



LUND UNIVERSITY

Vildagliptin Enhances Islet Responsiveness to Both Hyper- and Hypoglycemia in Patients with Type 2 Diabetes.

Ahrén, Bo; Schweizer, Anja; Dejager, Sylvie; Dunning, Beth E; Nilsson, Peter; Persson, Margaretha; Foley, James E

Published in:
Journal of Clinical Endocrinology and Metabolism

DOI:
[10.1210/jc.2008-2152](https://doi.org/10.1210/jc.2008-2152)

2009

[Link to publication](#)

Citation for published version (APA):
Ahrén, B., Schweizer, A., Dejager, S., Dunning, B. E., Nilsson, P., Persson, M., & Foley, J. E. (2009). Vildagliptin Enhances Islet Responsiveness to Both Hyper- and Hypoglycemia in Patients with Type 2 Diabetes. *Journal of Clinical Endocrinology and Metabolism*, 94, 1236-1243. <https://doi.org/10.1210/jc.2008-2152>

Total number of authors:
7

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00



LUND UNIVERSITY
Faculty of Medicine

LUP

Lund University Publications

Institutional Repository of Lund University

This is an author produced version of a paper published in
The Journal of clinical endocrinology and metabolism.
This paper has been peer-reviewed but does not include
the final publisher proof-corrections or journal pagination.

Citation for the published paper:

Bo Ahrén, Anja Schweizer, Sylvie Dejager, Beth E Dunning,
Peter Nilsson, Margaretha Persson, James E Foley

Vildagliptin Enhances Islet Responsiveness to Both Hyper-
and Hypoglycemia in Patients with Type 2 Diabetes.

The Journal of clinical endocrinology and metabolism,
2009 Jan 27. [Epub ahead of print]

<http://dx.doi.org/10.1210/jc.2008-2152>

Access to the published version may
require journal subscription.

Published with permission from:
Endocrine Society

Vildagliptin Enhances Islet Responsiveness to Both Hyper- and Hypoglycemia in Patients With Type 2 Diabetes

Bo Ahrén, Anja Schweizer, Sylvie Dejager, Beth E. Dunning, Peter M. Nilsson, Margaretha Persson, James E. Foley

Department of Clinical Sciences, Lund University, Lund, Sweden (B.A., M.P.); Novartis Pharma AG, Basel, Switzerland (A.S.) and Rueil Malmaison, France (S.D.); PharmaWrite, LLC, Princeton, NJ, USA (B.E.D); University Hospital, Malmö, Sweden (P.M.N.); Novartis Pharmaceuticals, East Hanover, NJ, USA (J.E.F.)

Corresponding author:

Bo Ahrén, MD, PhD

Department of Clinical Sciences, Lund University

B11 BMC, Lund, Sweden

SE-221 84

Phone: +46462220758

Fax: +46462220757

E-mail: bo.ahren@med.lu.se

Running title: Vildagliptin and islet glucose sensing

Word count: 3574, 3 tables, 2 figures

Key terms: glucagon, hypoglycemia, type 2 diabetes, vildagliptin

This trial (NCT00390520) is registered with ClinicalTrials.gov.

Precis: We conclude that vildagliptin enhances α -cell responsiveness to both the suppressive effects of hyperglycemia and the stimulatory effects of hypoglycemia.

ABSTRACT

Context: Dipeptidyl peptidase-4 (DPP-4) inhibitors act by increasing plasma levels of glucagon-like peptide-1 (GLP-1) and suppressing excessive glucagon secretion in patients with type 2 diabetes (T2DM). However, their effects on the glucagon response to hypoglycemia are not established.

Objective: Assess effects of the DPP-4 inhibitor vildagliptin on α -cell response to hyper- and hypoglycemia.

Design: Single-center, randomized, double-blind, placebo-controlled, two-period crossover study of 28-d treatment, with a 4-wk between-period washout

Setting: Participants received study drug as outpatients.

Patients: Drug-naïve patients with T2DM and baseline $\text{HbA}_{1c} \leq 7.5\%$

Intervention: Vildagliptin (100 mg qd) or placebo

Primary Outcome Measure(s): 1) Change in plasma glucagon levels during hypoglycemic (2.5 mM glucose) clamp. 2) Incremental (Δ) glucagon area under the concentration-time curve from time 0 to 60 min ($\text{AUC}_{0-60\text{min}}$) during standard meal test. Before the study, it was hypothesized that vildagliptin would suppress glucagon secretion during meal tests and enhance the glucagon response to hypoglycemia.

Results: The mean change in glucagon during hypoglycemic clamp was 46.7 ± 6.9 ng/liter with vildagliptin treatment and 33.9 ± 6.7 ng/liter with placebo; the between-treatment difference was 12.8 ± 7.0 ng/liter ($P=0.039$), representing a 38% increase with vildagliptin. In contrast, the mean glucagon $\Delta\text{AUC}_{0-60\text{min}}$ during meal test with vildagliptin was 512 ± 163 ng/liter•min vs 861 ± 130 ng/liter•min with placebo; the between-treatment difference was -349 ± 158 ng/liter•min ($P=0.019$), representing a 41% decrease with vildagliptin.

Conclusions: Vildagliptin enhances α -cell responsiveness to both the suppressive effects of hyperglycemia and the stimulatory effects of hypoglycemia. These effects likely contribute to the efficacy of vildagliptin to improve glycemic control as well as to its low hypoglycemic potential.

Introduction

Vildagliptin is a potent and selective dipeptidyl peptidase-4 (DPP-4) inhibitor that improves glycemic control in patients with type 2 diabetes (T2DM) when given as monotherapy (1-3), or in combination with metformin (4), thiazolidinediones (5, 6), sulfonylureas (7), or insulin (8). By inhibiting DPP-4, vildagliptin increases plasma levels of the intact, active form of glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) (8-13). The glucose-dependent insulinotropic effects of both GLP-1 and GIP, as well as the glucagonostatic effect of GLP-1, are thought to underlie the therapeutic efficacy of vildagliptin (5, 6, 8-13).

The relative contributions of improvements in α - and β -cell function are debatable. However, the correlation between the suppression of the glucagon response to meal ingestion and the improved glucose tolerance after treatment with vildagliptin (9), and the observation that vildagliptin significantly reduced $1cA_{1c}$ (HbA_{1c} in patients with T2DM treated with high-dose (>80 U/d) insulin monotherapy (8) clearly indicate that the contribution of glucagon suppression is not negligible. Because of the critical role that glucagon plays in the prevention or correction of hypoglycemia (14), it was possible that suppressing glucagon secretion with vildagliptin would predispose insulin-treated patients to hypoglycemia. However, hypoglycemia was less frequent and less severe when vildagliptin was given as an add-on to insulin (vs placebo added to insulin), suggesting that vildagliptin may exert a protective effect against severe hypoglycemia (8). Accordingly, we hypothesized that vildagliptin would increase the glucagon response to hypoglycemia while suppressing glucagon during hyperglycemia in patients with T2DM. To test this hypothesis, the present study was performed with drug-naïve patients with T2DM and mild hyperglycemia ($HbA_{1c} \leq 7.5\%$). After 28-d treatment with vildagliptin (100 mg qd) or placebo, standard breakfast meal tests were performed, followed by stepped glucose clamps (7.5, 5.0, and 2.5 mmol/liter glucose).

Research Design and Methods

Study design. This was a single-center, double-blind, randomized, placebo-controlled, crossover study of 28-d treatment with vildagliptin (100 mg qd) with a 4-wk between-treatment washout period. Each patient

attended one screening visit (Week -4), during which inclusion/exclusion criteria were assessed. Eligible patients were randomized at visit 2 (Day 1) and expected to complete two treatment periods, receiving a different blinded study medication during each period (vildagliptin 100 mg qd and placebo, in random order). At the baseline (Day 1) visit of each treatment period, HbA_{1c}, fasting plasma glucose (FPG), and baseline safety assessments were made and study medication was dispensed for 4 weeks of outpatient treatment.

The test procedure (see below) was performed after an overnight fast, on Day 28 of the first treatment period. Study medication was then discontinued, and a 4-wk washout period occurred before the alternative treatment period. The test procedure was repeated on Day 28 of the second treatment period.

Study population. The study enrolled male and female patients (females of childbearing potential required to use a medically approved birth control method) aged ≥ 18 yr; with a body mass index (BMI) between 22 and 35 kg/m², inclusive; and $_{1c}HbA_{1c} \leq 7.5\%$. Enrollment required patients to have been diagnosed with T2DM at least 6 weeks prior to visit 1, to have received no oral antidiabetic drug (OAD) for at least 12 weeks prior to study entry, and to have never received an OAD for >3 consecutive months at any time in the past. These patients were considered to be representative of a drug-naïve population with mild hyperglycemia.

Patients were excluded if they had a history of type 1 or secondary forms of diabetes, acute metabolic diabetic complications, or evidence of significant diabetic complications. A history of significant cardiac arrhythmia, congestive heart failure (New York Heart Association [NYHA] class III or IV), or liver disease (ie, cirrhosis, chronic active hepatitis) also precluded participation, as did any significant laboratory abnormalities.

Study assessments. The test procedure (performed after overnight fast, following placement of a cannula in an antecubital vein) began with a standard breakfast meal test comprising 180 mL orange juice, 2 slices [60 g] bread; 30 g jam or preserves; 15 g butter or margarine; 120 mL whole milk (or

equivalent amount cheese plus 120 mL water); decaffeinated coffee or tea, supplying 500 kilocalorie (60% carbohydrate, 30% fat, 10% protein). This was followed by a 3-step hyperinsulinemic clamp (glucose 7.5, 5.0 and 2.5 mmol/liter; 45 min per step). A baseline blood sample was taken (time -20 min), then study medication was given 15 min before the meal was provided (time 0 min). A pre-meal blood sample was obtained (time -5 min), and the meal was consumed in 10 min. During the meal test, samples for determination of glucose, glucagon, insulin, and C-peptide were obtained at times -20, -5, 15, 30, 45, 60, 90, 115, and 120 min (with time 120 min serving as baseline for the hyperglycemic step of the clamp). Samples for measurement of intact (active) GLP-1 were obtained at times -20, 30, 60, and 90 min. No further samples were obtained due to limitations on the volume of blood withdrawn.

For the stepped clamp, patients received a primed, continuous infusion of insulin (600 pmol/m²/min, 0-4 min; 500 pmol/m²/min, 4-7 min; 400 pmol/m²/min, 7-10 min, 300 pmol/m²/min thereafter), with glucose infused at a variable rate to achieve three glycemic plateaus: 1) hyperglycemic step of 45 min at 7.5 mmol/liter glucose (times 120 to 165 min), 2) euglycemic step of 45 min at 5.0 mmol/liter glucose (times 165 to 210 min), and 3) hypoglycemic step of 45 min at 2.5 mmol/liter glucose (times 210 to 255 min). After the hypoglycemic step, the insulin infusion was discontinued; glucose was infused if necessary, so that all subjects “began” recovery with a plasma glucose level of 3.2 mmol/liter; subsequently plasma glucose levels were allowed to recover.

In addition to frequent samples for real-time glucose measurements used to adjust the glucose infusion rate (performed by the glucose dehydrogenase technique with technique with a HemoCue[®] device [HemoCueAB, Ängelholm, Sweden]), during the stepped clamps, samples for determination of glucose were obtained every 5 min from time 120 to 255 min, then at 10-min intervals until time 315 min. Samples for measurement of insulin, glucagon, and C-peptide were obtained at times 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270, and 285 min. Additionally, during the hypoglycemic step, samples for measurement of epinephrine, norepinephrine, cortisol, and pancreatic polypeptide (PP) were obtained at times 210, 225, 240, 255, and 285 min.

HbA_{1c} and FPG were measured on Day 1 and Day 28 of both treatment periods. Vital signs and body weight were measured and safety laboratory assessments were made at every study visit.

Assays. All assessments of samples obtained during the test procedure except GLP-1, catecholamines and cortisol were made at Lund University. Plasma glucose was measured with the glucose oxidase method. Glucagon concentrations were analyzed with double-antibody RIA using guinea pig antihuman glucagon antibodies specific for pancreatic glucagon. Insulin concentrations were analyzed with double-antibody RIA technique using guinea pig antihuman insulin antibodies. PP was determined with double-antibody RIA using rabbit antihuman PP antibodies (all Linco Res. Inc., St Charles, MO). Norepinephrine and epinephrine concentrations were determined by high-performance liquid chromatography. Cortisol was determined with the Beckman Coulter Access[®] Immunoassay System (Fullerton, CA). These analyses were performed by the Department of Clinical Chemistry, University Hospital, Malmö.

GLP-1 was measured at Wuxi PharmaTech Co (Shanghai, China) by ELISA with an N-terminally directed antiserum, thus detecting only intact, biologically active GLP-1. HbA_{1c}, FPG, and safety laboratory assessments were made by Covance (Geneva, Switzerland). Assays were performed according to standardized and validated procedures and good laboratory practice.

Data analysis. The insulin secretory rate (ISR) was estimated by deconvolution of C-peptide levels. From samples obtained during the standard meal test, the total and incremental areas under the curve (AUC and Δ AUC) for the times 0 to 60 min, 0 to 90 min, and 0 to 120 min were calculated by the trapezoidal method for all analytes. Insulin secretion corrected for glucose ($ISR/G = ISR \text{ AUC} \div \text{glucose AUC}$) was used as an index of β -cell function. During the hypoglycemic clamp step, the change from baseline (time 210 min) to endpoint (time 255 min) was calculated for all analytes. During the euglycemic and hypoglycemic clamp steps, insulin sensitivity was estimated by the glucose infusion rate divided by the mean plasma insulin level.

Between-treatment differences in each of the aforementioned variables were made with paired *t* tests in the completers population. Because the “direction” of change in each parameter was predicted (hypothesized) before study inception, one-sided tests were performed, with a 0.05 significance level.

Ethics and good clinical practice. The protocol was approved by the ethics committee of Lund University, Sweden, and all subjects gave written informed consent before entering the study. The study was conducted using good clinical practice and in accordance with the Declaration of Helsinki.

Results

Patients studied. Thirty-two patients were screened, 30 were randomized (15 to each treatment sequence), and 25 patients comprised the completers population, defined as all randomized patients who received at least one dose of study drug and had a valid assessment of the primary variable at the end of each treatment period. Of the 30 randomized patients, 2 had no valid primary efficacy assessment during the double-blind treatment period, 2 had only one valid primary efficacy assessment during the double-blind treatment period, and 1 had hyperglucagonemia (pre-meal glucagon levels >200 ng/liter during both treatment periods); thus, these 5 subjects were excluded from the completers population. **Table 1** summarizes the demographic and baseline characteristics of the completers population.

Patients were all Caucasian and predominantly male, with a mean age, BMI, disease duration, and baseline $_{1c}HbA_{1c}$ of approximately 66 years, 28 kg/m², 6 years, and 6.3%, respectively. Patients randomized to treatment sequence A (vildagliptin, then placebo) had somewhat higher baseline levels of HbA_{1c} and FPG, and somewhat longer disease duration than did patients randomized to treatment sequence B (placebo, then vildagliptin). However, these modest differences should be of no significance because every patient received both treatments, and there was a 4-wk washout period between treatments. **Standard meal tests.** **Figure 1** depicts the time-courses of glucose, GLP-1, glucagon, and ISR during the standard meal test that was performed immediately before the stepped glucose clamp. It may be appreciated that relative to placebo, vildagliptin treatment was associated with lower FPG and postprandial glucose levels and fasting and postprandial glucagon levels, with greatly enhanced fasting and postprandial plasma levels of intact GLP-1, but essentially no effect on absolute ISR.

Table 2 summarizes the integrated responses to the standard meal. Vildagliptin significantly increased the GLP-1 response and significantly decreased the glucagon response to meals, whether expressed as total secretion, or the incremental response, integrated over the first hour of sampling, or the entire post-meal sampling period. Similarly, postprandial glucose levels were significantly decreased during vildagliptin treatment.

The incremental ISR was significantly increased when integrated over the first 60 min, but the absolute ISR was unaffected if the total AUC was considered, regardless of the time interval used, and the incremental insulin response integrated over the 2-h post-meal period showed only a slight trend toward an increase with vildagliptin vs placebo administration. In contrast, insulin secretion relative to glucose was significantly increased, whether integrated over the first 60 min or the entire 2-h post-meal sampling period, and whether the total responses or the incremental responses were considered. Again, the percentage changes in the incremental AUCs were greater than the percentage changes in the total AUCs.

Hyperinsulinemic stepped glucose clamps. **Figure 2** depicts plasma glucose and glucagon levels, and the ISR during the hyperinsulinemic stepped glucose clamps initiated immediately after the standard meal tests on Day 28 of both treatment periods. It can be seen that, although plasma glucose levels were significantly lower in the vildagliptin treatment period during the hyperglycemic step, plasma glucose levels were well matched during the euglycemic and hypoglycemic steps, and plasma glucose levels recovered in <1 h in both treatment periods. Plasma glucagon levels were suppressed during the hyperglycemic step in the vildagliptin treatment period, despite significantly lower plasma glucose levels, and remained suppressed during the euglycemic step. However, during the hypoglycemic clamp step, plasma glucagon levels increased from a significantly lower level at time 210 min, to a level slightly higher and not significantly different at the end of the hypoglycemic clamp (time 255 min) during vildagliptin treatment when compared with placebo administration. Thus, as reported in **Table 3**, the increase in glucagon during hypoglycemia with vildagliptin (change = 46.7 ± 6.9 ng/liter) was significantly greater than the increase during hypoglycemia with placebo (change = 33.9 ± 6.7 ng/liter, $P=0.039$ vs vildagliptin).

The ISR was significantly higher during vildagliptin treatment from times 165 to 255 min of the stepped clamp; (ie, during euglycemia and hypoglycemia) (**Figure 2**). However, during hypoglycemia with vildagliptin treatment, ISR decreased from 151.5 to 81.9 pmol/m²/min (change = -69.6 ± 6.6 pmol/m²/min), whereas with placebo during hypoglycemia, ISR decreased from 134.0 to 71.7 pmol/m²/min (change = -62.3 ± 6.9 pmol/m²/min, $P=0.011$ vs vildagliptin, **Table 3**). Additionally, insulin

sensitivity as quantified by the glucose infusion rate/mean insulin level was significantly greater during the euglycemic step with vildagliptin (0.73 ± 0.46 mg•liter/pmol•min) than with placebo (0.62 ± 0.46 mg•liter/pmol•min, $P=0.011$). Insulin sensitivity was unchanged during the hypoglycemic clamp step (data not shown).

Table 3 summarizes the changes from time 210 to time 255 min in plasma glucagon, ISR, and plasma epinephrine, norepinephrine, cortisol, and PP (ie, the pancreatic, sympathoadrenal, and parasympathetic nervous system responses to hypoglycemia). The increase in plasma glucagon during hypoglycemia was significantly greater, and the suppression of insulin secretion was significantly more pronounced during vildagliptin than placebo administration. The between-treatment differences in the hypoglycemia-induced changes in glucagon and insulin secretion represent a 38% increase in the α -cell response (ie, increase in glucagon) and a 12% increase in the β -cell response (ie, suppression of ISR) with vildagliptin relative to placebo. Although the sympathoadrenal responses (epinephrine, norepinephrine, cortisol) to hypoglycemia did not differ between treatment periods, there was a strong trend toward enhanced PP release during hypoglycemia with vildagliptin vs placebo (between-treatment difference = 51.6 ± 31.5 pmol/liter, $P=0.057$).

Glycemic control. Four-wk treatment with vildagliptin also improved glycemic control, despite low baseline levels of HbA_{1c} (~6.3%). In patients receiving placebo, FPG increased ($\Delta = 0.3 \pm 0.1$ mmol/L) while FPG decreased in patients receiving vildagliptin ($\Delta = -0.5 \pm 0.1$ mmol/liter, $P < 0.001$ vs placebo). Similarly, HbA_{1c} increased slightly in patients receiving placebo ($\Delta = 0.1 \pm 0.1\%$) and decreased in those receiving vildagliptin ($\Delta = -0.2 \pm 0.1\%$, $P=0.002$ vs placebo).

Safety and tolerability. The overall adverse event (AE) profiles during treatment with vildagliptin were similar to those during placebo administration. No specific AE was reported by more than 3 patients during either treatment period, and the only AE reported by 3 patients was nasopharyngitis, which occurred during both treatment periods (ie, during placebo and during vildagliptin). No serious AE (SAE) was experienced by any patient during vildagliptin treatment; during placebo administration, 3 patients experienced an SAE: one case of appendicitis, one of infective arthritis, and one myocardial infarction.

Discontinuations due to an AE were limited to one case of decreased appetite in a patient receiving vildagliptin, and the myocardial infarction experienced by a patient receiving placebo. There were no hypoglycemic events or asymptomatic low blood glucose reported during either treatment.

Discussion

Glucagon secretion is stimulated by hypoglycemia and suppressed by hyperglycemia; in healthy subjects, the glycemic threshold for stimulation of glucagon release is approximately 4 mmol/liter. Numerous factors in addition to glucose can influence glucagon secretion and potentially contribute to the pathophysiology of glucagon secretion that occurs in T2DM (15). For example, epinephrine, sympathetic and parasympathetic neurotransmitters, amino acids, and several gut hormones (eg, cholecystokinin, gastrin-releasing peptide) stimulate glucagon secretion, whereas free fatty acids and ketones, gut hormones (eg, GLP-1, secretin), as well as locally released insulin and pancreatic somatostatin inhibit glucagon secretion. Indeed, it has been suggested that locally released insulin mediates the suppressive effects of hyperglycemia on glucagon secretion (16).

Abnormalities of glucagon secretion occur in patients with impaired glucose tolerance (IGT) and T2DM, and most abnormalities, if not all, may actually reflect an impairment of α -cell glucose sensing (15, 17, 18), (ie, an impaired ability of glucose to suppress glucagon secretion) (15, 17, 18).

A new class of OADs, the DPP-4 inhibitors, has been developed for the treatment of T2DM (19). The efficacy of DPP-4 inhibitors, such as vildagliptin, is attributable in part to a GLP-1-mediated glucagonostatic effect (10, 11, 20). However, if these agents suppressed glucagon secretion under all conditions, they could predispose patients to hypoglycemia; this, however, has not been observed. In fact, all clinical experience with DPP-4 inhibitors to date suggests that they have a low propensity to induce hypoglycemia. An earlier study in healthy volunteers suggested that short-term infusion of the incretin mimetic exenatide augmented the glucagon response to severe hypoglycemia (21), and another study found that vildagliptin added to high-dose insulin therapy in patients with T2DM actually decreased the incidence and severity of hypoglycemia (8). Consequently, we hypothesized that vildagliptin would

enhance the ability of the α -cell to sense and respond appropriately to changes in plasma glucose concentrations.

To test this hypothesis we examined the influence of 28-d treatment with vildagliptin (100 mg qd) on the glucagon response to both hyperglycemia and hypoglycemia in patients with T2DM and mild hyperglycemia (HbA_{1c} =6.3%). As expected, and shown previously (9-12), vildagliptin suppressed inappropriate glucagon secretion during meals. Further, plasma glucagon levels remained suppressed not only during the hyperglycemic step, but also during the euglycemic step of the clamp. Thus, the enhanced response to hypoglycemia during vildagliptin treatment reflected solely the significantly lower initial levels (at time 210 min). Nonetheless, the present finding of an increase from a significantly lower level to a slightly higher glucagon level during the hypoglycemic step with vildagliptin vs placebo clearly indicates that the α -cell response to hypoglycemia was not impaired. Indeed, the finding that the increment in plasma glucagon concentrations during hypoglycemia with vildagliptin was 38% higher than with placebo could be interpreted as an enhanced response. Since the duration of each glucose step was only 45 min, and an apparent “steady-state” hypoglycemia was maintained for only 10 min, it remains to be determined if a more sustained period of hypoglycemia would reveal a truly enhanced response with significantly higher glucagon levels with vildagliptin vs placebo.

Several additional findings from this study may shed light on the mechanisms underlying the greater increase in glucagon with vildagliptin vs placebo. During the hypoglycemic step of the clamp, the timing and degree of hypoglycemia were essentially identical during the two treatment periods, and the increases in epinephrine, norepinephrine, and cortisol also were unaffected by vildagliptin treatment. This suggests that, as intended, equivalent “stress” was induced by hypoglycemia during the two treatment periods. However, activation of the parasympathetic nervous system appears to have been augmented by vildagliptin treatment. Circulating PP levels are considered to be an index of parasympathetic nervous system activity (22), and, in the present study, the mean PP response to hypoglycemia increased by >80% during vildagliptin treatment; the trend approached, but did not achieve, statistical significance.

The strong trend toward an increase in the PP response to hypoglycemia with vildagliptin treatment suggests that increased vagal activity may mediate, or contribute to, the enhanced glucagon response. This would be consistent with the growing body of literature suggesting that many of the actions often ascribed to circulating GLP-1 may in fact be mediated by neuronal mechanisms (23-28).

The present study also found that the decrease of ISR during hypoglycemia was more pronounced with vildagliptin than with placebo, ie, ISR decreased from a significantly higher level at time 210 min to an identical level at 285 min with the change (reduction) in ISR during hypoglycemia being significantly greater with vildagliptin than with placebo. To our knowledge, this is the first demonstration that an agent presumably acting through GLP-1 receptor signaling enhances the effectiveness of low glucose levels to suppress insulin secretion. Although some would argue that this observation supports the concept that glucose control of glucagon secretion is mediated by local insulin levels (29-31), we have recently shown that vildagliptin suppresses postprandial glucagon levels in insulinopenic patients with type 1 diabetes (32). Thus, although enhanced suppression of insulin secretion may have contributed to the enhanced glucagon response to hypoglycemia seen with vildagliptin treatment, understanding the mechanisms underlying the effects on both insulin and glucagon secretion will require further study. Independent of the exact underlying mechanisms, the present study provides evidence that DPP-4 inhibition with vildagliptin improves (or restores) the ability of both α - and β -cells to sense and respond appropriately to changes in plasma glucose levels.

Vildagliptin (100 mg qd) was efficacious at decreasing FPG and HbA_{1c} as in all previous studies of >4-wk duration (1-3, 6-9, 33-36). In addition, as in previous trials, vildagliptin generally increased plasma levels of intact GLP-1, reduced prandial glucose and glucagon, and increased insulin secretion relative to glucose when these measures were made. These changes were judged to be independent of the patient's severity of disease or treatment duration (9-11, 13, 37-40).

In summary, the present study demonstrated that in patients with T2DM, the DPP-4 inhibitor vildagliptin improved the ability of both α - and β -cells to sense and respond appropriately to hypoglycemia. The concurrent strong trend toward an increased PP response to hypoglycemia suggests

that vagal mechanisms may be involved. Improved islet glucose sensing/responsiveness likely underlies the low propensity of DPP-4 inhibitors to elicit hypoglycemia.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Michele Valentin, who provided operational support for this study.

REFERENCES

1. **Pi-Sunyer FX, Schweizer A, Mills D, Dejager S** 2007 Efficacy and tolerability of vildagliptin monotherapy in drug-naïve patients with type 2 diabetes. *Diabetes Res Clin Pract* 76:132-138
2. **Schweizer A, Couturier A, Foley JE, Dejager S** 2007 Comparison between vildagliptin and metformin to sustain reductions in HbA_{1c} over one year in drug-naïve patients with type 2 diabetes. *Diabet Med* 24:955-961
3. **Scherbaum WA, Schweizer A, Mari A, Nilsson PM, Lalanne G, Jauffret S, Foley JE** 2008 Efficacy and tolerability of vildagliptin in drug-naïve patients with type 2 diabetes and mild hyperglycemia. *Diabetes Obes Metab* 10:675-682
4. **Bosi E, Camisasca RP, Collober C, Rochotte E, Garber AJ** 2007 Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin. *Diabetes Care* 30:890-893
5. **Garber AJ, Schweizer A, Baron MA, Rochotte E, Dejager S** 2007 Vildagliptin in combination with pioglitazone improves glycaemic control in patients with type 2 diabetes failing thiazolidinedione monotherapy: a randomized, placebo-controlled study. *Diabetes Obes Metab* 9:166-174
6. **Rosenstock J, Baron MA, Camisasca RP, Cressier F, Couturier A, Dejager S** 2007 Efficacy and tolerability of initial combination therapy with vildagliptin and pioglitazone compared to component monotherapy in patients with type 2 diabetes. *Diabetes Obes Metab* 9:175-185
7. **Garber AJ, Foley JE, Banerji MA, Ebeling P, Gudbjornsdottir S, Camisasca RP, Couturier A, Baron MA** 2008 Effects of vildagliptin on glucose control in patients with

type 2 diabetes inadequately controlled with a sulphonylurea. *Diabetes Obes Metab* 10:1047-1056

8. **Fonseca V, Schweizer A, Albrecht D, Baron MA, Chang I, Dejager S** 2007 Addition of vildagliptin to insulin improves glycaemic control in type 2 diabetes. *Diabetologia* 50:1148-1155
9. **Ahrén B, Landin-Olsson M, Jansson P-A, Svenson 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
10. **Azuma K, Radikova Z, Mancino J, Toledo FG, Thomas E, Kangani C, Dalla MC, Cobelli C, Holst JJ, Deacon CF, He Y, Ligueros-Saylan M, Serra D, Foley JE, Kelley DE** 2007 Measurements of Islet Function and Glucose Metabolism With the DPP-4 Inhibitor Vildagliptin in Patients With Type 2 Diabetes. *J Clin Endocrinol Metab* 93:459-464
11. **Balas B, Baig MR, Watson C, Dunning BE, Ligueros-Saylan M, Wang Y, He YL, Darland C, Holst JJ, Deacon CF, Cusi K, Mari A, Foley JE, DeFronzo RA** 2007 The Dipeptidyl Peptidase IV Inhibitor Vildagliptin Suppresses Endogenous Glucose Production and Enhances Islet Function after Single Dose Administration in Type 2 Diabetic Patients. *J Clin Endocrinol Metab* 92:1249-1255
12. **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

13. **Rosenstock J, Foley JE, Rendell M, Landin-Olsson M, Holst JJ, Deacon CF, Rochotte E, Baron MA** 2008 Effects of the DPP-4 inhibitor vildagliptin on incretin hormones, islet function, and postprandial glycemia in subjects with impaired glucose tolerance. *Diabetes Care* 31:30-35
14. **Cryer PE** 1993 Glucose counterregulation: prevention and correction of hypoglycemia in humans. *Am J Physiol* 264:E149-E155
15. **Ahrén B, Foley JE, Dunning BE** 2005 Alpha cell function in health and disease: influence of glucagon-like peptide-1. *Diabetologia* 48:1700-1713
16. **Greenbaum CJ, Havel PJ, Taborsky GJ, Jr., Klaff LJ** 1991 Intra-islet insulin permits glucose to directly suppress pancreatic A cell function. *J Clin Invest* 88:767-773
17. **Dunning BE, Gerich JE** 2007 The role of α -cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. *Endocr Rev* 28:253-283
18. **Ahrén B, Larsson H** 2001 Impaired glucose tolerance (IGT) is associated with reduced insulin-induced suppression of glucagon concentrations. *Diabetologia* 44:1998-2003
19. **Ahrén B** 2007 Dipeptidyl peptidase-4 inhibitors: clinical data and clinical implications. *Diabetes Care* 30:1344-1350
20. **Ahrén B, Foley JE** 2008 The islet enhancer vildagliptin: mechanisms of improved glucose metabolism. *Int J Clin Pract Suppl*:8-14
21. **Degn KB, Brock B, Juhl CB, Djurhuus CB, Grubert J, Kim D, Han J, Taylor K, Fineman M, Schmitz O** 2004 Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. *Diabetes* 53:2397-2403

22. **Schwartz TW, Holst JJ, Fahrenkrug J, Jensen SL, Nielsen OV, Rehfeld JF, de Muckadell OB, Stadil F** 1978 Vagal, cholinergic regulation of pancreatic polypeptide secretion. *J Clin Invest* 61:781-789
23. **Meier JJ, Kemmeries G, Holst JJ, Nauck MA** 2005 Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. *Diabetes* 54:2212-2218
24. **Knauf C, Cani PD, Perrin C, Iglesias MA, Maury JF, Bernard E, Benhamed F, Gremeaux T, Drucker DJ, Kahn CR, Girard J, Tanti JF, Delzenne NM, Postic C, Burcelin R** 2005 Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *J Clin Invest* 115:3554-3563
25. **D'Alessio DA, Sandoval DA, Seeley RJ** 2005 New ways in which GLP-1 can regulate glucose homeostasis. *J Clin Invest* 115:3406-3408
26. **Vahl TP, Tauchi M, Durler TS, Elfers EE, Fernandes TM, Bitner RD, Ellis KS, Woods SC, Seeley RJ, Herman JP, D'Alessio DA** 2007 Glucagon-like peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. *Endocrinology* 148:4965-4973
27. **Knauf C, Cani PD, Kim DH, Iglesias MA, Chabo C, Waget A, Colom A, Rastrelli S, Delzenne NM, Drucker DJ, Seeley RJ, Burcelin R** 2008 The role of CNS GLP-1 receptors in enteric glucose sensing. *Diabetes* 57:2603-2612
28. **Ahrén B** 2004 Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *Am J Physiol Regul Integr Comp Physiol* 286:R269-R272

29. **Gosmanov NR, Szoke E, Israelian Z, Smith T, Cryer PE, Gerich JE, Meyer C** 2005 Role of the decrement in intraislet insulin for the glucagon response to hypoglycemia in humans. *Diabetes Care* 28:1124-1131
30. **Hope KM, Tran PO, Zhou H, Oseid E, Leroy E, Robertson RP** 2004 Regulation of α -cell function by the beta-cell in isolated human and rat islets deprived of glucose: the "switch-off" hypothesis. *Diabetes* 53:1488-1495
31. **Raju B, Cryer PE** 2005 Loss of the decrement in intraislet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin-deficient diabetes: documentation of the intraislet insulin hypothesis in humans. *Diabetes* 54:757-764
32. **Foley JE, Ligueros-Saylan M, He YL, Holst JJ, Deacon CF, Dunning BE, Leone-Jones A, Yu T, Kelley DE** 2008 Effect of vildagliptin on glucagon concentration during meals in patients with type 1 diabetes. *Horm Metab Res* 40:727-730
33. **Pratley RE, Jauffret-Kamel S, Galbreath E, Holmes D** 2006 Twelve-week monotherapy with the DPP-4 inhibitor vildagliptin improves glycemic control in subjects with type 2 diabetes. *Horm Metab Res* 38:423-428
34. **Ristic S, Byiers S, Foley J, Holmes D** 2005 Improved glycaemic control with dipeptidyl peptidase-4 inhibition in patients with type 2 diabetes: vildagliptin (LAF237) dose response. *Diabetes Obes Metab* 7:692-698
35. **Rosenstock J, Baron MA, Dejager S, Mills D, Schweizer A** 2007 Comparison of vildagliptin and rosiglitazone monotherapy in patients with type 2 diabetes: a 24-week, double-blind, randomized trial. *Diabetes Care* 30:217-223
36. **Scherbaum WA, Schweizer A, Mari A, Nilsson PM, Lalanne G, Wang Y, Dunning BE, Foley JE** 2008 Evidence that vildagliptin attenuates deterioration of glycaemic control

during 2-year treatment of patients with type 2 diabetes and mild hyperglycaemia. *Diabetes Obes Metab* 10:1114-1124

37. **He YL, Bullock JM, Deacon CF, Holst JJ, Dunning BE, Ligueros-Saylan M, Foley JE, Wang Y** 2007 Pharmacodynamics of vildagliptin in patients with type 2 diabetes during OGTT. *J Clin Pharmacol* 47:633-641
38. **Mari A, Scherbaum WA, Nilsson PM, Lalanne G, Schweizer A, Dunning BE, Jauffret S, Foley JE** 2008 Characterization of the influence of vildagliptin on model-assessed {beta}-cell function in patients with type 2 diabetes and mild hyperglycemia. *J Clin Endocrinol Metab* 93:103-109
39. **Matikainen N, Mänttari S, Schweizer A, Ulvestad A, Mills D, Dunning BE, Foley JE, Taskinen M-R** 2006 Vildagliptin therapy reduces postprandial intestinal triglyceride-rich lipoprotein particles in patients with type 2 diabetes. *Diabetologia* 49:2049-2057
40. **Utzschneider KM, Tong J, Montgomery B, Udayasankar J, Gerchman F, Marcovina SM, Watson CE, Ligueros-Saylan MA, Foley JE, Holst JJ, Deacon CF, Kahn SE** 2008 The dipeptidyl peptidase-4 inhibitor vildagliptin improves {beta}-cell function and insulin sensitivity in subjects with impaired fasting glucose. *Diabetes Care* 31:108-113

TABLE 1. Demographic and baseline characteristics of the completers population

Mean $\pm$ SD or n (%)	Sequence A (vildagliptin 100 mg qd/placebo)	Sequence B (placebo/vildagliptin 100 mg qd)	All
N	14	11	25
Age (y)	66.6 $\pm$ 6.4	64.1 $\pm$ 3.7	65.5 $\pm$ 5.4
Age category			
<65 y	5 (35.7)	6 (54.5)	11 (44.0)
$\geq$ 65 y	9 (64.3)	5 (45.5)	14 (56.0)
Male	13 (92.9)	9 (81.8)	22 (88.0)
Caucasian	14 (100.0)	11 (100.0)	25 (100.0)
BMI (kg/m ²)	28.1 $\pm$ 2.7	27.6 $\pm$ 3.5	27.9 $\pm$ 3.0
HbA _{1c} (%)	6.4 $\pm$ 0.6	6.2 $\pm$ 0.6	6.3 $\pm$ 0.6
Median (min, max)	6.6 (5.3, 7.1)	6.2 (5.3, 7.3)	6.25 (5.6, 9.3)
FPG (mmol/liter)	7.7 $\pm$ 1.2	7.2 $\pm$ 0.7	7.5 $\pm$ 1.0
Median (min, max)	8.0 (5.6, 9.3)	7.3 (5.9, 8.4)	7.35 (5.6, 9.3)
Disease duration (y)	6.7 $\pm$ 7.9	4.2 $\pm$ 4.0	5.6 $\pm$ 6.5

TABLE 2. Integrated responses to meals

Mean $\pm$ SE	Placebo (n=25)	Vildagliptin (n=25)	<i>P</i> value*	% change
GLP-1 (pmol/liter•min)				
Total AUC ₀₋₆₀	379 $\pm$ 101	678 $\pm$ 159	0.012	+79%
Δ AUC ₀₋₆₀	119 $\pm$ 106	334 $\pm$ 37	0.036	+180%
Total AUC ₀₋₉₀	558 $\pm$ 155	1003 $\pm$ 240	0.002	+80%
Δ AUC ₀₋₉₀	169 $\pm$ 108	488 $\pm$ 52	0.008	+89%
Glucagon (ng/liter•min)				
Total AUC ₀₋₆₀	8930 $\pm$ 356	8060 $\pm$ 403	0.007	-10%
Δ AUC ₀₋₆₀	861 $\pm$ 130	512 $\pm$ 163	0.019	-41%
Total AUC ₀₋₁₂₀	16878 $\pm$ 650	15244 $\pm$ 713	0.005	-9%
Δ AUC ₀₋₁₂₀	740 $\pm$ 242	148 $\pm$ 274	0.025	-80%
Glucose (mmol/liter•min)				
Total AUC ₀₋₆₀	535 $\pm$ 17	499 $\pm$ 17	0.001	-7%
Δ AUC ₀₋₆₀	125 $\pm$ 8	107 $\pm$ 8	0.007	-15%
Total AUC ₀₋₁₂₀	1048 $\pm$ 93	952 $\pm$ 40	<0.0001	-9%
Δ AUC ₀₋₁₂₀	227 $\pm$ 22	168 $\pm$ 22	< 0.0001	-26%
ISR (pmol/m²)				
Total AUC ₀₋₆₀	11258 $\pm$ 1051	11349 $\pm$ 1028	0.418	+1%
Δ AUC ₀₋₆₀	4295 $\pm$ 486	4797 $\pm$ 384	0.043	+12%
Total AUC ₀₋₁₂₀	26976 $\pm$ 2282	26846 $\pm$ 2372	0.448	-1%
Δ AUC ₀₋₁₂₀	10351 $\pm$ 1143	13908 $\pm$ 1116	0.167	+7%
ISR/G (β-cell function, pmol/m²/mM)				
Total AUC ₀₋₆₀	21.7 $\pm$ 2.2	23.4 $\pm$ 2.3	0.039	+8%
Δ AUC ₀₋₆₀	37.5 $\pm$ 4.7	54.5 $\pm$ 7.2	<0.0001	+45%
Total AUC ₀₋₁₂₀	2.7 $\pm$ 2.5	29.1 $\pm$ 2.7	0.037	+9%
Δ AUC ₀₋₁₂₀	75.7 $\pm$ 11.7	151.5 $\pm$ 42.5	0.037	+100%

*By one-tailed paired *t* test (for absolute, not percentage change)

TABLE 3. Pancreatic, sympathoadrenal, and parasympathetic responses* to hypoglycemia

mean $\pm$ SE	Placebo (n=25)	Vildagliptin (n=25)	Difference	<i>P</i> value**
Glucagon (ng/liter)	33.9 $\pm$ 6.7	46.7 $\pm$ 6.9	12.8 $\pm$ 7.0	0.039
ISR (pmol/m ² /min)	-62.3 $\pm$ 6.9	-69.6 $\pm$ 6.6	-7.3 $\pm$ 3.0	0.011
EPI (nmol/liter)	2.12 $\pm$ 0.51	2.00 $\pm$ 0.42	-0.12 $\pm$ 0.29	0.684
NE (nmol/liter)	0.99 $\pm$ 0.19	0.67 $\pm$ 0.30	-0.32 $\pm$ 0.30	0.305
Cortisol (nmol/liter)	76.6 $\pm$ 22.7	88.4 $\pm$ 24.3	11.9 $\pm$ 22.8	0.607
PP (pmol/liter)	63.2 $\pm$ 14.6	114.8 $\pm$ 29.3	51.6 $\pm$ 31.5	0.057

*Changes from time 210 min to time 255 min, **By paired *t* test

Figure Legends

Figure 1. Plasma glucose (panel A), GLP-1 (panel B), glucagon (panel C), and insulin secretory rate (ISR, panel D) during standard breakfast meal tests performed on Day 28 of treatment with vildagliptin (closed triangles) or placebo (open circles). Mean $\pm$ SE, n=25 per treatment. * P <0.05 or better vs placebo.

Figure 2. Plasma glucose (panel A), glucagon (panel B), and insulin secretory rate (ISR, panel C) during hyperinsulinemic, stepped glucose clamps performed on Day 28 of treatment with vildagliptin (closed triangles) or placebo (open circles). Mean $\pm$ SE, n=25 per treatment. * P <0.05 or better vs. placebo.



