REVIEW

Gut adaptation and the glucagon-like peptides

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The glucagon-like peptides GLP-1 and GLP-2 are synthesised and then released from enteroendocrine cells in the small and large intestine. GLP-1 promotes efficient nutrient assimilation while GLP-2 regulates energy absorption via effects on nutrient intake, gastric acid secretion and gastric emptying, nutrient absorption, and mucosal permeability. Preliminary human studies indicate that GLP-2 may enhance energy absorption and reduce fluid loss in subjects with short bowel syndrome suggesting that GLP-2 functions as a key regulator of mucosal integrity, permeability, and nutrient absorption. Hence GLP-2 may be therapeutically useful in diseases characterised by injury or dysfunction of the gastrointestinal epithelium.

SUMMARY

The glucagon-like peptides are synthesised in and released from enteroendocrine cells in the small and large intestine. Glucagon-like peptide 1 (GLP-1) promotes efficient nutrient assimilation via effects on food intake, gastric emptying, stimulation of insulin secretion, and control of islet proliferation. Glucagon-like peptide 2 (GLP-2), a 33 amino acid peptide cosecreted with GLP-1, regulates energy absorption via effects on nutrient intake, gastric acid secretion and gastric emptying, nutrient absorption, and mucosal permeability. GLP-2 secretion is stimulated by nutrients, and plasma levels of circulating GLP-2 are elevated in the setting of intestinal injury. GLP-2 is enzymatically inactivated by dipeptidyl peptidase IV by cleavage at the position 2 alanine, hence the native peptide has a $t_{1/2}$ of minutes in vivo. Exogenous administration of GLP-2 promotes expansion of the mucosal epithelium via stimulation of crypt cell proliferation and inhibition of crypt and enterocyte apoptosis, leading to an increase in mucosal surface area. Administration of GLP-2 in the setting of experimental intestinal injury reduces the extent of mucosal damage in both the small and large intestine. GLP-2 augments the endogenous adaptive response to small bowel resection and stimulates nutrient absorption in the normal and injured mucosal epithelium. The actions of GLP-2 are mediated by a recently identified G protein coupled receptor expressed in endocrine cells and enteric neurones of the stomach, small bowel, and colon. Preliminary human studies demonstrate that GLP-2 may enhance energy absorption and reduce fluid loss in subjects with short bowel syndrome. The available evidence suggests that GLP-2 functions as a key regulator of mucosal integrity, permeability, and nutrient absorption and hence GLP-2 may potentially be therapeutically useful in diseases characterised by injury or dysfunction of the gastrointestinal epithelium.

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INTRODUCTION

Following the development of radioimmunoassays for pancreatic glucagon by Unger and colleagues in the early 1960s, glucagon-like immunoreactivity (GLI) was detected not only in pancreatic extracts but also in the small and large intestine. GLI detected by radioimmunoassay, also known as "enteroglucagon", was heterogeneous with multiple molecular forms detected in both gut extracts and the circulation. Subsequent studies demonstrated a correlation between increased levels of circulating GLI or "enteroglucagon" and proliferation or adaptation of the intestinal mucosa. Furthermore, several patients with glucagonomas were reported that exhibited small bowel villus hyperplasia that receded following tumour resection. Although glicentin is weakly trophic for the intestinal mucosa, GLP-2 was recently identified as the proglucagon derived peptide (PGDP) with the greatest intestinotrophic activity. This review discusses the scientific evidence linking PGDPs with intestinal adaptation, and summarises experimental data that establish GLP-2 as a physiological regulator of mucosal epithelial homeostasis.

GLUCAGON, GLICENTIN, AND GLUCAGON-LIKE IMMUNOREACTIVITY

Glucagon, a 29 amino acid peptide hormone, is synthesised in and secreted from pancreatic endocrine A cells. Antisera directed against the mid- and carboxy terminal region of pancreatic glucagon commonly cross react with larger immunoreactive forms of GLI in both plasma and tissue extracts from canine, porcine, and human intestine.[1 2] Although estimates from gel chromatography originally led to designation of glicentin as a peptide of ~100 amino acids, isolation and sequencing of glicentin from porcine intestine, taken together with the cloning of proglucagon cDNAs,[3 4] demonstrated that glicentin is composed of 69 amino acids, including the 29 amino acid sequence of glucagon, as shown in fig 1.[5] The carboxy terminal end of glicentin, containing glucagon and the eight amino acid extension, was designated oxyntomodulin following the demonstration that this 37 amino acid peptide stimulated cAMP formation in the rat gastric fundus.[6]

Abbreviations: GLP-1, GLP-2, glucagon-like peptides 1 and 2; GLI, glucagon-like immunoreactivity; PGDP, proglucagon derived peptide; IP-1, IP-2, intervening peptides 1 and-2; DP IV, dipeptidyl peptidase IV; PKA, protein kinase A; GLP-2R, GLP-2 receptor.

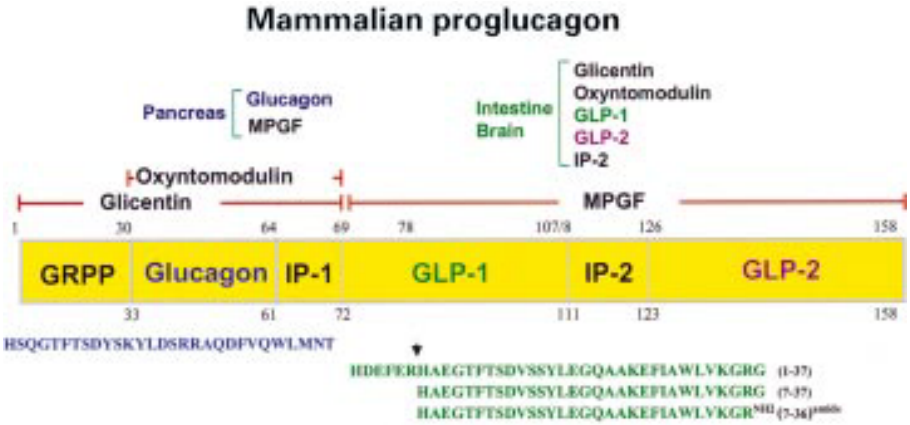

Figure 1 Structure and processing of proglucagon. The structural organisation of mammalian proglucagon and the proglucagon derived peptides are shown. The numbers above and below proglucagon refer to the corresponding amino acid sequence boundaries of the different peptides. The amino acid sequences of glucagon (blue) and GLP-1 (green) are illustrated. GLP-1, GLP-2, glucagon-like peptides 1 and 2 respectively; IP-1, IP-2, intervening peptides 1 and 2, respectively; MPGF, major proglucagon fragment.

GLUCAGONOMAS, ENTEROGLUCAGON, AND INTESTINAL GROWTH

A link between increased circulating levels of the PGDPs and intestinal mucosal proliferation was first described in a 44 year old woman with an endocrine tumour in the kidney who presented with constipation, oedema, nausea, abdominal distension, and occasional vomiting.[7] Mild glucose intolerance and steatorrhoea was detected and a barium enema revealed faecal retention but normal colonic mucosa. In contrast, the small bowel follow through demonstrated thickened mucosal folds and delayed intestinal transit times. A surgical biopsy of the mid-jejunum revealed villous enlargement, and at laparotomy a 7 cm endocrine tumour of the right kidney was detected that exhibited immunopositivity for glucagon. Plasma glucagon was more than 10-fold higher than the upper limit of normal, and glucagon immunoreactivity was also detected in tumour extracts.[7] Several weeks following removal of the tumour, constipation disappeared, intestinal transit time normalised, and the morphology of the small bowel mucosal epithelium returned to normal. Analysis of tumour derived glucagon immunoreactivity by gel chromatography and radioimmunoassays demonstrated a major peak of immunoreactivity that eluted in the same position as the high molecular weight fraction of enteroglucagon from human ileal extracts.[8] The authors speculated that either glucagon or a related tumour derived substance was responsible for the observed changes in the motility and mucosa of the small bowel.

Identification of a possible link between gut derived enteroglucagon(s) and intestinal growth was followed by an important series of studies by Dowling, Bloom, and others establishing an association between intestinal epithelial injury or resection, and increased circulating levels of enteroglucagon or glicentin in rats.[9–13] Furthermore, suppression of plasma enteroglucagon following somatostatin infusion reduced the rate of crypt cell proliferation in the ileum.[14] Evidence that the elusive intestinotrophic factor acted in a humoral endocrine manner derived from experiments demonstrating that increased circulating levels of enteroglucagon correlated with enhanced crypt cell proliferation in exteriorised Thiry-Vella fistulae following small bowel resection.[15] Intestinal resection was consistently associated with increased circulating levels of the PGDPs and upregulation of intestinal proglucagon mRNA transcripts in the intestinal remnant.[16–19]

Injury to the gastrointestinal epithelium is also associated with increased circulating levels of the PGDPs in human subjects. Patients with jejunoileal bypass procedures exhibited elevations in circulating enteroglucagon, hyperplasia in the intestinal remnant, and preservation of the mucosal epithelium in the bypassed intestinal segment.[20] Acute tropical sprue and coeliac disease with small bowel villus atrophy and carbohydrate malabsorption was associated with markedly elevated levels of circulating enteroglucagon in the fasting state, with elevation in enteroglucagon related to the extent of nutrient malabsorption.[21 22] Small bowel, but not colonic, resection was associated with increased circulating levels of enteroglucagon in human subjects.[23] The contribution of the colon to circulating enteroglucagon was further illustrated in patients with ileostomies that exhibited reduced circulating levels of plasma enteroglucagon.[24]

Additional studies of glucagonoma patients confirmed the association between increased circulating levels of the PGDPs and intestinal mucosal epithelial hyperplasia. A 39 year old male with necrolytic migratory erythema and diabetes exhibited giant duodenal villi on endoscopy. Circulating plasma hyperglucagonaemia and a large endocrine tumour of the pancreas was subsequently detected[25] and multiple molecular forms of glucagon immunoreactive peptides were observed in the patient's plasma using column chromatography and glucagon radioimmunoassays. Villous hypertrophy, delayed intestinal transit, and increased thickness of the small bowel epithelium have also been reported as radiological signs in patients with pancreatic glucagonomas.[26 27] Hence small intestinal mucosal proliferation may be more common than previously appreciated in subjects with glucagonomas, depending on the pattern of tumour specific post-translational processing and the degree to which the small bowel is carefully examined by endoscopy, biopsy, or imaging studies.

Despite the correlation between intestinal injury or resection, increased levels of circulating PGDPs, and subsequent mucosal regrowth and gut adaptation in both rodents and human subjects, the identity of the specific PGDP with intestinotrophic activity remained elusive. Following isolation of proglucagon cDNAs from pancreatic, brainstem, and intestinal cDNA libraries (fig 1), the potential complexity of PGDP synthesis and secretion became evident.[3 4 28–30] Although the nucleotide sequence of mammalian proglucagon mRNA transcripts is identical in pancreas, intestine, and brain, tissue specific post-translational processing (fig 1) results in the liberation of a different profile of PGDPs in each tissue.[31 32] Hence intestinal PGDPs, originally termed "enteroglucagons", include not only glicentin and oxyntomodulin but also two glucagon-like peptides, GLP-1 and GLP-2. Furthermore, two intervening or spacer peptides, IP-1 and IP-2, are also liberated following proglucagon processing in the intestine.

Although experimental evidence links increased circulating levels of glicentin or "enteroglucagon" with intestinal adaptation and enhanced mucosal growth, purified rat glicentin did

Table 1 Alignment of glucagon-like peptide 2 amino acid sequences from different species

Species	Glucagon-like peptide 2		
Human (*Homo sapiens*)	HADGSFSDEMNTILDNLAAR	DFINWLIQTK	ITD
Cow (*Bos taurus*)	HADGSFSDEMNTVLDSLATR	DFINWLIQTK	ITD
Dog (*Canis familiaris*)	HADGSFSDEMNTVLDTLATR	DFINWLLQTK	ITD
Hamster (*Mesocricetus auratus*)	HADGSFSDEMNTILDSLATR	DFINWLIQTK	ITD
Rat (*Rattus norvegicus*)	HADGSFSDEMNTILDNLATR	DFINWLIQTK	ITD
Mouse (*Mus musculus*)	HADGSFSDEMSTILDNLATR	DFINWLIQTK	ITD
Guinea pig (*Cavia porcellus*)	HADGSFSDEMNTILDNLATR	DFINWLIQTK	ITD
Degu (*Octodon degus*)	HADGSFSDEMNTVLDNLATK	DFINWLIQTK	ITD
Pig (*Sus scrofa*)	HADGSFSDEMNTVLDNLATR	DFINWLLHTK	ITD
Lizard (*Heloderma suspectum*)	HADGTFTSDYNQLLDDIATQ	EFLKWLINQK	VTQ
Chicken (*Gallus gallus*)	HADGSFTSDINKILDDMAAK	EFLKWLINTK	VTQ
Bullfrog (*Rana catesbeiana*)	HADGSFTSDFNKALDIKAAQ	EFLDWIINTP	VKE
Leopard frog (*Rana pipiens*)	HADGSFTSDFNKALDIKAAQ	EFLDWIINTP	VKE
African clawed frog (*Xenopus laevis*)	HADGSFTNDINKVLDIIAAQ	EFLDWVINTQ	ETE
Amphiuma (*Amphiuma tridactylum*)	HADGSFTSDINKVLDTIAAK	EFLNWLISTK	VTE
Rainbow trout I (*Oncorhynchus mykiss*)	HVDGSFTSDVNKVLDSLAAK	EYLLWVMTSK	TSG
Rainbow trout II (*Oncorhynchus mykiss*)	HVDGSFTSDVNKVLDSLAAK	EYLLWVMTSK	TSG
Sea lamprey I (*Petromyzon marinus*)	HAE—DVNALLDRTMAK	TFIEWLEKQN	SNDQTD
Sea lamprey II (*Petromyzon marinus*)	HSDGSFTNDMNVMLDRMSAKNFLEWLKQQG		RG

not consistently stimulate proliferation of small bowel epithelial cells,[33] and infusion of monoclonal antibodies directed against the mid N terminal region of glucagon (that cross reacts with glicentin) failed to attenuate the adaptive ileal response to 70% small bowel resection in rats.[34] Furthermore, attempts at implicating specific sub fragments of glicentin as mediators of the intestinotrophic response gave conflicting responses, depending on the experimental systems studied.[35 36] Nevertheless, the available data supported a role for a circulating gut derived factor related to the PGDPs as a mediator of intestinal adaptation, as reviewed by Taylor and Fuller.[37] Furthermore, the intestinotrophic effects of disparate peptide growth factors such as bombesin, epidermal growth factor, and keratinocyte growth factor have also been associated with upregulation of circulating enteroglucagon in rats in vivo.[14 38 39]

GLUCAGON-LIKE PEPTIDE 2: STRUCTURE, SYNTHESIS, AND SECRETION

Given the inconclusive evidence in support of a role for glicentin as the PGDP with intestinotrophic activity, subsequent experiments examined whether the intestinal PGDPs GLP-1 or GLP-2 stimulate intestinal mucosal epithelial proliferation. Following the observation that mice harbouring subcutaneous glucagonomas developed small bowel hyperplasia, the intestinotrophic effects of individual PGDPs were evaluated in vivo.[40] Administration of GLP-1 to mice twice daily for 10 days had no effect on small bowel mass whereas treatment with either glicentin or GLP-2 significantly increased small bowel weight. Nevertheless, in a direct comparison of glicentin and GLP-2 action, the greatest intestinotrophic effect was observed with GLP-2.[40] These observations provided the rationale for a series of studies examining whether GLP-2 might represent the long sought after PGDP with intestinotrophic activity in vivo.

GLP-2 is a 33 amino acid peptide that exhibits considerable sequence identity in mammals but less conservation of amino acid sequence in lower vertebrates (table 1). The observation that anglerfish pancreatic proglucagon cDNAs encoded for GLP-1 but did not contain a GLP-2 sequence cast doubt on the potential biological importance of GLP-2.[4 41] Subsequent studies demonstrated that fish, chicken, and reptile proglucagons do contain a GLP-2 sequence but tissue specific RNA splicing generates unique proglucagon mRNA transcripts encoding GLP-2 in the intestine but not in the pancreas.[42 43] These findings clearly account for the previous inability to detect the GLP-2 sequence in anglerfish islet cDNAs.

Analysis of circulating molecular forms of GLP-2 in rats and humans demonstrates the presence of intact GLP-2 (1–33) and N terminally cleaved GLP-2 (3–33).[44–48] The presence of an alanine at position 2 of both GLP-1 and GLP-2 renders these peptides excellent substrates for the aminopeptidase dipeptidyl peptidase IV (DP IV)[49] and inhibition of DP IV activity augments the trophic properties of GLP-2 in the rat small bowel.[50] Indeed, rats exhibit comparatively greater DP IV activity than mice, and wild-type GLP-2 is less active in rats compared with mice when administered in comparable 0.1 mg/kg dosing regimens due to DP IV mediated degradation.[49] In contrast, GLP-2 analogues engineered to be resistant to DP IV are considerably more potent than the native GLP-2 molecule in vivo.[49 51]

The presence of nutrients in the gastrointestinal tract constitutes the primary stimulus for GLP-2 secretion in both rodents and humans.[44 45 47 48] As might be inferred from previous studies demonstrating nutrient dependent secretion of glicentin and GLP-1,[52 53] circulating levels of GLP-2 are low in the fasted state and increase rapidly following nutrient ingestion.[44 45 47] Rats with experimental diabetes exhibit small intestinal mucosal hyperplasia and increased circulating levels of GLP-2. Following treatment with insulin, levels of GLP-2 and the thickness of the small bowel epithelial mucosa revert towards normal.[54 55] Similarly, the intestinotrophic effects of short chain fatty acid infusion are associated with increased circulating levels of GLP-2 in rats.[56]

> "The presence of nutrients in the gastrointestinal tract constitutes the primary stimulus for GLP-2 secretion in both rodents and humans"

The kidney plays an important role in the degradation of both GLP-1 and GLP-2.[57] Circulating levels of total GLP-2 immunoreactivity are increased in patients with renal failure,[44] and clearance of both GLP-2 (1–33) and the degradation resistant h[Gly^2]-GLP-2(1–33) was significantly reduced in nephrectomised rats.[58] Following intravenous administration of GLP-2(1–33) to human subjects, the elimination $t_{1/2}$ was ~ 7.2 minutes, and ~69% of the biologically intact peptide remained undegraded 60 minutes following subcutaneous injection of 400 μg of synthetic GLP-2 (1–33).[48] Hence, although GLP-1 and GLP-2 are structurally related and share common degradation and clearance mechanisms involving DP IV and the kidney, GLP-2 exhibits a longer $t_{1/2}$ in vivo.

As GLP-2 secreting enteroendocrine L cells are most abundant in the ileum and colon, regional gastrointestinal disease may be associated with perturbations in the synthesis and secretion of GLP-2. Circulating levels of fasting plasma GLP-2 (1–33) were increased in patients with active Crohn's disease or ulcerative colitis, in association with a relative increase in the ratio of intact GLP-2 (1–33) versus the N terminally cleaved GLP-2 (3–33), and a decrease in levels of plasma DP IV activity.[46]

"Regional gastrointestinal disease may be associated with perturbations in the synthesis and secretion of GLP-2"

An intact colon is an important determinant of the levels of circulating GLP-2. Patients with less than 140 cm of remnant small bowel but with a colon in continuity had significantly elevated levels of fasting GLP-2.[59] In contrast, short bowel patients with a jejunostomy lacking a colon exhibited normal basal levels but markedly impaired meal stimulated levels of GLP-2.[60] Taken together, these data suggest that injury to the intestinal epithelium is associated with upregulated synthesis and secretion of GLP-2, even in the absence of recent nutrient ingestion.

PHYSIOLOGY OF GLP-2 ACTION

Initial studies of GLP-2 action focused on the trophic role of this peptide in the stimulation of small bowel growth. Exogenous GLP-2 administration increased wet weights in the rodent jejunum and ileum, in association with enhanced crypt cell proliferation, reduced apoptosis in the enterocyte and crypt compartments, and increased thickness of the epithelial mucosa.[40 51 61 62] The small bowel villus hyperplasia induced by GLP-2 treatment was associated with increased expression of mucosal digestive enzymes and normal to enhanced absorptive function, as assessed in nutrient tolerance testing in mice.[63] Similarly, a 14 day intravenous infusion of GLP-2 in normal rats enhanced galactose and glycine absorption and increased mucosal protein and DNA content in the small intestine.[64] Despite the importance of nutrients for GLP-2 secretion and growth of the intestinal mucosa, GLP-2 administration is not associated with increased or decreased food consumption over a 10 day period in mice.[62] Intriguingly, although GLP-2 exhibits a short $t_{1/2}$ in vivo, administration of a single injection of GLP-2 every other day was sufficient to promote enhanced small bowel weight in mice after seven injections over a 14 day treatment period.[62] Hence continuously elevated levels of GLP-2 are not required for the intestinotrophic actions of GLP-2 in vivo.

The most rapid action of GLP-2 yet described is stimulation of intestinal hexose transport. Both GIP and GLP-2 increased the rate of glucose transport in basolateral membrane vesicles isolated from the rat jejunum.[65] The effect of GLP-2 on hexose transport in brush border membrane vesicles was detectable within 30 minutes of peptide infusion and maximal by 60 minutes, and was associated with an increase in sodium dependent phloridzin binding and SGLT-1.[66] The effect of GLP-2 on upregulation of SGLT-1 in vesicles was blocked by brefeldin A, an inhibitor of protein translocation, and by wortmannin, an inhibitor of PI 3-kinase. Despite the actions of GLP-2 on intestinal vesicle glucose transporters in rats, GLP-2 infusion had no effect on blood glucose in pigs[67] or mice.[63] GLP-2 infusion also rapidly reduced hypoglycaemia induced antral motility in the pig in a dose dependent manner, with an ED_{50} of 35 pM.[67] Modestly supraphysiological levels of GLP-2 (mean 115±8 pM) had no impact on basal gastric acid secretion but inhibited sham feeding induced gastric acid secretion in normal human subjects.[68]

GLP-2 administration is associated with both structural and functional changes in the gut mucosal epithelium. Small bowel epithelial cells from mice treated with h[Gly^2]-GLP-2 for 10 days appear significantly narrower and longer, and the length of enterocyte microvilli are significantly increased.[69] The GLP-2-treated epithelium exhibited reduced conductance, decreased flux of Cr-EDTA, and reduced serosal to mucosal flux of Na^+, consistent with a reduction in paracellular permeability. Furthermore, the transcellular flux of horseradish peroxidase was also significantly reduced in Ussing chamber studies of intestinal mucosa taken from h[Gly^2]-GLP-2-treated mice,[69] with the effects on tissue conductance and horseradish peroxidase flux detectable within four hours of GLP-2 administration in vivo.

GLP-2, INTESTINAL ADAPTATION, AND EXPERIMENTAL INTESTINAL INJURY

The proliferative antiapoptotic and proabsorptive actions of GLP-2 have led to examination of its potential therapeutic efficacy in animal models of intestinal disease. Co-infusion of GLP-2 and parenteral nutrition prevented mucosal atrophy, and significantly attenuated loss of protein and DNA content in the small bowel but not in the colon of parenterally fed rats.[70] Furthermore, GLP-2 treated rats exhibited significantly increased villus height and total mucosal thickness in the duodenum, jejunum, and ileum.

"The proliferative antiapoptotic and proabsorptive actions of GLP-2 have led to examination of its potential therapeutic efficacy in animal models of intestinal disease"

The intestinotrophic properties of GLP-2 were also observed in parenterally fed tumour bearing rats. Co-infusion of GLP-2 and parenteral nutrition increased intestinal mass and DNA content in the small bowel but had no effect on the mass of the colon or on tumour growth.[71] These observations suggest that the importance of enteral nutrition for optimal mucosal growth in the small intestine may be dependent in part on stimulation of GLP-2 secretion by luminal nutrients.

The enterotrophic actions of GLP-2 have been examined in rat models of 75–80% jejunoileal resection. Circulating levels of GLP-2 are increased in rats following intestinal resection[72]; rats treated with h[Gly^2]-GLP-2 following intestinal resection exhibited no differences in food intake or body weight gain over the 21 day treatment period.[73] Although saline treated rats exhibited a significant degree of intestinal adaptation in the absence of exogenous GLP-2, h[Gly^2]-GLP-2 treatment produced significant increases in segmental and mucosal wet weights following six and 21 days of peptide administration. Furthermore, crypt-villus height and mucosal sucrase activity were significantly increased in the jejunum of h[Gly^2]-GLP-2 treated rats following intestinal resection. Finally, h[Gly^2]-GLP-2 reversed the significant decrement in urinary xylose excretion by the end of the 21 day treatment period.[73] These results established that administration of GLP-2 in rats significantly augments the endogenous adaptive response to intestinal resection.

The biological actions of GLP-2 have been examined in animal models of inflammatory bowel disease and intestinal injury. Administration of h[Gly^2]-GLP-2 to mice with dextran sulphate induced colitis significantly reduced weight loss, attenuated small bowel shortening, enhanced mucosal area and integrity, and significantly decreased interleukin 1 expression and disease activity scores in the injured colon of CD1 and BALB/C mice.[74] The reparative and protective effects of GLP-2 are also evident in the setting of small bowel injury as a GLP-2 analogue increased mucosal protein and DNA

content and significantly reduced mortality in rats following superior mesenteric artery ischaemia.[75] Similarly, treatment of transgenic rats that develop spontaneous gastrointestinal inflammation with a 14 day systemic infusion of intravenous GLP-2 markedly decreased intestinal damage scores in the small and large intestine, and reduced expression of tumour necrosis factor α and interferon γ in the inflamed colon.[76]

The importance of GLP-2 pre-administration for prevention of intestinal injury was elucidated in studies of mice with indomethacin induced enteritis. h[Gly^2]-GLP-2 decreased intestinal damage and significantly reduced mortality when administered prior to, concomitant with, or following indomethacin administration.[77]

"A major locus of GLP-2 action appears to be reduction of apoptosis in the crypt compartment"

Remarkably, administration of GLP-2 prior to intestinal injury was the most effective regimen for reduction of indomethacin associated mortality. Mice treated with h[Gly^2]-GLP-2 exhibited preservation of small bowel length, significantly increased villus height, reduced small bowel ulceration and epithelial damage scores, decreased expression of mucosal epithelial cytokines, and stimulation of crypt cell proliferation. A major locus of GLP-2 action appears to be reduction of apoptosis in the crypt compartment, as crypt apoptosis is markedly induced following indomethacin administration and significantly reduced in GLP-2 treated mice.[77] The decreased mortality in indomethacin treated mice given h[Gly^2]-GLP-2 may also be explained in part by the significant reduction in circulating bacteraemia in GLP-2 treated mice with intestinal injury.

A reduction in intestinal apoptotic damage following chemotherapy administration was also observed following GLP-2 treatment of mice administered 5'-fluorouracil or irinotecan. Pretreatment of mice with h[Gly^2]-GLP-2 prior to chemotherapy significantly improved survival and decreased apoptosis in specific crypt compartments.[78] Irinotecan treated mice administered h[Gly^2]-GLP-2 exhibited reduced intestinal damage scores and a significant reduction in the number of positive bacterial cultures of mesentery, liver, spleen, and blood. Furthermore, mice harbouring syngeneic CT-26 colonic adenocarcinomas treated with cyclical irinotecan alone or with irinotecan and concomitant h[Gly^2]-GLP-2 exhibited significantly enhanced survival yet comparable tumour lysis following concomitant h[Gly^2]-GLP-2 administration.[78]

"The available evidence supports distinct roles for both the antiapoptotic and regenerative components of GLP-2 action in the prevention of and recovery from acute experimental intestinal injury"

h[Gly^2]-GLP-2 also improved histological parameters of intestinal injury in rats treated with 5-fluorouracil.[79] Taken together, the available evidence supports distinct roles for both the antiapoptotic and regenerative components of GLP-2 action in the prevention of and recovery from acute experimental intestinal injury.

THE GLP-2 RECEPTOR AND MECHANISMS OF GLP-2 ACTION

Understanding the pleiotropic effects of GLP-2 on the stomach and small and large bowel requires a detailed analysis of downstream mediators of GLP-2 action in the gastrointestinal epithelium. A cDNA encoding a putative GLP-2 receptor (GLP-2R) was isolated from hypothalamic and intestinal cDNA libraries that exhibits considerable sequence identity with related members of the glucagon-GLP-1 receptor superfamily. The human GLP-2R was localised to chromosome 17p13.3 and was predicted to encode a 550 amino acid G protein coupled receptor that is likely processed to yield a mature 486 amino acid receptor protein.[80] To date, no evidence for alternative RNA splicing or multiple GLP-2R genes or isoforms has been reported.[80 81]

The transfected GLP-2R specifically recognises GLP-2, but not glucagon, GLP-1, exendin-4, and GIP or related peptides, with an increase in cAMP accumulation.[80 82 83] In contrast, no increase in intracellular calcium accumulation was observed in BHK cells expressing a transfected rat GLP-2R.[82] Although GLP-2 stimulated immediate early gene expression in quiescent BHK-GLP-2R fibroblasts, a significant increase in cell proliferation was detected only with pharmacological concentrations (100 nM) of GLP-2.[82] Similarly, Caco-2 cells exhibited increased cell proliferation following exposure to pharmacological concentrations of GLP-2 (10 μM) but whether these cells express the endogenous GLP-2R remains unclear.[84]

The finding that GLP-2 administration prevented apoptosis induced by non-steroidal anti-inflammatory drugs[77] or chemotherapeutic agents in rodent gastrointestinal epithelium in vivo[78] prompted analysis of the direct antiapoptotic actions of GLP-2 in vitro. Cells expressing the transfected GLP-2R exhibit resistance to apoptotic cell death following exposure to cycloheximide.[85] Despite the importance of cAMP and presumably protein kinase A (PKA) for GLP-2 mediated signal transduction,[80 82] the effects of GLP-2 on cycloheximide induced cell death, cytochrome c release, and cleavage of caspase-3, caspase-8, and poly(ADP-ribose) polymerase were PKA independent.[85] GLP-2 also exhibits modest direct antiapoptotic activity in cells exposed to chemotherapeutic agents in vitro.[78]

A combination of RNAse protection, northern blotting, and reverse transcription-polymerase chain reaction analyses have demonstrated that GLP-2R is expressed in a tissue specific manner in the stomach, and in both the small and large intestine.[80 81] Remarkably, antisera directed against GLP-2R localised expression to enteroendocrine cells in the human stomach, small bowel, and colon.[81] Although the majority of gut endocrine cell subpopulations do not express GLP-2R, all GLP-2R immunopositive cells in the human gastrointestinal epithelium were identified as endocrine cells. In contrast, GLP-2R immunopositive gut endocrine cells have not been observed in rodents, and in situ hybridisation experiments have localised murine GLP-2R to enteric neurones.[86]

GLP-2R mRNA transcripts have also been localised to multiple regions of the rat and mouse central nervous system.[81 87] Intracerebroventricular but not peripheral injection of GLP-2 transiently inhibits feeding behaviour in rodents.[63 87 88] Pharmacological blockade or genetic disruption of GLP-1 receptor signalling augments the anorectic actions of GLP-2 in mice but not rats,[88] suggesting that both GLP-1 and GLP-2 may interact to regulate satiety.[87] Whether GLP-2R expression is perturbed in the setting of obesity or gastrointestinal disease has not yet been determined.

GLP-2R is also expressed in the fetal and neonatal rodent gut, raising the possibility that a functional GLP-2-GLP-2R axis plays a role in development and/or functional maturation of the gastrointestinal epithelium.[89] The neonatal rat intestine responds to exogenous GLP-2 administration with an increase in small and large bowel length and small bowel growth, demonstrating that the neonatal rat gut is functionally responsive to exogenous GLP-2.[89]

"Although GLP-2 is trophic for the neonatal gut, whether GLP-2 is important for gut development remains unclear"

Concomitantly, GLP-2 infusion in premature parenterally fed piglets enhanced intestinal protein and DNA accretion, prevented protein degradation, and reduced apoptosis in the small bowel.[90] Although GLP-2 is trophic for the neonatal gut, whether GLP-2 is important for gut development remains unclear, as mice with a dominant negative mutation of the pax6 transcription factor exhibit almost complete loss of GLP-2 producing enteroendocrine cells yet exhibit normal embryonic development of the small and large bowel epithelium.[91] Similarly, circulating GLP-2 was not detectable in 98 day gestation fetal pigs, and GLP-2 infusion did not stimulate intestinal growth in fetal pigs.[92] Hence it seems unlikely that GLP-2R signalling is important for development of the gastrointestinal tract.

GLP-2 AND HUMAN SHORT BOWEL SYNDROME

Although studies examining the therapeutic potential of GLP-2 in experimental injury of the rodent intestinal epithelium appear promising, clinical experience with GLP-2 in the setting of human gastrointestinal disease is limited. GLP-2 has been administered to eight patients with short bowel syndrome who received two injections of human GLP-2 (1–33), 400 μg subcutaneously for 35 days. Four of the patients were dependent on parenteral nutrition (mean remnant bowel length 83 cm) and four were not (mean remnant length 106 cm). At the end of the treatment period, GLP-2 treated patients exhibited improved energy absorption, increased body weight and lean body mass, decreased fat mass, and increased crypt height and villus depth.[93] Although the relative magnitude of changes in these parameters was small, the positive results suggest that further examination of the potential therapeutic role of GLP-2, or more potent degradation resistant GLP-2 analogues, in patients with intestinal failure may be warranted.

GLP-2: UNANSWERED QUESTIONS AND FUTURE RESEARCH DIRECTIONS

Although studies over the past five years have considerably expanded our understanding of the effects of exogenous GLP-2 administration, the relative physiological importance of endogenous GLP-2 for gastric emptying and acid secretion, small intestinal proliferation and apoptosis, nutrient absorption, and epithelial permeability remains unknown. Similarly, the contribution of GLP-2 to the control of mucosal proliferation in the colon is unclear. More definitive studies using GLP-2 receptor antagonists, GLP-2 immunoneutralising antisera, or GLP-2 receptor knockout mice are clearly needed to define the role of GLP-2 in the biology of the gastrointestinal epithelium.

Similarly, as studies to date of GLP-2 receptor signalling have primarily utilised cell lines transfected with the GLP-2 receptor cDNA, information on signalling mechanisms utilised by the endogenous GLP-2R in non-immortalised intestinal cells is lacking. Furthermore, GLP-2R localisation in diseased segments of the human intestine has not been examined, and the cellular localisation of GLP-2R expression in the fetal gut has not been reported. Similarly, the precise physiological role of endogenous GLP-2 in the central nervous system, and its contribution to peptidergic networks regulating food intake requires further study.

Although preliminary experience with GLP-2 administration in patients with short bowel syndrome appears promising, only a few patients have been studied to date, the responses observed were modest, and the study was limited to a 35 day treatment period. Larger studies of patients with and without colons, carried out for longer durations are required to more fully assess the therapeutic potential of GLP-2 in a diverse population of subjects with short bowel syndrome.

> "Whether GLP-2 may be useful for the treatment of patients with inflammatory bowel disease, or the prevention of chemotherapy induced mucositis, will require further study"

Furthermore, the proliferative and antiapoptotic effects of GLP-2, although demonstrated primarily in the small bowel, suggest that careful surveillance for the development of hyperplastic lesions in the colons of subjects treated with GLP-2 for prolonged periods of time is warranted. Whether GLP-2 may be useful for the treatment of patients with inflammatory bowel disease, or the prevention of chemotherapy induced mucositis, will require further study in properly designed and controlled clinical trials. The recent progress in understanding the physiological roles of GLP-2 and its companion peptide GLP-1 in the intake, absorption, and disposal of nutrients has fostered considerable interest in the potential of these gut derived hormones for the treatment of intestinal diseases and diabetes.[94] As both of these molecules are currently being evaluated in clinical trials, we should soon have answers to questions raised about the potential therapeutic efficacy of the glucagon-like peptides in the treatment of human disease.

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