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Biologic Properties and Therapeutic Potential of Glucagon-like Peptide-2

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ABSTRACT. *Background:* Glucagon-like peptide-2 (GLP-2), a 33 amino acid, proglucagon-derived peptide with intestinotrophic activity, is secreted from enteroendocrine cells in the small and large intestine. *Methods:* This review describes recent advances in our understanding of GLP-2 physiology from rodent experiments *in vivo*. *Results:* GLP-2 administration induces mucosal epithelial proliferation in small and large bowel and stomach. GLP-2 is rapidly degraded by the enzyme dipeptidyl peptidase IV (DPP-IV) to produce the biologically inactive form GLP-2(3–33), however, GLP-2 analogs that confer resistance to DPP-IV exhibit enhanced biologic activity *in vivo*. GLP-2–treated bowel retains normal to enhanced functional absorptive capacity. Furthermore, GLP-2 infusion prevents total parenteral nutrition (TPN)-associated intestinal hypoplasia, and enhances bowel adaptation and nutrient absorption in rats following small bowel resection. GLP-2 also reverses weight loss and improves histologic and biochemical parameters of disease activity in mice with experimental colitis. *Conclusions:* GLP-2 is an intestine-derived peptide with intestinotrophic properties that may be therapeutically useful in diseases characterized by intestinal damage or insufficiency. (*Journal of Parenteral and Enteral Nutrition* **23:**S98–S100, 1999)

Mammalian proglucagons encode 2 glucagon-like peptides, (GLP), GLP-1 and GLP-2, that are contained within a common proglucagon precursor that is identical in brain, pancreas, and gut. Although the absence of GLP-2 from anglerfish pancreatic proglucagon cDNA was originally interpreted as evidence for a diminished biologic importance of GLP-2, subsequent studies demonstrated that GLP-2 is present in fish proglucagons,[1] and proglucagon mRNA transcripts encoding GLP-2 are generated in fish intestine via tissue-specific mRNA splicing. The amino acid sequence of GLP-2 is highly conserved across species, with rat and human GLP-2 differing by only 1 amino acid.

The intestinal proglucagon-derived peptides (PGDPs), comprising glicentin, oxyntomodulin, GLP-1, and GLP-2 are secreted from the small and large bowel following food ingestion. GLP-2 is secreted from the human intestine following nutrient ingestion with levels rising from approximately 151 to 225 pmol/L 2 hours after a mixed meal.[2] GLP-2 levels also increase significantly, although to a lesser degree, after ingestion of a donut and coffee in human volunteers, consistent with a highly sensitive nutrient-dependent regulation of human intestinal PGDP secretion.[3]

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Biologic Activity of GLP-2 in Normal Rodents

Considerable experimental evidence, from both rodent and human studies, links intestinal adaptation to increased production and secretion of the intestinal PGDPs.[4–8] Transplantable glucagonomas in mice were utilized for analysis of the effects of increased circulating levels of PGDPs on bowel growth. Subcutaneous implantation of pancreatic or intestinal glucagon-producing cell lines resulted, after 3 to 4 weeks, in markedly increased circulating levels of the PGDPs *in vivo*.[9] All mice harboring subcutaneous glucagonomas developed small bowel hyperplasia,[10] consistent with the previously described correlation between PGDPs and small bowel growth. Subsequent administration of individual synthetic PGDPs to mice by daily subcutanous injection demonstrated that GLP-2 was the intestinal PGDP with significant intestinotrophic activity.[10]

After release from the enteroendocrine cell, GLP-2 is cleaved at the N-terminus by the enzyme dipeptidyl peptidase IV (DPP-IV),[3,11] the identical peptidase shown to be important for GLP-1 inactivation *in vivo*.[12,13] As DPP-IV is highly expressed in a crypt to villous gradient in the mucosal epithelium,[14] the biologic activity of GLP-2 may be locally regulated in part via DPP-IV–mediated inactivation. Although the clearance of GLP-2 has not been extensively studied, current data indicate that the kidney may also be an important determinant of GLP-2 clearance in the rat.[15]

The observations that native GLP-1 is rapidly cleaved by DPP-IV, taken together with the similarity of GLP-1 and GLP-2 at the amino-terminus, have stimulated interest in the development of more potent

GLP-1 and GLP-2 analogues.[16] Consistent with the importance of DPP-IV for inactivation of GLP-2, native GLP-2 appears to be comparatively more intestinotrophic in rats with a genetic mutation in the DPP-IV enzyme.[11] Furthermore, a DPP-IV–resistant GLP-2 analogue exhibited a greater degree of intestinotrophic activity in normal rats, compared with similar doses of native GLP-2. Human GLP-2 has also been shown to be metabolized by DPP-IV both *in vivo* and *in vitro*.[3] These observations clearly indicate that DPP-IV–mediated degradation of GLP-2 is an important determinant of GLP-2 bioactivity *in vivo*.

GLP-2 administration to mice produces significant increases in small bowel mass in 10- to 14-day experiments,[17] however, significantly increased small bowel mass is evident after only 4 days of GLP-2 treatment.[10] The minimal duration of and concentration requirement for GLP-2 treatment and subsequent induction of the intestinal growth response is not known. Nevertheless, administration of a single dose of GLP-2 every other day for 2 weeks induced a significant increase in murine small bowel mass.[17] Furthermore, biologic activity of GLP-2 in the gut is apparent within 30 minutes of IV GLP-2 infusion, as assessed by increased brush border membrane glucose transport secondary to increased glucose transporter translocation.[18,19]

The principal histologic change detected after GLP-2 administration is an increase in the thickness of the small intestinal mucosal villus epithelium. In contrast, no significant change is detected in the intestinal muscle layer.[10,17] The mechanisms underlying GLP-2-mediated bowel growth include stimulation of crypt cell proliferation[10] and inhibition of enterocyte apoptosis.[20] After cessation of GLP-2 administration, intestinal weight reverts to normal after several days,[20] suggesting an ongoing requirement for GLP-2 for maintenance of enhanced mucosal epithelial thickness *in vivo*.

GLP-2 in Rodent Models of Experimental Disease

The development of intestinal hyperplasia in rodents with experimental diabetes correlates well with increased endogenous intestinal GLP-2 synthesis.[21] Circulating plasma GLP-2 increases after the induction of experimental diabetes with streptozotocin, in parallel with increasing thickness of the mucosal epithelium. Furthermore, treatment of diabetic rats with insulin prevented intestinal mucosal hyperplasia, in association with decreased circulating levels of GLP-2 and decreased ileal concentrations of the PGDPs.[21]

The small bowel mucosal hypoplasia that accompanies total parenteral nutrition (TPN) in rodents was completely prevented in TPN-treated rats coinfused with GLP-2 for 6 days,[22] which strongly suggests that GLP-2 may be a major component of the nutrient-dependent regulation of mucosal epithelial proliferation. The GLP-2 effects on the mucosal epithelium were most notable in the small bowel, as GLP-2 infusion did not increase mean colon weight in the TPN-treated rats. These observations are consistent with previous findings[23] that the small bowel epithelium may be relatively more sensitive to trophic effects of GLP-2 *in vivo*.

Although the small bowel, specifically the jejunum, appears to be comparatively more sensitive to the growth-promoting effects of GLP-2, an increase in colon weight and protein content was observed in mice treated with GLP-2, 2.5 μg twice daily for 10 days.[20] Subsequent experiments demonstrated that both native GLP-2 and a more potent human GLP-2 analogue, h[Gly2]-GLP-2, induced significant increases in large bowel weight and crypt depth in mice.[23,24] Furthermore, GLP-2, in combination with growth hormone, insulin-like growth factor-1 (IGF-1), epithelial growth factor (EGF), or IGF-2 significantly augmented the intestinotrophic response, in both small and large bowel.[23]

Therapeutic Potential of GLP-2

The induction of small intestinal growth in GLP-2–treated rodents raises the possibility that GLP-2 may be a useful therapeutic adjunct in diseases associated with epithelial damage and defective nutrient absorption. To address the physiologic function of GLP-2–induced mucosa, we assessed the functional expression of enzymes in control and GLP-2–treated murine small bowel.[25] The levels of enzyme activity, normalized to total protein content, of maltase, sucrase, lactase, γ-glutamyltransferase, and DPP-IV were normal in GLP-2–treated bowel. The capacity of GLP-2–treated intestine for functional nutrient absorption was also assessed with nutrient challenge tests. These experiments demonstrated that GLP-2–treated mice exhibit normal to enhanced protein, carbohydrate and fat absorption,[25] providing important functional evidence that GLP-2–induced bowel growth is not associated with perturbation in the functional capacity of the intestine for nutrient absorption *in vivo*.

Experimental small bowel resection (SBR) has previously been shown to be associated with increased circulating levels of the PGDPs, and induction of proglucagon gene expression in the intestinal remnant. A potential role for GLP-2 in the augmentation of intestinal adaptation that follows intestinal resection was demonstrated in rats following major small bowel resection. GLP-2 treatment decreased intestinal permeability and significantly augmented epithelial hyperplasia, mucosal weight, sucrase activity, and absorptive capacity after 75% jejuno-ileal resection.[26] These studies raise the possibility that GLP-2 may be useful for enhancing intestinal absorptive function in patients with intestinal resection.

A role for GLP-2 in healing compromised intestinal mucosa is suggested by studies demonstrating markedly reduced weight loss, and improved histologic indices of disease activity in GLP-2–treated mice with dextran sulfate–induced colitis.[27] Remarkably, GLP-2–treated mice also exhibited reduced levels of interleukin-1 gene expression in the inflamed colon. These observations suggest that the biologic properties of GLP-2 that lead to enhanced repair of damaged mucosal epithelium may also be of potential therapeutic benefit in the setting of intestinal inflammation, findings that await confirmation in future animal studies.

Despite the recent expansion of our knowledge underlying GLP-2 actions *in vivo*, the precise mechanism(s) underlying the biologic effects of GLP-2 remain unknown. Given that GLP-2 shows strong homology to structurally related peptides such as GLP-1, glucagon, and GIP, it seems reasonable to predict that the GLP-2 receptor will also be a new member of the G-protein linked, seven transmembrane domain receptor superfamily.[28–30] Whether the effects of GLP-2 on bowel growth are direct, via GLP-2 receptors expressed locally on crypt stem cells, or indirect, via induction of one or more intestinal growth factors, remains to be determined. Furthermore, as the biologic activity of GLP-2 was originally described in experiments using hypothalamic and pituitary membranes,[31] it is likely that extraintestinal effects of GLP-2, particularly in the central nervous system, await elucidation. Taken together, the expanding biologic activities of the glucagon-like peptides, along with their potential utility for the treatment of human disease, seem likely to engender considerable interest and ongoing research into the physiology and mechanism of action of the PGDPs *in vivo*.

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A GLP-2 receptor has now been cloned. CPNAS 96: 1569–1573, 1999.

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