0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(11):4165–4171

doi: 10.1210/jc.2006-1932

The Dipeptidyl Peptidase 4 Inhibitor Vildagliptin Does Not Accentuate Glibenclamide-Induced Hypoglycemia but Reduces Glucose-Induced Glucagon-Like Peptide 1 and Gastric Inhibitory Polypeptide Secretion

Andrea El-Ouaghlidi, Erika Rehring, Jens J. Holst, Anja Schweizer, James Foley, David Holmes, and Michael A. Nauck

Diabeteszentrum (A.E.-O., E.R., M.A.N.), D-37431 Bad Lauterberg im Harz, Germany; Department of Medical Physiology (J.J.H.), Panum Institute, University of Copenhagen, DK-2200 Copenhagen, Denmark; and Novartis Pharma AG (A.S., J.F., D.H.), CH-4002 Basel, Switzerland

Background/Aims: Inhibition of dipeptidyl peptidase 4 by vildagliptin enhances the concentrations of the active form of the incretin hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP). The present study asked whether vildagliptin accentuates glibenclamide-induced hypoglycemia or affects endogenous secretion of GLP-1 and GIP after an oral glucose tolerance test.

Methods: There were 16 healthy male subjects studied on four occasions after an overnight fast in a double-blind, four-way crossover study. In random order, vildagliptin (100 mg) or placebo, with and without glibenclamide (5 mg), was administered 30 min before 75 g oral glucose. Blood was sampled to measure glucose, and total (sum of active and inactive) GLP-1 and GIP. Statistical evaluation was done using repeated-measures ANOVA.

Results: Glibenclamide provoked hypoglycemia (≤1.9 mM), but this was not accentuated by the simultaneous administration of vildagliptin ($P = 0.25$). The integrated incremental responses of total GLP-1 were reduced by vildagliptin by 72% (with glibenclamide) and 48% (without glibenclamide) (effect of vildagliptin: $P < 0.0001$; glibenclamide: $P = 0.31$; interaction: $P = 0.26$). Similarly, integrated incremental responses of total GIP were reduced by vildagliptin by 26 and 21%, with and without glibenclamide, respectively (vildagliptin: $P = 0.017$; glibenclamide: $P = 0.44$; interaction: $P = 0.69$).

Conclusions: Sulfonylurea-induced hypoglycemia after the oral administration of glibenclamide is not accentuated by the coadministration of vildagliptin. This may be explained by a negative feedback regulation of GLP-1 and GIP secretion that limits the degree to which the active incretin levels are enhanced. **(*J Clin Endocrinol Metab* 92: 4165–4171, 2007)**

INHIBITORS OF THE proteolytic enzyme dipeptidyl peptidase 4 (DPP-4) (1) have recently attracted attention because of their potential as oral antidiabetic agents (2, 3). They prevent the rapid proteolytic degradation and inactivation of intact, biologically active glucagon-like peptide 1 (GLP-1) [and gastric inhibitory polypeptide (GIP) as well], and, thus, increase the postprandial plasma concentrations of intact GLP-1/GIP by approximately 2-fold. In pigs, Deacon *et al*. (4, 5) demonstrated with valine pyrrolidide as a DPP-4 inhibitor and exogenous peptide that the proportion of intact GIP and GLP-1 could be increased to values of close to 100% of total GIP and GLP-1, respectively, and that the insulin response to a glucose infusion could thus be significantly enhanced.

Administration of the DPP-4 inhibitor NVP-DPP728 to dogs has acutely reduced the meal-induced secretion of total GLP-1 and GIP (6). These results are in line with data from the perfused pig intestine in which exogenous GLP-1 was able to reduce the secretion of glucagon-like peptide 2 (7). Because glucagon-like peptide 2 is a product of the same gene expressed in the same cells (L cells), this most likely indicated a feedback mechanism modulating GLP-1 (L cell) secretion. However, this has not been described in human studies.

In patients with diabetes, treatment with the DPP-4 inhibitor vildagliptin reduces fasting and postprandial glucose concentrations, suppresses meal-related glucagon responses, and enhances insulin secretion relative to ambient glucose concentrations (8–10). Similar findings have been reported with other DPP-4 inhibitors (11, 12).

DPP-4 inhibitors can be expected to be used in combination with other oral antidiabetic agents, including sulfonylureas. A potential interaction of effects on insulin secretion may cause hypoglycemia. Therefore, the primary objective of the present clinical study was to investigate any possible potentiation of hypoglycemia induced by glibenclamide through a coadministration of vildagliptin. Healthy subjects were chosen because they were regarded as particularly susceptible to hypoglycemia under these conditions. We also studied the secretion of GIP and GLP-1 in response to a single dose of the DPP-4 inhibitor to determine whether there was any evidence for a negative feedback mechanism as hypothesized previously. Preliminary data have been published in abstract form (13, 14).

First Published Online August 14, 2007

Abbreviations: DPP-4, Dipeptidyl peptidase 4; GEC, gastric emptying coefficient; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Subjects and Methods

Study protocol

The study protocol was approved by the ethics committee of the Ärztekammer Niedersachsen on May 15, 2001. Written informed consent was obtained from all participants before the study.

Subjects

There were 16 healthy male volunteers studied. Their characteristics are shown in Table 1. They had no personal history of diabetes or gastrointestinal disease, and none had a first-degree relative with type 2 diabetes. Standard clinical chemistry and hematology laboratory values were in the normal range (liver enzymes, serum creatinine, blood count, and TSH). Total cholesterol was 4.2 ± 0.8 mmol/liter (normal, 3.2–5.2), high-density lipoprotein cholesterol was 1.3 ± 0.2 mmol/liter (normal, 0.9–1.6), low-density lipoprotein cholesterol was 2.4 ± 0.7 mmol/liter (normal, 1.3–4.1), and triglycerides were 1.0 ± 0.5 mmol/liter (normal, 0.5–2.3).

Design/study drugs

After screening, each subject participated in four examinations started in the morning in the fasting state, separated by at least 1 wk (d 0, 7, 14, and 21). Vildagliptin (100 mg tablet), glibenclamide (5 mg tablet), both, or placebo was administered in randomized order. If no vildagliptin or likewise, if no glibenclamide was given, matching placebo tablets were administered. An oral glucose tolerance test (75 g glucose equivalents in 300 ml; Roche, Mannheim, Germany) was performed 30 min after drug administration. Vildagliptin and glibenclamide tablets were provided by Novartis Pharmaceuticals Corp. (East Hanover, NJ). There was 10 mg ^{13}C-acetate (Löns-Apotheke, Buchholz, Germany) added to the oral glucose drink to assess gastric emptying by measuring ^{13}C-CO_2 in expired breath samples.

Blood specimens

Venous blood was drawn into chilled tubes containing EDTA, aprotinin (Trasylol; 20,000 KIU/ml, 180 μl/9 ml blood; Bayer AG, Leverkusen, Germany), and Diprotin A (10 mM, 90 μl/9 ml blood), and kept on ice. In addition, 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 was measured using an insulin antibody coated microtiter well (Insulin ELISA) from DRG Instruments GmbH (Marburg, Germany) on a PersonalLab semiautomatic analyzer (BioChem ImmunoSystems, Freiburg, Germany). Intraassay coefficients of variation were approximately 4%. Human insulin was used as standard.

C-peptide was measured using C-peptide antibody coated microtiter wells (C-peptide ELISA) from DRG Instruments GmbH, on a PersonalLab semiautomatic analyzer. Intraassay coefficients of variation were approximately 6%. Human C-peptide was used as standard.

Total GIP was determined as previously described (15), using antiserum R 65 (final dilution 1:150,000), and synthetic human GIP for tracer preparation and as standard. The experimental detection limit was less than 1 pmol/liter. Antiserum R 65 binds near the C terminus of the GIP molecule. Intraassay coefficients of variation were approximately 6%, and interassay coefficients of variation were less than 8%.

Total concentrations of GLP-1, including the DPP-4 breakdown products GLP-1, 9–36 amide and 9–37, were measured using the nonspecific antiserum 2135 directed against the midportion of the GLP-1 molecule, as described (16, 17).

Pancreatic glucagon was measured using porcine antibody 4305 in ethanol-extracted plasma, as previously described (18). The detection limit was less than 1 pmol/liter. Intraassay coefficients of variation were 6.7%, and interassay coefficients of variation were 16%.

Cortisol was measured using cortisol antibody coated microtiter wells (EIAgen Cortisol) from BioChem Immunosystems (Adaltis, Bologna, Italy) on a PersonalLab semiautomatic analyzer. Intraassay coefficients of variation were approximately 4%. Human cortisol was used as standard.

Gastric emptying. The velocity of gastric emptying was assessed by following the concentrations of ^{13}C-CO_2 in breath samples after the ingestion of ^{13}C-acetate as a water-soluble source of ^{13}C. Breath samples were taken into gas-tight plastic bags (volume, ~300 ml) at 0, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after ingesting oral glucose/^{13}C-acetate. ^{13}C-CO_2 in breath samples was determined by infrared absorptiometry (Wagner-Analysentechnik, Achim, Germany). The method of deriving gastric emptying rates from breath $^{13}CO_2$ concentrations has been described by Ghoos *et al.* (19) and adapted to liquids by Braden *et al.* (20). In detail, the cumulative appearance of ^{13}C was fitted to the equation

$$Y = M \times (1 - e^{-k \times t})^{\beta}$$

Based on the estimation of M, k, and β, parameters characterizing gastric emptying (lag time, half-emptying time) were calculated. The fact that M represents a value asymptotically approached when emptying of the stomach is complete allows the calculation of gastric content over time by expressing Y as a fraction of M. For details, see Ref. 21.

As an overall index of the velocity of gastric emptying, the gastric emptying coefficient (GEC) was calculated as suggested by Ghoos *et al.* (19). For the calculation of the GEC, the percent ^{13}C-CO_2 recovery per hour relative to the dose of ^{13}C-CO_2 administered was fitted to:

$$Y = a \cdot t^{b} \cdot e^{-c \cdot t},$$

where t = time and a, b, and c are coefficients. The GEC was calculated according to

$$GEC = ln(a),$$

where In(a) is the natural logarithm of the coefficient (a).

Statistics

Subject characteristics are reported as mean ± SD. Results are reported as mean ± SEM. Integration was performed using the trapezoidal rule. Integrated incremental responses only describe changes above baseline.

Repeated-measures ANOVA (Statistica 5.0; StatSoft, Tulsa, AZ) was performed on all parameters determined over the whole duration of the experiments, considering the paired (crossover) design. Independent variables were treatment with vildagliptin (*vs.* placebo) and with glibenclamide (*vs.* placebo), and subject (as a random variable). Dependent variables were either time courses of outcome variables as depicted in the figures (repeated-measures design) or integrated values (areas under the curve). As results, treatment effects of vildagliptin and glibenclamide and their interaction with time and/or each other are reported in the figures and tables. If a significant difference regarding treatment effects for vildagliptin or glibenclamide, or a significant interaction of these treatment effects with time ($P < 0.05$) were documented, results at single time points were compared by ANOVA using the same independent

TABLE 1. Participant characteristics (n = 16)

Parameter	Value	Range
Race		
No. of Caucasians (%)	15 (93.8)	
No. of Blacks (%)	1 (6.3)	
Mean age ± SD (yr)	29 ± 10	18–52
Mean BMI ± SD (kg/m^2)	25.3 ± 2.8	22.1–30.5
Mean fasting plasma glucose ± SD (mmol/liter)	4.5 ± 0.3	4.0–5.2
Mean 2-h plasma glucose ± SD (mmol/liter)[a]	4.5 ± 0.8	3.3–6.0

BMI, Body mass index.
[a] After 75-g oral glucose.

variables. *P* values less than 0.05 were taken to indicate significant differences.

Results

Glucose concentrations and insulin secretion after oral glucose

The ingestion of 5 mg glibenclamide enhanced insulin and C-peptide responses ($P < 0.0005$ for both) and provoked hypoglycemia occurring at approximately 120–240 min after the ingestion of glucose (Fig. 1). Vildagliptin alone did not enhance insulin secretory responses if compared with placebo (Fig. 1, B and C), whereas vildagliptin in addition to glibenclamide led to somewhat higher insulin and C-peptide responses than glibenclamide alone. These differences were not significant (Fig. 1, B and C, and Table 2). The degree of hypoglycemia was similar with glibenclamide alone, and with glibenclamide and vildagliptin administered simultaneously. This was the case, no matter whether: 1) the individual glucose nadir (2.6 ± 0.1 mmol/liter with glibenclamide alone *vs.* 2.6 ± 0.1 mmol/liter with glibenclamide/vildagliptin; vildagliptin: $P = 0.15$; glibenclamide: $P < 0.0001$; interaction: $P = 0.19$); or 2) reductions in integrated glucose concentration below the baseline (vildagliptin: $P = 0.31$; glibenclamide: $P < 0.0001$; interaction: $P = 0.96$) were compared.

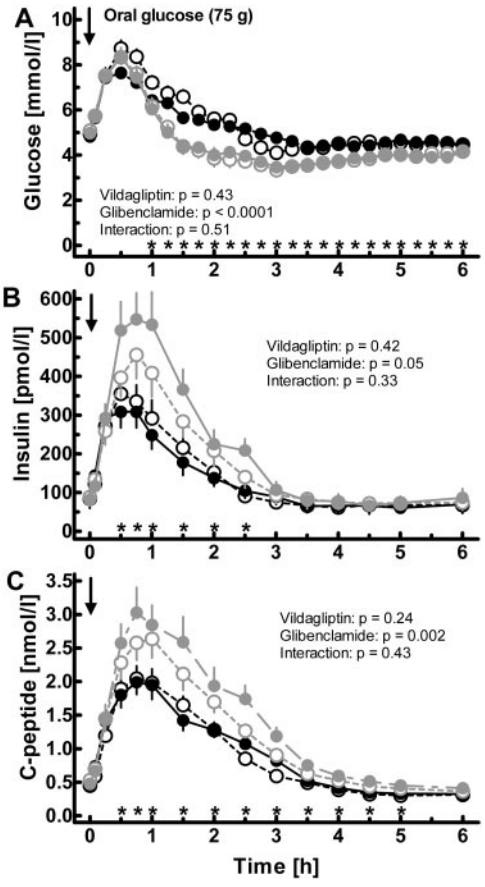

FIG. 1. Concentrations of glucose (A), insulin (B), and C-peptide (C) over 6 h after an oral glucose load (75 g in 300 ml, *arrow*) 30 min after the oral intake of placebo (O- - - -O), 100 mg vildagliptin (●—●), 5 mg glibenclamide (○- - - -○), or 100 mg vildagliptin and 5 mg glibenclamide (◍—◍) in 16 healthy subjects. Experiments with and without vildagliptin are shown as *full vs. open symbols*, experiments with glibenclamide are shown in *gray*, whereas experiments without glibenclamide are shown in *black*. Mean ± SEM (n = 16). *Asterisks* indicate time points with a significant difference due to the administration of glibenclamide.

Total GLP-1 responses after oral glucose

Total GLP-1 concentrations increased after oral glucose, from baseline concentrations of 10.0 ± 1.7 to 25.6 ± 4.6 pmol/liter after 15 min with placebo (Fig. 2A). In both experiments with vildagliptin (with or without additional glibenclamide), the integrated increments in GLP-1 were reduced by 72% (in the absence of glibenclamide) and 48% (with glibenclamide) (vildagliptin: $P < 0.0001$). Glibenclamide had no influence on the pattern of GLP-1 responses after oral glucose (glibenclamide: $P = 0.31$), and there was no interaction of the effects (interaction: $P = 0.26$). Unfortunately, not enough blood samples with adequate additives were available for the determination of intact, biologically active GLP-1.

Total GIP responses after oral glucose

Total GIP concentrations increased after oral glucose, from 9.5 ± 3.4 to 43.9 ± 4.7 pmol/liter 30 min after glucose ingestion (Fig. 2B). In both experiments with vildagliptin, there was a reduction in the integrated increments in GIP by 26% (in the absence of glibenclamide) and 21% (with glibenclamide) (vildagliptin: $P = 0.017$). Glibenclamide had no effect on GIP secretion (glibenclamide: $P = 0.44$) ,and there was no interaction of the two treatments (interaction: $P = 0.69$).

Counter-regulatory hormone responses

Integrated increments of glucagon and cortisol were calculated during hypoglycemic counter-regulation (2–6 h) (Table 2) that started when the positive glycemic excursion ended approximately 120 min after glucose ingestion. The increase in glucagon (Fig. 3A) and cortisol (Fig. 3B) was accentuated in experiments with glibenclamide, and mirrored the occurrence of sulfonylurea-induced hypoglycemia (Fig. 1A). Vildagliptin had no significant influence on glucagon ($P = 0.83$) or cortisol ($P = 0.21$) responses.

Gastric emptying

There was no difference in the time course of gastric emptying among the four experiments (Fig. 4). Along this line, the primary parameters M, k, and β estimated by nonlinear regression analysis did not differ significantly (details not shown). However, there were subtle, statistically significant but clinically probably irrelevant, influences of vildagliptin on the GEC ($P < 0.05$), an overall index of the velocity of gastric emptying (Table 3), and a trend to longer gastric emptying half-time that was, however, not significant ($P = 0.08$). Glibenclamide had no measurable influence on the velocity of gastric emptying (Fig. 4 and Table 3).

Discussion

The present results indicate that glibenclamide, administered with an oral glucose load to healthy human subjects,

TABLE 2. Integrated incremental increases during oral glucose load

Parameter	Experiment				Significance (*P* values)		
	Placebo	Vildagliptin	Glibenclamide	Vildagliptin + glibenclamide	Related to the effect of vildagliptin	Related to the effect of glibenclamide	Interaction
C_{min} glucose (mmol/liter)	3.6 ± 0.11	3.9 ± 0.09	2.6 ± 0.14	2.6 ± 0.09	0.15	<0.0001	0.19
0- to 6-h glucose (mmol·l^{-1}·h)[a]	2.4 ± 0.6	3.2 ± 0.8	3.2 ± 1.1	1.8 ± 1.1	0.31	<0.0001	0.96
0- to 6-h insulin (pmol·l^{-1}·h)[a]	300 ± 75	235 ± 64	411 ± 82	713 ± 122	0.32	<0.0005	0.07
0- to 6-h C-peptide (nmol·l^{-1}·h)[a]	2.5 ± 0.4	2.7 ± 0.3	3.6 ± 0.6	5.2 ± 0.6	0.10	<0.0005	0.18
2- to 6-h glucagon (pmol·l^{-1}·h)[b]	606 ± 89	655 ± 153	1272 ± 223	1152 ± 217	0.83	<0.01	0.65
2- to 6-h cortisol (pmol·l^{-1}·h)[b]	292 ± 108	118 ± 33	1174 ± 190	1081 ± 234	0.21	<0.0001	0.98
0- to 6-h GLP-1 (pmol·l^{-1}·h)[a]	1577 ± 169	439 ± 169	1559 ± 177	808 ± 169	<0.0001	0.31	0.26
0- to 6-h GIP (pmol·l^{-1}·h)[a]	4637 ± 408	3448 ± 408	4148 ± 427	3287 ± 408	<0.05	0.44	0.69

Mean ± SEM (n = 16).
[a] Integrated incremental (above baseline values) responses between 0 min (baseline before glucose ingestion) and 6 h.
[b] Integrated incremental (above baseline values) responses between 2 h (*i.e.* when the lowest plasma glucose concentrations occurred and counter-regulatory responses started) and 6 h.

leads to hypoglycemia but that this is not further aggravated by the coadministration of a DPP-4 inhibitor, vildagliptin. Our results indicate that, at least after single doses, the combination of glibenclamide and vildagliptin is safe, and does not lead to unexpected interactions with respect to insulin secretory responses (Fig. 2 and Table 2), counter-regulatory hormone secretion in response to sulfonylurea-induced hypoglycemia (Fig. 3 and Table 2), or the occurrence of or accentuation of hypoglycemia (Fig. 2). It is likely that the risk of sulfonylurea-induced hypoglycemia would be even lower in patients with type 2 diabetes because it requires a brisk increase in insulin secretion (22), which is diminished or absent in patients with type 2 diabetes (23, 24). Because our study was by intention performed under conditions characterized by marked hypoglycemia induced by glibenclamide (Fig. 2A), any potential worsening due to vildagliptin would be expected to produce rather severe hypoglycemia, thus underscoring the potential clinical relevance of our findings. One still needs to observe carefully the clinical consequences of a combined administration of sulfonylureas and DPP-4 inhibitors in patients with type 2 diabetes because results from studying healthy volunteers cannot entirely replace safety assessments in the true target population.

The secretion of both incretin hormones in response to a single administration of the DPP-4 inhibitor vildagliptin, as indicated by total GLP-1 and total GIP concentrations (Fig. 1 and Table 2), was reduced significantly to a degree great enough to be of biological importance. This finding is in line

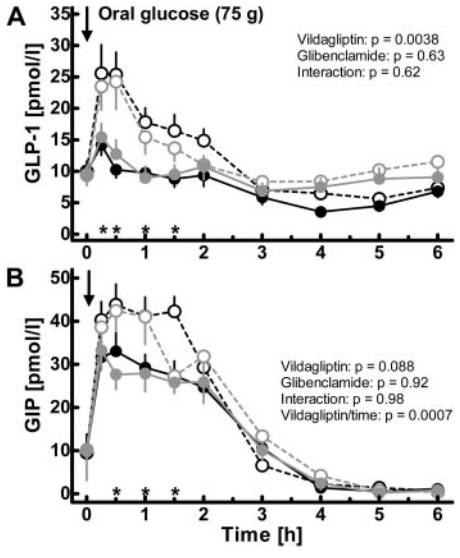

FIG. 2. Concentrations of total GLP-1 (A) and total GIP (B) over 6 h after an oral glucose load (75 g in 300 ml, *arrow*) 30 min after the oral intake of placebo, 100 mg vildagliptin, 5 mg glibenclamide, or 100 mg vildagliptin and 5 mg glibenclamide in 16 healthy subjects. Mean ± SEM (n = 16). The symbols used and details of the statistical analysis are identical to the description in the legend to Fig. 1. Statistical analysis was performed with repeated-measures ANOVA (treatment with vildagliptin and/or glibenclamide *vs.* placebo as independent variables). *P* values for treatment effects with vildagliptin and glibenclamide *vs.* placebo and their interaction are reported. *Asterisks* indicate time points with a significant difference due to the administration of vildagliptin.

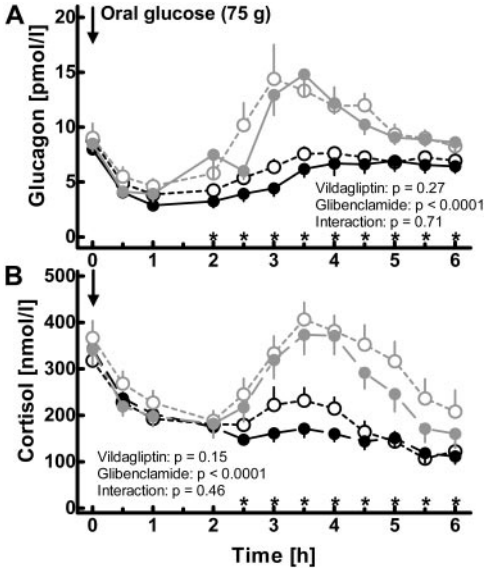

FIG. 3. Concentrations of glucagon (A) and cortisol (B) over 6 h after an oral glucose load (75 g in 300 ml, *arrow*) 30 min after the oral intake of placebo, 100 mg vildagliptin, 5 mg glibenclamide, or 100 mg vildagliptin and 5 mg glibenclamide in 16 healthy subjects. Mean ± SEM (n = 16). The symbols used and details of the statistical analysis are identical to the description in the legend to Fig. 1. *Asterisks* indicate time points with a significant difference due to the administration of glibenclamide.

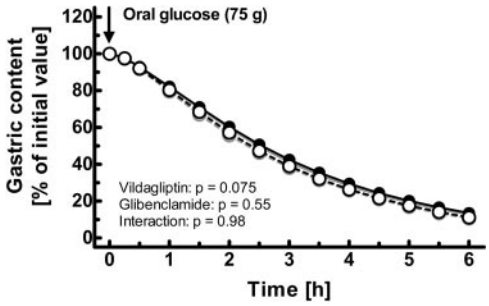

FIG. 4. Gastric emptying as percentage of the initial gastric content over 6 h after an oral glucose load (75 g in 300 ml, *arrow*) 30 min after the oral intake of placebo, 100 mg vildagliptin, 5 mg glibenclamide, or 100 mg vildagliptin and 5 mg glibenclamide in 16 healthy subjects. Mean ± SEM (n = 16). The symbols used and details of the statistical analysis are identical to the description in the legend to Fig. 1. Due to similarities in results (see Table 3 for further statistical analysis), some symbols are invisible (covered by other symbols).

with previous data in dogs published by Deacon *et al*. (6) but is at variance with data in diabetic *fa/fa* Zucker rats published by Balkan *et al*. (25). In both these studies, the postprandial response of intact, biologically active GLP-1 was enhanced by DPP-4 inhibition. In a third study (in diabetic mini pigs), no significant change in the secretion of GIP and GLP-1 was described (26). Our study is the first description of a reduction in the prandial GLP-1 response, as measured by levels of total GLP-1, in the presence of DPP-4 inhibition in human subjects. The magnitude of this effect is not trivial, amounting to a 72% reduction in integrated incremental responses of total GLP-1 (Fig. 1 and Table 2). Recently, similar findings have been reported with sitagliptin, another DPP-4 inhibitor (12), although the degree of suppression was smaller. Different DPP-4 inhibitors may differ in their potency to reduce L-cell secretion, and the type of nutrients (oral glucose *vs.* mixed meals) may determine the magnitude of the response.

The reduction in GLP-1 secretion (Fig. 2A) in response to DPP-4 inhibition may be an important finding for several reasons. First, the acute reduction of L-cell secretion after nutrient stimulation in response to DPP-4 inhibition suggests a feedback inhibition in the sense that higher concentrations of intact, biologically active GLP-1 suppress L-cell secretion triggered by nutrients like oral glucose ingestion. Because we have not measured intact GLP-1 in the present study, our assumptions regarding the behavior of intact GLP-1 concentrations rely on published data, which uniformly have described a significant augmentation of the nutrient-related responses to approximately double the values observed with placebo with vildagliptin (8, 10, 27) and sitagliptin (12). We do not doubt that our data regarding total GLP-1 (Fig. 2A) are compatible with similar findings in the present study, had intact, biologically active GLP-1 been measured. However, it is not known whether the elevated intact GLP-1 concentration after DPP-4 inhibition is sensed by the L cell itself or elsewhere, such as afferent nerve endings of the parasympathetic autonomous nervous system (28, 29), or, more likely, somatostatin-producing D cells in the neighborhood of L cells, that could influence GLP-1 secretion by paracrine action (7). It will be of interest to study this effect with more prolonged inhibition of DPP-4, especially considering the fact that the potential feedback inhibition was evident only hours after the administration of a single dose of vildagliptin (Fig. 2). It remains to be determined whether this effect will remain the same, or be of greater or lesser magnitude over longer periods of time.

Second, the inhibition of the secretion of total GLP-1 and total GIP in response to vildagliptin raises the questions as to whether the increases in intact, biologically active GLP-1 and GIP are sufficient to explain the antidiabetic effects that have been seen with DPP-4 inhibition (30). The degree by which intact GLP-1 is elevated is modest (8, 25, 30–32). Previous studies with exogenous GLP-1 have suggested that variations of GLP-1 concentrations within the physiological range have little if any effect on insulin secretion, and that pharmacological concentrations of GLP-1 are needed to stimulate insulin secretion and normalize plasma glucose in type 2 diabetic patients (33). Deacon *et al*. (5), using a fixed dose of exogenous GLP-1 in combination with DPP-4 inhibition, found an elevation of at least 5-fold in intact, biologically active GLP-1 (and no change in total GLP-1) in pigs. Our study suggests that the increments in intact GLP-1 that can be measured after DPP-4 inhibition in a therapeutic situation are smaller in comparison to the experimental conditions used by Deacon *et al*. (5). Such findings have raised a debate as to whether GLP-1 is the main or only mediator of antidiabetic effects of DPP-4 inhibition (29, 30). It remains open whether venous plasma concentrations of GLP-1 are representative for its biological actions because concentrations in intestinal capillaries or in the hepatoportal region may be of particular importance (29, 34). It has, for example, been shown (35) that the postglucose load portal levels, which are believed to be relevant to the islet actions of GLP-1, are 60% higher than arterial levels in dogs. In addition, it needs to be studied whether similar results would be obtained in type 2 diabetic subjects as well.

Our results further indicate that a reduction in the secretion of GLP-1 is not compensated for by an oversecretion of GIP (Fig. 2 and Table 2). On the contrary, GIP secretion itself was significantly reduced, although the magnitude of the

TABLE 3. Parameters characterizing the velocity of gastric emptying after oral glucose loads

Parameter	Experiment (active drugs)				Significance (*P* values)		
	Placebo	Vildagliptin	Glibenclamide	Vildagliptin + glibenclamide	Related to the effect of vildagliptin	Related to the effect of glibenclamide	Interaction
Half-emptying time (min)	143 ± 5	154 ± 5	141 ± 7	151 ± 6	0.08	0.69	0.92
Lag time (min)	63 ± 4	68 ± 4	65 ± 6	68 ± 3	0.39	0.77	0.78
GEC (U)	3.23 ± 0.04	3.14 ± 0.03	3.25 ± 0.08	3.12 ± 0.04	0.044	0.97	0.68

Mean ± SEM (n = 16). In one subject treated with placebo, the GEC could not be calculated because nonlinear regression analysis failed to estimate the parameters a, b, and c. See *Subjects and Methods* for details.

effect was smaller than for GLP-1. This appears to correlate with the degree of augmentation of intact GLP-1 and/or GIP concentrations in response to DPP-4 inhibition (4, 5).

The reduction in the oral glucose-induced secretion of GLP-1 and GIP (Fig. 1 and Table 2) was demonstrated both in experiments with and without the coadministration of glibenclamide. The effect was of similar magnitude under both conditions. This attests to the robustness of the effect and indicates that glibenclamide had no influence by itself. The fact that glibenclamide, although effectively producing hypoglycemia (Fig. 2), did not change the secretion of GLP-1 or GIP does not support the idea that L and/or K cells express sulfonylurea receptors and/or ATP-dependent potassium channels, as suggested (36), or at least not that they are important in initiating or regulating L-cell secretory behavior under the conditions studied here. It may be interesting to speculate that it is the feedback inhibition of GLP-1 secretion that partially protects from hypoglycemia when using the combination of oral antidiabetic agents studied here. In this connection, it is also interesting to note that the concomitant administration of a sulfonylurea with a GLP-1 mimetic increases the incidence of hypoglycemia (37).

DPP-4 inhibition with vildagliptin caused marginal changes in gastric emptying (Fig. 4), most prominently a significant change in the GEC. This may indicate that intact GLP-1 concentrations were elevated sufficiently to cause this effect, but not to the degree previously achieved with exogenous GLP-1 (21). This finding may be viewed as being at variance with a previous observation in cynomolgus monkeys (31). However, these changes were rather small as well. The fact that GLP-1 secretion was reduced by a feedback mechanism (Fig. 2 and Table 2) probably contributed to minimizing the potential deceleration in gastric emptying (30).

In the present study, vildagliptin, alone or in combination with glibenclamide, had no significant effect on parameters characterizing insulin secretion, glucagon, or glucose concentrations (Figs. 1 and 3, and Table 2). Only a trend was seen for vildagliptin added to glibenclamide toward an augmentation of insulin secretion relative to the study with glibenclamide alone. This may be viewed as a discrepancy to studies describing antidiabetic actions along with a reduction in glucagon responses and compelling evidence of an augmentation in glucose-stimulated insulin secretion in patients with type 2 diabetes (8, 32). The obvious differences are that our study was performed with a single administration of vildagliptin, rather than after continued treatment for at least 4 wk, and that healthy subjects were studied compared with type 2 diabetic patients. It had been noted previously with another DPP-4 inhibitor, P 32/98, that a single dose was without significant effects on the same parameters (38). Therefore, DPP-4 inhibitors may significantly differ in their activity comparing healthy and diabetic populations. This could mean that if conditions could be found under which vildagliptin has a more profound influence on glycemia and/or insulin, this would be a better protocol to test our hypothesis. However, we do not know conditions that appear better suited for this purpose.

In conclusion, the combination of glibenclamide with the DPP-4 inhibitor vildagliptin does not provoke more hypoglycemia after oral glucose than does glibenclamide alone. A single dose of vildagliptin administered before an oral glucose load greatly reduces the total GLP-1 secretory response, suggesting feedback inhibition of GLP-1 secretion involving sensing of intact, biologically active GLP-1.

Acknowledgments

We thank B. Nawroth and E. Herold for their excellent technical assistance. We also thank Mrs. S. Petrick for preparing figures and for secretarial assistance.

Received September 6, 2006. Accepted August 2, 2007.

Address all correspondence and requests for reprints to: Prof. Dr. med. Michael Nauck, Diabeteszentrum Bad Lauterberg, Kirchberg 21, D-37431 Bad Lauterberg im Harz, Germany. E-mail: Michael.Nauck@diabeteszentrum.de.

Disclosure Statement: A.E.-O. and E.R. have nothing to declare. A.S., J.F., and D.H. are employees of Novartis Pharma. M.A.N. has received consulting and speaker's honoraria, and grant support from Novartis Pharma. J.J.H. has received lecture fees and grant support from Novartis Pharma.

References

1. **Mentlein R** 1999 Dipeptidyl-peptidase IV (CD26)–role in the inactivation of regulatory peptides. Regul Pept 85:9–24
2. **Holst JJ, Deacon CF** 1998 Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. Diabetes 47:1663–1670
3. **Deacon CF, Holst JJ** 2002 Dipeptidyl peptidase IV inhibition as an approach to the treatment and prevention of type 2 diabetes: a historical perspective. Biochem Biophys Res Commun 294:1–4
4. **Deacon CF, Danielsen P, Klarskov L, Olesen M, Holst JJ** 2001 Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and potentiates its insulinotropic and antihyperglycemic effects in anesthetized pigs. Diabetes 50:1588–1597
5. **Deacon CF, Hughes TE, Holst JJ** 1998 Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig. Diabetes 47:764–769
6. **Deacon CF, Wamberg S, Bie P, Hughes TE, Holst JJ** 2002 Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. J Endocrinol 172:355–362
7. **Hansen L, Hartmann B, Mineo H, Holst JJ** 2004 Glucagon-like peptide-1 secretion is influenced by perfusate glucose concentration and by a feedback mechanism involving somatostatin in isolated perfused porcine ileum. Regul Pept 118:11–18
8. **Åhren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A** 2004 Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab 89:2078–2084
9. **Åhren B, Gomis R, Standl E, Mills D, Schweizer A** 2004 Twelve- and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. Diabetes Care 27:2874–2880
10. **Mari A, Sallas WM, He YL, Watson C, Ligueros-Saylan M, Dunning BE, Deacon CF, Holst JJ, Foley JE** 2005 Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed β-cell function in patients with type 2 diabetes. J Clin Endocrinol Metab 90:4888–4894
11. **Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, Snyder K, Hilliard D, Tanen M, Tanaka W, Wang AQ, Zeng W, Musson D, Winchell G, Davies MJ, Ramael S, Gottesdiener KM, Wagner JA** 2005 Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther 78: 675–688
12. **Herman GA, Bergman A, Stevens C, Kotey P, Yi B, Zhao P, Dietrich B, Golor G, Schrodter A, Keymeulen B, Lasseter KC, Kipnes MS, Snyder K, Hilliard D, Tanen M, Cilissen C, De Smet M, de Lepeleire I, Van Dyck K, Wang AQ, Zeng W, Davies MJ, Tanaka W, Holst JJ, Deacon CF, Gottesdiener KM, Wagner JA** 2006 Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. J Clin Endocrinol Metab 91: 4612–4619
13. **El-Ouaghlidi A, Rehring E, Schweizer A, Holmes D, Nauck MA** 2003 The dipeptidyl peptidase IV inhibitor LAF237 does not accentuate reactive hypoglyaemia caused by the sulfonylurea glibenclamide administered before an oral glucose load in healthy subjects. Diabetes 52(Suppl 1):A118 (Abstract 507-P)
14. **El-Ouaghlidi A, Rehring E, Holst JJ, Schweizer A, Holmes D, Nauck MA** 2004 Reduced increments in total glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) in plasma after a single dose of the dipeptidyl

peptidase-4 inhibitor LAF237 before an oral glucose load in healthy subjects. Diabetologia 47(Suppl 1):A (Abstract 786)
15. **Krarup T, Madsbad S, Moody A, Regeur L, Faber O, Holst J, Sestoft L** 1983 Diminished immunoreactive gastric inhibitory polypeptide response to a meal in newly diagnosed type I diabetics. J Clin Endocrinol Metab 56:1306–1312
16. **Ørskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ** 1994 Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 43:535–539
17. **Vilsbøll T, Krarup T, Deacon CF, Madsbad S, Holst JJ** 2001 Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes 50:609–613
18. **Holst JJ** 1982 Evidence that peak II GLI or enteroglucagon is identical to the C-terminal sequence (residues 33–69) of glicentin. Biochem J 207:381–388
19. **Ghoos YE, Maes BD, Gaypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G** 1993 Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology 104:1640–1647
20. **Braden B, Adams S, Duan LP, Orth KH, Maul FD, Lembcke B, Hör G, Caspary WF** 1995 The [13C]acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. Gastroenterology 108:1048–1055
21. **Meier JJ, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, Nauck MA** 2003 Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. J Clin Endocrinol Metab 88:2719–2725
22. **Toft-Nielsen M, Madsbad S, Holst JJ** 1998 Exaggerated secretion of glucagon-like peptide-1 (GLP-1) could cause reactive hypoglycaemia. Diabetologia 41:1180–1186
23. **Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D** 1984 Diminished B cell secretory capacity in patients with non-insulin-dependent diabetes mellitus. J Clin Invest 74:1318–1328
24. **Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Frank BH, Galloway JA, Van Cauter E** 1988 Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. N Engl J Med 318:1231–1239
25. **Balkan B, Kwasnik L, Miserendino R, Holst JJ, Li X** 1999 Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7–36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. Diabetologia 42:1324–1331
26. **Olholm Larsen M, Rolin B, Ribel U, Wilken M, Deacon CF, Svendsen O, Gotfredsen CF, Carr RD** 2003 Valine pyrrolidide preserves intact glucose-dependent insulinotropic peptide and improves abnormal glucose tolerance in minipigs with reduced β-cell mass. Exp Diabetes Res 4:93–105
27. **Åhren B, Simonsson E, Larsson H, Landin-Olsson M, Torgeirsson H, Jansson P-A, Sandquist M, Bavenholm P, Efendic S, Eriksson JW, Dickinson S, Holmes D** 2002 Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. Diabetes Care 25:869–875
28. **Hansen L, Hartmann B, Bisgaard T, Mineo H, Jorgensen PN, Holst JJ** 2000 Somatostatin restrains the secretion of glucagon-like peptide-1 and -2 from isolated perfused porcine ileum. Am J Physiol Endocrinol Metab 278:E1010–E1018
29. **Holst JJ, Deacon CF** 2005 Glucagon-like peptide-1 mediates the therapeutic actions of DPP-IV inhibitors. Diabetologia 48:612–615
30. **Nauck MA, El-Ouaghlidi A** 2005 The therapeutic actions of DPP-IV inhibition are not mediated by glucagon-like peptide-1 (for debate). Diabetologia 48:608–611
31. **Dardik B, Valentin M, Schwartzkopf C, Gutierrez C, Stevens D, Russell ME, Edwin V, Hughes T** 2003 NVP-LAF237, a dipeptidyl peptidase IV inhibitor, improves glucose tolerance and delays gastric emptying in obese insulin resistant Cynomolgus monkeys. Diabetes 52(Suppl 1):A322 (Abstract 1292-P)
32. **Åhren B, Pacini G, Foley JE, Schweizer A** 2005 Improved meal-related beta-cell function and insulin sensitivity by the dipeptidyl peptidase-IV inhibitor vildagliptin in metformin-treated patients with type 2 diabetes over 1 year. Diabetes Care 28:1936–1940
33. **Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W** 1993 Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 91:301–307
34. **Burcelin R, Da Costa A, Drucker D, Thorens B** 2001 Glucose competence of the hepatoportal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. Diabetes 50:1720–1728
35. **Dardevet D, Moore MC, Neal D, DiCostanzo CA, Snead W, Cherrington AD** 2004 Insulin-independent effects of GLP-1 on canine liver glucose metabolism: duration of infusion and involvement of hepatoportal region. Am J Physiol Endocrinol Metab 287:E75–E81
36. **Gribble FM, Tucker SJ, Seino S, Ashcroft FM** 1998 Tissue specificity of sulfonylureas. Studies on cardiac and β-cell K_{ATP} channels. Diabetes 47:1412–1418
37. **Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A, Kim D, Baron AD** 2003 Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patients with type 2 diabetes. Diabetes Care 26:2370–2377
38. **Hoffmann T, Iwanow K, Wolf R, Demuth H, Nauck M** 2002 The important effect of meal stimulated incretin secretion on blood sugar regulation could not be improved by the DP IV-inhibitor P 32/98 in healthy subjects. Diabetologia 45(Suppl 2):A176 (Abstract 543)

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.