Vagal afferents are essential for maximal resection-induced intestinal adaptive growth in orally fed rats

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Nelson, David W., Xiaowen Liu, Jens J. Holst, Helen E. Raybould, and Denise M. Ney. Vagal afferents are essential for maximal resection-induced intestinal adaptive growth in orally fed rats. Am J Physiol Regul Integr Comp Physiol 291: R1256–R1264, 2006; doi:10.1152/ajpregu.00247.2006—Small bowel resection stimulates intestinal adaptive growth by a neuroendocrine process thought to involve both sympathetic and parasympathetic innervation and enterotrophic hormones such as glucagon-like peptide-2 (GLP-2). We investigated whether capsaicin-sensitive vagal afferent neurons are essential for maximal resection-induced intestinal growth. Rats received systemic or perivagal capsaicin or ganglionectomy before 70% midjejunoileal resection or transection and were fed orally or by total parenteral nutrition (TPN) for 7 days after surgery. Growth of residual bowel was assessed by changes in mucosal mass, protein, and DNA, and histology. Both systemic and perivagal capsaicin significantly attenuated by 48–100% resection-induced increases in ileal mucosal mass, protein, and DNA in rats fed orally. Villus height was significantly reduced in resected rats given capsaicin compared with vehicle. Sucrase specific activity in jejunal mucosa was not significantly different; ileal mucosal sucrase specific activity was significantly increased by resection in capsaicin-treated rats. Capsaicin did not alter the 57% increase in ileal proglucagon mRNA or the 150% increase in plasma concentration of bioactive GLP-2 resulting from resection in orally fed rats. Ablation of spinal/splanchnic innervation by ganglionectomy failed to attenuate resection-induced adaptive growth. In TPN rats, capsaicin did not attenuate resection-induced mucosal growth. We conclude that vagal afferents are not essential for GLP-2 secretion when the ileum has direct contact with luminal nutrients after resection. In summary, vagal afferent neurons are essential for maximal resection-induced intestinal adaptation through a mechanism that appears to involve stimulation by luminal nutrients.

capsaicin; glucagon-like peptide-2; parenteral nutrition; proglucagon; sucrase

THE MECHANISMS UNDERLYING intestinal adaptation to bowel resection are largely unknown. This multifactorial process that results in adaptive growth and improved function of the residual bowel is believed to be regulated by luminal nutrients, hormones, neural pathways, and pancreaticobiliary secretions (1, 6, 44, 47). Furthermore, the interactions between luminal nutrients and neuroendocrine signals in regulating intestinal adaptation are poorly understood. Glucagon-like peptide-2 (GLP-2) has emerged as a key hormonal mediator of intestinal adaptive growth (13).

GLP-2 is a peptide derived from tissue-specific posttranslational processing of proglucagon in the endocrine L cells of the ileum and colon (7, 43). The presence of luminal nutrients is the primary stimulus for GLP-2 secretion (48). Luminal nutrients are thought to stimulate GLP-2 secretion by direct contact with ileal and colonic L cells and by an indirect duodenal-ileal neural pathway (15, 40). Plasma concentration of bioactive GLP-2 and ileal proglucagon mRNA expression are positively correlated with resection-induced mucosal growth after midjejunooileal resection in rats (10). Additionally, immunoneutralization of endogenous GLP-2 inhibits resection-induced growth in residual rat ileum (36) and adaptive intestinal growth in diabetic rats (25). Endogenous GLP-2 is also a key regulator of compensatory refeeding-induced growth in mice subjected to fasting followed by refeeding (42). When given to individuals with short bowel syndrome, scubaneous administration of GLP-2 improves villus height and crypt depth, intestinal energy absorption, and ultimately lean body mass (28, 29). Taken together, these observations suggest a role for GLP-2 in intestinal adaptation although the pathway and mechanism of action are unclear.

Despite evidence demonstrating the intestinotrophic effects of GLP-2, there is uncertainty regarding the specific cellular location of the GLP-2 receptor (GLP-2R) in the gastrointestinal tract (7, 20). The trophic actions of GLP-2 are thought to be mediated indirectly by paracrine and/or neural pathways because the GLP-2R does not appear to be expressed by normal crypt cells or enterocytes (17). The GLP-2R has been localized to murine (4) and porcine (20) enteric neurons, subepithelial myofibroblasts (35, 37), and human enteroendocrine cells (49).

Vagal or spinal sensory innervation of the gastrointestinal tract may modulate intestinal secretion of proglucagon-derived peptides such as GLP-1 and GLP-2. For example, duodenal nutrients stimulate ileal release of GLP-1, which is cosecreted with GLP-2, and vagotomy completely blocks this effect in rats with gut transections (40). In contrast, selective in vivo stimulation of the vagus nerve has no effect on secretion of either GLP-1 or GLP-2 in pigs (5, 23). However, transmitters of the enteric nervous system, notably ACh, increase GLP-1 secretion (2, 3, 23). The selective roles of vagal or spinal afferent innervation in GLP-2 responses and resection-induced adaptive growth have not been thoroughly investigated.

This study was conducted to further understanding of the role of the vagal afferent pathway in the regulation of resection-induced intestinal adaptation. Our overall goal was to test the hypothesis that vagal afferents are required for maximal resection-induced intestinal growth by administering capsaicin...
to induce functional ablation of primary extrinsic afferent innervation to the gastrointestinal tract before midjejunouileal resection. Capsaicin, the excitotoxic vanilloid from peppers (26), is used to study afferent innervation of the gastrointestinal tract (27, 38, 46). To further the interpretation of our experiments with capsaicin, we used selective ganglionectomy to rule out input from spinal/splanchnic innervation in resection-induced intestinal growth. The objectives of this study were twofold: first, to determine if vagal afferents are essential for maximal intestinal adaptive growth after 70% midjejunouileal resection in rats and second, to determine if this effect of vagal afferents is dependent on the presence of exogenous luminal nutrients by maintaining rats with total parenteral nutrition (TPN) after 70% midjejunouileal resection.

MATERIALS AND METHODS

Animals and Experimental Design

We conducted a series of four experiments to determine if capsaicin-sensitive vagal afferents are required for resection-induced intestinal mucosal growth in rats subjected to 70% midjejunouileal resection and to elucidate the interaction of GLP-2 with the vagal afferent pathway. The University of Wisconsin-Madison and University of California-Davis Institutional Animal Care and Use Committees approved the animal facilities and protocol. Male Sprague-Dawley rats (Harlan, Madison, WI and San Diego, CA) initially weighing 150–200 grams (6–7 wk old) were housed in stainless steel wire bottom or plastic shoebox cages with unlimited access to water. The animal facilities were maintained at 22°C on a 12:12-h light-dark cycle. All animals were acclimated to the facilities for 5–7 days while being fed a standard rodent diet ad libitum. For the 2 days before intestinal surgery, rats were fed a fiber-free semipurified diet ad libitum to clean the bowel before surgery (Vital; donated by Ross Laboratories, Columbus, OH; see Ref. 10).

We used three approaches, systemic capsaicin, perivagal capsaicin, or ganglionectomy, to manipulate vagal and spinal innervation to the intestine before subjecting rats to 70% midjejunouileal resection. First, rats were pretreated with subcutaneous systemic injections of capsaicin or vehicle 8 days before surgery. Rats were maintained with TPN or oral feeding in two separate experiments for 7 days after surgery. Each experiment, TPN or oral, had a 2 × 2 factorial design with the following treatment groups: transection + vehicle, resection + vehicle, transection + capsaicin, and resection + capsaicin (n = 4–7 rats/transaction group and n = 7–10 rats/resection group). Second, rats were pretreated with perivagal capsaicin application 3 days before midjejunouileal resection. Rats were fed orally for 7 days after midjejunouileal resection. This experiment was a 2 × 2 factorial design with the following treatment groups: transection + vehicle, resection + vehicle, transection + capsaicin, and resection + capsaicin (n = 4–7 rats/group). Third, rats underwent ganglionectomy of the celiac/superior and inferior mesenteric ganglia or sham surgery immediately before transection or resection surgery and were then fed orally for 7 days. This experiment was a 2 × 2 factorial design with the following treatment groups: transection + sham surgery, resection + sham surgery, transection + ganglionectomy, and resection + ganglionectomy (n = 4–6 rats/group).

Surgical Procedures and Animal Care

Systemic capsaicin. Rats were pretreated with systemic capsaicin administered by subcutaneous injection 8 days before transection or resection surgery to partially ablate function of the extrinsic capsaicin-sensitive afferents (26, 39). Capsaicin (Sigma, St. Louis, MO) was dissolved (25 mg/ml) in 10% ethanol, 10% Tween 80 (Sigma), and 80% (0.9%) saline (by volume). Rats were anesthetized by inhalation of isoflurane (IsoFlo; Abbot Laboratories, North Chicago, IL) via an anesthesia machine before receiving capsaicin in the following three separate injections: t₀, 25 mg/kg body wt; t₁/₂h, 50 mg/kg body wt; and t₂h, 50 mg/kg body wt, to deliver a total dose of 125 mg/kg body wt. Control rats received injections of vehicle alone. Because of the acute local effect of capsaicin injection, bupivacaine (0.1 mg) was infiltrated at the site before injection. Capsaicin induced an immediate transient reduction in food intake and body weight. Within 3 days, all animals were eating and had regained all weight lost postinjection.

Perivagal capsaicin. To ablate only vagal afferents, rats were pretreated with perivagal capsaicin application 3 days before either intestinal surgery. Capsaicin was applied directly to the left and right cervical vagus as previously described (38). Briefly, rats were pretreated with atropine sulfate (1 mg/kg) and then anesthetized with pentobarbital sodium (60 mg/kg ip). Through a midline neck incision, the left vagus nerve was exposed, freed from the carotid artery and vein, and isolated using paraffilm. Capsaicin was dissolved in warmed Tween 80 (Fisher) and sonicated for ~5 min. Olive oil was added, and the entire solution was vortexed briefly. The nerve was wrapped in a cotton wool pledget, and then one drop of capsaicin (1%) or vehicle (10% Tween 80 in olive oil) was applied for 30 min; the nerve was swabbed, and capsaicin was reapplied every 10 min (total dose <1 mg/rat). After 30 min, the pledget was removed, and the area was thoroughly rinsed with saline. The procedure was then repeated on the right vagus nerve.

Ganglionectomy. Sympathetic denervation was achieved by surgical resection of the celiac/superior mesenteric ganglia and inferior mesenteric ganglion. After a midline abdominal incision, the junction of the celiac and superior mesenteric arteries with the dorsal aorta was identified, and the ganglia that lie in between the two arterial branches were removed. In addition, the vessels were stripped of nerve fibers. The inferior mesenteric artery was identified, and the ganglion was removed.

Seventy percent midjejunouileal resection after recovery from capsaicin or ganglionectomy. On the day of intestinal surgery, rats were anesthetized either by inhalation of isoflurane via an anesthesia machine or by intraperitoneal injection of ketamine (20 mg), acepromazine (2 mg), and xylazine (4 mg). Animals underwent 70% midjejunouileal resection or transection as described previously (10). 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 jejunum and ileum were reconnected by an end-to-end anastomosis using 6-0 silk suture. Transected animals received a single cut and anastomosis 15 cm proximal to the cecum. Animals were given 5 ml of saline intraperitoneally for fluid resuscitation. Resected animals lost ~5 grams of intestine because of surgery. After closure of the abdominal incision, the TPN catheter was placed in the superior vena cava via the external jugular vein (TPN rats) as previously described (11). Rats received oxymorphone (0.18 mg/kg body wt) every 6 h for 24 h after surgery for pain management (18), and ampicillin (200 mg/kg body wt) every 12 h for 48 h after surgery as a prophylactic antibiotic.

Immediately after surgery (day 0), TPN infusion was begun to provide the sole source of nutrition (TPN animals), or diet was provided (oral animals). TPN rats were given a nutritionally complete TPN solution providing 32% nonprotein energy from fat and 68% nonprotein energy from dextrose (11). The TPN infusion rate was gradually increased over the first 2 days after surgery with full infusion provided on days 2–6. Orally fed rats received either a semipurified diet with a macronutrient profile comparable to TPN (11) or pelleted certified rodent diet 5001 (Lab Diet, St. Louis, MO).

Intestinal Composition and Histology

Rats were anesthetized and killed by exsanguination from the heart. The entire small and large bowel, liver, and kidneys were removed for analysis. Intestine adjacent to the anastomosis (1 cm on either side) was discarded. The small bowel was sectioned into duodenum, pylo-
CGRP was measured in the stomach. In the perivagal capsaicin experiment, related peptides. For systemic capsaicin and ganglionectomy experiments, containing 40–50% activity was measured in ileal mucosal homogenates using the protein assay; Pierce Chemicals, Rockford, IL) and DNA (30) content. Jejunal sucrase activity (8) was assayed from the same section as RNA extraction. Fixed tissue for histology was paraffin embedded, cut into 5-μm sections, and stained with hematoxylin and eosin for histomorphology as previously described (19).

Biochemical Analyses

Calcitonin gene-related peptide. Ablation of vagal afferents was confirmed by measuring calcitonin gene-related peptide (CGRP) using a commercial RIA with an antibody raised against rat CGRP (cross-reactivity for rat CGRP = 100%; for rat CGRP II = 78.6%; Phoenix Pharmaceuticals, Belmont, CA; Table 1). Determination of CGRP in the stomach was used to confirm functional ablation of the extrinsic capsaicin-sensitive afferent innervation in rats given systemic capsaicin or undergoing surgical removal of the spinal afferent innervation by celiac/superior mesenteric ganglionectomy because these treatments disrupt spinal afferent neurons. CGRP in the stomach is entirely of vagal afferent origin (38). Determination of CGRP concentration in the trachea was used to confirm functional ablation of vagal afferent innervation in rats treated with perivagal capsaicin because CGRP in the trachea is entirely of vagal afferent origin (38).

Plasma bioactive GLP-2 (1–33). Blood was collected in chilled tubes containing a final concentration of 1 mg/ml EDTA, 0.1 mM Diprotin A (MP Biomedicals, Aurora, OH), and 0.01 mM aprotinin (Calbiochem, La Jolla, CA). Plasma was isolated by centrifugation at 1,000 g for 15 min at 4°C and was stored at −70°C until GLP-2 measurement. Plasma bioactive GLP-2 was quantified by RIA using an antibody specific to the NH₂ terminus of GLP-2 (24).

Dipeptidyl peptidase-IV activity. Dipeptidyl peptidase-IV (DPP-IV) activity was measured in ileal mucosal homogenates using the discontinuous direct photometric method of Nagatsu et al. (34). Tubes containing 40–50 μl water, 10 μl ileal mucosal homogenate, and 1 μmol (500 μμl of 3 mM) glycylproline-p-nitroaniline tosylate substrate were incubated at 37°C for 30 min. Absorbance was read at 385 nm, and the amount of p-nitroaniline liberated per minute was used to determine enzyme activity based on the absorbance of a 150-nmol p-nitroaniline standard. One unit of DPP-IV activity is defined as the amount of enzyme that hydrolyses 1 μmol of substrate/min. Data were calculated per milligram of protein (specific activity) or centimeter of ileal mucosa (segmental activity) to distinguish between effects due to specific expression and mucosal growth.

Proglucagon mRNA

Total RNA was extracted from intact ileum using TRIzol reagent (GIBCO-BRL Life Technologies, Grand Island, NY) and quantified spectrophotometrically at 260 nm. Integrity was confirmed by ethidium bromide staining of 18S and 28S rRNA on an agarose-formaldehyde gel. Quantification of proglucagon mRNA was done using a Northern Max kit (Ambion, Austin, TX). RNA samples (20-μg aliquots) were fractionated on a 1% agarose-formaldehyde gel containing ethidium bromide. 18S and 28S rRNA was quantified by densitometry (Optiquant; Packard Instruments, Meriden, CT) of Polaroids of the ultraviolet-exposed gel before Northern blot analysis. RNA was transferred to a nitrocellulose membrane (Brightstar-Plus; Ambion) and hybridized to an antisense RNA probe synthesized as previously described (10). Proglucagon bands were visualized by exposing the blots to phosphorscreens (Packard Instruments). Quantification was performed using OptiQuant; band intensity for each sample was normalized to 18S rRNA expression.

Statistical Analyses

Treatment groups in each of the four resection experiments were compared by two-way ANOVA to identify main effects resulting from resection and capsaicin or ganglionectomy and their interactions (SAS; SAS Institute, Cary, NC). Individual differences between treatment groups were determined by one-way ANOVA followed by the protected least-differences technique. All data are presented as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Body Weight and CGRP

There were no significant differences in baseline body weight in any of the experiments. In each of the four experiments, rats gained weight (12.6 ± 4.5 g/7 days) after transection, and within the three capsaicin studies there were no significant differences in final body weight between the capsaicin- and vehicle-treated groups (218–255 g). Furthermore, there was no evidence of diarrhea in the orally fed rats treated with capsaicin. These data indicate that rats received adequate oral or parenteral nutrition throughout the studies.

CGRP immunoreactivity was measured to confirm functional ablation of afferent innervation from spinal and vagal origin. Systemic capsaicin significantly reduced CGRP immunoreactivity by ~65–75% in the stomach of both orally fed and TPN rats (Table 1). CGRP in the trachea of rats treated with perivagal capsaicin showed a significant 41% decrease resulting from capsaicin treatment. Ganglionectomy also significantly decreased stomach CGRP by ~53% compared with sham surgery. These data confirm that capsaicin and ganglionectomy treatments induced partial functional ablation of afferent innervation.

Mucosal Adaptive Growth

Systemic capsaicin. Significant resection-induced mucosal adaptive growth was present throughout the small bowel in rats fed orally or by TPN after resection compared with control transected rats. Growth was most pronounced in the residual ileum. The residual ileum of resected oral rats showed the greatest growth with 103–182% increases in mucosal dry mass,
protein, and DNA (Fig. 1). Growth of the ileum in resected TPN rats was \( \sim 50\% \) of that observed in resected oral rats, and capsaicin did not attenuate increased mucosal mass or cellularity resulting from resection. However, in orally fed rats, systemic capsaicin significantly attenuated resection-induced increases in mucosal mass (55%), protein (62%), and DNA (100%) as reflected in significant interaction (2-way ANOVA) between surgery and capsaicin treatment. This attenuation of increased ileal mucosal cellularity with capsaicin showed a significant correlation with reduction in the concentration of CGRP in the stomach (\( r^2 = 0.28 - 0.60; \ P = 0.002 - 0.05 \)). Overall, the ability of capsaicin to inhibit resection-induced intestinal growth in oral rats, but not in TPN rats, the remaining studies were carried out only in orally fed rats.

The colon did not show adaptive growth due to resection without capsaicin treatment, as previously reported (10). However, we were surprised to note 28–76% greater \( (P < 0.05) \) colonic mass and concentrations of protein and DNA in the colon of capsaicin-treated resected rats compared with capsaicin-treated transection rats. This suggests that capsaicin induced colonic growth in orally fed resected rats.

**Perivagal capsaicin.** Resection induced significant adaptive growth in the residual ileum of orally fed rats based on 145–255% increases in mucosal dry mass, protein, and DNA. Perivagal application of capsaicin attenuated mucosal growth in orally fed rats to a similar extent as that noted with systemic capsaicin treatment (Fig. 2). Capsaicin significantly attenuated resection-induced increases in ileal mucosal mass (48%), protein (71%), and DNA (100%) as reflected in significant interaction (2-way ANOVA) between surgery and capsaicin treatment. Because systemic and perivagal capsaicin treatments produced nearly identical mucosal growth responses, the remaining data are presented as combined data for systemic and perivagal treatments.

**Histology and sucrase activity.** Consistent with resection-induced mucosal growth, resection significantly increased ileal villus height and crypt depth in control rats treated with vehicle (Table 2). Attenuation of resection-induced mucosal growth resulting from capsaicin treatment resulted in a significant reduction in villus height in resected rats given capsaicin.
compared with resected rats given vehicle. Capsaicin treatment increased crypt depth in transection controls. Thus conclusions about crypt depth cannot be made with respect to capsaicin treatment in resected rats.

Resection significantly increased sucrase segmental activity (U/cm) in jejunal and ileal mucosa in both capsaicin- and vehicle-treated rats (Fig. 3). The relative resection-induced increase in sucrase activity was dramatically greater in the jejunum than in the ileum (40% increase vs. 500% increase), although absolute sucrase activity in transection controls was 10-fold higher in the jejunum than in the ileum. Sucrase specific activity (U/mg protein) was not significantly different due to resection or capsaicin treatment. Interestingly, ileal sucrase specific activity was significantly increased by resection in rats treated with capsaicin; ileal sucrase specific activity was not significantly different in vehicle-treated transected and resected rats.

**Ganglionectomy.** Unlike capsaicin treatment, ganglionece- tomy failed to attenuate resection-induced adaptive growth in jejunum or ileum (Table 3). However, rats developed significant diarrhea with reduced food intake and loss of body weight after ganglionectomy, and all rats were switched to a low-residue diet. These alterations in postsurgical feeding may have reduced resection-induced mucosal growth. There were no significant differences in plasma concentration of GLP-2 among groups (transsection = 27 ± 4; resection = 49 ± 10; pmol/l; P = 0.3831). Interestingly, jejunum showed greater adaptive growth than ileum in the ganglionection rats, possibly related to the low-residue diet resulting in less undigested nutrients reaching the ileum.

**Proglucagon mRNA and Plasma GLP-2 Concentration**

Ileal proglucagon mRNA expression and plasma concentration of bioactive GLP-2 were determined in rats treated with capsaicin or vehicle and maintained with oral feeding after transection or resection. Capsaicin treatment (systemic or perivagal) in transection control rats did not alter proglucagon expression or plasma GLP-2 concentration. Resection showed a significant main effect (P = 0.002) to increase ileal proglucagon mRNA expression by 57% in vehicle- and capsaicin-treated rats (Fig. 4). In parallel, resection significantly (P = 0.003) increased plasma bioactive GLP-2 by 150% in the vehicle- and capsaicin-treated rats. In summary, capsaicin failed to significantly attenuate resection-induced increases in ileal proglucagon mRNA or plasma GLP-2 in orally fed rats despite significant attenuation of resection-induced ileal mucosal growth.

**DPP-IV Activity**

DPP-IV is a serine protease enzyme that deactivates GLP-2 by cleavage at the NH2-terminal alanine residue to GLP-2-(3–33) (43). Resection significantly increased ileal segmental DPP-IV activity (U/cm) by 100–150%; capsaicin significantly attenuated the resection-induced increase in DPP-IV activity (Fig. 5). DPP-IV specific activity (U/mg protein) showed no significant differences among groups. Thus segmental differences in DPP-IV activity are consistent with increased mucosal cellularity due to resection and the attenuation of resection-induced growth due to capsaicin.

**DISCUSSION**

The interactions between luminal nutrients and neuroendo- crine signals in the stimulation of intestinal adaptive growth are poorly understood; however, GLP-2 is thought to be a key hormonal mediator (6, 10, 13, 21, 36, 42). We have investigated how partial functional ablation of vagal afferent input

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<tr>
<th>Treatment</th>
<th>Villus Height, μm</th>
<th>Crypt Depth, μm</th>
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<tbody>
<tr>
<td>Veh + Tran</td>
<td>358±24*</td>
<td>131±6*</td>
</tr>
<tr>
<td>Veh + Res</td>
<td>500±40†</td>
<td>154±5*</td>
</tr>
<tr>
<td>Cap + Tran</td>
<td>379±24*</td>
<td>142±4*</td>
</tr>
<tr>
<td>Cap + Res</td>
<td>516±49‡</td>
<td>146±6*</td>
</tr>
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</table>

Values are means ± SE; n = 9–13 rats. Veh, vehicle; Tran, transection; Res, resection; Cap, capsaicin. Means with different superscripts are significantly different. Two-way ANOVA indicated a significant resection main effect for villus height (P < 0.001) and significant interaction between capsaicin and resection for crypt depth (P = 0.0432).

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Fig. 2. Ileal mucosal dry mass (A), protein (B), and DNA (C) in rats treated with vehicle or perivagal capsaicin and maintained with oral feeding for 7 days after transection (Trans) or resection (Resec). Perivagal capsaicin attenuated resection-induced increases in mass, protein, and DNA (48–100%, P = 0.0001–0.0009). Values are means ± SE; n = 4–7 rats/group. Means with different superscripts are significantly different (P < 0.05).
impacts resection-induced intestinal adaptive growth. Our results demonstrate for the first time that capsaicin-sensitive vagal afferents are essential for maximal, nutrient-stimulated adaptive growth after 70% midjejunoileal resection in rats. Moreover, ablation of vagal afferents does not reduce ileal proglucagon expression or plasma concentration of bioactive GLP-2, suggesting that vagal afferent innervation is not essential for GLP-2 synthesis or release in rats fed orally after 70% midjejunoileal resection.

The essential role of the vagal afferents in resection-induced adaptive growth was demonstrated in a series of four experiments using 70% midjejunoileal resection: systemic capsaicin/oral feeding; systemic capsaicin/TPN; perivagal capsaicin/oral feeding; and ganglionectomy/oral feeding. Each experiment showed significant resection-induced mucosal growth throughout the small intestine based on increases in villus height and crypt depth, mucosal concentrations of protein and DNA, and mucosal sucrase activity. Systemic capsaicin significantly attenuated resection-induced ileal growth in orally fed rats by 48–100% based on mucosal mass and concentrations of protein and DNA. Consistent with the mucosal chemistry data, a significant decrease in villus height suggests a decrease in mucosal surface area in resected rats treated with capsaicin. Our observation that capsaicin treatment increased crypt depth in transection controls is unexplained, but it suggests that vagal afferent innervation may modulate, and possibly decrease, basal enterocyte proliferation. Moreover, capsaicin treatment increased the proportion of ileal mucosal protein that expresses sucrase activity based on a significant increase in sucrase specific activity in resected rats.

Table 3. Intestinal adaptive growth in rats receiving ganglionectomy or sham laparotomy and maintained with oral feeding for 7 days after transection or resection surgery

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Jejunum</th>
<th>Ileum</th>
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<tbody>
<tr>
<td></td>
<td>Mass, mg/cm</td>
<td>Protein, mg/cm</td>
</tr>
<tr>
<td>Sham + Tran</td>
<td>9.9±2.2*</td>
<td>5.0±0.6*</td>
</tr>
<tr>
<td>Sham + Res</td>
<td>20.0±2.0†</td>
<td>8.2±0.6†</td>
</tr>
<tr>
<td>Gang + Tran</td>
<td>10.5±1.9*</td>
<td>4.7±0.5*</td>
</tr>
<tr>
<td>Gang + Res</td>
<td>14.9±1.7†</td>
<td>6.6±0.5†</td>
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</table>

Two-way ANOVA, P values

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<tbody>
<tr>
<td>Gang</td>
<td>0.258</td>
<td>0.1322</td>
<td>0.0106</td>
</tr>
<tr>
<td>Res</td>
<td>0.003</td>
<td>0.0007</td>
<td>0.0003</td>
</tr>
<tr>
<td>Gang × Res</td>
<td>0.158</td>
<td>0.3232</td>
<td>0.3379</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4–6 rats/group. Gang, ganglionectomy. Means with different superscripts in the same column are significantly different, P < 0.05.

Fig. 3. Sucrase segmental activity (A) and specific activity (B) in jejunum and ileum in rats treated with vehicle or capsaicin and maintained with oral feeding for 7 days after transection or resection. Values are means ± SE; n = 9–15 rats/group. Means with different superscripts are significantly different (P < 0.05).
treated with capsaicin compared with vehicle. Taken together, the data suggest that vagal afferent innervation may play a role in enterocyte proliferation and differentiation.

Given that systemic capsaicin induces functional ablation of several unmyelinated and thinly myelinated afferents, an additional experiment was conducted. Capsaicin was applied directly to the cervical vagus (i.e., perivagal application) to ablate only vagal afferents. Both systemic and perivagal capsaicin treatment significantly attenuated resection-induced growth to the same extent based on mucosal mass, chemistry, and villus height. This strongly indicates that capsaicin-sensitive vagal afferents are key mediators of adaptive growth.

An experiment utilizing celiac/superior and inferior mesenteric ganglionectomies further elucidated the role of the spinal innervation on resection-induced adaptive growth. Ganglionectomy-associated diarrhea and modest resection-induced mucosal growth in controls complicated the interpretation. However, ganglionectomy did not impair resection-induced growth, which suggests that spinal nerves do not play a significant role in resection-induced adaptive growth after midjejunooileal resection.

Together, these experiments demonstrate that afferents, and specifically vagal but not spinal afferents, are key mediators of maximal resection-induced intestinal adaptive growth. Only one publication, to our knowledge, has investigated an association between the vagus nerve and resection-induced intestinal adaptation (31). The protocol used surgically deafferented pigs that underwent 30% distal jejuneumectomy and were fed orally for 28 days. The resection-induced increase in mucosal dry mass was significantly attenuated in deafferented pigs. These data support our finding that vagal input is essential for maximal resection-induced intestinal adaptive growth.

An interesting observation in the present study was that vagal afferents are essential for maximal resection-induced adaptive growth only in orally fed rats, and not in rats treated with systemic capsaicin and nourished with TPN after 70% midjejunooileal resection. Both oral and TPN rats exhibited significant resection-induced adaptive growth, although growth in TPN rats was only 50% of that noted in oral rats. This supports previous findings that luminal nutrients are a major stimulus for mucosal cell proliferation and intestinal adaptation (10, 32). Luminal nutrients increase pancreaticobiliary secretions, gut neuronal/vagal activity, splanchnic blood flow, and secretion of hormones that may stimulate gut growth directly or indirectly (6, 14, 21).

In a sense, the TPN experiment served as a negative control for the effects of vagal afferent innervation in the current study because the presence of luminal nutrients is the primary stimulus for activation of vagal afferents and GLP-2 secretion (15). Thus it is not surprising that capsaicin did not attenuate the modest resection-induced growth observed in TPN rats because stimulation of vagal afferents is diminished during TPN. The mechanisms of resection-induced mucosal growth are likely independent of the vagal pathway in TPN rats. In summary, these data suggest that luminal nutrients are part of

Fig. 4. Ileal proglucagon mRNA expression (A) and plasma bioactive glucagon-like peptide (GLP)-2 concentration (B) in vehicle- or capsaicin-treated rats maintained with oral feeding for 7 days after transection or resection. Resection caused a significant increase in ileal proglucagon message in both vehicle- and capsaicin-treated rats (main effects: capsaicin, P = 0.3159; resection, P = 0.0019; capsaicin × resection, P = 0.6435) and a significant 133–169% increase in plasma GLP-2 in vehicle- and capsaicin-treated rats (capsaicin, P = 0.7360; resection, P = 0.0029; capsaicin × resection, P = 0.7049). Inset: bands from a representative Northern blot analysis of ileal proglucagon mRNA. Values are means ± SE; n = 5–8 rats/group. Means with different superscripts are significantly different (P < 0.05).

Fig. 5. Ileal dipeptidyl peptidase-IV (DPP-IV) segmental activity (U/cm) in vehicle- or capsaicin-treated rats maintained with oral feeding for 7 days after transection or resection. Resection caused a significant 156% increase in ileal DPP-IV activity (capsaicin, P = 0.01298). Values are means ± SE; n = 5–8 rats/group. Means with different superscripts are significantly different (P < 0.05).

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the mechanism by which vagal afferent input maximizes resection-induced mucosal growth.

Data from the present study demonstrate that functional ablation of vagal afferents does not significantly reduce endogenous GLP-2 responses under conditions where unabsorbed nutrients are present in the ileum. In rats fed orally for 7 days after resection, we observed a significant 57% increase in ileal proglucagon mRNA and a significant 150% increase in plasma concentration of bioactive GLP-2. Systemic and peri-vaginal capsaicin each failed to attenuate increases in either proglucagon expression or plasma GLP-2 levels. These data agree with previous findings demonstrating resection-induced ileal growth is associated with increases in ileal proglucagon mRNA (33, 41, 45) and both proglucagon and bioactive plasma GLP-2 (10). The ability of capsaicin to attenuate ileal mucosal growth without inhibiting resection-induced increases in proglucagon or GLP-2 suggests that functional ablation of vagal afferents does not reduce adaptive growth by altering GLP-2 synthesis or secretion. Rather we speculate that luminal nutrients stimulate vagal afferents as part of a vago-vagal loop that modulates the intestinoendocrine actions of GLP-2 at the level of the GLP-2R. Further studies are planned to address the effects of vagal innervation on GLP-2 action.

The stimulus for intestinal secretion of GLP-1 and GLP-2 is thought to occur in two phases, an acute first peak of secretion within 15–30 min after ingestion of a meal and a second sustained peak within 60–90 min (15). The first peak of secretion is associated with a proximal-distal neuroendocrine loop involving the enteric nervous system and the afferent and efferent vagus nerves in the rat (40). The second peak of secretion is induced by direct contact between luminal nutrients, especially lipid and carbohydrate, and ileal L cells (48). In the current study, resection-induced increases in plasma GLP-2 likely reflect direct stimulation of GLP-2 secretion due to the presence of nutrients in the ileum. Thus, when luminal nutrients are present in the ileum to directly stimulate secretion of GLP-2, vagal afferent innervation is not essential. Another explanation is that vagal innervation does not control secretion of GLP-1 and GLP-2 as supported by studies demonstrating that electrical stimulation of the peripheral vagus in vagotomized pigs has no effect on plasma concentrations of GLP-1 and GLP-2 (23).

The proglucagon-derived peptides GLP-1 and GLP-2 are both subject to extensive NH2-terminal degradation by the serine protease DPP-IV present in the crypt-villus axis and in plasma (12). Over one-half of the newly secreted GLP-1 is degraded before it enters the circulation in association with DPP-IV present in the endothelium of the capillary beds in close proximity to GLP-1- and GLP-2-secreting L cells (22). We noted increased segmental but not specific activity of DPP-IV in ileal mucosa in association with resection-induced increases in mucosal cellularity and plasma bioactive GLP-2. These findings confirm an earlier study in our laboratory showing increased ileal specific and segmental DPP-IV activity in rats 48–72 h after 70% midjejunoleileal resection (9). In contrast, a decrease in ileal DPP-IV mRNA was noted in rats at 2, 4, and 7 days after midsmall bowel resection, leaving only 5 cm of residual ileum (16). These data suggest that changes in intestinal DPP-IV activity may help determine circulating levels of bioactive GLP-2 after resection; however, additional research is needed.

In summary, interactions between luminal nutrients and neuroendocrine signals play a key role in the regulation of intestinal adaptive growth after bowel resection. The present study shows that vagal capsaicin-sensitive afferents and non spinal afferents are essential for maximal resection-induced intestinal growth, through a mechanism that appears to involve stimulation by luminal nutrients. Taken together, this research demonstrates the interaction between vagal afferents and luminal nutrients in maximizing resection-induced mucosal growth. Additional research is needed to elucidate the role of the vagal pathway in intestinal growth and GLP-2 synthesis and action in different physiological models.

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