Dipeptidyl Peptidase IV Inhibition Reduces the Degradation and Clearance of GIP and Potentiates Its Insulinotropic and Antihyperglycemic Effects in Anesthetized Pigs

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Glucose-dependent insulinotropic peptide (GIP) is known to be degraded by dipeptidyl peptidase IV (DPP IV), forming an inactive metabolite, but the extent of the enzyme’s role in regulating the biological activity of GIP in vivo is still largely unknown. In nonfasted anesthetized pigs given an intravenous infusion of GIP, the intact peptide (determined by a novel NH2-terminally directed radioimmunoassay) accounts for only 14.5 ± 2.5% of total immunoreactivity. This is increased (to 40.9 ± 0.9%, P < 0.0001) by coadministration of valine-pyrrolidide (a specific DPP IV inhibitor) at a dose that completely inhibits plasma DPP IV activity. The plasma t1/2 of intact GIP is prolonged by the inhibitor (from 3.3 ± 0.3 to 8.1 ± 0.6 min; P < 0.001), whereas the t1/2 for COOH-terminal immunoreactivity is unaffected (13.2 ± 0.5 and 11.5 ± 0.8 min, pre- and postinhibitor). Measurement of arteriovenous concentration differences revealed that the liver, kidney, and extremities are the main sites of removal of exogenous intact GIP (organ extractions, 28.0 ± 2.2, 26.3 ± 5.7, and 21.8 ± 3.0%, respectively). These organ extractions are reduced (P < 0.02) but not eliminated (kidney and extremities) by valine-pyrrolidide (to 6.5 ± 4.6, 14.1 ± 3.1, and 13.9 ± 2.4%, respectively). Valine-pyrrolidide potentiates the insulinotropic effect of GIP (P < 0.02), resulting in an enhanced glucose disappearance rate (k, from 8.0 ± 0.5 to 15.5 ± 2.2%/min; P < 0.01) and a reduction in the glucose excursion after an intravenous glucose load (area under the curve, from 133 ± 23 to 75 ± 9 min · mmol/l; P < 0.05). These results suggest that DPP IV plays an important role in GIP metabolism but is not the sole enzyme responsible for its NH2-terminal degradation. Nevertheless, DPP IV inhibition increases the proportion of intact peptide sufficiently to enhance its insulinotropic and antihyperglycemic effects. Diabetes 50:1588–1597, 2001

Glucose-dependent insulinotropic peptide (GIP), also known as gastric inhibitory polypeptide, is secreted from the K-cells in the upper small intestine, in response to the presence of nutrients, especially fats (1). Together with the structurally related peptide, glucagon-like peptide 1 (GLP-1), released from the intestinal L-cell, GIP is considered an incretin hormone (2).

In vitro studies have shown GIP to be a substrate for the enzyme dipeptidyl peptidase IV (DPP IV) (3,4), resulting in the formation of a truncated inactive metabolite, GIP(3–42) (5). However, because all previously described GIP assays crossreact with both intact GIP and the metabolite, measurement of circulating concentrations of the biologically active form of the peptide has been hampered, so that the relevance of DPP IV for regulation of GIP activity in vivo has been difficult to investigate fully. The recent development of a novel assay directed toward the intact NH2-terminus of GIP has identified GIP(3–42) as a major component of endogenous GIP immunoreactivity in human plasma (6), suggesting an important role for the enzyme in GIP metabolism.

Only a few studies have focused on identifying sites of GIP degradation. In vitro studies using isolated perfused rat livers demonstrated a negligible hepatic extraction of exogenous immunoreactive GIP (7). Similar findings were obtained by comparing the portal-to-peripheral venous ratios (7) or arteriovenous concentration differences (8) for endogenous GIP in conscious dogs. Measurement of peripheral GIP in uremic subjects or patients with chronic renal failure pointed to the kidney as being important for the removal of GIP (9,10), as did the finding that GIP clearance was reduced in nephrectomized rats (11). More direct evidence of renal involvement was obtained from determination of arteriovenous concentration differences in man (11) and dog (9). However, because all these studies relied on antisera that were directed toward the central or COOH-terminal sequence in GIP, no conclusions can be drawn regarding clearance of intact biologically active GIP.

Based on the pivotal role of DPP IV in inactivating GLP-1, DPP IV inhibitors have been proposed as a new therapy for the treatment of diabetes (12,13). Several animal studies have been published showing that this...
approach does lead to an improvement in glucose tolerance (14–16), but—although a role for GLP-1 has been demonstrated—the contribution of GIP has only been speculated. The present study was undertaken to assess the effect of DPP IV in regulating the biological activity of exogenously infused GIP and to see whether inhibition of the enzyme will enhance the insulinotropic effect of the peptide. In addition, simultaneous blood samples were collected during the infusions to allow determination of arteriovenous concentration differences across different organs using radioimmunoassays (RIAs) specific for each end of the molecule to identify the sites of elimination of circulating GIP. The ability of this approach to reveal metabolic degradation of GIP has been validated previously by high-performance liquid chromatography (HPLC) (6).

RESEARCH DESIGN AND METHODS

Anesthetized pig. Nonfasted Danish LYY-strain pigs (33–40 kg) were used. Before premedication with midazolam (0.5 mg/kg, Dormicum; Roche, Basel, Switzerland) and ketamine (10 mg/kg, Ketaminol; Veterinaria AG, Zurich, Switzerland), animals were anesthetized with intravenous administration of α-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 vein in the left ear for peptide infusion, and in a vein in the right ear for administration of glucose and valine-pyrrolidide. Synthetic and stored at 20°C until analysis. To verify that the GIP in the infusate was the intact NH2-terminus of GIP and cross-reacts less than 0.1% with GIP(3–42) or with the structurally related peptides GIP(1–36)-amide, GLP-1(9–36)-amide, GLP-2(3–33), GLP-2(3–33), or glucagon at concentrations of up to 100 pmol/l. For all bovine, human GIP (Peninsula Laboratories Europe, St. Helens, Merseyside, U.K.) was used as standard; radiolabeled GIP was obtained from Amersharm Pharma Biotech (Little Chalfont, Buckinghamshire, U.K.). Separation of bound peptide from free peptide was achieved using plasma-coated charcoal. Plasma samples were extracted with ethanol (70% vol/vol; final concentration), resulting in recoveries of 85% and intra-assay variations of <6%.

Insulin immunoreactivity was measured in unextracted plasma using antiserum 2004 (23), and glucagon immunoreactivity was determined after ethanol extraction, using the COOH-terminally directed antiserum 4305, which measures glucagon of pancreatic origin (23).

Calculations and statistical analysis. The plasma t1/2 of GIP was calculated by log-linear regression analysis of peptide concentrations (after subtraction of endogenous values) in samples collected after the end of the infusion. The metabolic clearance rate (MCR) of GIP was calculated from the actual infusion rate for each animal divided by the plateau plasma concentration, after subtraction of basal values. Results are expressed per kilogram body weight. The incremental area under the curve (AUC) for glucose and insulin were calculated using the trapezoidal method, after subtraction of the basal concentrations measured in samples before the start of each GIP infusion. Based on the period between the removal of the catheter from the jugular vein, the AUCs were calculated after subtraction of the concentrations in the 35-min samples collected before administration of glucose. The disappearance rate (k) for glucose was calculated using the formula where [GIP-IR]carotid artery is the concentration of GIP immunoreactivity in the carotid artery at time t, [GIP-IR]cava is the concentration of GIP immunoreactivity in the cava at time t, [GIP-IR]0 is the concentration of GIP immunoreactivity in the carotid artery, and k is the disappearance rate of GIP in the plasma.

For each animal, the extraction of exogenous GIP across the hind limb, kidney, and portal bed was calculated as a ratio, defined as

\[
\frac{\text{[(GIP-IR)}_{\text{carotid artery}} - \text{[(GIP-IR)}_{\text{cava}}\text{]} / \text{[(GIP-IR)}_{\text{carotid artery}}\text{]}}
\]

where [(GIP-IR)carotid artery] is the concentration of GIP immunoreactivity in the carotid artery and [(GIP-IR)cava] is the concentration of GIP immunoreactivity in the cava.

For the liver, the hepatic blood supply was calculated on the assumption that 75% originates from the portal vein and 25% is of arterial origin. The extraction across the lungs was defined as

\[
\frac{\text{[(GIP-IR)}_{\text{pulmonary artery}} - \text{[(GIP-IR)}_{\text{carotid artery}}\text{]}} / \text{[(GIP-IR)}_{\text{pulmonary artery}}\text{]}
\]

where [(GIP-IR)pulmonary artery] is the concentration of GIP immunoreactivity in the pulmonary artery.

Data are expressed as means ± SE and were analyzed using GraphPAD InStat software (version 1.13; San Diego, CA) and Statistica software (StatSoft, Tulsa, OK). Two-factor analysis of variance (ANOVA) for repeated measures with post-hoc analysis was used to analyze time-course curves before and after DPP IV inhibition. The t1/2, k, AUC, arteriovenous concentration differences, and MCR were analyzed using ANOVA (when appropriate) and two-tailed Student’s t tests for paired and nonpaired data. One-sample
RESULTS

Comparison of two successive GIP infusions. Similar concentrations of intact and total GIP were measured during the first and second infusions of GIP alone (Fig. 1), and the percentage of GIP that remained intact was unchanged (13.2 ± 0.4 and 11.9 ± 0.5%, first and second infusions, respectively). Similarly, there were no significant differences in blood glucose or insulin profiles after the first GIP/glucose infusion compared with the second (Fig. 1; glucose k1, 10.5 ± 2.8 s vs. 9.8 ± 0.9%/min).

DPP IV inhibition. Administration of valine-pyrrolidide (300 μmol/kg) completely inhibited plasma DPP IV activity for the remainder of the experiment.

GIP pharmacokinetics. HPLC analysis of infusate samples confirmed that the GIP eluted as a single peak with the same retention time as intact GIP (1-42) and that no degradation took place in the infusate during the course of the experiment.

Plasma GIP concentrations measured by NH2-terminal assay (intact peptide) were lower (P < 0.001) at all points than concentrations determined with the COOH-terminal assay (intact + NH2-terminally degraded peptide). Treatment with the DPP IV inhibitor had no effect on COOH-terminal immunoreactivity, but NH2-terminal GIP levels increased significantly (P < 0.001) after valine-pyrolidide (Fig. 2). During infusion of GIP alone, the intact peptide accounted for 14.5 ± 0.9% of total immunoreactivity (AUC), and this was increased to 40.9 ± 2.5% (P < 0.0001) in the presence of valine-pyrrolidide.

When GIP was infused alone, the plasma t1/2 of the intact peptide (determined by NH2-terminal RIA) was shorter than that determined by COOH-terminal assay (3.3 ± 0.3 vs. 13.2 ± 0.5 min; P < 0.001). However, in the presence of valine-pyrrolidide, the NH2-terminal t1/2 was prolonged to 8.1 ± 0.6 min (P < 0.001 compared with NH2-terminal t1/2 for GIP alone). The COOH-terminal t1/2 during DPP IV inhibition (11.5 ± 0.8 min) did not differ from the COOH-terminal t1/2 before administration of valine-pyrrolidide.

There was a significant difference in the MCR determined with the two assays when GIP was infused alone (7.19 ± 0.93 and 51.45 ± 8.91 ml · kg⁻¹ · min⁻¹, COOH-terminal and NH2-terminal, respectively; P < 0.001), but this difference disappeared when GIP was infused in the presence of valine-pyrolidide (8.31 ± 1.01 and 19.83 ± 2.73 ml · kg⁻¹ · min⁻¹, COOH-terminal and NH2-terminal, respectively; P > 0.05). The COOH-terminal MCR was unaffected by coinfusion of the inhibitor, whereas the NH2-terminal MCR was significantly shortened (P < 0.001) compared with GIP alone.

GIP extraction. Plasma concentrations measured during the GIP infusion without and with DPP IV inhibition are shown in Table 1, and the regional extraction is shown in Fig. 3. No extraction across the lungs was detected with either assay when either GIP was infused alone or in the presence of valine-pyrrolidide. Significant extraction across the hind limb was detected with both NH2-terminal and COOH-terminal assays when GIP was infused alone, and there was a trend (P = 0.07) for this to be higher when determined with the NH2-terminal assay. There was no effect on COOH-terminal hind-limb extraction when valine-pyrrolidide was coinfused, but NH2-terminal extraction was significantly (P < 0.02) reduced. Similarly, both assays detected significant extraction across the renal bed when GIP was infused alone, but in this case, the extraction detected by both assays was reduced by DPP IV inhibition (P < 0.02). Extraction of GIP across the splanchnic bed was detected only by the COOH-terminal assay in the absence of DPP IV inhibition, but when the inhibitor was included, no extraction was detected with either assay. The significant hepatic extraction detected by the NH2-terminal assay was abolished by coinfusion of valine-pyrrolidide (P < 0.002). In contrast, no significant hepatic extraction could be detected with the COOH-terminal assay when GIP was infused alone, but a small extraction was detected after DPP IV inhibition.

GIP pharmacodynamics. GIP concentrations determined...
with the COOH-terminal assay were still decreasing (because of the previous infusion) when the inhibitor was administered and continued to decrease after valine-pyrrolidide was administered (39.61 pmol/l before the inhibitor, 35.61 pmol/l at 15 min, and 31.63 pmol/l at 30 min after the inhibitor). In contrast, valine-pyrrolidide stopped and even reversed the decrease in NH2-terminal GIP concentrations (8.61 pmol/l before the inhibitor, 9.61 pmol/l at 15 min, and 11.61 pmol/l at 30 min after the inhibitor), reflecting elevated levels of intact endogenous GIP (Fig. 2). This was accompanied by a small reduction in blood glucose (5.40 ± 0.4 mmol/l before the inhibitor, decreasing to 4.80 ± 0.4 mmol/l at 30 min after the inhibitor, \( P < 0.005 \) [Fig. 4]; AUC 321 ± 61 min · mmol/l, \( P < 0.05 \) vs. 0), a transient trend toward an increase in insulin (58 ± 14 pmol/l before the inhibitor, peaking at 77 ± 21 pmol/l at 15 min after the inhibitor, \( P > 0.05 \) [Fig. 5]; AUC 321 ± 180 min · pmol/l, \( P > 0.05 \) vs. 0), and a decrease in plasma glucagon (14 ± 2 pmol/l before the inhibitor, decreasing to 10 ± 2 pmol/l at 15 min after the inhibitor, \( P < 0.01 \) [Fig. 6]).

**Comparison of two successive GIP infusions in the absence and presence of DPP IV inhibition.** In comparison with the first infusion of GIP alone, the glucose excursion in response to intravenous administration of glucose was reduced (\( P < 0.05 \)) when GIP was infused in the presence of valine-pyrrolidide, resulting in a smaller AUC in the period after the glucose load (Fig. 4; Table 2).

**FIG. 2.** Plasma GIP immunoreactivity in blood samples from the carotid artery, measured with COOH-terminally (●) and NH2-terminally (□) directed RIAs. Animals received a 57-min intravenous infusion of GIP (2 pmol · kg\(^{-1} \) · min\(^{-1} \)). Valine-pyrrolidide (val-pyd; 300μmol/kg) was given during minutes 38–47 of each GIP infusion. Data are means ± SE; \( n = 6 \). The horizontal arrows indicate the periods of the infusions. NH2-terminal immunoreactivity is lower than COOH-terminal immunoreactivity at all time points (\( P < 0.001 \)). The COOH-terminal GIP profiles before (minutes 0–127) and after (minutes 180–327) the inhibitor are not significantly different, but the NH2-terminal GIP profile is increased after the inhibitor (\( P < 0.001 \)).

**TABLE 1**

<table>
<thead>
<tr>
<th>Plasma concentrations of exogenous GIP during intravenous infusion</th>
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<tr>
<td><strong>COOH-terminal immunoreactivity (pmol/l)</strong></td>
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<tr>
<td><strong>Before</strong></td>
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<tr>
<td>Carotid artery</td>
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<tr>
<td>Femoral vein</td>
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<tr>
<td>Renal vein</td>
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<td>Portal vein</td>
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<td>Pulmonary artery</td>
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<td>Hepatic blood flow</td>
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<td>Hepatic vein</td>
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Date are means ± SE of individual plasma concentration determined during intravenous infusion of GIP before and after administration of valine-pyrrolidide (300 μmol/kg); \( n = 6 \). Hepatic blood supply was calculated on the assumption that 75% originates from the portal vein and 25% is of arterial origin. *\( P < 0.05 \); †\( P < 0.01 \); ‡\( P < 0.001 \); difference from carotid artery and between hepatic vein and hepatic blood supply. NS, not significant.
This was mainly due to the increased disappearance rate for glucose in the presence of the inhibitor (GIP alone, 8.0 ± 0.5%/min; GIP + valine-pyrrolidide, 15.5 ± 2.2%/min; P < 0.01). The presence of the inhibitor resulted in a small increase in insulin secretion even before the start of the glucose infusion, and this was greatly potentiated (P < 0.02) after the glucose load was administered (Fig. 5, Table 2). Infusion of GIP alone had no effect on plasma glucagon before the glucose load, but there was a modest suppression in response to the glucose (from 10.3 ± 1.5 to 8.2 ± 1.6 pmol/l; P < 0.001). Plasma glucagon concentrations decreased after valine-pyrrolidide administration (from 13.5 ± 1.9 to 10.0 ± 1.6 pmol/l; P < 0.05), but this decrease was reversed after the GIP infusion started, reaching 16.2 ± 1.7 pmol/l. DPP IV inhibition potentiated the decrease in glucagon in response to the glucose challenge (to 6.5 ± 0.5 pmol/l; P < 0.01 [Fig. 6]), but after the second GIP infusion was ended, plasma glucagon levels rebounded and exceeded basal values (P < 0.05).

**DISCUSSION**

In the present study, valine-pyrrolidide was used to examine the role of DPP IV in regulating the degradation and biological activity of GIP. The results show that inhibition of the enzyme reduces the clearance of the peptide and results in increased levels of intact, biologically active GIP. This is associated with enhancement of its insulinotropic activity and results in a reduction in the glucose excursion after an intravenous glucose load.

Although it has been known since 1993 that GIP is susceptible to degradation by DPP IV in vitro (3), producing a metabolite, GIP(3–42), which lacks significant insulinotropic activity (5), only a few studies have provided specific data pointing to the relevance of the enzyme for in vivo regulation of GIP bioactivity. Thus, Kieffer et al. (24), using HPLC to follow degradation of radiolabeled GIP in rats, showed that the peptide was rapidly cleaved at the NH₂-terminus in normal animals but not in a DPP IV–deficient strain, suggesting a role for the enzyme in vivo. Further support came from a study using a DPP IV–resistant GIP analog, which resulted in improved glycemic control compared with native GIP (25,26). However, direct assessment of the extent of GIP degradation has been hampered by the lack of specific analyses, because all reported assays also crossreact with the NH₂-terminally truncated metabolite GIP(3–42). The recent development of an NH₂-terminally directed GIP assay (6) coupled with the use of a specific DPP IV inhibitor has, for the first time, made it possible to follow GIP degradation in vivo and fully assess the degree to which DPP IV influences the peptide’s biological activity.

During infusion of GIP alone, the intact peptide, as

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**FIG. 3. Regional extraction of GIP during intravenous infusion determined by COOH-terminal (R65) and NH₂-terminal (171) RIAs before and after administration of valine-pyrrolidide (val-pyd). The extraction is the ratio calculated by dividing the arteriovenous concentration difference by the arterial concentration. The hepatic blood supply was calculated on the assumption that 75% originates from the portal vein and 25% is of arterial origin. Data are means ± SE of individual extractions across each organ calculated for each animal; n = 6. * P < 0.05, ** P < 0.01, *** P < 0.001, difference from 0. A positive balance indicates a net extraction of GIP.**

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During infusion of GIP alone, the intact peptide, as
determined using the NH2-terminal assay, accounts for only \( \approx 15\% \) of the overall immunoreactivity. This suggests that greater NH2-terminal degradation may occur in pigs, because a recent study in humans, using the same assays, showed that intact GIP accounts for 37% of total immunoreactivity after intravenous infusion (6). The plasma \( t_{1/2} \) for both NH2-terminal and COOH-terminal immunoreactivity were also lower in the pig (3 and 13 min, respectively) compared with humans (7 and 17 min, respectively) (6), which may indicate that GIP is cleared more quickly in pigs than in humans. Inclusion of the DPP IV inhibitor significantly increased the proportion of intact GIP, but it is worth noting that full protection was not attained. This is in contrast to the situation for GLP-1, in which valine-pyrrolidide completely prevented NH2-terminal degradation of exogenous GLP-1 (27), but the reason for this difference is not clear. The same inhibitor was used in both studies, and inhibition of the enzyme was complete for the duration of the experiment. The possibility exists, therefore, that GIP may be a substrate for other enzymes that cleave the peptide in the NH2-terminal portion and are not inhibitable by valine-pyrrolidide, an inhibitor known to be highly specific for DPP IV.

Sites of GIP extraction were identified from differences in arteriovenous concentrations across the various organ beds. Changes in these differences after DPP IV inhibition were interpreted to indicate a role of the enzyme at these sites. However, it cannot be totally excluded that DPP IV inhibition per se could lead to a change in blood flow across the different organ beds.

Determination of the arteriovenous concentration differences revealed that the kidney is a major site of GIP extraction, in agreement with previously reported findings in humans (9–11), dogs (9), and rats (11). This was detected with both assays, consistent with the peptide being filtered and undergoing proteolysis within the renal tubules. However, DPP IV inhibition significantly reduced renal clearance detected with both NH2-terminal and COOH-terminal assays. It is likely that GIP is degraded by DPP IV in the renal capillary endothelium. This would be prevented by the inhibitor, thereby explaining the reduced clearance detected by the NH2-terminal assay. The reduced clearance detected with the COOH-terminal assay can be interpreted as indicating that NH2-terminal cleavage is necessary before a later stage of degradation can occur; thus, preservation of the NH2-terminus of the peptide possibly affords protection from other subsequent enzymatic attacks.

The other major site of GIP removal is the liver, where the NH2-terminal assay revealed that cleavage in the NH2-terminal part of the peptide occurs. However, no significant extraction is detected with the COOH-terminal assay, confirming the negative findings of earlier studies in rats (7) and dogs (7,8), in which assays of similar specificity were used. In the presence of the enzyme inhibitor, NH2-terminal extraction decreased to undetectable levels, indicating the involvement of DPP IV. This is fully consistent with the location of the enzyme in high concentration on hepatocytes (28). However, with DPP IV inhibition, a modest COOH-terminal extraction was detected. This is more difficult to explain, but because direct comparison of COOH-terminal extraction before and after the inhibitor revealed no effect of valine-pyrrolidide, it may simply be a coincidental finding.
Extraction across the hind limb, representing degradation by connective, supportive, and muscular tissues and their capillary supply, was detected by both assays. This was reduced by valine-pyrrolidide, indicating a role of DPP IV in sites such as the capillary endothelium (29). However, significant NH₂-terminal extraction was still detectable, which again suggests that other enzymes are involved. It has been suggested that neutral endopeptidase 24.11 may play a role in GLP-1 degradation (17,30), but this seems less likely in the case of GIP because GIP was reported to be a poor substrate for the endopeptidase (30). Thus, the main sites of GIP extraction were identified as the kidney, liver, and extremities. If one takes into account the relative cardiac output (passing through the lungs and being distributed with 25% to the kidneys, 33% to the liver, and 25% to the extremities), then the liver is more important in terms of whole-body clearance of exogenous GIP, followed by the kidneys and the extremities. However, because the endogenous peptide travels first to the liver before being distributed to the remainder of the body, the liver becomes quantitatively much more important in terms of extraction of endogenous GIP.

The use of a DPP IV inhibitor clearly confirms that the enzyme does play an important role in the in vivo regulation of GIP activity, as is also seen with the other incretin hormone, GLP-1 (27), with inhibition of the enzyme leading to a marked improvement in the insulinotropic action of GIP. It cannot be totally excluded that the experimental design per se may contribute to this effect, in that increased insulin secretion occurred with the second GIP glucose infusions (in the presence of the inhibitor). However, this seems unlikely, because the glucose and insulin responses to two successive infusions of GIP alone were of a similar magnitude.

Valine-pyrrolidide treatment resulted in an increase in the proportion of intact biologically active GIP and was accompanied by a transient increase in insulin secretion even before the glucose load. This could be due to the increased levels of intact GIP having a modest stimulatory effect on the β-cell at a time when blood glucose levels may have been just above the threshold below which GIP ceases to be insulinotropic (31), but it is also likely that enhanced levels of intact endogenous GLP-1 contribute to the effect. After the glucose load, the presence of valine-pyrrolidide significantly enhanced insulin secretion and led to a reduction in the glucose excursion, with a doubling of the disappearance rate for glucose. GIP also lowers blood glucose by directly influencing pancreatic secretion (32,33), but there is evidence that it also has extrapancreatic effects. GIP receptors have been identified in several tissues (34,35), and there are reports that GIP promotes glucose uptake in muscle (36) and fat (37–39). GIP has also been indicated to increase the affinity of the insulin receptor (40), giving rise to the suggestion that GIP can modulate the effects of insulin by directly altering target tissue sensitivity to insulin (38), thereby contributing to the effective uptake of glucose postprandially (39,41). GIP has been shown to enhance insulin-mediated glucose disposal in sheep (42), rats (25), and mice (26), although a study in humans failed to show an effect (43). Therefore, it is possible that the increased glucose clearance seen in the present study in pigs after DPP IV inhibition may be due to the increased levels of intact GIP.

FIG. 5. Plasma insulin concentrations measured before, during, and after intravenous infusions of GIP (2 pmol · kg⁻¹ · min⁻¹) given alone (minutes 0–150) or during (minutes 150–327) DPP IV inhibition. Valine-pyrrolidide (val-pyd; 300 µmol/kg) was given 90 min after the end of the first GIP infusion, and after an additional 30 min, the second GIP infusion was started. Glucose infusions (0.2 g/kg) were given during minutes 38–47 of each GIP infusion. Data are means ± SE; n = 6. The horizontal arrows indicate the periods of the infusions. The plasma insulin profile obtained before valine-pyrrolidide treatment (minutes 0–147) is significantly lower (P < 0.02) than that obtained after administration of valine-pyrrolidide (minutes 180–327).
stimulating insulin secretion and directly or indirectly improving peripheral glucose uptake.

Several studies have now examined the effects of DPP IV inhibitors on glucose homeostasis and demonstrated that they result in improved glucose tolerance (14–16). Although the role of GLP-1 in mediating this effect has been shown by specific analysis of intact and total GLP-1, revealing that DPP IV inhibition protects the peptide from NH₂-terminal degradation (15,27), the lack of suitable methodology has, until now, meant that the contribution of GIP could only be a matter of speculation. As the present study shows, DPP IV inhibition does prevent at least part of the NH₂-terminal degradation of GIP, resulting in increased concentrations of the bioactive form of the peptide and improving both insulin secretion and glucose excursion. It can, therefore, be concluded that the mechanism of action of DPP IV inhibitors involves protection of the intact forms of both incretin hormones.

GLP-1 inhibits glucagon secretion both in vivo and in isolated pancreata (44), and basal levels of GLP-1 have been reported to play a role in the tonic inhibition of the pancreatic α-cell, either directly or indirectly via somatostatin or insulin (45). In contrast, GIP has a glucagonotropic effect in isolated α-cells (46), isolated pancreatic islets (47,48), perfused rat pancreata (49), and porcine pancreata (J. J. Holst, unpublished observations) and after intravenous infusion in rats (50) and humans (51). In the present study, infusion of GIP alone had no effect on plasma glucagon concentrations, possibly because concentrations of the intact peptide were insufficient. Moreover, a fall in glucagon concentrations was seen when the DPP IV inhibitor was given. This was also seen in our previous study with GLP-1 (27) and could be attributed to the increased concentrations of endogenous intact GLP-1 arising after DPP IV inhibition having an inhibitory effect on α-cell secretion. However, once the second GIP infusion was started, the increased levels of intact GIP arising from inhibition of DPP IV activity reverse the decrease by

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**TABLE 2**

Incremental AUCs for glucose and insulin attained during infusions of GIP alone or with inhibition of DPP IV

<table>
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<tr>
<th>Period (min)</th>
<th>Incremental AUC for glucose (min · mmol/l)</th>
<th>Incremental AUC for insulin (min · pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GIP alone</td>
<td>GIP + Val-pyd</td>
</tr>
<tr>
<td>0–35</td>
<td>6 ± 2</td>
<td>1 ± 4</td>
</tr>
<tr>
<td>0–147</td>
<td>116 ± 22</td>
<td>63 ± 28</td>
</tr>
<tr>
<td>35–147</td>
<td>133 ± 23</td>
<td>75 ± 9</td>
</tr>
</tbody>
</table>

Date are means ± SE; n = 6. Animals received 57-min intravenous infusions of GIP (2 pmol · kg⁻¹ · min⁻¹) alone or in the presence of a DPP IV inhibitor. Valine-pyrrolidide (300 μmol/kg) was given 90 min after the end of the first GIP infusion, and after an additional 30 min, the second GIP infusion was started. Glucose infusions (0.2 g/kg) were given during minutes 38–47 of each GIP infusion. “Period” refers to the time after the start of each GIP infusion.
stabilizing glucagon secretion. The apparent paradox, whereby GIP potentiates the reduction in glucagon levels in response to the glucose load, could be a consequence of an indirect effect caused by the inhibitory effect of GIP-induced insulin secretion (52).

In summary, our results demonstrate that GIP is degraded in a tissue-specific manner, with the liver, kidney, and extremities being the main sites of extraction. DPP IV is not the sole enzyme capable of inactivating GIP, but the use of a DPP IV inhibitor confirms that the enzyme does play a major role in regulating the insulinoetric and antihyperglycemic effects of GIP in vivo.

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DPP IV INHIBITION AND GIP