Symposium: Glucagon-Like Peptide 2: Function and Clinical Applications

Dual Regulation of Cell Proliferation and Survival via Activation of Glucagon-Like Peptide-2 Receptor Signaling[1,2]

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ABSTRACT Peptide hormones regulate cell viability and tissue integrity, directly or indirectly, through activation of G-protein–coupled receptors via diverse mechanisms including stimulation of cell proliferation and inhibition of cell death. Glucagon-like peptide-2 (GLP-2) is a 33 amino acid peptide hormone released from intestinal endocrine cells following nutrient ingestion. GLP-2 stimulates intestinal crypt cell proliferation leading to expansion of the gastrointestinal mucosal epithelium. Exogenous GLP-2 administration attenuates intestinal injury in experimental models of gastrointestinal disease and improves intestinal absorption and nutritional status in human patients with intestinal failure secondary to short bowel syndrome. GLP-2 also promotes mucosal integrity via reduction of injury-associated apoptosis in the intestinal mucosa and directly reduces apoptosis in cells expressing the GLP-2 receptor in vitro. Hence, the regenerative and cytoprotective properties of GLP-2 contribute to its therapeutic potential for the treatment of patients with intestinal disease. J. Nutr. 133: 3708–3711, 2003.

KEY WORDS: • *glucagon-like peptide-2* • *apoptosis* • *GPCR* • *intestine*

Glucagon-like peptide 2 (GLP-2)[4] is produced in and secreted from enteroendocrine L cells following posttranslational processing of proglucagon by prohormone convertase 1/3 (1–3). Intestinal proglucagon-derived peptides (PGDP) liberated with GLP-2 include oxyntomodulin, glicentin, GLP-1 and two intervening peptides, IP-1 and IP-2 (**Fig. 1**) (4,5). GLP-2 is also produced in the brainstem. Circulating levels of GLP-2 are low in the fasting state and increase following nutrient ingestion (6–8). GLP-2 has a half-life of minutes due principally to rapid inactivation following cleavage by dipeptidyl peptidase IV in vivo (6,9,10), and in part due to renal clearance (11,12).

Stimulation of cellular proliferation by GLP-2

Small bowel villus hyperplasia detected in human patients with proglucagon- producing tumors suggests that one or more specific PGDP exhibit intestinal growth factor-like activity (13–15). GLP-2 administration to rodents increases small bowel weight and mucosal crypt and villus height (9,16–19). Subsequent studies demonstrated that GLP-2 increases intestinal nutrient absorption (17,20,21), decreases motility (22,23), enhances barrier function (18), increases mucosal hexose transport and digestive enzyme expression (17,24–26) and decreases gastric acid secretion (27). Although the small bowel mucosa is comparatively more sensitive to the trophic effects of GLP-2, the administration of degradation-resistant GLP-2 analogues or the wild-type molecule also promotes mucosal growth in the large bowel (19,28).

The lack of specific potent GLP-2 antagonists has hampered delineation of the physiological importance of endogenous GLP-2. GLP-2 (3–33) exhibits both weak antagonist and partial agonist activity (29), complicating its use for elucidation of physiological GLP-2 actions. Upregulated circulating levels of the PGDP including GLP-2, have been detected in untreated diabetic rats, and may be implicated in the genera-

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[4] Abbreviations used: BHK, baby hamster kidney; BHK-rGLP-2R, BHK cells stably transfected with the rat GLP-2 receptor; cAMP, cyclic adenosine monophosphate; CRE, cAMP-response element; GLP-2, glucagon-like peptide-2; GSK-3, glycogen synthase kinase-3; h[Gly2]GLP-2, degradation-resistant GLP-2 analogue; PGDP, proglucagon-derived peptides; PKA, protein kinase-A; PI3K, phosphatidylinositol 3-kinase.

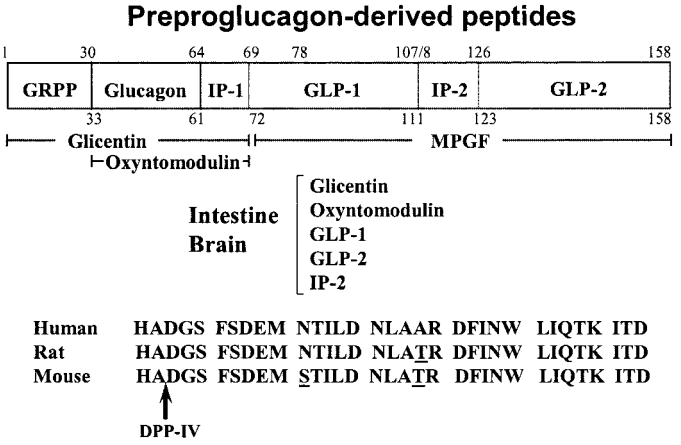

FIGURE 1 Structure of proglucagon depicting the proglucagon-derived peptides. The amino acid sequences of mouse, rat and human GLP-2. Nonconserved residues divergent from the human GLP-2 sequence are underlined. The site of cleavage at the position 2 alanine by the enzyme dipeptidyl peptidase IV (DPP-IV) is shown with an arrow.

tion of increased intestinal mass observed in diabetic rodents (30,31). Levels of circulating GLP-2 increase rapidly following rat small bowel resection (32) and immunoneutralization of GLP-2 using polyclonal antisera partially attenuates the intestinal growth response in diabetic rats (31). GLP-2 also increases intestinal weight and villus height in neonatal pigs (33). The actions of GLP-2 in the brain are less clear; although intracerebroventricular GLP-2 administration modestly inhibits food intake in rats and mice, the physiological importance of GLP-2 as an anorexic peptide is uncertain (34,35).

The increase in bowel weight and mucosal thickness following GLP-2 administration is due principally to the stimulation of crypt cell proliferation, leading to lengthening of the intestinal villi and a modest expansion of the crypt compartment (16,19,36,37). Microscopic analysis of GLP-2–treated small bowel mucosa reveals a considerable increase in the number of microvilli, providing yet another mechanism for expansion of the mucosal absorptive surface area (18). Rodents treated with GLP-2 exhibit increased DNA and protein content in the small bowel and colon (21,38) and an increased crypt cell proliferation rate in the small intestine (36). GLP-2-induced crypt cell proliferation in the large bowel has been observed in parenterally, but not orally fed rats (19). Significantly increased small bowel weight can be maintained with repeated daily subcutaneous administration of GLP-2 for at least 12 wk; cessation of peptide treatment results in normalization of bowel mass within days of peptide withdrawal (36).

The intestinotrophic actions of GLP-2 are largely indirect, consistent with the localization of GLP-2 receptors to murine enteric neurons and human enteroendocrine cells (39,40). Although the downstream mediators of GLP-2 action on growth and apoptosis remain unknown, the GLP-2–dependent stimulation of intestinal glucose uptake and blood flow is blocked by N^{ω}-nitro-L-arginine methyl ester, implicating a role for nitric oxide in the transduction of specific GLP-2 signals (41). At pharmacological doses, GLP-2 stimulates cell proliferation measured by increased [^{3}H]-thymidine incorporation in intestinal cell lines not shown to express the cloned GLP-2 receptor in vitro (42,43). Similarly, GLP-2 promotes cell proliferation in baby hamster kidney (BHK) cells stably expressing a transfected rat GLP-2 receptor (44) and in primary cultures of rat astrocytes derived from the cerebral cortex (45).

Cytoprotective properties of GLP-2

The increase in bowel mass following GLP-2 administration to normal rodents may be attributed in part to a decrease in the number of apoptotic cells in the intestinal mucosa (36). Nevertheless, the ability of GLP-2 to prevent cell death is more readily evident following induction of experimental intestinal injury. A degradation-resistant GLP-2 analogue (h[Gly2]GLP-2) (6,9,28) significantly diminishes the severity of Dextran sulfate-induced colitis in mice. h[Gly2]GLP-2-treated mice exhibit enhanced preservation of mucosal integrity accompanied by an increase in intestinal mass largely as a result of increased cellular proliferation (46). In contrast, nonsteroidal anti-inflammatory agent-induced murine enteritis is markedly attenuated via effects on both mucosal cell proliferation and reduction of cell death. h[Gly2]GLP-2 reduces the number of apoptotic cells in the crypt compartment following indomethacin administration, in association with reduced mucosal cytokine expression, decreased myeloperoxidase activity and marked diminution in bacterial translocation (47).

The development of apoptotic mucosal cell death following administration of chemotherapeutic agents can also be reduced by concomitant or prior treatment with h[Gly2]GLP-2. Mice treated with h[Gly2]GLP-2 and either irinotecan or 5′-fluoruracil exhibit increased survival, reduced histological evidence of disease and a highly significant reduction in positional crypt compartment apoptosis (48). The trophic and antiapoptotic actions of GLP-2 have also been demonstrated in rodents and pigs following withdrawal of enteral nutrition. GLP-2 infusion prevents the development of mucosal hypoplasia in the small bowel of normal and tumor-bearing rats (49,50). Similarly, GLP-2 administration to premature pigs maintained on total parenteral nutrition reduced proteolysis and crypt cell apoptosis in the small bowel (51).

The GLP-2 receptor

The GLP-2 receptor (GLP-2R) was cloned from rat and human intestinal and hypothalamic cDNA libraries and is a member of the class B glucagon-secretin–like G-protein coupled receptor superfamily (52,53). The receptor exhibits high sequence homology with related members of the superfamily including the GLP-1, glucagon and glucose-dependent insulinotropic polypeptide receptors (52). Activation of GLP-2 receptor signaling results in an increase in intracellular cyclic adenosine monophosphate (cAMP), activation of cAMP-dependent protein kinase-A (PKA), an increase in cAMP-response element (CRE) and AP-1 dependent transcription, and an increase in expression of immediate early genes (44,52). The GLP-2R is localized to a subset of human enteroendocrine cells (39), murine enteric neurons (40) and specific regions of the murine and rat central nervous system (34,35).

Signaling through the GLP-2 receptor directly inhibits cell death in transfected heterologous cell lines treated with chemical inducers of apoptosis. GLP-2 inhibits cycloheximide-induced apoptosis in a cAMP-dependent, PKA-, mitogen-activated protein kinase-, and phosphatidylinositol 3-kinase (PI3K)-independent manner in BHK cells stably transfected with the rat GLP-2 receptor (BHK-rGLP-2R) (54). GLP-2 reduces caspase-3 and -8 activation and poly(ADP-ribose) polymerase cleavage following incubation of BHK:rGLP-2R cells with cycloheximide, irinotecan or LY294002, a specific PI3-kinase inhibitor (48,54,55). GLP-2 also inhibits cycloheximide and LY294002-induced mitochondrial cytochrome c release and the caspase-dependent cleavage of β-catenin and

Akt induced by inhibition of PI3K (54,55). Similarly, GLP-2 reduces activation of glycogen synthase kinase-3 (GSK-3) and the mitochondrial association of the proapoptotic molecules Bad and Bax in BHK-rGLP-2R cells treated with LY294002 (55). Interestingly, in contrast to the PKA-independent reduction of cycloheximide-induced apoptosis, GLP-2 inhibits LY294002-induced apoptosis in a PKA-dependent manner (55), illustrating that the cytoprotective effects of GLP-2R signaling are mediated through multiple pathways depending on the apoptotic stimulus.

GLP-2 and the treatment of intestinal disease

Due in part to its cytoprotective and regenerative properties, GLP-2 and dipeptidyl peptidase IV-resistant GLP-2 analogues are currently being evaluated for the treatment of human intestinal disease. Administration of GLP-2 or h[Gly2]GLP-2 attenuates intestinal injury in diverse experimental models, including acute necrotizing pancreatitis (56), acute burn injury (57) and ischemia-reperfusion injury (58). Similarly, GLP-2 infusion decreases the severity of inflammatory bowel injury (46,47,59) or chemotherapy-induced enteritis (48,60).

In contrast to the evidence for beneficial effects of GLP-2 in experimental models of gut injury, very limited information is available about the potential therapeutic actions of GLP-2 in human subjects. Eight patients with intestinal failure secondary to short bowel syndrome were treated twice daily with subcutaneous injections of wild-type GLP-2 (for 35 d). GLP-2 treatment improved nutrient absorption, increased body weight, delayed gastric emptying and increased bone mass (61,62). Hence, the available data suggests that the proabsorptive beneficial effects of GLP-2 noted in preclinical studies may also be detected in short term studies in human subjects. Whether GLP-2 will also prove to be effective in reducing intestinal injury or enhancing gut repair in patients remains unknown pending further clinical evaluation of GLP-2 in humans.

Although much has been learned about GLP-2 action over the past 7 y, the mechanisms responsible for the pleiotropic effects of GLP-2 in the gastrointestinal tract are poorly defined. The precise chemical identity and subtype of the enteric neurons and endocrine cells that express the GLP-2 receptor remains obscure. Similarly, it seems likely that multiple distinct second mediators transduce the diverse actions of GLP-2 in the stomach, and both small and large bowel, yet little is known about the molecules and second messengers activated or repressed following GLP-2R activation. Finally, the physiological importance of various GLP-2 actions, ideally defined through the use of specific GLP-2 receptor antagonists and/or murine models with inactivating mutations in the GLP-2/GLP-2R axis, remains to be elucidated. The emerging physiological importance and therapeutic potential of GLP-2 suggests that the answers to many of these questions will be pursued vigorously by multiple investigators with complementary experimental approaches.

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