THE YALE JOURNAL OF BIOLOGY AND MEDICINE 65 (1992), 1-15

Plasma and Tissue Alterations of Peptide YY and Enteroglucagon in Rats After Colectomy

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Received February 18, 1991

Peptide YY (PYY) and enteroglucagon are produced by endocrine cells of the colonic mucosa. PYY inhibits upper gastrointestinal motility, and enteroglucagon is trophic for small bowel mucosa. Adaptive increase in the production and release of these peptides may improve functional results after colorectal resections. We hypothesized that if segments of the colon were resected, then production and release of PYY and enteroglucagon would increase in the remaining segments of bowel. Animals which underwent colonic transections and partial resections had transient elevations of PYY up to 250 ± 80 pmol/L, which dropped to control group levels in the second week following surgery. Rats with an abdominal colectomy had significantly greater PYY levels than all other groups from the third (208 ± 30 pmol/L) to the thirty-eighth (100 ± 16 pmol/L) week of the study. Circulating levels of enteroglucagon were elevated to 156 ± 35 pmol/L in rats with a right hemicolectomy during the first week following surgery. Enteroglucagon levels did not significantly vary in the other groups studied. Both tissue PYY (413 ± 33 pmol/gram) and tissue enteroglucagon (171 ± 17 pmol/gram) were significantly elevated in the rectums of the rats with an abdominal colectomy, as compared to all other groups. The elevated tissue levels may thus account for the ability to maintain elevated plasma PYY. Double immunogold labeling of endocrine cells in the colorectal tissue for PYY and enteroglucagon revealed both peptides within the same endocrine cells and secretory granules. These studies support the hypothesis that circulating levels of PYY are elevated after major colonic resections and suggest that L-type endocrine cells may participate in adaptive responses which improve intestinal function following colonic surgery.

INTRODUCTION

The colon acts as an endocrine organ by release of such bioactive peptides as peptide YY (PYY) and enteroglucagon. PYY and enteroglucagon are contained within mucosal endocrine cells, called L cells [1,2,3,4,5]. These peptides are primarily located within the distal gastrointestinal tract [3,6,7,8,9,10,11]. PYY inhibits upper gastrointestinal tract motility, while enteroglucagon is trophic for small bowel mucosa. Thus, the colon contains peptides which influence proximal gastrointestinal function.

Tatemoto and Mutt first isolated PYY by means of its C-terminal amide from porcine small bowel [12,13]. It is a 36 amino acid straight-chain peptide, named peptide YY because of its N- and C-terminal tyrosine (Y) residues [13]. Infusion of PYY into healthy human subjects significantly inhibits gastric acid and pepsinogen secretion, delays gastric emptying, and inhibits small bowel motility [14,15]. In

Abbreviations: GLI: glucagon-like immunoreactivity PYY: peptide YY

human surgical specimens, Adrian et al. found the highest concentrations of PYY within the terminal ileum, large bowel, and up to 480 pmol/gram in the rectum, in contrast to the less than 6 pmol/gram of PYY within the stomach, duodenum, and small bowel [9,11]. In rats, Miyachi et al. found tissue levels of PYY were greatest in the distal half of the colon (449 pmol/gram of tissue) and less than 9 pmol/gram in tissue proximal to the ileum [10].

Unger et al. first detected enteroglucagon in canine gastric mucosa, using a cross-reacting pancreatic glucagon radioimmunoassay, in 1961 [16]. This enteric source of pancreatic glucagon-like immunoreactivity, or enteroglucagon, consists of a 69 amino acid peptide, glicentin/proglucagon, and a more biologically active 37 amino acid C-terminal degradation product, oxyntomodulin [6,17,18]. The 29 amino acid sequence of pancreatic glucagon is contained within the N-terminal portion of oxyntomodulin [17]. Enteroglucagon, as the term is used within this paper, describes both the glicentin and oxyntomodulin detected by current radioimmunoassay techniques [8]. Circulating enteroglucagon levels strongly correlate with enhanced growth rate of the small bowel mucosa, following small bowel resection [19,20,21,22], and enteroglucagon-secreting tumors are associated with villous hypertrophy [23]. Enteroglucagon does not display the glycogenolytic and hyperglycemic properties of pancreatic glucagon [24,25,26]. Using human surgical specimens, Ghatei et al. found the greatest tissue concentration in the ileum (275 pmol/gram) as compared to the jejunum (58 pmol/gram) and colon (79 pmol/gram) [8]. In rats, Kervran et al. found that enteroglucagon distribution was similar, with considerably more peptide present in the ileum (197 pmol/gram), as compared to the duodenum (23 pmol/gram) and cecum (71 pmol/gram) [6].

Partial and total large bowel resections are frequently performed each year for patients with colon cancer, inflammatory bowel disease, and familial polyposis coli. Functional results are often poor in the early post-operative period but improve with time. Typically, water absorption is diminished and stool frequency is increased. Function slowly improves, reaching a stable plateau one year after surgery. Presumably, compensatory mechanisms evolve, in the year following operation, which lead to improved water absorption and decreased stool frequency. The mechanisms which account for this improved function, however, have not been characterized.

In previous studies, we have demonstrated that postprandial plasma levels of PYY and ileal pouch tissue levels of PYY and enteroglucagon were elevated one year after total proctocolectomy and ileal pouch-anal anastomosis [27]. It is possible that this increase in PYY and enteroglucagon represents an adaptive response which serves to slow small bowel transit and to promote mucosal growth. Both of these responses may contribute to the improving functional results which evolve in the year following this operation.

In the present study, we speculated that an adaptive response in plasma and tissue levels of PYY and enteroglucagon similar to that observed after total proctocolectomy and ileo-anal anastomosis might occur after partial or total resections of the colon. We hypothesized that if segments of the colon were resected, then production and release of PYY and enteroglucagon would increase in the remaining segments of bowel. Therefore, the specific aims of this study were to measure changes in plasma and tissue levels of PYY and enteroglucagon following partial and subtotal colectomy in rats. In addition, immunogold double-labeling for both PYY and enteroglucagon of colonic endocrine cells was examined to determine if, in rats, these peptides

co-localized within the same endocrine cells and the same secretory granules. Co-localization of PYY and enteroglucagon within the same cells and secretory granules would suggest that any observed increase in plasma or tissue levels of these peptides represented a generalized L-type endocrine cell response.

METHODS

Operation

The protocols used in this study were approved by the Animal Investigations Committee of the institutions. Male Wistar rats (Charles River Laboratories) were anesthetized with an intramuscular injection of ketamine HCl (75 mg/kg). An abdominal laparotomy was performed. The bowel was transected or resected, and appropriate vessels were ligated and divided. Five milliliters of 0.9 percent NaCl were administered intraperitoneally during the abdominal closure in order to maintain a positive fluid balance. The rats were allowed immediate access to water and allowed to feed 12 hours later.

Calculation of Weight and Survival

Whole-body weights (grams) of the rats were recorded at the time of blood sampling. Weight measurements of the rats in the first week were subtracted from the weight of each subsequent week to establish the weight increases occurring; these were also calculated as a percentage of the week 1 body weight.

The percentage survival of animals was measured from the end of the first week of the study in order to discount acute surgical complications from the calculation.

Serial Blood Sampling

All blood samples (1.5 ml) were taken by tail vein phlebotomy, in tubes containing aprotinin (0.1 mg) and EDTA (2 mg). After centrifugation (1,200 rpm, five minutes, 4°C), plasma was separated and stored at −20°C until peptide analysis could be performed.

Tissue Sampling

At sacrifice, tissue samples (approximately one gram) were removed from the terminal ileum, ascending colon, descending colon, rectum, and anastomosis site for peptide content analysis. The tissue samples were weighed and then suspended in 10 ml/g tissue weight of 0.5 M glacial acetic acid in polypropylene tubes and boiled in a water bath for 15 minutes, according to the methods of Adrian et al. [11]. These extracts were then stored at −20°C for later peptide analysis.

IMMUNOGOLD DOUBLE-LABELING OF ENDOCRINE CELLS

The cellular localization of PYY and enteroglucagon in rat colorectal tissue was investigated, using immunogold double-labeling techniques [28]. Tissues from normal rat colon and rectum were fixed in a mixture of 4 percent formaldehyde and 0.5 percent glutaraldehyde in phosphate buffer, pH 7.4, and subsequently dehydrated and embedded in Lowicryl K4M (Polysciences, Inc., Warrington, PA) or Epox 812 (Fullam Inc. Latham, NY). Thin sections were placed on formvar-coated nickel grids and incubated on drops of antibody solutions. Primary incubation was carried out with a mixture of *(1)* rabbit anti-PYY antiserum number B52 (Milab, Malmo,

Sweden) and *(2)* mouse monoclonal anti-glucagon antibody number GLU-001 (Novo Biolabs, Danbury, CT). The anti-glucagon antibody was directed against both pancreatic glucagon and enteroglucagon and thus revealed glucagon-like immunoreactivity (GLI). Antibody binding sites were detected by a secondary incubation with a mixture of *(1)* goat-anti-rabbit IgG-gold (GAR G5; Jansson, Piscataway, NJ) and *(2)* goat-anti-mouse IgG-gold (GAMIgG G15; Jansson). Sections were contrasted with aqueous uranyl acetate and viewed in a Philips 300 electron microscope. Control sections were incubated with primary antisera adsorbed with an excess of synthetic PYY or glucagon.

Radioimmunoassays—PYY

Concentrations of PYY were measured, using a well-characterized radioimmunoassay, which has been previously validated for rat PYY [11,29]. The antibody (Y-21) was raised in a rabbit immunized with pure, unconjugated, natural PYY. This antiserum is added to give a final dilution of 1:128,000, a concentration which binds 50 percent of 1 fmol iodinated PYY. PYY is iodinated by conventional chloramine T oxidation and labeled PYY purified using high-resolution, reverse-phase, high-pressure liquid chromatography. The assay detects changes between adjacent tubes of 0.4 fmol with 95 percent confidence.

Enteroglucagon

This assay system has previously been validated for rat enteroglucagon and described in detail [3,8]. Total glucagon immunoreactivity was measured, utilizing an antibody (GL-77) which fully recognizes pancreatic glucagon and enteroglucagon, the antibody being N-terminally directed. Pancreatic glucagon was measured utilizing the antibody RCS-5, which recognizes only pancreatic glucagon, the antibody being C-terminally directed. In the isolated venous effluent samples and rat resection tissue samples, there was no potential source of pancreatic glucagon, and the enteroglucagon concentrations could then be measured directly with antibody GL-77. In plasma samples, the pancreatic glucagon is subtracted from the total glucagon immunoreactivity to derive a value for enteroglucagon. The assay shows no significant cross-reaction with related peptides (secretin, VIP, PHI, GRF, and GIP) and detects changes of 0.2 fmol/tube with 95 percent confidence.

Statistical Methods

The data analysis was performed using the *SYSTAT* computer software package [30]. Plasma and tissue peptide measurements were subjected to a Bartlett's test for homogeneity of the variances. For those comparisons with a significant F ($p < 0.05$), data were tested by a Tukey test in order to determine significant differences between the control group and the experimental groups. Plasma peptide levels were compared at each time point. Tissue peptide levels were compared at each sampling site; tissue peptide levels were also compared between the different sampling sites, within each experimental group.

Experimental Design

The rats were divided into various experimental groups. Control rats underwent no surgery. Two groups of rats underwent transections and the bowel was reapproximated with no loss of tissue. The first group underwent a jejunal transection 10 cm

TABLE 1
The Increase in Body Weight (Grams) from the First Week of the Study for the Control, Transection, and Resection Groups of Rats

Surgery	*n*	Weeks After Surgery					
		1	2	3	6	12	Sacrifice
Controls	7	0	37 ± 2 (22)	64 ± 3 (38)	129 ± 3 (76)	187 ± 4 (110)	223 ± 13 (131)
Jejunal transection	6	0	36 ± 3 (19)	59 ± 4 (31)	127 ± 4 (68)	177 ± 5 (95)	223 ± 5 (120)
Colonic transection	7	0	27 ± 10 (13)	51 ± 10 (25)	94 ± 10 (45)	129 ± 11 (61)	169 ± 11 (79)
Right colectomy	5	0	46 ± 7 (31)	72 ± 10 (50)	135 ± 12 (93)	190 ± 15 (132)	226 ± 14 (155)
Left colectomy	7	0	34 ± 1 (18)	65 ± 3 (34)	126 ± 7 (66)	175 ± 5 (92)	218 ± 7 (115)
Subtotal colectomy	10	0	14 ± 4 (11)	25 ± 5 (19)	64 ± 7 (45)	102 ± 7 (73)	168 ± 12 (120)

Results are presented as mean ± SEM. The percentage increase in body weight over the initial weight is indicated in parentheses.

from the ligament of Trietz, and the second group underwent a colon transection between the right and middle colic arteries. Three groups of rats underwent bowel resections and the remaining bowel was anastomosed. In the right hemicolectomy group, the distal ileum was transected 2 cm from the ileocecal valve and the colon transected just proximal to the middle colic artery. In the left hemicolectomy group, the rectum was divided so as to preserve the distal two-thirds of the rectum and the colon transected just distal to the middle colic artery. In the abdominal colectomy or subtotal colectomy group, the rectum was divided as in the left hemicolectomy group and the terminal ileum divided 2 cm from the ileocecal valve.

Blood samples from a group of normal rats ($n = 18$) were used to establish basal levels of the peptides, so as not to compromise survival of those rats used in the surgical model. Blood samples were taken at one, two, three, six, 12, and 38 weeks following surgery. Tissue samples were taken at sacrifice. In addition, three groups of normal rats were bled weekly for one, two, and three weeks in a staggered manner in order to establish whether the potential stress associated with the bleeding procedure itself had any effects on peptide levels.

RESULTS

Change in Body Weight of Rats Following Colon Resections

The weight changes which occurred in the rats over the course of the study are shown in Table 1. All groups gained considerable body weight with each subsequent time point in the study.

Long-Term Survival of Rats Following Surgery

The percentage of animals surviving from week 1 until the end of the study at 38 weeks was: 87 percent of controls, 100 percent of jejunal transections, 64 percent of colon transections, 56 percent of right hemicolectomies, 78 percent of left hemicolectomies, and 77 percent of subtotal colectomies. There was no relationship of survival to the amount of bowel resected.

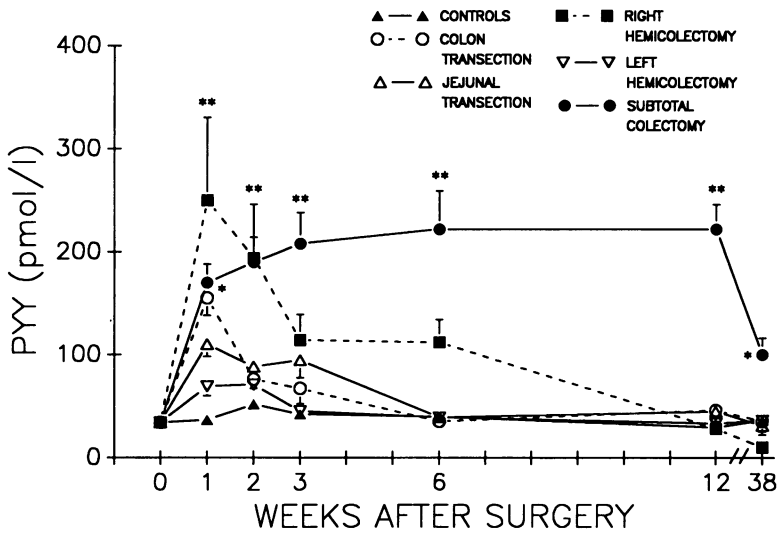

FIG. 1. The plasma PYY levels (mean ± SEM) of the rats following colon transection, jejunal transection, right hemicolectomy, left hemicolectomy, and subtotal colectomy as compared to a control group. When significant differences existed between the mean plasma levels for the transection or resection groups and that for the controls, as shown by a Bartlett's test of homogeneity and a Tukey test for significant differences between the means, this fact has been indicated by * for $p < 0.05$ and ** for $p < 0.01$.

Serial Blood Sampling of Rats for Post-Surgical Peptide Levels

The plasma PYY levels for control, transection, and resection groups are shown in Fig. 1. The colon transection (155 ± 17 pmol/L; Tukey, $p < 0.05$), right hemicolectomy (250 ± 80 pmol/L; Tukey, $p < 0.01$), and subtotal colectomy (170 ± 18 pmol/L; Tukey, $p < 0.05$) groups had significantly elevated (Bartlett's, $p < 0.0001$) plasma PYY levels in week 1 over that of controls. The PYY levels in the colon transection group decreased by week 2, but elevation of PYY persisted until week 2 for the right hemicolectomy group (Bartlett's, $p < 0.0001$; Tukey, $p < 0.01$). In contrast to the other groups, PYY levels in the subtotal colectomy group continued to rise following surgery and were significantly elevated over that of controls from week 2 (190 ± 24 pmol/L) until week 12 (222 ± 24 pmol/L) (Bartlett's, $p < 0.0001$; Tukey, $p < 0.01$). At sacrifice (38 weeks), the PYY levels in the subtotal colectomy group had fallen considerably, but they were still significantly elevated over those of all other groups (100 ± 16 pmol/L; Bartlett's, $p < 0.0001$; Tukey, $p < 0.05$).

The plasma enteroglucagon levels in control, transection, and resection groups are shown in Fig. 2. Plasma enteroglucagon was significantly elevated in the right hemicolectomy group as compared to controls during week 1 only (156 ± 35 pmol/L; Bartlett's, $p < 0.001$; Tukey, $p < 0.01$). The enteroglucagon levels of the other groups did not significantly differ (Bartlett's) from those of the controls. Within each group there was some elevation in plasma levels of enteroglucagon during the first three weeks as compared to the latter part of the study.

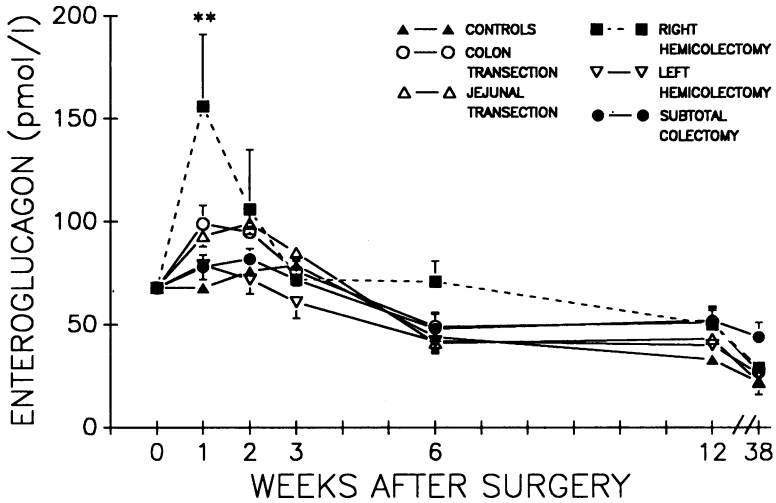

FIG. 2. The plasma enteroglucagon levels (mean ± SEM) of the rats following colon transection, jejunal transection, right hemicolectomy, left hemicolectomy, and subtotal colectomy as compared to a control group. When significant differences existed between the mean plasma levels for the transection or resection groups and that for the controls, as shown by a Bartlett's test of homogeneity and a Tukey test for significant differences between the means, this fact has been indicated by * for $p <$ 0.05 and ** for $p < 0.01$.

Effect of Serial Blood Sampling on Plasma Peptide Levels in Control Rats

Three groups of control rats underwent serial blood sampling in a staggered manner for three weeks in order to examine the effects of the potential stress due to weekly phlebotomy on circulating peptide levels. These results are shown in Table 2. There were no significant differences in plasma levels of PYY or enteroglucagon between the various sampling times within groups 1 or 2, or between the groups at each time point (Bartlett's).

TABLE 2
Serial Blood Sampling of Control Rats

		PYY Weeks			Enteroglucagon Weeks		
Group	*n*	1	2	3	1	2	3
1	6	37 ± 4	52 ± 7	42 ± 8	68 ± 3	76 ± 4	79 ± 10
2	6	NA	38 ± 7	26 ± 4	NA	75 ± 3	60 ± 4
3	6	NA	NA	28 ± 6	NA	NA	62 ± 6

PYY and enteroglucagon plasma levels were measured (pmol/liter). Results are shown as means ± SEM. Groups 2 and 3 were not sampled on the weeks marked NA. There were no significant differences between the various sampling times within groups, or between groups at each time point, as shown by Bartlett's test.

NA = not applicable

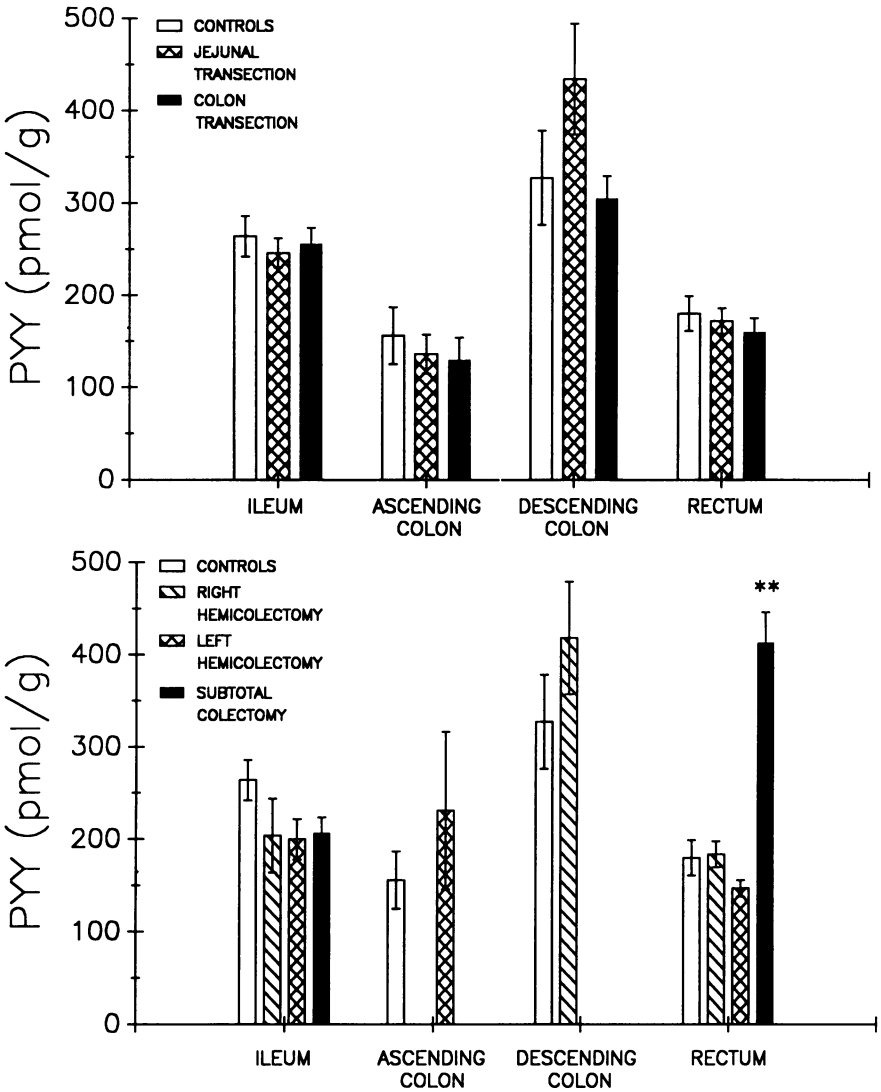

FIG. 3. The extracted tissue PYY content (pmol/gram of tissue) in samples of tissue from the (**A**) control and transection groups and from the (**B**) control and resection groups. Results are shown as mean ± SEM. When differences of mean PYY tissue content of one group varied significantly from that of controls, according to a Bartlett's test of homogeneity and a Tukey test for significant differences between the means, this fact has been indicated by ** for $p < 0.01$.

Peptide Extractions of Tissues from Resected Rats

The results of the peptide extractions of the tissues are shown in Figs. 3 (PYY) and 4 (enteroglucagon), for *(a)* controls compared to transections and *(b)* controls compared to resections. In tissue samples of the control group, the PYY tissue content was highest in the descending colon (327 ± 51 pmol/g), followed by the terminal ileum (264 ± 24 pmol/g), rectum (180 ± 19 pmol/g), and the ascending colon (156 ± 31 pmol/g). The transection groups were similar. The differences

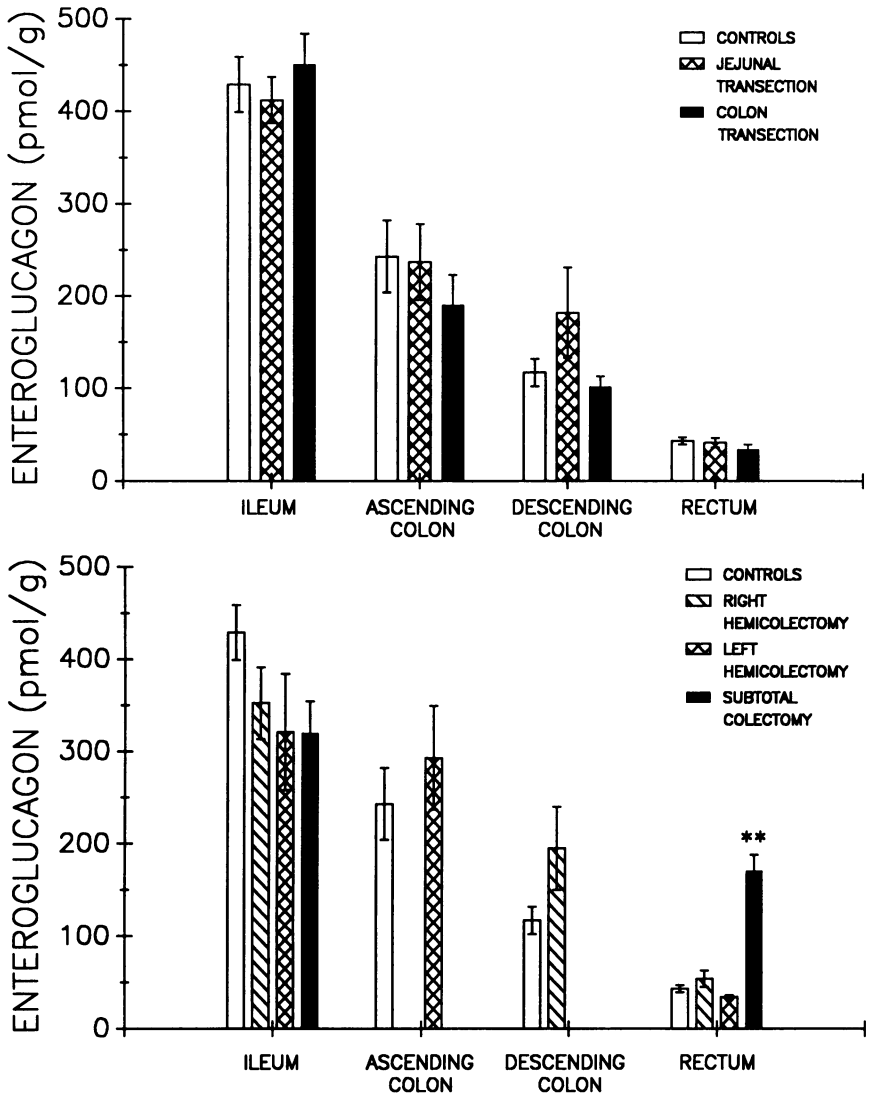

FIG. 4. The extracted tissue enteroglucagon content (pmol/gram of tissue) in samples of tissues from the (**A**) control and transection groups and from the (**B**) control and resection groups. Results are shown as mean ± SEM. When differences of mean enteroglucagon tissue content of one group varied significantly from that of controls, according to a Bartlett's test of homogeneity and a Tukey's test for significant differences between the means, this fact has been indicated by ** for $p < 0.01$.

within groups were only significant for the jejunal transection group, in which that of the descending colon was significantly greater than all of the other segments (413 ± 33 pmol/g; Bartlett's, $p < 0.0001$; Tukey, $p < 0.01$). When comparing mean PYY tissue contents of a particular bowel segment to that of the control group, only the rectal tissue of the subtotal colectomy group, which was two- to threefold greater than that of all other experimental groups, was significantly different (Bartlett's, $p < 0.0001$; Tukey, $p < 0.01$). Although PYY content in the segments of colon

which remained in the two hemicolectomy groups was greater than that of the controls, the differences were not significant. The rectal and ileal tissue PYY contents were similar to or less than those of the controls for the two hemicolectomy groups.

The tissue content of enteroglucagon was greatest in the terminal ileum (429 ± 30 pmol/g) and decreased in the ascending colon (243 ± 39 pmol/g), descending colon (117 ± 15 pmol/g), and rectum (43 ± 4 pmol/g) in the control group. The results were similar for the other groups. Specifically, the enteroglucagon content was significantly greater (Bartlett's, $p < 0.001$; Tukey, $p < 0.01$) in the terminal ileum than the other bowel segments, in the control, colonic transection, and subtotal colectomy groups, and also in the jejunal transection group (Bartlett's, $p < 0.0001$; Tukey, $p < 0.05$). Within all groups, the rectal tissue contained significantly less (171 ± 17 pmol/g; Bartlett's, $p < 0.01$; Tukey, $p < 0.01$) enteroglucagon than the other bowel segments. When enteroglucagon levels of extracted bowel segments from different groups were compared to those of the controls, only the rectal sample from the subtotal colectomy group, which was three- to fourfold greater than all the other groups, was significantly elevated (Bartlett's, $p < 0.0001$; Tukey, $p < 0.01$). Although enteroglucagon content in the segments of colon which remained in the two hemicolectomy groups was greater than that of the controls, the difference was not significant. The rectal and ileal tissue enteroglucagon contents were similar to or less than those of the controls for the two hemicolectomy groups.

Immunogold Double-Labeling of Endocrine Cells

Specific immunogold labeling was observed over epithelial endocrine cells both in proximal colon and rectum. PYY and glucagon-like immunoreactivity (GLI) could easily be distinguished from each other because of the different sizes of gold particles. All cells labeled positively for PYY ($n = 21$) also labeled positively for GLI. These cells contained round to slightly oval granules with a diameter of 100–200 nm and were identified as L cells. The majority of PYY and GLI labeling was observed over the granules in the same cells. This labeling was found to co-exist in a majority of the granules. Enterochromaffin cells ($n = 40$) displayed no specific labeling. When antisera were absorbed with an excess of the corresponding antigen, no labeling was observed over L cells. Control sections showed no cross-reactivity between the two immunogold detection systems.

DISCUSSION

We studied the serum and tissue levels of PYY and enteroglucagon in rats following various large bowel resections. Plasma PYY levels following abdominal (subtotal) colectomy were elevated more than fivefold over controls, and remained significantly elevated throughout the 38 weeks of the study. The animals which underwent transections or partial resections showed only transient increases in circulating PYY levels, which quickly dropped to levels comparable to the control group. In contrast to the plasma PYY changes, circulating levels of enteroglucagon did not change significantly, with the exception of the right hemicolectomy group. The tissue levels of PYY (two- to threefold greater) and enteroglucagon (three- to fourfold greater) in the rectums of the rats with an abdominal colectomy were significantly elevated as compared with all other experimental groups. Similarly, there was a trend toward elevation of PYY and enteroglucagon in the remaining

colonic tissue in the two partially resected groups. Finally, immunogold double-labeling studies co-localized PYY and glucagon-like immunoreactivity (GLI) within the same colonic L cells of the rectum and colon and within the same secretory granules.

Plasma PYY levels were significantly elevated in the subtotal colectomy and right hemicolectomy groups. One explanation for this response is the continued presence of a significant intraluminal release stimulus to the endocrine tissue. For example, infusion of bile salts and fatty acids into the lumen of the colon has been shown to elevate plasma PYY [31,32]. More specifically, in studies with the isolated rabbit colon, bile salts stimulated the release of PYY [33]. Following subtotal colectomy and right hemicolectomy, with the loss of the terminal ileum and proximal colon, larger quantities of bile salts reach the distal gut mucosa [34]. In the right hemicolectomy group, after a number of weeks, the PYY levels returned to control levels, and, in the subtotal colectomy groups, the PYY levels also began to fall by 38 weeks. Perhaps there may be some ability of the small bowel or rectum to compensate for the resected ileum and cecum. In contrast to these groups, the left hemicolectomy group did not have ileum and cecum resected, and there was no protracted elevation of peptides. Thus, alterations of bile salts in the enteric contents arriving at the distal bowel may be modulating the release of PYY.

In the groups transected in the jejunum and in the mid-colon, significant transient increases in circulating levels of plasma PYY occurred during the first week following surgery. A rise and fall of lesser magnitude was also observed for the plasma levels of enteroglucagon during the same time period. Savage et al. observed a similar transient rise in plasma levels of both peptides in rats having undergone a small bowel resection [29]. Interestingly, the transient elevations of peptides, observed in the first week of our studies, involved both PYY and enteroglucagon. The later elevations of circulating peptides in the subtotal colectomy and right hemicolectomy groups involved PYY only. The transient increases of both peptides may reflect temporary disruption of gastrointestinal transit, allowing unabsorbed nutrients in the distal gut [32,33,35,36]. The later elevations of plasma PYY may be a response to a more PYY-specific releasing stimulus.

Both tissue PYY and enteroglucagon were elevated in the rectums of the subtotal colectomy animals. There was also a trend toward elevation of peptide content in the remaining colonic tissue of the partially resected groups, although this did not reach significant levels. These changes in tissue peptide content suggest that a compensatory mechanism may exist in the remaining endocrine tissue following resection. A hyperfunctional state has been morphologically described by Buchan et al. in the enteroglucagon-producing L cells following small bowel resection in rats [37]. This state was characterized by increases in the morphologic area occupied by the rough endoplasmic reticulum, but no significant change in cell size. Plasma enteroglucagon was elevated in these animals [37]. Thus, it is possible that the elevated tissue and circulating PYY observed in our study may be due to a similar hyperfunctional state of the L cells producing PYY.

The PYY and glucagon-like immunoreactivity (GLI) found within the same colorectal L cells of rats indicates co-localization of PYY and enteroglucagon. Our studies utilized an anti-glucagon antibody directed against both pancreatic glucagon and enteroglucagon; however, more than 99 percent of GLI in the rat colon represents enteroglucagon [6]. Thus, the immunogold labeling indicates that PYY

and enteroglucagon are produced within the same endocrine cells of the rat colon and rectum.

The co-localization of PYY and enteroglucagon within the same colorectal L cells of rats was in agreement with previous studies. In immunocytochemical studies of serial sections of colon tissue, PYY and enteroglucagon co-localized in some endocrine cells in rats and man [1,3]. Previous electron microscope work with immunogold revealed both peptides within the same endocrine cells in cats and man [5]. In our study, PYY and enteroglucagon co-localized in all cells examined, but this finding does not exclude the possibility that separate populations of cells containing only PYY or glucagon exist.

In the subtotal colectomy group, both PYY and enteroglucagon content were elevated in the rectum, while only a rise in plasma PYY occurred. The elevation in rectal tissue content of both PYY and enteroglucagon could be explained by the co-localization of PYY and enteroglucagon in the same L cells. The differential rise of plasma PYY in these rats could indicate separate mechanisms of release. In isolated colon experiments, for example, there was selective release of enteroglucagon in response to intraluminal oleic acid and release of PYY in response to intraluminal bile salt [33]. Alternatively, the two peptides may be cleared at different rates.

Our results are similar to those observed in human studies. Besterman et al. observed no significant difference in fasting and postprandial enteroglucagon levels between patients with colectomy ($n = 9$) and controls [38]. Our study also showed no significant change in plasma enteroglucagon following colectomy. In other human studies, Adrian et al. observed a significantly greater PYY incremental integrated response to a meal, in colectomy patients ($n = 5$) compared with controls, but did not find significant differences in fasting PYY levels and mean PYY levels [39]. These findings were somewhat different from those of our studies, which showed that fasting plasma PYY levels were elevated following colectomy. Perhaps the difference in findings may be due to the variable amount of time after surgery in the human studies. Furthermore, the data of both of the earlier human studies is more difficult to interpret because of the inability to control for the amount of bowel resected and because of the use of inflammatory bowel disease patients. Inflammatory bowel disease alters plasma and tissue levels of both PYY and enteroglucagon [40,41,42]. Thus, our studies and data from previous human studies indicate that PYY levels are elevated while enteroglucagon levels are unchanged, following large bowel surgery.

Resection of distal segments of gut which contain PYY may hinder digestion and absorption of food in the small bowel. Infusion of fat into the terminal ileum in man slows both gastric emptying and intestinal transit [43]. The mechanism which mediates this effect has been called "the ileal brake." Since PYY inhibits gastric acid and pepsinogen secretion, gastric emptying and small bowel transit, it has been proposed that PYY mediates this mechanism [44]. PYY may be released after meals from the terminal ileum as well as the colon by postprandial increases of plasma levels of cholecystokinin or by other neural mechanisms [45]. Our study did not identify the mechanism which leads to increased plasma levels of PYY and tissue levels of PYY and enteroglucagon following subtotal colectomy. These studies, however, suggest that the colon may play a role in the mediation of the "ileal brake mechanism" and that loss of the entire colon compromises this mechanism through loss of tissue which normally releases PYY and enteroglucagon following meals. It is

possible that, as a result of shortened transit time, proximal digestion and absorption is hindered and delivery to the distal gut increased of factors within the succus entericus which stimulate PYY and enteroglucagon synthesis and release by L-type endocrine cells. Elevated levels of PYY then improve proximal digestion and absorption while enteroglucagon may promote increased mucosal growth, leading to returned normal function of the ileal brake mechanism. Additional studies, however, will be required to test this possibility directly.

It is unlikely that the plasma and tissue elevations of PYY and enteroglucagon observed in our studies reflect impaired health of or physiologic stress on the rats. When using growth and survival as a marker for the general metabolic state of the rats, the data indicate that all groups of rats were in an anabolic state. All experimental rats continued to gain substantial amounts of weight throughout the study. Similarly, the survival rate of the subtotal colectomy group (77 percent) from the first week through the thirty-eighth week was at least as good as the right and left hemicolectomy groups (56 percent and 78 percent, respectively). Furthermore, when the effect of stress from serial blood sampling on plasma peptide levels was tested, there were no significant differences observed.

This study demonstrated that plasma and tissue levels of the colonic hormones PYY and enteroglucagon are altered following colonic resections. These results suggest that functional outcome following colonic resections may be effected by subsequent growth and function of colonic endocrine cells. Further study of these and other colonic peptides may yield useful information to apply in the treatment of patients with inflammatory bowel disease or patients who have undergone small and large bowel resections.

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