Colonic short chain fatty acids mediate jejunal growth by increasing gastrin

K J Reilly, W L Frankel, A M Bain, J L Rombeau

Abstract
Colonic infusion of short chain fatty acids (SCFAs) is trophic to rat jejunum and is associated with raised jejunal gastrin concentration. This study examined the hypothesis that the jejunal trophic effects of colonic SCFAs are mediated in part by gastrin. Forty six adult rats underwent caecectomy to reduce endogenous production of SCFA, ileocolonic anastomosis, and placement of a colonic infusion catheter. SCFA (70 mM acetate, 35 mM propionate, 20 mM butyrate) or saline were continuously infused into the colon for seven days. Rats received either a gastrin receptor blocker (L-365,260) or a control solution and animals were killed on day 8. SCFA infused into the colon acted systemically to significantly improve jejunal structure and increase jejunal gastrin concentrations. Gastrin receptor blockade abolished effects of SCFA on jejunal DNA, protein, crypt cell proliferation, and gastrin. Gastrin blockade did not reduce SCFA induced augmentation of villous height or crypt depth. It is concluded that the jejunal trophic effects of colically infused SCFA are mediated in part by gastrin.

Methods

EXPERIMENT I
Male Sprague-Dawley rats weighing 200 to 250 grams (Charles Rivers Laboratories, Portage, MI) were housed in individual cages and fed rat chow and water ad libitum to acclimatise at least five days before the start of the experiment. This study was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania.

Rats were placed in individual cages with raised wire floors to limit intake of bedding material and maintained in a light, humidity, and temperature controlled environment. All animals were fed fibre free diets containing 5% w/w kaolin (Dyets, Bethlehem, PA) for five days before surgery. Water was provided ad libitum throughout the study.

On the day of surgery, rats were anaesthetised with pentobarbital (50 mg/kg body weight intraperitoneally), the ileum and colon were transected 1 cm from the caecum, and the caecum was removed to reduce endogenous SCFA production. An end to side anastomosis was performed between the terminal ileum and the proximal ascending colon with interrupted 6-0 polypropylene suture. An infusion catheter (internal diameter 0-03 inches, Baxter, McGaw Park, IL) was placed into the proximal end of the colon and secured with a 5-0 purse string suture, tunnelled subcutaneously, exteriorised at the interscapular area, and connected to a spring and swivel device (Instech, Polymouth Meeting, PA) to permit concurrent infusion and ambulation within the cage.

Rats (n=46) were randomly assigned to receive either a gastrin receptor blocker, L-365,260 (5 mg/kg/d in 0-9% NaCl; Merck, Cincinnati, OH), or control (vehicle, 0-9% NaCl) by twice daily gavage. L-365,260 is a high affinity (Kᵢ=1.1±0.4 nM) non-peptide antagonist of gastrin/CKK-B receptors. After oral administration, L-365,260 has an onset of action of less than five minutes and a half life of 8–12 hours. Oral L-365,260 has been shown to consistently antagonise the action of gastrin on acid secretion in rats in a dose dependent fashion. The first dose of gastrin receptor blocker or control was given two hours preoperatively and the last dose was given two hours before the rats were killed.

Postoperatively, animals were assigned to one of two colonic infusion groups: SCFA (70 mM acetate, 35 mM propionate, 20 mM butyrate, pH 6-1; Sigma, St Louis, MO) or saline (Sorensen’s phosphate buffer, iso-osmolar with SCFA solution, pH 6-1). Infusions

Short chain fatty acids (SCFAs) are the one to six carbon volatile fatty acids, predominantly acetate, propionate, and butyrate. They are produced in the colon by microbial fermentation of dietary polysaccharides and are the major luminal organic anions in the human and rat colon. Importantly, butyrate is the preferred oxidative fuel of the colonocyte. SCFAs are trophic to rat colon and jejunum when given by colonic infusion. The trophic effects of SCFAs on the colon require direct, mucosal contact, while SCFAs act on the jejunum by a systemic mechanism involving the autonomic nervous system. Previous investigation from our laboratory has shown that both the parasympathetic and sympathetic divisions of the autonomic nervous system must be intact for colonic SCFA mediated jejunal trophism to occur. Additionally, colonic SCFA infusion is associated with increased jejunal gastrin concentrations, which may be important in mediating jejunal trophism. The purpose of this experiment was to discover if gastrin mediates the trophic effects of colically infused SCFA on rat jejunum.

Keywords: short chain fatty acids, gastrin, enteroglucagon.

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were delivered continuously at 1·5 ml/h by syringe infusion pump (Harvard Apparatus, South Natick, MA). The selection of SCFA concentrations and rate of infusion was based upon the physiological concentrations of each SCFA in the rat colon and previous experience with the specified rate.1–3 8–12

All animals were permitted access to water and a fibre free diet containing 5% w/w kaolin beginning 24 hours postoperatively. After seven days of infusion and an overnight fast, animals were killed with sodium pentobarbital (50 mg/kg intraperitoneally) and rapid cardiac exanguination. Animals who received gastrin receptor blocker were killed 12 hours later than control rats because of an unavoidable laboratory emergency. Plasma gastrin concentration was determined by radioimmunoassay using corresponding human peptides as standards.21 22 Jejunal samples were harvested for determination of wet weight, histology, and crypt cell proliferation rate by bromodeoxyuridine incorporation (Amersham Proliferation Kit, Arlington Heights, IL).23 24 Twenty well oriented crypts and villi per animal were randomly selected and measured for villous height and crypt depth.25 Jejunal mucosa was harvested from 10 cm of proximal jejunum and analysed for DNA and protein contents.26 27 Gastrin was extracted from jejunal tissue samples by boiling in 0·1 M acetic acid for 20 minutes.23 28 Plasma and tissue gastrin samples were frozen at −70°C and gastrin concentrations were determined by radioimmunoassay (ARUP, Salt Lake City, UT).23 24

<table>
<thead>
<tr>
<th>Jejunal weight (mg/cm)</th>
<th>Plasma gastrin (pg/ml)</th>
</tr>
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<tbody>
<tr>
<td>Saline/control</td>
<td>67·6 (1·5)</td>
</tr>
<tr>
<td>SCFA/control</td>
<td>73·0 (3·6)*</td>
</tr>
<tr>
<td>Saline/GRB</td>
<td>78·1 (3·0)</td>
</tr>
<tr>
<td>SCFA/GRB</td>
<td>80·0 (2·6)</td>
</tr>
</tbody>
</table>

Data are mean (SEM); *p<0·05 v saline/control.

### EXPERIMENT 2

Nineteen male Sprague-Dawley rats were maintained preoperatively as described in Experiment 1. Rats were anaesthetised with pentobarbital (50 mg/kg body weight intraperitoneally) and underwent caecectomy, ileocolic anastomosis, and placement of an end colonic infusion cather as described previously. The animals were assigned to one of two groups (SCFA, saline), and infusions were delivered as described in Experiment 1. After seven days of infusion and an overnight fast, the animals were killed, and a blood sample obtained for plasma enteroglucagon concentration. Jejunal tissue concentrations of enteroglucagon were determined after tissue extraction23 28 by radioimmunoassay and subtraction of specifically measured pancreatic glucagon from total N-terminal glucagon immunoreactivity.28–31 These determinations were performed by Drs Bloom and Ghatei, Hammersmith Hospital, London.

### STATISTICAL ANALYSIS

For Experiment 1, randomised two way analysis of variance was performed with the Systat software (Systat, Chicago, IL). If significant treatment effects were found, a protected least significant differences test was performed for multiple comparisons. Complete one way analysis of variance was performed in Experiment 2 using the Systat software.

All data are presented as mean (SEM). Significance values are stated in Figure or Table legends for each end point investigated.

### Results

#### Body weight

Control rats who received SCFA gained significantly more weight than control rats who received saline (12·4 (4·2) g v 2·7 (3·7) g, p<0·01). In addition, both groups of rats who received gastrin blocker (saline/gastrin receptor blocker and SCFA/gastrin receptor blocker) gained significantly more weight than saline/control rats (16·5 (2·0) and 18·7 (2·7) v 2·7 (3·7) g, p<0·01).

#### Jejunal weight (Table 1)

Rats who received SCFA/control had significantly increases (p<0·03) in jejunal weight when compared with rats who received saline/control. In the gastrin receptor blocker groups, SCFA did not increase jejunal weight.

#### Mucosal DNA and protein (Figs 1, 2)

Both jejunal mucosal DNA and protein content were significantly increased (p<0·01) in control rats who received SCFA compared with saline. In the gastrin receptor blocker groups, SCFA did not change either DNA or protein content.

#### Crypt cell proliferation (Fig 3)

SCFA increased crypt cell proliferation in control rats but not in those who received gastrin receptor blocker (p<0·02). In addition, rats who received SCFA/gastrin receptor blocker

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![Figure 1: Augmentation of jejunal mucosal DNA content by SCFAs in control (vehicle, 0·9% NaCl) rats when compared with saline. No increase in jejunal DNA content is seen after SCFA administration in rats who received gastrin receptor blocker (GRB). Data are mean (SEM); *p<0·01 v saline/control.](image-url)
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blocker had a significant increase in crypt cell proliferation when compared with SCFA/control (p<0.05).

Jejunal and plasma gastrin (Fig 4, Table I)
There was a significant increase in jejunal tissue gastrin concentrations in SCFA/control rats vs saline/control rats (p<0.001). No significant differences were seen in gastrin receptor blocker rats who received SCFA or saline (Fig 4). There were no significant differences in plasma gastrin concentrations among groups (Table I).

Histological measurements (Table II)
There was a significant increase in jejunal villous height in rats who received SCFA/gastrin receptor blocker vs saline/gastrin receptor blocker (p<0.03). Significant increases were seen in crypt depth in SCFA rats who received either control or gastrin receptor blocker (p<0.03) when compared with same group saline rats.

EXPERIMENT 2

Plasma and tissue enteroglucagon concentrations (Table III)
There were no significant differences between groups who received SCFA when compared with those who received saline.

Discussion
This study corroborated previous findings that colonically infused SCFAs are trophic to rat jejunum\(^8\) and are associated with increased jejunal gastrin concentrations.\(^9\) Gastrin receptor blockade abolished most jejunotrophic effects of SCFAs, therefore, local binding of gastrin mediates, in part, SCFA induced jejunotrophism. SCFA administration did not significantly change plasma gastrin concentrations or tissue or plasma enteroglucagon concentrations.

Jejunal growth and proliferation in response to colonomically administered SCFA in control groups was shown by increased jejunal weight, mucosal DNA and protein contents, crypt cell proliferation rate, and crypt depth. Villous height was augmented by SCFA administration in the control group, although not to a significant degree (p=0.055). SCFA significantly increased concentrations of jejunal gastrin, while not affecting plasma gastrin values. Gastrin receptor blockade with L-365, 260, a selective gastrin/CK-B receptor antagonist was used to discover if jejunotrophic effects of SCFAs are mediated directly by tissue gastrin.\(^13\) Gastrin receptor blockade abolished SCFA induced increases in jejunal weight, DNA, protein, and crypt cell proliferation, without changing SCFA effects on crypt depth and villous height. Neither SCFAs nor gastrin receptor blocker administration affected plasma gastrin concentrations. We hypothesize that SCFAs may stimulate upregulation of gastrin receptors on jejunal proliferative cells, thereby increasing jejunal gastrin binding without affecting plasma gastrin concentrations. Alternatively, there may be increased G cell gastrin synthesis as well as increased jejunal gastrin binding, with no net change in plasma gastrin concentration. Previous investigations of gastrin action on the small intestine have frequently shown wide variations in plasma gastrin\(^32\) and small intestinal gastrin concentrations have rarely been measured.

Figure 2: Increase in jejunal mucosal protein content by SCFAs in control (vehicle, 0.9% NaCl) rats when compared with saline. No increase in jejunal protein content is seen after SCFA administration in rats who received gastrin receptor blockers (GRB). Data are mean (SEM); *p<0.01 v saline/control.

Figure 3: Effects of SCFAs and gastrin receptor blocker (GRB) on jejunal crypt cell proliferation. SCFAs increased jejunal crypt cell proliferation in control rats when compared with saline; crypt cell proliferation was also increased in both gastrin receptor blocker groups when compared with saline/control. Additionally, crypt cell proliferation was greater in the SCFA/gastrin receptor blocker group when compared with SCFA/control group. Data are mean (SEM); *p<0.02 v saline/control; tp<0.05 v SCFA/control.

Figure 4: SCFA infusion significantly increased jejunal tissue gastrin content in control (vehicle, 0.9% NaCl) rats when compared with saline. No effect of SCFAs on gastrin content is seen in rats that had received gastrin receptor blocker (GRB). Data are mean (SEM); *p<0.001 v saline/control.
The results of this investigation renew interest in the enterotropic effects of gastrin. Gastrin administration stimulates growth throughout the gastrointestinal tract with the exception of salivary glands, oesophagus, and gastric antrum.\textsuperscript{34 35} Gastrin clearly regulates growth of the acid producing (oxyntic) portion of the stomach\textsuperscript{34 36-40} where it stimulates DNA, RNA, and protein synthesis.\textsuperscript{35 37 41} Exogenous gastrin also augments enteroendocrine cell proliferation in duodenal and colonic mucosa\textsuperscript{39 41-44} and increases acinar cell counts, organ weight, and RNA concentrations in the pancreas.\textsuperscript{45 46}

Gastrin mediation of small intestinal growth (beyond the duodenum) is controversial. Gastrin increases weight, DNA synthesis, RNA and protein contents, and expands the crypt cell proliferation zone in rat small intestinal mucosa.\textsuperscript{34 41 42 46-50} In a rat fed a ketotic transplant model, exogenously administered gastrin-17 significantly increases intestinal DNA concentration and absorptive capacity.\textsuperscript{51} In vitro, gastrin stimulates DNA synthesis in all intestinal tissue\textsuperscript{34 43 47} as well as in the intestinal crypt IEC-6 and human cancer LoVo cell lines.\textsuperscript{52 53} Several findings, however, argue against a role for gastrin in small intestinal trophism.\textsuperscript{32 33 45 54} Refeeding starved rats results in an increase in crypt cell production rate in the proximal small intestine, but does not correlate with plasma gastrin concentrations (tissue values were not measured).\textsuperscript{55 56} Chronic pentagastrin injection in rats increases duodenal weight but does not change structure or function of the jejunum.\textsuperscript{45} Additionally, induced endogenous or exogenous hypergastrinaemia in rats has no enterotropic effects outside the oxyntic stomach.\textsuperscript{54}

Evidence of a direct effect of gastrin on small intestinal growth is debatable; however, gastrin probably has a role in the maintenance of intestinal mucosal integrity, as noted by studies relating decreased endogenous gastrin concentrations to intestinal mucosal atrophy. For example, pentagastrin treatment of starved animals prevents decreases in small intestinal protein and RNA content in the rat. In parenterally fed rats, intravenous pentagastrin administration prevents small intestinal weight loss and restores most structural and functional variables to normal.\textsuperscript{37 57} Because endogenous production of gastrin is decreased in both starvation and intravenous alimentation, and exogenous gastrin prevents the atrophic changes associated with these conditions, gastrin seems vital for maintaining structural integrity in the small intestine.\textsuperscript{34 36 57}

Enteroglucagon producing tumours are associated with tremendous proliferation in intestinal mucosa\textsuperscript{58} and enteroglucagon is the peptide hormone most strongly associated with intestinal mucosal growth.\textsuperscript{30 31 59 60} Fermentable fibre has been shown to stimulate crypt cell production rate in the distal small intestine and colon and plasma enteroglucagon concentrations correlate with the degree of epithelial cell proliferation in these regions.\textsuperscript{55 56} No significant differences were seen in either tissue or plasma enteroglucagon concentrations with colonic SCFA administration in this experiment; therefore, enteroglucagon is not an important mediator of colonic SCFA induced growth of the jejunum.

The mechanism(s) by which colonic infusion of SCFAs promotes jejunal growth clearly involves gastrin. The failure of gastrin receptor blockade to abrogate all effects of SCFAs on jejunal structure (crypt depth, villous height) is consistent with other reports showing varied effects of gastrin on small intestinal growth and function. Alternatively, this may be experimental aberration, which would not be reproducible. Finally, the autonomic nervous system may modulate gastrin's trophic action on the jejunum, possibly by changing local blood flow or receptor expression. Previous investigations in our laboratory show that both divisions of the autonomic nervous system must be intact for colonic SCFA to induce jejunoatrophy in the rat.\textsuperscript{61}

We postulate that the autonomic nervous system transmits the SCFA induced nervous signal from the colon to the central nervous system, which then elaborates a secondary

<table>
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<tr>
<th>TABLE II</th>
<th>Effect of SCFAs and gastrin receptor blocker (GRB) on jejunal histology</th>
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<tbody>
<tr>
<td>Villous height (μm)</td>
<td>Crypt depth (μm)</td>
</tr>
<tr>
<td>Saline/control</td>
<td>712.4 (30-2)</td>
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<tr>
<td>SCFA/control</td>
<td>795.0 (31-0)*</td>
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<tr>
<td>Saline/GRB</td>
<td>784.8 (28-2)</td>
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<tr>
<td>SCFA/GRB</td>
<td>868.0 (20-8)†</td>
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Data are mean (SEM); *p<0.03 v saline/control; †p<0.03 v saline/GRB.

<table>
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<tr>
<th>TABLE III</th>
<th>Effect of SCFAs and gastrin receptor blocker (GRB) on tissue and plasma enteroglucagon</th>
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</thead>
<tbody>
<tr>
<td>Tissue (pmol/l)</td>
<td>Plasma (pmol/l)</td>
</tr>
<tr>
<td>Saline/control</td>
<td>6.08 (6.8)</td>
</tr>
<tr>
<td>SCFA/control</td>
<td>5.72 (6.5)</td>
</tr>
</tbody>
</table>

Data are mean (SEM).
neural signal, hormone or growth factor that stimulates jejunal growth (Fig 5). This study shows that gastrin, but not enteroendocrine, may be a secondary messenger in transmitting the SCFA induced trophic signal to the jejunal.

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