The “cryptic” mechanism of action of glucagon-like peptide-2

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Submitted 31 January 2011; accepted in final form 25 April 2011


The intestinal hormone glucagon-like peptide-2 (GLP-2) is currently in clinical trials as a therapeutic for a variety of intestinal insufficiencies and diseases (www.npsp.com). Central to the beneficial effects of GLP-2 on the gut is its biological action to increase intestinal growth, executed through the enhancement of crypt cell proliferation and inhibition of apoptosis (20, 23). These functions of GLP-2 assist in the maintenance of the physiological barrier from the external environment and facilitate digestion and absorption of ingested nutrients. However, GLP-2 also regulates the size and integrity of the gut following insult, as well as in response to disease and altered nutrient status. Nonetheless, despite highly localized expression of the GLP-2 receptor (GLP-2R) in the intestine (6, 28, 56, 77), the mechanisms underlying the regulatory actions of GLP-2 have proven to be complex, involving multiple downstream mediators (20). Recent advances have also linked this hormonal peptide to the intestinal microbiota, novel intrinsic factors, and putative stem cells. This review will focus on recent progress in our understanding of the complex mechanism of action of GLP-2 in the gastrointestinal tract.

Intestinal Bioactivities of GLP-2

Initial reports of intestinal hypertrophy in humans with GLP-2-overexpressing tumors (26, 66) as well as the demonstration of intestinotropic effects of exogenous GLP-2 in rodents (19) identified this proglucagon-derived peptide as a potent stimulator of intestinal growth. In intestinal endocrine L cells, posttranslational cleavage of the proglucagon precursor molecule by prohormone convertase-1/3 liberates the glucagon-like peptides (GLP-21–33 and GLP-1) along with several other bioactive peptides (17). Nutrient intake is the primary stimulus for GLP-2 secretion into circulation, after which degradation occurs via dipeptidylpeptidase (DPP)-IV-mediated cleavage to GLP-21–33, followed by renal clearance (67, 73). Hence the synthesis of DPP-IV-resistant GLP-2 analogs, such as Gly2-GLP-2 and teduglutide (Gattex), has both extended the half-life and improved efficacy in the context of pharmacological delivery of GLP-2.

A large number of studies have demonstrated that exogenously administered GLP-2 is tropic for the small intestine and, to a lesser extent, the colon (19, 21, 39, 56). Evidence from animal models demonstrates that GLP-2 treatment significantly increases intestinal weight through increased cellularity of the epithelial layer of the intestinal wall. This is mediated through stimulation of intestinal epithelial cell (IEC) proliferation in the crypts and inhibition of apoptosis in both the crypt and villus compartments and results in increased crypt-villus height. Ultimately, the actions of exogenously administered GLP-2 in...
rodents serve to increase the functional surface area of the mucosa, as evidenced by enhancements in both digestive enzyme activity and facilitated nutrient absorption (9, 15, 42).

Importantly, notable intestinal actions of GLP-2 have also been demonstrated in humans. Hence, chronic administration of either GLP-2 for 6 wk or teduglutide for 3 wk (0.03–0.15 mg·kg\(^{-1}\)·day\(^{-1}\)) to subjects with short bowel syndrome (SBS) increases crypt-villus height and mitotic index and reduces fecal output in association with increased absorption of enteral nutrients (39, 40). Similarly, chronic treatment with teduglutide (for 20–24 wk, 0.05 mg·kg\(^{-1}\)·day\(^{-1}\)) reduced the need for parenteral nutrition by 20% or more in 63% of SBS patients (38). Interestingly, statistical reductions were not observed in this study with a higher dose of teduglutide (0.1 mg·kg\(^{-1}\)·day\(^{-1}\)), although the decrease in parenteral volume was equal between the two groups. A pilot study investigating the effects of teduglutide (0.05–0.2 mg·kg\(^{-1}\)·day\(^{-1}\)) in patients with Crohn’s disease is also suggestive of beneficial effects to increase intestinal mucosal mass and/or mucosal healing, as assessed through measurements of plasma citrulline concentration (10). However, although a trend toward increased clinical responses and/or remission was observed in these subjects, there were no differences in their Crohn’s Disease Activity Index. This was suggested to be due to a lack of power in the study; however, it also remains possible that there is a difference in the response to GLP-2 or its analogs between humans with Crohn’s disease and animal models of intestinal inflammation. Furthermore, the findings suggested that both the low (0.05 mg·kg\(^{-1}\)·day\(^{-1}\)) and the high (0.2 mg·kg\(^{-1}\)·day\(^{-1}\)) dose of teduglutide were more effective than the intermediate dose (0.1 mg·kg\(^{-1}\)·day\(^{-1}\)). Although the reasons for the apparent discrepancies in teduglutide effectiveness are not clear, previous studies in normal mice have indicated that the intestinotrophic effects of GLP-2 are dose dependent (70). Hence, further studies in humans to determine the exact relationship between the dose of teduglutide and clinical effectiveness are required.

Finally, the growth-promoting effects of GLP-2 in mice and those of teduglutide on nutrient absorption in humans with SBS are reversed upon withdrawal of treatment (40, 70). Thus teduglutide will likely require chronic administration, and potential long-term adverse effects cannot be discounted, as discussed in more detail below.

Interestingly, although exogenously administered GLP-2 produces a robust effect on gut growth and function, the tropic actions of the endogenously produced peptide appear to be relatively more modest. The importance of endogenous GLP-2 has largely been elucidated through the use of a GLP-2R antagonist (e.g., the GLP-2 metabolite, GLP-2\(^{3–33}\)), the GLP-2R knockout mouse model, or immunoneutralization techniques. Of note, although the GLP-2 metabolite, GLP-2\(^{3–33}\) acts as an antagonist at lower concentrations, it also functions as a partial agonist at increased doses, with significant tropic effects observed in both the small and large bowel following administration of higher amounts of GLP-2\(^{3–33}\) (69).

Hence, caution must be taken in interpreting the results of studies using the GLP-2R antagonist, and appropriate controls must always be included. Nonetheless, administration of GLP-2\(^{3–33}\) for either 24 h or 4 wk demonstrated a physiological role for GLP-2 in basal intestinal growth, reducing small bowel weight and decreasing crypt-villus height; these changes occurred in the absence of any detectable changes in proliferation but, at least in the 24-h model, were associated with increased IEC apoptosis (36, 63). Although these findings stand in contrast to the report of normal intestinal weight, crypt-villus height, and proliferative index in the GLP-2R null mouse (3, 4), it is possible that chronic adaptation occurs in these animals to maintain basal intestinal growth. Endogenous GLP-2 also plays a role in the adaptive intestinal growth that occurs in mice and rats in response to oral refeeding after a period of nutrient deprivation, as demonstrated with use of both the GLP-2 antagonist and GLP-2R null mice (4, 54, 63). Finally, immunoneutralization of GLP-2 reduces the adaptive intestinal growth that is associated with experimental type 1 diabetes in rats (32), although these findings were not recapitulated in streptozotocin-diabetic GLP-2R knockout mice (3). Hence, the tropic effects of endogenous GLP-2 appear to be related to adaptation of the intestine in response to varying nutrient intake, although the relative importance of this role appears to vary with the species and/or the model utilized. Furthermore, the cellular mechanism of action of GLP-2 to increase intestinal growth remains a subject of intense interest, as discussed in more detail below.

In addition to the growth-promoting properties of GLP-2, several recent studies have begun to address the pathways by which GLP-2 increases intestinal function. Long known to enhance triolein absorption (9), treatment of mice and hamsters with GLP-2 has now been linked to increased plasma triglyceride and cholesterol levels, through a CD36-dependent mechanism that results in stimulation of intestinal apoB48 secretion following an oral fat load (35). Studies in pigs and humans have also demonstrated that GLP-2 increases mesenteric blood flow (8, 28, 29), thus providing another mechanism to facilitate digestion and absorption of nutrients. Finally, the effects of GLP-2 to increase barrier function (12, 13) have been confirmed in murine models of both Type 1 and Type 2 diabetes (the nonobese diabetic and ob/ob mouse, respectively) (14, 30), and the mechanism whereby these effects are transduced has begun to be elucidated. Thus administration of a prebiotic to ob/ob mice not only promoted GLP-2 synthesis, but also resulted in GLP-2-dependent upregulation of the tight junction proteins, zonula occludens-1 and occludin (14).

Because of the beneficial effects of GLP-2 on the gastrointestinal growth and function, teduglutide (Gattex) has recently completed clinical trials (phase 3) for the treatment of SBS, is currently under investigation for Crohn’s disease (phase 2), and is also in preclinical development for gastrointestinal mucositis and pediatric indications (http://www.apsp.com; Refs. 10, 39, 40). Indeed, unlike other mitogenic factors, the apparent intestinal specificity of GLP-2 renders this peptide attractive for use in conditions of intestinal dysfunction. Nonetheless, because of our incomplete knowledge of the mechanism of action of GLP-2 on the intestine, many current studies are focused on the cellular mechanism of GLP-2.

**Rationale Underlying Indirect Actions of GLP-2: The GLP-2R**

The bioactivities of GLP-2 are transduced through a G protein-coupled receptor (GPCR) belonging to the glucagon-secretin class B receptor family (53). Distribution of the GLP-2R appears to be largely restricted to the intestinal tract, although limited expression has been also detected in the lung
However, despite a broad knowledge of the intestinal actions of GLP-2, mechanistic studies have been limited because of the fact that the GLP-2R is not localized to the epithelial cells, the major site of the proliferative and cytoprotective actions of GLP-2 (6, 28, 56, 77). Rather, the GLP-2R is expressed in the intestinal subepithelial myofibroblasts (ISEMFs) that underlie the epithelium, dispersed enteroendocrine cells, and the enteric nervous system. It was therefore proposed that GLP-2 exerts its intestinotropic actions indirectly, via downstream mediators deriving from GLP-2R-expressing cells (77). Although there currently exist few in vitro cell models that allow for the study of such a complex system, numerous in vivo studies have now demonstrated that GLP-2 indeed elicits a variety of its intestinal effects indirectly, through several paracrine mediators.

**Downstream Mediators, the List Keeps Growing**

Several studies originally described keratinocyte growth factor (KGF) and endothelial nitric oxide synthase (eNOS) as mediators involved in GLP-2-induced colonic growth and intestinal blood flow, respectively (28, 29, 56). However, more recent studies have focused on the insulin-like growth factors (IGFs) (21, 22, 46, 48, 54), the ErbB network (4, 76) and vasoactive intestinal polypeptide (VIP) (64) as key players in the tropic actions of GLP-2 (Table 1).

The IGFs, IGF-1 and IGF-2, are mitogenic peptides with functions encompassing cellular proliferation, survival, and differentiation (49). Importantly, the IGFs and GLP-2 share many similar biological actions in the gut (18, 21, 51, 55). The rationale for the involvement of IGF-1 in the intestinotropic effects of GLP-2 has been previously reviewed by Dubé and Brubaker (20). Briefly, studies performed in IGF-1 and IGF-2 knockout mice determined that IGF-1 and, to a lesser extent, IGF-2, is required for the tropic effects of GLP-2 on both the small and large intestine (Fig. 1; Ref. 21). Hence, there was a dramatic lack of response to GLP-2 administration in IGF-1 global knockout mice, in terms of crypt cell proliferation, crypt-villus length, and intestinal weight. In contrast, the role of IGF-2 in the intestinotropic effects of GLP-2 was more modest and appeared to be restricted to mucosal surface area. In further support of a requirement for IGF-1 in the actions of GLP-2 are findings that GLP-2 increases IGF-1 mRNA transcript levels in murine and rat intestine, as well as in cultures of murine ISEMF cells, and induces IGF-1 secretion by fetal rat intestinal cells in vitro (21, 46, 48). Nelson et al. (54) have also demonstrated that mucosal growth upon reintroduction of luminal nutrients in the rat not only is associated with elevated jejunal IGF-1 mRNA levels but also is partially blocked by administration of GLP-23–33. Nonetheless, it is important to recognize that IGF-1 is produced in many tissues in addition to the intestine and also circulates in the bloodstream (74). Hence, more studies are required to determine whether the intestine

![Table 1. Indirect mediators of the intestinal actions of GLP-2](http://www.ajpgi.org/)

<table>
<thead>
<tr>
<th>Indirect Mediator</th>
<th>Demonstrated Intestinal Site of Action</th>
<th>References</th>
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<tbody>
<tr>
<td>ErbB ligands</td>
<td>Jejunal mucosa</td>
<td>76</td>
</tr>
<tr>
<td>Insulin-like growth factor-1</td>
<td>Jejunal, ileal, colonic mucosa</td>
<td>21, 22</td>
</tr>
<tr>
<td>Insulin-like growth factor-2</td>
<td>Jejunal, colonic mucosa</td>
<td>21</td>
</tr>
<tr>
<td>Keratinocyte growth factor</td>
<td>Colonic mucosa</td>
<td>56</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Mucosal blood vessels</td>
<td>28, 29</td>
</tr>
<tr>
<td>Vasoactive intestinal polypeptide</td>
<td>Ileal, colonic mucosa</td>
<td>64</td>
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and hypothalamus (53, 77). However, despite a broad knowledge of the intestinal actions of GLP-2, mechanistic studies have been limited because of the fact that the GLP-2R is not localized to the epithelial cells, the major site of the proliferative and cytoprotective actions of GLP-2 (6, 28, 56, 77). Rather, the GLP-2R is expressed in the intestinal subepithelial myofibroblasts (ISEMFs) that underlie the epithelium, dispersed enteroendocrine cells, and the enteric nervous system. It was therefore proposed that GLP-2 exerts its intestinotropic actions indirectly, via downstream mediators deriving from GLP-2R-expressing cells (77). Although there currently exist few in vitro cell models that allow for the study of such a complex system, numerous in vivo studies have now demonstrated that GLP-2 indeed elicits a variety of its intestinal effects indirectly, through several paracrine mediators.

![Fig. 1. Model for the proposed mechanism of action of glucagon-like peptide-2 (GLP-2) on intestinal crypt cells. The GLP-2 receptor (R) is expressed by intestinal subepithelial myofibroblasts (ISEMF), enteroendocrine cells including the L-type endocrine cell, and enteric neurons. Following nutrient-induced release into the circulation, GLP-2 acts via GLP-2R binding on ISEMF cells, leading to the release of ISEMF-derived insulin-like growth factor-1 (IGF-1) and, likely, other growth factors, such as ErbB ligands. IGF-1 then binds to the IGF-1R located on crypt epithelial cells, where it may transactivate the ErbB receptor, leading to cell proliferative responses including phosphatidylinositol-3-kinase/Akt and β-catenin signaling. The role of the IGF-1R and/or ErbB receptor expressed by the ISEMF cells has not been delineated but may mediate autocrine effects of ISEMF-derived factors, leading to the release of other growth factors and/or support factors for the underlying lamina propria (67a).](http://www.ajpgi.org/)
GLP-2 reduces intestinal inflammation and damage via activation of submucosal enteric neurons, recent evidence indicates that GLP-2.

IGF-1R and the ErbB receptor in the proliferative response to clear the enteric system (1, 27, 41, 58). Studies in other cell models have also shown a loss of GLP-2-induced crypt cell proliferation in these animals (K. J. Rowland and P. L. Brubaker, unpublished data). Collectively, these findings indicate important roles for both IGF-1 and the IEC IGF-1R in the acute (e.g., in fasting-refeeding), as well as the chronic intestinal tropic actions of GLP-2 (Fig. 1).

In addition to the IGFs, ErbB ligands have recently generated interest as downstream mediators of GLP-2-induced intestinotropic actions. The ErbB network is a potent proliferative system that contributes to the maintenance of intestinal mucosal growth and function (34). The ErbB ligands epiregulin and neuregulin were found to be upregulated in the murine small intestine 1 and 4 h after GLP-2 treatment (76). Similarly, administration of GLP-2 or epidermal growth factor (EGF) increased mRNA expression of the ErbB ligands, amphiregulin, epiregulin and HB-EGF, as well of the immediate early genes, c-fos, egr-1, and phlda-1 within full-thickness intestinal sections. Furthermore, the ErbB receptors were shown to be required for the chronic intestinal proliferative and growth effects of GLP-2, as determined through the use of a pan-ErbB inhibitor. More recently, EGF administration was also found to rescue the lack of adaptive regrowth in the reined GLP-2R knockout mouse (4). In contrast, using the ErbB receptor inhibitor gefitinib, others have reported that the positive effects of GLP-2 on intestinal weight and crypt-villus length are independent of ErbB signaling (31). Nonetheless, collectively, the data suggest the involvement of an ErbB ligand-Erb signaling pathway in the proliferative actions of GLP-2.

How the findings on the ErbB axis can be reconciled with the data on the IGF-1-IEC-IGF-1R pathway remains unclear (Fig. 1). Recent data has indicated that GLP-2 treatment increases IGF-1 but not ErbB ligand mRNA transcript levels in ISEM cultures (46), suggesting that the ErbB system lies downstream of the IGF-1 network. Conversely, exogenous EGF but not IGF-1 rescued the growth deficit in refed GLP-2R null mice (4), suggesting that IGF-1 lies downstream of EGF/Erbb signaling. Nonetheless, it has been well established that the IGF-1R can transactivate ErbB receptors, and vice versa, demonstrating the existence of cross-talk between these two pathways (1, 27, 41, 58). Studies in other cell models have also indicated that the two pathways demonstrate codependence (16). Additional studies utilizing cell-specific models are clearly required to delineate the exact relationship between the IGF-1R and the ErbB receptor in the proliferative response to GLP-2.

Finally, in association with expression of the GLP-2R on submucosal enteric neurons, recent evidence indicates that GLP-2 reduces intestinal inflammation and damage via activation of VIP-producing neurons (64). In a variety of rat and mouse models of inflammatory bowel disease, GLP-2 treatment reduces the levels of inflammatory cytokines (i.e., IFN-γ, TNF-α, IL-1β), and increases the production of the anti-inflammatory cytokine, IL-10, in association with increased numbers of VIP-expressing neurons in the submucosal plexus of the small intestine. However, in contrast to the well-established mitogenic effects of GLP-2 in the normal bowel, Sigalet et al. (64) observed a GLP-2-mediated decrease in epithelial proliferation rates in these inflammatory models, with a reduction in inflammation-induced mitogenesis toward normal levels. Nonetheless, treatment with either GLP-2 or VIP improved weight loss and reduced intestinal damage in these animals, and antagonism of the actions of VIP prevented the anti-inflammatory actions of GLP-2. Finally, despite the initial findings on IL-10 (64), as noted above, studies in the IL-10 null model of colitis demonstrated that GLP-2-mediated decreases in mucosal inflammation and crypt cell proliferation occur through an IL-10-independent mechanism. Rather, the anti-inflammatory actions of GLP-2 in this model involved activation of suppressor of cytokine signaling-3 signaling in IECs, and an IL-6-mediated increase in IL transducer and activator of transcription-3 in colonic mucosal scrapings (37). Hence, although VIP is an essential regulator and downstream mediator of GLP-2 in rodent models of inflammatory bowel disease, the exact pathway by which these effects are modulated remains unclear.

Collectively, therefore, multiple studies now indicate that the actions of GLP-2 on the intestine are complex, involving multiple mediators (e.g., eNOS, ErbB ligands, IGF-1, IGF-2, KGF, and VIP), the roles of which are both cell-type specific and dependent on the physiological/pathophysiological state of the organism.

Downstream Signaling Pathways

Although there have been numerous advances recently in the understanding of downstream mediators of GLP-2 action by using in vivo models, these studies do not provide information regarding intracellular signaling mechanisms of the GLP-2R. Thus a number of different in vitro models have been used to examine GLP-2R signaling. Similar to other class B GPCRs, ligand binding in heterologous cells expressing the transfected GLP-2R results in a dose-dependent increase in cAMP and activation of PKA, cAMP response element-binding protein and AP-1 (53, 78). Furthermore, GLP-2 has a small, PKA-independent proliferative effect on cells transfected with the GLP-2R and decreases apoptosis through inhibition of glycogen synthase kinase-3β and Bcl-2-associated death promoter (75, 78). However, the results of studies using heterologous cell models are not entirely consistent with data from homologous intestinal cell models. Thus, although primary rat mucosal cells, intestinal muscle strips, and fetal rat intestinal cells, all of which express the endogenous GLP-2R, respond to GLP-2 treatment with an increase in cAMP levels (2, 21, 63, 71), a recent study with primary ISEM cells, which also naturally express the GLP-2R, demonstrated no effect of GLP-2 on cAMP levels (2, 21, 63, 71). A similar lack of effect of GLP-2 on cAMP levels was also reported for HeLa cells, a model that also expresses the endogenous GLP-2R (44). Furthermore, unexpectedly, the stimulatory effect of GLP-2 on IGF-1 mRNA transcript levels in the ISEM cells
was found to be dependent upon phosphatidylinositol-3-kinase (PI3K)/Akt, a pathway that had not previously been linked to GLP-2R activation in vitro (46). Indeed, although GLP-2 also activates PI3K/Akt signaling in IEC cells in vivo (11, 15, 22), this is presumed to be downstream of the cellular mediators, rather than because of direct signaling by the GLP-2R. This hypothesis is supported by findings that both the IGF-1R and ErbB receptors stimulate PI3K/Akt signaling in IEC cells (57, 62). Similarly, although the canonical Wingless (cWnt)/β-catenin signaling pathway, an integral system for cell cycle regulation, has recently been implicated in the effects of GLP-2 on the crypt cell in vivo (22), activation of this pathway must be mediated indirectly, possibly through the IEC-IGF-1R (K. J. Rowland and P. L. Brubaker, unpublished data), rather than through direct linkage to the GLP-2R. This notion is also supported by the finding that PI3K/Akt signaling activates β-catenin in intestinal stem and progenitor cells via phosphorylation at Ser552 (33, 45), through a mechanism involving Ras activation (50) and GSK3β phosphorylation (25). Therefore, although treatment with GLP-2 in vivo activates a number of signaling pathways in the IEC cells, there is currently only limited information about GLP-2R signaling in the actual target cells of this hormone in the intestine, namely the ISEMFs, with nothing reported to date on the signaling pathways activated by GLP-2 in either intestinal neurons or endoendocrine cells.

Putative Stem Cell Involvement

The stimulatory effect of GLP-2 on crypt cell proliferation appears to be temporally regulated, as bromodeoxyuridine uptake is increased in cell positions ~3–10 from the base of the crypt following acute (e.g., 6 h) administration of GLP-2 (76), whereas expression of the proliferative marker Ki-67 is increased in cell positions ~15–20 in response to chronic (e.g., 10 days) GLP-2 treatment (21). Furthermore, the results of several studies have suggested that GLP-2 may also regulate intestinal stem cells (ISC), which are localized in cell positions 1–4 (e.g., Lgr5-positive cells), at or near 4 (e.g., DCAMKL1-expressing cells) or 5–10 (e.g., Musashi-1-containing or progenitor cells). For example, an instrumental study examining the effects of GLP-2 on ISCks revealed that pretreatment of mice with GLP-2 protects progenitor/ISCs from radiation damage (7). GLP-2 administration for 10 days also increases the number of putative ISCks and absorptive progenitor cells, as determined by a somatic mutation-progenitor assay (6). Chronic administration of GLP-2 to mice also increases the number of musashi-1-positive cells within the crypt base and the enriched stem cell zone (21). Although musashi-1 is not a definitive ISC marker, these results collectively imply that GLP-2 exerts a positive effect on early progenitor cells. Furthermore, GLP-2 administration immediately after ileocecal resection in mice transiently increases P-β-catenin552-positive putative ISCks and this is temporally correlated with increased jejunal IGF-1 mRNA expression (24). Finally, GLP-2 activates cWnt signaling in intestinal crypt cells (22), a pathway known to be critical in the regulation of ISC proliferation (5, 33, 61). Together, these findings suggest that GLP-2 positively regulates the proliferation of both ISC and transit-amplifying cells, although it remains unknown whether these effects are context specific and/or are different in healthy gut compared with an injury model.

Although it has been suggested that GLP-2 preferentially increases the number of absorptive enterocytes rather than goblet cells (6, 72), others have reported an increase in the number of mucin-positive cells following chronic GLP-2 administration (2). Conversely, no change in cell distribution has been detected in the IEC-IGF-1R null mouse (K. J. Rowland and P. L. Brubaker, unpublished data). Thus, in addition to lengthening of the villi and crypts through stimulation of progenitor and/or stem cells in the crypt, GLP-2 treatment may alter the distribution of differentiated epithelial cells, although this effect may be model specific.

Proliferation of Normal and Neoplastic Tissue?

The therapeutic advantage of the intestinal-specific actions of GLP-2 make this hormone an attractive candidate over other gut growth factors that also exhibit extraintestinal tropic actions, such as IGF-1. However, as with any growth factor, the potential for carcinogenic effects of chronic GLP-2 treatment must not be overlooked.

Several studies have examined the effects of GLP-2 treatment on cancer initiation and/or progression in rodents. In the first of such studies, methylyating carcinogen (dimethylhydrazine)-induced colonic tumors were increased in mice treated with long-acting Gly2-GLP-2 compared with untreated control groups (68). Interestingly, Gly2-GLP-2 specifically increased the number of small, medium, and large polyps in these animals, with medium and large polyps characterized as pedunculated nonmalignant adenomas, whereas native GLP-2 only increased the number of small polyps, none of which were malignant. The potential carcinogenic effect of GLP-2 has also been studied in mice that were pretreated with the carcinogen azoxymethane (36). Azoxymethane is a rare dietary carcinogen that has been established for use as a model of “sporadic” colon cancer; however, the exact relevance of this model to colon cancer induction in humans remains uncertain. Nonetheless, this study demonstrated not only a significant increase in the number of colonic aberrant crypt foci (ACF), but also of the more dysplastic, mucin-depleted foci. Furthermore, the promoting-effect on ACF was prevented by treatment with GLP-23–33, implicating endogenous GLP-2 in the development of colonic dysplasia. Together, these findings suggest that GLP-2 increases tumor initiation as well as progression in the dimethylhydrazine and azoxymethane models of sporadic murine colon cancer. Although the mechanism of this action has not been established, one possibility is the regulatory relationship between the cWnt and PI3K/Akt signaling pathways, both of which have been implicated in the pathogenesis of colorectal cancer (52). However, in contrast to these findings, other findings indicate that GLP-2 does not modulate tumor growth in vitro or in vivo. For instance, treatment of stable colon cancer cell lines transfected with the GLP-2R did not affect cell proliferation or survival (43). Furthermore, injection of these cells into nude mice followed by treatment with GLP-2 did not induce tumor growth. Finally, GLP-2 did not modulate tumor growth in APC−/− (adenomatous polyposis coli; multiple intestinal neoplasia) mice, which have an activating mutation in APC causing increased cWnt signaling and are, thus, a model of familial colon cancer.
(43). Thus, despite the beneficial effects of GLP-2 and its long-acting derivative, teduglutide, in the treatment of gastrointestinal disease, the potential for GLP-2 to induce carcinogenesis, albeit controversial, is an important issue that warrants further long-term investigation.

**Concluding Remarks**

The physiological and potential pathophysiological roles of GLP-2 and its complex interplay with downstream factors are beginning to be elucidated, particularly in terms of proliferative actions on the gut. Although the development of in vitro systems that recapitulate the in vivo setting would allow for accelerated mechanistic discoveries, recent studies with mouse deletion models are contributing to our understanding of GLP-2 action. Nonetheless, additional tissue-specific models will be required to unravel the complex paracrine and neurocrine signaling induced by GLP-2, as well as how each of these interacts in a temporal and spatial manner to effect intestinal growth. Although alterations in intestinal gene expression profiles occur in response to nutrient availability (60, 65), gut adaptation also occurs consequent to intestinal inflammation, as well as to alterations in the microbiome, diabetes, and other inflammatory stressors such as obesity and oxidative stress, and the mechanism of action of GLP-2 in these settings of intestinal adaptation remains poorly understood. Importantly, future studies should aim to discover not only novel therapeutic usages of GLP-2 but, moreover, the exact mechanisms of action of this intestinotropic hormone to avoid potential adverse outcomes such as enhanced carcinogenic risk.

**GRANTS**

K. J. Rowland is supported by a Doctoral Award from the Canadian Institutes of Health Research (CIHR) in partnership with the Canadian Digestive Health Foundation and P. L. Brubaker by the Canada Research Chair in Gastroenterology and Digestive Health. K. J. Rowland is supported by a Doctoral Award from the Canadian Institutes of Health Research (CIHR) in partnership with the Canadian Digestive Health Foundation and P. L. Brubaker by the Canada Research Chair in Gastroenterology and Digestive Health.

**DISCLOSURES**

P. L. Brubaker has received consulting fees on the topic of GLP-2 from Regeneron and Zealand.

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