Glucagon-like peptide-2 induces intestinal adaptation in parenterally fed rats with short bowel syndrome

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Glucagon-like peptide-2 induces intestinal adaptation in parenterally fed rats with short bowel syndrome. Am J Physiol Gastrointest Liver Physiol 286: G964–G972, 2004. First published February 12, 2004; 10.1152/ajpgi.00509.2003.—Glucagon-like peptide-2 (GLP-2) is an intestinal trophic enteroendocrine peptide that is associated with intestinal adaptation following resection. Herein, we investigate the effects of GLP-2 in a total parenteral nutrition (TPN)-supported model of experimental short bowel syndrome. Juvenile Sprague-Dawley rats underwent a 90% small intestinal resection and jugular catheter insertion. Rats were randomized to three groups: enteral diet and intravenous saline infusion, TPN only, or TPN + 10 μg·kg⁻¹·h⁻¹ GLP-2. Nutritional maintenance was isocaloric and isonitrogenous. After 7 days, intestinal permeability was assessed by quantifying the urinary recovery of gavaged carbohydrate probes. The following day, animals were euthanized, and intestinal tissue was processed for morphological and crypt cell proliferation (CCP) analysis, apoptosis (caspase-3), and expression of SGLT-1 and GLUT-5 transport proteins. TPN plus GLP-2 treatment resulted in increased bowel and body weight, villus height, intestinal mucosal surface area, CCP, and reduced intestinal permeability compared with the TPN alone animals (P < 0.05). GLP-2 treatment induced increases in serum GLP-2 levels and intestinal SGLT-1 expression (P < 0.01) compared with either TPN or enteral groups. No differences were seen in the villus apoptotic index between resection groups. Enterally fed resected animals had a significant decrease in crypt apoptotic indexes compared with nontreated animals. This study demonstrates that GLP-2 alone, without enteral feeding, stimulates indexes of intestinal adaptation. Secondly, villus hypertrophy associated with adaptation was predominately due to an increase in CCP and not to changes in apoptotic rates. Further studies are warranted to establish the mechanisms of action and therapeutic potential of GLP-2.

Short bowel syndrome (SBS) typically occurs following removal of >50% of the small intestine. An inadequate intestinal surface area results in chronic difficulties with nutrient malabsorption (14, 42, 52, 56). The residual intestine does adapt by increasing both intestinal surface area and absorptive enzymatic capacity, which increases nutrient absorptive capacity per unit length of intestine (14, 42, 56). The increase in surface area is primarily due to an increase in the rate of intestinal crypt cell proliferation, which, in turn, results in an increase in villus size and, likely, secondarily increases bowel diameter and possibly length (14, 35, 56). The factors stimulating this compensatory regulation of absorptive capacity are poorly understood, but they appear to be driven largely by the provision of enteral nutrients, predominantly fat (6, 42, 48, 51, 52, 56). This is particularly relevant following massive small bowel resection (>80% of bowel length), because patients often cannot tolerate significant amounts of enteral nutrients and are therefore reliant on total (or partial) parenteral nutrition (TPN). Unfortunately, there are adverse consequences associated with TPN maintenance such as intestinal atrophy, electrolyte and metabolic alterations, increased intestinal permeability, sepsis, and cholestasis (6, 48, 51, 53). In the pediatric population, TPN cholestasis and secondary liver dysfunction are major causes of morbidity and mortality (4, 32, 42, 53); thus the development of a therapeutic strategy that could enhance the intestinal adaptive response and reduce the requirements for TPN would be invaluable.

Glucagon-like peptide-2 (GLP-2), a 33-amino acid member of the pituitary adenylate cyclase-activating peptide/glucagon superfamily (15, 41), has been shown to be trophic to the entire gastrointestinal tract; it is a potential candidate for the treatment of gastrointestinal disorders associated with inadequate mucosal function (15). GLP-2 is secreted from ileal and colonic enteroendocrine L cells in response to both proximal enteric neuronal signaling and the presence of luminal nutrients (21, 38, 39, 57) and is involved in the regulation of small intestinal motility, epithelial permeability, crypt cell proliferation, and nutrient absorption (23, 25, 28, 40, 43, 50). Mechanistically, GLP-2 activates a specific G protein-coupled receptor (30, 34) that has been localized to the murine enteric neuronal system (2); however, there are conflicting data suggesting that GLP-2 acts on intestinal epithelial cells directly (55, 58, 59).

Numerous studies (5, 16, 17, 50) demonstrate that GLP-2 administration results in intestinal hypertrophy as determined by increases in villus height, crypt depth, protein content, and bowel weight. GLP-2 has also been shown to increase the activity of specific sugar transport proteins (SGLT-1 and GLUT-2), intestinal blood flow, and glucose uptake (10, 20). Additionally, GLP-2 treatment reduces mucosal atrophy associated with TPN administration and augments the adaptive growth response following small bowel resection (7, 8, 9, 19, 22, 49).

The description of the intestinal trophic effects of GLP-2 suggests that it may be involved in the process of intestinal adaptation. In rats, we have previously shown a significant correlation between serum GLP-2 levels and the occurrence of intestinal adaptation (28). In these studies, GLP-2 release was greatly increased following intestinal resection; these levels were highly correlated both with the degree of nutrient malab-

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absorption and the morphological features of intestinal adaptation. However, studies aimed at proving the hypothesis that GLP-2 directly stimulates the adaptive process are limited by the lack of knockout models, pharmacological blockers, or neutralizing antibodies (15). Accordingly, following a massive intestinal resection leaving remnant ileum, we compared the adaptation stimulating effects of enteral nutrition with TPN or TPN plus exogenous GLP-2. We show that in animals maintained with TPN following intestinal resection, the addition of GLP-2 alone was sufficient to trigger the classic morphological and functional features of postresection adaptation.

MATERIALS AND METHODS

Animals and Nutritional Maintenance

Animal studies were conducted under the guidelines established by the Canadian Council of Animal Care following the approval of the Animal Care Committee at the University of Calgary. Male Sprague-Dawley (SD) rats weighing 250–275 g were housed in metabolic cages and acclimated to their environment for 10 days before experiments. Animals were maintained under standardized temperature, humidity, and 12:12-h light-dark cycles. All animals were fed a liquid diet (Ensure Plus, Abbott Laboratories, Saint-Laurent, PQ), 75.5 kilocalories/day for 3 days before surgery. With our previously described methods (44), animals underwent a 90% proximal small intestinal resection, leaving 10 cm of intestinal ileum anastomosed to the jejunum 2 cm below the ligament of Treitz. All procedures were done following an overnight fast using halothane anesthetic and aseptic technique. Animals received preoperative and postoperative cefazolin throughout the study (50 mg·kg⁻¹·day⁻¹; Novapharm, Toronto, ON). Procedures were done with the aid of an operating microscope. Bowel anastomoses were completed with interrupted sutures of 6-0 silk (Davis-Geck, Peterborough, ON), and the abdomen was closed with a 4-0 absorbable suture. The animals were allowed to recover for 24 h, were reanesthetized, weighed, and an intravenous catheter was inserted into the right jugular vein using a silastic catheter, outer diameter 1.6 mm (Bentec Medical, Woodland, CA), tunneled out of the back, and attached to a standard free swivel device (Harvard Laboraboties, Boston, MA). TPN was delivered by a multichannel syringe pump (Pump 22 infusion pump, Harvard Apparatus). All animals were maintained with 0.9% normal saline solution for the first 12 h and then randomly assigned to a treatment group for 8 days. All animals received isocaloric/isonitrogenous nutritional support consisting of 75.5 kcal·rat⁻¹·day⁻¹; 2.8 g protein, 2.0 g fat, and 11.4 g carbohydrate. The enterally fed resected animals were maintained with a 1 ml/h 0.9% saline infusion through their intravenous catheter and received 64 ml/day of a liquid diet (Ensure Plus, Abbott Laboratories). A nontreated group was also included; these animals were fed using an identical regimen of 64 ml of the liquid diet for 8 days and did not receive any surgery. The parenterally maintained animals received 54 ml/day of TPN [Travasol, 10% amino acid (Abbott Laboratories)]. A nontreated group was also included; these animals received an intraperitoneal injection of 50 mg/kg 5-bromo-2′-deoxyuridine (BrDU; Sigma-Aldrich, St. Louis, MO) 1 h before death. After 60 min, the rats were anesthetized with an intravenous injection of ketamine (10 mg/kg), and blood was immediately drawn by cardiac puncture for GLP-2 determinations. Animals were then euthanized with an overdose of pentobarbital sodium (50 mg/kg iv, Huntingdon Laboratories, Toronto, ON), and tissues were harvested.

In Vivo Absorption/Permeability Evaluations

Intestinal absorption of 3-0 methylglucose (3-0 MG), mannitol (Man), and lactulose (Lac) was determined as previously described (31, 44). Rats were preconditioned to handling by daily sham gavage during the acclimatization period. On day 7, the animals were gavaged with a 2-ml solution containing 60 mg 3-0 MG, 40 mg Man, and 60 mg Lac (352 mosM). Subsequently, the urine output was collected over the next 20 h, the volume was recorded, and samples were frozen for analysis. The samples were batch analyzed for the presence of the carbohydrate probes by HPLC. In brief, the urine was filtered through a 0.4-μm filter, diluted, deionized, and then injected on to a Dionex MA-1 ion-exchange column (Dionex, Sunnyvale, CA). The sugars were eluted with NaOH at a flow rate of 0.4 ml/min with concentrations ranging from 400–600 μM/l and the peaks detected using pulse and amperometric detection on a Dionex HPLC and quantitated as peak areas. Calibration was performed daily with authenticated samples. The data are reported as absorbance, reflecting the percentage of the gavaged amount recovered in the urine.

Tissue/blood collections. On day 8, following the completion of the urine studies, animals received an intraperitoneal injection of 50 mg/kg 5-bromo-2′-deoxyuridine (BrDU; Sigma-Aldrich, St. Louis, MO) 1 h before death. After 60 min, the rats were anesthetized with an intravenous injection of ketamine (10 mg/kg), and blood was immediately drawn by cardiac puncture for GLP-2 determinations. Animals were then euthanized with an overdose of pentobarbital sodium (50 mg/kg iv, Huntingdon Laboratories, Toronto, ON), and tissues were harvested.

Gross morphology and histology. Gross intestinal morphology was assessed in all groups as previously described (31, 44). Briefly, the mesentery was opened, and with the use of a standardized tension, bowel length was measured from the anastomotic site to the ileoecal valve along the antimesenteric border: the proximal and distal intestinal circumference was measured 2 cm below the ligament of Treitz and 2 cm above the ileoecal valve. In the nontreated group, the terminal 10 cm of ileum above the ileoecal valve were analyzed. The distal ileal tissue was quickly dissected, opened along the mesenteric border, and rinsed in cold saline. The intestinal remnant was weighed, and samples for morphological analysis were harvested 2 cm below the anastomosis and fixed in 10% formalin. The adjacent aboral 4 cm of small bowel and a portion of the ascending colon were frozen for protein analysis. Paraffin sections were cut at 6 μm of thickness and stained with hematoxylin and eosin for analysis.
GLUCAGON-LIKE PEPTIDE-2 INDUCES INTESTINAL ADAPTATION

Table 1. Body and organ weights

<table>
<thead>
<tr>
<th>Group</th>
<th>Small Bowel Weight, g</th>
<th>Small Bowel %BWT</th>
<th>Colon %BWT</th>
<th>Body Weight, % change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>0.98 ± 0.04</td>
<td>0.28 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>2.33 ± 0.6</td>
</tr>
<tr>
<td>Enteral</td>
<td>2.37 ± 0.1†</td>
<td>0.83 ± 0.04†</td>
<td>0.42 ± 0.08</td>
<td>−2.88 ± 0.9†</td>
</tr>
<tr>
<td>TPN</td>
<td>1.2 ± 0.08†</td>
<td>0.46 ± 0.05†</td>
<td>0.34 ± 0.04</td>
<td>−11.8 ± 2.0†</td>
</tr>
<tr>
<td>TPN + GLP-2</td>
<td>2.1 ± 0.09†</td>
<td>0.81 ± 0.04†</td>
<td>0.52 ± 0.09</td>
<td>−4.24 ± 0.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Total parenteral nutrition (TPN) only group compared with enteral and TPN + glucagon-like peptide-2 (GLP-2) groups. †P < 0.01. Nontreated control animals compared with enterally fed resected and TPN + GLP-2 groups (†P < 0.05). Small bowel weight and measurement were assessed from the anastomotic site in the remnant ileum to the ileocecal valve. Groups/daily nutrients: nontreated animals received no surgery and were fed 75.5 kcal/day of Ensure plus (Abbott laboratories); Enteral orally fed 75.5 kcal/day Ensure plus; TPN, received 75.5 kcal of nutrients intravenously; TPN + GLP-2, same as TPN with the addition of 10 μg·kg−1·day−1 of GLP-2 iv. %BWT, tissue assessed as a percentage of final body wt.

then stained with hematoxylin and eosin. An observer that was blinded to the origin of the intestinal tissue assessed morphology. For each sample slide, microscopic measurements of villus height, villus width, crypt depth, and number of villi per 100 μm were recorded from immunostained well-oriented villi/crypt units (33). The serosal and villus surface area was used to calculate intestinal surface area using methods discussed previously (33, 44).

Crypt cell proliferation. Crypt cell proliferation rates were quantified using BrdU as an active cell division marker (11). Deparaffinized sections were denatured with 1 M HCl for 1 h at 60°C and then blocked for 2 h with a PBS-blocking solution containing 10% normal goat serum and 1% BSA. The ileal tissue was then incubated in a 1:200 dilution of monoclonal mouse BrdU primary antibody (Sigma) in the same blocking solution at 4°C overnight. Crypt cells incorporating BrdU were detected by immunofluorescence with a Cy3-conjugated anti-mouse IgG secondary (Jackson Immunoresearch Laboratories, West Grove, PA). Ten consecutive well-oriented crypts per slide were reviewed by an observer blinded as to tissue source and were reported as the number of BrdU staining cells per crypt.

Apoptotic index. To assess treatment effects on apoptosis in the three resection groups, tissue levels of activated caspase-3 were quantified using Western blot analysis of mucosal homogenates: all four groups had caspase-3 localized using immunohistochemistry.

Western blot analysis. Previously snap-frozen mucosal scraping samples were thawed, homogenized in 2.5 mM EDTA, and then aliquots were placed in Laemmli’s sample buffer (Sigma). The protein concentration was determined by Lowry assay (26). Protein samples were separated by SDS-polyacrylamide gel electrophoresis and electro transferred onto polyvinylidene difluoride transfer membranes (Amersham Pharmacia Biotechnology, Buckinghamshire, England). The resulting blot was blocked with 5% nonfat dry milk in PBS containing 0.05% Tween 20 and then incubated for 48 h at 4°C in a 1:1000 dilution of antiactive caspase-3 antibody (BD PharMingen) or a 2% BSA/PBS blocking buffer (control). After several washings, the slides were incubated with a 1:25 dilution of HRP-conjugated anti-rabbit IgG for 2 h at room temperature. Diaminobenzidine (Vector kit) was added to visualize activated caspase-3. Slides were counterstained with Mayer’s hematoxylin and analyzed. Counts are reported as caspase-3-positive staining cells per crypt or villus.

Nutrient transporters. To assess treatment effects on intestinal transporter capacity after resection, tissue levels of SGLT-1 and GLUT-5 in the three resection groups were quantified using Western blot analysis. The tissue preparation was identical as described for caspase-3 Western blot analysis. Blots were incubated for 24 h at 4°C with 1:5,000 anti-SGLT-1 antibody or 1:2,500 anti-glut 5 (Chemicon). The membrane was then washed and incubated with HRP-anti-rabbit IgG at 1:3,000 (Amersham) for 2 h at room temperature with detection methods as described above.

Serum GLP-2 Quantification

Blood draws were completed in the morning (16 h postprandial in the enterally fed animals) to assess basal GLP-2 levels. The blood was drawn into heparinized syringes, transferred into chilled EDTA tubes, and centrifuged (in <10 min from original draw) at 2,500 × g for 10 min at 4°C. The serum was collected and placed into cryovials containing 1 mM PMSF (in 90% ethanol), quickly frozen on dry ice, and stored at −70°C until analysis. With the use of previously described methods (49), total GLP-2 levels (1–33 and 33–33) were measured using an ELISA based on a polyclonal antibody specific for native GLP-2. The ELISA uses a capture antibody (rabbit polyclonal, ALE 0303, NPS Pharmaceuticals, Mississauga, ON) raised against the first 18 residues of native GLP-2 (ELISA GLP-2 analysis done in a blinded fashion courtesy of NPS Pharmaceuticals).

Statistical Methods

All data are expressed as means ± SE. The comparisons among groups were done using a one-way ANOVA followed by a Tukey’s multiple-comparison posttest. Statistical analysis was completed using Graph Pad Prism version 3.0 software (Instat, San Francisco, CA).

RESULTS

There were no significant differences in fasting total GLP-2 levels between the TPN and enterally resected groups, whereas TPN plus GLP-2 infusion caused a significant increase in the basal level of GLP-2. In the TPN-treated animals, GLP-2 levels were 0.6 ± 0.1 ng/ml; in fasted (basal level) enterally treated animals, 0.7 ± 0.1 ng/ml; and in GLP-2-supplemented TPN animals, 6.0 ± 1.2 ng/ml. For comparative purposes, in a previous study (28), we have shown that enterally fed animals that had received a 90% intestinal resection using the identical

Table 2. Functional measures of adaptation

<table>
<thead>
<tr>
<th>Group</th>
<th>3-O-MG, %</th>
<th>Mannitol, %</th>
<th>Lactulose, %</th>
<th>L/M Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>87.9 ± 5.1</td>
<td>5.7 ± 0.7</td>
<td>0.8 ± 0.2</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Enteral</td>
<td>79.9 ± 2.6</td>
<td>8.2 ± 0.9</td>
<td>1.5 ± 0.4</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>TPN + GLP-2</td>
<td>79.7 ± 0.6</td>
<td>8.3 ± 1.1</td>
<td>2.0 ± 0.3</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>TPN</td>
<td>77.6 ± 4.2</td>
<td>7.6 ± 1.6</td>
<td>2.6 ± 0.5†</td>
<td>0.31 ± 0.02†</td>
</tr>
</tbody>
</table>

Values are means ± SE. 3-O methylglucose (3-O-MG), mannitol, and lactulose values reported as %absorption. †P < 0.01 vs. non-treated group; †P < 0.05 vs. enterally resected group; †P < 0.05 vs. all other groups. Groups: nontreated control animals received no surgery and 75.5 kcal Ensure Plus daily; Enteral, orally fed 75.5 kcal Ensure Plus; TPN, received 75.5 kcal of nutrients intravenously; TPN + GLP-2, same as TPN with the addition of 10 μg·kg−1·day−1 of GLP-2. L/M, lactulose/mannitol as a ratio.
experimental protocol applied in this study had postprandial GLP-2 levels of 2.6 ± 1.2 ng/ml.

In general, the enterally fed resected animals and the TPN plus GLP-2 animals showed remarkably similar intestinal adaptive changes (Table 1). Over 8 days, the enterally fed resected and TPN plus GLP-2 animals had a slight decrease in weight, whereas TPN-only maintained animals had a significant loss of weight, despite receiving equivalent nutritional input (Table 1). TPN-only maintained animals also had a significant decrease in small bowel weight both in absolute and relative terms compared with the other resection groups. There was a significant increase in small bowel weight in both the enterally fed resected and TPN plus GLP-2 animals, as well as an increase in colon weight relative to body weight in the TPN plus GLP-2 animals, when compared with the nontreated animals (Table 1). There were no differences in kidney or liver weight between groups (kidney weight as %body wt: nontreated, 0.41 ± 0.01; enteral, 0.44 ± 0.01; TPN, 0.49 ± 0.04; TPN + GLP-2, 0.52 ± 0.09. Liver weight as %body weight: nontreated, 3.46 ± 0.11; enteral, 3.37 ± 0.16; TPN, 4.11 ± 0.29; TPN + GLP-2, 4.03 ± 0.18).

Intestinal Permeability

Enteral resection and TPN plus GLP-2 animals showed no changes in active 3-0 MG or paracellular permeability (Lac/Man ratio). However, TPN alone animals showed a significant increase in intestinal permeability as measured by increased Lac clearance and Lac/Man ratios (Table 2; P < 0.01). There were no significant differences in the other intestinal permeability parameters between the enterally fed resected and TPN plus GLP-2 animals.

Gross and Microscopic Morphology

Gross and microscopic morphology parameters are outlined in Table 3. After 7 days, enterally fed resected and TPN plus GLP-2 animals had growth in the remnant ileal segment, as noted by significant increases in villus height, small bowel length, and small bowel surface area, when compared with the nontreated bowel group. There were no significant differences between enterally resected and TPN plus GLP-2 animals compared with nonresected controls (measured over the distal 10 cm of ileum) or TPN-alone animals (Table 3).

Crypt Cell Proliferation

Ileal crypt cell proliferation changes as measured by BrdU immunoreactivity are shown in Fig. 3. A and B. Crypt cell

Fig. 2. Ileal morphology. Histology sections (hematoxylin and eosin) representative of nontreated (A), TPN (B), enterally fed resected (C), and TPN + GLP-2 distal ileum (D). TPN-maintained rats (B) had a significant reduction in villus height compared with enterally fed resected (C) and TPN + GLP-2 (D) rats (Table 3). There was no difference between enteral (C) and TPN + GLP-2 (D) rats. Representative photos visualized at magnification: ×10.
proliferation was significantly reduced in the TPN-alone animals compared with the enterally fed resected and TPN plus GLP-2 animals. GLP-2 increased crypt cell proliferation to values that were not significantly different from normally adapting, enterally fed resected animals.

**Active Caspase-3 Expression**

No significant difference in apoptosis was seen in mucosal homogenates, villus compartment, or crypt region between any of the resected groups as assessed both by immunoreactivity to active caspase-3 (Fig. 4, A and B) or by Western blot analysis (Fig. 5). There was a significant decrease in the apoptotic index in the crypt region of enterally fed resected animals compared with the nonresected controls (Fig. 4B, left; P < 0.05).

**Nutrient Transporters**

The TPN plus GLP-2 group had significantly increased SGLT-1 protein expression in ileal mucosal scrapings compared with both the TPN and enterally fed resected rats (Fig. 6). There were no measurable differences in SGLT-1 protein expression between enterally fed resected and TPN-maintained animals. There was a trend to increased GLUT-5 transporter protein expression with GLP-2 treatment (Fig. 7; P < 0.06).

**DISCUSSION**

In this study, we have demonstrated that GLP-2 infusion is able to stimulate the morphological and functional parameters of intestinal adaptation in the absence of enteral nutrients. GLP-2 administration to TPN-maintained resected rats prevented bowel and body weight loss, sustained normal morphological adaptation and epithelial barrier function, stimulated ileal crypt cell proliferation, and increased the protein expression of the SGLT-1 cotransporter. Previously, we have reported (28) that in enterally maintained animals, the degree of nutrient malabsorption following resection correlates with an increase in postprandial serum GLP-2 levels and that this rise is further correlated with intestinal adaptation. The present study demonstrates that the trophic effects of GLP-2 occur in the absence of enteral nutrients.

Both the permeability to the synthetic disaccharide Lac and the Lac/Man ratio were significantly increased following TPN therapy. This noninvasive test is useful for measuring changes...
in paracellular diffusion of molecules across the intestinal barrier, because an increased amount recovered in the urine following an oral dose indicates abnormal intestinal permeability (27, 31). Infusions of GLP-2 were able to reverse TPN-induced increases in intestinal permeability, suggesting that GLP-2 helps to maintain epithelial barrier function.

The effects on animal weight with GLP-2 treatment in this model are notable. In previous work from our laboratory with this model (43), we have shown that the remnant ileum undergoes dramatic adaptive upregulation of nutrient absorption by 7–10 days postresection, with a weight profile similar to that seen in the present study. In the present study, we were surprised to see that the TPN-alone animals lost significantly more weight than the enterally fed or TPN plus GLP-2 animals, despite receiving identical nutrients. Typically, weight gain is equivalent in rats maintained with isocaloric nutrients delivered intravenously or enterally (24). Although GLP-2 is directly trophic to the small bowel and colon, the actual weight gain of these two organs in the TPN plus GLP-2 group was several grams, whereas the net difference in animal weight was on the order of 15 g in the GLP-2-treated animals. The mechanisms underlying these observations are not clear but may speak to an improvement in nutrient handling or metabolism induced by GLP-2.

Crypt cell proliferation as assessed by BrdU incorporation was significantly reduced in the TPN rats compared with the enterally fed resected and TPN plus GLP-2 rats. The infusion of GLP-2 in the absence of enteral nutrients was able to increase crypt cell proliferation rates to values similar to the normally adapting, enterally fed resection animals. This is an important finding, because it is well established that the intestinal epithelium is constantly replaced by cell replication within the crypt region followed by a migration of these descendants onto the small intestinal villus epithelium (36). Thus, after injury, the typical patterns of epithelial differentiation are reestablished by the migration and differentiation of cells from the crypt (11, 36). In the present study, the stimulated increase of crypt cell proliferation rates appears to be the principal mechanism by which GLP-2 increased villus height and absorptive mucosal surface area. This observation supports the findings of Bjerknes and Cheng (2), who noted that GLP-2 stimulation increased the production of crypt cell progenitors in mice. Additionally, Potten et al. (37) reported that the administration of ALX-0600, a GLP-2 synthetic analog, increased the height and area of small intestinal crypts and resulted in an increase in crypt cell survival following radiation exposure. However, little is understood about the mechanisms involved in the hormonal and/or neural regulation of epithelial

**Fig. 4.** A: active caspase-3 immunoreactivity in the crypt region of the terminal ileal mucosa. Representative of active caspase-3 staining (arrows) in the distal ileum of nontreated (a), enterally fed resected (b), TPN (c), and TPN + GLP-2 groups (d). Note the reduction in caspase-3-immunopositive cells in resected animals (b–d) compared with nontreated (a) tissue. As described in MATERIALS AND METHODS, anti-active caspase-3 antibody visualized with horseradish peroxidase-conjugated anti-rabbit IgG. Slides were counterstained with Mayer’s hematoxylin. Counts were reported as caspase-3-positive staining cells per crypt. B: active caspase-3 immunoreactivity in the crypt (left) and villus (right) region of the terminal ileal mucosa. There was a significant reduction in the apoptotic index in the crypt region of enterally fed resected animals vs. nontreated animals (*P < 0.05). Data expressed as means ± SE active caspase-3 immunoreactive cells per crypt or villus.
cell replication, differentiation, or migration in normal or wounded intestine.

Studies (6, 7, 12) have shown that TPN feeding induces intestinal apoptosis and decreases enterocyte proliferation in intestinally intact rats, whereas massive intestinal resection increases both the rate of crypt cell and enterocyte apoptosis (35, 45). Notably, in the current study, there was no difference in activated caspase-3 expression, a key mediator of apoptosis in mammalian cells, among the enterally fed resected, TPN only, or the TPN plus GLP-2-maintained animals. The present study used a marker of the final stages of the apoptotic cascade and so should directly reflect any GLP-2-related effects on apoptotic signaling in the crypt or villus compartment of the intestinal mucosa. Furthermore, previous studies examined animals with an intact bowel, whereas we assessed resection-induced intestinal adaptation. We found that the animals that received a resection and were enterally fed had a significant decrease in their crypt apoptotic index when compared with nontreated, intact animals, and there was a trend in the other resected animal groups to a similar decrease in crypt region apoptosis. This supports the suggestion of previous workers that adaptation to resection reduces apoptosis (35).

Others have shown (4) that twice-daily administration of GLP-2 to mice over 10 days significantly reduced jejunal apoptosis when compared with nontreated controls. In contrast, our results show that GLP-2 administration to 90% resected, TPN-maintained rats did not reduce ileal apoptosis when compared with resected, nontreated, enterally fed and/or TPN-alone rats. This could be a result of the difference in apoptotic markers used in the different studies, species, or surgical model differences, or may reflect differential responses attributable to the jejunal and ileal regions. Because no differences were noted in the remnant ileum, this suggests that changes in apoptotic signaling did not contribute to an accumulation of cells in the villus in either TPN plus GLP-2 or nutrient-stimulated adaptation in the ileum of enterally fed resected rats. However, these studies were done only over a short time and require confirmation over a longer, more clinically relevant time course.

The significant upregulation of ileal SGLT-1 protein expression following intestinal resection and GLP-2 infusion over 8 days is an important finding. It has been shown that GLP-2 induces both an increased insertion of both SGLT-1 and GLUT-2 into the apical and basolateral membranes, respectively (1, 9). This suggests a mechanism whereby GLP-2 could increase nutrient transport into the cell via upregulation of apical SGLT-1 transporters as well as increasing the exit of nutrients from the enterocyte via increased activity of the GLUT-2 transporter. This also corroborates the findings noted in a human trial using a GLP-2 analog for treatment of SBS. These patients had significantly increased SGLT-1 mRNA abundance in the mucosa of jejunal biopsies (47). Previous
investigators have demonstrated that GLP-2 administration in mice results in a differential expression of crypt progenitors that favors columnar rather than mucous cells (2). The columnar cells are known to be the source of the surface absorptive epithelial cells at the apex of the villus; thus the preferential selection of columnar cells following GLP-2 stimulation may give rise to a larger population of cells capable of generating SGLT-1.

A second possibility is that SGLT-1 receptor expression is enhanced directly by GLP-2 interactions with the enterocyte. Recently, it has been shown that the stimulation of an uncharacterized “sensor” initiates a G protein-coupled receptor (GPCR) cAMP-dependent signaling pathway that upregulates SGLT-1 receptor expression independent of glucose metabolism (18). Other supportive evidence proposes that the mucosal GLP-2 receptor is linked to the activation of a cAMP/protein kinase A-dependent, growth-promoting pathway in vitro (55). We theorize that GLP-2 initiates a cascade involving a GPCR linked to a cAMP-PKA-dependent pathway, which results in enhanced SGLT-1 expression and the increase in functional SGLT-1 transporters. This requires further study.

This mechanistic pathway may also be involved in the enigma of how GLP-2 activates the intestinal epithelium. It has been demonstrated that GLP-2 acts, at least in part, through a G protein-coupled receptor localized to enteroendocrine cells in the human gastrointestinal tract (30, 34), suggesting that GLP-2 actions are mediated directly through receptors on the enterocyte. Recent supportive data demonstrated that intestinal mucosal cells isolated from rat intestine express mRNA for the GLP-2 receptor and that these epithelial cells respond to GLP-2 stimulation via a cAMP-dependent pathway resulting in increased thymidine incorporation (55). Others (2, 54) report that in murine species, the GLP-2 receptor is not located on the enterocyte but is localized to the neuronal elements of the enteric nervous system. In one of these studies, the administration of GLP-2 to mice stimulated a rapid increase in c-Fos expression, a marker of neuronal activation, in the myenteric plexus followed shortly by an increase in crypt cell c-Fos expression. The GLP-2-stimulated increase in c-Fos expression was abolished by the topical coadministration of a blocker (tetrodotoxin) of neuronal activation (2). In a separate study (29), we have found that short-term administration of GLP-2 to rats stimulates a significant increase in c-Fos expression in both the myenteric and submucosal plexus. These findings are suggestive of a novel pathway of hormonal-neural signaling in which the administration of GLP-2 activates enteric neuronal activity, stimulating the release of as yet undefined mediators that induce crypt cell proliferation. An additional possibility is that GLP-2 actions are mediated through an uncharacterized GLP-2-sensitive receptor, because additions of the ligand have been shown to prolong the survival of cultured IEC-18 cells despite the fact that these cells do not express the characterized GLP-2 receptor and have no neuronal input (46).

These results demonstrate that the infusion of GLP-2 can stimulate intestinal adaptation in TPN-maintained SBS animals. GLP-2 therapy also maintained epithelial barrier function and increased both crypt cell proliferation and weight gain in the absence of enteral nutrients, supporting the development of GLP-2 into a therapeutic strategy that could enhance intestinal adaptation and reduce the consequences of TPN. The results of the present study do not provide further information as to the potential mechanistic pathways by which GLP-2 induces the adaptive changes noted; however, the extent of the increases in mucosal mass and nutrient transporter activity noted with GLP-2 treatment underscores its potential clinical relevance. Clearly, there is a very significant and biologically important effect of GLP-2 on intestinal function and whole animal physiology that strongly supports further research into the use of GLP-2 as a therapy.

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