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Exogenous Glucagon-Like Peptide-2 (GLP-2) Augments GLP-2 Receptor mRNA and Maintains Proglucagon mRNA Levels in Resected Rats

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Abstract

Background—Glucagon-like peptide-2 (GLP-2) is a nutrient-dependent proglucagon-derived hormone that stimulates intestinal adaptive growth. Our aim was to determine whether exogenous GLP-2 increases resection-induced adaptation without diminishing endogenous proglucagon and GLP-2 receptor expression.

Methods—Rats underwent transection or 70% jejunioileal resection ± GLP-2 infusion (100 µg/kg body weight/d) and were fed a semipurified diet with continuous infusion of GLP-2 or saline by means of jugular catheter. After 7 days, body weight, mucosal cellularity (dry mass, protein and DNA), crypt–villus height, and crypt cell proliferation (by bromodeoxyuridine staining) were determined. Plasma bioactive GLP-2 (by radioimmunoassay), proglucagon and GLP-2 receptor mRNA expression (by Northern blot and real-time reverse transcriptase quantitative polymerase chain reaction) were measured. GLP-2 receptor was colocalized to neuroendocrine markers by immunohistochemistry.

Results—Low-dose exogenous GLP-2 increased mucosal cellularity and crypt–villus height in the duodenum, jejunum, and ileum; enterocyte proliferation in the jejunal crypt; and duodenal and jejunal sucrase segmental activity. Plasma bioactive GLP-2 concentration increased 70% upon resection, with an additional 54% increase upon GLP-2 infusion in resected rats ($P < .05$). Ileal proglucagon mRNA expression increased with resection, and exogenous ileum GLP-2 failed to blunt this response. Exogenous GLP-2 increased ileum GLP-2 receptor expression 3-fold in resected animals and was colocalized to vasoactive intestinal peptide-positive and endothelial nitric oxide synthase-expressing enteric neurons and serotonin-containing enteroendocrine cells in the jejunum and ileum of resected rats.

Conclusions—Exogenous GLP-2 augments adaptive growth and digestive capacity of the residual small intestine in a rat model of mid–small bowel resection by increasing plasma GLP-2

concentrations and GLP-2 receptor expression without diminishing endogenous proglucagon expression.

Keywords

intestinal failure; intestinal adaptation; GI hormones; short bowel syndrome; bowel resection

Massive resection of the intestine without adequate intestinal adaptation may lead to short bowel syndrome (SBS). SBS includes a complex of symptoms related to inadequate nutrient and water absorption and increased GI transit that are associated with malnutrition, vitamin deficiencies, and dehydration. This condition, which affects approximately 30,000 people in the United States, often requires parenteral nutrition for survival, a modality that is costly and leads to excessive morbidity and mortality due to its various complications.¹ While increasingly effective, the current surgical treatments for SBS, intestinal transplantation and intestinal lengthening procedures have been restricted to a small number of patients due to scarce donor organs and limited indications.^{2,3} Thus, there is a need to develop alternative therapies, such as utilization of intestinotrophic hormones, for individuals with SBS.

Glucagon-like peptide-2 (GLP-2) is a 33-amino acid hormone that is derived from proglucagon, a peptide of 160 amino acids that undergoes tissue-specific posttranslational processing in the enteroendocrine L cells of the ileum and colon.^{4,5} GLP-2 secretion is primarily stimulated by luminal nutrients, but plasma concentrations of GLP-2 and ileum proglucagon gene expression have also been shown to increase following massive intestinal resection.^{6–10} Moreover, endogenous GLP-2 has been correlated with postresection intestinal adaptation in animal models of mid–small bowel resection both in the presence and absence of luminal nutrients, and this postresection adaptation is blocked by immunoneutralization of GLP-2.^{8,11–13}

GLP-2 is a key mediator of resection-induced intestinal adaptive growth. GLP-2 administration augments the adaptive response to massive intestinal resection in animal models receiving oral feedings alone and in SBS models using parenteral nutrition, or parenteral nutrition plus supplemental enteral nutrients.^{14–17} In human studies, GLP-2 improves absorption and nutrition parameters in patients with reduced endogenous GLP-2 secretion due to massive bowel resection and SBS.¹⁸ Clinical trials using teduglutide, a GLP-2 analogue resistant to degradation by dipeptidyl peptidase IV, have shown safety and evidence of adaptation in patients with SBS.¹⁹ Given its promise as a novel treatment modality for intestinal failure, we sought to determine whether exogenous GLP-2, administered at a low dose, enhances the intestinotrophic response to massive small bowel resection without down-regulating endogenous proglucagon and GLP-2 receptor expression as well as plasma GLP-2 concentrations in orally fed rats.

Materials and Methods

Animals and Experimental Design

We investigated the effect of exogenous GLP-2 on the intestinotrophic response to massive small bowel resection in the rat. The animal facilities and research protocols were approved by the University of Wisconsin Madison Institutional Care and Use Committee. Male Sprague-Dawley rats (Harlan, Madison, WI) initially weighing 175–200 g were housed in individual stainless steel cages with unlimited water access. The animal facilities were maintained at 22°C on a 12:12-hour light–dark cycle. All animals were acclimated to their new environment for 5–7 days while being fed semipurified elemental powdered diet ad libitum.²⁰ Rats were randomly assigned to the following treatment groups using a 2 × 2

factorial design (n = 8–11 per group in 3 blocks): transection alone, resection alone, transection with GLP-2 infusion, and resection with GLP-2 infusion.

Surgical Procedures and Animal Care

On the day of surgery, rats were anesthetized by inhalation of isoflurane (Isoflo; Abbot Laboratories, North Chicago, IL) by means of an anesthetic machine. After anesthesia, rats underwent a 70% mid-jejunoileal resection or transection as described previously.⁸ Briefly, 70% of the small intestine was resected from 15 cm distal to the ligament of Treitz to 15 cm proximal to the cecum. The remaining 30 cm of small intestine was repaired primarily with an end-to-end anastomosis using 6-0 silk suture. Transected animals received a single transection and primary anastomosis 15 cm proximal to the cecum. Five milliliters of intraperitoneal saline was given for fluid resuscitation. Resected animals lost approximately 5 g of intestine due to surgery. The peritoneum was closed with absorbable suture, and the abdominal skin incision was closed with wound clips. After abdominal closure, an IV catheter was placed in the superior vena cava by means of the external jugular vein as previously described.²⁰ Rats received oxymorphone (0.18 mg/kg body weight) every 6 hours for 24 hours after surgery for analgesia, and ampicillin (200 mg/kg body weight) was administered every 12 hours for 48 hours postoperatively as perioperative prophylaxis.²¹

Rats infused with GLP-2 received human GLP-2 (33 amino acids, preproglucagon 126–158; CA Peptide Research Inc, Napa, CA) as a 100 µg/kg body weight/d continuous infusion for 7 days after surgery. This was the lowest dose of GLP-2 found to return GLP-2 plasma concentrations in parenteral nutrition-fed animals back to those values seen in orally fed rats.²² Rats not receiving GLP-2 were infused with vehicle (0.9% phosphate buffered saline) as a continuous infusion. All rats were fed a semipurified diet throughout the 7-day study. At 7 days after surgery, the rats were anesthetized with isoflurane and killed by exsanguination, at which time the entire small and large intestine, liver, and kidneys were removed for analysis.

Intestinal Composition and Histology

After removal, 1 cm of intestine on either side of the anastomosis was removed and discarded. The remaining intestine was sectioned into duodenum, jejunum (15 cm distal to ligament of Treitz), ileum (15 cm proximal to the cecum), and colon. All segments were flushed with ice-cold saline and placed on a chilled glass plate for further sectioning. The first 2 cm of each section were used for measuring wet and dry mucosal mass. The third cm was fixed in 10% formalin for 24 hours, transferred to 70% ethanol, paraffin-embedded, cut into 5-µm sections, and stained with hematoxylin and eosin for histomorphology as previously described.²³ The next 2 cm were used to determine mucosal protein (bicinchoninic acid protein assay; Pierce Chemicals, Rockford, IL) and DNA content, along with sucrase activity.^{24,25} The remaining sections from each segment of bowel were snap-frozen intact in liquid nitrogen and stored at –70°C for RNA extraction.

Jejunal Proliferation

Jejunal epithelial cell proliferation was quantified using bromodeoxyuridine (BrdU) as an S-phase marker. Briefly, transected and resected animals receiving GLP-2 infusion were injected with BrdU (0.2 mg/kg body weight) 1 hour before sacrifice and jejunal sections were prepared as previously described.²⁶ Ten consecutive well-oriented crypts per slide were identified, and the number and position of BrdU labeled cells on 1 side of each crypt were counted by a single observer blinded to the treatment group assigned to each slide. Proliferation index was calculated as the number of BrdU-labeled cells/total number of cells per crypt column.

Plasma Bioactive GLP-2

Blood was collected in chilled tubes containing 1 mg of ethylenediaminetetraacetic acid, 0.1 mmol Diprotin A/L (MP Biomedicals, Aurora, OH), and 0.01 mmol aprotinin/L (Calbiochem, La Jolla, CA). Plasma bioactive GLP-2 was quantified by radioimmunoassay with the use of an antibody specific to the N-terminus of GLP-2.⁴

RNA Extraction and Quantification of Proglucagon and GLP-2 Receptor (GLP-2R) mRNA

Total RNA was extracted from intact ileum using the TRIzol reagent (Gibco BRL Life Technologies, Grand Island, NY). Quantification of proglucagon mRNA was done using the Northern Max kit (Ambion, Austin, TX).²⁰ Proglucagon and 18S bands were quantified by light densitometry using OPTIQUANT version 03.00 software (Packard Instruments, Meriden, CT).

Proglucagon and GLP-2R mRNA expression was measured in a 2-step reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) using SYBR Green detection method. Ten micrograms of jejunum and ileum total RNA was treated with DNase (TURBO DNA-free kitTM, Ambion) to eliminate genomic DNA and reverse transcribed using random hexamers (High-Capacity cDNA Reverse Transcription Kit; Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Control reactions without reverse transcriptase were performed to confirm the specificity of transcription reaction. cDNA was diluted to a concentration of 10 ng/μL based on a template titration assay and real-time qPCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). No reverse transcriptase control and no template control reactions were done with every assay to ensure the specificity of the reaction and absence of any contamination. Sequences for primers were as follows (Integrated DNA Technologies, Inc, Coralville, IA).

Proglucagon: the forward primer was 5'-GAA TTC ATT GCT TGG CTG GT-3' and the reverse primer was 5'-TTC CTC AGC TAT GGC GAC TT-3', making a 72-bp size amplicon.

GLP-2R: the forward primer was 5'-CGA CGA CCA AGT TCA AGG AT-3' and the reverse primer was 5'-TCC ATT GGC AAA GCC ATA CT-3', making a 109-bp size amplicon.

A 4-step hot-start real-time PCR was performed using Applied Biosystems 7000 Real-Time PCR instrument (Applied Biosystems) with conditions as follows: step 1, 50°C for 2 minutes; step 2, 95°C for 10 minutes; step 3, 50 cycles of 95°C for 0.15 minute followed by 55°C for 1 minute; step 4 (dissociation), 95°C for 0.15 minute, 60°C for 0.20 minute, and 95°C for 0.15 minute. The dissociation step was performed to confirm the uniformity of amplicon size and absence of any primer dimers. The 18S qPCR was performed under similar conditions using QuantumRNATM 18S Internal Standards (Ambion). Equal efficiency of amplification was verified for all assays using the serial dilutions technique. Data were analyzed using 7000 system software (Applied Biosystems) and relative quantification was done using $\Delta\Delta C_{(t)}$ method with 18S as the internal control and fed group as reference control.²⁷

Immunohistochemistry

Tissue from rats in each treatment group was used to colocalize the GLP-2R with neuroendocrine markers in the jejunum and ileum via fluorescence immunohistochemistry. Slides were prepared as previously described.²⁸ Cryostat tissue sections (10μm) of jejunum and ileum were mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA). Slides were dried for 3 minutes on a warming table, washed with phosphate buffered saline (PBS)

for three 5-minute intervals, and then blocked with 10% goat serum (GS; Vector Laboratories, Burlingame, CA), in 0.2% Triton X-100, and 0.1% bovine serum albumin in PBS (ICC buffer) at room temperature for 30 minutes. The slides were then incubated in ICC buffer with NGS overnight at 4°C with a primary rabbit antibody raised in our lab against the N-terminal of the rat GLP-2R diluted at 1:1000. The next day, sections were washed with PBS and then incubated with 1:400 Cy3 labeled goat anti-rabbit Fab fragment in ICC buffer and incubated 1 hour at room temperature. Sections were then washed with PBS, incubated in unconjugated goat anti-rabbit IgG fab fragments (10µg/mL), washed with PBS and then with ICC buffer, and incubated overnight at 4°C with one of the following second step primary antibodies provided by Dr Brownfield: anti-chromogranin A antibody (1:1000); anti-serotonin (5-HT) antibody (1:1000); anti PGP 9.5 antibody (1:10); anti-vasoactive intestinal peptide (VIP; 1:200); anti-endothelial-derived nitric oxide synthase (eNOS; 1:1000). The slides were then rinsed in PBS 3 times for 20 minutes each and incubated with FITC conjugated goat anti-rabbit or goat anti-mouse IgG antibodies (where appropriate) and incubated at room temperature 1 hour. Slides were then washed with PBS twice for 15 minutes at room temperature and then once overnight at 4°C. Slides were then mounted under Polymount^T (Polysciences) with cover slips. Immunostained sections were viewed and photographed using a Nikon Eclipse E600 microscope equipped with a Spot^T digital camera (Diagnostic Instruments, Sterling Heights, MI).

Statistical Analyses

SAS version 8.2 (SAS Institute, Cary, NC) was used for statistical analysis. The differences between treatment groups were examined by 1-way and 2-way ANOVA followed by the protected least-significant-differences technique. General linear models were used to analyze the main effects of the 2 treatments (resection and GLP-2), block effects, and their interactions. Data from gels were analyzed using the linear mixed-effects model and statistics were performed on log-transformed data for proglucagon mRNA because residual plots of this data set indicated unequal variance among groups. All values are presented as means $\pm$ standard error; P .05 was considered statistically significant.

Results

Body Weight

Changes in final body weight are shown in Figure 1. There were no significant differences in body weight between treatment groups before surgery and on the day of surgery mean body weight was 246 ± 16 g. Transected rats given GLP-2 had a significantly greater final body weight than did transected rats given vehicle alone, whereas the anabolic effects of GLP-2 was not significant in resected animals. Resection also decreased weight gain. Food intake was not significantly different across the treatment groups.

Intestinal Adaptive Growth

Rats given resection alone showed adaptive growth of the residual jejunum and ileum as demonstrated by significantly increased mucosal dry mass and concentrations of protein and DNA (Figures 2 and 3). Resection also led to significantly increased duodenal and colonic dry mass and duodenal mucosal protein concentration (Figure 4, colon data not shown). The infusion of exogenous GLP-2 led to an additional significant increase in the concentrations of protein and DNA in jejunal mucosa.

Mid-small bowel resection resulted in significantly greater villus height and crypt depth in rat duodenum, jejunum, and ileum, as did treatment with GLP-2 in animals that underwent transection control surgery (Table 1). Rats that underwent resection with GLP-2 infusion showed an additional significant increase in villus height in duodenum, jejunum, ileum, and

crypt depth in ileum. For jejunal–villus height, a significant interaction was observed ($P < .05$) between treatment with resection and GLP-2.

Sucrase Activity

Both resection and exogenous GLP-2 significantly increased sucrase segmental activity ($\mu\text{mol}/\text{min}/\text{cm}$) in duodenal and jejunal mucosa (Figures 5A and 5B). Resection increased jejunal sucrase activity by 65% and GLP-2 administration led to an additional 59% increase. Resection significantly increased sucrase segmental activity in ileal mucosa, however, unlike in the jejunum, there was no significant additional increase with GLP-2 infusion (Figure 5C). Sucrase specific activity ($\mu\text{mol}/\text{min}/\text{mg}$ protein) in duodenal, jejunal and ileal mucosa was not significantly changed by resection or GLP-2 administration (data not shown).

Enterocyte Proliferation in the Jejunum

Jejunal crypt cell proliferation as measured by BrdU staining was significantly increased by GLP-2 administration in resected rats when compared with animals that underwent resection alone (Table 2). The number of BrdU-labeled cells was increased by 79% and the proliferation index was increased by 90% in rats undergoing resection plus GLP-2 infusion compared with resection controls.

Plasma Bioactive GLP-2 and Expression of Proglucagon mRNA

There was a significant increase in plasma bioactive GLP-2 with exogenous GLP-2 administration in both transected and resected rats when compared with saline-infused controls. The concentration of bioactive GLP-2 in plasma increased 70% with resection, and increased an additional 54% with GLP-2 infusion in resected rats (Figure 6A).

Mid–small bowel resection resulted in a significant (5-fold) increase in ileum proglucagon mRNA expression when compared with transection control surgery using Northern blot analysis (Figure 6B). Interestingly, exogenous GLP-2 led to an additional 89% increase in ileum proglucagon mRNA expression in resected rats, which was statistically significant. Of note, this increase in ileum proglucagon mRNA expression in response to exogenous GLP-2 was not seen in rats undergoing transection surgery.

Real-time quantitative RT-PCR confirmed the significant increase in ileum proglucagon mRNA expression in response to mid–small bowel resection shown by Northern blot analysis, although not to the same magnitude (Figure 6C). RT-PCR, however, did not show a significant difference in ileum proglucagon expression with resection plus GLP-2 administration compared with resection alone.

GLP-2R mRNA Expression and Receptor Localization

There was no significant difference in jejunal GLP-2R expression in response to resection (Figure 7A); however, GLP-2 administration had a significant main effect to increase GLP-2R. In contrast, there was a significant 3-fold increase in GLP-2R expression in the ileum of resected rats treated with GLP-2 compared to resected controls given saline (Figure 7B). There was also a 39% increase in GLP-2 expression in the ileum of resected saline animals compared to transected controls that did not reach statistical significance. Moreover, resection significantly increased GLP-2R mRNA levels in animals treated with resection compared with transection.

We used our anti-rat GLP-2R antibody to localize GLP-2R immunoreactivity in the small intestine of rats undergoing mid–small bowel resection. GLP-2R was colocalized to cells immunoreactive to the enteroendocrine cell-specific protein chromogranin A as well as the

neurotransmitter 5-HT, demonstrating that GLP-2R is expressed in serotonin-expressing enteroendocrine cells in rat jejunum and ileum (Figure 8, ileum data not shown). In addition, colocalization of GLP-2R protein to cells immunoreactive to the neuron-specific marker PGP 9.5 as well as the VIP and eNOS proteins confirms GLP-2R expression in VIP- and eNOS-expressing enteric neurons in rat jejunum and ileum (Figure 9, ileum data not shown).

Discussion

Administration of intestinotrophic hormones such as GLP-2 may lead to improved therapies for SBS. This study demonstrates for the first time that low-dose exogenous GLP-2 augments the intestinal adaptation induced by mid-small bowel resection without diminishing endogenous GLP-2 and proglucagon responses. Treatment with low-dose GLP-2 significantly increased mucosal cellularity and crypt-villus height in the duodenum, jejunum, and ileum, and enterocyte proliferation in the jejunal crypt. In addition, resection led to an increase in sucrase segmental activity in duodenum, jejunum, and ileum, with GLP-2 administration inducing a further increase in the duodenum and jejunum. This result is consistent with other reports that show increased intestinal function after GLP-2 administration.^{29,30} The increase in sucrase segmental activity (activity per centimeter of intestine), and not specific activity (activity per milligram of protein), suggests that the increase in sucrose metabolism is due to mucosal hyperplasia and epithelial growth, not increased expression of the enzyme.

When comparing the response between treatment groups, the addition of GLP-2 to transection surgery increased mucosal dry mass, protein, and DNA in the duodenum and jejunum, but not ileum, and the addition of GLP-2 to resection surgery increased mucosal protein, DNA, and sucrase activity in jejunum, but not ileum. Furthermore, resection and GLP-2 treatment induced a synergistic response (based on significant statistical interaction) resulting in greater villus height and mucosal surface area in jejunum, with a trend toward a synergistic increase in crypt depth. Thus, resection alone stimulates adaptation in both the proximal and distal small intestine, and the addition of GLP-2 to resection further augments growth and function in the proximal but not distal intestine. This differential growth is consistent with previous reports that show a trophic response to post-resection GLP-2 administration in the proximal intestine, but not the ileum.¹⁷ The mechanism is unclear, but may reflect that GLP-2 has both paracrine and endocrine effects on target tissues. Since ileum produces proglucagon, it may have a higher local tissue concentration of GLP-2 in response to resection than jejunum, with GLP-2 providing a primarily paracrine stimulus. Jejunum does not make proglucagon and shows greater expression of GLP-2R relative to ileum, and thus may be more responsive than ileum to the endocrine stimulus of exogenous GLP-2.^{4,5,31}

This phenomenon may also have clinical significance, as patients with intestinal failure due to a proximal bowel resection may not be as responsive to GLP-2 treatment as those whose failure is a result of a distal bowel resection. In fact, rats that have undergone proximal resection have been shown to have a 3-fold increase in plasma GLP-2 levels, as compared to a 2-fold increase in distally resected animals.⁹

This study uses a low dose of GLP-2 (100 µg/kg body weight/d) compared with similar studies in which GLP-2 was also given to animals undergoing bowel resection, with doses ranging from approximately 200 to 240 µg/kg body weight/d.^{16,17} This dose was chosen based on a dose response study conducted in parenteral nutrition-fed rats as the lowest dose that would restore plasma GLP-2 concentrations to those of orally fed rats.²² The dose was previously shown to induce adaptation in a parenteral nutrition-dependent rat model of SBS.¹⁴ However, this is the first study to confirm that a low dose of GLP-2 induces

adaptation in a resected, orally fed model. This finding is significant because the model of mid-small bowel resection used in this study leaves residual ileum in situ, providing a larger potential source of endogenous GLP-2 production. Despite this greater potential source of endogenous GLP-2, there was still an adaptive response to low-dose exogenous GLP-2 comparable to that in other studies using a similar extent of resection but a higher dose of GLP-2.

The mechanism behind increased plasma GLP-2 levels after resection likely involves increased production of the GLP-2 precursor, proglucagon, rather than decreased GLP-2 deactivation by the serine protease enzyme dipeptidyl peptidase IV (DPP-IV).³³ Indeed our study confirmed increased expression of proglucagon mRNA in ileum following resection. One aim of this study was to assess whether exogenous GLP-2 down-regulates endogenous proglucagon expression. Using two different methods of mRNA quantification, RT-qPCR and Northern blot analysis, we have shown that this is not the case. As this is the first study to measure proglucagon expression in orally-fed resected rats treated with GLP-2, comparison studies are needed to confirm our finding of no decrease in proglucagon expression in resected rats given a low-dose of GLP-2.

We have also demonstrated for the first time that ileum GLP-2R expression is increased with resection. Of note, resection combined with exogenous ileum GLP-2 leads to a 3-fold increase in ileum GLP-2R mRNA levels when compared with resection controls. That GLP-2R expression undergoes a significant increase only at the higher plasma GLP-2 levels associated with resection combined with GLP-2 suggests a threshold plasma concentration at which GLP-2R expression is up-regulated. This may have clinical significance in regard to the timing of GLP-2 administration in intestinal failure. Human studies to date treating SBS with GLP-2 analogs have only included patients who are 1–20 years from their last resection.^{19,34} Given that GLP-2 administered perioperatively in our study markedly increased GLP-2R expression, the optimal time to initiate GLP-2 therapy may be in the immediate postresection period in patients deemed to be at risk of intestinal failure due to the extent of their resection and the residual length and function of their remaining bowel. Animal studies in which GLP-2 treatment is initiated at different time points after resection are needed to determine whether the adaptive response to GLP-2 is time-dependent.

GLP-2R expression is known to be greater in the jejunum compared to the ileum as noted in this study.³⁵ Thus, our observation of no increase in GLP-2R expression due to resection in the jejunum may be a reflection of this increased baseline receptor density. Likewise, the ileum showed a trend for greater GLP-2R expression with resection. Importantly, GLP-2 infusion did not decrease GLP-2R expression in the jejunum, further evidence that exogenous GLP-2 does not down-regulate the endogenous adaptive response.

In this study, we show for the first time in a rat resection model that the GLP-2R is colocalized with serotonin-producing enteroendocrine cells and endothelial nitric oxide synthase and vasoactive intestinal peptide-expressing enteric neurons in the jejunum and ileum. This is consistent with findings in other studies that used unresected pig and mouse models.^{36,37} The GLP-2R has also been identified in the brain and on vagal afferents, enteroendocrine cells, and enteric neurons in the unresected rat jejunum and ileum; in enteric neurons in a rat model of inflammatory bowel disease; and in subepithelial myofibroblasts in mice.^{28,38,39} Of interest, the GLP-2R is not found on GI epithelial cells, which are the cells most responsive to the trophic actions of GLP-2. This finding suggests the presence of a downstream mediator linking the GLP-2R on enteroendocrine cells, enteric neurons, and subepithelial myofibroblasts to its target tissue, the intestinal epithelium. Recent evidence suggests that insulin-like growth factor (IGF-1), an intestinotrophic peptide hormone that stimulates intestinal adaptation after bowel resection and mucosal hyperplasia

in parenteral nutrition-fed animals, may play such a mediating role.^{20,23,40} Further studies are needed to elucidate the mechanism of this potential link between GLP-2 and IGF-1.

In summary, exogenous GLP-2 increases plasma GLP-2 concentration, GLP-2R mRNA expression, and augments adaptive growth and digestive capacity of the residual small intestine, while maintaining endogenous proglucagon expression in a rat model of mid-small bowel resection. These findings support the potential clinical use of GLP-2 as a gut-specific trophic agent that enhances intestinal adaptation after massive bowel resection without abrogating the endogenous intestinal adaptive response.

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Figure 1.

Increase in body weight (g) at 7 days after transection or resection surgery in rats treated with GLP-2 or saline vehicle. Values are means $\pm$ SE; $n = 8-11$. $*P < .05$. Main effects of resection and GLP-2 and their interaction, as indicated by 2-way ANOVA, are shown at top right. Res, resection; GLP-2, glucagon-like peptide-2; $R \times G$, interaction between resection and GLP-2 treatments.

Figure 2.

Jejunal mucosal dry mass (A), protein (B), and DNA (C) in rats subjected to transection or resection surgery and treatment with GLP-2 or saline vehicle. Values are means $\pm$ SE; $n = 8-11$. $*P < .05$. Main effects of resection and GLP-2 and their interaction, as indicated by 2-way ANOVA, are shown at top right of each graph. Res, resection; GLP-2, glucagon-like peptide-2; $R \times G$, interaction between resection and GLP-2 treatments.

Figure 3.

Ileal mucosal dry mass (A), protein (B), and DNA (C) in rats subjected to transection or resection surgery and treatment with GLP-2 or saline vehicle. Values are means $\pm$ SE; $n = 8-11$. * $P < .05$. Main effects of resection and GLP-2 and their interaction, as indicated by 2-way ANOVA, are shown at top right of each graph. Res, resection; GLP-2, glucagon-like peptide-2; R $\times$ G, interaction between resection and GLP-2 treatments.

Figure 4.

Duodenal mucosal dry mass (A), protein (B), and DNA (C) in rats subjected to transection or resection surgery and treatment with GLP-2 or saline vehicle. Values are means $\pm$ SE; $n = 8-11$. $*P < .05$. Main effects of resection and GLP-2 and their interaction, as indicated by 2-way ANOVA, are shown at top right of each graph. Res, resection; GLP-2, glucagon-like peptide-2; R $\times$ G, interaction between resection and GLP-2 treatments.

Figure 5.

Sucrase segmental activity in (A) duodenum, (B) jejunum, and (C) ileum in rats subjected to transection or resection surgery and treated with GLP-2 or saline vehicle. Values are means $\pm$ SE; $n = 8-11$. $*P < .05$. Main effects of resection and GLP-2 and their interaction, as indicated by 2-way ANOVA, are shown at top right of each graph. Res, resection; GLP-2, glucagon-like peptide-2; $R \times G$, interaction between resection and GLP-2 treatments.

Figure 6.

Plasma bioactive GLP-2 (A) and ileal proglucagon mRNA expression using Northern blot analysis (B) and real-time quantitative RT-PCR (C) in rats subjected to transection or resection surgery and treated with GLP-2 or saline vehicle. Data were analyzed using $\Delta\Delta C_{(t)}$ method with 18S RNA as internal control and expressed relative to transection plus saline group. Efficiency of primers was checked using the serial dilution method. Values are means $\pm$ SE; $n = 8-11$. $*P < .05$. Main effects of resection and GLP-2 and their interaction, as indicated by 2-way ANOVA, are shown at top right of each graph. Res, resection; GLP-2, glucagon-like peptide-2; R $\times$ G, interaction between resection and GLP-2 treatments.

Figure 7.

GLP-2R mRNA expression in (A) jejunum and (B) ileum in rats subjected to transection or resection surgery and treated with GLP-2 or saline vehicle. Data were analyzed using $\Delta\Delta C_{(t)}$ method with 18S RNA as internal control and expressed relative to transection plus saline group. Values are means $\pm$ SE; $n = 8-11$. * $P < .05$. Main effects of resection and GLP-2 and their interaction, as indicated by 2-way ANOVA, are shown at top right of each graph. Res, resection; GLP-2, glucagon-like peptide-2; GLP-2R, glucagon-like peptide-2 receptor; $R \times G$, interaction between resection and GLP-2 treatments.

Figure 8.

Photomicrographs at $\times 40$ of GLP-2R immunoreactivity in fixed sections of jejunum in rats 7 days after undergoing mid-small bowel resection. Cross-section through crypt epithelium (A) and villus epithelium (B) showing cells with the characteristic shape of enteroendocrine cells (EC) that are immunoreactive for the GLP-2R (in red). The same crypt and villus EC cells were immunoreactive to chromogranin A (B, in green) and serotonin (E, in green), respectively. The GLP-2R protein was colocalized with the EC-specific marker chromogranin A (C, in yellow) and the neurotransmitter serotonin (F, in yellow), confirming GLP-2R colocalization on serotonin-containing enteroendocrine cells in the jejunum of resected rats. GLP-2R, glucagon-like peptide 2 receptor; Chro A, chromogranin A; 5-HT, serotonin.

Figure 9.

Photomicrographs at $\times 40$ of glucagon-like peptide-2 receptor (GLP-2R) immunoreactivity in fixed sections of jejunum in rats 7 days after undergoing mid-small bowel resection. Cross-section through the jejunal myenteric plexus shows enteric neurons that are immunoreactive for the GLP-2R (A, D, and G in red). The same enteric neurons were immunoreactive to protein gene product 9.5 (PGP 9.5, B), vasoactive intestinal peptide (VIP, E), and endothelial nitric oxide synthase (eNOS, H), respectively (all in green). The GLP-2R protein was colocalized with the neuron-specific marker PGP 9.5 (C, in yellow), the neurotransmitter VIP (F, in yellow), and e-NOS (I, in yellow) confirming GLP-2R colocalization on VIP-positive, nitric oxide-expressing enteric neurons in the jejunum of resected rats.

Table 1

Small Bowel Histology at 7 Days After Resection

Treatment mcm	Duodenum		Jejunum		Ileum	
	Villus Height μm	Crypt Depth μm	Villus Height μm	Crypt Depth μm	Villus Height μm	Crypt Depth μm
T + Saline	772 $\pm$ 26 ^c	182 $\pm$ 4 ^b	547 $\pm$ 15 ^c	128 $\pm$ 3 ^b	381 $\pm$ 15 ^d	123 $\pm$ 3 ^c
R + Saline	921 $\pm$ 30 ^b	200 $\pm$ 7 ^a	748 $\pm$ 25 ^b	157 $\pm$ 5 ^a	627 $\pm$ 32 ^b	141 $\pm$ 4 ^b
T + GLP-2	976 $\pm$ 20 ^{ab}	206 $\pm$ 5 ^a	733 $\pm$ 40 ^b	160 $\pm$ 7 ^a	467 $\pm$ 11 ^c	142 $\pm$ 3 ^b
R + GLP-2	1037 $\pm$ 40 ^a	208 $\pm$ 5 ^a	842 $\pm$ 27 ^a	170 $\pm$ 4 ^a	724 $\pm$ 34 ^a	154 $\pm$ 4 ^a
2-way ANOVA, <i>P</i> values						
Resection	.0004	.0533	.0003	.0013	< .0001	.0009
GLP-2	< .0001	.0028	< .0001	< .0001	.0003	< .0001
R $\times$ G	.2512	.2320	.0457	.0801	.7742	.6331

T, transection; R, resection; G and GLP-2, glucagon-like peptide 2; R $\times$ G, interaction between resection and GLP-2 treatments. Values are mcm expressed as means $\pm$ SE; n = 8–16. Means with different superscripts in the same column are significantly different, *P* < .05.

Table 2

Enterocyte Proliferation in the Jejunal Crypt at 7 Days After Resection

Treatment	Cells per Crypt Column, <i>n</i>	Mitotic Cells per Crypt Column, <i>n</i>	Mitotic Index, %
Resection + saline	27.3 ± 0.9	2.8 ± 0.2 *	10 ± 1.2 *
Resection + GLP-2	26.7 ± 0.8	5.0 ± 0.5	19 ± 1.0

Values are expressed as means ± SE; *n* = 8–9.

*
P = .0013 based on *t* test.