Gut hormones in adaptation

S R BLOOM

From the Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London.

SUMMARY The presence of a circulating factor affecting gut growth can be surmised from the findings in gut isolated from the main food stream and not under direct nutritional influence. Thus when a Thiry Vella fistula is constructed and the crypt cell production rate counted in the fistula it can be shown to correlate with the degree of resection of the main bowel left in continuity. The only hormones which become raised in a similar pattern are enteroglucagon and peptide tyrosine tyrosine (PYY). Enteroglucagon has been shown to be part of preproglucagon, which contains in addition oxyntomodulin, glucagon like peptide 1 1-37 and 6-36NH₂ and glucagon like peptide 2. These form the main candidates for the 'hormone of gut growth'. Peptide tyrosine tyrosine has been tested by direct administration over 12 days, matching the natural rise, but no effect on crypt cell production rate was seen. Glucagon like peptide 1 1-37 was similarly tested and also found to produce no effect. It remains to test the other members of the glucagon family to confirm or refute the hypothesis that one of them is the enigmatic small gut growth factor.

It is well recognised that after damage, for example by infection, the growth rate of the intestinal mucosa increases rapidly and that after small intestinal resection the residual intestine hypertrophies. The mechanism by which this adaptive growth is controlled is less clear. One possibility is that it involves, perhaps only in part, a circulating hormonal factor. Further this is most likely to come from the gut itself, perhaps downstream from the region damaged or lost.

Methods

ANIMALS

In an attempt to investigate the relative importance of local factors, such as luminal nutrition and pancreaticobiliary secretions, as opposed to putative circulating hormonal factors a series of rats were studied in which a Thiry Vella fistula was fashioned. The external fistula comprised the proximal 75% of the small bowel, measured from the ligament of Treitz, with the remaining bowel continuity being restored by an end to end anastomosis. The fistula was thus excluded from luminal nutrition and pancreaticobiliary secretions. In a second group of fistula rats a cannula was, in addition, inserted into the superior vena cava *via* the internal jugular vein and exteriorised at the back of the neck. A third series of rats had merely a jejunal transection and formed

the control groups. All oral food was excluded from the second group which were only fed intravenously and gained slightly more weight than the other two groups. After 12 days all the animals were killed. Gut mucosal cell proliferation was determined by the stathmokinetic method using vincristine and crypt cell microdissection to assess the crypt cell production rate.¹ In the control animals (transected) the crypt cell production rate (CCPR) in the terminal ileum was 16.8 ± 0.9 cells per crypt per hour. In the Thiry Vella fistula group (with 75% bypassed gut) the rate in the terminal ileum was much greater at 52 ± 8 . In the intravenously fed group, who had also undergone 75% gut bypass, the rate was not significantly different from the controls, however, at 18 ± 5 . This showed, yet again, the direct or indirect importance of luminal nutrition. In the Thiry Vella fistula itself the CCPR in the intravenously fed animals was 16.0 ± 1.5 , not different from the normal terminal ileum in the control animals, but significantly less than the CCPR in the Thiry Vella fistula of the orally fed rats (23.8 ± 2.8 , $p < 0.01$).² Thus in these experiments there is a weak effect of a circulating factor acting on the Thiry Vella fistula, which appeared to be dependent on luminal nutrition for its release from the bowel in continuity. In a further similar series of experiments a smaller Thiry Vella fistula was constructed allowing groups of rats with 25%, 50%, 75%, and 90% resection of the bowel in continuity. Once again the Thiry Vella fistula was found to have a lower basal crypt cell production rate (10 ± 1) less

Address for correspondence: Professor S R Bloom, Dept of Medicine, RPMS, Hammersmith Hospital, Du Cane Road, London W12 0HS

Fig. 1 Plasma enteroglucagon responses to a 530 calorie test breakfast in 12 patients with acute diarrhoea and 13 healthy controls (from 21).

than the ileum in continuity (16 ± 2). The CCPR in the fistula rose, however, with increasing resection of the bowel in continuity and had doubled in the 90% resection group to 35 ± 2 ($p < 0.001$).³ This clearly showed the likely presence of a circulating factor affecting mucosal growth but gave no information on its possible interaction with luminal nutrients at the target site as the studied Thiry Vella fistula received no luminal stimulant. It is conceivable, but unproven, that even a small amount of luminal nutrient stimulation might have greatly enhanced the effectiveness of the putative circulating growth factor or factors on the fistula mucosa.

Discussion

In 1971 a patient with intestinal mucosal hypertrophy was studied⁴ who had a resectable enteroglucagon producing tumour⁵ after which the hypertrophy disappeared. Enteroglucagon was therefore proposed as 'growth hormone to the small intestine'.⁶ Subsequently enteroglucagon was found to correlate with crypt cell production rate in a number of animal models of intestinal adaptation.⁷⁻¹³ In the two Thiry Vella fistula models, mentioned above, enteroglucagon concentrations rose in proportion to the Thiry Vella fistula increase in crypt cell production rate.^{14,15} Although a preliminary study suggested a direct trophic effect of partially purified enteroglucagon on cultured guinea pig jejunal mucosa¹⁶ no definitive study has yet firmly proven the trophic action of enteroglucagon. In man there is extensive correlation between a rise in enteroglucagon and circumstances where increased enterocyte turnover would be expected – for example, after gut resection, acute infective diarrhoea (Fig. 1), jejunioileal bypass, coeliac disease, tropical malabsorption, cystic fibrosis and, in the newborn, after initiation of enteral feeding.¹⁷⁻²⁷

Recently the human pre-pro-glucagon sequence has been derived and two additional glucagon like peptides discovered (Fig. 2).²⁸ The first of these (GLP1) was also found in the angler fish pre-pro-glucagon²⁹ and its sequence was approximately equally conserved to that of pancreatic glucagon itself. Thus over five hundred million years the forces of evolution have retained, virtually unchanged, two peptides with similar sequence suggesting that both must be necessary for survival. Enteroglucagon was sequenced in 1981³⁰ and shown to be composed of 69 amino acids and to contain the entire sequence of pancreatic glucagon towards its C-terminal terminus. In the alpha cell of the pancreas pre-pro-glucagon undergoes post-translational enzymic processing to cleave out pancreatic glucagon, no enteroglucagon being formed.³¹ In contrast the two glucagon-like peptides, GLP1 and GLP2, are mostly not separated

Fig. 2 Scheme showing the regions of the pre-pro-glucagon molecule.

Fig. 3 Plasma PYY concentrations during a 530 calorie test breakfast in 12 patients with acute infective diarrhoea and 15 healthy controls (from 39).

Fig. 4 PYY concentrations before and after a 530 calorie test breakfast in 18 subjects who had previously undergone an ileal resection, 9 subjects who had previously undergone a colonic resection and 12 healthy controls (from 42).

in the alpha cell³² and are secreted as a large conglomerate peptide. In the EG cell of the gut mucosa the pre-pro-glucagon molecule is differently cleaved. Pancreatic glucagon is not formed but is contained within enteroglucagon,³³ while GLP1 6-36NH₂ and GLP2 are separately produced and secreted.³⁴ As the main body of 'evidence' linking enteroglucagon with gut growth is by correlation and GLP1 is coreleased with enteroglucagon, it is clearly possible that GLP1 itself might be the true growth factor. We have administered GLP-1 1-37 (pre-pro-glucagon 72-108) chronically to a group of eight rats, using an Alzet mini pump implanted subcutaneously in a dose of 5 pmol/kg/min over 12 days without producing any effect on intestinal CCPR (plasma GLP-1 increment of 141 ± 29 pmol/l). Unfortunately not all forms

have been tested as the amounts of the various GLPs that would be required to produce significant long term effects in animals would, at the present time, be very expensive. Preliminary tests on isolated cultures have not proved positive but the GLP's action has not yet been exhaustively investigated.

Only the gut glucagon system correlates well with gut growth and hitherto other hormonal systems, including gastrin, cholecystokinin, gastric inhibitory polypeptide, motilin, neurotensin, secretin, and other local gut peptides have not been observed to be associated with this phenomenon. Recently a further gastrointestinal peptide was isolated and termed peptide tyrosine tyrosine (PYY using the IUPAC shorthand nomenclature).³⁵ This peptide was found to be present in endocrine cells of the intestinal mucosa, where it is costored with the gut glucagon peptides in at least a proportion of the endocrine cells.³⁶ In man it was found to have a very similar distribution to enteroglucagon, being present in high concentrations in the mucosa of the large bowel and distal small intestine.³⁷ It was not found elsewhere in the body. It was also found to be released by long chain fatty acids and carbohydrates, in a similar manner to enteroglucagon. Indeed in all the bowel diseases which caused a rise in enteroglucagon in man PYY was also found to be very raised (Fig. 3 and Fig. 4).^{38,39} Unlike enteroglucagon, which is not yet freely available in its 69 amino acid form, PYY has been synthesised in bulk and its human pharmacology tested. Short term infusions were found to significantly suppress gastric acid secretion. Indeed a plasma PYY increment of 27 ± 2 pmol/l caused a 90% reduction in the incremental gastric secretion response to pentagastrin in volunteers. This blood level had no influence on duodenal juice volume, output of bicarbonate, trypsin or bilirubin during low dose secretin and cholecystokinin infusions. At physiological doses PYY also inhibits gastric emptying and independently inhibits intestinal transit time.⁴⁰ The question therefore arose as to whether PYY might be a trophic influence on the intestinal mucosa. To investigate this rats were studied after 75% proximal small bowel resection and this produced a rise in plasma PYY from 28 ± 3.1 to 85 ± 12.3 pmol/l ($p < 0.001$), this change being similar in magnitude to that of plasma enteroglucagon. Indeed plasma PYY concentrations correlated both with crypt cell production rate and plasma enteroglucagon levels. In a second study, using the Alzet minipump, PYY or saline was infused over a 12 day period to two groups of rats. No significant change in intestinal wet weight, CCPR or bowel appearance was noted. From this it was concluded that while PYY correlated with the intestinal trophic response, it was not itself responsible for it.⁴¹ Nonetheless the inhibition of intestinal

transit and gastric emptying rate produced by physiological concentrations of PYY might well assist in the adaptive process, following gut resection or other causes of impaired digestive or absorptive function.

Thus it seems likely that a circulating hormonal factor or factors stimulating intestinal mucosal growth does exist and that it is of pathophysiological importance. At the present time correlative studies with known gastrointestinal hormonal peptides suggest that enteroglucagon, or perhaps the newly identified glucagon like peptides, would best fit this role. Indeed it is difficult to produce circumstances in which a trophic influence on the gut is seen in which enteroglucagon (and presumably the glucagon like peptides) are not raised. Although the novel peptide, PYY, which may be coproduced by the mucosal enteroglucagon endocrine cell, also correlates well with intestinal trophic influences, it fails to stimulate growth when directly administered to rats. Although the circumstances of administration may have, in some way, been inadequate to show a trophic influence, these experiments make PYY less likely than the gut glucagons to fulfill the role of trophic hormone on present evidence. Nonetheless, until the gut glucagons can be directly tested, no firm conclusions can be reached.

References

- Wright NA, Appleton DR. The metaphase arrest technique. *Cell Tissue Kinet* 1980; **13**: 643–63.
- Sagor GR, Ghatei MA, Al-Mukhtar MYT, Wright NA, Bloom SR. Evidence for a humoral mechanism after small intestinal resection. *Gastroenterology* 1983; **84**: 902–6.
- Gornacz GE, Ghatei MA, Al-Mukhtar MYT, *et al.* Plasma enteroglucagon and CCK levels and cell proliferation in defunctioned small bowel in the rat. *Dig Dis Sci* 1984; **29**: 1041–9.
- Gleeson MH, Bloom SR, Polak JM, Henry K, Dowling RM. An endocrine tumour in kidney affecting small bowel structure, motility and absorptive function. *Gut* 1971; **12**: 733–82.
- Bloom SR. An enteroglucagon tumour. *Gut* 1972; **13**: 520–3.
- Bloom SR. Gastrointestinal hormones (I): pancreatic polypeptide, motilin, gastric inhibitory peptide, neurotensin, enteroglucagon and others In: Gray CH, James VHT eds, *Hormones in blood*, London: Academic Press, 1979: 321–56.
- Jacobs LR, Bloom SR, Dowling RH. Response of plasma and tissue levels of enteroglucagon immunoreactivity to intestinal resection, lactation and hyperphagia. *Life Sci* 1981; **29**: 2003–7.
- Sagor GR, Al-Mukhtar MYT, Ghatei MA, Wright NA & Bloom SR. The effect of altered luminal nutrition on cellular proliferation and plasma concentrations of enteroglucagon and gastrin after small bowel resection in the rat. *Br J Surg* 1982; **69**: 14–18.
- Sagor GR, Ghatei MA, O'Shaughnessy DJ, Al-Mukhtar MYT, Wright NAG, Bloom SR. Influence of somatostatin and bombesin on plasma enteroglucagon and cell proliferation after intestinal resection in the rat. *Gut* 1985; **26**: 89–95.
- Miazza BM, Al-Mukhtar MYT, Salmeron M, *et al.* Hypenteroglucagonaemia and small intestinal mucosal growth after colonic perfusion of glucose in rats. *Gut* 1985; **26**: 518–24.
- Al-Mukhtar MYT, Sagor GR, Ghatei MA, Bloom SR, Wright NA. The role of pancreatico-biliary secretions in intestinal adaptation after resection, and its relationship to plasma enteroglucagon. *Br J Surg* 1983; **70**: 398–400.
- Gornacz GE, Al-Mukhtar MYT, Ghatei MA, Sagor GR, Wright NA, Bloom SR. Pattern of cell proliferation and enteroglucagon response following small bowel resection in the rat. *Digestion* 1984; **29**: 65–72.
- Koopmans HS, Ferri G-L, Sarson DL, Polak JM, Bloom SR. The effects of ileal transposition and jejunoileal bypass on food intake and GI hormone levels in rats. *Physiol Behav* 1984; **33**: 601–9.
- Sagor GR, Ghatei MA, Al-Mukhtar MYT, Wright NA, Bloom SR. Evidence for a humoral mechanism after small intestinal resection. *Gastroenterology* 1983; **84**: 902–6.
- Gornacz GE, Ghatei MA, Al-Mukhtar MYT, *et al.* Plasma enteroglucagon and CCK levels and cell proliferation in defunctioned small bowel in the rat. *Dig Dis Sci* 1984; **29**: 1041–9.
- Uttenthal LO, Batt RM, Carter MW, Bloom SR. Stimulation of DNA synthesis in cultured small intestine by partially purified enteroglucagon. *Regul Peptides* 1982; **3**: 84.
- Besterman HS, Bloom SR, Sarson DL, *et al.* Gut-hormone profile in coeliac disease. *Lancet* 1978; **1**: 785–8.
- Besterman HS, Cook GC, Sarson DL, *et al.* Gut hormones in tropical malabsorption. *Br Med J* 1979; **1**: 1252–5.
- Besterman HS, Adrian TE, Mallinson CN, *et al.* Gut hormone release after intestinal resection. *Gut* 1982; **23**: 854–61.
- Besterman HS, Adrian TE, Bloom SR, *et al.* Pancreatic and gastrointestinal hormones in chronic pancreatitis. *Digestion* 1982; **24**: 195–208.
- Besterman HS, Christofides ND, Welsby PD, Adrian TE, Sarson DL, Bloom SR. Gut hormones in acute diarrhoea. *Gut* 1983; **24**: 665–71.
- Bloom SR. Hormonal changes after jejuno-ileal bypass and their physiological significance. In: Maxwell JD, Gazet J-C, Pilkington TR, eds, *Surgical management of obesity*, London: Academic Press, 1980: 115–23.
- Allen JM, Penketh ARL, Adrian TE, *et al.* Adult cystic fibrosis: postprandial response of gut regulatory peptides. *Gastroenterology* 1983; **85**: 1379–83.
- Bloom SR, Royston CMS, Thomson JPS. Enteroglucagon release in the dumping syndrome. *Lancet* 1972; **2**: 789–91.
- Lucas A, Adrian TE, Christofides N, Bloom SR,

- Aynsley-Green A. Plasma motilin, gastrin, and enteroglucagon and feeding in the human newborn. *Arch Dis Child* 1980; **55**: 673–7.
- 26 Christofides ND, Mallet E, Gbatei MA, Lee YC, Bloom SR. Plasma enteroglucagon and neurotensin in infantile pyloric stenosis. *Arch Dis Child* 1983; **58**: 52–55.
 - 27 Sarson DL, Scopinaro N, Bloom SR. Gut hormone changes after jejunoileal (JIB) or biliopancreatic (BPB) bypass surgery for morbid obesity. *Int J Obesity* 1981; **5**: 471–80.
 - 28 Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human pre-pro-glucagon gene. *Nature* 1983; **304**: 368–71.
 - 29 Lund PK, Goodman RH, Habener JF. Pancreatic pre-pro-glucagons are encoded by two separate mRNAs. *J Biol Chem* 1981; **256**: 6515–8.
 - 30 Thim L, Moody AJ. The primary structure of porcine glicentin (pro-glucagon). *Regul Peptides* 1981; **2**: 139–50.
 - 31 Gbatei MA, Uttenthal LO, Bryant MG, Christofides ND, Moody AJ, Bloom SR. Molecular forms of glucagon-like immunoreactivity in porcine intestine and pancreas. *Endocrinology* 1983; **112**: 917–23.
 - 32 Uttenthal LO, Ghigione M, George SK, Bishop AE, Polak JM, Bloom SR. Molecular forms of glucagon-like peptide-I in human pancreas and glucagonomas. *J Clin Endocrinol Metab* 1985; **61**: 472–9.
 - 33 Gbatei MA, Uttenthal LO, Christofides ND, Bryant MG, Bloom SR. Molecular forms of human enteroglucagon in tissue and plasma: plasma responses to nutrient stimuli in health and in disorders of the upper gastrointestinal tract. *J Clin Endocrinol Metab* 1983; **57**: 488–95.
 - 34 George SK, Uttenthal LO, Ghigione M, Bloom SR. Molecular forms of glucagon-like peptides in man. *Febs Lett* 1985; **192**: 275–8.
 - 35 Tatemoto K, Mutt V. Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature* 1980; **285**: 417–8.
 - 36 Ali-Rachedi A, Varndell IM, Adrian TE, *et al.* Peptide YY (PYY) immunoreactivity is co-stored with glucagon-related immunoreactants in endocrine cells of the gut and pancreas. *Histochemistry* 1984; **80**: 487–91.
 - 37 Adrian TE, Ferri G-L, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone peptide YY. *Gastroenterology* 1985; **89**: 1070–7.
 - 38 Adrian TE, Long RG, Fuessl HS, Bloom SR. Plasma peptide YY (PYY) in dumping syndrome. *Dig Dis Sci* 1985; **30**: 1145–8.
 - 39 Adrian TE, Savage AP, Bacarese-Hamilton AJ, Wolfe K, Besterman HS, Bloom SR. Peptide YY abnormalities in gastrointestinal diseases. *Gastroenterology* 1986; **90**: 379–84.
 - 40 Savage AP, Adrian TE, Carolan G, Chatterjee VK, Bloom SR. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. (Submitted to Gut).
 - 41 Savage AP, Gornacz GE, Adrian TE, *et al.* Is raised plasma peptide YY after intestinal resection in the rat responsible for the trophic response? *Gut* 1985; **26**: 1353–8.
 - 42 Russell RCG, Bloom SR, Jones S. The interpretation of plasma gastrin results – an explanation. *Br J Surg* 1975; **62**: 157.