Regulation of the biological activity of glucagon-like peptide 2 in vivo by dipeptidyl peptidase IV

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Species-specific differences in the enzymatic inactivation of peptides is an important consideration in the evaluation of therapeutic efficacy. We demonstrate that glucagon-like peptide 2 (GLP-2), shown to be highly intestinotrophic in mice, promotes an increase in intestinal villus height but has no trophic effect on small bowel weight in rats. The reduced intestinotrophic activity of GLP-2 in rats is attributable to inactivation by the enzyme dipeptidyl peptidase IV (DPP-IV). GLP-2(1-33) was degraded to GLP-2(3-33) following incubation with human placental DPP-IV or rat serum but not by serum from DPP-IV-deficient rats. Administration of rat GLP-2 to DPP-IV-deficient rats was associated with markedly increased bioactivity of rat GLP-2 resulting in a significant increase in small bowel weight. A synthetic GLP-2 analog, r[Gly^2]GLP-2, with an alanine to glycine substitution at position 2, was resistant to cleavage by both DPP-IV and rat serum in vitro. Treatment of wild-type rats with r[Gly^2]GLP-2 produced a statistically significant increase in small bowel mass. DPP-IV-mediated inactivation of GLP-2 is a critical determinant of the growth factor-like properties of GLP-2.

Keywords: protein modeling, protease inactivation, growth factor, intestine, therapeutic

The differentiated cell types of the small bowel epithelium serve an important physiological function in the regulation of nutrient and electrolyte absorption. The rapid turnover and renewal of the intestinal mucosal epithelium provides a unique model for the analysis of the molecular factors important for cell proliferation and apoptosis. A number of peptide growth factors have been shown to be trophic for the small bowel in vivo, and exert their effects in general via stimulation of crypt cell proliferation and inhibition of apoptosis[1]. Whereas many epithelial growth factors such as growth hormone, various interleukins, the insulin-like growth factors, and keratinocyte growth factor stimulate the proliferation of a diverse number of epithelial cell types, peptide hormones such as gastrin or neurotensin appear to exert their growth-promoting effects in a more tissue-restricted manner.

The peptides coencoded with glucagon in the proglucagon gene have long been implicated in the control of intestinal growth and regeneration. The clinical reports of patients that presented glucagon-producing tumors and villus hyperplasia of the small bowel[2,3] were followed by experimental observations linking intestinal growth with increased production and secretion of the proglucagon-derived peptides[4]. Furthermore, major small bowel resection in rats is associated with increased expression of the proglucagon gene in the intestinal remnant[5], and bowel resection in humans is associated with increased release of enteroglucagon following a test meal[6], providing further albeit indirect, evidence that links intestinal adaptation with enhanced production of the proglucagon-derived peptides (PGDP) in vivo. Nevertheless, despite numerous studies demonstrating an association between proglucagon gene expression and intestinal growth, the identity of the PGDP with intestinotrophic properties remained unclear.

To elucidate the relationship between peptides encoded within proglucagon and small bowel growth, we established a series of transplantable glucagonomas in nude mice. Elevation of the circulating PGDPs in tumor-bearing mice[7] was consistently associated with the induction of crypt cell proliferation leading to significantly increased small bowel mass[8]. The PGDP that exhibited the greatest intestinal growth-promoting activity in a murine assay was identified as glucagon-like peptide 2 (ref. 8), a 33-amino acid peptide liberated in the enteroendocrine L cells of the small and large bowel.

GLP-2 administered subcutaneously to mice for 10–14 days induced a 1.5–2-fold increase of the mass of the small bowel. Comparable doses of GLP-2 (on a per weight basis) administered to rats did not result in an increase in small bowel weight. As all of these experiments were carried out with rat GLP-2, species-specific differences in the biological activities of these peptides did not account for the differential bioactivity in mice versus rats. We show that GLP-2 is inactivated by the enzyme DPP-IV (EC 3.4.14.5) following N-terminal cleavage at the position 2 alanine, and that the DPP-IV-mediated peptide inactivation is a major determinant of the intestinotrophic activity of GLP-2 in vivo.

Results

GLP-2 administration to mice stimulates crypt cell proliferation leading to increased mass of the intestinal mucosa[8]. Administration of rat GLP-2 via daily subcutaneous injection to mice in doses of 10–200 μg/kg consistently results in the induction of increased small bowel mass after 10–14 days[8,9]. To ascertain whether GLP-2 would exhibit similar growth-promoting properties in rats, we administered rat GLP-2 (either 2.5 or 25 μg in a 10% gelatin formulation subcutaneously twice daily for 10 days) to 200 to 250 gram Fischer 344 rats. GLP-2 treatment was associated with an

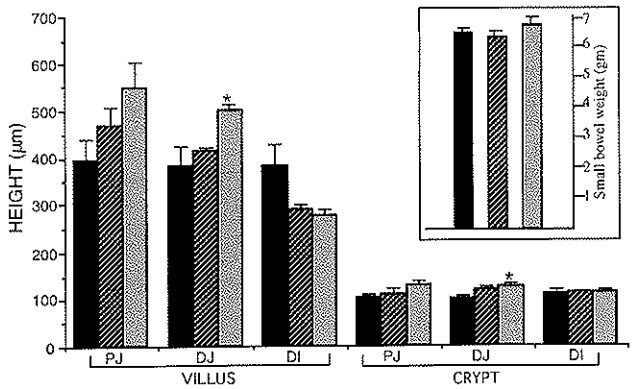

Figure 1. Effect of GLP-2 in wild-type Fischer rats. Rat GLP-2 (2.5 ▨ or 25 μg ■ in 10% gelatin) or 10% gelatin alone (control ■) was administered subcutaneously, every 12 hours for 10 days to male Fischer rats (200–250 grams, n = 3 per group). The villus and crypt height was assessed by microscopy from multiple sections (n = 10 sections per rat) of proximal jejunum (PJ), distal jejunum (DJ), and distal ileum (DI). * = p<.05.

increase in villus height in the proximal and distal jejunum ($p<0.05$) but not in the ileum (Fig. 1). A small increase in crypt depth was also detected in the jejunum following GLP-2 treatment. In contrast to the increase in mucosal epithelial height no change in small bowel weight was observed following treatment with either dose of GLP-2 (Fig. 1).

The difference in the effect of GLP-2 on the height of the intestinal mucosal epithelium compared to the small bowel mass in normal rats suggested that GLP-2 may be considerably less intestinotrophic in rats compared to mice[8]. Structurally related members of the glucagon peptide superfamily such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) contain a penultimate N-terminal alanine residue and are inactivated by the enzyme DPP-IV[10,11]. Nevertheless, GIP and GLP-1 are biologically active when administered to rats. One explanation for the markedly reduced biological activity of GLP-2 in rats may be due to species-specific differences in the inactivation of GLP-2 by DPP-IV, as GLP-2, like GIP and GLP-1, also contains a His-Ala dipeptide sequence at the N-terminus.

Native rat GLP-2 was incubated with human placental DPP-IV for 24 hours at 37°C, following which peptides were examined by HPLC. Incubation of peptide in the absence of DPP-IV was not associated with the generation of significant amounts of detectable cleavage products (Fig. 2A). In contrast, analysis of rat GLP-2 following incubation with DPP-IV demonstrated the presence of a second major peak (Fig. 2B) that eluted in the identical position as synthetic GLP-2(3-33). The identity of the peak as GLP-2(3-33) was confirmed by a combination of mass spectrometry and amino acid analysis. To determine if GLP-2 undergoes a similar DPP-IV-mediated cleavage to GLP-2(3-33) in rat plasma, we incubated rat GLP-2 for 24 hours with serum from wild-type rats or DPP-IV(-) rats. The HPLC profile of immunoreactive rat GLP-2 following incubation with wild-type rat serum showed a major peak that eluted in the identical position as synthetic GLP-2(1-33) (Fig. 3A). A second less abundant, but clearly detectable peak was observed that eluted in the identical position of synthetic GLP-2(3-33). In contrast, following incubation of rat GLP-2 with serum from a DPP-IV(-) rat, minimal amounts of immunoreactive GLP-2(3-33) were detected (Fig. 3B).

To test the hypothesis that DPP-IV is a major determinant of GLP-2 activity in vivo, we examined the biological effects of GLP-2 in DPP-IV(-) rats. These rats, derived from a normal colony of Fischer 344 rats, exhibit decreased DPP-IV activity in vivo due to translation of a mutant form of the protein with decreased biological activity[12,13]. In contrast to the results obtained following GLP-2 administration to wild-type Fischer rats (Fig. 1), rat GLP-2 (25 μg twice a day) induced a statistically significant increase in both the height of the mucosal epithelium and the mass of the small bowel (Fig. 4, $p<0.05$–0.01 and $p<0.0001$, GLP-2 vs. control, for mucosal height and small bowel weight, respectively). These data demon-

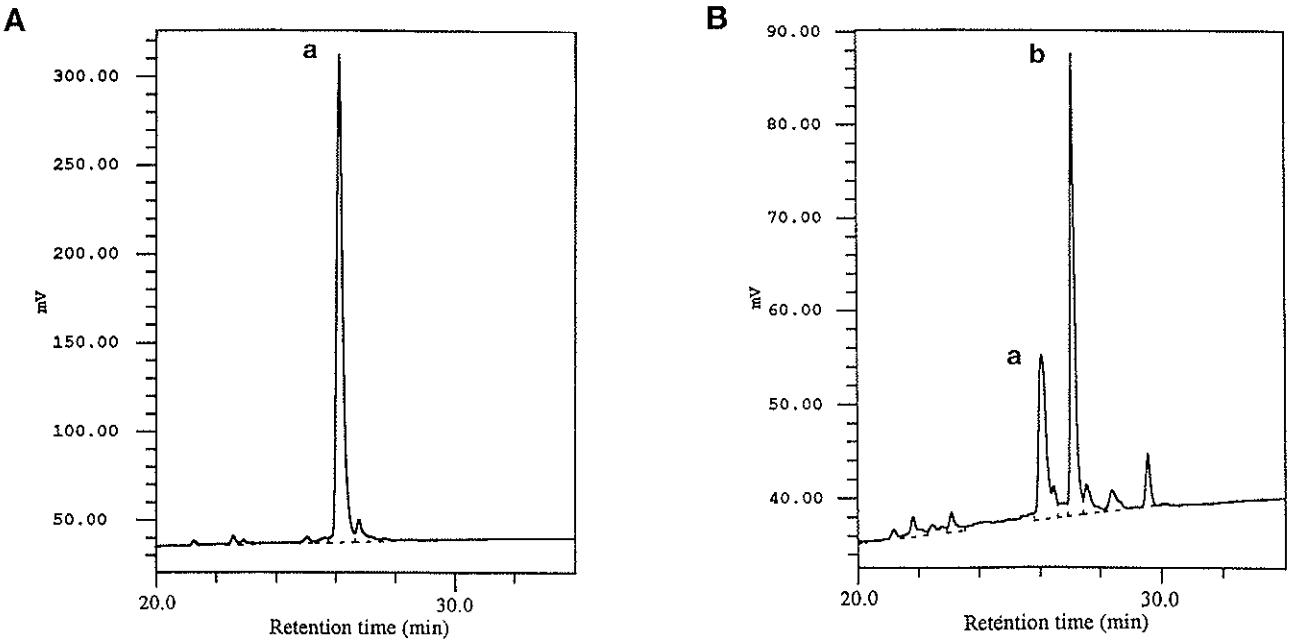

Figure 2. HPLC chromatogram of native rat GLP-2 before (A) and after (B) treatment with DPP-IV. Incubation with human placental DPP-IV was at 37°C for 24 hours. The peaks marked *a* and *b* correspond to the elution positions of synthetic rat GLP-2(1-33) and GLP-2(3-33), respectively. The identities of peaks *a* and *b* were confirmed by mass spectrometry and amino acid sequence analysis.

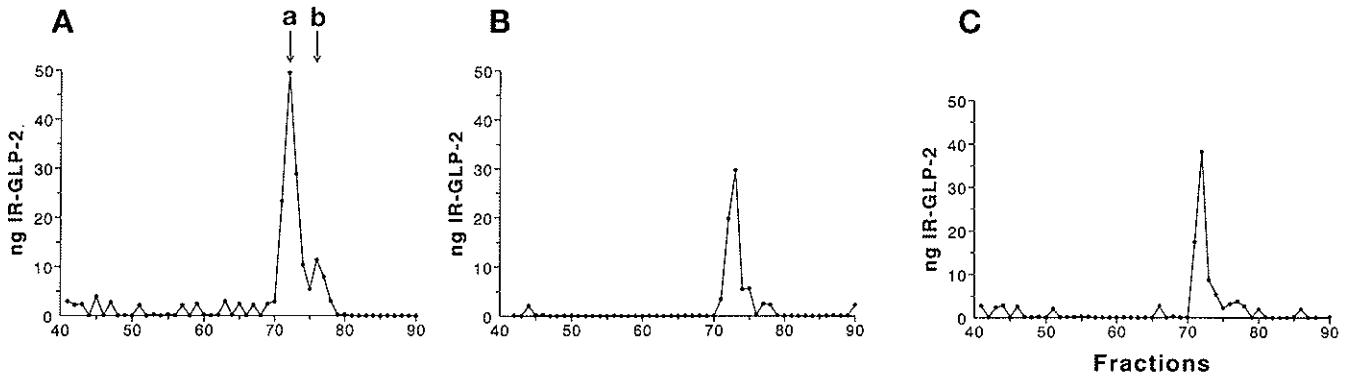

Figure 3. Rat GLP-2(1-33) (A and B) or r[Gly2]GLP-2 (0.1 μg peptide) was incubated in separate experiments in serum from control rats (A and C) or DPP-IV(-) rats (B), for 24 hours. Peptides were then separated by HPLC and analyzed by RIA. Peaks *a* and *b* indicate the elution positions of synthetic rat GLP-2(1-33) and GLP-2(3-33), respectively. Data shown is representative of n = 5 experiments.

strate significantly increased intestinotrophic activity of GLP-2 in DPP-IV(-) compared to wild-type rats and are consistent with the hypothesis that expression of the enzyme DPP-IV in wild-type rats is critically important for inactivation of GLP-2 and hence modulation of its bioactivity in vivo.

The demonstration that the enzymatic inactivation of GLP-2 may be a limiting determinant of GLP-2 action prompted us to determine whether amino acid substitutions that confer resistance to DPP-IV cleavage might render the GLP-2 molecule more bioactive in wild-type rats in vivo. A rat GLP-2 analog containing a glycine for alanine substitution at position 2 was synthesized and incubated with human DPP-IV in vitro. No detectable cleavage of r[Gly2]GLP-2 to GLP-2(3-33) was observed (Fig. 5), consistent with the notion that the penultimate alanine is a major determinant of DPP-IV recognition and cleavage[14]. Similarly, incubation of r[Gly2]GLP-2 with rat serum followed by HPLC and RIA demonstrated minimal cleavage to GLP-2(3-33) in vitro (Fig. 3C). Taken together, these observations demonstrate that r[Gly2]GLP-2 is resistant to DPP-IV-mediated cleavage and as a result, raise the possiblity that this analog may be comparatively more bioactive than the native peptide in vivo.

To determine the intestinotrophic properties of r[Gly2]GLP-2 in vivo, wild-type and DPP-IV(-) Fischer rats were treated for 10 days with twice daily injections of 25 μg of r[Gly2]GLP-2, following which the rats were sacrificed for analysis of small bowel weight and histology. In contrast to the negative results obtained with the native rat peptide, r[Gly2]GLP-2 treatment of wild-type rats was associated with a significant increase in both villus height ($p<0.05–0.0001$) and small bowel mass ($p<.0001$) (Fig. 6). r[Gly2]GLP-2 was also slightly more intestinotrophic (as assessed by analysis of small bowel weight and histology) than native rat GLP-2 in DPP-IV(-) rats (Fig. 4). The combination of in vitro and in vivo analyses suggests that DPP-IV is a major determinant of GLP-2 bioactivity.

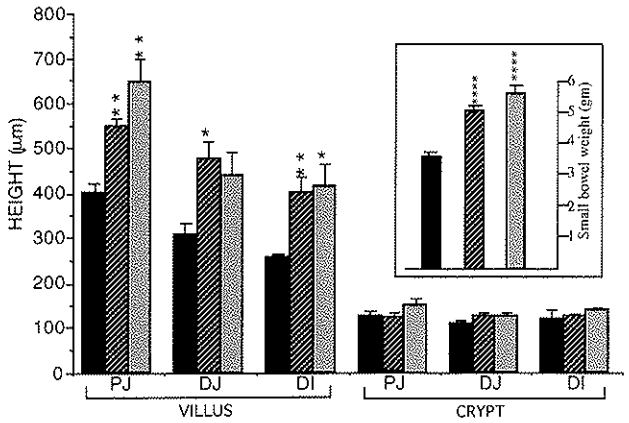

Figure 4. GLP-2 induces small bowel growth in DPP-IV(-) rats. DPP-IV(-) male Fischer rats (~80–95 grams, n = 3 per group) were treated with saline alone (control ■), or rat GLP-2 ▨, or r[Gly2]GLP-2 ■ 25 μg, twice daily for 10 days. Histological analysis of the small bowel proximal jejunum (PJ), distal jejunum (DJ), and distal ileum (DI) was carried out as described in Experimental protocol. *p<0.05, **p<0.01, ****p<0.0001

Discussion

The glucagon peptide superfamily comprises a number of peptides that contain an alanine residue at position 2, consistent with the known recognition cleavage sites (alanine or proline) for the protease DPP-IV. As the majority of these peptides require an intact N-terminus for full biological activity, cleavage of the peptides at the N-terminus generally inactivates the biological activity in vivo. For example, native growth hormone-releasing hormone (1-44) or (1-40) is rapidly inactivated by DPP-IV to generate the GRH(3-44) or GRH(3-40) peptides. Hence, a major component of plasma immunoreactive GRH is in fact not the mature intact peptide but the N-terminally degraded, biologically inactive forms that exhibit a longer circulating $t_{1/2}$ in vivo[15]. GRH analogs that can be synthesized are resistant to DPP-IV and exhibit greater stability and a longer half-life in vivo[15-17].

Circulating forms of GLP-1 are also rapidly cleaved by DPP-IV to generate GLP-1(9-36) amide (and possibly GLP-1(9-37)), peptides that are biologically inactive in vivo[10,11,18]. The observation that GLP-1(9-36) amide may function as an antagonist at the GLP-1 receptor[19] suggests that DPP-IV may play an important role in determining the ratio of circulating agonist/antagonist in vivo, and hence may be a key determinant regulating signalling at the level of the GLP-1 receptor.

The relative degree of peptide degradation by DPP-IV appears to be substantially greater for GLP-1 than for GLP-2. More than 60% of GLP-1 was cleaved after only a 30 minute incubation with human plasma at 37°C[11]. Consistent with in vitro data demonstrating the rapid degradation of GLP-1 at the N-terminus, a substantial proportion of circulating immunoreactive GLP-1 in human plasma from fasting individuals is actually the N-terminally truncated GLP-1(9-36) amide, which represents the majority of immunoreactive GLP-1 in the postprandial state[11]. In contrast, we observed less than 10% degradation of GLP-2 after a much longer 24-hour incubation with rat plasma in vitro. Similarly, the major molecular form detected after an 8-hour incubation of [^{125}-I]GLP-1(7-36) amide with DPP-IV was the N-terminally cleaved [^{125}-I]GLP-1(9-36) amide[10] whereas the major form of GLP-2 detected

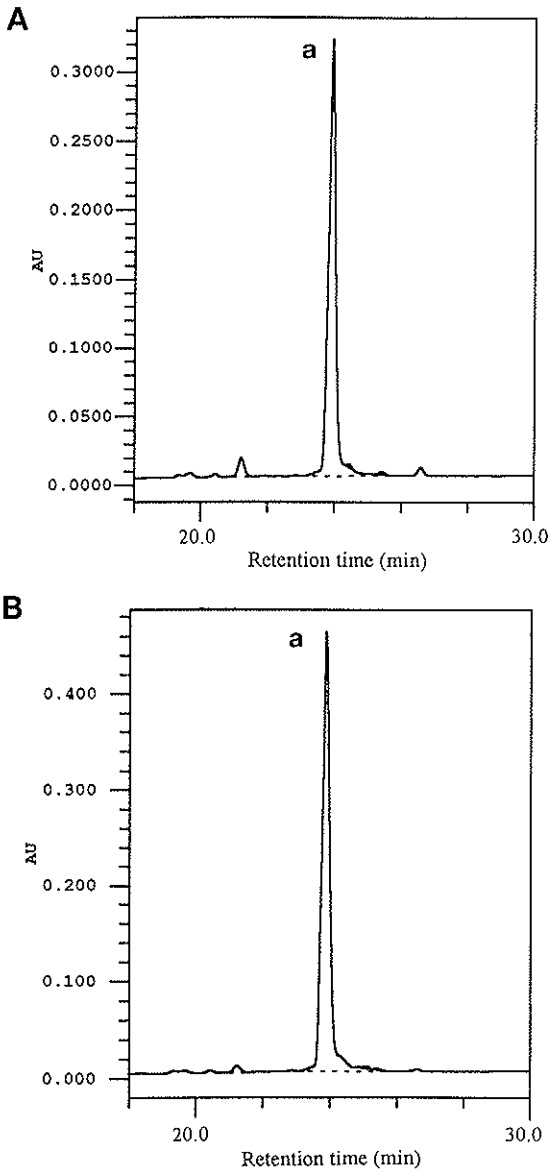

Figure 5. HPLC chromatogram of r[Gly2]GLP-2 before (A) and after (B) treatment with DPP-IV at 37°C for 24 hours. The peak marked *a* corresponds to the elution position of synthetic r[Gly2]GLP-2. The identity of peak *a* was confirmed by mass spectrometry and amino acid sequence analysis.

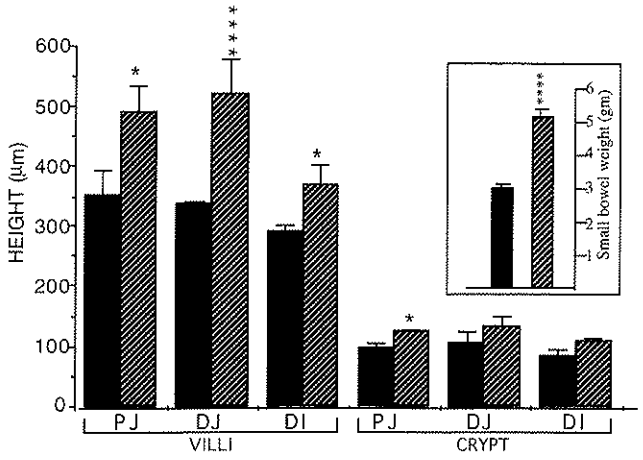

Figure 6. Effect of r[Gly2]GLP-2 on rat small bowel growth. Male Fischer rats (~60 grams at the start of the experiment, n = 3 per group) were injected with 20 µg r[Gly2]GLP-2 ▨ in 0.5 ml saline subcutaneously twice daily (or saline alone, control ■) for 10 days. Histological analysis of the small bowel proximal jejunum (PJ), distal jejunum (DJ), and distal ileum (DI) was carried out as described in Experimental protocol. *p<0.05, ****p<0.0001

after a 24-hour incubation with DPP-IV was the intact 33-amino acid peptide. These data imply that the relative stability of GLP-2 in vivo may be somewhat greater than that observed for GLP-1.

The importance of DPP-IV for the control of GLP-1 degradation is supported by studies demonstrating reduced N-terminal degradation of GLP-1 following infusion of GLP-1 into the DPP-IV-deficient rat[10]. The DPP-IV-deficient rat, derived from a Japanese strain of Fischer 344 rats[12], contains mRNA transcripts for DPP-IV but reduced levels of DPP-IV protein and enzymatic activity[12,13,20] due to translation of abnormal isoforms that fail to be processed to the biologically active mature glycosylated enzyme[21]. Although our in vitro data suggests that GLP-2 may be relatively less susceptible to degradation by DPP-IV compared to GLP-1, analysis of the molecular forms of GLP-2 in rat plasma demonstrate a considerable amount of circulating GLP-2(3-33), consistent with DPP-IV-mediated cleavage of this peptide in vivo (data not shown). Consistent with the importance of DPP-IV for the inactivation of GLP-2, we did not observe significant induction of small bowel growth following twice daily administration of GLP-2 to normal rats. Administration of pharmacological amounts of GLP-2 to rats may not necessarily be associated with increased concentrations of biologically active intact peptide, perhaps due to relatively increased DPP-IV activity in the rat. In contrast, treatment of DPP-IV-deficient rats with unmodified rat GLP-2 was associated with a statistically significant increase in small bowel mass, providing clear evidence in support of a key role for DPP-IV in the regulation of GLP-2 activity in vivo.

The serine protease DPP-IV is expressed in a broad range of tissues and at very high levels in the kidney[22]. DPP-IV activity has also been localized to the small intestine of both rats and humans, with the highest levels detected in the jejunum and ileum[23,24]. Although the precise cellular target for GLP-2 activity has not been identified, the demonstration that GLP-2 stimulates crypt cell proliferation suggests that GLP-2 may exert its effects in part via a direct effect on crypt stem cells. Intriguingly, a crypt to villus gradient of DPP-IV has been described in the rat jejunum, with low levels of DPP-IV in crypt cells increasing progressively in differentiated enterocytes[25]. Local region-specific differences in intestinal DPP-IV expression may modulate the activity of GLP-2 by preferentially inactivating the peptide at different sites along the crypt to villus axis.

The GLP-2-mediated increase in villus height, but not small bowel weight, suggests that the amount of biologically active circulating GLP-2 in these experiments was slightly below the threshold required to induce changes in small bowel mass. Consistent with this hypothesis, we have observed that mice treated with a low dose of GLP-2, 250 ng every 12 hours, exhibit a statistically significant increase in jejunal crypt plus villus height but not in small bowel weight[9]. In contrast, larger doses of GLP-2 increased both villus height and small bowel weight in mice in vivo[9].

The detection of GLP-2(3-33) as the major cleavage product generated following incubation of GLP-2 with DPP-IV has implications for interpreting data from radioimmunoassays that employ antisera specific for the mid or carboxyterminal region of GLP-2. At the present time little information is available about the circulating forms of GLP-2 immunoreactivity, however a single study

has reported that GLP-2 levels rise following food ingestion in normal human volunteers[26]. The antisera used for this study was raised against 34-amino acid synthetic human GLP-2 (proglucagon 126-159), however the GLP-2 epitopes recognized by these antisera have not yet been reported. Taken together, the data reported here further extend the central role of DPP-IV in the regulation of the biological activity of the glucagon-like peptides and suggests that GLP-2 analogs exhibiting DPP-IV resistance may potentially be therapeutically useful in vivo.

Experimental protocol

All chemicals were from Sigma Chemicals (St.Louis, MO) or Baxter Travenol Canada (Toronto, Ontario). Rat GLP-2 was obtained from the American Peptide Company (Sunnyvale, CA) or from California Peptide Research (Napa, CA) and was greater than 99% pure by HPLC. Rat (Gly2)GLP-2(1-33) and rat GLP-2(3-33) were obtained from California Peptide Research and were 97% pure by HPLC. Diprotin A was purchased from Novabiochem (San Diego, CA) or Sigma. Human placental dipeptidyl peptidase-IV (specific activity 5,000 mU/mg protein) was purchased from Calbiochem (San Diego, CA). Trasylol was obtained from Bayer (Toronto, Ontario) and EDTA was from ACP Chemical (Montreal, Quebec).

Animals. Fischer rats were obtained from Charles River Laboratory (St. Constant, Quebec). A small colony of DPP-IV negative rats[10,12] was established following procurement of the animals from Ray Pederson, University of British Columbia (Vancouver, BC). The rats were not restricted by diet or activity during the experiment and were housed 2–3/cage under a light/dark cycle of 12 h. Animals were fasted overnight (12–14 h) prior to sacrifice.

Animal and Tissue Processing. Rats were anesthetized with CO_2 and the small intestine was removed from the peritoneal cavity (from pylorus to cecum), cleaned, weighed and measured. For comparative purposes, sections from each animal were obtained from similar anatomical positions, ~16, 38, and 65 cm from the gastroduodenal junction for proximal and distal jejunum and ileum, respectively. Each small bowel fragment was opened longitudinally on its antimesenteric border, sectioned, and then placed in 10% formalin (v/v) overnight.

Histological sections 5 µm thick were cut, stained with hematoxylin and eosin and used for micrometry and morphometric analysis as previously described[6]. Intestinal micrometry was performed using a Leitz (Wetzar, Germany) microscope with a video camera connected to a computer monitor. The microscope was calibrated at 4×, 10×, and 25× magnification and the same microscope was used for all evaluations. Crypt and villus height was measured by examining at least 10 longitudinally oriented villi from each slide for proximal and distal jejunum and distal ileum and is expressed in µm ± SEM.

DPP-IV cleavage of rat GLP-2 and r[Gly2]GLP-2. Peptide solutions were prepared at a concentration of 0.2 mg/ml in 40 mM PBS, pH 6.5. A 2.5 µl aliquot (0.125 mU) of human DPP-IV was added to each 50 µl aliquot (10 µg) of peptide solution, and the mixtures were incubated at 37°C for 24 h. The enzyme incubations were quenched by addition of 50 µl (200 µg) of a 4 mg/ml solution of Diprotin A in PBS. Following incubation of peptides with DPP-IV in vitro, a 100 µl aliquot of the quenched incubation mixture was injected onto a Rainin Dynamax C18 (250×4.6 mm) column. The samples were eluted using a linear solvent gradient [30–60% B; solvent A = 0.1% (v/v) TFA in water and solvent B = 0.1% TFA in acetonitrile] at a flow rate of 1 ml/min. Elution was monitored by absorbance at 214 nm.

Blood for in vitro incubations was collected from wild-type Wistar and Fischer rats, following which peptides (0.1 µg) were incubated with 1 ml of serum for 24 h at 37°C. The reaction was terminated by addition of 110 µl TED (Trasylol:EDTA:Diprotin A; [5000 KIU/ml:1.2 mg/ml:0.1 mM). The sample was then mixed with 2 ml of of 1% (v/v) TFA, pH adjusted to 2.5 with diethylamine and applied to a C_{18} Sep Pak cartridge (Waters Associates, Bedford, MA). The peptides were eluted with 3.0 ml 80% (v/v) isopropanol in 0.1% (v/v) TFA and stored at -70 C prior to radioimmunoassay. Peptides were separated by HPLC as described above, using a C_{18} uBondapak column (Waters) with a flow rate of 1.5 ml/min. Fractions were collected every 0.3 min.

GLP-2 Radioimmunoassay. RIA for GLP-2 was carried out using an antiserum (UTTH-7) that recognizes the midsequence of GLP-2 (amino acids 25–30) and cross-reacts equally with GLP-2(1-33) and GLP-2(3-33) (data not shown). The detection limits of the assay were 10–2000 pg/tube; the intra and interassay variations were 3.6% and 8.3%, respectively.

Statistical Analysis. Statistical significance was calculated by ANOVA using a SAS program (Statistical Analysis Systems, Cary, NC) for IBM computers.

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1. Taylor, R.G. and Fuller, P.J. 1994. Humoral regulation of intestinal adaptation. *Baillieres Clin. Endocrinol. and Metab.* **8**:165–183.
2. Gleeson, M.H., Bloom, S.R., Polak, J.M., Henry, K., and Dowling, R.H. 1971. Endocrine tumour in kidney affecting small bowel structure, motility, and absorptive function. *Gut* **12**:773–782.
3. Stevens, F.M., Flanagan, R.W., O'Gorman, D., and Buchanan, K.D. 1984. Glucagonoma syndrome demonstrating giant duodenal villi. *Gut* **25**:784–791.
4. Bloom, S.R. and Polak, J.M. 1982. The hormonal pattern of intestinal adaptation [a major role for enteroglucagon]. *Scand. J. Gastroenterol.* **17**:93–103.
5. Fuller, P.J., Beveridge, D.J., and Taylor, R.G. 1993. Ileal proglucagon gene expression in the rat: characterization in intestinal adaptation using in situ hybridization. *Gastroenterology* **104**:459–466.
6. Besterman, H.S., Adrian, T.E., Mallinson, C.N., Christofides, N.D., Sarson, D.L., Pera, A., et al. 1982. Gut hormone release after intestinal resection. *Gut* **23**:854–861.
7. Ehrlich, P., Tucker, D., Asa, S.L., Brubaker, P.L., and Drucker, D.J. 1994. Inhibition of pancreatic proglucagon gene expression in mice bearing subcutaneous endocrine tumors. *Am. J. Physiol. Endocrinol. Metab.* **267**:E662–E671.
8. Drucker, D.J., Ehrlich, P., Asa, S.L., and Brubaker, P.L. 1996. Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proc. Natl. Acad. Sci. USA* **93**:7911–7916.
9. Tsai, C.-H., Hill, M., and Drucker, D.J. 1997. Biological determinants of intestinotrophic properties of GLP-2 in vivo. *Am. J. Physiol.* **272**:G662–G668.
10. Kieffer, T.J., McIntosh, C.H.S., and Pederson, R.A. 1995. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* **136**:3585–3596.
11. Deacon, C.F., Johnsen, A.H., and Holst, J.J. 1995. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J. Clin. Endocrinol. Metab.* **80**:952–957.
12. Watanabe, Y., Kojima, T., and Fujimoto, Y. 1987. Deficiency of membrane-bound dipeptidyl aminopeptidase IV in a certain rat strain. *Experientia* **43**:400–401.
13. Thompson, N.L., Hixson, D.C., Callanan, H., Panzica, M., Flanagan, D., Faris, R.A., et al. 1991. A Fischer rat substrain deficient in dipeptidyl peptidase IV activity makes normal steady-state RNA levels and an altered protein. *Biochem. J.* **273**:497–502.
14. Bongers, J., Lambros, T., Ahmad, M., and Heimer, E.P. 1992. Kinetics of dipeptidyl peptidase IV proteolysis of growth hormone-releasing factor and analogs. *Biochim. Biophys. Acta* **1122**:147–153.
15. Frohman, L.A., Downs, T.R., Heimer, E.P., and Felix, A.M. 1989. Dipeptidyl peptidase IV and trypsin-like enzymatic degradation of human growth hormone-releasing hormone in plasma. *J. Clin. Invest.* **83**:1533–1540.
16. Lance, V.A., Murphy, W.A., Sueiras-Diaz, J., and Coy, D.H. 1984. Super-active analogues of growth hormone-releasing factor (1-29)-amide. *Biochem. Biophys. Res. Commun.* **119**:265–272.
17. Frohman, L.A., Downs, T.R., Williams, T.C., Heimer, E.P., Pan, Y.-C.E., and Felix, A.M. 1986. Rapid enzymatic degradation of growth hormone releasing factor by plasma in vivo and in vivo to a biologically inactive product cleaved at the NH_2-terminuss. *J. Clin. Invest.* **78**:906–913.
18. Mentlein, R., Gallwitz, B., and Schmidt, W.E. 1993. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* **214**:829–835.
19. Knudsen, L.B. and Pridal, L. 1996. Glucagon-like peptide-1-(9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36) amide after in vivo administration to dogs and it acts as an antagonist on the pancreatic receptor. *Eur. J. Pharmacol.* **318**:429–435.
20. Thompson, N.L., Hixson, D.C., Callanan, H., Panzica, M., Flanagan, D., Faris, R.A., et al. 1991. A Fischer rat substrain deficient in dipeptidyl peptidase IV activity makes normal steady-state RNA levels and an altered protein. Use as a liver-cell transplantation model. *Biochem. J.* **273**:497–502.
21. Erickson, R.H., Suzuki, Y., Sedlmayer, A., and Kim, Y.S. 1992. Biosynthesis and degradation of altered immature forms of intestinal dipeptidyl peptidase IV in a rat strain lacking the enzyme. *J. Biol. Chem.* **267**:21623–21629.
22. Hildebrandt, M., Reutter, W., and Gitlin, J.D. 1991. Tissue-specific regulation of dipeptidyl peptidase IV expression during development. *Biochem. J.* **277**:331–334.
23. Darmoul, D., Voisin, T., Couvineau, A., Rouyer-Fessard, C., Salomon, R., Wang, Y., et al. 1994. Regional expression of epithelial dipeptidyl peptidase IV in the human intestines. *Biochem. Biophys. Res. Commun.* **203**:1224–1229.
24. Bai, J.P.F. 1994. Distribution of brush-border membrane peptidases along the rat intestine. *Pharm. Res.* **11**:897–900.
25. Darmoul, D., Rouyer-Fessard, C., Blais, A., Voisin, T., Sapin, C., Baricault, L., et al. 1991. Dipeptidyl peptidase IV expression in rat jejunal crypt-villus axis is controlled at mRNA level. *Am. J. Physiol.* **261**:G763–G769.
26. Orskov, C. and Holst, J.J. 1987. Radio-immunoassays for glucagon-like peptides 1 and 2 (GLP-1 and GLP-2). *Scand. J. Clin. Lab. Invest.* **47**:165–174.