SD</th>
<th>ISR, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14 ± 2</td>
<td>34 ± 12</td>
<td>125 ± 14</td>
<td>513 ± 172</td>
</tr>
<tr>
<td>B</td>
<td>14 ± 2</td>
<td>34 ± 12</td>
<td>125 ± 14</td>
<td>513 ± 172</td>
</tr>
<tr>
<td>C</td>
<td>14 ± 2</td>
<td>34 ± 12</td>
<td>125 ± 14</td>
<td>513 ± 172</td>
</tr>
<tr>
<td>D</td>
<td>14 ± 2</td>
<td>34 ± 12</td>
<td>125 ± 14</td>
<td>513 ± 172</td>
</tr>
<tr>
<td>E</td>
<td>14 ± 2</td>
<td>34 ± 12</td>
<td>125 ± 14</td>
<td>513 ± 172</td>
</tr>
<tr>
<td>F</td>
<td>14 ± 2</td>
<td>34 ± 12</td>
<td>125 ± 14</td>
<td>513 ± 172</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the present study, we analyzed the possible role of the gut hormones GIP, GLP-1, and GLP-2 in the inappropriate glucagon response to OGTT observed in patients with T2DM, using IIGI (which in itself results in suppression of glucagon secretion in T2DM) plus separate and combined intravenous infusions of the three hormones. We were able to mimic the inappropriate OGTT-induced glucagon response using IIGI plus combined infusion of GIP, GLP-1, and GLP-2, and we show that glucagon suppression during IIGI is potentiated by intravenous GLP-1 and “overruled” and reversed to hypersecretion by intravenous GIP. IIGI and concomitant intravenous GLP-2 elicited glucagon responses in the midrange between IIGI and OGTT.

The unexpected finding that diminished glucagon suppression is observed in patients with T2DM during OGTT but not following isoglycemic intravenous glucose administration (20, 21, 23, 30) is still unexplained. A “lighter version” of the phenomenon has been observed in healthy subjects, especially when larger oral glucose loads are applied (23, 27). Both glucose and insulin are known to have potent glucagonostatic effects (15, 17, 37, 40, 47) and have been proposed to be the main responsible factors for the glucagon suppression observed in healthy subjects following administration of glucose. In T2DM, reduced α-cell sensitivity to glucose and insulin combined with reduced insulin secretion have been proposed to constitute the most important mechanisms behind the hyperglucagonemic component of the disease (4, 12, 49). However, recent findings of isolated glucagon hypersecretion during
OGTT and glucagon suppression during IIGI in patients with T2DM (20, 21, 23, 30) question that changes in concentration and/or effect of glucose and/or insulin are responsible for the inappropriate glucagon secretion following oral glucose stimulation in these patients. First, the differential glucagon responses (during oral vs. iv glucose) occur during isoglycemic conditions, ruling out differences in plasma glucose concentrations as the culprit. Second, the inappropriate suppression of glucagon occurs during OGTT when insulin secretion is higher compared with IIGI (20, 21, 23, 30). Thus differences in glucose or α-cell secretion during the two glucose stimuli do not seem to explain the OGTT-induced hypersecretion of glucagon. In 2007, we (20) and Meier et al. (27) proposed that glucagonotropic/glucagonostatic gastrointestinal hormones secreted in response to oral glucose might play a role in the different glucagon responses to OGTT and IIGI. This hypothesis was tested in the present study.

It is well acknowledged that the concentrations of endogenous GIP, GLP-1, and GLP-2 are severalfold higher in the intestinal and the portal circulation compared with the peripheral circulation (11). Therefore, the infusions of GIP, GLP-1, and GLP-2 were designed to reach high physiological plasma concentrations (measured peripherally) to ensure high enough plasma concentrations in the intestinal and portal circulation so that both endocrine and potential neural pathways (originating from the small intestinal wall or the portal circulation) regulated by glucagonotropic/glucagonostatic gastrointestinal hormones secreted in response to oral glucose might play a role in the different glucagon responses to OGTT and IIGI. This hypothesis was tested in the present study.

Table 3. Glucagon

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Mean baseline, pM</td>
<td>11.3 ± 2.5</td>
<td>9.1 ± 1.2</td>
<td>8.5 ± 0.8</td>
<td>8.7 ± 1.5</td>
<td>9.2 ± 1.6</td>
<td>9.2 ± 1.3</td>
<td>0.161</td>
</tr>
<tr>
<td>AUC, pM × 3 h</td>
<td>1,422 ± 191</td>
<td>1,144 ± 178</td>
<td>991 ± 127</td>
<td>900 ± 185</td>
<td>1,124 ± 169</td>
<td>1,054 ± 155</td>
<td>0.220</td>
</tr>
<tr>
<td>iAUC, pM × 30 min</td>
<td>−1 ± 13</td>
<td>−27 ± 10,*;C</td>
<td>45 ± 15,*;BD</td>
<td>−45 ± 12,*;C</td>
<td>−14 ± 10</td>
<td>−8 ± 15</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Data are mean values ± SE. Mean baseline concentrations, AUC values, and iAUC values (0–30 min) for glucagon during 50-g OGTT (day A) and IIGI with infusions of saline (day B), GIP (day C), GLP-1 (day D), GLP-2 (day E), or a combination of the three hormones (day F) in patients with type 2 diabetes. Significant (P < 0.05) differences between individual responses are indicated († followed by the letter of the day compared with). *Significant differences from 0.
Glucagon following OGTT is not a result of disturbed GLP-1 secretion (8) or effect (6). GLP-2 has been shown to stimulate glucagon secretion in rats and in healthy human subjects at both physiological and supraphysiological doses (8, 29, 45). However, an effect of GLP-2 on glucagon release has to our knowledge not been reported in patients with T2DM; and the insignificant effect of GLP-2 on glucagon responses observed in this study, combined with unpublished data showing normal postprandial GLP-2 responses in patients with T2DM (F. K. Knop, K. J. Hare, J. Pedersen, J. J. Holst, and T. Vilsbøll, unpublished observation), suggests that OGTT-induced hypersecretion of glucagon in T2DM is not a consequence of exaggerated secretion or effect of GLP-2. GIP has, in addition to its insulinotropic effect (which is severely compromised in T2DM (31)), been shown to possess glucagonotropic properties, both in healthy subjects and in patients with T2DM (2, 28, 31, 48). This bifunctional role of GIP is believed to prevent reactive hypoglycemia and provide a “smooth landing” for plasma glucose concentrations following postprandial rises. Previous studies have mainly looked at glucagon release in response to supraphysiological levels of GIP (2, 31, 48), or at the effect of GIP in healthy subjects during euglycemic conditions (28). In the present study, we show that plasma GIP concentrations corresponding to physiological postprandial levels of GIP have a clear glucagonotropic effect in patients with T2DM when plasma glucose rises in the same way as during oral ingestion of glucose. This effect coincided with a significant early phase insulin response (excluding falling insulin as a factor), supporting the notion that GIP has a direct effect on pancreatic α-cells, as suggested by studies performed in isolated perfused rat pancreata (38). Interestingly, elevated GIP responses have been reported in patients with T2DM (14) and in their healthy offspring (35). Furthermore, glucose homeostatic dysregulation in healthy subjects results in exaggerated OGTT-induced GIP responses (5), and gestational diabetes mellitus is associated with increased postprandial GIP responses, which normalize following delivery and reversion to normal glucose tolerance (L. Bonde, T. Vilsbøll, T. Nielsen, J. Svare, J. J. Holst, S. Larsen, and F. K. Knop, unpublished observations). Thus, potentially, glucagonotropic GIP signaling combined with increased GIP secretion in T2DM may play a hitherto overlooked causal role in the inappropriate glucagon responses of T2DM.

The present design cannot rule out that other mechanisms (for instance, neuronal stimulation of glucagon release via nerve fibers in the intestinal mucosa, secretion of glucagon from endocrine cells in the small intestinal mucosa, or reduced renal glucagon extraction) could be involved in the diminished glucagon suppression after oral ingestion of glucose. However, the fact that we, by IIGI and concomitant infusion of the gastrointestinal hormones GIP, GLP-1, and GLP-2 (normally secreted in response to ingestion of nutrients, including glucose), were able to reproduce the inappropriate glucagon response observed in patients with T2DM during OGTT suggests that these gastrointestinal hormones play a significant role. The exact mechanisms by which these peptides exert their effects on the α-cells are not fully elucidated, and the present study does not allow us to discern between direct or indirect effects of the hormones on pancreatic α-cells.

A potential limitation of isoglycemic clamp studies is that, by design, the oral glucose challenge has to be carried out before the IIGI, entailing a potential risk of an order effect. In the present study, the five different IIGIs were performed in randomized order following the initial OGTT experiment, eliminating potential order effects of the hormone infusions. Nevertheless, like others (27), we observed that fasting glucagon levels tended to be highest on the first experimental day (OGTT). However, the inappropriate glucagon response was still apparent when glucagon values were expressed as incremental AUC. Therefore, the observed differences in the suppression of glucagon secretion between OGTT and IIGI are unlikely to be due to an order effect.

The isoglycemic clamp technique was developed to quantify the incretin effect (32, 39). The incretin effect is defined as the potentiation of insulin secretion exerted by the incretin hormones released after ingestion of nutrients (OGTT). In the present study, the incretin effect amounted to 6%, a severe reduction compared with obese healthy subjects (41%) (24). Interestingly, when we calculated the incretin effect of the individual and the combined infusion, we observed incretin effects equaling the incretin effect during OGTT when GLP-1 was infused (days D and F) [in accordance with the impaired but not eliminated insulinitropic effect of GLP-1 in patients with T2DM (48)] and negative incretin effects when individual GIP or GLP-2 infusions were applied (in accordance with the lost insulinitropic effect of GIP in T2DM and the lack of effect of GLP-2 on insulin secretion).

In conclusion, our results indicate that the inappropriate hyperglucagonemic response to orally ingested glucose in type 2 diabetes may be dependent on the release of the intestinal hormones, GIP, GLP-1, and GLP-2, with GIP playing the predominant role in this pathophysiological trait.

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DISCLOSURES

The authors have nothing to disclose.

REFERENCES


