Glucagon-Like Peptide 2: A Nutrient-Responsive Gut Growth Factor

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ABSTRACT Glucagon-like peptide 2 (GLP-2) is a 33-amino acid peptide derived from the tissue-specific, post-translational processing of the proglucagon gene expressed in the intestinal enteroendocrine L-cell. The primary stimulus for GLP-2 secretion is nutrient intake, and involves direct luminal stimulation of the L-cell as well as indirect enteroendocrine and neural mechanisms. The biological activity of GLP-2 in circulation is regulated by the proteolytic cleavage of the N-terminus by dipeptidylpeptidase IV. Several studies have shown that GLP-2 has specific trophic effects on the small and large intestine, which are mediated by stimulation of cell proliferation and inhibition of apoptosis and proteolysis. GLP-2 also has been shown to suppress gastric motility and acid secretion, increase hexose transport activity and suppress food intake, specifically when infused centrally. The actions of GLP-2 are mediated by a G-protein–linked, membrane receptor (GLP-2R) that is localized largely to the gastrointestinal tract, but also is found in the brain. The secretion of GLP-2 and expression of the GLP-2R are present in the late gestation fetus. However, the developing intestine does not become responsive to the trophic effect of GLP-2 until after birth. Based on its efficacy in preventing atrophy and stimulating growth in the neonatal gut, GLP-2 may be a promising therapeutic adjuvant for treatment of infants with compromised gut function.


KEY WORDS: cell proliferation • apoptosis • gut hormone • enteral nutrition • neonate

In the last five years, glucagon-like peptide 2 (GLP-2) has emerged as one of the most intriguing and potent modulators of intestinal growth and function. GLP-2 is a member of a family of glucagon-like peptides, which have a variety of important biological functions not only within the gastrointestinal (GI) tract, where they are produced, but also in the body as a whole in terms of carbohydrate metabolism and appetite regulation. Although the biological function of pancreatic glucagon is well established, the precise structural identity and function of gut glucagon, or “enteroglucagon,” has until recently remained obscure. An early clue that glucagon-like peptides have intestinal trophic actions was the report of small bowel hyperplasia in patients with glucagonomas (1). Drucker and others (2) reproduced this phenomenon in nude mice implanted with glucagonomas, and established that the effect was due to GLP-2. Several subsequent studies (3–5) have suggested that GLP-2 is a gut peptide with trophic actions that are highly specific for the GI tract. Moreover, GLP-2 is currently being developed for therapeutic clinical use; it recently was approved for treatment of gastrointestinal disease in adults. The aim of this review is to provide an overview of the biological functions of GLP-2 and to highlight the gaps in our understanding of its physiologic relevance and mechanism of action, especially in the developing neonate.

Gene Expression. Glucagon-like peptide 2, a product of the proglucagon gene, and belongs to a superfamily of genes that code for gastrointestinal regulatory peptides including secretin, glucose-dependent insulinotropic peptide (GIP) and vasoactive intestinal peptide. In the intestine, GLP-2 is produced as a 33-amino acid peptide derived from post-translational processing of the proglucagon polypeptide in enteroendocrine “L” cells, which are located predominantly in the distal small intestine and colon (5,6). There is relatively high homology (87–97%) in the GLP-2 peptide sequence among the species reported, including humans, pigs, cows and rats. Other products of the proglucagon gene in the intestine and brain are glucagon-like peptide 1, glicentin and oxyntomodulin. In the A-cells of the pancreatic islets, glucagon is the major post-translational product of the gene. It is noteworthy that GLP-2 is produced from proglucagon expressed in regions of the brain (4). The tissue-specific processing of the proglucagon-derived peptides is explained largely by the differential expression of specific prohormone convertases in the intestinal L-cells and pancreatic A-cells. Much more is known about the molecular regulation of proglucagon expression in pancreatic A-cells than in the enteroendocrine L-cells (7). However, studies in rodents have demonstrated the presence of intestinal proglu...
cagon expression during fetal and early neonatal development (8). Other studies have identified specific transcription factors and aspects of the promoter sequence that are important for the early development and tissue-specific regulation of proglucagon gene expression (9,10).

**Secretion.** Many of the factors that regulate the secretion of GLP-2 can be inferred from our understanding of GLP-1 secretion; both peptides appear to be secreted in parallel and are derived from the same gene, precursor peptide and cell type. The primary stimulus for GLP-2 secretion is enteral nutrient intake, especially carbohydrate and lipid (11) (Fig. 1). In adult humans after an oral feeding, there is a biphasic increase in the circulating GLP-2 concentration; a rapid initial increase occurs within 15 min, followed by a second increase after ~1 h. Our studies in neonatal animals have also shown that the circulating GLP-2 concentration increases approximately fourfold within 1 h after an oral feeding and is positively correlated with the level of enteral intake (12,13). It is remarkable that although intestinal secretions of GLP-1 and GLP-2 are stimulated by a meal, the pancreatic secretion of glucagon is effectively suppressed, a fact that highlights the tissue-specific regulation of proglucagon processing and secretion. Although direct exposure of the L-cell to luminal nutrients stimulates GLP-2 secretion, the rapid increase in the circulating concentration after feeding, coupled with the largely distal localization of these cells, suggests the involvement of other indirect mechanisms. Indeed, evidence suggests that nutrient-mediated secretion of GLP-2 involves both endocrine stimulation, mediated by GIP released from enteroneodocrine K-cells, and neural reflexes involving gastrin-releasing peptide (GRP) (14). Moreover, there is evidence that short-chain fatty acids (SCFA) stimulate ileal proglucagon expression and GLP-2 secretion, and thus provide a partial explanation for the intestinal trophic effect of SCFA infusion and dietary fiber consumption (15,16). The stimulation of SCFA production arising from fermentation of malabsorbed dietary carbohydrate may be a triggering mechanism for GLP-2 secretion, and hence may act as an important trophic signal in intestinal adaptation after massive small bowel resection. Consistent with this concept are study findings indicating that the resection of the distal small bowel and colon significantly diminishes GLP-2 secretion due to loss of GLP-2–producing tissue and leads to intestinal failure (17). GLP-2 secretion may also be regulated developmentally, based on evidence that GLP-2 is present at low levels in fetal plasma and the fact that the concentration increases progressively during early neonatal development (8,18). It is interesting to note that the rate of intestinal growth and its trophic response to enteral nutrition also increase during early postnatal development, raising the possibility that GLP-2 secretion may be involved. The marked increases in the proportion of dietary carbohydrate and fiber associated with weaning may upregulate GLP-2 secretion and partially mediate the increased gut growth and cell proliferation observed during this developmental transition.

**Biological Activity and Metabolism.** An important regulatory aspect of GLP-2 activity and metabolism is its rapid rate of degradation and renal clearance once secreted from the intestinal l-cell (19). GLP-2 is secreted as a 33-amino acid peptide, but is rapidly degraded at an N-terminus site to GLP-2 [3–33] in circulation, in large part, by dipeptidylpeptidase IV (DPP-IV). This results in a relatively short half-life (7 min) for full-length GLP-2 [1–33] compared with the truncated GLP-2 [3–33] form (27 min) (5). The truncated GLP-2 [3–33] is believed to be largely biologically inactive (20). To prolong its half-life and enhance the biological activity of GLP-2, the exquisite sensitivity to DPP-IV has been exploited experimentally using a synthetic GLP-2 peptide analog h[Gly2]-GLP-2, with an N-terminus substitution of glycine for alanine (3,4) and specific inhibitors of DPP-IV activity, such as valine-pyrrolidide (20), both of which increase the potency of GLP-2. The potency of GLP-2 is also greater in mice than in rats due to the species differences in DPP-IV activity. It is important to note that the DPP IV enzyme activity is relatively abundant in the intestinal mucosa and thus may have an important influence on the biological activity of GLP-2 even before it reaches the systemic circulation (5). Furthermore, the intestinal activity of DPP IV is higher in neonatal than adult animals, and higher in the distal than proximal gut; however, it is not known whether this alters the biological effect of GLP-2 in neonates.

**Physiologic and Metabolic Effects.** The peptide sequence of GLP-2 was deduced initially after the proglucagon gene sequence was reported by Bell and others (21). However, the biological function of GLP-2 was virtually unknown until the seminal studies by Drucker and others (2) revealed the marked intestinal trophic effects of GLP-2. Their initial report and a series of subsequent studies showed that chronic treatment with GLP-2 has potent and specific trophic effects on the GI tract that are characterized grossly by increased tissue mass and mucosal thickness and morphologically by increased villous length and crypt depth. In vivo studies in humans and pigs have demonstrated that GLP-2 infusion acutely inhibits gastric secretion and motility, an effect that is common among the other peptides secreted from the intestinal l-cell, namely GLP-1 and PYY (5). As such, GLP-2 may be implicated together with these peptides in the “ileal brake” phenomenon (6).

There are conflicting reports concerning the effects of GLP-2 treatment on intestinal digestive enzyme and nutrient transport. In some cases, GLP-2 has been found to increase the activities and expression of hydrodases, such as sucrase-isomaltase, maltase-glucosaminase, lactase and aminopeptidase N (18,22,23). However, some studies in rodents and neonatal pigs indicate that GLP-2 enhances intestinal hexose transport by modulating the activity and localization of GLUT2 and SGLT1 (24,25,26), whereas others found expression of these transporters to be decreased by GLP-2 (22). Furthermore,
studies show that GLP-2 increases amino acid transport (25,27). Yet, in vivo kinetics studies suggest only modest increases in nutrient absorption in GLP-2–treated mice (22). In addition to the reported effects of GLP-2 on intestinal absorptive function, the increased mucosal growth and villous surface area that occur with GLP-2 treatment may have an important role in gut barrier function. Indeed, GLP-2 has been shown to reduce the permeability of the intestine to macromolecules, decrease bacteria translocation and suppress the local expression of proinflammatory cytokines (4,28,29).

GLP-2 may have limited effects on systemic or whole-body metabolism, given evidence that its actions are confined largely to the GI tract. Indeed, there is no reported evidence that systemic GLP-2 administration affects food intake, growth rate or metabolism. Yet, it is interesting that central administration of GLP-2 into the lateral cerebral ventricle suppresses food intake in rats and thus, like GLP-1, it may be implicated in appetite regulation (30). We have recently found that GLP-2 stimulates intestinal protein anabolism in neonatal piglets receiving total parenteral nutrition (TPN) by suppressing proteolysis, whereas protein synthesis was unaffected (31). However, whether GLP-2 affects any aspect of whole-body protein, carbohydrate or lipid metabolism remains to be determined. Thus, from a physiologic perspective, the general picture that has emerged suggests that GLP-2 acts to slow the ingestion and transit of food through the GI tract, while increasing the absorption of nutrients from the small intestine.

**Cellular Actions of GLP-2 Receptor.** The cellular actions of GLP-2 are mediated initially by binding to the GLP-2 receptor (GLP-2R). The recent cloning of cDNAs encoding the human and rat GLP-2R indicate that it is a G-protein–linked, seven-transmembrane-domain receptor with homology to glucagon, GLP-1 and GIP receptors (4). The human GLP-2R is encoded by a single gene localized to chromosome 17p13.3. The initial cloning of the GLP-2R was performed using hypothalamic cDNA libraries; however, efforts to quantify expression based on a ribonuclease protection assay detected mRNA transcripts primarily in the GI tract. A recent report has confirmed the tissue-specific distribution of the GLP-2R mRNA, using Northern analysis and reverse transcription-polymerase chain reaction (RT-PCR), demonstrating expression in the gut, brain and the lung (32). This report has shed light on the more critical issue of localization within the GI tract using immunohistochemistry, and provides evidence of GLP-2R immunopositivity localized to a limited number of enteroendocrine cells within the stomach and the large intestine. That finding is supported by the fact that RT-PCR analysis failed to detect GLP-2R transcripts among several intestinal epithelial cells lines studied. In contrast, another recent report demonstrated that GLP-2 treatment stimulated proliferation of colonic epithelial (Caco-2) cells, albeit at concentrations that were ~100-fold greater than the circulating concentration in plasma (33). Yet another group found that injection of 125I-GLP-2 into rats resulted in specific binding of labeled GLP-2 localized diffusely along the villous epithelium (34). Thus, the definitive cellular and regional localization of the GLP-2R within the GI tract remains to be established. However, if indeed the GLP-2R is localized to enteroendocrine cells, this implies that there is a secondary signal or signals that mediate the biological action on surrounding epithelial cells via a paracrine mechanism. An additional interpretation of these recent reports is that GLP-2 clearly acts by an endocrine mechanism because it is secreted from cells that are predominantly localized in the distal regions of the GI tract, yet its effects occur throughout the GI tract.

Given the limited and, to some extent, conflicting recent findings concerning the localization of the GLP-2R, it is perhaps not surprising that our knowledge of its intracellular signaling mechanisms is also in its infancy. On the basis of the known sequence information and initial biochemical characterization, the GLP-2R is a G-protein–linked membrane receptor, which activates a cyclic AMP, protein kinase A (PKA)-dependent pathway when transfected into baby hamster kidney (BHK) fibroblasts (35). However, the coupling of GLP-2R activation with downstream cellular events that mediate increased proliferation and cell survival has not been established. The studies with transfected BHK fibroblasts suggest that GLP-2 actions are not mediated by PKA, phosphatidylinositol 3 kinase (PI-3 kinase) or mitogen-activated protein (MAP) kinase pathways. However, the GLP-2–dependent stimulation of thymidine uptake in Caco-2 cells was suppressed in a dose-dependent manner by inhibitors of both the PI 3-kinase and MAP-kinase pathways (33). Thus, it is uncertain whether these signaling pathways that are apparently activated by GLP-2 in Caco-2 cells, but not in transfected BHK fibroblasts, are indeed present in normal intestinal epithelial cells. This again raises the critical question of the existence of a secondary signal that may act via a heterologous, paracrine cellular mechanism between enteroendocrine and other intestinal epithelial cells.

An aspect of GLP-2 function in which we have been especially interested is the ontogeny of GLP-2R expression and the onset of GLP-2 responsiveness during early mammalian development. A recent report in rodents (8) and our studies in pigs (unpublished results) indicate that the GLP-2R is expressed during fetal and neonatal development. These findings are consistent with evidence of trophic and functional effects of GLP-2 in suckling rat pups and in TPN-fed piglets delivered preterm and at term. Interestingly, however, in fetuses given GLP-2 infusions in utero for 6 d, there was no stimulation of intestinal growth or enhanced development of digestive hydrolase expression despite the presence of the GLP-2R transcript in the GI tract (18). In our neonatal piglets studies (31), we found that although GLP-2 potently blocked the mucosal villous atrophy normally induced by TPN, this was mediated by suppression of apoptosis and proteolysis. In contrast, the intestinal trophic effect of enteral nutrition was mediated by a suppression of apoptosis and a stimulation of cell proliferation and protein synthesis. Thus, although it is clear that restoring the circulating GLP-2 concentration to supraphysiologic levels can maintain near-normal intestinal growth in the absence of any enteral nutrient stimulus, the trophic effect was mechanistically different than that of enteral nutrition. This finding raises a critical, yet unresolved, question regarding the physiologic importance of GLP-2, given that most of the studies reported have administered supraphysiologic doses of GLP-2. The development of experimental approaches to block endogenous GLP-2 action via immunoneutralization, peptide antagonist and targeted disruption of the GLP-2R gene should provide answers to this question. However, it appears that during fetal life, GLP-2 is not essential for intestinal development, based on recent evidence from newborn Pax6 mutant mice with deficient intestinal endocrine cell development and proglucagon expression (10).

**Therapeutic Potential.** Since the original report of the
intestinotrophic actions of GLP-2 by Drucker and colleagues (2), a number of studies have demonstrated the therapeutic efficacy of GLP-2 and the protease-resistant h[Gly2]-GLP-2 analog in several models of intestinal dysfunction, injury and insufficiency, including TPN (31,36), massive small bowel resection (37), colitis and nonsteroidal anti-inflammatory drug-induced enteritis (4), inflammatory bowel syndrome (29), ischemic bowel (38) and chemotherapy-apneic injury (39). The suppression of intestinal cytokine expression (3,29) and proteolytic activity (31) may be key cellular mechanisms whereby GLP-2 ameliorates gut injury and inflammation. The fact that GLP-2 effectively stimulates intestinal growth, while slowing proximal bowel motility and secretion, makes it a promising therapeutic option for patients with short-bowel syndrome. In fact, the protease-resistant GLP-2 analog, h[Gly2]-GLP-2, also referred to as ALX-0600, was recently granted orphan drug status by the Food and Drug Administration and is in Phase II clinical trial for treatment of short-bowel syndrome in adults. Moreover, in a recent study in short-bowel patients, GLP-2 treatment increased nutrient absorption and weight gain (40). Thus, provided that GLP-2 treatment is safe, other clinical applications may be anticipated, including treatment of infants with compromised intestinal function.

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