

# Is raised plasma peptide YY after intestinal resection in the rat responsible for the trophic response?

A P SAVAGE, G E GORNACZ, T E ADRIAN, M A GHATEI,  
R A GOODLAD, N A WRIGHT, AND S R BLOOM

*From the Departments of Medicine and Histopathology, Royal Postgraduate Medical School, Hammersmith Hospital, London*

**SUMMARY** The relationship between the adaptive response and plasma PYY concentrations after small bowel resection has been investigated. Seventy five per cent proximal small bowel resection resulted in a rise in plasma PYY at six days from  $28 \pm 3.1$  to  $85 \pm 12.3$  pmol/l ( $p < 0.001$ ) and this difference was maintained to 48 days. Plasma PYY correlates both with crypt cell production rate (CCPR) in the ileum and with plasma enteroglucagon levels. In a second study, PYY or saline was infused over a 12 day period. There were no significant changes in intestinal wet weight or CCPR in any part of the bowel studied. This indicates that it is unlikely that PYY exerts a major trophic effect on the gastrointestinal tract.

Peptide YY (PYY) is a recently discovered 36 amino acid polypeptide hormone first isolated from porcine small bowel which shares considerable sequence homology with pancreatic polypeptide.<sup>1,2</sup> It has been localised to the mucosal endocrine cells of the distal small bowel and colon in man and the rat.<sup>3-5</sup> Peptide YY has been shown to have profound effects on gastrointestinal motor activity inhibiting jejunal and colonic motility in the cat<sup>3</sup> and the interdigestive contractile activity of dog stomach.<sup>6</sup> In man, PYY has been shown to inhibit pentagastrin stimulated gastric acid and pepsin secretion.<sup>7</sup> It is therefore likely that PYY is an important new circulating gastrointestinal hormone.

Enteroglucagon is also localised to the distal small bowel with maximal concentrations in the terminal ileum and lower levels in the colon.<sup>8</sup> Plasma enteroglucagon concentrations are raised in patients with active coeliac disease<sup>8</sup> small intestinal resection<sup>9</sup> and jejunio-ileal bypass,<sup>10</sup> all conditions in which there is a hyperplastic response in functioning normal bowel. In addition there is evidence from studies in the rat that enteroglucagon is trophic to the small bowel.<sup>11,12</sup> Because of the similarity in distribution between PYY and enteroglucagon, we have investigated whether PYY is coreleased with enteroglucagon into the circulation after small

intestinal resection and correlated PYY concentrations with crypt cell production rate (CCPR) as a measure of the proliferative status of the small bowel. In addition, we have investigated the effect of chronic infusion of PYY on intestinal wet weight and CCPR.

## Methods

### ANIMALS

Male Wistar rats weighing between 250 and 300 grams were used for all studies. Animals were housed in animal quarters with 12 hour day/night cycle in open wire bottom cages. Water and a standard pelleted rat diet (Labsure Animal Foods, Poole, Dorset) was given *ad libitum* except that food was withheld for 12 hours before being killed. All operations were carried out under intramuscular Hypnorm (fentanyl and fluanisone) 0.5 ml/kg and intraperitoneal diazepam 0.5 mg/kg anaesthetic. Animals were weighed preoperatively and on death, and weight gain expressed as the difference between these two weights.

In the time course study, 96 rats were divided into two groups, half undergoing 75% proximal small bowel resection and half undergoing jejunal transection. Bowel continuity was restored by end to end anastomosis using 5/0 black silk. Eight animals from each group were killed at 1.5 days, three days, seven days, 12 days, 24 days, and 48 days after surgery by ether anaesthetic and cardiac puncture exsanguina-

Address for correspondence: Professor S R Bloom, Department of Medicine, The Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS.

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tion. Blood samples were placed in heparinised tubes containing 0.2 ml Trasylol (4000 KIU aprotinin) and plasma separated after centrifugation and stored at  $-20^{\circ}\text{C}$  pending assay. The small bowel and colon were dissected free of mesentery, opened longitudinally and washed with ice cold normal saline. A 5 cm segment of terminal ileum was placed in Carnoy's fixative for estimation of crypt cell production rate (CCPR). In the second study, two groups of eight rats underwent subcutaneous implantation of Alzet mini-osmotic pumps model 2002 (Alza Co Ltd). In the first group, pumps were filled with a solution of PYY (Bachem, USA) 1.5 mg in 2.5 ml saline+1% human serum albumin (HSA). The pump delivered  $0.44 \mu\text{l/h}$  for 14 days giving a calculated infusion rate of  $5 \text{ pmol/kg/min}$ . In the control group the pumps were filled with sterile normal saline+1% HSA. Animals were killed on the 12th postoperative day as above and plasma separated and stored at  $-20^{\circ}\text{C}$ . The stomach, duodenum, small bowel, colon, and pancreas were dissected free. Bowel was opened longitudinally, washed in ice cold saline and blotted dry. Weight of these organs was recorded. Samples for CCPR were taken from the duodenum 8 cm from the pylorus, mid small bowel and midcolon.

#### CRYPT CELL PRODUCTION RATE

A stathmokinetic method was used to assess the rate of production of cells in the crypts of Lieberkuhn.<sup>13,14</sup> At 9 am on the morning before being killed each animal received an intra-peritoneal injection of vincristine (Oncovin, Eli Lilly, Basingstoke)  $1 \text{ mg/kg}$  which arrests cell division in metaphase. Animals were killed from 30 minutes to three hours after injection. Samples of bowel for crypt cell production rate were fixed in Carnoy's solution for four hours and then transferred to 70% ethanol. The tissue was stained with the Feulgen reaction, individual crypts dissected free under a dissecting microscope and the number of metaphases per crypt in 10 crypts from each sample were counted. The crypt cell production rate and its standard error was determined by linear regression analysis for individual values against time.

#### PYY AND ENTEROGLUCAGON ASSAY

Plasma PYY was measured by a standard radioimmunoassay using a method described in detail elsewhere.<sup>15</sup>  $^{125}\text{I}$  PYY was prepared by the chloramine T method and purified using high performance liquid chromatography. Rabbit antiserum to PYY was raised using unconjugated PYY in Freund's adjuvant. This antibody (Y21) shows no detectable cross reaction with bovine PP, human PP, avian PP, neurotensin, glicentin or VIP in

concentrations up to  $500 \text{ pmol/tube}$ . Porcine NPY showed a slight cross reaction of 0.04%. The assay detects changes between adjacent plasma samples of  $2.5 \text{ pmol PYY/l}$  with 95% confidence.

Enteroglucagon was measured by subtracting the level of pancreatic glucagon measured with a specific antiserum (RCS5) from total glucagon immunoreactivity measured with an antibody that fully cross reacts with glicentin.<sup>8,16</sup> The detection limit of the assay was  $12 \text{ pmol/l}$ .

#### CHROMATOGRAPHY

Gel filtration chromatography was done on pooled plasma from the 75% resected animals at 12 days and on pooled plasma from the PYY infused group of animals. A 1 ml sample was loaded onto a  $100 \times 1.5 \text{ cm}$  column packed with Sephadex G-50 superfine (Pharmacia) and eluted at  $5.5 \text{ ml/hour}$  with  $0.06 \text{ M}$  phosphate buffer pH 7.4 with  $10 \text{ mM}$  EDTA,  $7.5 \text{ mM}$  sodium azide and  $50 \mu\text{M}$  bovine serum albumin. The internal column markers used for indicating void volume ( $V_0$ ) and total volume ( $V_t$ ) were dextran blue and  $\text{Na}^{125}$  respectively. Pure natural porcine PYY was used to determine the elution position of PYY. The elution position ( $V_e$ ) was expressed as elution co-efficient ( $K_{av}$ ) where  $K_{av} = V_e - V_0 / V_t - V_0$ .<sup>17</sup>

#### STATISTICAL METHODS

Students  $t$  test for unpaired data was used for intergroup comparison. Results are expressed as mean  $\pm$  standard error of the mean (SEM). Correlations are calculated by linear regression analysis.

#### Results

##### WEIGHT GAIN AND FOOD INTAKE

In the time course study, both groups regained their preoperative body weight between 12 and 24 days. After 12 days, the transected group gained significantly more weight than the resected group. Food intake was greater in the resected group from six to 24 days but not at 48 days (Fig. 1). There were no differences in food intake or weight gain between the PYY and the saline infused group (Table).

##### PLASMA PYY AND ENTEROGLUCAGON

There was no significant difference in plasma PYY between resected ( $58.2 \pm 8.4 \text{ pmol/l}$ ) and the transected group ( $43.4 \pm 5.7 \text{ pmol/l}$ ) at 1.5 days. Thereafter PYY concentrations in the transected group fell to  $28.6 \pm 3.1 \text{ pmol/l}$  and rose in the resected group to  $84.8 \pm 12.3 \text{ pmol/l}$  at six days ( $p < 0.001$ ) and these levels were maintained for 48 days (Fig. 2). Plasma enteroglucagon was raised 2.5 times in the resected group at 1.5 days and rose to maximal levels at 12

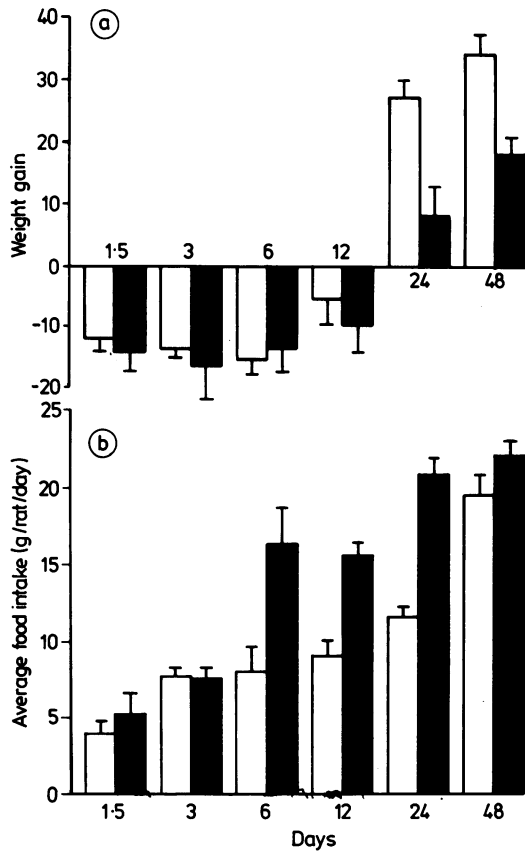


Fig. 1 (a) Weight gain and (b) food intake in the time course study (mean  $\pm$  SEM). Solid bars=resected animals; open bars=controls animals.

Table Weight gain, food intake, CCPR and wet weight of intestinal organs in Alzet pump PYY infused animals and saline infused animals.

	PYY infused	Saline infused
Weight gain (grams)	44.7 $\pm$ 2.5	45.6 $\pm$ 5.0
Food intake (grams/day)	19.5 $\pm$ 0.4	19.7 $\pm$ 0.4
PYY plasma concentration (pmol/l)	141 $\pm$ 24.3	14 $\pm$ 3.9
CCPR(cells/crypt/h)		
Duodenum	24.5 $\pm$ 6.4	24.4 $\pm$ 7.4
Mid small bowel	18.9 $\pm$ 10.1	15.4 $\pm$ 3.2
Colon	10.0 $\pm$ 1.9	6.9 $\pm$ 2.1
Wet weight (grams)		
Stomach	1.39 $\pm$ 0.04	1.39 $\pm$ 0.03
Duodenum	0.84 $\pm$ 0.05	0.74 $\pm$ 0.03
Small bowel	5.35 $\pm$ 0.08	5.30 $\pm$ 0.14
Colon	1.30 $\pm$ 0.04	1.29 $\pm$ 0.05
Pancreas	1.04 $\pm$ 0.07	1.19 $\pm$ 0.05

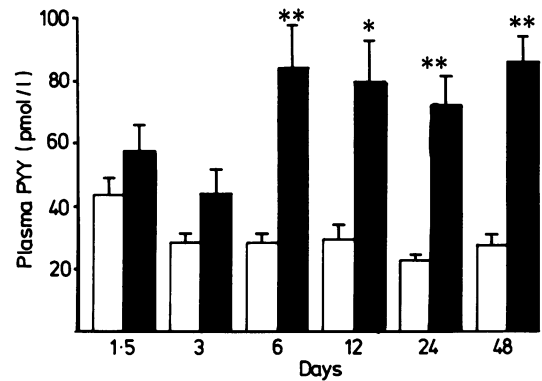


Fig. 2 Plasma PYY (mean  $\pm$  SEM) at the six time points after 75% small bowel resection (solid bars) or control transection (open bars). \* =  $p < 0.005$ , \*\* =  $p < 0.001$ .

days, these levels being maintained for 48 days (Fig. 3). Plasma PYY concentrations in the PYY infused groups were 141  $\pm$  24.3 pmol/l while control levels were 14  $\pm$  3.9 pmol/l ( $p < 0.001$ ).

#### CHROMATOGRAPHY

Eighty per cent of PYY immunoreactive material in the 75% resected animals eluted on Sephadex G-50 as a single major peak at  $K_{av}$  of 0.52 (Fig. 4 upper profile). The remaining immunoreactivity eluted as a small higher molecular weight peak. The major peak coeluted with the immunoreactive peak from the PYY infused group of animals (Fig. 4 lower panel) and with pure natural porcine PYY standard.

#### CRYPT CELL PRODUCTION RATE

The changes in CCPR in the time course study are

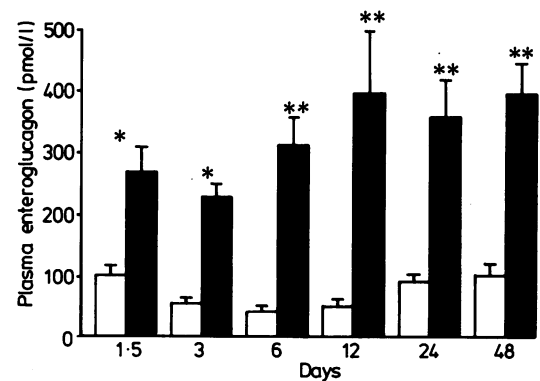


Fig. 3 Plasma enteroglucagon (mean  $\pm$  SEM) after 75% bowel resection (solid bars) or transection (open bars). \* =  $p < 0.005$ , \*\* =  $p < 0.001$ .

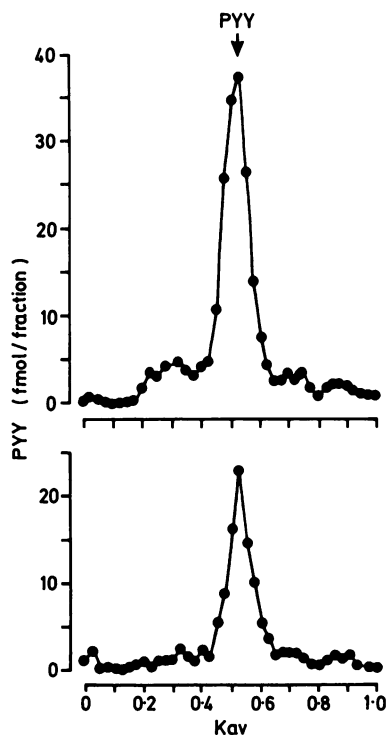


Fig. 4 Sephadex G-50 column profile of pooled plasma from 75% resection animals at 24 days postoperatively (upper) and Alzet pump PYY infused animals (lower). The arrow marks the elution position of pure PYY standard. Recovery from the column was 87% and 82% respectively.

shown in Fig. 5. Terminal ileal CCPR in the resected group rose from  $29.8 \pm 3.9$  cells/crypt/h at 1.5 days to a maximum of  $50.6 \pm 5.7$  cells/crypt/h at 12 days and fell to  $41.5 \pm 5.5$  cells/crypt/h at 48 days. Crypt cell production rate in transected animals were 45% of resected values at 1.5 days and 26% at 12 days. Figure 6 shows the close correlation between PYY, enteroglucagon and CCPR. There were no significant changes in CCPR in the PYY infused animals compared with the saline infused animals in the duodenum, mid small bowel or colon. (Table).

### Discussion

In the time course study we have shown that plasma PYY is raised almost three fold after 75% small intestinal resection over transection levels. This response parallels the rise in enteroglucagon after resection. After transection, however, PYY concentrations do not reach a steady level until three days and remain consistently raised more than 1.5 times

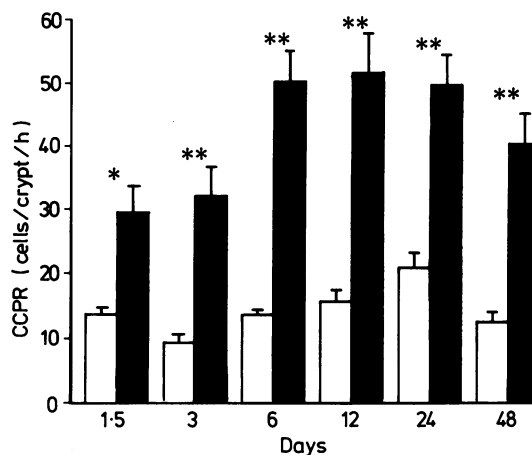


Fig. 5 Crypt cell production rate (CCPR) ± SEM after intestinal resection (solid bars) or transection (open bars). \* =  $p < 0.005$ , \*\* =  $p < 0.001$ .

the level seen in the saline infused group of animals which did not undergo either laparotomy or jejunal transection. Whilst luminal factors<sup>18 19</sup> and pancreatico-biliary secretions<sup>20-22</sup> have been shown to be important in the maintenance of normal cell turnover in the small intestine, these factors cannot explain the hyperplasia seen proximal to resected bowel. Limited adaptation also occurs in isolated Thiry-Vella loops after resection of the remaining bowel<sup>23</sup> and in the small bowel of parabiotic animals coupled to animals who have had a bowel resection<sup>24 25</sup> suggesting a humoral mechanism. Enteroglucagon has been proposed as a trophic hormone to the gastrointestinal tract after a report of a patient with an enteroglucagon producing tumour who exhibited marked intestinal hyperplasia and diminished intestinal transit time, these changes reversing on removal of the tumour.<sup>26 27</sup> Further supportive evidence that enteroglucagon may be this humoral factor comes from the finding that enteroglucagon concentrations are raised in patients with coeliac disease,<sup>8</sup> small intestinal resection<sup>9</sup> and jejuno-ileal bypass<sup>10</sup> in all of which there is a hyperplastic response in remaining or functioning small bowel. Previous studies in rats have also shown a close association between plasma enteroglucagon and intestinal proliferative status.<sup>11 12 28</sup> In the time course study, this close association is confirmed. Peptide YY also shows a highly significant correlation with both CCPR and enteroglucagon, however, the correlation between enteroglucagon and CCPR being marginally better than between PYY and enteroglucagon (Fig. 6). While a positive correlation does not prove that there is a causal relationship

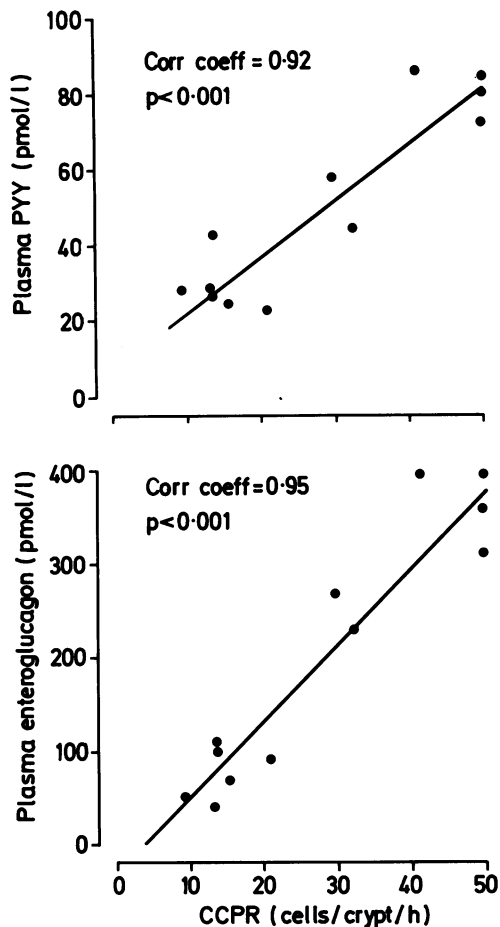


Fig. 6 Plasma PYY (upper) and enteroglucagon (lower) plotted against crypt cell production rate for each of the time points and their regression lines.

between either enteroglucagon or PYY and intestinal proliferative status, it does indicate that such a relationship may exist. Partly purified enteroglucagon has been shown to increase the incorporation of  $^3\text{H}$  thymidine into DNA in cultured small intestine.<sup>29</sup> Pure rat enteroglucagon, however, has not yet been isolated and definition of its role in the maintenance of intestinal cell mass must await this.

In the second study, we have shown that infusion of PYY over 12 days had no significant effect on intestinal wet weight or proliferative status of the gastrointestinal tract. There were no changes in wet weight of the stomach, duodenum, small bowel, colon, or pancreas. Though there was a slight increase in CCPR in the PYY infused animals, this was not significant at any site and while CCPR is a

very robust indicator of change in proliferative status, its precision is such that these differences are no greater than the normal variation of the method. Changes in wet weight of adapted bowel after resection have been reported to reach plateau levels at 12 days<sup>30</sup> and if the slight increase in CCPR shown did reflect a trophic response, weight changes would also be expected. These observations indicate that it is unlikely that PYY exerts a major trophic effect on the gastrointestinal tract and enteroglucagon remains a favoured candidate for this role.

Peptide YY was first isolated from porcine small bowel and has now been synthesised. We have shown that more than 80% of endogenous rat PYY immunoreactivity and synthetic porcine PYY in the PYY infused animals coeluted with natural porcine PYY standard and as rat PYY shares immunoreactivity with porcine PYY, there may be no major sequence differences though confirmation of this will have to await its isolation and purification. The trophic response is only one feature of intestinal adaptation. Other aspects include functional adaptation of the mucosa to increase absorption<sup>18</sup> and a slowing of intestinal transit to allow prolonged contact between food and mucosa.<sup>31</sup> Further studies are required to assess whether PYY mediates control of intestinal transit in the adaptive response following intestinal resection, a possible role in view of the powerful inhibitory effects of PYY on gastrointestinal motility in experimental animals.<sup>36</sup>

In conclusion, we have shown that plasma PYY is raised after small intestinal resection and that this rise correlates with enteroglucagon concentrations and CCPR. Infusion of PYY for 12 days, however, did not result in a significant increase in wet weight or CCPR and this would suggest that it is unlikely that PYY exerts a major trophic effect on the gastrointestinal tract.

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