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

Glucagon-Like Peptide-2 and Intestinal Adaptation: An Historical and Clinical Perspective[1]

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ABSTRACT Of the many models of intestinal adaptation, the structural and functional changes seen in the residual small bowel following jejunectomy or ileectomy are the most predictable and best studied. There are three major mechanisms for these adaptive phenomena: changes in *i*) luminal nutrition, *ii*) pancreatico-biliary secretions and *iii*) hormonal factors. Observations in a unique patient with an "enteroglucagon"-secreting tumor of the kidney associated with massive small bowel enlargement, provided the strongest evidence, at that time (>30 y ago), in favor of hormonal factors. When the patient's renal tumor was removed, the markedly increased circulating concentrations of the glucagon-like peptide (now presumed to be GLP-2) returned to normal–as did her intestinal anatomy. Subsequent studies showed that there are increased tissue and plasma enteroglucagon (and recently GLP-2) levels in many animal models of intestinal adaptation. This, and anecdotal evidence from three other case reports, coupled with contemporary studies of GLP-2, strongly suggest that this glucagon-like peptide is a potent, but not the sole, enterotrophin. J. Nutr. 133: 3703–3707, 2003.

KEY WORDS: • *intestinal adaptation* • *small bowel resection* • *glucagon-like peptide 2* • *human history*

The results of studies from several laboratories around the world have defined the multiple effects of the glucagon family of peptides, and particularly of glucagon-like peptide-2 (GLP-2),[3] on the gastrointestinal (GI) tract [reviewed recently in (1,2)]. GLP-2 inhibits gastric acid secretion, enhances intestinal sugar transport and slows gastric emptying (1). Arguably however, the most important property of GLP-2 in the GI tract is its enterotrophic effect (3–5) and, therefore, its potential therapeutic role in patients with intestinal insufficiency secondary to extensive disease or resection of the small bowel (6).

This enterotrophic effect falls within the orbit of intestinal adaptation; a topic which has been studied extensively during the past 40–50 y. The purpose of this contribution, therefore, is to review selected aspects of the history of intestinal adaptation, with particular emphasis on the role of GLP-2. It also recalls lessons drawn from clinical observations in patients with "enteroglucagon"-secreting tumors (7–11), and in those with adaptive mucosal changes in the intestinal mucosa, particularly as a result of extensive small bowel resection.

Historical perspective

The first reported case of successful small bowel resection in man is attributed to the French surgeon, Koeberlé. In his 1881 case report (12), he describes how the operation lasted more than 3 h and notes that the patient recovered satisfactorily "with recourse to the antiseptic techniques of Lister." This report was followed, over the next 30 y, by accounts of small bowel resection in animals (13,14). At that time, the principal preoccupation was with the maximum length of small bowel that could be removed that was still compatible with life. The most significant early account of intestinal adaptation by the Baltimore surgeon, Flint, was in 1912 when he clearly demonstrated "marked villous hypertrophy in remaining small intestine following extensive resection in dogs" (14). Then in 1929, Brenizer (15) asked ". . . the most important question: does functional recovery (16) depend upon compensatory hypertrophy of the remaining small intestine?" He went on to answer his own rhetorical question by suggesting, speculatively, that "functional recoveries in man, as in dogs, are [indeed] likely to depend on compensatory hypertrophy."

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[3] Abbreviations used: CCK, cholecystokinin; GI, gastrointestinal; GLP-2, glucagon-like peptide-2.

The author's interest in this field began some 40 y ago with clinical studies of patients who had undergone massive small bowel resection (16). This often resulted in diarrhea, progressive weight loss and malnutrition secondary to malabsorption of fat and other major nutrients. Most of these patients had lost varying lengths of distal small intestine, with the most extensive resections also involving portions of the jejunum. However, in patients with extensive small bowel resection, the presence of some or all of the residual colon was particularly important in determining a successful outcome (17). Apart from studies designed to optimize the clinical management of these patients, and metabolic balance studies to determine the pattern of their nutrient malabsorption, the first questions addressed were: *i*) are there structural and functional changes in the residual small bowel, and *ii*) are there regional differences in the adaptive response of the proximal and distal small intestine? In other words, our initial aim was to define the phenomena of intestinal adaptation, not only in humans but also in a number of animal species, particularly in the rat.

Intestinal adaptation in humans

Intestinal dilatation. In patients who have undergone intestinal resection, barium studies often show obvious dilatation of the residual intestine that is unrelated to other causes such as mechanical obstruction (16). Similar observations have been made in patients who have undergone small bowel by-pass, followed some time later by direct measurements of intestinal caliber during clinically-indicated laparotomy. These studies confirmed that the small bowel which remained in continuity was indeed dilated in contrast to the narrow and contracted bowel excluded from luminal stimuli (18). This dilatation alone increases the intestinal absorptive surface area, independent of changes in crypt:villus architecture.

Adaptive mucosal hyperplasia. After distal small bowel resection in humans, the adaptive mucosal hyperplasia in the jejunum is relatively modest (compared to ileal adaptation following proximal resection). Because most patients with small bowel resection have undergone removal of the distal intestine, quantitative morphometric measurements have largely been confined to per-oral biopsies from the jejunum. Nonetheless, the results of these studies show that after ileectomy there is a small, but statistically significant, increase in jejunal villus height (19).

Segmental mucosal hyperfunction. For ethical and logistical reasons, there have been very few measurements of segmental absorptive function in the jejunum of patients who have undergone distal small bowel resection. However, several reports show that there is, indeed, enhanced absorption per unit length of residual jejunum, when compared with that in matched controls (16).

Intestinal adaptation in experimental animals

Results obtained with experimental animal models confirm that structural and functional adaptation occurs in the remnant intestine after both jejunectomy and ileectomy, but particularly in the ileum following proximal small bowel resection (20). Indeed, the ileal changes following jejunectomy are, undoubtedly, the most predictable and widely studied in the field of intestinal adaptation (19).

Macroscopic changes. After jejunectomy, there is obvious dilatation of the residual ileum. This is most marked immediately distal to the anastomotic site (the first part of the ileum to receive a richer than normal supply of luminal or topical nutrients), and decreases gradually towards the ileo-caecal region (20). In contrast, after ileal resection the jejunal remnant shows only limited dilatation, which increases gradually in an ab-oral direction to reach a maximum just before the anastomosis (20).

Microscopic changes. The histological changes in the mucosa of the small bowel remnant are even more impressive and consistent. There are taller villi and deeper crypts, although for the most part, the ratio of villus height:crypt depth does not change. This contrasts with the intestinal response to injury or disease where, as a repair mechanism, there is also increased cell proliferation but an altered villus height:crypt depth ratio with deep crypts but short villi (19). Although there have been occasional reports of changes in the structure of individual epithelial cells and their organelles, such as microvilli (21), most investigators find that the size of the individual enterocyte does not change during adaptation. This means that the taller villi and deeper crypts are associated with increased numbers of cells (hyperplasia) rather than increased size of cells (hypertrophy).

Changes in cell proliferation. The key cytokinetic event in adaptive intestinal mucosal hyperplasia is an increase in the crypt cell production rate (22). This is associated with increases in the crypt cell mitotic rate, mitotic index and migratory rate. As a result of these changes, the total time taken from cell birth in the crypt to cell shedding at the villus tip, may not be greatly changed (23). Some authors believe that, as a result of rapid cell migration, the villi become populated with functional immature cells (19). This has important implications for the parameters used to express results of intestinal transport/absorption studies in the adapted intestine. Although early studies of cytokinetics in intestinal adaptation focused mainly on cell proliferation (23), more recent studies have shown that there are also changes in apoptosis. The results of these studies show that apoptosis is reduced in the residual intestine after small bowel resection (24).

Changes in intestinal digestive and absorptive function. The combination of intestinal dilatation and epithelial hyperplasia increases the absorptive surface area. It is not surprising, therefore, that segmental perfusion studies show corresponding increases in the absorption of most nutrient substrates, when the results are expressed per unit length intestine (19). With digestive enzymes there is no consistent pattern of results, regardless of the method of data expression. If any change occurs during adaptation, the digestive and absorptive function of individual cells from the adapted intestine diminishes, in keeping with the concept that the villi become populated with functional immature cells. Nonetheless, for some enzymes such as the disaccharidases, there may be substrate-enzyme induction with increases in enzyme specific activity in the adapted intestine (25).

Mechanisms for intestinal adaptation

A detailed discussion of the various experimental models used in the past to define the mechanisms for intestinal adaptation is beyond the scope of this review. Moreover, since the broad conclusions drawn from these studies have been summarized above and elsewhere (19,26), the present discussion is confined to the "bottom-line" messages about the principal mechanisms for intestinal adaptation. When taken together, the results of these studies suggest that three factors play a major mechanistic role in intestinal adaptation, namely: *i*) luminal (or topical) nutrition, *ii*) pancreatico-biliary secretions and *iii*) hormonal factors. Neural factors and changes in regional blood flow to the intestine may also be important but they fall outside the scope of this review.

Luminal nutrition. In general, excess luminal nutrition (whether absolute or relative to normal) stimulates intestinal mucosal hyperplasia whereas the intestine deprived of luminal nutrition becomes hypoplastic. Indeed, luminal nutrients also play a major role in the adaptive response to small bowel resection (27). Based on the results of studies in which the ileal adaptive response to jejunectomy was compared in orally- and parenterally-fed animals, we concluded (almost certainly simplistically), "no luminal nutrition, no adaptation" (27).

In fact, there are several objections to this naive conclusion. First, at a cellular and molecular level, the earliest adaptive changes occur in the remnant intestine within hours of resection independent of whether or not there is luminal nutrition (28). Second, in the experimental model of lactation, the jejunal adaptive response occurs equally in the presence or absence of luminal nutrients. In lactating, and thus hyperphagic, rats there is striking mucosal hyperplasia and hyperfunction (29) that occurs equally in jejunal segments by-passed from continuity (and therefore excluded from luminal nutrients and pancreatico-biliary secretions) as in intact jejunum (29). The results of this study clearly show that, in lactation at least, luminal nutrition is not all-important in the adaptive response. These findings also extended the suspicion raised in other experiments [reviewed in (19,26,28)] that circulating hormonal factors might also be important.

Despite these caveats, the importance of luminal nutrition in the maintenance of normal small bowel structure and function is inescapable. Further evidence in support of luminal nutrition came from studies of intestine deprived of this stimulus, either as a result of a Thiry-Vella by-pass (30) or in animals receiving total parenteral nutrition (TPN) (27,31). As noted above, in both these situations the absence of exogenous luminal nutrition leads to obvious mucosal hypoplasia and hypofunction. Indeed, the TPN model in experimental animals has been used extensively to study the effects of putative enterotrophins (including GLP-2) infused parenterally. Furthermore, specific luminal nutrients, such as ingested carbohydrates and fats, appear to be important in stimulating the release of GLP-2 and in promoting adaptive mucosal hyperplasia in the small intestine (32). A possible sequence showing schematically how luminal nutrients might trigger an intestinal adaptive response is illustrated in **Figure 1**.

Pancreatico-biliary secretions. The simple but classical studies by Altmann and colleagues (33) clearly showed that exocrine pancreatic secretions (and to a lesser extent bile) are trophic to self-emptying loops of ileum. During TPN, not only the gut but also the pancreas becomes hypoplastic (34) leading us to postulate that the intestinal mucosal hypoplasia of TPN could be secondary to a reduction in enterotrophic pancreatic secretions—rather than to the exclusion of exogenous luminal nutrition. Indeed, dogs maintained with TPN alone showed the expected intestinal mucosal hypoplasia. In contrast, dogs which also received intravenous cholecystokinin (CCK) and secretin showed no such hypoplasia; instead, their villus height remained unchanged before and after 6 wk of total parenteral nutrition(35). The conclusion from this limited study was that the combination of CCK and secretin was potentially enterotrophic, although we could not exclude an effect mediated indirectly through the exocrine pancreatic secretions. Nonetheless, this provided one further link in the chain of evidence implicating hormonal factors in intestinal adaptation.

Hormonal factors. In the midst of studying intestinal adaptation in animal models, serendipitous observations in a unique patient who had a renal tumor associated with massive small bowel enlargement (7) provided the strongest evidence

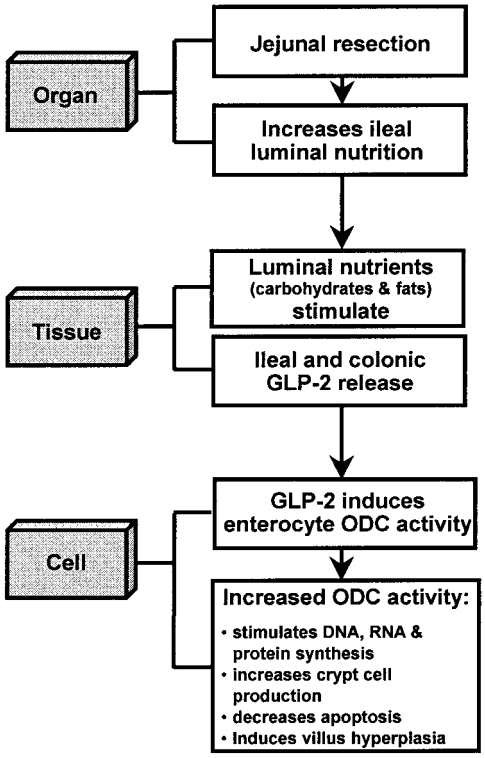

FIGURE 1 Hypothetical sequence explaining how, at the organ, tissue and cellular levels, jejunectomy might stimulate adaptive ileal mucosal hyperplasia through luminal nutrients and GLP-2 release. Abbreviations: GLP-2, glucagon-like peptide-2; ODC, ornithine decarboxylase.

at that time, now over 30 y ago, in favor of enterotrophic hormones.

Clinical and historical perspectives of "the enteroglucagon story." In parallel with his routine clinical duties, a young research collaborator, Dr Michael Gleeson, had been studying intestinal adaptation in animals (23,30). We were investigating a 44-y-old German woman who presented with hypoproteinemic edema. Despite the fact that she suffered from slow-transit constipation, we found that she also had steatorrhea and impaired glucose tolerance (7).

Investigation of hypoproteinemic edema. We found no abnormalities of hepatic or renal function to explain her hypoproteinemia and therefore undertook barium studies of the GI tract to exclude protein-losing enteropathy. To our surprise, this showed a very abnormal small bowel that was markedly dilated with coarse mucosal folds. Because these radiological findings did not conform to any known clinical entity, the patient underwent an exploratory laparotomy. This confirmed massive generalized enlargement of the small intestine. Although there was no obvious local pathology, a full thickness surgical biopsy was taken from the jejunum to exclude such diseases as a diffuse lymphoma. None was found and the histology of the surgical biopsy was basically normal with the important exception those the villi were huge, measuring significantly more than that in matched controls. Indeed, the hyperplastic villi were easily visible to the naked eye.

Recurrent urinary tract infection. During her postoperative recovery, fate took a hand. The patient developed several unexplained bouts of urinary tract infection. These precipitated an intravenous pyelogram which, in turn, generated a second clinical surprise. There was a large (7 cm) tumor

occupying the middle third of the right kidney. Gleeson made an inspired leap of imagination and speculated that this was no ordinary hypernephroma, but rather a hormone-secreting tumor that was responsible for the massive intestine enlargement.

Peptide hormone secreting renal tumor. In the days before ultrasonography, computerized tomography, MRI and positron-emission tomography scans, and before the widespread use of guided-needle biopsies or fine-needle aspiration, we decided that the kidney should be explored surgically. Gleeson organized a team of photographers, pathologists and gut hormone experts to attend the operation. His prediction was correct; the histopathology, electron microscopy and immunofluorescent studies showed that the tumor consisted of sheets and ribbons of epithelial cells that were full of secretory granules. By today's standards, the antisera available to measure the different fractions of the proglucagon-derived peptides were limited (8). However, by using two different immunoassays that measure total glucagon and pancreatic glucagon separately, our collaborators, Drs. Bloom and Polak, were able to estimate "enteroglucagon" levels in pre- and postnephrectomy serum and in the freshly excised renal tumor tissue (7). They confirmed by immunofluorescence that the tumor "lit up" brilliantly with antiglucagon antiserum. Bloom also showed that the preoperative circulating levels of plasma enteroglucagon were high, but rapidly returned to normal after the tumor was removed (7). And in support of his enlightened clinical speculation, Gleeson demonstrated that the radiological appearance of the small intestine also reverted to normal (7). Intraperitoneal injections of simple saline extracts of the tumor in mice, compared with saline alone, resulted in marked enlargement in the recipient mouse intestine (36).

Over the years, there have been three other reports of thickened folds in the small bowel mucosa and/or giant villi that proved to be due to malignant enteroglucagon-secreting tumors (9–11). Although the cumulative evidence strongly favored an enterotrophic role for the gut glucagon family of peptides, for many years proof was lacking. Indeed, the hypothesis was challenged by Gregor and colleagues (37) when they developed antisera to a fraction of the gut glucagon family and gave it parenterally to animals with intestinal adaptation. Although they were able to prevent the rise in circulating "enteroglucagon" levels, the antisera had no effect on the intestinal mucosal hyperplasia (37). Although this negative evidence was also indirect, it raised serious doubts about the significance of the previous findings. It was not until the full sequence of the proglucagon-derived peptides was characterized (38), and the definitive studies by Drucker and colleagues (3,4) carried out, that it was proved once and for all that GLP-2 was the missing link in these clinical experiences.

Future considerations

There are several alternative enterotrophins to GLP-2 and at this stage it is far from clear that GLP-2 is the most potent of the intestinal growth stimulating peptides. Studies determining how much of the spontaneous adaptive response to small bowel resection is due to GLP-2 versus other enterotrophins are eagerly anticipated. Equally important questions pertain to receptor and signaling biology, tissue specificity of the hyperplastic response, mucosal epithelial kinetics and practical consideration regarding optimal dosing criteria. Perhaps the answers to some or all of these questions will form the basis for another symposium on GLP-2 at a future Experimental Biology meeting.

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