Influence of somatostatin and bombesin on plasma enteroglucagon and cell proliferation after intestinal resection in the rat

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SUMMARY The possible relationship between enteroglucagon and cellular proliferation in a rat model of intestinal adaptation after suppression and stimulation of enteroglucagon by somatostatin and bombesin has been investigated. Forty eight rats were divided into three groups of 16 animals, each group being further sub-divided into eight animals having intestinal resection and eight having intestinal transection. Group 1 was given somatostatin to suppress enteroglucagon, group 2 was given bombesin to stimulate enteroglucagon and group 3 (control group) had neither peptide. All animals were killed 12 days after operation. Circulating enteroglucagon and crypt cell production rate (CCPR) in the terminal ileum were measured. After administration of somatostatin (group 1) both CCPR and plasma enteroglucagon were lower after resection than controls (group 3) (p<0.001). Transected rats receiving somatostatin showed a reduction in both plasma enteroglucagon and CCPR, but only the fall in enteroglucagon was statistically significant (p<0.001). Transected rats receiving bombesin (group 2) had raised plasma enteroglucagon and CCPR compared with the control group (group 3) (P<0.005) but there was no significant further rise in these already raised parameters in resected animals. This study indicates that cell proliferation in the rat small bowel after surgery can be influenced by regulatory peptides. The changes in enteroglucagon corresponded closely with changes in CCPR, and this peptide remains a favoured candidate for the humorally mediated trophic influence on the small bowel.

The adaptive hyperplastic changes seen in the residual intestine after extensive small bowel resection have been shown to be closely related to oral nutritional intake following surgery. That luminal nutrition is the sole factor in initiating this response has been questioned and evidence is accumulating to suggest that a humoral mechanism may also be involved in this process. Gastrin was proposed as a candidate for such a role but this has not been substantiated and it now seems unlikely that gastrin has any major trophic effect on the small bowel. Furthermore, enteric mucosal hyperplasia has not been recorded in conditions of hypergastrinaemia such as the Zollinger-Ellison syndrome or pernicious anaemia. A number of other regulatory peptides have been investigated for a role as an enterotropin in intestinal adaptation. Although cholecystokinin (CCK) and secretin given together to dogs on total parenteral nutrition prevented the villous hypoplasia associated with total parenteral nutrition, when each peptide was given separately to rats, no such effect was noted. During lactation, adaptive changes are seen in by-passed Thiry-Vella jejunal loops, suggesting that humoral factors may be responsible for these effects, and although prolactin seemed an obvious candidate, this hormone has been shown not to play a part in bowel adaptation during lactation. Ornithine decarboxylase, corticosteroids, epidermal growth factor and hypothalamic/anterior pituitary hormones have all been investigated and shown to play a possible role in intestinal adaptation.

The report of an enteroglucagon producing renal tumour in a patient who exhibited small bowel...
thickening and increased villous height\textsuperscript{19, 20} prompted the suggestion that this peptide may be a candidate humoral agent involved in intestinal adaptation. Further studies\textsuperscript{21–23} have provided additional circumstantial evidence that entero-
glucagon is trophic to the small bowel. This study was, therefore, designed to investigate the entero-
cyte proliferation after small bowel resection and transection, under conditions of stimulation and 
hibition of plasma entero-glucagon release by other gastrointestinal regulatory peptides.

\textbf{Methods}

\textbf{ANIMALS}

Forty eight male Wistar rats, weighing 200–250 g at the time of surgery, were used in the study. Diazepam and Hypnorm (fentanyl and fluanisone Janssen Pharmaceutical) by injection, were used as anaesthetic. Half of the rats had a 75% proximal small bowel resection, measured distally from the ligament of Treitz, while the other half had a jejunal transection immediately below the ligament of Treitz, with reanastomosis. Bowel anastomoses were fashioned with 6/0 black silk. Animals were allowed water and food \textit{ad libitum}. Food was in the form of pelleted rat diet (Labsure Animal Foods, Poole, Dorset). All animals were killed with ether 12 days after surgery as changes in CCPR reach a plateau at this time.\textsuperscript{24} Standard segments of small bowel (see below) were taken for estimation of crypt cell production rate and blood was taken by direct cardiac puncture for entero-glucagon radio-
immunoassay. Both the food intake and the body weight changes over the duration of the experiment were measured daily.

Animals were divided into three groups of 16, each group comprising eight rats with 75% proximal small bowel resection and eight rats with jejunal transection. Group 1 was given long-acting somato-
statin (Des-AA\textsuperscript{1, 2, 4, 5, 12, 13}[D-Trp\textsuperscript{8}]-SS)\textsuperscript{25} 100 \mu g per 100 \mu l rat plasma subcutaneously twice a day for the last seven days of the experiment, a dose which preliminary experiments had suggested would approximately halve basal plasma entero-glucagon concentrations. Group 2 rats were given bombesin (Bachem Co, California) for a similar period. As this peptide is rapidly cleared after injection, it was administered continuously via the Alzet osmotic minipump, model 2001 (Scientific Marketing Associated, London), which was implanted subcutaneously in the back of the animals' neck under anaesthesia. Each pump, which delivers a constant flow at a rate of 1 \mu l/hour for seven days was filled with a solution of bombesin, 96 \mu g per 100 \mu l normal saline. This dose was ascertained in preliminary experiments to approximately double basal plasma entero-glucagon concentrations. Group 3 animals were given a minipump containing saline only over the last seven days of the experiment.

\textbf{CELL PROLIFERATION}

The crypt cell production rate (CCPR) was used as an index of cellular proliferation in the small bowel. Vincristine was used to arrest dividing cells in the metaphase so that the number of such cells could be counted.\textsuperscript{26} At 09.30 hours on the day of killing the animals, each rat was given vincristine sulphate (Oncovin, Eli Lilly & Co Ltd, Basingstoke, UK) in a dose of 1 mg/kg body weight, by intraperitoneal injection. The first rat was killed 30 minutes after the injection and each subsequent animal was killed at 20 minute intervals thereafter. The ileocaecal valve was taken as a reference point and 20 mm lengths of bowel, at a fixed point 20 mm from the ileocaecal junction, were removed from each rat. The tissues were initially fixed in Carnoy's fluid for four hours and then transferred to 75% ethanol. The tissues were stained with Feulgen stain and the mucosa was stripped from the muscle coat. Individual crypts were then dissected out under the dissecting microscope. The number of metaphases in each crypt were counted and the mean of the metaphase counts of 10 crypts were taken as the reading for each individual rat. The number of cells arrested in metaphase per crypt was then plotted against time after vincristine administration. The crypt cell production per hour (CCPR) for the group is given by the slope of the regression line, fitted by method of least squares.\textsuperscript{27, 28}

\textbf{PLASMA ENTEROGLUCAGON}

Two assays were carried out, one for total glucagon-
like immunoreactivity (R59) which reacts fully with pure porcine entero-glucagon (glicentin) and one for pancreatic glucagon, using a relatively specific C-
terminally-reacting antisera, RCS5, which gives zero readings in plasma after total pancreatec-
tomy.\textsuperscript{29} The entero-glucagon concentration was then obtained by subtracting the small concentration of pancreatic glucagon from that of total glucagon. Changes of 10 pmol/l entero-glucagon in the plasma could be detected with 95\% confidence.

\textbf{STATISTICAL METHODS}

The Student's \textit{t} test for paired data was used for the group analysis and results were expressed as a mean and standard error of the mean.

\textbf{Results}

\textbf{FOOD INTAKE AND BODY WEIGHT}

There was no significant difference in food intake
Enteroglucagon and intestinal adaptation

per day between the three groups (21±3 g, 23±2 g, and 24±3 g respectively). At the end of the experiment all rats had regained their preoperative body weights, without significant differences occurring between the groups.

CCPR AND PLASMA ENTEROGLUCAGON

In the control animals (group 3) there was an increase in CCPR/h in the terminal ileum from 16.8±0.9 in transected to 49.2±4.9 in resected animals (p<0.001) (Table). Similarly, plasma enteroglucagon increased from 99.1±9.6 pmol/l in transected to 667±70 pmol/l in resected rats (p<0.001).

After administration of somatostatin (group 1), rats with intestinal resection showed a fall in CCPR/h to 15.4±1.0 compared with controls (group 3) (p<0.001) (Table). Similarly, there was a fall in plasma enteroglucagon after resection to 73±9 pmol/l compared with group 3 (p<0.001). Although transected rats given somatostatin did show a fall in plasma enteroglucagon, 26.3±8.9 pmol/l compared with group 3 (p<0.001), the change in CCPR was not significant.

After administration of bombesin (group 2) transected rats showed a rise in CCPR from 11.8±0.9 in animals (group 3) to 24.5±1.9 (p<0.005) (Table). Similarly plasma enteroglucagon also rose from 99.1±9.6 pmol/l in group 3 to 218±34 pmol/l (p<0.005). Bombesin treated resected rats, however, showed no alteration in CCPR and plasma enteroglucagon, compared with group 3 resected animals.

Discussion

Although it seems certain that luminal nutrition is necessary for the adaptive response after extensive small bowel resection, there is considerable evidence that humoral mechanisms may also participate in the production of these changes. Thus the prevention of hypoplasia seen in isolated Thiry-Vella fistulae after jejunectomy and the adaptive changes in the ileum after colonic resection, where there is no change in the amount of nutrition reaching this part of the bowel, suggests that mechanisms, other than luminal nutrition, must be operative in these models. When two groups of intravenously-fed rats underwent either 75% proximal small bowel resection or jejunal transection, the resected rats showed a limited but significant increase in cell turnover in the residual bowel compared with transected animals, indicating that factors other than luminal nutrition must be responsible for these adaptive changes.

More direct evidence for a humoral mechanism in the adaptive process after small bowel resection is found in a study where rats were linked in vascular parabiosis for 48 hours. Transection and jejunal resection in one partner, resulted in a limited increase in uptake of tritiated thymidine in the unoperated parabiont, though this response was weaker than the direct changes seen in the resected animal.

Interest in enteroglucagon as a trophic agent in small bowel mucosa began with the finding of an enteroglucagon producing tumour in a patient who was found to have delayed gastrointestinal transit, gross thickening of the small bowel mucosa, with mucosal hyperplasia and an increase in the diameter of the bowel. These abnormalities reverted to normal after tumour resection with reduction of the plasma enteroglucagon concentrations. Furthermore, plasma enteroglucagon is markedly raised postprandially in a number of situations where there is also cellular proliferation in small bowel mucosa, such as untreated coeliac disease, after jejunoileal bypass and after small bowel resection. The enteroglucagon producing cells have a distal distribution in the gut, being found in greatest concentration in the distal ileum, and to a lesser extent in the colon. These cells are stimulated directly by carbohydrates and long chain triglycerides and it has been suggested that in situations where proximal failure of absorption of nutrients occurs, such as after proximal small bowel resection, the enteroglucagon cells in the distal gut are stimulated by the relatively undigested chyme coming into contact

Table  Plasma enteroglucagon concentrations and ileal crypt cell production rates (CCPR) in transected or resected rats receiving somatostatin, saline or bombesin (mean±SEM, n=8).

<table>
<thead>
<tr>
<th></th>
<th>CCPR</th>
<th>Plasma enteroglucagon (pmol/g)</th>
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<tbody>
<tr>
<td></td>
<td>Transected</td>
<td>p</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>15.2±0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Control</td>
<td>16.8±0.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Bombesin</td>
<td>24.5±1.9</td>
<td>0.001</td>
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In some text, CCPR/h was used to express the concentration of hormones, which stands for "eugloglucagon concentration per hour".
with them. This peptide may be responsible for stimulating enterocyte turnover and thus enhancing small intestinal absorptive capacity. This hypothesis is supported by strong circumstantial evidence in a number of models of adaptation, where good close correlation between circulating enteroglucagon concentrations and crypt cell production rate is seen. Both these parameters were equally influenced by the amount of orally ingested nutrients. When rats with isolated Thiry-Vella fistulae were nourished, either intravenously or orally, the latter group was found to have significantly less hypoplasia in the isolated fistula, with correspondingly greater plasma enteroglucagon concentrations and it is possible that the increased enteroglucagon released from the bowel of the orally fed rats was the circulating factor that helped to enhance cell turnover in the isolated loop. Long chain triglycerides given intragastrically have been shown to promote small bowel adaptation after resection in parenterally fed rats and as triglycerides are one of the major stimuli for enteroglucagon release, it provides further circumstantial evidence that this may play a part in bringing about these adaptive changes, although clearly, other mechanisms such as stimulation of the exocrine pancreas, the secretion of which appears to have a trophic influence on small bowel, may also be operating. Eight days after pancreatico-biliary bypass (PBD), plasma enteroglucagon concentrations were high, correlating with the adaptive bowel changes, while at three months after this procedure, although the degree of intestinal thickness was unchanged, the plasma enteroglucagon concentrations had fallen, suggesting that this peptide may be more important in initiating the adaptive response than maintaining it. The results of this study are, however, in contrast with the findings in a time course experiment, where rats with small bowel resection were studied at 1½, 3, 6, 12, 24, and 48 days after surgery. Plasma enteroglucagon and CCPR were markedly raised at 1½ days and continued to rise in a closely correlated manner for the full period studied suggesting that at least in this model where cell production rate was measured, enteroglucagon may also be associated with the maintenance of the adaptive response.

In the present study, somatostatin and bombesin were administered over the last seven days of the study, these peptides having the effect of inhibiting (in the case of somatostatin) and stimulating (in the case of bombesin) enteroglucagon release. Because of the long acting nature of the somatostatin given, it was not necessary to administer this peptide by continuous pump infusion as was the case with bombesin. The dose of somatostatin was calculated for body weight from previous infusion studies of this peptide in man, while the bombesin dose was derived from the study in which 2.5 pmol/kg/min of amphibian bombesin was found to stimulate the release of enteroglucagon and both were checked in pilot studies for their effect on enteroglucagon. Besides its effect on enteroglucagon, somatostatin has a wide range of inhibitory actions on other gastrointestinal functions, such as gastric and pancreatic secretion, gut motility, and blood flow, and the secretion of other peptide hormones. Likewise, the effects of bombesin are opposite to those of somatostatin, stimulating the various gastrointestinal functions mentioned above. There are, therefore, numerous mechanisms by which these two peptides could have had their effect on CCPR in this study. As the food intake in the three groups was similar, luminal nutrition was probably not responsible for these changes. Weight changes were not significantly different in the groups so it is unlikely that the major gastrointestinal functions were grossly impaired. It may well be, therefore, that the changes seen after somatostatin and bombesin administration could be mediated, at least in part, via their effect on enteroglucagon. Our data suggest that there may be a limiting concentration for CCPR and enteroglucagon, above or below which it is not possible to induce further changes, that is, stimulation with bombesin or suppression with somatostatin.

The results in this study, are in agreement with previous work, showing that shortening the bowel produces compensatory hyperplasia in the residual intestine and also supports the hypothesis that humoral factors may be operative. The mechanisms of intestinal adaptation are complex and multifactorial, with luminal nutrition, intestinal secretions and humoral factors probably all contributing. While it is possible that more than one humoral factor is implicated, the findings in this study suggest that enteroglucagon could be regarded as one of the favoured candidates, an interpretation also supported by the findings in another recent study, in which low dose partially purified rat enteroglucagon produced a 50% increase in a DNA synthesis in cultured guinea-pig jejunal mucosal cells. Direct infusion studies in animals, using a more purified enteroglucagon preparation, are now required to provide conclusive evidence as to the possible trophic role of enteroglucagon.

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Enteroglucagon and intestinal adaptation


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