

# Dipeptidyl Peptidase IV Inhibition Potentiates the Insulinotropic Effect of Glucagon-Like Peptide 1 in the Anesthetized Pig

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Glucagon-like peptide 1 (GLP-1) has been proposed as a new therapeutic agent in the management of diabetes because of its glucose-dependent stimulation of insulin secretion, but this is limited by its rapid degradation in vivo by dipeptidyl peptidase IV (DPP IV). In nonfasted anesthetized pigs, valine-pyrrolidide (a stable and selective inhibitor of DPP IV), at a dose that reduced plasma DPP IV activity by more than 90%, increased both the amount of intact GLP-1 in the basal state (from  $5 \pm 1$  to  $18 \pm 7$  pmol/l;  $P < 0.05$ ) and the proportion remaining undegraded during an infusion (from  $21.0 \pm 1.3$  to  $102.3 \pm 4.5\%$ ;  $P < 0.0001$ ). This was associated with a prolonged plasma half-life for the intact peptide (from  $1.0 \pm 0.1$  to  $3.2 \pm 0.2$  min;  $P < 0.0005$ ). In the basal (nonfasted) state, valine-pyrrolidide potentiated the effect of intravenous GLP-1 on the incremental area under the curve (AUC) for glucose ( $-0.50 \pm 0.91$  to  $-2.83 \pm 0.59$  20 min  $\cdot$  mmol  $\cdot$  l $^{-1}$ ;  $P < 0.05$ ) and insulin ( $23.8 \pm 30.5$  to  $332.5 \pm 99.6$  20 min  $\cdot$  pmol  $\cdot$  l $^{-1}$ ;  $P < 0.05$ ). When an intravenous glucose load was given during the GLP-1 infusion, valine-pyrrolidide augmented the insulin response (AUC,  $2,086.2 \pm 600.9$  to  $6,247.0 \pm 1443.9$  40 min  $\cdot$  pmol  $\cdot$  l $^{-1}$ ;  $P < 0.05$ ). These results suggest that by reducing GLP-1 degradation, DPP IV inhibition potentiates the insulinotropic effect of GLP-1 and may, therefore, be a viable approach to the management of diabetes. *Diabetes* 47:764–769, 1998

**G**lucagon-like peptide 1 (GLP-1) arises from tissue-specific posttranslational processing of the glucagon precursor in the intestinal L-cell (1). Numerous studies have shown that when given to healthy subjects, GLP-1 potently influences glycemic levels as well as insulin and glucagon concentrations (1,2), effects which are glucose dependent (3,4). Moreover, it is also effective in patients with diabetes (5,6), normalizing blood glu-

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AUC, area under the curve; AMC, 7-amino-4-methylcoumarin; DPP IV, dipeptidyl peptidase IV; GLP-1, glucagon-like peptide 1;  $t_{1/2}$ , half-life; HPLC, high-performance liquid chromatography; GIP, glucose-dependent insulinotropic polypeptide; RIA, radioimmunoassay.

cose levels in type 2 diabetic subjects (7) and improving glycemic control in type 1 patients (8), raising the possibility of its use as a therapeutic agent.

GLP-1 is, however, metabolically unstable, having a plasma half-life ( $t_{1/2}$ ) of only 1–2 min in vivo (9–11). The aminopeptidase dipeptidyl peptidase IV (DPP IV) is particularly important to this rapid metabolism (9,12,13), with the resulting metabolite, GLP-1(9-36) amide, circulating at approximately the same concentration as the intact peptide in the basal/fasting state (13). Exogenously administered GLP-1 is also rapidly degraded (14). This metabolic instability, together with the formation of a metabolite [GLP-1(9-36) amide] that can antagonize the effects of GLP-1(7-36) amide (15), may limit the therapeutic potential of native GLP-1.

The availability of specific inhibitors raises the possibility of influencing GLP-1 metabolism by DPP IV in vivo, with preliminary reports suggesting that DPP IV inhibition improves glucose homeostasis in rats (16–18). Although the effects of the inhibitors on GLP-1 levels were not directly assessed, it was speculated that reduced GLP-1 degradation may have been responsible. The present study aimed to assess the role of DPP IV in glucose homeostasis and the possibility of enhancing the effect of exogenous GLP-1. GLP-1 pharmacokinetics and pharmacodynamics were examined, with and without concomitant DPP IV inhibition by valine-pyrrolidide, in anesthetized pigs given an intravenous glucose load. Pigs were chosen partly because of their resemblance to humans in terms of gastrointestinal physiology (19,20) and partly because a large animal model allows parallel determination of blood glucose, insulin, and glucagon concentrations, as well as both intact and total GLP-1 in the same samples.

## RESEARCH DESIGN AND METHODS

**DPP IV inhibitor.** Valine-pyrrolidide (Fig. 1) was generously provided by Dr. Orin Tempkin (Novartis Pharmaceuticals, East Hanover, NJ). It is a competitive inhibitor of DPP IV with a  $K_i$  of approximately 0.4  $\mu$ mol/l (21). The compound is highly selective for DPP IV relative to other proteases and is essentially inactive against other enzymes, including dipeptidyl peptidase II, aminopeptidase P, post-proline-cleaving enzyme, trypsin, and elastase (data not shown). This selectivity for DPP IV relative to other enzymes cleaving adjacent to proline residues is derived from the presence of the primary basic amine functionality of valine, while the conformationally constrained pyrrolidide group confers selectivity for DPP IV (a postproline-cleaving enzyme) relative to other aminopeptidases.

**Anesthetized pig.** Nonfasted, Danish LYY strain pigs (30–35 kg) were used. After premedication with midazolam (0.5 mg/kg, Dormicum; Roche, Basel, Switzerland) and ketamine (10 mg/kg, Ketaminol; Veterinaria, Zurich, Switzerland), animals were anesthetized with intravenous  $\alpha$ -chloralose (66 mg/kg; Merck, Darmstadt, Germany) and ventilated with intermittent positive pressure. Catheters were placed in the right carotid artery for sampling of arterial blood, in a left ear vein for peptide infusion, and in a right ear vein for glucose and valine-pyrrolidide administration. After surgical preparation, animals were heparinized

and left undisturbed for 30 min. Anesthesia was maintained with additional chloralose as necessary.

Six animals were used, each receiving two intravenous infusions of GLP-1, one before and one after administration of valine-pyrrolidide. Synthetic GLP-1(7-36) amide (Peninsula Laboratories Europe, St. Helens, U.K.), dissolved in 0.9% NaCl containing 1% human serum albumin (Behringwerke, Marburg, Germany), was infused at a rate of  $5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 40 min using a syringe pump, commencing at time 0. An intravenous glucose infusion ( $0.2 \text{ g/kg}$ ; 50% solution) was administered between min 21–30. Arterial blood samples (4 ml) were taken at -10, 0, 5, 10, 15, 20, 25, 30, 32, 35, 37, and 40 min from the start of the infusion. After 40 min, the GLP-1 infusion was stopped, and further blood samples were taken at 1, 2, 5, 7, 10, 15, 20, 30, 40, and 50 min. Sixty minutes after the cessation of the GLP-1 infusion, valine-pyrrolidide ( $300 \text{ } \mu\text{mol/kg}$ , dissolved in 0.9% NaCl) was given as a bolus intravenous injection over 2 min. Blood samples were taken at 5-min intervals for 30 min, after which the second GLP-1 infusion was started, and the protocol was repeated for blood sampling and the glucose infusion.

Blood glucose was measured immediately (One Touch II; Lifescan, Lyngby, Denmark). Blood samples were collected into chilled tubes containing EDTA ( $7.4 \text{ mmol/l}$  final concentration), aprotinin ( $500 \text{ kallikrein inhibitory equivalents/ml}$  blood; Novo Nordisk, Bagsvaerd, Denmark), and diprotin A ( $0.1 \text{ mmol/l}$  final concentration; Bachem, Bubendorf, Switzerland) for hormonal analysis, and into heparinized tubes for DPP IV activity determination. They were kept on ice until centrifugation at  $4^\circ\text{C}$ . Plasma was separated and stored at  $-20^\circ\text{C}$  until analysis.

**Hormonal analysis.** Plasma samples were assayed for GLP-1 using radioimmunoassays (RIAs) specific for each end of the molecule.  $\text{NH}_2$ -terminal immunoreactivity was measured using antiserum 93242 (22), which has a cross-reactivity of  $\sim 10\%$  with GLP-1(1-36) amide and of  $<0.1\%$  with GLP-1(8-36) amide and GLP-1(9-36) amide. The detection limit is  $5 \text{ pmol/l}$ . High-performance liquid chromatography (HPLC) supports the use of RIAs with this specificity for determination of intact GLP-1 (13). COOH-terminal immunoreactivity was determined using antiserum 89390 (23), which has an absolute requirement for the intact amidated COOH-terminus of GLP-1(7-36) amide and cross-reacts  $<0.01\%$  with COOH-terminally truncated fragments and  $83\%$  with GLP-1(9-36) amide. For all assays, the intra-assay coefficient of variation was  $<6\%$ . Plasma samples were extracted with 70% ethanol (vol/vol, final concentration) before assay, giving recoveries of  $75\%$  (24). Insulin immunoreactivity was measured in unextracted plasma using antiserum 2004 (24), and glucagon immunoreactivity was determined after ethanol extraction, using the COOH-terminally directed antiserum 4305, which measures glucagon of pancreatic origin (24).

**DPP IV activity determination.** DPP IV activity was assessed using assay conditions modified from a previously published method (25). In brief,  $5 \text{ } \mu\text{l}$  aliquots of plasma were added to 96-well flat-bottom microtiter plates (Falcon, Oxnard, CA), followed by  $5 \text{ } \mu\text{l}$  of  $80 \text{ mmol/l}$   $\text{MgCl}_2$  in incubation buffer ( $25 \text{ mmol/l}$  HEPES,  $140 \text{ mmol/l}$  NaCl,  $1\%$  RIA-grade bovine serum albumin, pH 7.8). After a 5-min incubation at room temperature, the reaction was initiated by addition of  $10 \text{ } \mu\text{l}$  of incubation buffer containing  $0.1 \text{ mmol/l}$  substrate (H-glycine-proline-AMC; AMC is 7-amino-4-methylcoumarin; Bachem, King of Prussia, PA). The plates were incubated for 20 min in the dark at room temperature, after which the reaction was stopped by the addition of  $20 \text{ } \mu\text{l}$  25% acetic acid. The fluorescence was measured using a CytoFluor II fluorimeter (PerSeptive Biosystems, Framingham, MA; excitation  $380 \text{ nm}$ , emission  $460 \text{ nm}$ ; gain setting 65). A fluorescence-concentration curve of free AMC was generated using  $0\text{--}50 \text{ } \mu\text{mol/l}$  solutions of AMC in assay buffer for interpolation of substrate consumption (catalytic activity in  $\text{nmol}$  substrate

cleaved/min). Values were expressed as percent of predose levels to correct for differences in DPP IV activity between animals.

**Statistical analysis.** The plasma  $t_{1/2}$  of GLP-1 was calculated by  $\log_e$ -linear regression analysis of peptide concentrations (after subtraction of endogenous values) in samples collected after the end of the infusion. The incremental areas under the curve (AUCs) for glucose and insulin were calculated using the trapezoidal method, after subtraction of the basal concentrations measured in samples before the start of each GLP-1 infusion. Additionally, for the period immediately after the glucose load, the AUCs were calculated after subtraction of the concentrations in the 20-min samples taken before glucose administration. The fractional clearance ( $k$ ) for glucose was calculated using the formula  $k = 0.693 / t_{1/2}$ .

Data are expressed as means  $\pm$  SE and were analyzed by repeated measures analysis of variance and two-tailed  $t$  tests for paired data as appropriate, using GraphPAD InStat, version 1.13 (San Diego, CA). Values of  $P < 0.05$  were considered to be significant.

## RESULTS

**DPP IV inhibition.** Preliminary analyses in the first three animals showed that basal DPP IV activity ( $15.7 \pm 2.5 \text{ mU/ml}$ ) was reduced by  $>90\%$  within 5 min of valine-pyrrolidide ( $300 \text{ } \mu\text{mol/kg}$ ) administration and that this effect was maintained throughout the rest of the experiment (Fig. 1). This dose of the inhibitor was, therefore, used for the remainder of the study.

**GLP-1 pharmacokinetics.** Plasma concentration curves for GLP-1, measured with  $\text{NH}_2$ -terminal (intact peptide) and COOH-terminal (intact +  $\text{NH}_2$ -terminally degraded peptide) RIAs, are shown in Fig. 2. During infusion of GLP-1 alone, the intact peptide accounted for only  $21.0 \pm 1.3\%$  of the total immunoreactivity (plateau values). This proportion was significantly ( $P < 0.0001$ ) increased to  $102.3 \pm 4.5\%$  during GLP-1 infusion in the presence of the DPP IV inhibitor.

When GLP-1 was infused alone, the plasma  $t_{1/2}$  of the intact peptide (determined by  $\text{NH}_2$ -terminal RIA) was significantly shorter than that determined by COOH-terminal RIA ( $1.0 \pm 0.1$  vs.  $3.2 \pm 0.2 \text{ min}$ ;  $P < 0.0005$ ). However, when GLP-1 was infused after valine-pyrrolidide administration, its  $\text{NH}_2$ -terminal  $t_{1/2}$  was significantly prolonged to  $3.2 \pm 0.2 \text{ min}$  ( $P < 0.0005$  compared with  $\text{NH}_2$ -terminal  $t_{1/2}$  for GLP-1 alone), which did not differ from the COOH-terminal  $t_{1/2}$  calculated during DPP IV inhibition ( $3.3 \pm 0.2 \text{ min}$ ). In contrast, there was no difference between the COOH-terminal  $t_{1/2}$  for GLP-1 calculated with or without DPP IV inhibition.

**GLP-1 pharmacodynamics.** Valine-pyrrolidide itself had no effect on endogenous GLP-1 levels measured by COOH-terminal RIA ( $15 \pm 3 \text{ pmol/l}$  before the inhibitor,  $14 \pm 2$

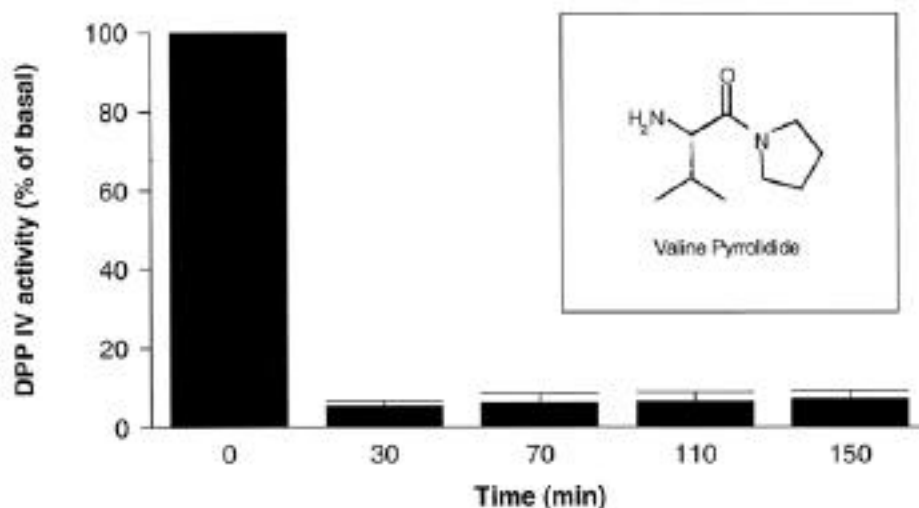


FIG. 1. Plasma DPP IV activity measured before and after administration of valine-pyrrolidide ( $300 \text{ } \mu\text{mol/kg}$ ) in the first three animals. This dose of inhibitor was used for the remainder of the study. Results are expressed as percentage of the basal activity, which was  $15.7 \pm 2.5 \text{ mU/ml}$ . Data are means  $\pm$  SE. The inset shows the structure of the inhibitor.

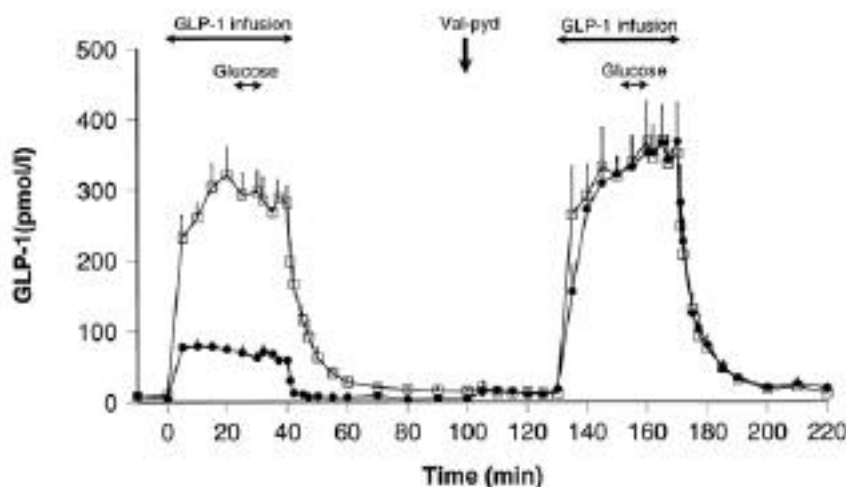


FIG. 2. Plasma GLP-1 immunoreactivity in blood sampled from the carotid artery, measured with COOH-terminally (□) and NH<sub>2</sub>-terminally (●) directed RIAs. Animals received a 40-min intravenous infusion of GLP-1 (7-36)amide (5 pmol · kg<sup>-1</sup> · min<sup>-1</sup>). Sixty minutes after the end of the first GLP-1 infusion, valine-pyrrolidide (Val-pyd) (300 μmol/kg) was given, and after a further 30 min, a second GLP-1 infusion was initiated. An intravenous glucose load (0.2 g/kg) was administered during min 21–30 of each GLP-1 infusion. Data are means ± SE; n = 6. The horizontal arrows indicate the periods of the infusions. NH<sub>2</sub>-terminal immunoreactivity is significantly lower than COOH-terminal immunoreactivity at all time points before valine-pyrrolidide administration (*P* < 0.05), except for -10 min. After valine-pyrrolidide (min 105–220), there is no difference between NH<sub>2</sub>- and COOH-terminal immunoreactivity at any time point.

pmol/l at 10 min, and 12 ± 2 pmol/l at 30 min after inhibitor administration). However, it increased endogenous GLP-1 concentrations measured by NH<sub>2</sub>-terminal RIA (from 5 ± 1 pmol/l before the inhibitor to 16 ± 3 pmol/l by 10 min, and reaching 18 ± 5 pmol/l by 30 min after inhibitor administration; *P* < 0.05), reflecting elevated intact peptide levels (Fig. 2). This was accompanied by a small reduction in blood glucose (4.9 ± 0.2 mmol/l before the inhibitor to 4.7 ± 0.2 mmol/l by 30 min; *P* < 0.05; Fig. 3), a transient increase in plasma insulin (28 ± 3 pmol/l before the inhibitor, peaking at 40 ± 2 pmol/l by 10 min; *P* < 0.05; Fig. 4), and a fall in plasma glucagon (12 ± 3 pmol/l before the inhibitor to 10 ± 3 pmol/l after 15 min; *P* < 0.05; Fig. 5).

Pilot studies (*n* = 3) revealed no significant differences in blood glucose or insulin profiles after the first GLP-1/glucose infusion compared with the second (glucose AUC, 83.10 ± 20.09 vs. 89.48 ± 24.88 80 min · mmol · l<sup>-1</sup>; insulin AUC, 2,447.0 ± 529.0 vs. 2,053.0 ± 134.7 80 min · pmol · l<sup>-1</sup>). Therefore, in subsequent studies, the effects of valine-pyrrolidide on the responses to GLP-1 (the second challenge) were com-

pared directly with those obtained in the same animal after the first GLP-1/glucose infusion.

Blood glucose levels did not differ significantly when GLP-1 was infused with or without valine-pyrrolidide (Fig. 3), and the fractional clearance of glucose was not affected (GLP-1 alone, 9.1 ± 0.7%/min; GLP-1 + valine-pyrrolidide, 8.1 ± 0.4%/min). In the period before the glucose load was administered, however, GLP-1 with concomitant DPP IV inhibition resulted in a reduction in the glucose AUC (Table 1). In the presence of valine-pyrrolidide, the GLP-1 infusion elicited a transient, small, but significant, increase in plasma insulin concentrations (Fig. 4) even before the start of the glucose infusion (concomitant with the fall in the glucose AUC). Once the glucose infusion was initiated, the insulinotropic effect of GLP-1 was greatly enhanced by DPP IV inhibition (Fig. 4), and this was reflected in the elevated AUC for insulin (Table 1). Plasma glucagon was also slightly suppressed by GLP-1 alone, even before the start of the glucose infusion, falling further once the glucose was given and returning toward basal values after the GLP-1 infusion

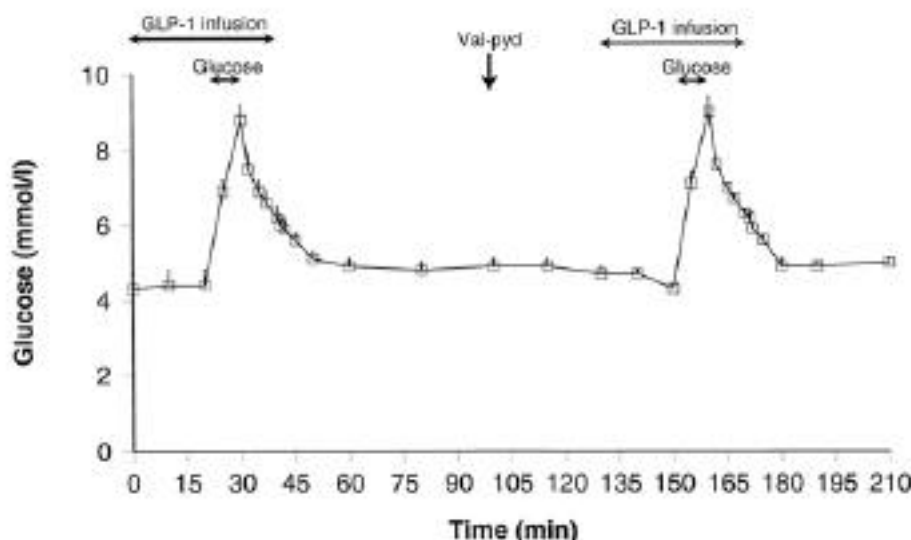


FIG. 3. Blood glucose concentrations measured before, during, and after 40-min intravenous infusions of GLP-1 (7-36)amide (5 pmol · kg<sup>-1</sup> · min<sup>-1</sup>) given alone (min 0–100) or during (min 100–210) DPP IV inhibition. Valine-pyrrolidide (Val-pyd) (300 μmol/kg) was given 60 min after the end of the first GLP-1 infusion, and after a further 30 min, the second GLP-1 infusion was initiated. An intravenous glucose load (0.2 g/kg) was administered during min 21–30 of each GLP-1 infusion. Data are means ± SE; n = 6. The horizontal arrows indicate the periods of the infusions. There is no significant difference between the blood glucose profile obtained before (min 0–80) and after (min 130–210) valine-pyrrolidide treatment.

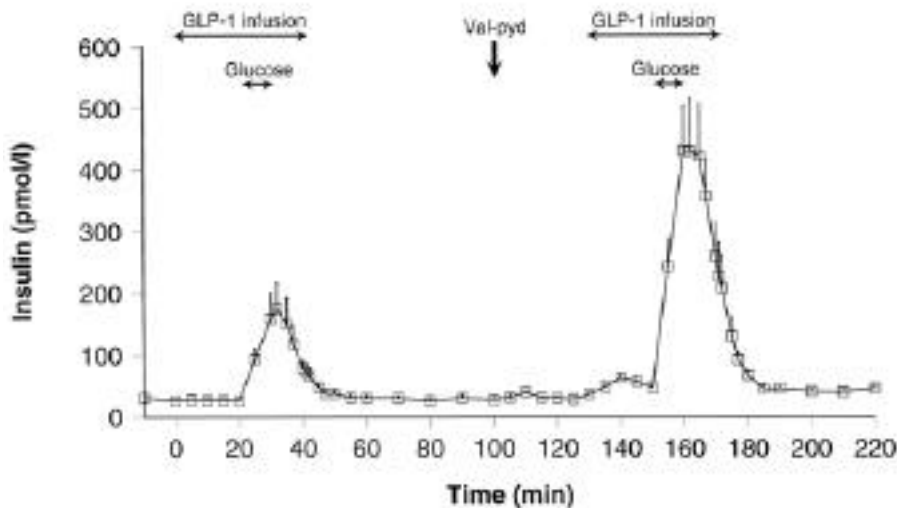


FIG. 4. Plasma insulin concentrations measured before, during, and after 40-min intravenous infusions of GLP-1 (7-36)amide ( $5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) given alone (min 0–100) or during (min 100–220) DPP IV inhibition. Valine-pyrrolidide (Val-pyd) ( $300 \text{ } \mu\text{mol/kg}$ ) was given 60 min after the end of the first GLP-1 infusion, and after a further 30 min, the second GLP-1 infusion was initiated. An intravenous glucose load ( $0.2 \text{ g/kg}$ ) was administered during min 21–30 of each GLP-1 infusion. Data are means  $\pm$  SE;  $n = 6$ . The horizontal arrows indicate the periods of the infusions. The plasma insulin profile obtained before (min 0–90) valine-pyrrolidide treatment is significantly lower ( $P < 0.05$ ) than that obtained after (min 130–220) valine-pyrrolidide.

was ended (Fig. 5). With valine-pyrrolidide, the glucagonostatic effect of GLP-1 was potentiated, but on cessation of the GLP-1 infusion, glucagon levels rebounded and exceeded basal values (Fig. 5).

#### DISCUSSION

In this study, the influence of GLP-1 on the responses to an intravenous glucose load was examined with or without simultaneous administration of valine-pyrrolidide, the selective, metabolically stable inhibitor of DPP IV. The results clearly show that both the insulinotropic and glucagonostatic effects of GLP-1 can be enhanced by DPP IV inhibition, confirming the importance of this enzyme in the regulation of GLP-1 activity in vivo.

Until recently, DPP IV-mediated degradation of GLP-1 has largely been overlooked, primarily because most assays do not distinguish between the intact peptide and the  $\text{NH}_2$ -terminally truncated metabolite. Since the first report that GLP-1 was a substrate for DPP IV (12), we and others have shown this enzyme to be the most important for GLP-1 degradation

in plasma (9,13), with other serum proteases having, at best, only a minor secondary role (26). DPP IV is also the main route for GLP-1 metabolism in vivo (9,10,14), resulting in a plasma  $t_{1/2}$  of only 1–2 min for biologically active GLP-1 (10,11). Moreover, exogenous GLP-1 is also extensively metabolized, with only ~20% remaining intact, regardless of route of administration (10,14,15,22). Together, these findings have led to the suggestion that to maximize the potential of GLP-1 as a therapeutic agent, some means of improving its duration of action should be sought. These have included the development of preparations or analogs of GLP-1 with a more retarded action (27,28) or which are resistant to DPP IV action (14) and the use of DPP IV inhibitors (14,16–18).

One of the first DPP IV inhibitors to be used to study GLP-1 metabolism was diprotin A, which, in vitro, completely prevents the peptide's degradation (12,13). However, early studies showed it was less effective in vivo (29), possibly because it is itself a substrate for the enzyme (30), but nevertheless, it was concluded that even partial inhibition of DPP IV in vivo can reduce GLP-1 metabolism. In the present study, valine-pyrro-

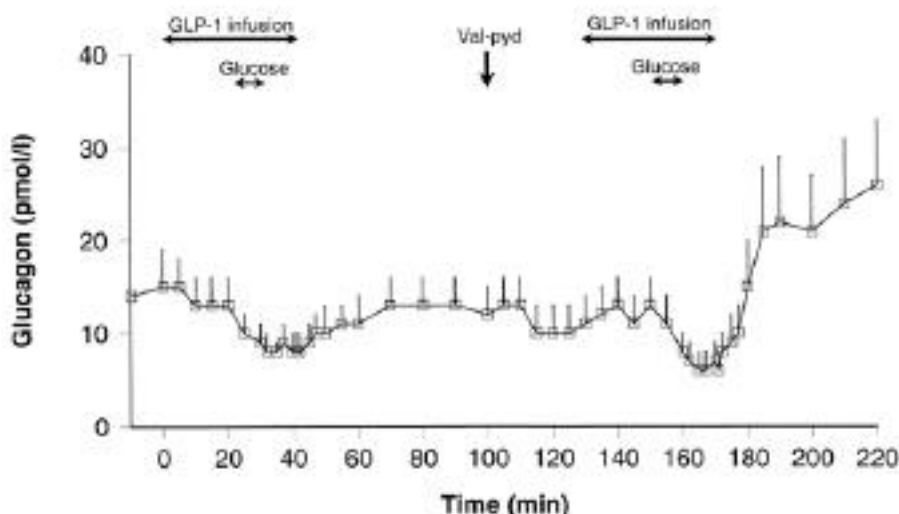


FIG. 5. Plasma glucagon concentrations measured before, during, and after 40-min intravenous infusions of GLP-1 (7-36)amide ( $5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) given alone (min 0–100) or during (min 100–220) DPP IV inhibition. Valine-pyrrolidide (Val-pyd) ( $300 \text{ } \mu\text{mol/kg}$ ) was given 60 min after the end of the first GLP-1 infusion, and after a further 30 min, the second GLP-1 infusion was initiated. An intravenous glucose load ( $0.2 \text{ g/kg}$ ) was administered during min 21–30 of each GLP-1 infusion. Data are means  $\pm$  SE;  $n = 6$ . The horizontal arrows indicate the periods of the infusions. There are no significant differences between the glucagon profiles before (min 0–90) or after (min 130–220) valine-pyrrolidide treatment, except at 37 min after the start of each GLP-1 infusion, where the glucagon concentration is significantly lower ( $P < 0.0005$ ) after valine-pyrrolidide, and at 90 min after the start of each GLP-1 infusion, where glucagon levels are elevated ( $P < 0.05$ ) after valine-pyrrolidide.

TABLE 1  
Incremental AUCs for glucose and insulin attained during infusions of GLP-1(7-36) amide alone or with inhibition of DPP IV

Period (min)	Incremental AUC for glucose (min · mmol · l <sup>-1</sup> )			Incremental AUC for insulin (min · pmol · l <sup>-1</sup> )		
	GLP-1 alone	GLP-1 + Val-pyd	<i>P</i> value	GLP-1 alone	GLP-1 + Val-pyd	<i>P</i> value
0–20	-0.50 ± 0.91	-2.83 ± 0.59	0.041	23.8 ± 30.5	332.5 ± 99.6	0.017
0–60	72.02 ± 6.80	55.87 ± 7.09	0.029	2154.7 ± 497.6	7110.9 ± 1500.7	0.012
0–80	81.85 ± 12.58	61.37 ± 8.84	0.124	2224.7 ± 449.7	7228.4 ± 1583.8	0.016
20–60	73.74 ± 6.88	72.58 ± 6.61	0.811	2086.2 ± 600.9	6247.0 ± 1443.9	0.020

Data are means ± SE; *n* = 6. Animals received 40-min intravenous infusions of GLP-1(7-36) amide (5 pmol · kg<sup>-1</sup> · min<sup>-1</sup>) given alone or with DPP IV inhibition. Valine-pyrrolidide (Val-pyd) (300 μmol/kg) was given 60 min after the end of the first GLP-1 infusion and, after a further 30 min, the second GLP-1 infusion was initiated. Glucose infusions (0.2 g/kg) were given during min 21–30 of each GLP-1 infusion. Period refers to the time after each GLP-1 infusion.

lidide decreased plasma DPP IV activity by >90% for the duration of the experiment, and preliminary reports indicated that a similar reduction may be obtained in rats (16–18). This degree of inhibition seems sufficient to prevent NH<sub>2</sub>-terminal degradation of GLP-1 in vivo; indeed, as the present study shows, with valine-pyrrolidide, plasma concentration curves obtained with the two GLP-1 assays are almost superimposable, with the NH<sub>2</sub>-terminal *t*<sub>1/2</sub> equaling that determined by the COOH-terminal assay. However, it does not exceed the COOH-terminal *t*<sub>1/2</sub>, indicating the role of non-DPP IV-mediated degradation in vivo, with renal metabolism likely to be important in eliminating both intact and degraded peptide (10).

The present study shows that the effects of GLP-1 can be enhanced by DPP IV inhibition. It could be argued that this is due to the inhibitor being given with the second GLP-1/glucose challenge; however, this seems unlikely, because pilot studies showed that glucose and hormonal responses to a second GLP-1/glucose challenge were of similar magnitude to the first. After valine-pyrrolidide administration, the small reductions in blood glucose and glucagon, with the transient increase in insulin, before the GLP-1 infusion, are consistent with the elevated levels of intact endogenous peptide. Once the GLP-1 infusion was started, concomitant DPP IV inhibition reduces GLP-1 degradation, causing further increases in insulin and a reduction in blood glucose, with significant potentiation of the insulinotropic and glucagonostatic effect of GLP-1 when the glucose load was given. However, in the period during and after the glucose load, blood glucose levels were not affected by DPP IV inhibition, even though insulin levels were elevated. This may be because glucose disposal in the pig is particularly rapid (31), as is also evident from the much higher fractional clearances of glucose in response to GLP-1 in the present study compared with humans (28,32). Thus, glucose elimination may already have reached a maximal rate in response to GLP-1 alone, so that no additional effect of the enhanced insulin levels was seen. The increase above basal glucagon values, seen with DPP IV inhibition toward the end of the experiment, remains unexplained, but similar, dose-related rebounds of glucagon were also seen in humans receiving GLP-1 with intravenous glucose (28). Taken together with the preliminary studies in rats (16–18), these results strongly suggest that DPP IV inhibition may improve glucose tolerance by reducing GLP-1 degradation. However, because the other incretin hormone, glucose-dependent insulinotropic polypeptide (GIP), is also a sub-

strate for DPP IV (12), it cannot be excluded that enhanced basal activity of GIP during DPP IV inhibition could contribute to the effect. It will be necessary to co-administer the inhibitor with a GLP-1 antagonist to confirm the relative importance of GLP-1.

Given the results obtained with DPP IV inhibitors, it might be expected that DPP IV-deficient rats would also have an exaggerated insulin response to an oral glucose load. However, somewhat surprisingly, they have normal glucose tolerance with no enhancement of the insulin response (33). This could be due to an adaptation to the lack of DPP IV compensating for the prolonged *t*<sub>1/2</sub> of the two incretins, GIP and GLP-1; and it was suggested that reduction in their secretion and/or desensitization of the pancreas to their effects may be responsible. Although this appeared to be true for GIP, it was not the case for GLP-1 (33). The resultant paradox of normal glucose tolerance in the face of apparently unimpaired GLP-1 secretion and pancreatic sensitivity, together with its longer-lived metabolic stability in DPP IV-deficient rats, remains unexplained, and it may turn out to be due to some other compensatory adaptation to the inherent lack of DPP IV in these animals.

Our results show that DPP IV inhibition in vivo prevents NH<sub>2</sub>-terminal degradation of GLP-1, extending the *t*<sub>1/2</sub> of the biologically active peptide, thereby potentiating the insulin response to intravenous glucose given with a GLP-1 infusion, and they suggest that enhancement of endogenous intact GLP-1 may explain the improved glucose tolerance seen after oral glucose without exogenous GLP-1 (16–18). This supports the proposal that DPP IV inhibitors may be useful in the treatment of diabetes, but additional studies are required to examine whether the beneficial effects are maintained over a longer period. However, as a note of caution, the incretin hormones are not the only substrates for DPP IV, and potential targets include growth hormone-releasing factor, neuropeptide Y, and several of the interleukins (34–36), among others. It remains to be seen whether these would be adversely affected by DPP IV inhibition, but, as an example, the successful introduction of angiotensin-converting enzyme inhibitors as antihypertensive agents was not limited, even though angiotensin I is not the only substrate for this enzyme. Clearly, further studies are needed to see whether the same applies to DPP IV inhibitors. Nonetheless, until these questions are resolved, DPP IV inhibition remains a viable approach to the man-

agement of diabetes, and its effects in suitable models should now be investigated.

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