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REVIEW

Gut hormones, and short bowel syndrome: The enigmatic role of glucagon-like peptide-2 in the regulation of intestinal adaptation

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Abstract

Short bowel syndrome (SBS) refers to the malabsorption of nutrients, water, and essential vitamins as a result of disease or surgical removal of parts of the small intestine. The most common reasons for removing part of the small intestine are due to surgical intervention for the treatment of either Crohn's disease or necrotizing enterocolitis. Intestinal adaptation following resection may take weeks to months to be achieved, thus nutritional support requires a variety of therapeutic measures, which include parenteral nutrition. Improper nutrition management can leave the SBS patient malnourished and/or dehydrated, which can be life threatening. The development of therapeutic strategies that reduce both the complications and medical costs associated with SBS/long-term parenteral nutrition while enhancing the intestinal adaptive response would be valuable.

Currently, therapeutic options available for the treatment of SBS are limited. There are many potential stimulators of intestinal adaptation including peptide hormones, growth factors, and neuronally-derived components. Glucagon-like peptide-2 (GLP-2) is one potential treatment for gastrointestinal disorders associated with insufficient mucosal function. A significant body of evidence demonstrates that GLP-2 is a trophic hormone that plays an important role in controlling intestinal adaptation. Recent data from clinical trials demonstrate that GLP-2 is safe, well-tolerated, and promotes intestinal growth in SBS patients. However, the mechanism of action and the localization of the glucagon-like peptide-2 receptor (GLP-2R) remains an enigma. This review summarizes the role of a number of mucosal-derived factors that might be involved with intestinal adaptation processes; however, this discussion primarily examines the physiology, mechanism of action, and utility of GLP-2 in the regulation of intestinal mucosal growth.

Key words: Short bowel syndrome; Glucagon-like peptide-2; Epidermal growth factor; Insulin-like growth factor-I; Parenteral nutrition; Total parenteral nutrition; Intestinal adaptation; Intestinal mucosa; Gut hormones; Enteric nervous system

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INTRODUCTION

Advances in surgical and medical care have made it possible for those with massive small bowel resections to survive. Unfortunately, a loss of greater than 50% of the small intestine often results in short bowel syndrome (SBS)[1-4]. SBS is characterized by the inefficient absorption of nutrients and fluids resulting in severe diarrhea, dehydration, electrolyte imbalances, nutrient deficiencies, weight loss, and frequently, a long-term dependence on parenteral nutrition[4-6]. In the pediatric population, total parenteral nutrition (TPN) maintenance-associated complications frequently result in sepsis, secondary liver failure, and high morbidity[5,7,8]. The development of therapeutic strategies that could reduce the consequences of SBS and long-term parenteral nutrition while enhancing the intestinal adaptive response would be valuable.

Numerous non-nutritional factors are potential stimulators of intestinal adaptation including peptide hormones, growth factors, and neuronally-derived components[9]. Some of the growth factors that have been shown to stimulate intestinal growth include glucagon-like peptide-2 (GLP-2), epidermal growth factor (EGF), growth hormone (GH), insulin-like growth factor-1 (IGF-1), neurotensin (NT), intestinal trefoil factor (ITF), transforming growth factor (TGF-α; TGF-β), and

Table 1 Growth factors as potential stimulators of intestinal adaptation

Mucosal growth factors and SBS			
Growth factor	**Amino acids /MW**	**Main source**	**GI receptor localization**
GLP-2	33 aa/3.8 kDa	Ileal and colonic EE L-cells, pancreatic α cells	GLP-2R; EE cells, myenteric/submucosal neurons of ENS
EGF	53 aa/6.4 kDa	Submandibular gland, duodenal Brunner's gland, epithelial and Paneth cells	EGFR/HER/ErbB, HER2/Neu/ErbB2, HER3/ErbB3, HER4/ErbB4
KGF (FGF-7)	194 aa/2.5 kDa	Fibroblast-like stromal cells in epithelial tissue	KGFR; a/bFGFR epithelial cells
Hb-EGF	86 aa/22 kDa	Smooth muscle cells	Activate all as listed for the EGFR
GH	191 aa/22 kDa	Liver	CRF type-1, PRL-R
TGF-α	50-53 aa/6.2-6.4 kDa	Epithelial cells	Activate all as listed for the EGFR
NT	13 aa/?	Ileum/colon >> proximal SB, EE N-cells	NTR1
PYY	36 aa/42 kDa	Ileal and colonic EE L-cells	Y1-R; EE cells, myenteric/submucosal neurons of ENS
GRP	14 aa/?	Neuroendocrine cells in the ENS, fibroblasts	GRPR antrum, upper GI tract
CCK	8, 33 aa/39 kDa	Duodenal EE I-cells	CCK_A >> CCK_B upper GI tissue myenteric/submucosal neurons of ENS
Gastrin	17 aa/21 kDa	Gastric antrum G-cells	CCK_B >> CCK_A
	34 aa/40 kDa	Pancreatic D-cells	upper GI tissue
IGF-I/II	I-70 aa/7.6 kDa, II-67 aa/7.4 kDa	Proximal SB >> distal, liver, Fibroblasts	IGF-IR and IGF-IIR epithelial cells, glial cells, fibroblasts

Many of these factors include peptide hormones, growth factors, and neurovascular components. SB, small bowel; ENS, enteric nervous system; EE, enteroendocrine; GLP-2, glucagon-like peptide-2; EGF, epidermal growth factor; Hb-EGF, heparin-binding EGF; KGF, keratinocyte growth factor (FGF-7); FGF, fibroblast growth factor; GH, growth hormone; CRF-1, cytokine-related family type-1 (JAK-STAT pathway); PRL, prolactin (subgroup of the CRF-1 superfamily); TGF-α, transforming growth factor-α; NT, neurotensin; PYY, peptide tyrosine tyrosine; GRP, gastrin-releasing peptide; CCK, cholecystokinin; IGF-I/II, insulin-like growth factor I/II.

fibroblast growth factor (FGF)[10]. Table 1 lists some of the mucosa-derived humoral factors that have been shown to promote intestinal adaptation following intestinal resection.

GLP-2 is a potential therapy currently under consideration for the treatment of gastrointestinal disorders that are associated with insufficient mucosal function. Evidence from both animal studies and clinical trials demonstrate that GLP-2 is a trophic hormone that plays an important role in the regulation of intestinal adaptation[11]. Under normal conditions, GLP-2 is secreted into the circulation in response to the ingestion of a mixed meal[12-15], particularly following one that contains carbohydrates, fatty acids, and fiber[16-18]. These studies have shown that following nutrient-induced release or exogenous administration, GLP-2 is involved in the regulation of cell proliferation, apoptosis, nutrient absorption, motility, as well as epithelial and intestinal permeability[15,19-25].

Initial pilot Phase II trials in SBS patients treated with teduglutide, a GLP-2 analog, observed that there were modest increases in fluid and nutrient absorption. Based upon the positive results of this trial, Phase III studies in adults with SBS were initiated. Subsequently, a recent article in *GUT* shows that teduglutide, using various dosing modalities over a 21 d experimental period, was safe, well tolerated, and promoted intestinal growth in SBS patients having either an end jejunostomy or a colon in continuity[26]. Moreover, teduglutide therapy in these SBS patients resulted in a significant increase in small intestinal villus height, crypt depth, and mitotic index. The authors state that the most significant clinical benefits of teduglutide in treating SBS patients were the reduction of intestinal wet weight excretion and in improving wet weight absorption[26]. There are several other clinical trials underway that are examining potential therapeutics for the treatment of SBS, unfortunately, Zorbitive® (hGH, glutamine) remains the only option presently available for prescriptive use.

This review summarizes the current understanding of a number of growth factors and their role in the treatment of SBS. As it is unlikely that there is just one specific growth factor responsible for maintaining mucosal growth, an important consideration is that there is likely some degree of regulation, transactivation, and crosstalk, between established stimulators of mucosal growth (i.e. epidermal growth factor, insulin-like growth factor-I, polyamines, GLP-2). Thus an understanding of the collective influence of these promoters of intestinal adaptation should not be dismissed. However, the primary focus of this discussion is to examine the physiology, mechanism of action, and utility of GLP-2 in the regulation of intestinal mucosal growth.

Discovery of GLP-2

The first indication that GLP-2 was trophic to the bowel was the observation that patients with glucagonomas had significantly elevated circulating enteroglucagon levels that was secondarily associated with intestinal mucosal hyperplasia[27,28]. The correlations between serum enteroglucagon levels and mucosal growth of the small intestine provided further evidence for the trophic effects of GLP-2[27]. A major breakthrough came in 1996 when it was shown that injection of proglucagon-producing tumors into nude mice resulted in significant mucosal hyperplasia[29]. Isolation of the various components of these proglucagon-producing tumors revealed that GLP-2 was the trophic factor responsible for the increased

mucosal growth.

Additional evidence for intestinotrophic properties of GLP-2 came from studies in rats and mice in which GLP-2 administration induced intestinal hypertrophy i.e., significant increases in protein and DNA content, villus height, and bowel weight[21,25,30,31]. Moreover, GLP-2 treatment reduced mucosal atrophy associated with TPN administration and augmented the adaptive growth response following massive small bowel resection[19-21,32]. Our studies showed that in parenterally maintained animals (no luminal nutrition), infusion with GLP-2 stimulated intestinal adaptation following small bowel resection[18]. Without the infusion of GLP-2, intestinal adaptation did not occur. Similarly, the immunoneutralization of endogenous circulatory GLP-2 reduced the intestinal post-resection adaptive response in rats and rabbits[33].

What is GLP-2?

GLP-2 is a member of the PACAP (Pituitary adenylate cyclase activating peptide)/Glucagon superfamily that includes the enteroglucagon/proglucagon-derived peptides (PGDP) that are produced in the L-cells of the small intestinal and colonic mucosa. The L-cells have been identified as the cellular origin of enteroglucagon (Figure 1)[24,34,35]. In 1983, the full sequence of hamster proglucagon, and later that year, the human sequence, were published revealing the sequences of GLP-1 and GLP-2[36,37]. GLP-2 is described as glucagon-like as it shares approximately 50% sequence homology with glucagon[37]. Sequencing of GLP-2 from human and pig intestine has confirmed the structure of GLP-2 as a 33 amino acid peptide that contains alanine at position 2 of the N-terminus (making it easily degraded by exopeptidases) and ends with a carboxy-terminal Asp residue[45]. GLP-2 and the other PGDPs are derived following the proteolytic cleavage and other enzymatic modifications of proglucagon in the organelles of the pancreatic α-cells and intestinal L-cells[38,39]. Interestingly, GLP-2 and the other products of post-translational processing (glicentin, GLP-1, oxyntomodulin) are produced in the intestinal L-cells, while glucagon and the major proglucagon fragment (MPGF) are produced in the α-cells of the pancreas[40]. They are formed from the same precursor molecule and therefore must be secreted in parallel. Differential expression of prohormone convertase (PC), the enzyme responsible for cleaving proglucagon into various hormonal products in the different cells, accounts for the different products. The PC2 enzyme, which has a greater abundance in the pancreatic α-cells, preferentially produces glucagon as its end product[41-43]. On the other hand, the PC1/3 enzyme, which is found in greater abundance in the intestinal L-cell, predominantly generates the glucagon-like peptides (GLP-1/2)[41]. Therefore, the differential processing of proglucagon in pancreatic α-cells by PC2, and by PC1/3 in the intestinal L-cell, might generate two unique hormonal products from the same precursor with each having a different physiological effect[43].

Initially, the GLP-2 sequence was identified in all mammalian cDNA, but not in other species. This led researchers to theorize that GLP-2 was a late evolutionary addition. Subsequent studies have demonstrated that fish, chickens, and lizards generate GLP-2 in the gut as a result of tissue-specific alternative RNA splicing of proglucagon RNA transcript[44].

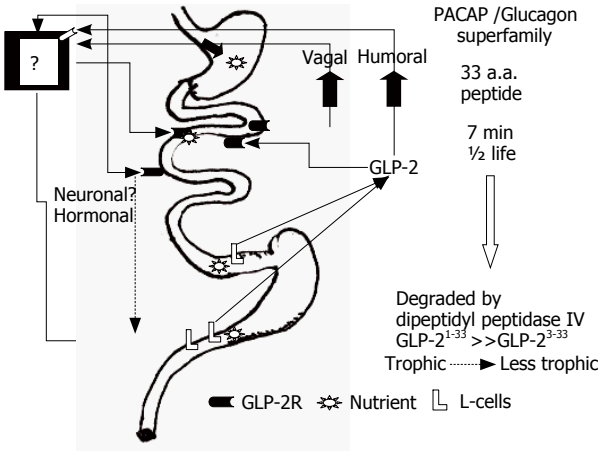

Figure 1 Potential pathways involved in the production, release, activity, and sites of action of GLP-2. GLP-2 produced by the L-cell is induced following the ingestion of a meal and can be induced either by direct stimulation of the L-cell, or potentially following the release of upstream neuronal /hormonal agonists. The GLP-2R has been localized to components of the enteric nervous system (ENS) and to regions of the brain, thus the growth-promoting effects associated with GLP-2 may involve interactions with the brain-gut axis. Potentially the ENS / vagal interface might be responsible for the early release of GLP-2.

Site of GLP-2 production

The distal regions of the small bowel and/or the large intestine are necessary for GLP-2 production. This was demonstrated in a study in which patients with SBS, without a colon in continuity, had significant reductions in their post-prandial circulatory GLP-2. Subjects that had 140 cm or less of small bowel, with colon in continuity, exhibited a significant increase in their serum GLP-2 levels[46,47]. Conversely, fasting and post-prandial serum GLP-2 levels were elevated in SBS patients (colon intact, no ileum) compared with sex- and age-matched controls[46]. The locale of the GLP-2-containing tissue in the human intestine is debatable, though it was recently reported that the number of immunoreactive GLP-2 cells increase in a proximal to distal distribution in normal colonic tissue[48].

However, there is debate as to the contribution of different regions of the bowel to total GLP-2 production. A 90% intestinal resection, leaving only 10 cm of distal ileum, results in a significant elevation in serum GLP-2 levels in the rat[15]. These elevations in circulatory GLP-2 correlate with several measurable parameters of intestinal adaptation[18]. As the remainder of the small bowel had been removed, this suggests that the distal ileum and/or the colon are the major sites of the L-cells, the cells responsible for the production of GLP-2 (Figure 1). Others describe that in the rat, TNBS (trinitrobenzene sulphonic acid) -induced ileitis resulted in no change in the number of GLP-2 immunoreactive cells in the ileum, yet colonic GLP-2 immunoreactivity was increased (personal communication, Jennifer O'Hara and Dr. K.A. Sharkey).

Interestingly, in a rat SBS model in which only 20 cm of jejunum was anastomosed directly to the ascending colon[49] (i.e., removal of 80% of the distal small bowel

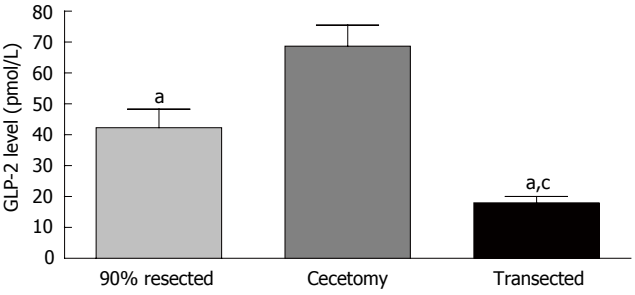

Figure 2 An experimental rat model of SBS-induces changes in circulatory GLP-2 levels. The distal regions of the small bowel and/or the large intestine are necessary for GLP-2 production. In these experiments, the entire proximal small bowel was removed; therefore the distal ileum and/or the colon are likely the cells responsible for the production of GLP-2 as all other bowel tissue had been removed. Resected = 90% intestinal resection leaving a 10 cm ileal remnant. CEC = cecetomy + removal of all but 20 cm of jejunum. The remnant jejunum was anastomosed to the ascending colon. $^{a}P < 0.05$ *vs* cecetomized animals; $^{c}P < 0.05$ *vs* 90% proximal resected animals that were enterally-fed (10 cm remnant ileum).

including the ileum and cecum), circulatory GLP-2 levels were significantly increased in comparison to both controls and the 90% resection animals with ileum intact (Figure 2). This strongly suggests that the colon is an important source of GLP-2. These findings emphasize the importance of determining if the increase in serum GLP-2 levels are the result of either an increase in the L-cell population, or alternatively, as a consequence of augmented GLP-2 production by the L-cells. Our ongoing experiments suggest, at least in this rat model of SBS, that increased GLP-2 production is the result of an increase in the colonic L-cell population (unpublished observations).

GLP-2 receptor and activity

Currently, the localization of the glucagon-like-2 receptor (GLP-2R) remains an enigma. Conflicting data in the literature report the GLP-2R to be located on enteroendocrine cells[50], on non-epithelial elements such as neuronal cells[51], and subepithelial myofibroblasts (Figure 1)[52]. Moreover, the localization of the GLP-2R in the bowel might be region-specific. In a recent study by Orskov *et al*, GLP-2 receptors were found throughout the small and large bowel in humans, mice, marmoset, and rat[52]. Furthermore, the intestinal region that had the greatest GLP-2R immunoreactivity was the proximal small bowel. These data were confirmed by both immunohistochemistry and *in situ* hybridization that showed that the GLP-2R was localized to cells lying beneath the basal membrane of enterocytes[52].

The specific GLP-2 receptor has been isolated, sequenced, and found to be expressed in both the gut and in the hypothalamus (Figure 1)[53]. GLP-2 binds to a 7 transmembrane (7-TM) G protein-coupled receptor that is comprised of 550 amino acids. In humans, the expression of the GLP-2R is localized to specific enteroendocrine cells in the stomach as well as the small and large bowel[53-55]. Currently there have been only two human cervical carcinoma cell lines, including HeLa cells, in which GLP-2R mRNA transcripts have been detected[56]: the majority of the signal pathway determinations have been carried out in cells transfected with the GLP-2R[55]. The

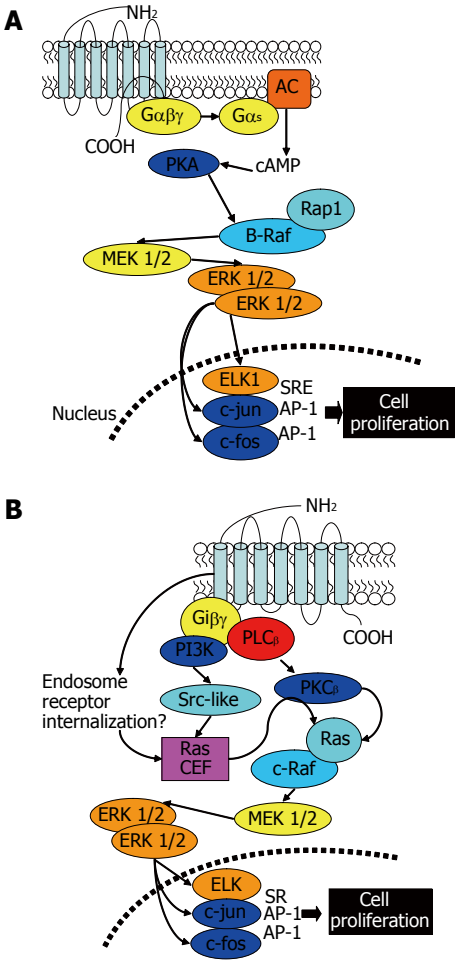

Figure 3 **A**: GLP-2 stimulation of the G_s-Coupled Receptor induced cell proliferation. The proposed GLP-2 /GLP-2R signaling pathway as characterized in cells that were transfected with the GLP-2R. See section on GLP-2 receptor-activity for description (pages 11-12); **B**: GLP-2 stimulation of the $G\alpha_i$-Coupled Receptor induced cell proliferation. Possible trafficking pathways triggered by GLP-2 in cells that tested negative for the characterized GLP-2R. Potentially, GLP-2 induces divergent signaling pathways which results in an increase in the rate of cell proliferation. See section on GLP-2 receptor-activity for description (pages 11-12).

enteroendocrine cells that do express the GLP-2R have also been found to express other gut hormones such as glucagon-like peptide-1, glucose dependent insulinotropic peptide, peptide YY, and serotonin[50]. PACAP and VIP share the same $VPAC_1$ receptor with equal affinity[34], thus it might be possible that other members of this family also share receptors.

There are a number of unanswered questions regarding the signaling mechanism involved in how GLP-2 stimulates mucosal growth. The initial studies on intracellular trafficking was done entirely in cell lines (BHK /COS) that had been transfected with the characterized GLP-2R[55,57]. These data showed that GLP-2/GLP-2R signaling involved classic G protein-coupled $G\alpha_s$ activation of adenylyl cyclase (AC), increased cAMP and PKA accumulation, and eventually ELK-1/c-fos/c-jun gene activation and increased cell proliferation (Figure 3A). Conversely, in epithelial cell lines devoid of the GLP-2R (as confirmed by both Western blot analysis and by the absence of mRNA transcripts), GLP-2 administration was still capable

of increasing cell proliferation[56,58,59]. Thymidine uptake as a measure of an increased proliferation was significantly inhibited following pre-incubation with pertussis toxin ($G\alpha_i$ inhibitor) or MAPK blockade, implying that $G\alpha_i$ or EGFR/TyrK trafficking pathways are involved (Figure 3B). These studies also revealed that, in comparison with the transfected cell lines in which increases in cAMP correlated with augmented growth, there was a significant correlation between increased cell proliferation and decreased cAMP accumulation.

Furthermore, the possibility that there are multiple signaling mechanisms for GLP-2 is suggested by recent observations regarding the divergent effects of GLP-2 administration on epithelial cell proliferation. These effects were dependent on both the cell line and the dose of GLP-2 administered. Two independent studies demonstrated that administration of GLP-2 results in the inhibition of proliferation of non-transformed small intestinal epithelial cell lines (IEC-6, IEC-18), while increasing proliferation of cancer-derived human colonic epithelial cell lines (Caco-2, Colo 320)[59,60]. Moreover, there can be divergent effects of GLP-2 on epithelial cell migration, as GLP-2 significantly increased migration in the small intestine-derived IEC-6/IEC-18 cells, but had no effect on epithelial cell migration in the colonic Caco-2 or the Colo 320 cells[60].

An article recently published in *Science* may have uncovered another important component of the GLP-2 signaling pathway[61]. It is established that the intestinal epithelium undergoes constant renewal along the crypt-villus axis and that the β-catenin/T cell factor (TCF) transduction pathway is involved in these proliferation/differentiation events[62-64]. These data reveal that GLP-2 treatment of mice increases β-catenin levels suggesting that β-catenin might be a downstream mediator of GLP-2-induced crypt cell proliferation. In addition, the expression of a cDNA encoding human R-spondin1 (hRSpo1) in KI chimeras led to a substantial increase in the diameter, length, and weight of the small intestine, diffuse thickening of the mucosa, and crypt epithelial hyperplasia[61]. Their data showed that the administration of hRSpo1 potently affects proliferation of the intestinal epithelium through activation of β-catenin; the activation of this pathway by hRSpo1 indicates that the effect might be directly stimulated by receptor-mediated binding. It is interesting that the expression of hRSpo1 in human intestinal enteroendocrine cells, consistent with the role of this protein as a crypt cell mitogen, is similarly localized to the expression of GLP-2[61]. This is certainly an exciting find in regards to mapping out potential signaling pathways involved in GLP-2-induced intestinal growth.

Collectively, this dichotomy suggests that there are divergent signaling pathways involved in how GLP-2 stimulates cell proliferation. Secondly, it begs the question: are other uncharacterized GLP-2R's or transactivation signaling mechanisms involved in the intestinotrophic effect of GLP-2 other than that initially described by Munroe *et al*[53]?

GLP-2 interactions with the ENS/CNS

There is evidence that the GLP-2R is expressed on both murine enteric neurons and cerebral cortex astrocytes[51,65]. Bjerknes and Cheng showed by *in situ* hybridization that the GLP-2R is located on nonepithelial elements of the enteric nervous system. These results were further verified by reverse-transcriptase polymerase chain reaction (RT-PCR) for the GLP-2R mRNA[51]. Moreover, the GLP-2R was located on neurons and not on glial cells. These experiments demonstrate that GLP-2 is capable of inducing *c-Fos* activity in enteric neurons in as little as 7 min, and achieved maximal expression by 15 min. After 90 min, the increase in GLP-2-induced neuronal *c-Fos* activity was followed by increased *c-Fos* activity in the crypt cells of the jejunum and colon. These observations suggest that GLP-2 stimulates the GLP-2R leading to an increased *c-Fos* expression in myenteric neurons (Figure 4), which is secondarily followed by both an increase in *c-Fos* expression in the crypt cells and the stimulation of columnar crypt cell growth. Tetrodotoxin (TTX) pre-treatment abolished *c-Fos* expression in the crypt cells revealing that GLP-2 signaling in the bowel might be dependent on a transduction pathway requiring enteric neurons[51]. Potentially the intestinotrophic property associated with GLP-2 involves a second downstream mediator that is released upon GLP-2's initial stimulation of the ENS (Figure 4).

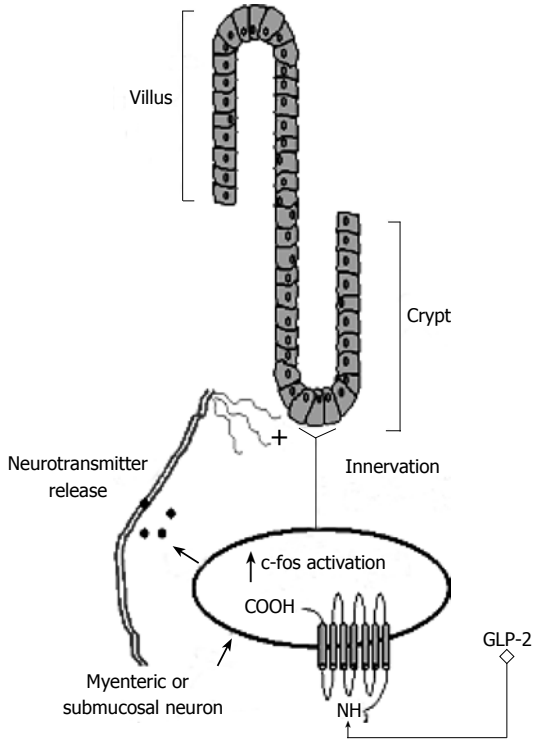

Figure 4 GLP-2 stimulation of the enteric nervous system. GLP-2 binds to 7-TM G protein-coupled receptors localized to components of the ENS. This results in a conformational change in the GLP-2R that eventually activates c-fos expression via the Gαs, AC, c-AMP pathway. This suggests that a rise in circulatory GLP-2 levelsmight stimulate the GLP-2R leading to an increased stimulation of the myenteric and/or submucosal neurons. Potentially the trophic effects on the gut mucosa associated with GLP-2 are the result of a second downstream mediator, either a neurotransmitter or direct innervation that is activated upon GLP-2's initial stimulation of the ENS localized GLP-2R.

The GLP-2R is also localized to regions of the hindbrain[66]. The dorsal medial hypothalamus (DMH) contains a dense plexus of GLP-2 immunoreactive fibers that express GLP-2 receptor mRNA (Figure 1). Though

research regarding GLP-2's involvement in neuronal activation is still in its infancy, it is interesting that GLP-2's effects on enteric /vagal pathways are remarkably similar to those characterized for cholecystokinin[67,68]. This possibility has led to discussions regarding GLP-2 as a factor involved in the regulation of feeding. There is evidence that GLP-2 administration can inhibit feed intake[66,69,70] however, other investigators report no effect on appetite or energy intake at physiological concentrations of GLP-2[71]. This area of GLP-2 physiology requires further investigation.

These observations suggest a novel pathway of GLP-2 action as perhaps either a meal-stimulated endogenous release of GLP-2 from the L-cell, or potentially, the therapeutic administration of GLP-2, could activate enteric neuronal activity, resulting secondarily in the release of downstream mediators capable of inducing crypt cell proliferation (Figure 4). Currently, no studies have been published in which the expression of the GLP-2R in the human enteric nervous system was examined.

GLP-2 receptor antagonists

An obstacle when examining mechanistic pathways involved in GLP-2-induced small bowel adaptation is the lack of effective GLP-2R antagonists. One possibility would be to use the primary degradation product ($GLP\text{-}2^{3\text{-}33}$) to see if the interaction with the GLP-2R could reduce the adaptive response to intestinal resection. These results might be difficult to interpret as the treatment of mice with either $GLP\text{-}2^{1\text{-}33}$ or $GLP\text{-}2^{3\text{-}33}$ induces significant growth in both the small and large bowel, though the growth response induced by the degradation product is reduced[72]. Furthermore, functional studies and binding data indicate that $GLP\text{-}2^{3\text{-}33}$ acts as both a partial agonist and as a partial antagonist on the GLP-2R[72]. However, there is recent data that demonstrates $GLP\text{-}2^{3\text{-}33}$ antagonizes the growth-promoting activities of $GLP\text{-}2^{1\text{-}33}$ *in vitro* and *ex vivo*[73]. Shin *et al* suggest that endogenous $GLP\text{-}2^{1\text{-}33}$ regulates the intestinal growth-promoting response via modulation of crypt-cell proliferation and villus apoptosis[73]. Collectively, these findings do not support the use of $GLP\text{-}2^{3\text{-}33}$ as a full antagonist of the GLP-2 receptor.

GLP-2 inactivation and metabolism

As mentioned earlier in this review, $GLP\text{-}2^{1\text{-}33}$ contains the amino acid alanine at position 2 thereby making it susceptible to degradation by the exopeptidase dipeptidyl peptidase IV (DPP-IV)[74]. For excellent reviews on the structural and functional properties of DPP-IV, see Mentlein or Lambeir[75,76]. The primary metabolism of $GLP\text{-}2^{1\text{-}33}$ by DPP-IV, which cleaves off two N-terminal amino acids, results in the formation of $GLP\text{-}2^{3\text{-}33}$, a potential receptor antagonist /agonist[72,73]. The elimination half-life of intact $GLP\text{-}2^{1\text{-}33}$ in humans is 7 min, whereas that of cleaved $GLP\text{-}2^{3\text{-}33}$ is 27 min[14], thus the cleavage of the NH_2 terminal may potentially disrupt the signal transduction pathway and reduce GLP-2's biological effectiveness. The administration of GLP-2 to DPP-IV deficient rats results in higher $GLP\text{-}2^{1\text{-}33}$ serum levels which correlate with a significant increase in small intestinal weight[74,77]. Similar increases in intestinal hypertrophy also occur in rats and mice administered a synthetic DPP-IV resistant GLP-2 analog (ALX-0600)[31,74] suggesting that the inactivation of $GLP\text{-}2^{1\text{-}33}$ by DPP-IV is a crucial limiting factor regarding GLP-2's trophic effect on the gut mucosa. In humans, the replacement of alanine in position 2 of the GLP-2 peptide with glycine, has been shown to extend the half life from approximately 7 min, as for GLP-2, to 0.9-2.3 h.[26] Thus, DPP-IV resistant GLP-2 analogues might be useful therapeutically to help improve mucosal regeneration.

In contrast, there is evidence that DPP-IV may not be the only factor important in the degradation of GLP-2[78]. Geier *et al* did not detect an observable role for DPP-IV in the regulation of GLP-2 in $DPP\text{-}IV^{-/-}$ mice, as there were no measurable differences in GLP-2 levels or plasma DPP-IV-like activity between $DPP\text{-}IV^{+/+}$ and $DPP\text{-}IV^{-/-}$ mice. Although $DPP\text{-}IV^{-/-}$ mice lack the *DPP-IV* gene, they do possess the genes for FAP, DP8, and DP9, and might have sufficient dipeptidyl peptidase levels to regulate GLP-2 cleavage. Moreover, the mice lacking the *DPP-IV* gene do not have either intrinsic resistance, or an enhanced rate of repair, in DSS-induced colitis. The authors concluded that residual dipeptidyl peptidase levels in the $DPP\text{-}IV^{-/-}$ mice results in insufficient $GLP\text{-}2^{1\text{-}33}$ bioavailability to protect these mice from DSS (dextran sodium sulfate) -induced damage The association between GLP-2 and DPP-IV family members will require further study.

Plasma concentrations of GLP-2 are not only influenced by the rate of secretion, but by the elimination or clearance rate. The kidney is important in the clearance of GLP-2 as there are increased circulatory levels in patients with renal failure[79,80]. In addition, experimental nephrectomy results in delayed clearance and increased circulating levels of GLP-2 in rats[81]. The increase in circulatory GLP-2 levels following nephrectomy is not surprising as it has been shown that the kidney is the site of greatest DPP-IV activity[75].

GLP-2 and intestinal inflammation

The treatment of animals with GLP-2 reduces harmful symptoms of inflammation associated with colitis as well as the mortality and the severity of both indomethacin and TNBS-induced enteritis[35,82,83]. Moreover, studies within our lab reveal that GLP-2 ameliorates inflammatory parameters associated with TNBS-induced ileitis (Gastroenterology abstract, T1530, 2005). These reductions in inflammation (decreased myeloperoxidase activity, interleukin-1β, and inducible nitric oxide synthase protein in mucosal tissue) 5 d post-TNBS occurred whether the GLP-2 was given simultaneously with TNBS, or 2 d post-TNBS injection. Others have observed that GLP-2 treatment initiated following chemotherapy enhances intestinal recovery[84]. A second report also showed the benefits of GLP-2 treatment as a preventative against cancer therapy-induced mucosal damage[85]. Teduglutide, a GLP-2 analog, given daily prior to whole body gamma-irradiation significantly increased crypt stem cell survival in mice when compared with vehicle-treated controls, though the protective effect was only observed when teduglutide was given prior to irradiation[85]. Collectively, these reports indicate that GLP-2 might be a useful therapeutic strategy in

the treatment of diseases associated with intestinal inflammation.

There are conflicting reports regarding the effect of intestinal inflammation on GLP-2 production, particularly on the site of GLP-2 production. Intuitively, any situation that depletes the GLP-2-producing L-cells could potentially reduce the ability of GLP-2 to repair the mucosa. This could be the case in Crohn's/colitis patients as inflammation of the intestine likely disrupts the L-cells. In a mouse-model of T-cell-induced inflammatory bowel disease (IBD), the amount of GLP-2 was significantly reduced in the colon[86], however, others report that TNBS-induced ileitis results in an increase in colonic GLP-2 immunoreactivity. (O'Hara and Sharkey, personal communications). Regarding the latter, perhaps an increase in local and circulatory GLP-2 is the result of IBD-induced diarrhea, thus increasing the nutrient load in the distal bowel, a well-described potent stimulus for inducing GLP-2 production[12,15,17]. Though speculative, perhaps increased growth and nutrient absorption in the proximal small intestine as a result of increased GLP-2 production would be successful in ameliorating inflammatory processes. Conversely, Schmidt *et al* found that both the fasting and meal-induced plasma levels of GLP-2 were not different between healthy controls and IBD patients[87]. This report suggests that L-cell secretion of GLP-2 is not altered in IBD. The discrepancies between these observations could be the result of model/species difference, or site-specific effects of inflammation in the bowel.

While GLP-2 is the most prominent enterotrophic peptide, there are a number of hormones that might be involved in the regulation of mucosal growth. These are briefly discussed as it is likely that processes involved in intestinal adaptation are regulated by a complex array of hormones, neuropeptides, and cytokines. A number of mucosal growth factors that might be useful for the treatment of short bowel syndrome are listed in Table 1.

Insulin-like growth factor-I

Another growth factor that might have a potential role in enhancing mucosal growth is insulin-like growth factor-I (IGF-I). IGF-I is produced mainly in the liver, but it is also synthesized locally in the gut[88]. There is evidence that IGF-I stimulates intestinal growth under experimental conditions of TPN therapy, intestinal resection, and radiation therapy[89]. In addition, the over-expression of the gene encoding for IGF-I is associated with increased small bowel length, small bowel weight, and crypt cell proliferation[90]. Conversely, others report that ileal IGF-I levels remain unchanged following intestinal resection[90,91].

Most of the circulating IGF-I is bound to IGF-binding proteins (IGFBPs) that potentially protect IGF-I from degradation thereby modulating the activity of IGF-I[92,93]. IGFBPs are capable of controlling the availability of circulating IGF-I to target tissues and are thus key components in the GH-IGF-I somatotropic axis. In humans, six IGFBPs have been identified and shown to modulate IGF-I actions differently[94]. Serum levels, jejunal tissue protein, and mRNA levels of IGF-I are reduced following small bowel resection[95]. One possibility is that there is a significant decrease in IGFBP-3 mRNA following intestinal resection, thus this rapid decrease in the IGFBP-3 could increase IGF-I bioavailability resulting in an enhanced adaptive response[96].

In SBS rats maintained on parenteral nutrition, IGF-I infusions induced jejunal hyperplasia and normalized ion transport[97]. Thus acute IGF-I treatments might prove to be beneficial in easing the transition from parental to enteral feeds thereby avoiding some of the long-term dilemmas associated with TPN therapy[98]. However, there is no significant evidence of serum or tissue concentration fluctuation of IGF-I following intestinal resection, therefore its utility as a treatment for SBS remains unclear.

Epidermal growth factor

Most of the EGF family of peptides (transforming growth factor-α, amphiregulin, heparin-binding EGF, epiregulin, betacellulin, neuregulin, neuregulin-2) are trophic to the gastrointestinal tract as they both stimulate crypt cell proliferation and suppress apoptosis[99]. There is evidence that EGF administration at the time of small bowel resection enhances the adaptive response. This appears to be accomplished by inducing mucosal hyperplasia and increasing nutrient absorption, while reducing intestinal permeability, weight loss, and apoptosis[49,100,101]. It is important to note that EGF therapy is most effective for intestinal adaptation when it is administered early following resection[102]. Very few studies have examined serum EGF levels following small bowel resection. Shin *et al* showed that small bowel resection does not change serum EGF levels, yet there was an increase in salivary and a decrease in urinary excretion of EGF[103]. Ileal epidermal growth factor receptor (EGFR) levels were significantly increased suggesting that the increased salivary EGF (significant endogenous source of EGF), enhanced EGF-R expression, and reduced urinary excretion of EGF, leads to an increase in ileal utilization of EGF following resection. Compared with control mice, the removal of the submandibular glands significantly attenuated the increase in villus height, total protein, and DNA content of the small bowel following massive small bowel resection[104].

It has also been suggested that intestinal adaptation following resection requires a functional EGFR[105]. Selective inhibition of the EGFR with an orogastric EGFR inhibitor (ZD1839, 50 mg/kg per day) results in impaired intestinal adaptation after small bowel resection[106,107]. There is also an impaired adaptive response to intestinal resection in waved-2 mice-mice that are predisposed to a naturally occurring mutation that results in a reduction in EGFR protein tyrosine kinase activity. *In vitro* studies demonstrate that serum from intestinal resection mice or rats is capable of stimulating a proliferative response in rat intestinal epithelial cells (IEC)[108]. This uncharacterized factor released into the serum of 7 d resected rats significantly increased EGF-R protein expression. Additionally, the proliferative response in IEC's was abolished following the addition of a specific EGFR inhibitor. It would be intriguing to explore if there is cross talk between GLP-2, which is significantly increased following intestinal resection, and the EGFR signaling pathway. The basis for this speculation was

discussed earlier and is depicted in Figure 3B.

PACAP and VIP

All but one of the members of the PACAP/glucagon superfamily, GIP, have been found as protein and/or mRNA in the brain and are thus classified as neuropeptides[34]. For an excellent review of this family of peptides, see Sherwood *et al*[34]. All of these peptides are found in the gut and signal through 7-TM G-protein coupled receptors. In humans, the present members include PACAP, glucagon, glucagon-like peptide-1, growth hormone releasing hormone, vasoactive intestinal peptide (VIP), secretin, glucose-dependent insulinotropic peptide (GIP), and GLP-2[34]. Both VIP and PACAP have a widespread distribution and are known to affect neural, circulatory, gastrointestinal, endocrine, and immune systems. There is evidence that VIP and PACAP administration reduces apoptosis and promotes the survival of neural cells by inducing *bcl-2* activity. Increased *bcl-2* activity is associated with an inhibition of caspase-3 activity and decreased cytoplasmic cytochrome C release[34]. Moreover, the BH4 domain of the pro-survival protein *bcl-2* both binds to the C-terminus of Apaf-1 on caspase-9, thus inhibiting caspase-induced apoptosis, as well as directly or indirectly preventing the release of cytochrome C from the mitochondria[109,110]. Similar results were attained following the activation of GLP-2R signaling by GLP-2 administration in transfected cells treated with cyclohexamide or irinotecan. These data show that there was an inhibition of apoptosis, reduced caspase activation, and decreased mitochondrial cytochrome C release[57,84]. An additional parallel of these peptides is revealed following recent studies suggesting that GLP-2 is a vasodilator[111,112]. PACAP and VIP are both established as potent vasodilators[34].

At physiological concentrations, VIP and GLP-2 are both ineffective in stimulating cell growth directly in cell culture. Perhaps the intact ENS might need to be activated first as VIP receptors, and likely GLP-2 receptors, are located on enteric neurons[34,51]. It would be interesting to examine the growth-promoting potential of the ENS on epithelial cells *in vivo*.

There are conflicting results regarding plasma and tissue levels of VIP following small bowel resection and in studies examining SBS. These differences may be species-specific as following intestinal resection, dogs and pigs have a significant reduction in VIP levels, whereas in humans, there is either an elevation or no change in plasma VIP levels[113-116]. There is no evidence that VIP and PACAP administration is directly trophic for the small bowel thus a role for VIP in small bowel resection in the rat has not been described.

Peptide YY and Cholecystokinin

Further study is required when looking at the relationship of other co-localized mediators that are released from the L-cell. Peptide tyrosine tyrosine (PYY) is a 36 amino acid gastrointestinal peptide present in endocrine L-cells of the ileal, colonic, and rectal mucosa[117-121]. PYY is co-localized to some degree with GLP-1/GLP-2 in the L-cells[122,123], moreover, it generates many of the physiological actions that are also attributed to GLP-2. There are data that both GLP-2 and PYY: slow gastric emptying and intestinal transit; production rates in rats and humans are increased following intestinal resection, production rates are influenced by luminal nutrients in the hindgut; release might be influenced by a proximal gut hormone or perhaps a neuroendocrine mechanism[51,124-133]. In support of the latter, our results examining postprandial GLP-2 release[15], together with reports of the meal-stimulated PYY response[133], demonstrate that there are significant increases in the serum levels of both peptides in less than 15 min. Intestinal transit times are not that rapid, thus these early increases probably are not attributable to luminal nutrients in the distal intestine. Neuroendocrine factor induction of the early release of PYY was described in a study by Greeley *et al*[121]. They administered a meal to dogs and then diverted the entire meal out of the proximal intestine via the creation of a stoma. Thus, there was no possibility of nutrient stimulation of PYY containing L-cells in the distal gut. Interestingly, there was still significant increase in plasma PYY levels. In these same dogs, the removal of the ileum, colon, and rectum, resulted in no increase in plasma PYY levels in response to a meal. These findings replicate a human study previously discussed in this paper, but instead of PYY, GLP-2 levels in patients that received a colectomy were significantly reduced[47]. This is certainly supportive of either a blood-borne hormone or a neural pathway in the generation of the early release of PYY and most assuredly, GLP-2. Furthermore, we have observed that rats with 20 cm of jejunum diverted out an abdominal stoma (i.e. no possibility of meal contents directly contacting the distal bowel) still had a significant increase in post-prandial GLP-2 levels-unpublished data-(Figure 5).

Several proximal gut hormones that are rapidly secreted following the ingestion of a meal have been tested (i.e., gastrin, glucose dependent insulinotropic peptide, secretin, neurotensin) and found to be ineffective in stimulating an increase in PYY[121]. Currently, no published literature has shown that these can stimulate GLP-2 release. One potential agonist, cholecystokinin (CCK), induces a dose-dependent release of PYY, and this CCK-induced PYY release, can be blocked by a CCK antagonist[121]. As CCK induces PYY production from L-cells, and having established that PYY and GLP-2 are co-localized, potentially, CCK might also be a foregut hormone responsible for stimulating the early release of GLP-2. It would be worthwhile to examine whether CCK also triggers GLP-2 secretion.

PYY administration has not shown that it is trophic to the gastrointestinal tract directly and it is unlikely that it is involved in the adaptive response following intestinal resection, though a reduction in intestinal transit may secondarily generate increased intestinal growth. These areas have not been explored. Both GLP-2 and PYY are inactivated by dipeptyl peptidases[75,76], thus additional studies of SBS should explore the relationship between serum and intestinal tissue levels of GLP-2, PYY, and DPP-IV.

Polyamines

Polyamines are present in prokaryotes, plants and animals. The polyamines putrescine and its derivatives spermidine and spermine are found in nearly all cells of higher eukaryotes[134,135]. The major role of polyamines in most cell types is to stimulate cell proliferation and thus is considered essential for life[135,136]. The inhibition of polyamine biosynthesis blocks cell growth[136,137]. The increase in intracellular polyamine levels and in the activity of ornithine decarboxylase (ODC), one of the rate-limiting enzymes in the strictly controlled polyamine biosynthetic pathway[138], are associated with rapid growth rates[139,140]. ODC activity in the rat small intestinal mucosa is increased following small bowel resection[134,138], parasitic or enteropathogenic bacteria-induced small intestinal inflammation or colitis[141,142], ischemia reperfusion[143], and following partial obstruction of the lumen. All of these instances are associated with an increase in mucosal growth. Of clinical relevance, mucosal ODC activity in the colon and rectum has been reported to be significantly higher in both ulcerative colitis and Crohn's disease patients[144]. In addition, evidence shows that polyamines are protective of DNA during the S-phase of the growth cycle[135,145] and in part may explain the protective effects of GLP-2 following the administration of chemotherapeutic agents.

There is a rise in plasma enteroglucagon levels that precedes the activation of ODC[143]. Thus it could be speculated that an increase in GLP-2 might initiate the activation of ODC leading to the repair of injured intestinal mucosa. If so, it would not be unreasonable for the growth promoting and protective role of GLP-2 in the small bowel to act through an ODC-dependent formation of polyamines. Considering that the trophic effects of many growth-promoting gut hormones are blocked following ODC or polyamine synthesis inhibition[134,138], it would not be surprising if this pathway is involved as a downstream effector of GLP-2 induced intestinal growth.

Intestinal carcinogenesis

A great deal of attention has focused on the potential for cancerous growth attributable as a side effect following the application of growth factors. Recent results cautioning restraint regarding the utility of GLP-2 was indicated as GLP-2, and to a greater degree, a GLP-2 analog, promoted an increase in the growth of mucosal neoplasms in mice pre-induced to form colonic tumours[146]. Furthermore, there was a significant increase in tumour load in the mice treated with either GLP-2, or the synthetic Gly2-GLP-2 analogue. There is also evidence that GLP-2R mRNA transcripts can be detected in two human cervical carcinoma cell lines[56]. But does the application of growth factors such as GLP-2 or EGF always trigger neoplastic growth? Could the application of growth factors prevent the development of intestinal neoplasms? Perhaps the primary factor involved in neoplastic growth is not necessarily the addition of the growth factor, but rather a dysfunction in downstream receptor (i.e., mutation) or signal transduction pathways. For example, there is evidence that mutations in cytoplasmic signaling elements involved in the EGFR signaling cascade result in an alteration in signal transduction events that lead to changes in the downregulation/internalization of the EGFR[147]. Perhaps what occurs in established oncogenic cells is that a mutation in an element of the signaling cascade prevents GLP-2- or EGF-induced receptor downregulation. This theory is supported by recent work that shows that an oncogenic form of c-Cbl/c-Cbl, which stabilizes and ubiquitinates the cytoplasmic region of the EGFR, is able to prevent EGF-induced EGFR down regulation thus bypassing degradative pathways[148]. The consequences of this mutation are a bypass of receptor internalization and desensitization processes, an induction in the over expression of the EGFR, and finally, aberrant cell growth. A downstream defect in trafficking could potentially be the primary cause of neoplastic, abnormal cell growth, not necessarily the application of the growth factor.

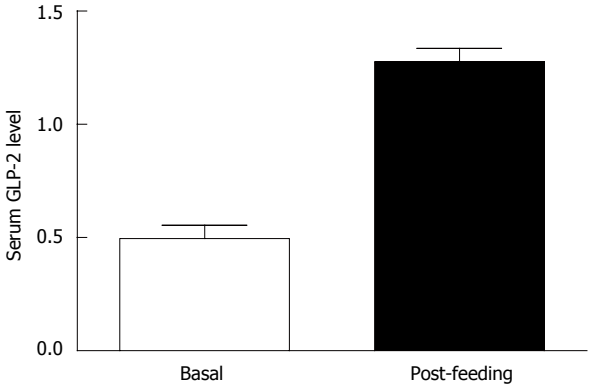

Figure 5 Neural or humoral mechanism involvement in the early release of GLP-2. The delivery of a meal significantly increases serum GLP-2 levels in rats with discontinuous small bowel (ANOVA, $P < 0.007$). The disruption of the continuity of the bowel (stoma creation) did not inhibit the initial nutrient-stimulated GLP-2 response. Thus, GLP-2 production is increased at least in part by neural and/or hormonal mechanisms. Serum GLP-2 levels reported as ng/mL.

In the case of the promotion of tumour size in mice following GLP-2 treatment as noted by Thulesen *et al*, or potentially as in the CaCO-2, T84, HeLa cells[56,58,59], what has occurred is that dysfunctional receptors and/or signal transduction pathways are already present within/on these cell types. As with any neoplastic condition, the application of growth factors is always a risk. Potentially the application of growth factors prior to the development of gastrointestinal neoplasms, such as during the early stages of Crohn's disease or ulcerative colitis, would be beneficial in halting the progress of prolonged inflammation. It is well established that prolonged intestinal inflammation often secondarily leads to the development of mutations, cell dysfunction, and carcinogenic growth.

CONCLUSIONS

The trophic effect of GLP-2 on the intestine demonstrates that it is possible to modify or accelerate the process of intestinal adaptation, which would potentially make GLP-2 useful for the treatment of short bowel syndrome. Evidence shows that increases in GLP-2 production following intestinal resection are significantly correlated with intestinal adaptation. The association between an increase in nutrient malabsorption and GLP-2 production

following intestinal resection has led to speculations that GLP-2 may act to control the nutrient absorptive capacity within the bowel. The increased availability of circulatory GLP-2 might then stimulate proximal mucosal growth resulting in enhanced nutrient absorptive capacity in the remaining intestinal remnant. Moreover, GLP-2 administration maintains epithelial barrier function and increases both crypt cell proliferation and weight gain in the absence of enteral nutrients. This supports the further development of GLP-2 as a therapeutic strategy that could enhance intestinal adaptation and reduce the consequences of parenteral feeding.

Although significant progress has been made towards elucidating the mechanisms mediating the trophic effects of GLP-2, several formidable challenges lie ahead. The biological and signaling role of the GLP-2R has not been fully defined, thus the mechanistic pathway (s) by which GLP-2 induces adaptive change in the small bowel presently remain elusive. Data showing that GLP-2 administration significantly increases cell proliferation in the absence of the characterized GLP-2R strongly suggest that GLP-2's trophic effect might also include activation of either an uncharacterized GLP-2R or the transactivation of other receptor/signaling paradigms. Another key goal should be in determining the mechanism involved in the early release of GLP-2 following a meal or in malabsorptive states. Lastly, an emergent area of interest is the potential of GLP-2 to induce stimulation of the ENS as an intermediate in the regulation of intestinal growth. Regardless, there is a very significant and biologically important effect of GLP-2 on intestinal function and whole animal physiology that strongly supports further research into the use of GLP-2 as a therapy. The future use of GLP-2 therapy for the treatment of intestinal disorders is an enticing prospect; however, the promise of these initial results must be tempered as we await confirmatory data from clinical trials.

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