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Tales beyond the Crypt: Glucagon-Like Peptide-2 and Cytoprotection in the Intestinal Mucosa

Intestinal function and mucosal epithelial integrity are regulated by the combined actions of different hormonal and neuronal signals. Enteral nutrition is an essential determinant of mucosal epithelial function, and peptide hormones released after nutrient ingestion regulate gastric motility, pancreatic and biliary secretions, nutrient transport, and the growth and differentiated actions of the mucosal epithelium. Examples of these gut-derived hormones include gastrin, glucose-dependent insulinotropic polypeptide, cholecystokinin, neurotensin, peptide YY, secretin, and the proglucagon-derived peptides glicentin, oxyntomodulin, glucagon-like peptide (GLP)-1, and GLP-2. Several of these peptides exhibit growth-promoting properties on the intestinal epithelial mucosa *in vivo*, such as glicentin, peptide YY, cholecystokinin, progastrin, and glycine-extended gastrin.

The identification of GLP-2 as a potent intestinotrophic hormone in rodents (1) sparked substantial interest in understanding how the biological actions of this peptide are transduced into growth signals for the gut mucosa. GLP-2 is a 33-amino-acid peptide hormone derived by posttranslational processing of proglucagon in enteroendocrine cells of both the small and large bowel and in specific regions of the brainstem. Circulating levels of GLP-2 rise rapidly after ingestion of nutrients, and the intact peptide is rapidly degraded to an inactive metabolite, GLP-2(3–33), via the enzyme dipeptidyl peptidase IV (2, 3). In addition to its potent trophic effects on the intestinal mucosa, GLP-2 inhibits gastric emptying and gastric acid secretion, stimulates intestinal barrier function (4), stimulates intestinal hexose transport (5), and enhances nutrient absorption in rodents and in human patients with short bowel syndrome (6).

GLP-2 appears to mimic at least some of the effects of enteral nutrition on the mucosal epithelium, as GLP-2 administration prevents the mucosal hypoplasia that develops in parenterally fed animals, in part by reducing proteolysis and crypt cell apoptosis (7, 8). Although a specific receptor for GLP-2 has been identified that is expressed in the small and large bowel (9–11), the mechanisms activated by GLP-2 that produce growth and inhibit apoptosis in the gut remain poorly understood.

The study by Burrin *et al.* (12) in this issue of *Endocrinology* addresses the cellular mechanisms by which GLP-2 induces cell survival and prevents intestinal atrophy in piglets maintained on parenteral nutrition. Burrin *et al.* demonstrate that constant administration of GLP-2 for 7 d at three different infusion rates reveals dose-dependent effects of GLP-2 on proliferation and cytoprotection in the absence of enteral nutrients. Although GLP-2 significantly inhibited cell death in the gut epithelium at lower infusion doses (2.5–5.0 nmol/kg), enhanced crypt cell proliferation and up-regulation of endothelial nitric oxide synthase expression was only observed at the highest GLP-2 infusion rate (10 nmol/kg). Furthermore, the authors provide evidence suggesting that the cytoprotective actions of GLP-2 in the intestinal mucosa were associated with reduced activation of caspase 3 and 6, increased Bcl-2 expression, phosphorylation of protein kinase B/Akt, and inactivation of the proapoptotic molecule glycogen synthase kinase-3. The authors suggest that physiological levels of GLP-2 achieved in the postprandial state, which approximate but are likely to be less than the circulating levels of GLP-2 obtained in the experimental group receiving the lowest infusion rate of the peptide, may act to maintain intestinal integrity and prevent cellular damage. In contrast, much higher circulating levels of GLP-2 are required to prevent parenteral nutrition-induced intestinal atrophy and stimulate mucosal proliferation. Thus, the antiapoptotic mechanisms engaged by the GLP-2 receptor (GLP-2R) appear more sensitive to lower plasma levels of GLP-2, whereas activation of the GLP-2R, leading to enhanced cell proliferation, apparently requires much higher levels of GLP-2.

This study provides new insights into the cellular mechanisms by which GLP-2 may protect the intestinal mucosa from damage *in vivo*. The GLP-2R, a G protein-coupled receptor (9) structurally related to but distinct from the glucagon and GLP-1 receptors, has been shown to exhibit a highly restricted pattern of expression in human enteroendocrine cells (10), murine enteric neurons (11), and in multiple regions of the rodent brain (13). Due to the relatively exclusive localization of the GLP-2R, efforts directed at identifying the mechanism(s) by which GLP-2 exerts its myriad effects in the gut epithelium are focused on the identification of indirect mediators of GLP-2 action liberated by the enteric nervous system or gut endocrine cells (Fig. 1).

The intestinotrophic effects of GLP-2 that result in expansion of the normal mucosal epithelium are largely due to stimulation of crypt cell proliferation and, to a lesser extent, inhibition of crypt cell apoptosis. These proliferative and cytoprotective effects of GLP-2 lead to lengthening of the intestinal villi and expansion of mucosal absorptive surface area, accompanied by a modest expansion of the crypt compartment. Because the GLP-2R does not appear to be directly expressed in normal crypt cells or enterocytes, proliferation and expansion of the mucosal epithelium is thought to occur by indirect mechanisms activated by GLP-2R signaling, possibly via stimulated release of presently unidentified growth and cytoprotective factors (Fig. 1). Although inhibition of cell death does not appear to contribute greatly to GLP-2-induced mucosal expansion in the healthy bowel, exogenous

Abbreviations: GLP, Glucagon-like peptide; GLP-2R, GLP-2 receptor.

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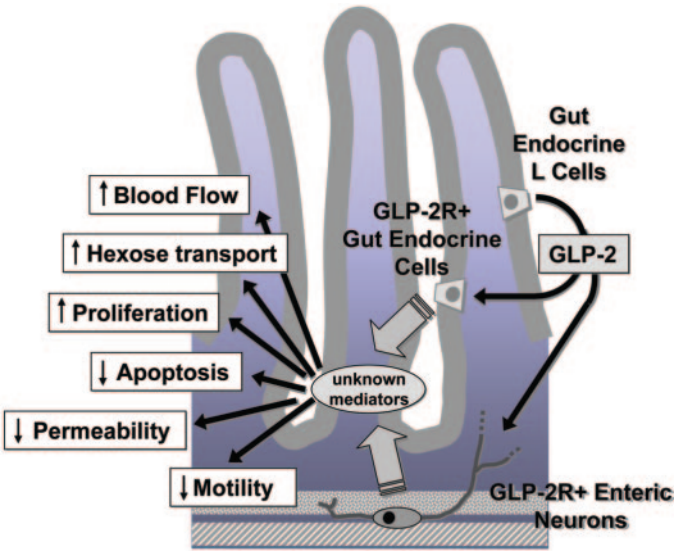

FIG. 1. Physiological actions of GLP-2 on the gastrointestinal mucosal epithelium. Upon release of GLP-2 from the intestinal L cells after nutrient ingestion, the peptide hormone is believed to act in a paracrine or endocrine manner on neighboring cells expressing the GLP-2 receptor. Given the precise localization of GLP-2R expression in enteroendocrine cells and enteric neurons, many of the physiological responses after GLP-2 receptor activation in the bowel are believed to be mediated via indirect mechanisms.

GLP-2 administration has been shown to inhibit crypt cell apoptosis, reduce mucosal injury, and prolong survival in rodent models of intestinal injury (14–16).

The current studies by Burrin *et al*. (12) demonstrate that the antiapoptotic signaling pathways activated by GLP-2 in the pig intestine *in vivo* are remarkably similar to those previously identified in studies using heterologous cell lines expressing the cloned GLP-2R (17). In addition, the differential dose dependence for GLP-2-regulated cytoprotection and cell proliferation in the pig intestine *in vivo* resembles data obtained in experiments examining the control of cell proliferation and cell survival in studies examining the direct actions of GLP-2 *in vitro*. Although the antiapoptotic consequences of GLP-2R signaling in BHK-GLP-2R cells or HeLa cells are evident at lower concentrations of peptide (20 nM) (17–19), GLP-2-induced cell proliferation requires more pharmacological doses of ligand (1–10 μM) *in vitro* (20–22).

Dysfunction and destruction of the intestinal mucosa is associated with a number of disease states including inflammatory bowel disease (such as Crohn's disease or ulcerative colitis), chemotherapy-induced enteritis, and necrotizing enterocolitis. Prolonged recurrent intestinal inflammation can result in extensive damage to the mucosal epithelium, leading in some instances to strictures, severe ulceration, abdominal pain, and infection ultimately requiring surgical resection of the injured segment of small or large bowel. Short-bowel syndrome, occurring as a result of extensive small bowel resection, is a condition characterized by severe malabsorption of nutrients, diarrhea, weight loss, and the frequent need for oral and parenteral nutrient supplementation. Currently, there are few therapies available for patients that act to enhance nutrient absorption and restore the functional integrity of the gut epithelium.

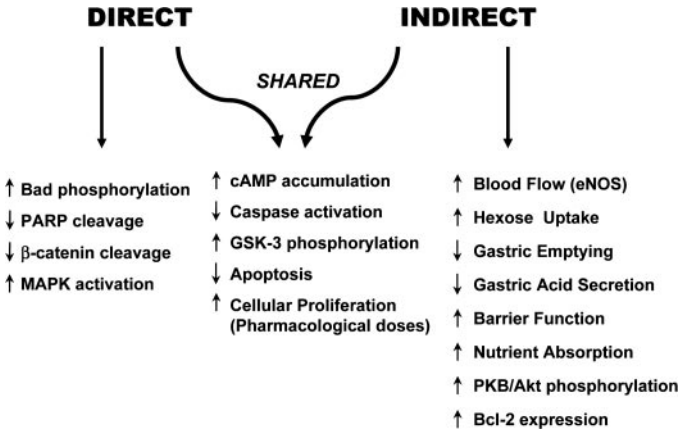

FIG. 2. Direct *vs.* indirect actions of GLP-2 signaling in the intestine. The consequences of GLP-2 receptor signaling have been investigated directly using cultured intestinal cells or cells transfected with the GLP-2R cDNA, or indirectly (*in vivo*) by monitoring the effects of GLP-2 administration in humans or using animal models. Given the data presented by Burrin *et al.* in this issue (12), many of the direct and indirect effects of GLP-2 receptor signaling appear to be similar.

Although the mechanisms by which GLP-2 elicits its cytoprotective and growth-promoting effects *in vivo* are still poorly understood, a degradation resistant GLP-2 analog, Teduglutide (NPS Pharmaceuticals, Salt Lake City, UT), is currently in late-stage clinical testing in human subjects with short bowel syndrome. Although there is still much to be learned about how GLP-2 produces its effects, it is encouraging to observe that data on GLP-2R action obtained through the use of *in vitro* cell models correlates well with observations made in animal models of GLP-2 action exemplified by the current study reported by Burrin *et al*. (12). Thus, it appears that signaling of the GLP-2R activates analogous signaling pathways in cells directly expressing the receptor as in cells indirectly responding to GLP-2 treatment (Fig. 2). Additional research is needed to identify the mechanisms by which GLP-2 indirectly regulates such a multitude of physiological processes in the normal and injured gastrointestinal mucosa. Nevertheless, it appears that studies using both *in vitro* cellular paradigms and more complex *in vivo* models of gastrointestinal physiology will provide useful and often complementary delineation of the downstream signaling pathways activated by the GLP-2R.

Jennifer L. Estall and Daniel J. Drucker
Departments of Laboratory Medicine and Pathobiology and Medicine
Banting and Best Diabetes Centre
Toronto General Hospital
University of Toronto
Toronto, Ontario, Canada M5G 2C4

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Address all correspondence and requests for reprints to: Dr. D. J. Drucker, Toronto General Hospital, Banting and Best Diabetes Centre, 200 Elizabeth Street MBRW4R-402, Toronto, Ontario, Canada M5G 2C4. E-mail: d.drucker@utoronto.ca.

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