A biological function for glucagon-like peptide-2

Mehboob A Hussain

Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Harvard Medical School, Wellman 320, 50 Blossom Street, Boston, Massachusetts 02114, USA

The cloning of the glucagon gene from anglerfish, rodents and humans ((1) and references therein) in the early 1980s yielded the surprising finding that the glucagon gene not only encoded the known peptide hormone glucagon, but also contained sequences for two additional peptides, which, because of their homology to glucagon, were named glucagon-like peptide (GLP)-1 and -2 (Fig. 1). The protein product of the gene, preproglucagon, is processed in a tissue-specific manner to glucagon and the major proglucagon fragment in pancreatic endocrine α-cells or to glicentin, oxyntomodulin. GLP-1, intervening peptide 2 (IP-2), and GLP-2 in intestinal L-cells and certain neurons of the brain (Fig. 1). GLP-1 has a high sequence homology to the incretin hormone, gastric inhibitory polypeptide (GIP), and thus, at the time of its identification, was already hypothesized to represent a second incretin hormone (1). Subsequent studies did, indeed, reveal an incretin effect of GLP-1 (2). Furthermore, GLP-1 has been shown to be involved in satiety control and gastric emptying (3, 4). GLP-2 is co-secreted with GLP-1. Fasting levels in human plasma are 151 ± 14 pmol/l and these increase to 225 ± 15 pmol/l by 2 h after a mixed meal (5).

In contrast, GLP-2, a 33-amino acid peptide, has only recently been ascribed a biological function, elucidated in a pioneering effort by Dr Drucker’s group in Toronto. The background of their studies were earlier observations that over-expression of the glucagon gene in rodents was associated with bowel growth and regeneration (6, 7). Furthermore, two reports of patients presenting with proglucagon-expressing tumors in the kidney, increased circulating concentrations of the proglucagon-derived peptides (PGDP), and small-bowel hypertrophy that regressed after tumor resection, suggested a link between peptide products derived from proglucagon and the control of small-bowel epithelium proliferation (8, 9). Small-bowel epithelial proliferation can also be reproduced in glucagon promoter–SV40 T antigen transgenic mice, which develop proglucagon-producing tumors and increased plasma concentrations of PGDP (10–12). When nude mice are injected subcutaneously with proglucagon-producing tumors, circulating PGDP are increased and the small bowel appears enlarged (13). To identify further which PGDP was leading to the intestinal enlargement, each PGDP was injected separately to normal mice. Whereas GLP-1 and IP2 had no effect, glicentin treatment led to a small increase in bowel weight. Remarkably, GLP-2 treatment resulted in a 50% increase in total small-bowel wet and dry weight. GLP-2 increased mucosal thickness, which was attributable to an increase in villus height; crypt depth and muscle thickness did not change. Markers for cell proliferation were increased in epithelial cells of the crypts and also extended to the lower portions of the villus surfaces. Remarkably, the effects of GLP-2 were confined to the crypt and villus epithelium, and no changes were detectable in other layers of the intestine or in other organs. Changes in intestinal epithelial proliferation markers were already evident after a single dose of GLP-2, and morphologic changes were detectable after a treatment period of as little as 4 days (13). Food intake and body weight were similar between control and GLP-2 treated animals, excluding the possibility that increased enteral nutrient ingestion attributable to GLP-2 may have trophic effects on gut epithelium (14).

Subsequent pharmacological studies revealed that GLP-2 treatment was associated, not only with increased proliferation of small-bowel epithelium, but also with reduced markers for apoptosis of these cells. The changes are found in young and in aged mice. Furthermore, the effects of GLP-2 are found after treatment for up to 12 weeks, suggesting that desensitization to the trophic effects of GLP-2 does not take place. After withdrawal of GLP-2, the morphological changes in intestinal mucosa regress to baseline status (15). Further, comparable doses of native rat and human GLP-2 are less intestinotrophic in vivo in rats than are GLP-2 derivatives containing amino acid substitutions that confer resistance to degradation by the enzyme, dipeptidyl peptidase IV (16).

In addition to the proliferative effects of GLP-2, a morphological differentiated state of the villus is reported to be maintained (13, 15). Functional assessments of several small-bowel epithelial enzymes revealed that parenteral GLP-2 administration was associated with increased activities of duodenal maltase, sucrase, lactase, glutamyl transpeptidase and dipeptidyl peptidase IV (17). Whether orally administered nutrients, which are possibly digested more rapidly during administration of GLP-2, are also resorbed at a higher rate remains unclear. Whereas one study demonstrated increased uptake of leucine and triolein but no changes in glucose or maltose uptake rates (17), other investigators have reported an increased activity of the sodium-dependent glucose transporter-1 after GLP-2 treatment (18, 19).

As mentioned above, an indirect effect of GLP-2 on gut epithelium by way of increasing food ingestion has
been excluded (14). However, GLP-2 treatment has been shown to prevent parenteral-nutrition-induced small intestinal hypoplasia in rats. Rats maintained on total parenteral nutrition for 6 days have a significant (approximately 30%) reduction in mass, DNA, and protein content of small and large intestine. GLP-2 administration was able to prevent hypoplasia in small intestine, but not in the colon. The muscular layers of the intestine remained unaffected by GLP-2 (20).

The combination of GLP-2 with other peptide growth factors previously known to exert trophic effects on the intestine (growth hormone (GH), insulin-like growth factors (IGF)-I and II, and epidermal growth factor) has yielded some additional insight into possible differences and somewhat additive effects of these various peptide hormones. Whereas GLP-2 appears to exert its trophic effects only on the intestine, the other factors (or their longer acting analogs) are known to have trophic effects on additional tissues. In the intestine, GLP-2 exerts its effects only on the epithelium, whereas GH and IGF-I have additional proliferative effects on submucosal muscular tissue. GLP-2 [in this cited study, a long acting human analog was used (16)] was the most potent of all peptides at the doses used in the study. However, administration of the various agents was not normalized for their in vivo molar concentrations. A combination of GLP-2 with GH or IGF-I exhibited a greater increase in histomorphological parameters of small intestinal growth. Administration of all five factors induced the largest increments in villus and crypt height, and in small and large bowel length and weight (21).

Experimental diabetes is associated with bowel growth. Whether GLP-2 has a role in this context was studied in rats made diabetic by streptozotocin (STZ) injection and treated with or without insulin for 3 weeks. Ileal concentrations of the intestinal proglucagon-derived peptides, glicentin + oxyntomodulin and GLPs 1 and 2, were significantly (57%) increased above those of insulin-treated controls. Similar increases in plasma concentrations of glicentin+oxyntomodulin (77%) and GLP-2 (91%) were seen in untreated STZ diabetes. Both wet and dry small intestinal weight increased by 74% in STZ diabetes. Villus height and crypt depth were significantly increased in untreated diabetic rat intestine. Insulin treatment prevented the changes in plasma GLP-2 and intestinal mass seen in untreated STZ diabetes (22).

The findings of the cited reports lend support to the long-held view that a humoral factor emanating from the fed intestine can reduce or prevent atrophy of bypassed (silent) portions of the intestine (Thiry-Vella loops of intestine) (23). Furthermore, early studies that documented an association between intestinal resection, injury, or inflammation and increased enteral proglucagon gene expression (24, 25) and increased concentrations of circulating intestinal PCDP may have pointed toward the role of GLP-2 (6). Whether GLP-2 is indeed increased in patients recovering from intestinal injury has not been reported. Intestinal hypertrophy as seen in patients with glucagonomas is quite likely to be solely attributable to GLP-2 produced by the tumors (8, 9).

It should be mentioned at this stage that the studies cited above all derive a role for GLP-2 when the peptide is administered in pharmacological doses. Although it is quite likely that GLP-2 has a role in maintaining small intestinal mucosal structure and function, strictly speaking, a physiological role for GLP-2 has yet to be established. Identification of the GLP-2 receptor and generation of rodent models lacking GLP-2 or the GLP-2 receptor (knockout) will further our understanding of the physiology of hormonal regulation of intestinal

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**Figure 1** The glucagon gene and its products. The glucagon gene consist of six exons (E-1 to E-6) that encode for preproglucagon. The protein that is translated from this transcript undergoes further post-translational tissue-specific processing, yielding as major products: glucagon in the pancreatic endocrine \(\alpha\)-cells and glucagon-like peptides-1 and -2 in intestine and brain (see text for further description).
organ size and function. Beyond the interesting aspects of the physiological role(s) and the cellular and subcellular effects of GLP-2 that remain to be elucidated, it is already tantalizing to speculate that a potential treatment modality for patients receiving parenteral nutrition, patients with short-bowel syndrome or those with inflammatory bowel disease may be available.

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References