# $K_{ATP}$ Channel Closure Ameliorates the Impaired Insulinotropic Effect of Glucose-Dependent Insulinotropic Polypeptide in Patients with Type 2 Diabetes

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**Objective:** The reduced incretin effect in subjects with type 2 diabetes is accompanied by a severely impaired insulinotropic effect of the incretin hormone glucose-dependent insulinotropic polypeptide (GIP). The $K_{ATP}$ channels of the $\beta$-cell appear to be essential for the function of GIP in mice, and mutations in the gene encoding these channels have been linked to the development of type 2 diabetes. With this study we therefore aimed at clarifying the role of $K_{ATP}$ channel malfunction in the impaired function of GIP.

**Research Design and Methods:** We examined 12 subjects with type 2 diabetes using a 2-h (15 mM) hyperglycemic clamp on 4 separate days with concomitant infusion of one of the following: GIP; GIP + 10 mg sulfonylurea (SU, glipizide) taken orally 1 h before the clamp; saline + 10 mg SU; or saline alone. Blood was sampled to measure plasma concentrations of glucose, intact GIP, insulin, C-peptide, and glucagon.

**Results:** Compared to the results of GIP alone, SU alone, or those results added together, coadministration of GIP and SU resulted in a more-than-additive increase in the peripheral insulin ($P = 0.002$) and C-peptide ($P = 0.028$) responses and furthermore, a more-than-additive increase in total ($P = 0.01$), early ($P = 0.02$), and late-phase ($P = 0.02$) insulin secretion.

**Conclusion:** We have demonstrated that inhibiting the $K_{ATP}$ channels of the diabetic $\beta$-cell acutely using SU significantly increases both the peripheral insulin response to GIP and GIP-induced insulin secretion, indicating an ameliorated insulinotropic effect of GIP. **(*J Clin Endocrinol Metab* 94: 603–608, 2009)**

The incretin effect is believed to be crucial in maintaining normal glucose homeostasis, and the reduced incretin effect in patients with type 2 diabetes (1) has become a key target in the treatment of the disease. The two incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), have been the subjects of extensive research, revealing striking abnormalities and functional differences in the type 2 diabetic state. GLP-1 shows a reduction in secretion and potency (2–4) but, importantly, a partly preserved insulinotropic activity when administered in higher doses. In contrast, GIP demonstrates an almost absent insulinotropic effect regardless of near-normal postprandial hormone secretion (5, 6). Strategies to restore the insulinotropic effect of GIP may potentially add another dimension to incretin-based treatments of type 2 diabetes.

Several genetic variations have been associated with increased risk of developing type 2 diabetes. Among the most frequent are

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Abbreviations: AUC, Area under the curve; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; iAUC, incremental AUC; ISR, insulin secretion rate; $K_{ATP}$ channel, ATP-sensitive potassium channel; SU, sulfonylurea.



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variations in the KCJN11 gene encoding the Kir6.2 subunit of the ATP-sensitive potassium channel ($K_{ATP}$ channel) of the $\beta$-cell (7). The $K_{ATP}$ channel couples changes in intracellular glucose metabolism to membrane depolarization and insulin secretion. It comprises a protein complex of two subunits: an inward-rectifier $K^+$ channel, Kir6.2, and the sulfonylurea (SU) receptor type 1. Mutation in either subunit disrupting the function of the channel obliterates the incretin effect of GLP-1 and GIP in mice (8, 9), and activating mutations in the KCJN11 gene have been shown to be associated with the development of neonatal diabetes, *i.e.* diabetes presenting within the first 3 months of life (10). Interestingly, closing the $K_{ATP}$ channels in these patients using SUs restores their insulin secretory capacity, with a higher insulin response to stimulation with oral glucose or a mixed meal compared with iv glucose—indicating restoration of the incretin effect (11). Furthermore, it has been shown that 1 month of SU treatment in patients with type 2 diabetes improved $\beta$-cell responsiveness to GIP. However, this might be a result of the general improvement in $\beta$-cell function due to the antidiabetic treatment (12).

In the present study, we therefore tested the hypothesis that $K_{ATP}$ channel malfunction might be the underlying cause of the lost insulinotropic effect of GIP. Through acute administration of SU with resulting $K_{ATP}$ channel closure, we hoped to improve $\beta$-cell sensitivity to GIP in a group of patients with type 2 diabetes.

## Subjects and Methods

### Subjects and study protocol

The study was approved by the Scientific-Ethical Committee of the Capital Region of Denmark, registration no. H-KA-05011), registered with the Danish Data Protection Agency (registration no. 2005-53-1097), and with ClinicalTrials.gov (ID: NCT00321321). The study was conducted according to the principles of the Helsinki Declaration II. Written informed consent was obtained from all participants before inclusion.

Twelve participants with type 2 diabetes (eight males) were studied [age, presented as median (range), 52 (45–70) yr; body mass index, 30 (25–36) kg/m$^2$; $HbA_{1c}$, 7.5 (7–8.6)%; and duration of diabetes, 29 (6–72) months]. Eleven subjects were on metformin monotherapy, and one subject was treated with diet only. None of the subjects included had a history of gastrointestinal surgery, positive measurements of islet cell autoantibodies and/or glutamate decarboxylase-65 autoantibodies, elevated liver enzymes (alanine aminotransferase or aspartate aminotransferase) twice the respective upper normal value, elevated serum creatinine concentration (>130 $\mu$M), albuminuria, or severe cardiovascular disease (New York Heart Association group III or IV).

All subjects were studied on four separate occasions with at least a 48-h interval between examinations. Antidiabetic medication was paused 1 wk before first examination and remained discontinued throughout the course of the study. The experiments were carried out in randomized order within a 2-wk period and consisted of four 2-h (15 mM) hyperglycemic clamps with continuous infusion of GIP (two occasions) and saline (two occasions). On 1 d with GIP infusion and on 1 d with saline infusion, 10 mg of SU (glipizide) was administered orally 1 h before the clamp.

After an overnight (10-h) fast, subjects were placed in a recumbent position, and a cannula was inserted in a dorsal hand vein. The hand was placed in a heating box (42 C) throughout the experiment to allow collection of arterialized blood samples. Another cannula was inserted in a contralateral cubital vein for glucose and hormone infusion. At time zero (0 min), a 50% glucose (wt/vol) bolus was infused during 1 min to increase plasma glucose to 15 mM. The glucose bolus was calculated as: (15 mM − fasting plasma glucose) × 35 mg glucose × body weight (in kilograms). At time 3 min, a continuous infusion of either GIP (4 pmol/kg body weight · min) or saline was initiated. Plasma glucose was measured bedside every 5 min during the 120-min clamp and maintained at a plasma concentration of 15 mM by an adjustable infusion of 20% glucose.

Arterialized blood was drawn at time −60, −30, −15, 0, 3, 5, 10, 20, 40, 60, 90, and 120 min on examination days with preceding SU administration (at time −60 min), and at time −15, −10, 0, 3, 5, 10, 20, 40, 60, 90, and 120 min on days without SU. Samples were distributed into chilled tubes containing EDTA plus aprotinin (500 KIU/ml blood; Trasylol; Bayer Corp., Leverkusen, Germany) and a specific dipeptidyl peptidase 4 inhibitor (valine pyrrolidide, final concentration = 0.01 mM; a gift from Dr. R. D. Carr, Novo Nordisk, Bagsværd, Denmark) for analysis of glucagon, GIP, and SU. For analysis of insulin and C-peptide, blood was drawn more frequently and distributed into chilled tubes containing heparin plus aprotinin (500 KIU/ml blood). All tubes were immediately cooled on ice and centrifuged for 20 min at 1200 × *g* and 4 C. Plasma for GIP and glucagon analyses was stored at −20 C, and plasma for insulin, C-peptide, and SU analyses was stored at −80 C until analysis. For bedside measurement of plasma glucose, blood was drawn every 5 min, distributed into fluoride tubes, and centrifuged immediately for 1 min at 7400 × *g* at room temperature.

Synthetic GIP for infusion was purchased from PolyPeptide Laboratories (Wolfenbüttel, Germany). The peptide was dissolved in sterilized water containing 2% human serum albumin (Human Albumin, guaranteed to be free of hepatitis-B surface antigen, hepatitis-C virus antibodies, and HIV antibodies; Statens Serum Institute, Copenhagen, Denmark) and subjected to sterile filtration. Appropriate amounts of peptide for each experimental subject were dispensed into glass ampoules and stored frozen under sterile conditions until the day of the experiment. The peptide was more than 97% pure and identical to the natural human peptide by HPLC, mass, and sequence analysis.

### Analytical procedures

#### *Glucose*

Plasma glucose concentrations were measured by the glucose-oxidase method, using a glucose analyzer (Yellow Springs Instrument Model 2300 STAT plus analyzer; YSI Inc., Yellow Springs, OH).

#### *GIP*

Intact biologically active human GIP was measured using antiserum no. 98171 (13).

#### *Glucagon*

The glucagon assay is directed against the C-terminal of the glucagon molecule (antibody code no. 4305) and, therefore, measures glucagon of mainly pancreatic origin (14). Neither glicentin nor oxyntomodulin cross-react, but proglucagon 1-61, which is mainly formed in the pancreas, does react fully in this assay (15, 16).

#### *Insulin and C-peptide*

Plasma insulin and C-peptide concentrations were measured using AutoDELFIA time-resolved fluoroimmunoassay (Wallac Oy, Turku, Finland) (17).

#### *SU*

Plasma concentrations of glipizide during the examinations in which the drug had been administered were measured as previously described (18, 19).

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### Calculations and statistical analyses

For insulin, C-peptide, and glucagon concentrations, area under the curve (AUC) values and incremental values (iAUC, *i.e.* baseline levels subtracted) were calculated using the trapezoidal rule. The individual iAUC from the examination with saline infusion was then subtracted from the individual iAUCs from examinations with GIP, GIP/SU, and SU and expressed as $\Delta iAUC_{GIP}$, $\Delta iAUC_{GIP/SU}$, and $\Delta iAUC_{SU}$, respectively. These values were used to calculate the insulin and C-peptide responses.

Prehepatic insulin secretion rate (ISR) was derived by deconvolution of peripheral C-peptide concentrations using a two-compartment model of C-peptide kinetics and population-based C-peptide kinetic parameters (17, 20, 21). ISR is expressed as picomoles per kilogram per minute. Total insulin secretion [$AUC_{120}$ (0 to 120 min)], early-phase insulin secretion [$AUC_{0-30}$ (0 to 30 min)], and late-phase secretion [$AUC_{30-120}$ (30 to 120 min)] were calculated from the ISR curves using the trapezoidal rule and expressed as picomoles per kilogram. The definition of the early-phase insulin response from 0–30 min was chosen after inspection of the ISR (Fig. 1C). To test for a synergistic effect of SU and GIP, the interaction effect for early, late, and total insulin secretion was calculated using the AUCs calculated from the ISR curve. The interaction effect was defined as the difference between the effect of GIP with and without the presence of SU, calculated as follows: with SU (GIP/SU minus SU), and without SU (GIP minus NaCl). This is identical to a calculation of the interaction effect using the difference between the effect of SU under the two conditions: with GIP (GIP/SU minus GIP), and without GIP (SU minus NaCl), which was also performed. The interaction effect is expressed as picomoles per kilogram. If the interaction effect is statistically different from zero (identical to a significant difference between the effect of GIP with and without SU), the effect of GIP/SU cannot be explained from the addition of effects of GIP alone and SU alone, *i.e.* GIP/SU has a more-than-additive effect on insulin secretion.

Statistical analyses were carried out using SPSS version 15 (SPSS Inc., Chicago, IL). Paired *t*-tests were used to test the interaction effects for the early phase, late phase, and total insulin secretion. All other tests were performed using Friedman's test to test for overall differences, and if a significant difference between experiments was identified ($P < 0.05$), Wilcoxon signed-rank test was used for paired comparisons.

## Results

### Glucose

During the hyperglycemic clamps (from 3 to 120 min), a plasma glucose concentration of 15 mM was achieved on all 4 experimental days (Fig. 1B), ranging from an average of 15.1 ±

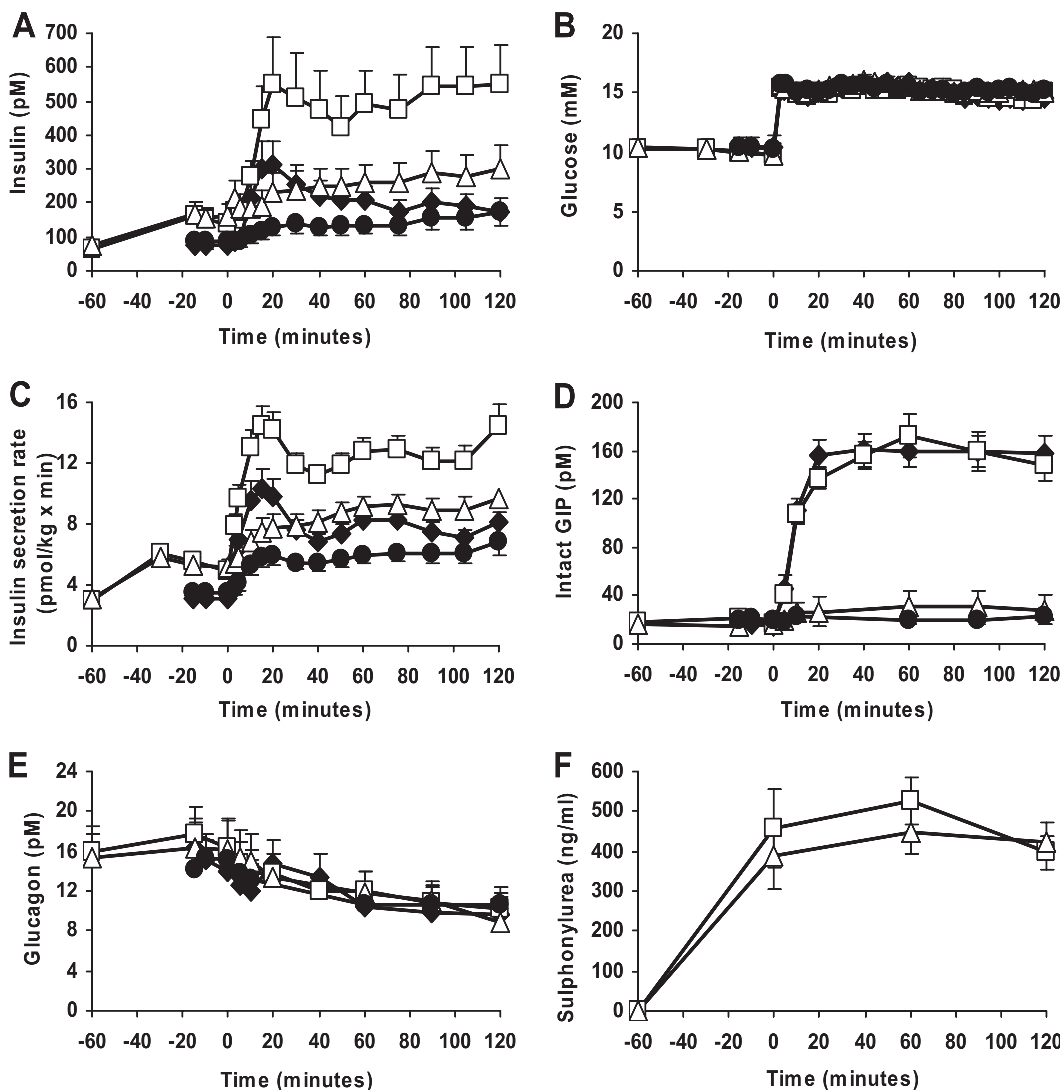


**FIG. 1.** A-F, Mean plasma concentrations of hormones and glucose during the four hyperglycemic clamps. Glipizide is shown for the 2 d with SU administration. ●, NaCl; ♦, GIP; △, SU; □,GIP/SU.

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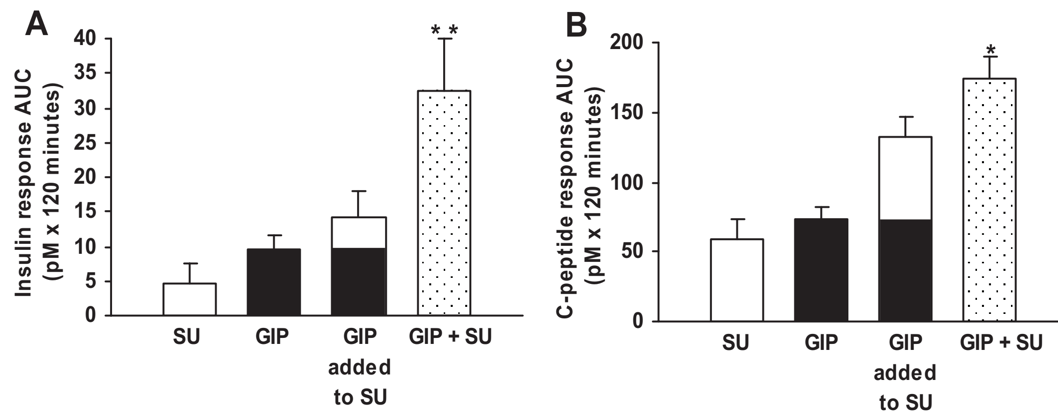


**FIG. 2.** Saline-subtracted ΔiAUC for the insulin (A) and C-peptide (B) response from the GIP, SU, and GIP/SU clamps. "GIP added to SU" displays the sum of the SU bar and the GIP bar. **, $P < 0.01$; *, $P < 0.05$ of paired comparisons.

0.3 to 15.4 ± 0.2 mM (mean ± SEM). To clamp the plasma glucose concentrations at 15 mM, we infused a significantly greater amount of glucose during the examination with GIP/SU compared with the examinations with GIP (101 ± 16.2 *vs.* 62.7 ± 11.4 g; $P = 0.003$), SU (101 ± 16.2 *vs.* 72.4 ± 9.5 g; $P = 0.03$), and saline (101 ± 16.2 *vs.* 49.4 ± 4.9 g; $P = 0.001$).

## GIP

Concentration curves of intact GIP are shown in Fig. 1D. During the two examinations with hormone infusion, plasma concentrations rose significantly to peak concentrations of 160 ± 14 (GIP) and 173 ± 18 (GIP/SU) pmol/liter ($P < 0.05$, mean ± SEM). There were no significant differences in mean plasma concentrations of intact GIP when comparing the two examinations with hormone infusion or the two examinations without hormone infusion.

## SU

Concentration curves of glipizide are shown in Fig. 1F. At 60 min, glipizide reached a maximal mean plasma concentration of 527 ± 59 ng/ml (mean ± SEM) on the GIP/SU day and 450 ± 57 ng/ml on the SU day, with no significant difference between the two examinations.

## Glucagon

The glucagon responses are shown in Fig. 1E. Calculations of AUC and decremental AUC showed no significant differences between experiments.

## Insulin, C-peptide, and interaction effect

Insulin concentration curves are shown in Fig. 1A. The insulin concentration curve from the examination with GIP/SU displayed a steep and significant rise in relation to the early-phase insulin response and a significant increase in mean concentration from the end of the early-phase to 120 min, indicating a late-phase insulin response. This was very different from the concentration curve from the examination with GIP infusion alone that displayed an early-phase response of lesser magnitude and a severely reduced late-phase insulin response. The GIP/SU insulin curve was also markedly different from the insulin curve from the examination with SU alone, which displayed a slow and gradual onset of the insulin response.

The insulin and C-peptide ΔiAUCs for the 3 d (GIP, SU, and GIP/SU) are shown in Fig. 2A and B, with a fourth bar showing the ΔiAUC from the GIP day ($\Delta iAUC_{GIP}$) added to the ΔiAUC from the SU day ($\Delta iAUC_{SU}$) for comparison. Statistical analyses showed that insulin $\Delta iAUC_{GIP/SU}$ was significantly greater than insulin $\Delta iAUC_{GIP}$ ($P = 0.002$) and insulin $\Delta iAUC_{SU}$ ($P = 0.002$). Insulin $\Delta iAUC_{GIP/SU}$ was also significantly greater than the sum of insulin $iAUC_{GIP}$ and insulin $\Delta iAUC_{SU}$ ($P = 0.002$). There was no significant difference between insulin $\Delta iAUC_{GIP}$ and insulin $\Delta iAUC_{SU}$ ($P = 0.14$). Calculations of the corresponding AUCs for the C-peptide responses (curves not shown) showed that, as for insulin, C-peptide $\Delta iAUC_{GIP/SU}$ was significantly greater than C-peptide $\Delta iAUC_{GIP}$ ($P = 0.002$) and C-peptide $\Delta iAUC_{SU}$ ($P = 0.003$), and that C-peptide $\Delta iAUC_{GIP/SU}$ was significantly greater than the sum of C-peptide $iAUC_{GIP}$ and C-peptide $\Delta iAUC_{SU}$ ($P = 0.028$). There was no significant difference between C-peptide $\Delta iAUC_{GIP}$ and C-peptide $\Delta iAUC_{SU}$ ($P = 0.35$).

The dynamic profiles of the ISRs are shown in Fig. 1C. Mean peak ISRs were found at time 15 min on examination days with GIP infusion: 10.3 ± 1.2 pmol/kg · min (GIP) and 14.4 ± 1.3 pmol/kg · min (GIP/SU); and at time 120 min on examination days without GIP: 9.7 ± 1.6 pmol/kg · min (SU) and 6.8 ± 0.9 pmol/kg · min (saline). The calculated interaction effects of GIP and SU on insulin secretion were: total insulin secretion, 223 ± 72 pmol/kg (mean ± SEM; $P = 0.01$); early-phase insulin secretion, 56 ± 21 pmol/kg ($P = 0.02$); and late-phase insulin secretion, 167 ± 58 pmol/kg ($P = 0.02$). Because the interaction effect was statistically different from zero in relation to all secretion periods, it follows that GIP/SU had a synergistic effect on total insulin secretion, early-phase insulin secretion, and late-phase insulin secretion.

# Discussion

The aim of this study was to clarify any potential involvement of β-cell $K_{ATP}$ channels in the GIP defect, and we found that co-

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administration of SU and GIP increased the GIP-induced insulin secretion in a more-than-additive fashion.

A markedly reduced late-phase insulin response to GIP is a well-known feature of the reduced incretin effect in patients with type 2 diabetes. The diminished ability of GIP to potentiate glucose-induced insulin secretion is most likely caused by a malfunction related directly to the $\beta$-cell because both secretion and elimination of the hormone in these subjects seem almost unaffected (22).

In a study of patients with type 2 diabetes and healthy control subjects, Vilsboll *et al.* (23) found an overall decreased, but yet preserved relative early-phase insulin response to GIP *vs.* GLP-1. This would indicate a common pathophysiology behind the reduced early-phase insulin responses to GIP and GLP-1, presumably made up partly by a reduction in functional $\beta$-cell mass, and possibly also by concomitant and uniform GIP and GLP-1 receptor down-regulation. In this respect, Xu *et al.* (24) have shown reversible induction of both GLP-1 and GIP-receptor down-regulation in a rat study employing long-term hyperglycemia. However, the same study also showed that short-term hyperglycemia in fact induces GIP receptor up-regulation simultaneously with GLP-1 receptor down-regulation. Furthermore, they found the overall insulin responses to GIP and GLP-1 to be similarly reduced, opposite to what has been found in earlier studies of subjects with type 2 diabetes (6, 23, 24). Lastly, subjects with type 2 diabetes display a partially preserved insulin response to GLP-1 in higher doses, as opposed to the almost complete lack of responsiveness to GIP (5). This would contradict general incretin receptor down-regulation as the sole cause of the reduced incretin effect. Considering strictly GIP receptor down-regulation, short-term hyperglycemia has been shown to reduce GIP receptor mRNA levels within a clonal $\beta$-cell line, to reduce GIP receptor expression in pancreatic islets, and to reduce the insulin response to GIP in hyperglycemic clamp studies in rats (25). But with a preserved early-phase insulin response to GIP relative to GLP-1, down-regulation of the GIP receptor as the single cause of the lost late-phase insulinotropic effect of GIP seems improbable.

It appears that the incretin effect is restored when patients with neonatal onset diabetes due to $K_{ATP}$ channel malfunction are treated with a SU compound (10). In the current study, we treated subjects with type 2 diabetes acutely with a SU compound and achieved a significant and synergistic increase in the insulin and C-peptide responses to GIP. This may indicate an association between the lost insulinotropic effect of GIP and the function of the $K_{ATP}$ channels. However, the precise mechanism by which SU ameliorates the impaired function of GIP in type 2 diabetes cannot be determined by this study, but it is most likely due to $K_{ATP}$ channel closure. With the administration of 10 mg glipizide, we achieved therapeutic concentrations of the drug and hereby presumably close to complete closure of the $K_{ATP}$ channels (26).

To avoid pitfalls and misinterpretations of $\beta$-cell function from changes in hepatic insulin extraction and C-peptide kinetics (27), we also estimated ISRs and the amount of insulin secreted over time. Again we found a significant and more-than-additive increase in insulin secretion in response to GIP after $K_{ATP}$ channel closure, in relation to the early-phase insulin secretion, the late-phase insulin secretion, and overall insulin secretion. During a hyperglycemic clamp in glucose-tolerant subjects, insulin secretion increases in a biphasic manner. There is a prominent first-phase insulin response during the first 10 min of the clamp, with a more gradual rise in insulin secretion during the remainder of the clamp, constituting the second-phase insulin response. As expected, in our diabetic patients the elevation of plasma glucose to 15 mM did not elicit a clear first-phase insulin response (28), and neither the GIP, GIP/SU, nor SU protocols triggered a "classical" first-phase insulin response, *i.e.* insulin secretion during the first 10 min. Instead, a slower response lasting about 30 min was observed in response to GIP in agreement with prior observations of a preserved early-phase response to GIP (23). This observation may in part be explained by the study design, where the GIP induction was delayed until 3 min after the induction of hyperglycemia. Therefore, the early-phase insulin response from 0–30 min may include some first-phase insulin response, but the primary contribution may in fact be the beginning of and the gradually increasing second-phase insulin response.

GIP has been shown to stimulate glucagon secretion in healthy subjects under normoglycemic conditions (29), whereas the effect in patients with diabetes and during hyperglycemia is more controversial. Likewise, SU has been shown to leave glucagon secretion unaltered, increased, or decreased, conditioned by the prevailing plasma glucose concentration (30–32). In contrast, it is well established that hyperglycemia inhibits glucagon secretion strongly, and in line with this we observed no significant effect of GIP and SU on the glucagon response to the glucose clamps. However, it is possible that the induced hyperglycemia may have masked any potential effects of GIP and/or SU.

To counteract problems associated with maximal or submaximal GIP-stimulated insulin secretion, we chose an infusion rate of GIP known to induce insulin secretion corresponding to the linear segment of the GIP-insulin dose-response curve (23, 33).

In a previous study we have shown that 4 wk of near-normalization of glycemic control (using insulin) in patients with type 2 diabetes in fact improves the insulinotropic effect of GIP (34). Furthermore, as mentioned, Meneilly *et al.* (12) showed an improvement in GIP action after a 4-wk treatment with a SU compound, which could be a combined effect of the $K_{ATP}$ channel blocker and an improvement in glycemic control. These results in combination with the findings in our present study would indicate that the GIP defect is reversible, that the cause is most likely multifactorial, and that it is probably related to the degree of glycemic control. Why GLP-1 in higher doses retains an insulinotropic effect in patients with type 2 diabetes in contrast to GIP remains unanswered.

In conclusion, we demonstrate a supraadditive effect of GIP/SU on $\beta$-cell secretion. This suggests a role of the $K_{ATP}$ channels in the impaired insulinotropic effect of GIP. However, the synergistic effect was related both to the early- and the late-phase of insulin secretion, and our study does therefore not answer the question of the primarily impaired late-phase response to GIP. Further studies are moreover required to investigate whether this

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effect of SU on GIP function is a specific trait of type 2 diabetes or whether it is related to the diabetic state as such.

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