Annu. Rev. Nutr. 2006. 26:391–411
doi: 10.1146/annurev.nutr.26.061505.111223

First published online as a Review in Advance on February 24, 2006

GLUCAGON-LIKE PEPTIDE-2

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Key Words intestine, enteroendocrine cell, GPCR, mucosa, apoptosis

■ **Abstract** Multiple peptide hormones produced within the gastrointestinal system aid in the regulation of energy homeostasis and metabolism. Among these is the intestinotrophic peptide glucagon-like peptide-2 (GLP-2), which is released following food intake and plays a significant role in the adaptive regulation of bowel mass and mucosal integrity. The discovery of GLP-2's potent growth-promoting and cytoprotective effects in the gastrointestinal (GI) tract stimulated interest in its use as a therapeutic agent for the treatment of GI diseases involving malabsorption, inflammation, and/or mucosal damage. Current research has focused on determining the physiological mechanisms contributing to the effects of GLP-2 and factors regulating its biological mechanisms of action. This chapter provides an overview of the biology of GLP-2 with a focus on the most recent findings on the role of this peptide hormone in the normal and diseased GI tract.

CONTENTS

REGULATION OF GLP-2 SYNTHESIS, SECRETION, AND BIOLOGICAL ACTION

Glucagon-like peptide-2 (GLP-2) is encoded within a large proglucagon precursor that also encodes the sequence of glucagon and GLP-1. Proglucagon is cleaved by prohormone convertase enzymes in a tissue-specific manner, with PC2 yielding glucagon and the major proglucagon fragment in the pancreas, or PC1 generating glicentin, oxyntomodulin, GLP-1, and GLP-2 in the gastrointestinal (GI) tract

and brain (Figure 1) (30, 42, 65). For a more detailed review of the regulation of proglucagon gene expression, see (33). Although the proglucagon-derived peptides (PGDPs) elicit a diverse set of biological actions, they all play a significant role in the regulation of nutrient absorption and/or the maintenance of energy homeostasis.

Glucagon secretion from islet α-cells is stimulated by hypoglycemia and suppressed following food intake, while GLP-1 and GLP-2 are released from intestinal L-cells in a 1:1 ratio following nutrient ingestion (32, 44, 86), primarily by meals rich in carbohydrates and lipids (85, 89, 91, 117). The short-chain fatty acid butyrate and GLP-2 exhibit overlapping actions within the gastrointestinal tract (102); supplementation of parenteral nutrition with butyrate in piglets has been shown to increase plasma GLP-2 concentrations (5, 103). Moreover, secretion of intestinal PGDPs is also regulated by glucose-dependent insulinotropic polypeptide (87), somatostatin (48), gastrin-releasing peptide (88), and neural stimuli (90) in a species-specific manner. For a more detailed review of the regulation of glucagon-like peptide secretion, see (12, 31, 38).

The biological half-life of circulating GLP-2 is relatively short (∼7 minutes in humans) due to extensive renal clearance and rapid degradation by the proteolytic enzyme dipeptidyl peptidase-4 (DPP-4) (49, 104). The importance of DDP-4 in the regulation of GLP-2 bioactivity was originally shown in rats, as administration of exogenous native GLP-2 had only a modest effect on bowel growth due to high levels of endogenous DDP-4 activity. Administration of a GLP-2 analogue lacking the N-terminal DPP-4 cleavage site (h[Gly-2]GLP-2) significantly increased bowel weight in these rats relative to administration of native GLP-2. Moreover, wild-type GLP-2 was comparatively more efficacious in the induction of bowel growth in rats deficient in DDP-4 activity (36). The metabolite produced as a result of DPP-4 cleavage, GLP-2 (3-33), was recently demonstrated to act as a competitive antagonist of the GLP-2 receptor (GLP-2R), and inhibited GLP-2- and nutrient-induced mucosal growth in rodents (96, 108). GLP-2 (3-33) may also act as a weak GLP-2R agonist at pharmacological concentrations. Thus, the biological actions of GLP-2 may be limited both by its rapid enzymatic degradation and renal clearance, and through competition with the products of its own degradation. Accordingly, the degradation-resistant analogue of GLP-2 (h[Gly-2]GLP-2, teduglutide) has been shown to have increased efficacy compared to wild-type GLP-2 in many experimental models (8, 34, 36) and is currently being investigated for the treatment of human gastrointestinal disease (58, 60).

THE GLP-2 RECEPTOR

The GLP-2 receptor was cloned from rat and human hypothalamic and intestinal cDNA libraries (75) and was classified as a member of the family B secretin-like subfamily of G protein-coupled receptors based on conserved structure. The GLP-2

receptor gene has been mapped to human chromosome 17p13.3 and encodes a 553 amino acid protein. The results of mutagenesis studies and Western blot analyses suggest that during translation, the rat and human GLP-2Rs are likely cleaved within the endoplasmic reticulum at a predicted signal peptide cleavage site located within the N-terminal domain, yielding a ~53 kDa protein after deglycosylation (40, 75). The GLP-2R recognizes GLP-2, but not related members of the glucagon peptide family, in a highly specific manner (28, 75).

GLP-2 receptor mRNA transcripts have been detected in the stomach, the small and large bowel, the brain, and the lung (71, 101, 121). mRNA transcripts were also found in RNA isolated from normal human cervix, human cervical cancer tissue, and cervical cell lines (63); however, the functional relevance of GLP-2R expression in the cervix or in cervical cancer is not yet known. Multiple experimental approaches have localized the GLP-2R to regions within the rodent central nervous system (CNS) including the hippocampus, hypothalamus, nucleus of the solitary tract, parabrachial nucleus, supra mammillary nucleus, substantia nigra, and cerebellum in the rat (71, 101), in addition to the medulla, amygdala, dentate gyrus, pons, cerebral cortex, and pituitary in the mouse (69). The GLP-2R has been localized to human enteroendocrine cells (121), murine enteric neurons (7), and intestinal myofibroblasts (78) (Figure 2). Given the lack of GLP-2R expression in enterocytes, it is likely that the proliferative and cytoprotective effects of GLP-2 observed in the bowel are indirect.

A role for keratinocyte growth factor (KGF) as a downstream mediator of GLP-2-stimulated colonic growth in mice has been suggested; however, immunoneutralization of KGF activity appeared to have no effect on GLP-2-induced mucosal growth within the small intestine (78). Moreover, GLP-2 and KGF exert differential effects on the bowel in a rat model of short bowel syndrome (113). The precise mechanisms by which GLP-2 stimulates bowel growth, particularly in the small bowel, where the most striking effects are seen, remain poorly understood.

Extensive efforts have been undertaken to identify an intestinal cell line that robustly expresses the endogenous GLP-2R; nevertheless, none have been found to date. HeLa cells have been shown to express the GLP-2R and exhibit a small but significant cAMP response following GLP-2 treatment (63); however, the majority of studies characterizing GLP-2R-regulated intracellular signaling pathways have utilized transfected cell lines. Agonist binding to the GLP-2R results in dose-dependent activation of adenylyl cyclase, increases in intracellular cAMP, and activation of PKA in fibroblasts expressing a heterologous rat or human GLP-2R, as well as in primary cell cultures from the CNS and the intestinal mucosa (63, 71, 75, 112, 122). Furthermore, GLP-2 activates c-fos in cells transfected with the GLP-2R (122) and in the murine intestinal mucosa (7). When expressed in HeLa cells, the GLP-2R also couples to the extracellular signal-regulated kinase (ERK1/2)-MAPK pathway in a $G\alpha_{i/o}$-, $G_{\beta/\gamma}$-, and Ras-dependent manner that does not appear to involve transactivation of the epidermal growth factor receptor (63).

Like many G protein-coupled receptors, the GLP-2R undergoes significant down-regulation of signaling upon acute ligand stimulation in vitro. This phenomenon, called receptor desensitization, occurs independently of receptor lipid raft-dependent internalization and results in a prolonged attenuation of receptor responsiveness to subsequent agonist stimulation (40). Mutagenesis studies demonstrate that the C-terminal domain of the receptor is not required for coupling to either the adenylyl cyclase or the (ERK1/2)-MAPK signaling cascades and is dispensable for acute, ligand-mediated receptor desensitization. However, independent activation of PKA can result in heterologous GLP-2R desensitization, which requires an intact GLP-2R C-terminus (39). Although the precise mechanisms regulating GLP-2R desensitization are not fully understood, the finding that the interaction with arrestin-2, a protein identified as a key modulator of GPCR desensitization, is not required for down-regulation of receptor signaling suggests that GLP-2R signaling (39) may be regulated by a unique, incompletely characterized pathway. Further studies are needed to address whether functional GLP-2R desensitization occurs in vivo either in response to short-term activation by endogenous GLP-2 or following persistent receptor signaling resulting from exogenous administration of long-acting GLP-2 analogues.

The mechanisms by which GLP-2 elicits its direct cytoprotective effects on cells appear to be dependent on the specific apoptotic stimulus and experimental model. GLP-2R activation in fibroblasts inhibits cycloheximide-induced apoptosis in a PKA-independent manner (119); however, PKA regulates the anti-apoptotic properties of GLP-2R signaling when apoptosis is induced either by inhibition of phosphoinositide 3-kinase (PI-3K) in transfected cells (63, 120) or by glutamate in cultured hippocampal neurons (71). Although the cellular mechanisms by which GLP-2 inhibits apoptosis are not fully understood, GLP-2 signaling inhibits the actions of the proapoptotic molecule glycogen synthase kinase-3 (GSK3) both in vitro and in vivo, in addition to directly inhibiting Bad in fibroblasts and increasing Bcl-2 expression within the neonatal pig intestine (17, 120).

Exogenous GLP-2 significantly increases the proliferation rate of enterocytes in vivo; nonetheless, the GLP-2R is not expressed within this cell population and there is inconclusive evidence on whether direct receptor activation stimulates cell division. Treatment of cultured colonic intestinal cells or astrocytes with GLP-2 results in increased cell proliferation (57, 92, 111). However, fibroblasts expressing the GLP-2R do not exhibit a significant mitogenic response following treatment with GLP-2 (63, 122), suggesting that increased cell proliferation is not a direct effect of GLP-2R activation. Furthermore, GLP-2 treatment inhibits proliferation in cultured epithelial cells from the small intestine, yet stimulates cell proliferation in cell lines derived from the large bowel (15), suggesting that GLP-2R activation of downstream mitogenic effectors may be cell-type- or tissue-specific. The proliferative effects of GLP-2 in cell culture systems are often observed in the absence of documented expression of the known GLP-2R (92), leaving open the possibility that GLP-2 may exert some of these effects through as yet undefined receptors and/or signaling pathways.

THE ACTIONS OF GLP-2 WITHIN THE GASTROINTESTINAL TRACT

Initial studies identifying GLP-2 as an intestinotrophic factor demonstrated that GLP-2 given twice daily to mice significantly increased small bowel weight and nutrient absorption (35). The increase in bowel mass was mainly attributable to an increase in crypt cell proliferation, leading to lengthening of the intestinal villi and a modest expansion of the crypt compartment (35, 36, 43, 110). These effects were primarily observed in the small intestine, although GLP-2-mediated colonic growth has also been observed (34, 43, 67, 78). The effects of GLP-2 within the bowel are reversible, as regression of bowel growth was observed following cessation of treatment (110). In addition to enhancing enterocyte proliferation, GLP-2 increases mucosal mass by inhibiting cell death within the intestinal crypt compartment. Although GLP-2 treatment was able to decrease enterocyte apoptosis in healthy mice (110), the cytoprotective effects of GLP-2 treatment are more evident in the setting of gastrointestinal injury or stress (9, 10, 17, 18, 96).

In addition to promoting expansion of the gastrointestinal mucosal surface area, GLP-2 exerts a number of other actions within the GI tract to promote energy absorption. GLP-2 stimulates the uptake of luminal nutrients including sugars and amino acids (14, 25, 61); enhances mucosal hexose transport possibly via increasing GLUT2 insertion in the jejunal-brush border (4, 25, 26); increases the expression of genes encoding nutrient transporters including the sodium-dependent glucose transporter 1 (SGLT-1) and immediate early genes such as PC2/TIS7 and c-fos (7, 24, 27, 54, 83, 84, 100); and increases the expression of multiple enzymes involved in digestion (14, 81) along the GI tract. GLP-2 administration also decreases gastric acid secretion (114) and inhibits antral gastric emptying (11, 13, 76, 115). Pharmacological doses of GLP-2 have been shown to decrease food intake in rodents when administered centrally (69, 101); however, reduced hunger or weight loss has not been observed in human patients receiving subcutaneous injections or intravenous infusions of either wild-type GLP-2 or the long-acting form of GLP-2, teduglutide (58, 76, 94, 98). GLP-2 also maintains mucosal integrity by enhancing intestinal barrier function and decreasing epithelial permeability in both the normal and injured bowel. Both wild-type GLP-2 and teduglutide reduced the conductance and flux of ions and macromolecules in the bowel within hours of treatment (6), and administration of GLP-2 reduces epithelial ion permeability within the setting of experimental allergy or stress (19, 20). Teduglutide also increased transepithelial resistance and decreased bacterial translocation in a rodent model of acute necrotizing pancreatitis (64).

GLP-2 AND THE MUCOSAL ADAPTIVE RESPONSE

The majority of GLP-2 actions have been elucidated following exogenous administration of GLP-2 or degradation-resistant GLP-2 analogues. Studies investigating the role of endogenous GLP-2 have shown that the peptide hormone plays a critical

role in the adaptive response to intestinal injury and/or stress. GLP-2 was shown to augment the growth and absorptive capacity of the intestine following massive bowel resection (95). Levels of GLP-2 are elevated in rodents and in some human patients with short bowel syndrome (29, 59, 68, 72, 106, 109). GLP-2 levels in rats postresection correlated with the magnitude of tissue loss and malabsorption, and an ongoing elevation of plasma GLP-2 was associated with maintenance of enhanced crypt cell proliferation (72). Oral nutrients may also further enhance the effects of GLP-2 in the setting of short bowel syndrome (29, 79). Consistent with the importance of GLP-2 in the adaptive growth response of the remnant gut mucosa, immunoneutralization of GLP-2 attenuated intestinal growth following massive small bowel resection in rats (80).

Marked intestinal hyperplasia has been observed following induction of diabetes in rats with streptozotocin (STZ). It was postulated that the increase in bowel mass was due to increased levels of circulating GLP-2 (41, 107), and treatment of diabetic rats with insulin reduced intestinal mucosal hyperplasia in association with reduced circulating levels of GLP-2 (41). Immunoneutralization of GLP-2 diminished STZ-induced intestinal growth in rats, providing further evidence that endogenous GLP-2 plays a role in this process (50). Furthermore, the GLP-2R antagonist, GLP-2 (3-33), abrogates the adaptive mucosal hyperplasia associated with re-feeding following an overnight fast in mice (96). Although enteral ingestion of nutrients stimulates the release of GLP-2 from endocrine cells, there are differences in the biological effects of enteral nutrition versus GLP-2 administration in pigs in the control of cell proliferation and apoptosis (18), suggesting that GLP-2 action alone cannot account for all of the intestinotrophic effects attributed to food ingestion. However, the effects of GLP-2 on apoptosis and proliferation may be dose-dependent, as lower levels of GLP-2 exert a cytoprotective effect in the pig intestinal tract, whereas higher pharmacological levels of GLP-2 are needed to stimulate cellular proliferation (17).

A number of studies have addressed a potential role for GLP-2 in the developing gastrointestinal system. GLP-2 (1-33) and the GLP-2R are detected in the fetal and neonatal rat intestine, with the levels of GLP-2R mRNA transcripts being comparatively higher within the developing GI tract relative to the adult GI tract. GLP-2 administration increased neonatal body weight and had trophic effects on the small and large bowel, demonstrating that GLP-2R signaling was functional in the neonatal rat intestine (70). GLP-2 rapidly increased nitric-oxide-dependent portal-vein blood flow rate, glucose uptake, and intestinal blood volume in total parenteral nutrition (TPN)-fed pigs, with increased mucosal blood flow specifically localized to the proximal small intestine, a region highly sensitive to the trophic effects of GLP-2 (99). GLP-2 stimulated an increase in both endothelial nitric oxide synthase protein levels and constitutive nitric oxide synthase activity, providing a possible mechanism for the observed effects on intestinal blood flow in TPN-fed piglets (46). Because decreased blood flow to the intestine may contribute to TPN-induced bowel hypoplasia (77), these data provide a complementary explanation for the ability of exogenous GLP-2 to maintain bowel mass in the absence of

enteral nutrients. GLP-2 alone had no effect on intestinal weight or lipid uptake in suckling or weanling rats, but it prevented the loss of body weight associated with glucocorticoid administration in suckling rats (55, 56). Furthermore, GLP-2 and dexamethasone given to lactating mother pigs enhanced lipid absorption in their suckling offspring. However, this combination therapy may be deleterious in later life as lipid malabsorption was detected post-weaning (56).

Infants with short bowel syndrome exhibit levels of postprandial GLP-2 that correlate with nutrient absorption capacity and were predictive of nutritional status (97). Similarly, children undergoing cancer treatment exhibit circulating levels of GLP-2 that correlate with the magnitude of enteral nutrient ingestion (2). Whether GLP-2 supplementation may be beneficial in infants with intestinal dysfunction has not yet been demonstrated. Although evidence suggests there is a role for GLP-2 in the intestinal adaptive response to mucosal injury in children and adults, there is some debate as to the physiological relevance of GLP-2 in fetal gut development because exogenous GLP-2 appears to have a variable or diminished intestinotrophic effect during intestinal development (for review, see 16).

GLP-2 ACTION IN THE SETTING OF GASTROINTESTINAL DISEASE

Because GLP-2 potently stimulates bowel growth, enhances absorptive function, and protects the intestinal mucosa from injury, it is not surprising that GLP-2 administration has been investigated as a potential treatment for human gastrointestinal disease. GLP-2 has proven to be beneficial in multiple animal models of short bowel syndrome, and teduglutide is currently in late-phase clinical trials in humans for treatment of short bowel syndrome. In preclinical studies, administration of teduglutide to rats with major small bowel resection restored nutrient absorptive capacity in the small bowel (95), increased villus and mucosal height in the jejunum, and improved mucosal glutathione redox status (113). Human patients with short bowel syndrome who received 400 micrograms subcutaneously twice a day for 35 days exhibited increased nutrient absorption, delayed gastric emptying, and increased body weight characterized by an increase in lean mass and a decrease in fat mass (58). Three weeks of once- or twice-daily administration of teduglutide to short bowel syndrome patients with varying colon lengths decreased fecal wet weight and fecal energy excretion, and increased villus height, crypt depth, and the mitotic index (60).

Initiation of TPN is often associated with significant bowel atrophy. As GLP-2 release in response to enteral nutrients is thought to play a significant role in the maintenance of bowel mass, it is hypothesized that administration of exogenous GLP-2 or stimulation of endogenous GLP-2 release may attenuate TPN-induced reductions in intestinal mucosal weight and function. Rats maintained on TPN supplemented with GLP-2 were shown to exhibit a reduction in villus hypoplasia within the small intestine (21, 22). Furthermore, TPN-fed rats with bowel resection

exhibited increased villus height and intestinal mucosal surface area, decreased intestinal permeability, and increased bowel mass and body weight in response to treatment with GLP-2. Similarly, GLP-2 mimicked the actions of enteral nutrition in its effects on intestinal structure, lactose digestive and hexose absorptive capacities, and hexose metabolism in TPN-fed piglets (27). In rats, the main mechanism of GLP-2 action in the setting of experimental bowel resection appeared to be primarily via the stimulation of crypt cell proliferation (73). In contrast, GLP-2 administration to TPN-fed piglets produced a decrease in mucosal proteolysis and an increase in bowel mass that was mainly attributable to antiapoptotic effects within the crypt compartment (17, 18). However, these differential effects on apoptosis and proliferation may also be attributable to differential sensitivity to circulating levels of GLP-2 (17).

The effects of GLP-2 on enhancing barrier function occur fairly rapidly, within minutes to hours. Teduglutide or native GLP-2 decreased bacterial colonization of the lymph nodes, pancreas, and the peritoneum in a rodent model of acute necrotizing pancreatitis (64); reduced the uptake of antigen, the antigen-induced secretory response, and the number of inflammatory cells in a mouse model of food allergy (20); and modestly decreased bacterial colonization of the lymph nodes following burn injury in rats (23). In a murine model of stress, GLP-2 ameliorated bacterial infiltration and decreased ion and macromolecule transport within regions of the small and large intestine (19). GLP-2 also reduced the number of mononuclear cells in the colonic mucosa of the stressed mice. The acute functional effects of GLP-2 on mucosal permeability have also been associated with subsequent ultrastructural changes in the murine intestinal mucosal (6).

Inflammatory bowel disease (IBD) may involve both the small and large bowel and produce changes in gut architecture, including crypt distortion and scarring and/or crypt abscesses. Because GLP-2 improves barrier function and nutrient absorption while concurrently stimulating bowel growth and regeneration, it may prove beneficial for the treatment and/or prevention of IBD. In multiple animal models of IBD, GLP-2 decreased weight loss and reduced intestinal damage by increasing cell proliferation and decreasing cell death, leading to functional restoration of mucosal integrity. In a mouse model of dextran sulfate-induced colitis, teduglutide reduced weight loss; increased colon length, mucosal area, and crypt depth; and reduced cytokine gene expression in the colonic mucosa (37). When enteritis was induced in mice by administration of the nonsteroidal anti-inflammatory drug indomethacin, teduglutide significantly improved survival, bowel integrity, and barrier function (9). GLP-2-treated mice exhibited fewer intestinal ulcerations, decreased systemic bacteremia, and decreased myeloperoxidase activity and cytokine induction in the small bowel. Similarly, in a rat model of IBD, intravenous GLP-2 administration reduced mucosal damage, histological lesion score, and the expression of the proinflammatory mediators TNF-α and IFN-γ (1). Similarly, rats with IBD receiving GLP-2 experienced decreased diarrhea and attenuated intestinal inflammation (3).

Although it is clear that GLP-2 is beneficial in rodent models of IBD, there is little data available on its effectiveness in the prevention or treatment of human

IBD. Interestingly, while GLP-2 levels are often increased in rodent models of gastrointestinal disease (29, 37, 68) and in human patients following bowel resection (72), no differences in meal-stimulated GLP-2 levels were observed in IBD patients versus healthy controls (93). However, it has been shown that although total circulating levels of GLP-2 may remain constant, the ratio of bioactive GLP-2 (1-33) to the inactive metabolite GLP-2 (3-33) appears higher in human subjects with either ulcerative colitis or Crohn's disease (116). This increase in active GLP-2 (1-33) may be due to decreased dipeptidyl peptidase (DDP)-4 activity observed in IBD patients (116). The potential benefits of teduglutide in human patients with IBD are currently being examined in clinical trials.

Gastrointestinal ischemia/reperfusion injury occurs when blood is recirculated into the small intestine following interruption of blood flow and is often the consequence of sepsis, hemorrhagic shock, vascular surgery, small bowel transplantation, or multiple organ failure. In the setting of ischemia/reperfusion injury in rats, teduglutide improved nutrient absorption and enhanced recovery of mucosal DNA content (82). Pretreatment of mice with GLP-2 prior to ischemia/reperfusion injury attenuated the intestinal histological damage and modestly increased villus height and crypt depth. Bacterial translocation and the production of reactive oxygen species were also reduced in GLP-2-treated mice, which may be due to increases in uncoupling protein 2 expression (45).

GLP-2 has been shown to exert beneficial effects when given prior to, during, and/or following the induction of intestinal damage (9, 66) and in transgenic models of established IBD (1, 3). Although GLP-2 may have therapeutic benefits at various stages of intestinal disease, the greatest therapeutic efficacy of GLP-2 has often been observed when the peptide was given prior to the induction of gut injury (9, 10, 37). Hence, the timing of GLP-2 administration relative to the onset of intestinal injury may influence its effectiveness. An alternative approach to exogenous GLP-2 administration involves the use of DDP-4 inhibitors to increase levels of bioactive GLP-2 (1-33). Although inhibition of DPP-4 enhances the intestinotrophic effects of exogenously administered GLP-2, DPP-4 inhibitors alone have not been shown to exert significant effects on levels of endogenous GLP-2 (51).

While it is apparent that GLP-2 has therapeutic benefits when administered alone, several studies have shown that GLP-2 may be administered in combination with other growth factors. Mice treated with h[Gly2]GLP-2 and either GH or IGF-I exhibited greater increases in histological parameters of small intestinal growth than did mice treated only with h[Gly2]GLP-2 (34). Experiments in parenterally fed rats demonstrated that concurrent treatment with both GLP-2 and epidermal growth factor resulted in greater small intestinal mass, cell proliferation rate, and crypt-villus area compared with results obtained when either hormone was given alone (62). However, GLP-2 may not always be more effective when coadministered with other therapeutic agents. Although GLP-2 decreased the severity of ulcerative colitis in mice when given concurrently with aminosalicylates, it failed to have beneficial effects when administered together with corticosteroids (66). These studies underline the need to evaluate the efficacy of GLP-2 given in conjunction with other drugs commonly used to treat GI disorders.

Gastrointestinal dysfunction often develops in patients receiving chemotherapy as the cytotoxic drugs inadvertently target the rapidly proliferating intestinal mucosa. As prophylactic treatment with GLP-2 can decrease the severity of chemically induced gastrointestinal damage and improve survival in multiple animal models, the cytoprotective potential of GLP-2 has been studied in animals receiving chemotherapy. When administered to mice prior to treatment with irinotecan hydrochloride or 5-fluorouracil, GLP-2 improved survival, decreased weight loss, reduced bacteremia, attenuated epithelial injury, and decreased chemotherapy-induced apoptosis within the intestinal crypt compartment (10). Although GLP-2 decreased cell death and protected the mucosa from cytotoxic damage in normal intestinal tissue, it did not interfere with the actions of irinotecan to reduce tumor size (10). In a subsequent study, biguanides were shown to promote the secretion of GLP-2, and the coadministration of the biguanide metformin with valine-pyrrolidide, a DDP-4 inhibitor, attenuated the loss of bowel wet weight induced by 5-fluorouracil (118). Consistent with a general cytoprotective effect of GLP-2 within the GI mucosa, teduglutide also protected cells within the murine small intestine from damage due to gamma-irradiation (8). Taken together, these studies provide support for the use of GLP-2 in the prevention of intestinal damage induced by various cytotoxic cancer treatments.

Because GLP-2 is a potent intestinotrophic growth factor, there remains the possibility that GLP-2 action may promote the formation of intestinal tumors or potentiate tumor growth. This is of particular concern in patients receiving chemotherapy for pre-existing cancer, as well as in patients with IBD, who have an increased risk for the development of colon cancer (74). Short-term GLP-2 treatment had no effect on tumor growth in rats with pre-existing large bowel tumors (22), and GLP-2 administration did not impair the effectiveness of chemotherapy to reduce tumor size in mice (10). However, following administration of the carcinogen 1,2-dimethylhydrazine to mice, one-month administration of a GLP-2 analogue increased the tumor load compared with saline-treated controls (105), with the tumor-promoting effects of native GLP-2 being significant only for small polyps. Hence, although the available evidence suggests that GLP-2 may potentiate tumor growth in the setting of carcinogen administration, whether GLP-2 alone potentiates tumor formation has not yet been demonstrated. Table 1 provides a summary of the physiological actions and therapeutic effects of GLP-2 in the setting of gastrointestinal disease.

EXTRAINTESTINAL ACTIONS OF GLP-2

During studies investigating the effects of GLP-2 in patients with short bowel syndrome, subjects receiving GLP-2 exhibited significant increases in bone density (47, 58). A five-week course of GLP-2 administration to patients with short bowel syndrome significantly increased both spinal and hip bone mineral density, which was accompanied by a decrease in markers of bone turnover (47). Subsequent

TABLE 1 The physiological and therapeutic effects of GLP-2 in the setting of GI disease*

Disease model	Species	Effect(s) of GLP-2 treatment	Reference
Short bowel syndrome	Rat	Restored absorptive capacity of bowel	(95)
		Increased villus and mucosal height	(113)
		Improved mucosal antioxidant capacity	
	Human	Increased nutrient absorption	(58, 60)
		Increased body weight	
		Delayed gastric emptying	
		Increased intestinal wet weight	(60)
		Decreased fecal excretion	
		Increased villus height, crypt depth, and mitotic index	
Total parenteral nutrition	Rat	Decreased villus shortening and mucosal thinning	(21, 22)
		Increased mucosal surface area and weight of bowel	(73)
		Increased body weight	
		Increased barrier function	
	Piglet	Decreased mucosal proteolysis and apoptosis	(17, 18)
		Increased bowel mass	
		Increased intestinal blood volume	(46, 99)
		Increased portal vein flow rate	
		Stimulated NOS production and activity	
		Maintenance of intestinal structure	(72)
		Maintenance of digestive and absorptive capacities	
Acute necrotizing pancreatitis	Rat	Decreased intestinal permeability	(64)
Food allergy	Mouse	Decreased uptake of antigen	(20)
		Diminished hypersensitivity reaction in bowel	
Burn injury	Rat	Reduced loss of bowel mass in response to burn	(23)
		Decreased immunosuppression	
Ischemia/reperfusion injury	Rat	Improved nutrient absorption	(82)
		Increased mucosal DNA content	
	Mouse	Increased villus height and crypt depth	(45)
		Decreased bacterial translocation	
		Decreased reactive oxygen species production	
		Increased UCP2 expression	

(*Continued*)

TABLE 1 (*Continued*)

Disease model	Species	Effect(s) of GLP-2 treatment	Reference
Irradiation	Mouse	Decreased apoptosis in the small bowel	(8)
Inflammatory bowel disease			
Dextran-induced colitis	Mouse	Improved survival Increased colon area Decreased cytokine expression	(37)
NSAID-induced enteritis	Mouse	Decreased lesion number Decreased intestinal permeability Reduced inflammatory response	(9)
Antigen-induced GI inflammation	Rat	Reduced mucosal damage Decreased expression of TNF-α and IFN-γ Decreased diarrhea Reduced inflammation	(1, 3)
Chemotherapy-induced mucosal damage	Mouse	Improved survival Decreased weight loss Reduced bacteremia Attenuated epithelial injury	(10)
Stress	Mouse	Improved intestinal barrier function	(19)

*Abbreviations: GI, gastrointestinal; GLP-2, glucagon-like peptide-2; IFN-γ, interferon-gamma; NSAID, nonsteroidal anti-inflammatory drugs; NOS, nitric oxide synthase; TNF-α, tumor necrosis factor-alpha; UCP2, uncoupling protein 2.

studies in fasting postmenopausal women showed that acute GLP-2 administration reduced bone resorption, evident by decreased levels of serum C-terminal telopeptide region of type I collagen and urine deoxypyridinoline (52, 53). However, the mechanisms by which GLP-2 inhibits bone resorption have not been characterized and the localization of the GLP-2R in bone has not yet been ascertained. Therefore, it is unclear whether the effects of GLP-2 on bone resorption and density are mediated via direct action on bone, or through indirect effects on nutrient absorption and mineral homeostasis.

The GLP-2R has been localized to multiple regions of the rodent CNS. Localization of the receptor within the dorsal medial hypothalamus (69, 101) suggested that GLP-2 may play a direct role in the regulation of hunger or satiety. Intracerebroventricular (ICV) administration of large amounts of GLP-2 inhibited food intake in rats (101), and pharmacological doses of ICV GLP-2 produced a modest, yet significant, inhibition of short-term food intake in mice (69). Whereas the inhibitory actions of ICV GLP-2 on food intake in rats were blocked by the GLP-1R antagonist exendin (9-39), similar experiments in wild-type mice failed to demonstrate a role for the GLP-1 receptor in the anorectic actions of GLP-2. Furthermore, the satiety effects of ICV GLP-2 were significantly potentiated in GLP-1R knockout mice (69). These discrepancies highlight possible species-specific

differences in GLP-2 action in the brain. Furthermore, studies in humans have not demonstrated a reduction in food intake following peripheral GLP-2 administration (58, 94, 98). As the GLP-2R has also been localized to multiple extrahypothalamic regions of the rodent CNS (69, 71), including areas involved in learning and memory, it seems likely that GLP-2 subserves additional roles within the brain.

As GLP-2 inhibits apoptosis in the gastrointestinal mucosal, the potential cytoprotective actions of GLP-2 have also been determined in the CNS. Treatment of cultured murine hippocampal neurons with teduglutide significantly inhibits glutamate-induced apoptosis (71). Hence, GLP-2R activation may also serve a cytoprotective role within the brain.

CONCLUSIONS AND FUTURE DIRECTIONS

With the prevalence of gastrointestinal dysfunction on the rise, there has been increasing interest in gaining a better understanding of the physiological processes that regulate gut physiology. Many gut-derived hormones serve as important regulators of digestion, nutrient absorption, and energy homeostasis. However, few of these hormones also demonstrate the striking intestinotrophic and reparative functions associated with GLP-2 action in the gastrointestinal system. The actions of GLP-2 are mediated via its cognate G protein-coupled receptor, which exhibits highly localized expression, high specificity of binding, and complex regulatory mechanisms that control signaling in vitro. All these factors make GLP-2 an attractive drug for the treatment or prevention of gastrointestinal disease in human subjects.

Although the GLP-2R has primarily been localized to cell populations within the GI tract, it is also found within specific regions of both the enteric and central nervous systems. Because GLP-2R signaling exerts antiapoptotic actions in many cellular systems, one can hypothesize that GLP-2 may also maintain neuronal integrity within the brain. However, little is currently known about the extraintestinal functions of GLP-2 or the potential effects of central locally synthesized GLP-2 in the brain. Understanding the mechanisms of GLP-2 action on nutrient absorption, cell survival, cell proliferation, and gut barrier function will help us to better understand the complex network of signals regulating intestinal function in the normal and injured GI tract.

ACKNOWLEDGMENTS

J. Estall was supported by a Doctoral Studentship Award from the Canadian Institutes for Health Research. GLP-2 research in the Drucker laboratory was supported by operating grants from the Canadian Institutes for Health Research and the Ontario Research and Development Challenge Fund. D. Drucker is supported by a Canada Research Chair in Regulatory Peptides. GLP-2 is the subject of a

licensing agreement between the University Health Network, the University of Toronto, D. Drucker, and NPS Pharmaceuticals Inc.

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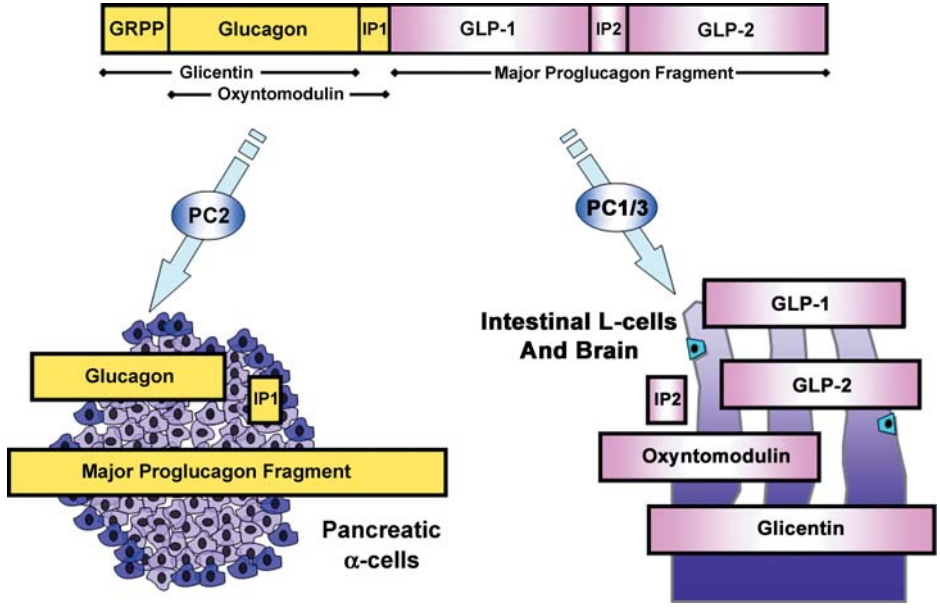

Figure 1 The proglucagon-derived peptides. The 160 amino acid proglucagon precursor encodes multiple biologically active peptides that are produced in a tissue-specific manner following enzymatic cleavage by prohormone convertases (PC). Cleavage by PC2 yields glicentin-related pancreatic polypeptide (GRPP), glucagon, intervening peptide 1 (IP1), and the major proglucagon fragment (MPGF) in the pancreatic α-cells. Cleavage by PC1/3 within intestinal L-cells and the brain produces glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), glicentin, oxyntomodulin, and intervening peptide 2 (IP2).

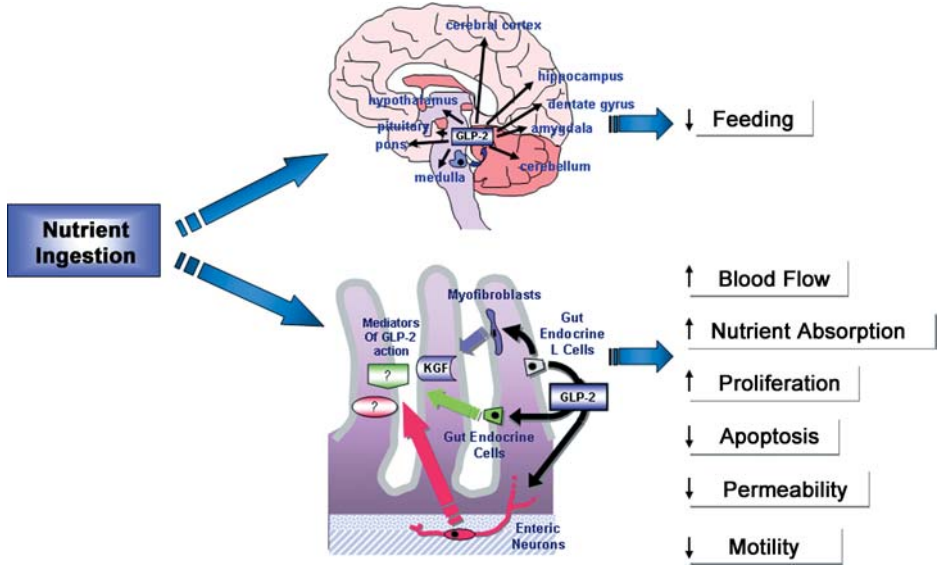

Figure 2 Physiological actions of GLP-2 within the gastrointestinal mucosa and the CNS. Upon release of GLP-2 from intestinal L-cells following nutrient ingestion, the peptide hormone is believed to act in a paracrine or endocrine manner on neighboring cells expressing the GLP-2 receptor. Given the precise localization of GLP-2R expression in enteroendocrine cells, myofibroblasts, and enteric neurons, many of the physiological responses following GLP-2 receptor activation in the bowel are believed to be mediated via indirect mechanisms following the release of currently unidentified mediators. The GLP-2R has also been localized to many different cell types within the CNS. Little is known about the consequences of GLP-2R activation within the brain; however, activation of the receptor in the hypothalamus may be responsible for the effects of central GLP-2 administration on feeding behavior.

Annual Review of Nutrition
Volume 25, 2005

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