Physiology and Pharmacology of the Enteroendocrine Hormone Glucagon-Like Peptide-2

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Abstract

Glucagon-like peptide-2 (GLP-2) is a 33-amino-acid proglucagon-derived peptide secreted from enteroendocrine L cells. GLP-2 circulates at low basal levels in the fasting period, and plasma levels rise rapidly after food ingestion. Renal clearance and enzymatic inactivation control the elimination of bioactive GLP-2. GLP-2 increases mesenteric blood flow and activates proabsorptive pathways in the gut, facilitating nutrient absorption. GLP-2 also enhances gut barrier function and induces proliferative and cytoprotective pathways in the small bowel. The actions of GLP-2 are transduced via a single G protein–coupled receptor (GLP-2R), expressed predominantly within the gastrointestinal tract. Disruption of GLP-2R signaling increases susceptibility to gut injury and impairs the adaptive mucosal response to refeeding. Sustained augmentation of GLP-2R signaling reduces the requirement for parenteral nutrition in human subjects with short-bowel syndrome. Hence GLP-2 integrates nutrient-derived signals to optimize mucosal integrity and energy absorption.

GLP-2 SYNTHESIS AND SECRETION

GLP-2: glucagon-like peptide-2

Proglucagon: the prohormone precursor encoding glucagon, oxyntomodulin, GLP-1, and GLP-2

PGDP: proglucagon-derived peptide

Enteroendocrine L cells: specialized gut endocrine cells that express the proglucagon (*Gcg*) gene and produce GLP-1 and GLP-2

Dipeptidyl peptidase-4 (DPP-4): the specialized exopeptidase that exists as both a membrane-spanning and a soluble form and that inactivates GLP-2 by cleavage at the position 2 alanine

h[Gly^2]-GLP-2 (also known as teduglutide): a degradation-resistant GLP-2 analog approved for the treatment of human short-bowel syndrome

Glucagon-like peptide-2 (GLP-2) is a 33-amino-acid proglucagon-derived peptide (PGDP) hormone liberated from proglucagon in enteroendocrine L cells in the small and large intestines. GLP-2 is also synthesized in the brain, predominantly in the brain stem, from where it may be transported to distal regions of the central nervous system to exert more-localized actions. The control of intestinal proglucagon (and hence GLP-2) expression is regulated by nutrients; food ingestion is the key stimulus for the induction of intestinal proglucagon gene expression and for the synthesis and secretion of the gut PGDPs. Nevertheless, intestinal PGDPs are also secreted at low basal rates even in the fasting state. There is limited information about the transcription factors and mechanisms critical for intestinal proglucagon gene transcription and for GLP-2 biosynthesis; *Pax6*, *Cdx2/3*, and *Pparβ/δ* are required for L cell development and/or intestinal proglucagon gene transcription in mice. Prohormone convertase 1 (PC1) liberates GLP-2 from proglucagon in the intestine; mice and humans with inactivating mutations in PC1 exhibit reduced levels of circulating mature GLP-2(1–33) and enteropathies (1, 2). Plasma GLP-2 levels rise rapidly following meal ingestion (3) through mechanisms requiring engagement of neural and endocrine circuits, as well as direct nutrient contact with L cells. Although localized predominantly to the distal gut, L cells have been identified more proximally in the jejunum. However, the relative contributions of proximal versus distal L cells to total PGDP secretion following meals and under different pathophysiological conditions are controversial and difficult to ascertain.

Plasma levels of intestinal PGDPs and GLP-2 rise rapidly in response to intestinal injury or major intestinal resection; nevertheless, the signals coupling sensing of gut injury to stimulation of L cell secretion remain obscure. Intact GLP-2(1–33) is cleaved within minutes to GLP-2(3–33) by dipeptidyl peptidase-4 (DPP-4) (4). Hence DPP-4-resistant GLP-2 analogs such as human [Gly^2]–GLP-2 (h[Gly^2]-GLP-2, or teduglutide) exhibit longer circulating half-lives and greater bioactivity relative to the native molecule (4). Inhibition of DPP-4 activity modestly potentiates the actions of native GLP-2, but treatment with DPP-4 inhibitors does not significantly expand the mucosal epithelium in normal rats (5). The predominant organ responsible for clearance of GLP-2 is the kidney, and circulating GLP-2 levels rise in the setting of renal impairment (6). Hence, dosing requirements for therapy with GLP-2R agonists such as teduglutide are reduced by ~50% in human subjects with moderate to severe renal impairment (7).

GLP-2 RECEPTOR

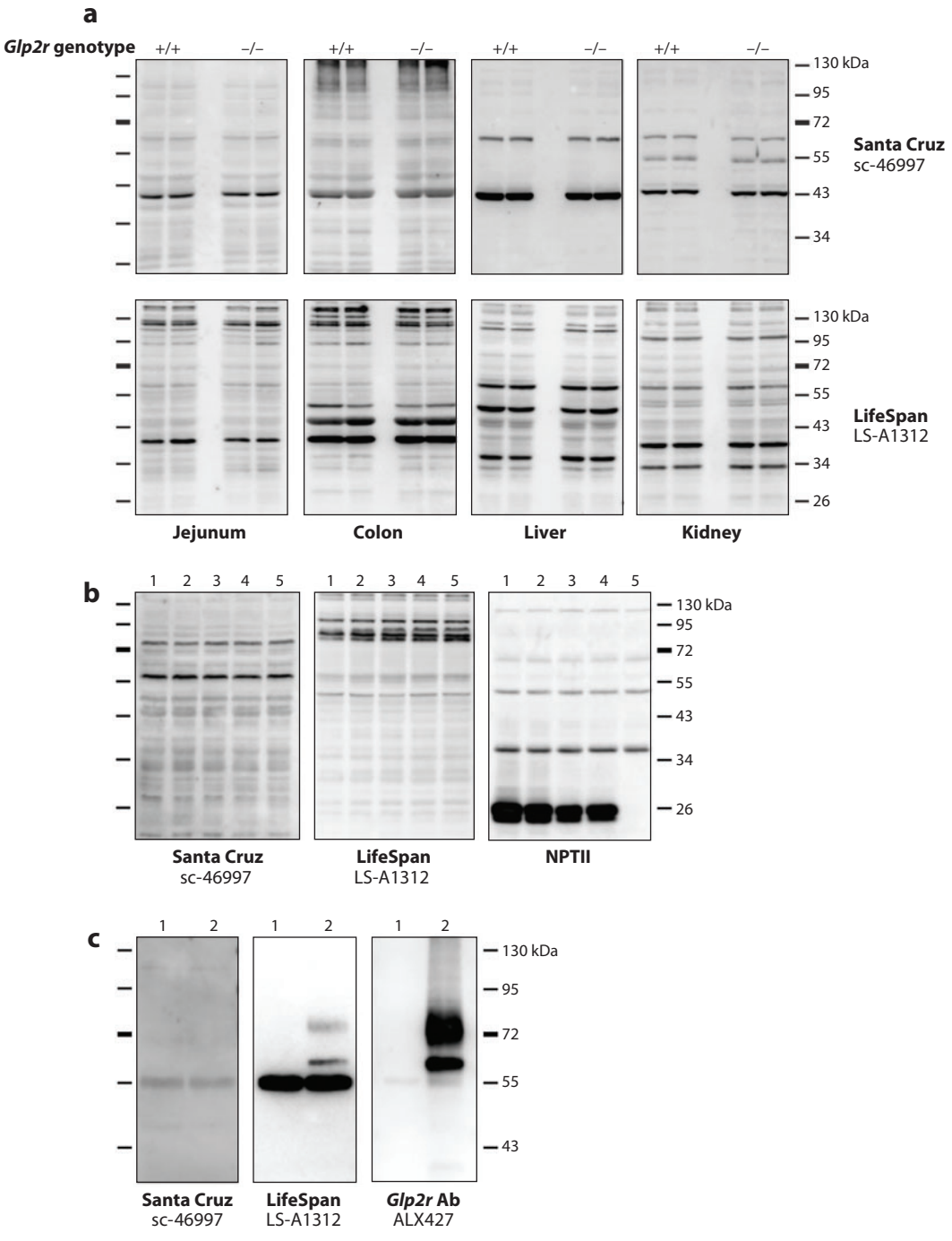
Most GLP-2 actions are mediated by a single G protein–coupled receptor (GPCR) (8), the GLP-2 receptor (GLP-2R), which exhibits considerable amino acid identity with related members of the class B GPCR superfamily that signal through adenylate cyclase (9). The human GLP-2R cDNA was cloned from intestinal and hypothalamic libraries and encodes a 553-amino-acid protein; the human *Glp2r* gene was localized to 17p13.3, a chromosomal region not yet identified as an intestinal disease susceptibility locus (8). A genome-wide association study examining the genetic determinants of fetal hemoglobin levels identified an association between variants in the *Glp2r* and fetal hemoglobin in erythrocytes of male subjects (10). The GLP-2R is highly selective for GLP-2 and reacts only weakly following exposure to equimolar concentrations of structurally related peptides such as glucagon, GLP-1, and glucose-dependent insulinotropic polypeptide (GIP) (11). Although GLP-2(3–33) is a pharmacological antagonist at the rodent GLP-2R, this peptide also exerts weak agonist activity (12).

LOCALIZATION OF GLP-2 RECEPTOR EXPRESSION

Glp2r mRNA transcripts have been localized to the gastrointestinal tract, central nervous system, and lung (8, 13). Within the gut, *Glp2r* mRNA transcripts are detected in the stomach and in both the small and large intestines. The precise sites of GLP-2R expression within the gastrointestinal tract in different animals and in humans are controversial. Immunocytochemical localization of GLP-2R expression was reported by using a polyclonal antiserum, ALX427, in human enteroendocrine cells (13). Subsequent studies employing in situ hybridization localized *Glp2r* mRNA transcripts to murine enteric neurons (14). A combination of in situ hybridization and immunocytochemistry identified subepithelial myofibroblasts as the principal GLP-2R+ cell type in rat, mouse, marmoset, and human small and large intestines (15). In contrast, Guan and colleagues (16) used a combination of immunocytochemistry and RNA isolated from laser capture microdissected tissue to identify GLP-2R expression in enteric neurons that coexpress vasoactive intestinal peptide (VIP) or endothelial nitric oxide synthase (eNOS) within the submucosal plexus and myenteric plexus. Multiple GLP-2R+ enteroendocrine cells were also identified by immunocytochemical techniques in human and pig intestines (16). Similarly, immunocytochemistry localized GLP-2R expression to vagal afferents in the nodose ganglia, enteroendocrine cells, enteric neurons, and nerve fibers in the rat myenteric plexus (17), and rat colon submucosal glial cells in culture also express an immunoreactive GLP-2R (18). Localization of GLP-2R-binding sites in human tissues and tumors has also been carried out by using receptor autoradiography. Analysis of 237 tumor and 148 non-neoplastic tissue samples detected GLP-2-binding sites in 68% of gastrointestinal stromal tumors and the intestinal myenteric plexus, with relatively more binding sites detected in the myenteric plexus from human subjects with Crohn's disease (19).

The divergent results obtained in studies of receptor localization likely reflect the low abundance of GLP-2R+ cells, species-specific differences in GLP-2R expression, and challenges with the sensitivity and specificity of methods and reagents (predominantly antisera) used to localize GLP-2R expression. Although several papers have described partial characterization of the sensitivity and specificity of various GLP-2R antisera (13, 16, 17), the precise attributes of each antiserum are often incompletely understood. Indeed, most reports employing antisera to detect the GLP-2R fail to validate the sensitivity or the specificity of the antisera by using appropriate positive and negative controls. Furthermore, we recently described major challenges surrounding the sensitivity and specificity of antisera used to detect the related GLP-1R, demonstrating that several commonly used GLP-1R antisera exhibit very poor sensitivity and a lack of specificity (20).

To better define the attributes of several commercially available antisera used to detect the GLP-2R, we assessed the sensitivity and specificity of two commonly used antisera by Western blot analysis of (*a*) tissue extracts from *Glp2r*+/+ and *Glp2r*−/− mice and (*b*) cells transfected with *Glp2r* cDNAs from three different species (**Figure 1**). Both antisera detected a large number of immunoreactive proteins in tissue extracts from jejunum and colon, tissues known to express the GLP-2R, as well as in two control tissues, liver and kidney, which do not express the GLP-2R (8, 13). Furthermore, the multiple proteins detected were indistinguishable in extracts from *Glp2r*−/− versus *Glp2r*+/+ mice. Hence, both antisera exhibit a complete lack of specificity. We next transfected the mouse, rat, or human *Glp2r* cDNAs into baby hamster kidney (BHK) fibroblasts and used the GLP-2R antisera in attempts to detect immunoreactive GLP-2R protein by conventional Western blot analysis. Neither commercially available antiserum was able to detect any of the three GLP-2R proteins (**Figure 1*b***). To greatly enhance the sensitivity of the detection method, we immunoprecipitated extracts by using an anti-FLAG antibody from cells transfected with the FLAG epitope–tagged human *GLP2R* cDNA. Immunoblotting revealed a faint GLP-2R-immunoreactive band with only one of the antisera, whereas the previously described ALX427



antisera (13) robustly detected the immunoprecipitated human GLP-2R (**Figure 1*c***). Hence these findings engender a further note of caution in regard to the interpretation of data obtained with incompletely characterized antisera used to detect the GLP-2R in immunocytochemical or immunoblotting analyses.

GLP-2 RECEPTOR SIGNAL TRANSDUCTION

GLP-2 directly increases cyclic AMP (cAMP) production in a broad range of GLP-2R-transfected cells (8, 21). Activation of GLP-2R signaling activates immediate-early-gene expression, increases levels of intracellular calcium, and induces the expression of cell survival genes and proteins (8, 21, 22). The direct prosurvival actions of GLP-2 in fibroblasts expressing a transfected GLP-2R are independent of Akt, $p90^{Rsk}$, or p70 S6 kinase activation and are linked to protein kinase A (PKA)-dependent inhibition of glycogen synthase kinase-3 activation (22). Although prolonged GLP-2R activation in vivo is not associated with tachyphylaxis (23), GLP-2R desensitization can be induced in heterologous cells in vitro, with reduced cell surface GLP-2R expression mediated through lipid raft–sensitive, clathrin-independent mechanisms (24). To date, studies show little evidence for substantial differences in rodent versus human GLP-2R signaling pathways.

GLP-2 also increased cAMP accumulation in dispersed neonatal brain stem cultures (25), in cultures of rat astrocytes (26), in fetal rat intestinal cell cultures (27), and in intestinal homogenates prepared from rat jejunum 2 h after injection of GLP-2 (28). Primary cultures of murine subepithelial myofibroblasts express the GLP-2R at low levels, and GLP-2 (10^{-8} M or 10 nM) transiently induced insulin-like growth factor 1 (*Igf1*) mRNA transcripts but had no effect on cAMP; Erk1/2 phosphorylation; intracellular calcium; or expression of *Epiregulin*, heparin-binding epidermal growth factor (*Hb-egf*), *ErbB1*, or *ErbB2* mRNA transcripts in the same experiments (29). Similarly, acute injection of h[Gly^2]-GLP-2 into fasted mice increased *Igf1* mRNA transcripts in jejunal mucosa through mechanisms sensitive to the phosphoinositide-3-kinase (PI3K) inhibitor wortmannin (29). Administration of GLP-2 in vivo induced rapid Akt phosphorylation in murine enterocytes of fasted mice (30), and GLP-2 directly increased levels of p-Akt and p-Erk in primary cultures of enteric neurons and glial cells from rat colonic submucosal enteric plexus (18). In contrast, GLP-2 did not increase cAMP levels in rat colonic myenteric neuron cultures (18). GLP-2 infusion in pigs increased jejunal eNOS expression in extracts from gut mucosa (16). GLP-2 also increased VIP expression within the rat ileal and colonic submucosal plexus through mechanisms sensitive to rapamycin and PD-98059 (18). Furthermore, GLP-2R

Figure 1

Analysis of the sensitivity and specificity of representative GLP-2 receptor (GLP-2R) antisera. (*a*) Western blot analysis with the indicated commercial GLP-2R antibodies of whole-tissue extracts (40 μg protein/lane) from jejunum, colon, liver, and kidney of two *Glp2r−/−* mice and two wild-type littermates. Molecular mass standards are indicated at the right. (*b*) BHK cells were left untransfected (lane 5) or were transiently transfected with the vector pcDNA3.1 alone (lane 1) or with the mouse (lane 2), rat (lane 3), or human (lane 4) *Glp2r* cDNA cloned into pcDNA3.1. Whole-cell extracts (40 μg protein/lane) were prepared 48 h after transfection and analyzed by immunoblotting with the indicated commercial GLP-2R antibodies. A rabbit polyclonal antibody against neomycin phosphotransferase II (NPTII, ∼26 kDa), encoded by pcDNA3.1, was used to assess the efficiency of the transfection. (*c*) Whole-cell extracts (450 μg protein) from BHK cells transfected with pcDNA3.1 (lane 1) or with an N-terminal FLAG epitope–tagged human *GLP2R* cDNA cloned into pcDNA3.1 (lane 2) were immunoprecipitated with an agarose-conjugated anti-FLAG M1 antibody. Immune complexes were next analyzed by Western blotting by using the indicated commercial GLP-2R antibodies and a previously characterized rabbit polyclonal GLP-2R antibody (ALX427). The ALX427 antiserum detected two immunoreactive GLP-2R proteins of ∼65 and ∼80 kDa, corresponding to differently glycosylated species of the GLP-2R. The human and rodent *Glp2r* cDNAs are predicted to encode proteins with a molecular mass of ∼53 kDa after signal peptide cleavage at a predicted consensus cleavage site. However, the mature GLP-2R proteins are ∼20 kDa larger because of posttranslational glycosylation (8, 12, 13, 17, 24).

signaling responses, including induction of VIP expression, were markedly attenuated in primary cultures from the ileal submucosal ganglia of *Pi3kγ−/−* mice (18). A small body of literature supports the existence of GLP-2-activated signaling mechanisms in cells that do not express the known GLP-2R. Whether these findings are attributable to smaller bioactive peptides derived from GLP-2 and/or to the existence of GLP-2R-independent signaling mechanisms requires further investigation. The diversity of GLP-2R signaling pathways and targets identified to date likely reflects differences in the experimental models, paradigms, and cell types under analysis.

Short-bowel syndrome (SBS): a clinical condition characterized by loss of fluids, electrolytes, and energy malabsorption arising in human subjects after resection of considerable amounts of the small bowel, with or without concomitant resection of the large bowel

GLP-2 ACTIONS WITHIN THE GASTROINTESTINAL TRACT

The first-described actions for GLP-2 encompassed the rapid induction of intestinal hexose transport (31) and the stimulation of crypt cell proliferation. Increased cell proliferation promotes expansion of the mucosal epithelium in the small and large intestines (32). GLP-2 also exerts antiapoptotic actions in the normal intestine and injured intestine, enhances barrier function, and selectively increases blood flow to the gastrointestinal tract (**Figure 2**).

Blood Flow

GLP-2 rapidly increased visceral blood flow and portal blood volume in pigs receiving parenteral nutrition (PN), whereas coinfusion of L-NAME (NG-nitro-L-arginine methyl ester) eliminated the GLP-2-stimulated induction of blood flow (33), consistent with the importance of nitric oxide as a downstream target for GLP-2 action (16). The induction of blood flow was significant in the pancreas and proximal gut (the duodenum and jejunum) and more prominent in the serosa versus the mucosa, whereas GLP-2 failed to increase blood flow in the porcine ileum or colon (34). Native GLP-2 also rapidly increased mesenteric blood flow in healthy human subjects (35), as well as in subjects with short-bowel syndrome (SBS), end-jejunostomy, and less than 200 cm of residual small bowel (36). The GLP-2-stimulated increases in blood flow were proportional to the length

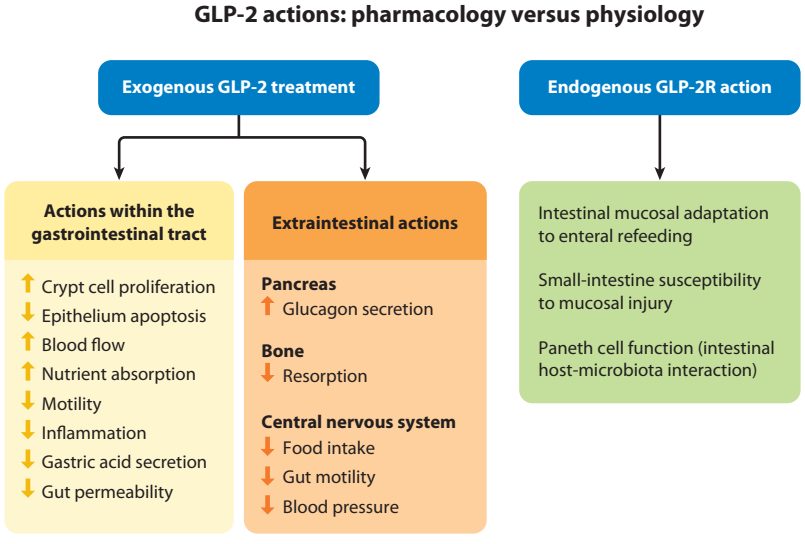


Figure 2

Pharmacological and physiological mechanisms of GLP-2 action.

of residual intestine. GLP-2 infusion in neonatal pigs increased intestinal blood flow, upregulated intestinal eNOS mRNA and protein, and enhanced intestinal eNOS phosphorylation at Ser1117. Whether induction of eNOS activity is always required for the GLP-2-dependent stimulation of blood flow remains uncertain (16).

Nutrient Absorption

Activation of GLP-2R signaling stimulates nutrient absorption and crypt cell proliferation and reduces apoptosis, leading to the rapid expansion of a proabsorptive mucosal epithelium (32, 37). Conversely, the absence of intestinal nutrients in rodents is associated with the rapid development of mucosal hypoplasia; infusion of GLP-2 reverses intestinal hypoplasia in the small bowels of parenterally fed rats in the absence of enteral nutrients, implying that GLP-2 may be the signal linking luminal nutrients to control of mucosal proliferation and apoptosis (38). The intestinotrophic actions of exogenous GLP-2 were also associated with the suppression of protein degradation in the small-bowel mucosa of parenterally fed pigs (39). Furthermore, the endogenous GLP-2R is essential for the mucosal adaptation to enteral refeeding, as mucosal regrowth was attenuated in wild-type mice administered the antagonist GLP-2(3–33) (12), and *Glp2r*−/− mice exhibited defective crypt cell proliferation and failed to significantly expand the small-bowel mucosal epithelium following refeeding (40) (**Figure 2**).

GLP-2 increases absorption of carbohydrates, amino acids, and lipids. The GLP-2-stimulated increases in nutrient absorption may be independent of simultaneous increases in blood flow; however, coadministration of the nonselective NOS inhibitor L-NAME eliminated GLP-2 induction of both blood flow and glucose uptake (33). Notably, GLP-2 augmented carbohydrate absorption in experimental models of small-bowel resection (41). GLP-2 rapidly augments the uptake of lipids and enhances triglyceride-rich-chylomicron secretion from the gut mucosa in mice through mechanisms requiring functional CD36 expression (42). Acute administration of GLP-2 also increased plasma triglyceride and free-fatty-acid levels in healthy human subjects during a test meal (43).

Gut Motility and Acid Secretion

Pharmacological levels of GLP-2 inhibit gastrointestinal motility and gastric acid secretion in rodents, pigs, and humans when infused at doses that achieve circulating GLP-2 levels that are generally higher than those obtained after meal ingestion. The effect of GLP-2 to inhibit small-bowel motility in rats and gastric emptying in humans is less potent than that observed with comparable infusions of GLP-1, and the inhibitory effects of GLP-2 and GLP-1 on gastric emptying are not additive (44). Although GLP-2 promotes smooth muscle relaxation and modulates electrical activity in the small bowel and colon, possibly through inhibition of cholinergic activity (45), the physiological relevance of endogenous GLP-2R signaling to small- and large-bowel motility is uncertain. Gastric emptying is accelerated in mice with targeted deletion of *Glp2r* in neurons expressing proopiomelanocortin (46), but whether neuronal GLP-2R activity is essential for the inhibitory effects of GLP-2 on motility in the stomach, small bowel, or large bowel has not been delineated.

Gut Permeability

GLP-2 rapidly decreases gut permeability and increases intestinal barrier function in normal rodents and in preclinical models of gut injury. GLP-2 reduces ionic conductance across the gut

epithelium and decreases transcellular migration of larger molecules such as horseradish peroxidase. Ultrastructural features evident following GLP-2R activation in mice include increased enterocyte length and a greater number of microvilli (47). The actions of GLP-2 to reduce gut permeability have also been demonstrated in experimental models of acute pancreatitis (48). The mechanisms recruited by GLP-2R signaling that rapidly reduce gut permeability have not been identified, and direct addition of GLP-2 to isolated intestinal segments has little or no effect on ionic conductance or barrier function. Pharmacological GLP-2 administration reduced gut permeability in *ob/ob* mice, whereas acute transient interruption of GLP-2R signaling using GLP-2(3–33) deteriorated gut barrier function, suggesting that modulation of GLP-2R signaling represents an important determinant of intestinal permeability (49). In contrast, systemic markers of inflammation and gut permeability were not enhanced in *ob/ob*:*Glp2r*−/− mice (50), and chronic administration of h[Gly^2]-GLP-2 for several months to nonobese diabetic mice, a model of type 1 diabetes that exhibits increased gut permeability, improved gut barrier function but failed to modify the incidence of type 1 diabetes (51). Similarly, gut permeability appears normal in *Glp2r*−/− mice (52). GLP-2 also reduces bacterial translocation in experimental models of gut injury or sepsis (48, 52, 53); whether this observation reflects direct effects on barrier function, augmentation of immune responses, or maintenance/restoration of epithelial integrity is challenging to resolve. Hence the significance of the GLP-2R for physiological and pharmacological control of intestinal barrier function remains uncertain.

EXTRAINTESTINAL ACTIONS OF GLP-2

Endocrine Pancreas

Exogenous GLP-2 administration to achieve plasma levels of more than 400 pmol/L rapidly increased plasma glucagon levels (**Figure 2**) without changing glucose levels in normal humans during both the fasting and postprandial states (43); the stimulatory effects of GLP-2 on glucagon levels were preserved in subjects with type 1 diabetes (54) but were diminished in human subjects with type 2 diabetes (55). GLP-2 infusion directly increased glucagon secretion from the perfused rat pancreas, consistent with a possible role for GLP-2Rs in islet α cells coupled to the control of glucagon secretion. Indeed, a 142-bp *Glp2r* cDNA product was generated by polymerase chain reaction in RNA from rat islets, and strong GLP-2R immunopositivity was detected by immunocytochemistry in islet α cells of rats and humans (56). In contrast, GLP-2 did not increase plasma glucagon levels under conditions of high or low glucose in mice or in isolated murine islets in vitro, and a full-length *Glp2r* mRNA transcript was not detected in RNA from murine islets (50). Furthermore, genetic disruption of the murine *Glp2r* is not associated with changes in fasting glucose, glucose tolerance, or plasma glucagon levels (50). Changes in glucagon levels or glycemia were not reported following acute or chronic GLP-2 or teduglutide administration in patients with SBS. Hence the importance of endogenous or exogenous GLP-2R signaling for the control of glucagon secretion and glucose homeostasis in different species requires further clarification.

Bone

Bone resorption increases in the fasted state, whereas meal ingestion is associated with reduction of bone resorption in rodents and humans, implicating a role for one or more gut-derived factors in bone remodeling. A 5-week course of 400 μg of native GLP-2 administered twice daily to human subjects with SBS significantly increased spinal bone mineral density, intestinal calcium absorption rates, and levels of plasma ionized calcium (57). Furthermore, a single subcutaneous

injection of GLP-2 in the morning reduced markers of bone resorption in plasma [serum C-terminal telopeptides of type 1 collagen (CTX)] and urine [deoxypyridinoline (DPD)], with no effect on markers of bone resorption (osteocalcin) in fasting postmenopausal women (58). Administration of GLP-2 in the evening to healthy postmenopausal women also reduced the nocturnal increase in serum CTX and produced modest but nonsignificant increases in osteocalcin levels (59). However, sustained GLP-2 administration (1.6 or 3.2 mg daily at 10 PM) to postmenopausal women for 14 days revealed persistent suppression of bone resorption [reduction in serum CTX but no effect on osteocalcin and PINP (type 1 procollagen N-terminal propeptide), markers of bone formation] (60). A longer (4-month), randomized, double-blind, placebo-controlled trial of GLP-2 administration in postmenopausal women (with doses ranging from 0.4 to 3.2 mg daily in the evening) demonstrated a persistent reduction in bone resorption (serum CTX) that was sustained at day 120 and a significant dose-dependent increase in total hip bone mineral density, but no change in spine bone mineral density or changes in markers of bone formation were detected (61). The precise mechanisms and cellular targets through which GLP-2 inhibits bone resorption require further elucidation.

ErbB superfamily: the group of structurally related membrane-spanning receptors, including the epidermal growth factor receptor, that transduce one or more signals linking GLP-2R activation to the promotion of intestinal growth

Central Nervous System

Intracerebroventricular administration of GLP-2 in rodents elicited a broad range of actions, including inhibition of food intake, preservation of cell survival, reduction of gut motility, and decreases in blood pressure (25, 46, 62) (**Figure 2**). In contrast, peripheral subcutaneous or intravenous GLP-2 administration to humans did not inhibit food intake or gastric emptying and had no significant effect on heart rate or blood pressure. GLP-2 regulates ion channel activity and stimulates astrocyte proliferation in vitro, but whether endogenous or exogenous central-nervous-system GLP-2R signaling modulates proliferative or cytoprotective actions in neural cells in vivo requires additional investigation.

MOLECULAR MEDIATORS OF GLP-2 RECEPTOR SIGNALING IN THE GASTROINTESTINAL TRACT

Keratinocyte Growth Factor

Multiple growth factors have been identified as targets of GLP-2 action, including keratinocyte growth factor (KGF) (15), IGF-1 (63), and the ErbB superfamily of ligands (64) (**Figure 3**). GLP-2R expression was localized to subepithelial myofibroblasts in the rat, marmoset, and human small and large intestines by immunocytochemistry and was colocalized with a surprisingly large number of cells immunopositive for KGF (15). Coadministration of GLP-2 and immunoneutralizing antisera against KGF demonstrated near complete attenuation of GLP-2-stimulated colonic growth in mice; in contrast, GLP-2 robustly increased the mass of the murine small bowel independently of concomitant KGF immunoneutralization (53). Whether GLP-2 increases KGF gene expression or circulating or intestinal levels of bioactive KGF remains unknown.

Insulin-Like Growth Factor 1

The observation that exogenous GLP-2 or IGF-1 increased nuclear β-catenin localization and c-*myc* and *sox-9* mRNA expression in the murine small bowel, together with attenuation of GLP-2 induction of nuclear β-catenin expression by concomitant administration of the IGF-1 receptor kinase inhibitor NVP-AEW541, implicated a role for IGF-1 as a downstream target of

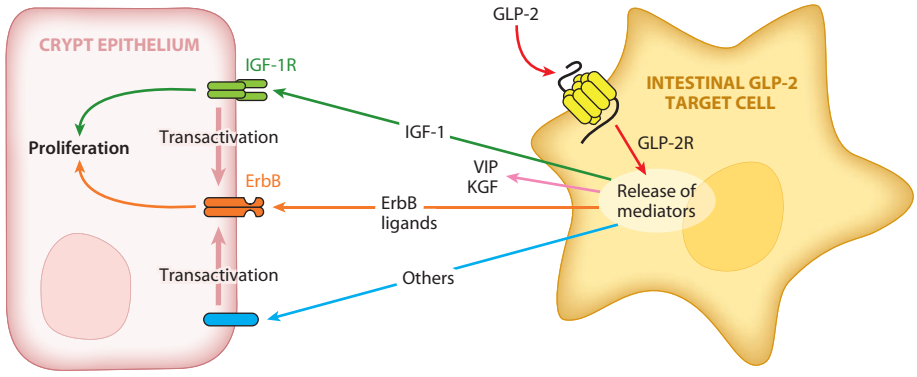


Figure 3

Key growth factor pathways transducing the intestinotrophic actions of GLP-2. IGF-1R and ErbB signaling mediates the intestinotrophic response to GLP-2. Abbreviations: KGF, keratinocyte growth factor; IGF-1, insulin-like growth factor 1; VIP, vasoactive intestinal peptide.

GLP-2 action (63) (**Figure 3**). Furthermore, GLP-2 directly increased levels of *Igf1* mRNA and immunoreactive IGF-1 protein in fetal rat intestinal cell cultures, and the intestinotrophic action of GLP-2 was attenuated in the small bowels of *Igf1*−/− mice (30, 63). The actions of GLP-2 to induce Akt phosphorylation in murine crypt cells were attenuated by NVP-AEW541 but were preserved in *Igf1*−/− mice, suggesting that factors other than IGF-1 contribute to GLP-2-mediated Akt activation (30).

Murali et al. (65) examined the GLP-2–IGF-1 axis in mice with combined inactivation of the IGF-binding protein (IGFBP)3 and 5 genes. *Igfbp3/5* knockout mice fail to exhibit a proliferative response to IGF-1 in the mouse jejunum (65). Nevertheless, GLP-2 significantly increased crypt cell proliferation, villous height, and crypt depth in *Igfbp3/5*−/− mice, indicating that IGF-1 action is not universally required for the intestinotrophic properties of GLP-2. Similarly, exogenous GLP-2 increased crypt depth, villus height, small-bowel weight, and mucosal cross-sectional area independently of any changes in crypt cell proliferation in the jejuna of mice with conditional deletion of the *Igf1r* from intestinal epithelium (66). However, the GLP-2-dependent induction of c-*myc* and nuclear β-catenin expression was attenuated in intestinal epithelium–specific *Igf1r*−/− mice. Hence IGF-1/IGF-1R signaling is required for a component of GLP-2 action in the gastrointestinal tract (**Figure 3**).

Epidermal Growth Factor and ErbB Ligands

Acute GLP-2 administration rapidly induced the expression of multiple ErbB ligands in the mouse jejunum (and colon), including mRNA transcripts for *amphiregulin*, *epiregulin*, *epigen*, *Hb-egf*, and *neuregulin-1* (64). In contrast, neither IGF-1 nor KGF increased the expression of ErbB ligands in the small or the large bowel. Furthermore, both EGF and GLP-2 produced similar induction of immediate early genes such as c-*fos*, *Egr-1*, and *Phlda-1* and proteins such as p-Erk1/2 and p-Akt in both the small and the large bowel, although the effects of EGF were more robust and rapid (64). Whereas some ErbB ligands such as TGF-α require processing by metalloproteinases prior to activation, coadministration of GM6001, a broad-spectrum matrix metalloproteinase inhibitor, did not abrogate the GLP-2-stimulated induction of ErbB ligands or immediate-early-gene expression. The intestinotrophic actions of GLP-2 were preserved in the *Egfr*$^{wa2/wa2}$ mouse and in mice coadministered EGFR inhibitors (64, 67, 68); hence the EGFR is not the primary

ErbB target for GLP-2 action. In contrast, the pan-ErbB inhibitor CI-1033 markedly attenuated the intestinotrophic actions of GLP-2 in the small bowel and colon (64).

Studies of refeeding-induced mucosal proliferation revealed the importance of ErbB signaling for the actions of endogenous GLP-2. The adaptive mucosal proliferative response to refeeding was markedly impaired in the proximal small bowels of *Glp2r−/−* mice, and exogenous administration of EGF, but not that of IGF-1, reversed the mucosal hypoplasia in the jejuna of refed *Glp2r−/−* mice (40). Consistent with a role for ErbB ligands in the transition from fasting to refeeding, levels of *amphiregulin*, *epiregulin*, *Hb-egf*, *Phlda-1*, and c-*fos* mRNA transcripts and p-Akt protein were induced in the guts of refed mice via mechanisms sensitive to ErbB inhibition via CI-1033 (40). Hence the endogenous GLP-2R controls the mucosal adaptation to refeeding via the activity of one or more ErbB ligands (**Figure 3**).

Similarly, GLP-2 rapidly and directly increased expression of mRNA transcripts encoding the *Egfr*, *ErbB2*, *ErbB3*, and *ErbB4* receptors and expression of mRNA transcripts for the cognate ligands encoded by *EGF*, *amphiregulin*, *epiregulin*, and *Hb-egf* in primary cultures from the rat submucosal enteric plexus (18). The identity of the key ErbB-related targets essential for the pleiotropic actions of GLP-2 in the gut requires further assessment.

GLP-2 ACTION IN EXPERIMENTAL MODELS OF GUT INJURY

GLP-2 and Preclinical Models of Cancer

Although sustained administration of GLP-2 does not appear to increase cancer incidence in non-sensitized preclinical models, prolonged GLP-2R activation in rodents pretreated with chemical carcinogens increases the size and number of intestinal polyps. Thulesen et al. (69) treated mice twice daily with 25 μg of native GLP-2 or h[Gly2]-GLP-2, following initiation of colonic tumor formation by using the carcinogen 1,2-dimethylhydrazine. Native GLP-2 administration for 1 month increased the number of small polyps, whereas h[Gly2]-GLP-2 treatment increased the number of small and large polyps. Histologically, the polyps were nonmalignant tubular adenomas. Similarly, administration of h[Gly^2]-GLP-2, at 1.5 μg twice daily for 4 weeks, to mice with azoxymethane (AOM)-induced aberrant crypt foci (ACF) significantly increased the numbers of ACF, whereas treatment with the GLP-2R antagonist GLP-2(3–33) reduced the development of ACFs (70). Notably, two mice developed intramucosal colonic tumors with histological features consistent with colonic adenocarcinoma.

Trivedi et al. (71) assessed the consequences of GLP-2R agonism in rats and mice with inflammation-associated colonic dysplasia and cancer. Rats were treated with a high-fat diet and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), and mice were administered dextran sulfate and treated with AOM. Increased numbers of ACF and colon cancers were detected in PhIP-treated rats that were administered h[Gly^2]-GLP-2. The number of colonic lesions exhibiting high-grade dysplasia was increased in mice with colitis following AOM administration and decreased following treatment with GLP-2(3–33) (71). In contrast, treatment of nude mice with implanted subcutaneous human colon cancers that expressed the human GLP-2R with 5 μg of GLP-2 twice daily for 6 weeks had no effect on tumor growth (72). Similarly, administration of 5 μg of GLP-2 daily for 7 weeks to $Apc^{Min+/-}$ mice did not increase the number or size of intestinal tumors, and genetic inactivation of the endogenous *Glp2r* in $Glp2r^{-/-}$:$Apc^{Min/+}$ mice did not modify tumor number, size, or location (72). Hence the ability of exogenous or endogenous GLP-2R signaling to modify the growth of preexisting intestinal tumors depends on the precise experimental model used.

Table 1 Preclinical models of intestinal disorders exhibiting amelioration in response to GLP-2[a]

■ TPN-induced gut hypoplasia
■ Major small-bowel resection and short-bowel syndrome
■ Colonic injury (dextran sulfate colitis)
■ Indomethacin-induced enterocolitis
■ TNBS-induced enteritis
■ Infectious enteritis
■ Antigen-induced inflammatory bowel disease
■ Burn- and radiation-induced enteritis
■ Vascular intestinal ischemia
■ Chemotherapy-induced mucositis
■ Immune-mediated hypersensitivity injury
■ Sepsis-induced mucosal dysfunction and reduced barrier function

[a]Abbreviations: TNBS, trinitrobenzene sulfonic acid; TPN, total parenteral nutrition.

CHEMICAL, ISCHEMIC, AND RADIATION INJURY IN THE GASTROINTESTINAL TRACT

Activation of GLP-2R signaling protects the small and large bowels in a broad spectrum of experimental models of intestinal injury (**Table 1**). GLP-2R agonists have been administered both prior to and after chemotherapy and chemical injury; the degree of mucosal protection was highly dependent on the specific model and timing of GLP-2 administration. Administration of h[Gly^2]-GLP-2 after a single injection of 5-fluorouracil (5-FU) improved villus height and crypt depth in rats, whereas administration of h[Gly^2]-GLP-2 for 6 days prior to and 3 days following 5-FU injection was less effective in preserving the crypt–villus axis (73). Similarly, Boushey and colleagues (53) demonstrated that administration of h[Gly^2]-GLP-2 for 3 days prior to and several weeks after administration of either 5-FU or irinotecan (IRT) resulted in significantly greater survival rates relative to survival rates of mice treated with chemotherapy alone. Enhanced survival was also detected in a subset of tumor-bearing mice treated with sequential courses of IRT and h[Gly^2]-GLP-2 for 3 weeks. h[Gly^2]-GLP-2 reduced bacterial septicemia, preserved gut histology, and decreased crypt compartment apoptosis in mice concomitantly treated with IRT (53). Similarly, a DPP-4-resistant GLP-2 analog (NNC103-0066) preserved villus height and intestinal weight and reduced apoptosis in the crypt compartment of mice treated with erlotinib and cisplatin (68).

As discussed above, GLP-2 augments intestinal blood flow in the absence of vascular injury. Several studies have examined the cytoprotective and regenerative properties of GLP-2 in the setting of ischemic intestinal injury. Administration of GLP-2 for 3 days by intravenous infusion after acute transient superior mesenteric artery (SMA) occlusion preserved mucosal DNA and protein content in the rat small bowel (74). Intestinal damage and bacterial translocation were also attenuated when GLP-2 [0.25 mg/(kg•day)] was given for 3 days prior to SMA occlusion in mice (75). Whether GLP-2 can preserve blood flow in atherosclerotic or inflamed blood vessels has not been tested.

Exogenous GLP-2 also reduces the severity of intestinal inflammation in the small and large bowels (**Figure 2**). h[Gly^2]-GLP-2 administration preserved mucosal histology, integrity, and colon length; decreased expression of inflammatory cytokines; and reduced weight loss in both CD1 and BALB/C mice with dextran sulfate colitis (76). Similarly, h[Gly^2]-GLP-2–treated mice

with dextran sulfate colitis displayed reduced intestinal damage, decreased disease activity scores, decreased weight loss, and improved survival, but the protective effects of GLP-2 were markedly attenuated by concomitant administration of methylprednisolone (77). h[Gly^2]-GLP-2 also enhanced survival, reduced intestinal injury, stimulated crypt cell proliferation, attenuated crypt compartment apoptosis, and decreased bacterial translocation in mice with indomethacin-induced small-bowel enteritis (78). GLP-2 had a therapeutic effect when administration was commenced prior to or after induction of gut injury with indomethacin and was associated with reduced expression of TNF-α, IL-2, IFN-γ, and IL-10 in the small bowel (78). Similarly, GLP-2 2G-XTEN, a long-acting GLP-2R agonist, improved body weight, decreased intestinal ulceration, preserved small-bowel mucosal histology, and reduced levels of TNF-α in rats with indomethacin-induced enteritis (79). Administration of GLP-2 prior to γ-radiation-induced intestinal injury also preserved epithelial mass and increased crypt stem cell survival in mice and rats (80, 81).

Major small-bowel resection (MSBR): an experimental preclinical model employing intestinal resection that is commonly used to mimic elements of short-bowel syndrome

The mechanisms mediating the anti-inflammatory actions of GLP-2 are likely indirect, either through maintenance of gut barrier integrity or through induction of anti-inflammatory mediators. GLP-2R expression or action has not been demonstrated in immune cells from bone marrow, spleen, thymus, or lymph nodes (51). Although IL-10 is not required for the therapeutic and anti-inflammatory actions of GLP-2 in the gut (82), Sigalet and colleagues (83) identified VIP as an important mediator of the anti-inflammatory effects of GLP-2. The anti-inflammatory actions of GLP-2 (reduced intestinal cytokine expression and decreased myeloperoxidase activity) in a rat model of trinitrobenzene sulfonic acid (TNBS)-induced colitis were attenuated by coadministration of the VIP hybrid antagonist Lys(1)-Pro(2,5)-Arg(3,4)-Tyr(6)VIP(7–28) (83); conversely, GLP-2 increased the number of VIP+ neurons in the ileal submucosal plexus. The anti-inflammatory actions of GLP-2 were more apparent when GLP-2 administration was commenced after the induction of gut injury. GLP-2 [0.05 mg/(kg·day)] selectively preserved the number of VIP+ neurons but had no effect on neuronal NOS, neurons, or glial cells in intestinal ganglia from rats with TNBS-induced colitis (84). GLP-2 also increased the expression of neuronal markers in cells cultured from the submucosal plexus ex vivo; such increases occurred through mechanisms sensitive to the inhibition of PI3K, mTOR, or Erk1/2 (18). Furthermore, enteric neuronal preparations isolated from *Pi3kγ−/−* mice failed to upregulate VIP expression in response to GLP-2 administration for 7 days ex vivo, yet exhibited a marked induction of mRNA transcripts for the ErbB ligands encoded by *amphiregulin*, *epiregulin*, and *Hb-egf* and for the *Egfr*, *ErbB2*, *ErbB3*, and *ErbB4* receptors (17). Nevertheless, VIP is not required for the GLP-2-dependent induction of ErbB ligands or for the stimulation of intestinal growth in mice (85).

Major Small-Bowel Resection

Endogenous circulating GLP-2 levels increase following major small-bowel resection (MSBR) (86), independently of the presence or absence of enteral nutrients (87). Scott and colleagues (41) administered h[Gly^2]-GLP-2 twice daily [0.1 mg/(kg·day)] for 21–40 days in rats after a 75% resection of the mid-small bowel. Teduglutide significantly increased parameters of adaptation (crypt + villus height, mucosal weight, xylose absorption) in the proximal jejunum, but not in the ileum (41). The actions of GLP-2 (maintenance of barrier function, augmentation of crypt cell proliferation and crypt + villus height, SGLT-1 expression) to enhance intestinal adaptation occurred independently of enteral feeding in parenterally fed rats following MSBR (88). Nevertheless, body weight, mucosal DNA and protein content, and villus height were greater after MSBR in rats that received both enteral nutrition and GLP-2 [0.1 mg/(kg·day) for 7 days] than in rats that received nutrition or GLP-2 alone (89). Interestingly, GLP-2 increased proglucagon (*Gcg*) gene expression

in the colons of parenterally fed rats after MSBR, implying that GLP-2 may upregulate its own expression (89). Similarly, increased levels of *Gcg* and *Glp2r* mRNA transcripts were observed in the ilea of enterally fed rats after 7 days of continuous GLP-2 [0.1 mg/(kg·day)] infusion (90). Perez and colleagues (91) examined the importance of endogenous GLP-2 for intestinal adaptation in rats after MSBR with and without concomitant administration of anti-GLP-2 antisera that commenced immediately after surgery and continued for 7 days. GLP-2 immunoneutralization reduced villus height and crypt depth and decreased the number of proliferating crypt cells. Continuous GLP-2 infusion [0.1 mg/(kg·day)] reduced mortality in rats after MSBR; consistent with the short biological half-life of GLP-2 and the rapid turnover of the gut epithelium, cessation of exogenous GLP-2 administration reversed the beneficial effects on protein and DNA content and rates of apoptosis in the remnant rat intestine after MSBR (92). Although most preclinical MSBR studies have shown beneficial effects of exogenous GLP-2 on intestinal adaptation, administration of hGLP-2 (1.6 mg/day for 7 days, then 0.8 mg/day for 5 weeks) to 4-week-old piglets after MSBR resulted in significantly decreased weight gain, independent of changes in food intake, compared with control pigs fed an enteral diet (93). Furthermore, GLP-2 had no effect on villus height or crypt depth in control animals or after MSBR. Hence further study of GLP-2 action in young pigs is required to explain why these results differ substantially from observations in rodents and from studies demonstrating enhanced nutrient absorption, stimulation of crypt cell proliferation, and inhibition of apoptosis in the porcine small bowel after GLP-2 administration (94).

Intestinal diversion carried out for the treatment of severe obesity, with or without concomitant diabetes, is associated with increased delivery of incompletely digested nutrients to the distal small bowel and increased secretion of GLP-1, GLP-2, and related gut peptides. Small-bowel hyperplasia has been observed in some preclinical models of intestinal bypass (95), raising the possibility that one or more gut peptides, including GLP-2, contribute to adaptive intestinal growth in this context. Paradoxically, although enhanced GLP-2 action would be predicted to augment nutrient absorption, bariatric surgery is universally associated with weight loss. Thus, the role of GLP-2 in the control of energy absorption in this setting requires more careful analysis.

PHYSIOLOGICAL ACTIONS OF ENDOGENOUS GLP-2 RECEPTOR SIGNALING

In contrast to the large number of pharmacological actions ascribed to activation of the GLP-2R, much less is known about the importance of basal GLP-2R signaling (**Figure 2**). Studies of GLP-2 physiology have employed immunoneutralizing antisera, the GLP-2R peptide antagonist GLP-2(3–33), and *Glp2r*−/− mice. Immunoneutralization of endogenous GLP-2 by administration of polyclonal GLP-2 antisera for 13 days attenuated the increase in cross-sectional area of the small-bowel mucosa without changes in the small-bowel weight of rats with streptozotocin-induced diabetes (96). However, no differences in small-bowel weight or crypt plus villus height were detected in *Glp2r*−/− mice with experimental diabetes or in *ob/ob*:*Glp2r*−/− mice (50). Ishizuka et al. (97) administered an anti-GLP-2 antiserum to 3-week-old weanling rats every other day for 2 weeks and detected reductions in body weight gain, small- and large-bowel weight, and small-bowel length and a significant decrease in the number of BrdU+ cells in the distal ileum.

Studies of wild-type mice and rats administered GLP-2(3–33) and *Glp2r*−/− mice have demonstrated the importance of basal GLP-2R signaling for the adaptive mucosal response to refeeding. Shin et al. (12) demonstrated that GLP-2(3–33) attenuated the increases in small-bowel weight, crypt + villus height, and crypt cell proliferation and blocked the reduction of apoptotic villus tip cells in the murine small bowel. GLP-2(3–33) also reduced small-bowel weight and crypt plus

villus height and increased villus tip apoptosis in randomly fed mice (12). Similarly, GLP-2(3–33) partially attenuated mucosal growth and decreased plasma levels of IGF-1 in refed rats (98). Consistent with these findings, refeeding did not increase small-bowel weight, crypt cell proliferation, or villus cell number in *Glp2r−/−* mice, and basal levels of mRNA transcripts for *Egf*, *Kgf*, and *Igf1r* were significantly lower in fasted *Glp2r−/−* mice (40).

Despite the actions of exogenous GLP-2 to increase small- and large-bowel mucosal mass, *Glp2r−/−* mice develop normally and exhibit normal small- and large-bowel weight, mucosal histology, small-bowel permeability, and normal numbers and localization of differentiated cell types in the small- and large-bowel epithelium (52). Nevertheless, these mice fail to exhibit induction of growth factor gene expression or small- and large-bowel growth in response to exogenous GLP-2 administration. Although the extent of large-bowel injury following dextran sulfate administration was similar in *Glp2r−/−* versus *Glp2r+/+* mice, loss of the GLP-2R conferred enhanced susceptibility to small-bowel injury and greater levels of septicemia following indomethacin administration. Furthermore, levels of small-bowel bacterial colonization were significantly higher, expression of Paneth cell gene products was reduced, and intestinal bactericidal activity was impaired in *Glp2r−/−* mice. Taken together, these findings reveal essential roles for GLP-2R signaling in adaptive mucosal growth, resistance to small-bowel injury, and microbial-mucosal defense mechanisms in mice (**Figure 2**).

SHORT-BOWEL SYNDROME IN HUMAN SUBJECTS

Native GLP-2 enhances energy absorption in human subjects with SBS (**Figure 4**). Energy balance was assessed in nine human SBS patients treated with placebo, intravenous GLP-1, and/or intravenous GLP-2; 1 pmol(kg·min) was administered for 72 h. Both GLP-1 and GLP-2 reduced excretion of fecal wet weight and decreased energy loss, but GLP-2 or GLP-1 alone only modestly enhanced energy absorption (99); the combination of GLP-1 plus GLP-2 was most effective in enhancing fluid absorption. Hence, although a proabsorptive effect can be detected rapidly following native GLP-2 administration in human subjects with SBS, the optimal dosing regimen, the time required to achieve maximal efficacy, and the mechanisms contributing to the proabsorptive actions require further study.

Administration of GLP-2 (400 μg subcutaneously twice daily for 35 days) in eight subjects with small-bowel resection and no residual colon significantly reduced gastric emptying and stomal output, improved intestinal energy absorption, and increased lean and fat mass and weight gain, independently of changes in small-bowel transit time (100). Histological evidence for increased crypt depth and villus height was also obtained from analysis of small-bowel biopsies. Spinal and hip bone mineral density and calcium absorption were increased, and markers of bone turnover were reduced, with a significant decrease in levels of deoxypyridinoline in GLP-2-treated subjects (57).

Native GLP-2 was administered (400 μg subcutaneously three times daily) in an open-label study to 11 subjects with SBS, 7 of whom had intestinal failure requiring either parenteral fluids and/or PN, with serial analysis of nutritional balance; parenteral nutritional support was kept constant. The most consistent finding for 8 subjects who completed the study was a reduction in fecal wet weight, with no significant changes in energy intake or absorption, intestinal mucosal morphology, or bone mineral density (101). GLP-2 improved multiple components of the quality-of-life evaluation instrument in most patients, but 2 patients with Crohn's disease discontinued therapy because of progressive abdominal discomfort (102).

The therapeutic actions of teduglutide, a degradation-resistant human GLP-2 analog, have been extensively examined in human subjects with SBS. Administration of teduglutide once or twice daily for 16 days improved energy absorption and decreased energy excretion in short-bowel

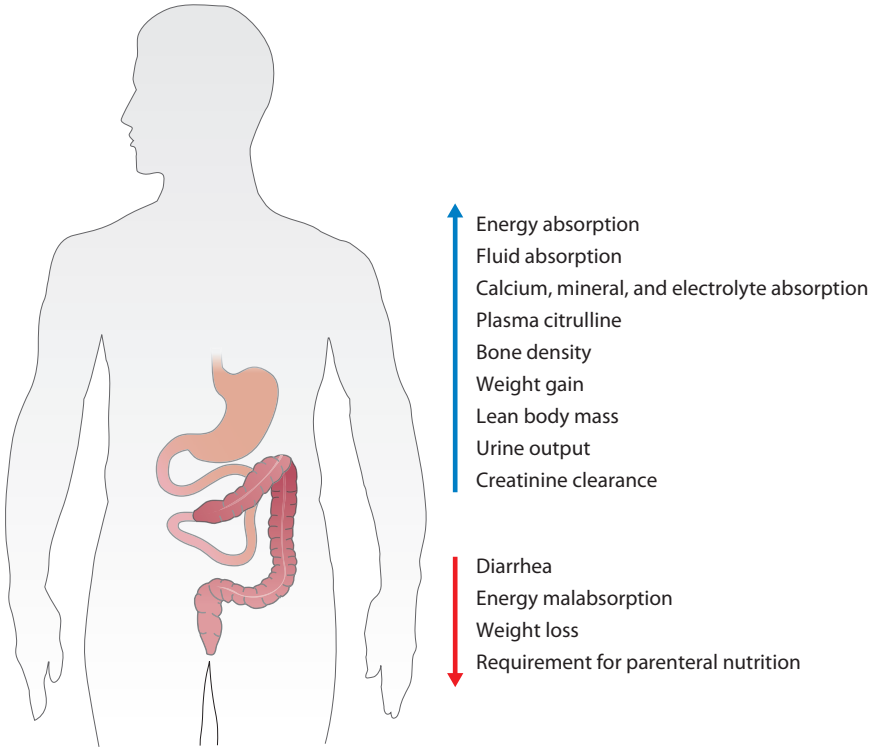


Figure 4

Actions of GLP-2 in human subjects with short-bowel syndrome.

patients; the improvements in energy absorption required ongoing teduglutide administration and were not evident 2–3 weeks following cessation of drug administration (103). A randomized, controlled clinical trial examined the efficacy of teduglutide administered to SBS patients once daily at doses of 0.05 or 0.1 mg/kg versus the efficacy of placebo over 26 weeks. Inclusion criteria included a history of stable SBS requiring intravenous fluid or PN support at least 3 times per week (104). PN weaning guidelines mandated no more than a 10% reduction in PN at 4-week intervals, with a concomitant requirement for an increase in urine volumes, consistent with maintenance of adequate hydration. Responders were defined as subjects who managed to reduce parenteral fluid requirements by 20% or more from baseline at weeks 20 and 24. Both doses of teduglutide were associated with a reduction of parenteral fluid volumes, but only the 0.05 mg/(kg·day) dose achieved a statistically significant 20% reduction in parenteral fluid requirements (104). In this experiment, 84 patients were randomized, and 83 were dosed. At the end of 24 weeks, 94%, 88%, and 77% of the placebo group, the 0.1 mg/(kg·day) group, and the 0.05 mg/(kg·day) group, respectively, completed the study. Three teduglutide-treated subjects, all of whom had been PN dependent for years prior to enrolling in the study, were completely weaned from PN. The reductions in PN were associated with reduced oral intake and increased urine volumes, consistent with maintenance or enhancement of hydration status. Teduglutide-treated subjects gained weight and exhibited increased plasma levels of citrulline and a significant increase in bone mineral content (104). Intestinal mucosal histology evaluated in a subset of patients demonstrated a significant increase in small-bowel villous height and colonic crypt depth.

The safety, tolerability, and efficacy of teduglutide were monitored for an additional 28 weeks in a blinded extension study of the same groups of subjects randomized to receive treatment with 0.05 or 0.1 mg/(kg·day) for an initial 24 weeks. In this study, 52 out of 56 subjects agreed to continue on teduglutide after completing the first 24-week study, and 43 completed a 1-year course of teduglutide (105). The most commonly reported treatment-associated adverse events were headache, nausea, vomiting, abdominal pain, nasopharyngitis, catheter sepsis, and urinary tract infection. Abdominal pain and injection site complaints were also common, as were reports of stomal site reaction or hypertrophy (105). Seven patients discontinued teduglutide because of persistent abdominal complaints. A progressive reduction in PN volume over 52 weeks was achieved in the teduglutide-treated subjects, most notably in the group receiving 0.05 mg/(kg·day) (4.9 L/week or a 52% reduction at week 52). Of subjects treated with 0.05 mg/(kg·day) and subjects treated with 0.1 mg/(kg·day), 68% and 52%, respectively, achieved at least a 20% reduction in PN volume by week 52 (105). Four weeks after drug cessation, PN requirements increased in subjects treated with 0.05 mg/(kg·day) teduglutide, but not in the group dosed with 0.1 mg/(kg·day). Hence the efficacy of teduglutide appears to be sustained over time, with many patients continuing to reduce PN requirements with ongoing treatment. Furthermore, a subset of patients, predominantly those with greater body mass index and residual small-bowel length, plus colon in continuity, can maintain a stable body mass index after discontinuation of teduglutide (106).

A second randomized, placebo-controlled clinical trial examined the efficacy of a single teduglutide dose, 0.05 mg/(kg·day), over 24 weeks in 86 subjects (43 in each arm) with SBS (107). PN was reduced if urine volumes were more than 10% greater than baseline. In this study, 63% of the teduglutide-treated group versus 30% of the placebo-treated subjects achieved at least a 20% reduction in PN volume at weeks 20 and 24. Adverse events were evenly distributed across groups, but plasma citrulline, a biomarker for intestinal mucosal mass, was significantly increased in the teduglutide-treated patients. Small-bowel length did not predict teduglutide responsivity, and a trend toward greater responsivity was observed in patients without a colon in continuity (107). At week 24, the mean PN volume reductions were 32% versus 21%, and the responder rate (20–100% reduction in PN) was 77% versus 46%, in the teduglutide- versus placebo-treated subjects, respectively. At 24 weeks, body weight was slightly increased (by 1 kg), and citrulline levels were significantly higher in subjects receiving teduglutide, whereas body weight was reduced (by 0.6 kg) in placebo-treated subjects. The most commonly reported adverse events in teduglutide-treated subjects were abdominal pain, nausea, stomal complications, and abdominal distention, and six patients developed non-neutralizing anti-teduglutide antibodies (107).

UNANSWERED QUESTIONS AND FUTURE DIRECTIONS

Prior to the description of the original biological actions of GLP-2 in 1996 (32), only a few dozen papers had described elements of GLP-2 biology, with early studies restricted largely to describing the synthesis, secretion, and circulating levels of GLP-2 in rodents and humans. Subsequently, several hundred papers have described multiple new aspects of GLP-2 biology, including metabolic, proabsorptive, anti-inflammatory, proliferative, antimicrobial, and cytoprotective actions in the gut mucosa. Surprisingly, however, despite the molecular cloning and characterization of the GLP-2R and the development of tools and mouse lines for analysis of loss of GLP-2 activity, the molecular mechanisms transducing the pleiotropic actions of GLP-2 in rare GLP-2R+ cells within the gut mucosa remain largely obscure.

Most GLP-2 actions described to date (summarized in **Figure 2**) are indirect and secondary to endocrine, paracrine, autocrine, and neural signals activated by the GLP-2R within, and possibly external to, the gut. The extraintestinal actions of GLP-2 remain even less defined, and the

importance of exogenous and endogenous GLP-2 action in the central and peripheral nervous systems remains uncertain. Many of the key actions of GLP-2 described in preclinical studies (**Figure 2** and **Table 1**) are conserved in humans, as teduglutide has demonstrated durable efficacy in enhancing energy absorption and reducing PN requirements in human subjects with SBS. Nevertheless, despite a pilot study of teduglutide action in human subjects with Crohn's disease (108), the therapeutic potential of GLP-2 action in miscellaneous human gastrointestinal disorders exhibiting pathological features modeled in many of the preclinical studies to date (**Table 1**) remains largely unexplored. Furthermore, the finding that sustained pharmacological GLP-2 administration promotes polyp and cancer cell growth in sensitized rodent models of carcinogen-induced neoplasia emphasizes the importance of careful screening and follow-up of human subjects treated with teduglutide. We have likely uncovered only a small subset of GLP-2 actions. Hence the current GLP-2 landscape will likely change substantially in the coming years as we collectively address the many unanswered questions surrounding the pleiotropic mechanisms of GLP-2 action in the gut and peripheral tissues.

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