GIP Does Not Potentiate the Antidiabetic Effects of GLP-1 in Hyperglycemic Patients With Type 2 Diabetes

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OBJECTIVE—The incretin glucagon-like peptide 1 (GLP-1) exerts insulinotropic activity in type 2 diabetic patients, whereas glucose-dependent insulinotropic polypeptide (GIP) no longer does. We studied whether GIP can alter the insulinotropic or glucagonostatic activity of GLP-1 in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS—Twelve patients with type 2 diabetes (nine men and three women; 61 ± 10 years; BMI 30.0 ± 3.7 kg/m^2; HbA_{1c} 7.3 ± 1.5%) were studied. In randomized order, intravenous infusions of GLP-1(7-36)-amide (1.2 pmol · kg^{-1} · min^{-1}), GIP (4 pmol · kg^{-1} · min^{-1}), GLP-1 plus GIP, and placebo were administered over 360 min after an overnight fast (≥1 day wash-out period between experiments). Capillary blood glucose, plasma insulin, C-peptide, glucagon, GIP, GLP-1, and free fatty acids (FFA) were determined.

RESULTS—Exogenous GLP-1 alone reduced glycemia from 10.3 to 5.1 ± 0.2 mmol/L. Insulin secretion was stimulated (insulin, C-peptide, $P < 0.0001$), and glucagon was suppressed ($P = 0.009$). With GIP alone, glucose was lowered slightly ($P = 0.0021$); insulin and C-peptide were stimulated to a lesser degree than with GLP-1 ($P < 0.001$). Adding GIP to GLP-1 did not further enhance the insulinotropic activity of GLP-1 (insulin, $P = 0.90$; C-peptide, $P = 0.85$). Rather, the suppression of glucagon elicited by GLP-1 was antagonized by the addition of GIP ($P = 0.008$). FFA were suppressed by GLP-1 ($P < 0.0001$) and hardly affected by GIP ($P = 0.07$).

CONCLUSIONS—GIP is unable to further amplify the insulinotropic and glucose-lowering effects of GLP-1 in type 2 diabetes. Rather, the suppression of glucagon by GLP-1 is antagonized by GIP. ***Diabetes*** **60:1270–1276, 2011**

Abnormalities in the secretion and insulinotropic activity of incretin hormones like gastric inhibitory polypeptide (also called glucose-dependent insulinotropic polypeptide [GIP]) and glucagon-like peptide 1 (GLP-1) have been described in patients with type 2 diabetes (1–4). In particular, GIP has been found to no longer potently stimulate insulin secretion in type 2 diabetes (5–7), even at high, supraphysiological doses/concentrations (7). This appears to be a problem of all types of diabetes (3,4), independent from their pathogenesis, and, therefore, has been blamed upon the common element, hyperglycemia (8). Although some recent studies have attempted to elucidate the mechanisms whereby hyperglycemia attenuates GIP receptor expression and the responsiveness of insulin secretory activity to GIP in the diabetic state (8,9), the phenomenon of a reduced insulinotropic activity in patients with type 2 diabetes is still not fully understood.

In contrast, GLP-1 is able to stimulate insulin secretion in type 2 diabetes (10–13) and has become the parent compound for the development of incretin-based antidiabetic drugs (14). This difference in the insulinotropic activity of the two incretins in patients with type 2 diabetes is a surprise, since the cellular mechanisms triggering insulin secretion are the same for both GIP and GLP-1 (15). Nevertheless, in animal experiments, GIP or its peptide analogs have been explored as glucose-lowering agents with some success (16–22), although none of these analogs has ever been developed as an antidiabetic drug for type 2 diabetes. It has become clear that GIP administered acutely does not augment insulin release to any great degree in patients with type 2 diabetes (6,7,23). This does not exclude a modification of insulin secretory activity primarily stimulated by other agents, as recently demonstrated for the coadministration of GIP and sulfonylureas (24). Thus we were interested in testing for a potential interaction between GIP and GLP-1, both administered exogenously, in patients with type 2 diabetes, especially because these incretin hormones interact in an additive manner in healthy human subjects (25). Preliminary results have been published in abstract form (26).

RESEARCH DESIGN AND METHODS

Study protocol. The study protocol was approved by the ethics committee of the Georg-August-Universität Göttingen on 9 June 2008 (registration number: 14/3/01) before the study. Written informed consent was obtained from all participants.

Patients. Twelve patients with type 2 diabetes were examined. Their characteristics are summarized in Table 1.

Design of the study. All participants underwent a screening examination and four additional tests on different days in randomized order. All antidiabetic medications (insulin and oral antidiabetic drugs) were discontinued 1 day before each experiment, and experiments were started in the morning after an overnight fast. Either placebo (vehicle: 0.9% NaCl with 1% human serum albumin), GIP (4 pmol · kg^{-1} · min^{-1}), GLP-1(7-36)-amide (1.2 pmol · kg^{-1} · min^{-1}), or a combination of both incretin hormones was infused over 360 min. This pharmacological dose of GLP-1 has previously been used to normalize fasting glycemia in patients with type 2 diabetes without side effects (10,11). The dose of GIP was expected to result in clearly high, pharmacological concentrations. In previous studies, even higher doses of GIP had been used (7), so that the dose chosen could be considered safe. Plasma glucose concentrations, parameters characterizing insulin secretion (insulin, C-peptide, insulin secretion rates determined by deconvolution analysis), glucagon, and free fatty acids were determined in blood drawn in the basal state and during the infusions. As a minimum, a day without study-related activities (normal eating rhythm, administration of usual antidiabetic treatment; Table 1) was allowed between experiments.

Human synthetic GLP-1 and GIP. Human synthetic GLP-1 and GIP (GMP grade, for human use) were obtained from PolyPeptide, Wolfenbüttel,

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Received 18 September 2010 and accepted 7 January 2011.
DOI: 10.2337/db10-1332
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TABLE 1
Subject characteristics of type 2 diabetic patients participating in the study

Parameter	Number	Mean ± SD	Range
Sex (female/male)	3/9		
Age (years)		61 ± 10	43–74
Duration of diabetes (years)		7 ± 6	2–22
HbA_{1c} (%)		7.3 ± 1.5	6–11.4
BMI (kg/m^2)		30.0 ± 3.7	24–33
Blood pressure (mmHg)			
Systolic		139 ± 10	120–160
Diastolic		81 ± 5	72–90
Creatinine (mg/dL)		0.9 ± 0.2	0.7–1.1
Cholesterol (mg/dL)			
Total		205 ± 51	135–297
LDL		123 ± 46	34–222
HDL		46 ± 10	30–55
Metformin (number, dose [mg/day])	8	2,200 ± 378	2,000–3,000
Glibenclamide (number, dose [mg/day])	3	4.7 ± 2.0	3.5–7.0
Bedtime insulin (number, dose [IU/day])	1	16	16
Intensive insulin therapy (number, dose [IU/day])	3	47.7 ± 40.0	16–103
Nephropathy	1		
Neuropathy	3		
Retinopathy	0		
Macroangiopathy	2		
Hypertension	9		
Fasting plasma glucose at screening (mmol/L)		8.2 ± 1.7	6.5–11.0
Fasting plasma glucose after antidiabetic therapy discontinuation (mmol/L)		10.1 ± 2.9	8.5–17.0

Germany, and prepared for the administration to human subjects by Pegasus Pharma, Hannover, Germany. This included sterile filtration, lyophylization, and packaging into sterile glass vials (GIP, 300 μg per vial; GLP-1, 150 μg per vial), labeling and analysis (amino acid composition and sequence, purity, solvent residues, sterility, etc.) and repeated examinations for stability. GIP and GLP-1 vials were stored in a refrigerator (4°C), opened for the experiments, and dissolved in vehicle (0.9% NaCl with 1% human serum albumin; Behring, Marburg, Germany, 20%, Lot. No. 5494441A). The concentration was calculated to allow for a common infusion rate (4 mL/h), adjusting for differences in body weight.

Blood specimens. Blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol; 20,000 KIU/mL, 180 μL per 9 mL blood; Bayer AG, Leverkusen) and kept on ice. A capillary sample taken from hyperemic ear lobes (Finalgon = Nonivamid 4 mg/g, Nicoboxil 25 mg/g) (~100 μL) was stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the immediate measurement of glucose. After centrifugation at 4°C, plasma for hormone analyses was divided into aliquots of 0.5 or 1 mL and stored frozen at −30°C.

Laboratory determinations. Glucose was measured using a glucose oxidase method with a Glucose Analyzer 2 (Beckman Instruments, Munich, Germany).

Insulin and C-peptide were determined by specific immunoassays as previously described (27). The concentrations of total GIP and GLP-1 were measured as previously reported (28,29).

Intact, biologically active GIP was measured as described (29) using an antiserum reacting with the NH_2-terminal portion of GIP. The experimental detection limit was <2 pmol/L. Intra- and interassay coefficients of variation are <6 and <12%, respectively.

Concentrations of intact, biologically active GLP-1 (sequence 7-36-amide) were determined using a sandwich radioimmunoassay as described (30). The experimental detection limit was <0.5 pmol/L. Intra- and interassay coefficients of variation are 2 and 5%, respectively.

Pancreatic glucagon was measured using porcine antibody 4305 in ethanol-extracted plasma, as previously described (31). The detection limit was <1 pmol/L. Intra-assay coefficients of variation were 6%, and interassay coefficients of variation were 16%.

Free fatty acids were assayed using reagents from Wako Chemicals, Neuss, Germany, on a Siemens (Dade Behring) Dimension Xpand Autoanalyser.

Statistics. Subject characteristics are reported as means ± SD. Results are reported as means ± SE. Integration was carried out using the trapezoidal rule. Integrated incremental responses describe changes above baseline; integrated decrements describe changes below baseline.

Insulin secretion rates were calculated from C-peptide concentrations using software ISEC version 3.4a, supplied by Dr. Roman Hovorka, London, U.K. Population-derived coefficients of transition between compartments were used as described (32–35).

Steady-state concentrations of incretin hormones were calculated as mean values between 60 and 360 min. Metabolic clearance rates for GLP-1 and GIP (total concentrations) were calculated dividing nominal infusion rates (per kilograms body wt and minute) by steady-state concentrations.

Repeated-measures ANOVA (Statistica 5.0; Statsoft, Tulsa, AZ) was performed on all parameters determined over the whole duration of the experiments. Independent variables were experimental conditions (placebo, GIP, GLP-1, or combined administration), patient (random variable), and time (fixed effects). As results, P values for treatment effects, changes with time, and their interaction are reported in the figures. If a significant difference regarding treatment, or a significant interaction of these treatment effects with time ($P < 0.05$), was documented, results at single time points were compared by one-way ANOVA, using the same independent variables; if P values were <0.05, this was followed by Duncan post hoc test to identify differences at individual time points between any of the treatments.

For integrated incremental or decremental changes, one-way ANOVA was used. In case of an overall significant difference ($P < 0.05$), Duncan post hoc test was used to identify differences between the groups.

P values <0.05 were taken to indicate significant differences.

RESULTS

Patients. Type 2 diabetic patients with fasting hyperglycemia were recruited for the current study. Hyperglycemia was more pronounced after discontinuing the antidiabetic medication for 1 day before each experiment ($P = 0.04$; Table 1).

Glucose. Plasma glucose concentrations slightly fell with placebo administration over the duration of the experiments (Fig. 1). With exogenous GIP, a slight reduction in glucose concentrations was observed. GLP-1, on the other hand, led to a normalization of glucose concentrations within 4 h, and normoglycemic values were maintained for the remainder of the experiment. The addition of GIP did not further lower glucose concentrations, nor was the reduction in glycemia observed earlier (Fig. 1). Integrated decremental glucose concentrations (Table 2) confirm the potent glucose-lowering activity of GLP-1 and the lack of activity of GIP, alone or in combination with GLP-1.

GIP and GLP-1 concentrations. Exogenous GIP elevated plasma concentrations of total and intact GIP, to steady-state levels of ~530 pmol/L for the total concentrations and 225 pmol/L for the concentrations of intact, biologically active GIP (Fig. 2). The coadministration of GLP-1 did not change the steady-state concentrations or metabolic clearance rate of GIP (Table 3). Exogenous GLP-1 elevated plasma concentrations of total and intact GLP-1, to steady-state levels of ~145 pmol/L for the total concentrations and 20 pmol/L for the concentrations of intact, biologically active GLP-1. The coadministration of GLP-1 did not change the steady-state concentrations or metabolic clearance rate of GLP-1 (Table 3).

Insulin secretory activity. Based on the analysis of insulin and C-peptide concentrations as well as insulin secretory rates, GLP-1 was able to potently stimulate insulin secretion, whereas GIP hardly did so (Fig. 1). Integrated increments in C-peptide were stimulated approximately threefold more with GLP-1 than with GIP ($P = 0.02$), and

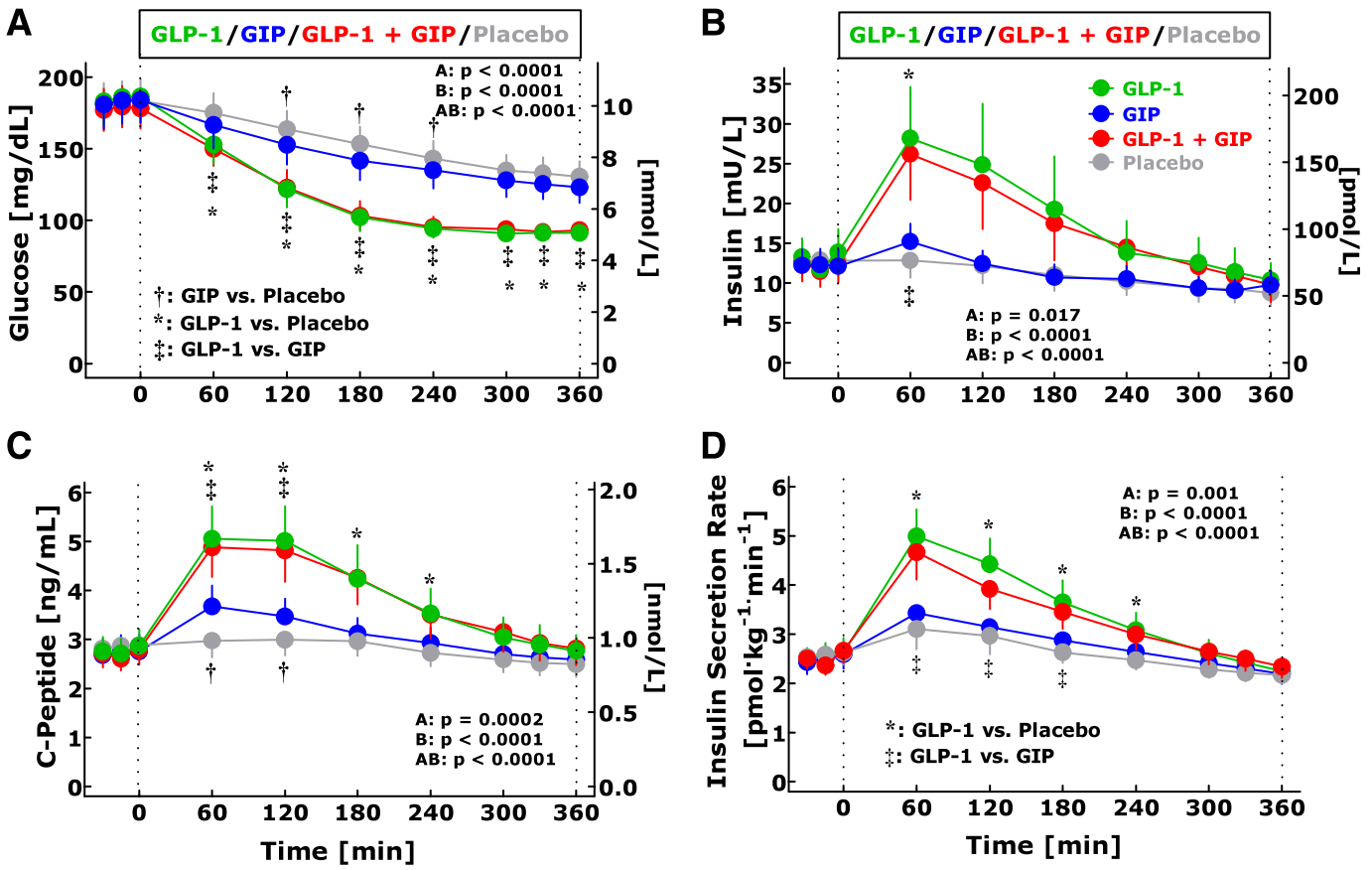

FIG. 1. Concentrations of capillary plasma glucose (*A*), and venous plasma concentrations of insulin (*B*), and C-peptide (*C*), and insulin secretion rates derived by deconvolution analysis (*D*) over 360 min of intravenous infusions of placebo (gray circles), GIP (blue circles), GLP-1 (green circles), or the combination of GIP and GLP-1 (red circles). Data are means ± SE. Statistical analysis was done by repeated-measures ANOVA (A, by group; B, by time; AB, interaction of group assignment and time). Symbols (see key) indicate significant differences between specific experiments. Definitions of symbols in *A* and *D* also apply to symbols in *B* and *C*.

the respective value for integrated insulin concentrations was approximately sixfold. The same pattern resulted from analyses of insulin secretory rates determined by deconvolution (Fig. 1).

Glucagon. Glucagon concentrations were suppressed significantly by exogenous GLP-1, whereas they tended to increase slightly with exogenous GIP (Fig. 3). Remarkably, the coadministration of GIP together with GLP-1 blunted the suppression of glucagon by GLP-1, which was no longer significant in the presence of elevated GIP concentrations.

Nonesterified fatty acids. Exogenous GLP-1 significantly reduced plasma concentrations of free fatty acids (Fig. 3), whereas exogenous GIP did not alter levels of free fatty acids, whether administered alone or in combination with GLP-1.

Adverse events. No nausea or vomiting, or any other significant side effect, was reported by any of the patients during or after the exogenous (intravenous) administration of GLP-1 and/or GIP.

DISCUSSION

Although pharmacological concentrations (approximately four- to sixfold higher than peak concentrations after nutrient stimulation [36,37]) of GLP-1 potently can augment

TABLE 2
Integrated incremental or decremental glucose, insulin, C-peptide, insulin secretion rates (deconvolution), glucagon, and free fatty acid concentrations after intravenous placebo, GLP-1, GIP, or GLP-1 plus GIP infusions in type 2 diabetic patients

Parameter	Relation to baseline	Placebo	GLP-1	GIP	GLP-1 plus GIP	Significance (*P* value)
Glucose (mmol · L^{-1} · min)	Below	−571 ± 85[b]	−1,370 ± 151[a,c]	−735 ± 93[b]	−1,230 ± 140[a,c]	<0.0001
Insulin (mU · L^{-1} · min)	Above	0.7 ± 0.3	13.9 ± 6.7	2.1 ± 0.6	12.6 ± 4.9	0.015
C-peptide (nmol · L^{-1} · min)	Above	12.9 ± 4.3[b]	141.0 ± 35.3[c]	54.1 ± 12.5	142.9 ± 32.0b[a,c]	<0.0001
Insulin secretion (pmol/kg body wt)	Above	82 ± 48	367 ± 80[a,c]	145 ± 34	321 ± 71[a]	0.002
Glucagon (pmol · L^{-1} · min)	Below	−1,111 ± 149[c]	−1,392 ± 232[c]	−554 ± 146[b,a]	−871 ± 182[b]	0.001
Nonesterified fatty acids (mmol · L^{-1} · min)	Below	−8 ± 4[b]	−34 ± 8[a,c]	−11 ± 3[b]	−34 ± 8[c]	0.0002

[a]Significant difference ($P < 0.05$) to placebo. [b]Significant difference ($P < 0.05$) to GLP-1. [c]Significant difference ($P < 0.05$) to GIP.

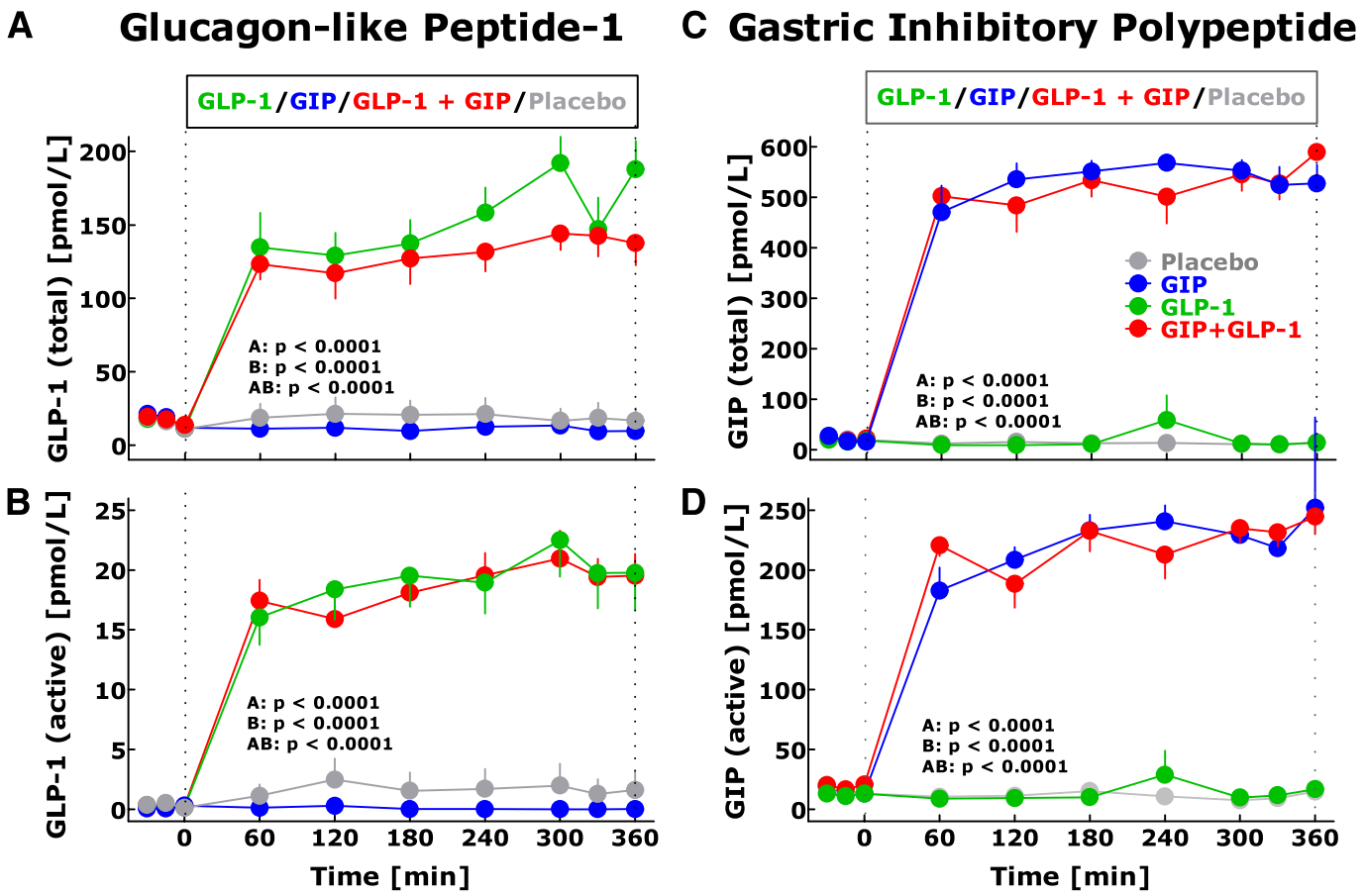

FIG. 2. Concentrations of GIP (total concentrations; *A*), intact GIP (*B*), GLP-1 (total concentrations; *C*), and intact GLP-1 (*D*) over 360 min of intravenous infusions of placebo (gray circles), GIP (blue circles), GLP-1 (green circles), or the combination of GIP and GLP-1 (red circles). Data are means ± SE. Statistical analysis was done by repeated-measures ANOVA (A, by group; B, by time; AB, interaction of group assignment and time). At all time points, GIP and GLP-1 concentrations were significantly elevated during infusion of the respective peptides.

insulin secretion in patients with type 2 diabetes (Fig. 1), leading to a normalization of plasma glucose concentrations within 4 h, GIP has only marginal effects on insulin secretion, with little consequence for glucose concentrations. These results confirm previous results, both with GLP-1 (10,11) and with GIP (5–7). The novel finding of our present study is the finding that coadministration of GIP in addition to GLP-1 does not further augment insulin secretory activity in patients with type 2 diabetes. Rather, the glucagon-suppressing activity of GLP-1 is counteracted by the simultaneous presence of high GIP concentrations (Fig. 3), in line with previous findings linking GIP to a stimulation of glucagon release (38). This could be because of the fact that GIP, like GLP-2, can directly stimulate the α-cell, whereas the GLP-1 effect to lower glucagon is indirect (via somatostatin). Apparently, these two mechanisms compete with each other, thus producing the effect observed (39). Similar mechanisms may be active in response to GLP-1 (suppression) and GIP (stimulation) in the current study.

The lack of interaction of GLP-1 and GIP in the stimulation of insulin secretion in patients with type 2 diabetes is in sharp contrast with similar experiments performed in healthy subjects (25) or islets from nondiabetic rodents (40), where an additivity of the insulinotropic effectiveness of GLP-1 and GIP has been described. It is not clear

TABLE 3
Steady-state concentrations and metabolic clearance rates for GLP-1 and GIP infused alone or in combination

Incretin	Steady-state concentration (pmol/L)	Significance (*P* value)	Metabolic clearance rate (mL · kg^{-1} · min^{-1})	Significance (*P* value)
GLP-1		0.36		0.50
Alone	155 ± 24		15.4 ± 7.4	
With GIP	132 ± 12		10.0 ± 1.0	
GIP		0.69		0.34
Alone	533 ± 23		8.0 ± 0.6	
With GLP-1	526 ± 33		8.0 ± 0.6	

Data are means ± SE. The analysis is based on total GLP-1 and GIP concentrations (RESEARCH DESIGN AND METHODS). Metabolic clearance rates were calculated by dividing infusion rates (pmol · kg^{-1} · min^{-1}) by steady-state concentrations (60–360 min) (pmol/L) (Fig. 2).

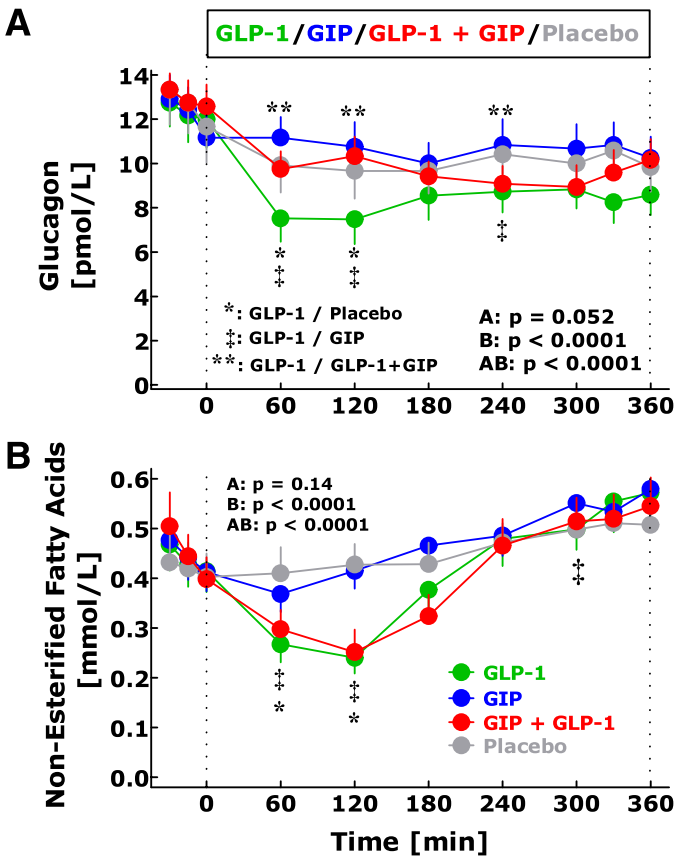

FIG. 3. Concentrations of glucagon (*A*) and free fatty acids (*B*) over 360 min of intravenous infusions of placebo (gray circles), GIP (blue circles), GLP-1 (green circles), or the combination of GIP and GLP-1 (red circles). Data are means ± SE. Statistical analysis was done by repeated-measures ANOVA (A, by group; B, by time; AB, interaction of group assignment and time). Symbols (see key) indicate significant differences between specific experiments. Definitions of symbols in *A* also apply to symbols in *B*.

whether this represents a true interaction on a molecular level or just the simultaneous activity of two independent processes. One possible explanation for the inability of GIP to augment the insulinotropic response to GLP-1 could be that the degree of stimulation was close to maximum with the dose of GLP-1 chosen alone, not allowing any further stimulation over and above what was observed with GLP-1. This, however, seems unlikely, since higher doses of GLP-1 have been able to elicit greater responses in published studies (41,42).

If physiological concentrations of GIP and GLP-1 were tested in healthy subjects, the majority of the insulin release in healthy subjects appeared to be because of GIP (25), whereas in other studies GLP-1 displayed a similar potential (43). However, in the experiments in type 2 diabetic patients described in the current study, GIP obviously could not contribute to insulin secretion to any significant extent. According to our study design, antidiabetic drugs were withdrawn to allow for a permissive degree of hyperglycemia, which should support the demonstration of antidiabetic actions of incretin hormones (10,44). Because hyperglycemia itself or any variation in the level of glycemia per se may play a role in the desensitization of the endocrine pancreas to GIP, this may, on the other hand, have precluded a better response to GIP. Additional experiments using patients made more normoglycemic at the time of stimulating insulin secretion may be helpful in that respect. It would also be of interest to study a more comprehensive dose range spanning from physiological to high pharmacological doses of GIP to more fully exclude any activity in type 2 diabetic patients.

It is important to note that the present experiments were performed in overnight-fasted patients with type 2 diabetes and that the results should not be extrapolated to the postprandial state, in which the deceleration of gastric emptying plays an important role regarding glucoregulatory actions of GLP-1 (45), but not GIP (46). Of interest, exogenous GIP has recently been described to worsen glycemic control after meal ingestion in patients with type 2 diabetes (47).

The doses and concentrations of both GLP-1 and GIP used in the current study were clearly supraphysiological. Regarding GIP, even higher doses (up to 16 pmol · kg^{-1} · min^{-1} [7]) have been used and were also found to be ineffective with respect to the stimulation of insulin secretion in patients with type 2 diabetes. When comparing the steady-state concentrations of intact (both COOH- and NH_2-terminal intact, full biological activity) versus total (including dipeptidyl peptidase-4 [DPP-4]–mediated degradation products) GIP and GLP-1, it is obvious that GLP-1 is the better substrate for DPP-4, with approximately one eighth remaining intact during continuous administration, as in previous studies (48). The corresponding figure for GIP is 40% remaining intact (29). GLP-1 and GIP do not interfere with each other's degradation or elimination in type 2 diabetic patients, as previously described in healthy subjects (Fig. 2 [25]). It also should be noted that the degree of normalization in fasting glycemia that can be achieved with exogenous GLP-1 is greater with intravenously infused GLP-1 in the current study and in previous publications (10,11), than typically can be achieved with the subcutaneous administration of GLP-1 receptor agonists approved for the treatment of type 2 diabetes (14).

The present results do not encourage the use of GIP as an antidiabetic agent meant to acutely lower glycemia in patients with type 2 diabetes. The fact that GIP and some of its analogs with presumed longer half-lives did improve the control of glycemia in rodent models of type 2 diabetes (16,18,19,21,49–52) may be because of the fact that the insulinotropic activity of GIP appears not to be impaired to the same degree as in human type 2 diabetes (53). Thus GIP analogs with promising results in rodents need to be tested in human patients before their value as potential antidiabetic agents can be estimated.

Limitations of our study are the rather small number of patients studied (however, with a very uniform result in all of them), the short duration of exposure to GIP (6 h), and the lack of patients with glucose concentrations closer to normal values. Because the incretin effect seems to be impaired once fasting glucose concentrations exceed 6 mmol/L (3,4), one could speculate that this provides indirect evidence that GIP only effectively stimulates insulin secretion when the fasting glucose concentrations remain below this threshold value. A recent study by Højberg et al. (54) showed an improvement in insulinotropic activity of GIP after an attempt to normalize glycemia with an intensified insulin regimen over 4 weeks. Nevertheless, the improvement in the response to GIP was small in size and, moreover, the antidiabetic treatment did not result in fasting glucose concentrations reaching a target of 6 mmol/L. It remains to be seen whether a more effective glucose-lowering

regimen will improve the insulinotropic activity of GIP to a greater extent or whether specific ways of lowering glucose concentrations (e.g., with GLP-1 or incretin-based medications) will trigger an improvement in how type 2 diabetic β-cells respond to GIP.

Assuming that GIP does not further potentiate the insulinotropic effects of GLP-1 and even antagonizes its glucagonostatic effects, the question arises why the DPP-4 inhibitors still exert potent effects on insulin and glucagon secretion although they raise the plasma levels of both incretin hormones. This apparent paradox might be resolved by the different GIP plasma levels achieved during GIP infusion in this study and during DPP-4 inhibitor treatment. Thus the intact GIP levels reached during DPP-4 inhibitor administration may not suffice to counteract the potent glucagonostatic effects of GLP-1. Nevertheless, in light of the present results it seems unlikely that raising GIP levels plays a relevant role for the antidiabetic actions of DPP-4 inhibitors.

In conclusion, GIP at a high, pharmacological dose does not augment insulin secretory responses to GLP-1, nor does it lead to more rapid lowering in glucose concentrations. The apparent counteraction of the glucagonostatic activity of GLP-1 is an adverse response of adding GIP to GLP-1 in terms of antidiabetic treatment. Taken together, our study testing the acute exposure to GLP-1 and GIP does not support any therapeutic effect of GIP in type 2 diabetic patients, both when administered as a single agent or in combination with GLP-1.

ACKNOWLEDGMENTS

The study was supported by a grant from Eli Lilly & Company (Indianapolis, IN).

No other potential conflicts of interest relevant to this article were reported.

N.M. researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. I.V. contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. L.D.K. and J.J.H. researched data, contributed to discussion, and reviewed and edited the manuscript. C.F.D. researched data and reviewed and edited the manuscript. M.T. and J.J.M. contributed to discussion and reviewed and edited the manuscript. M.A.N. researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript.

The expert technical assistance of B. Nawrodt and K. Rotenberger (Diabeteszentrum Bad Lauterberg) is acknowledged. Dr. V. Ng (University of Hong Kong) helped during the performance of the experiments and with data analyses.

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