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Peptides and epithelial growth regulation

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Summary. There is now considerable evidence implicating several peptides in the control of gastrointestinal epithelial cell proliferation and cell renewal. While some of these may act directly, many may be involved in regulating the powerful trophic effects of the intake and digestion of foold on the gut epithelium. – Several peptides have been associated with the regulation of intestinal cell proliferation. There is little doubt that gastrin is trophic to the stomach, but, its role in the rest of the gastrointestinal tract is debatable. Enteroglucagon has often been associated with increased intestinal epithelial proliferation, but at the moment all the evidence for this is circumstantial. The effects of peptide YY and bombesin warrant further study. The availability of recombinant epidermal growth factor (EGF) has recently enabled us to demonstrate a powerful trophic response to infused EGF throughout the gastrointestinal tract. The increasing availability of peptides will eventually allow the rigorous in vivo evaluation of the trophic role of these potentially very important peptides.
Key words. Peptides; gastrointestinal tract; epithelial cell proliferation; gastrin; enteroglucagon; peptide YY; bombesin, epidermal growth factor; cholecystokinin; somatostatin.

Introduction

In many ways the gastrointestinal epithelium is an ideal model for the study and investigation of the control of epithelial cell proliferation, as it is continuously and rapidly renewed with its cell division restricted to an anatomically discrete zone. It is also capable of adapting its rates of proliferation to a wide variety of physiological and other stimuli. The study of epithelial cell renewal is also of considerable importance since most tumours are of epithelial origin[76]. Three main mechanisms are generally considered to be involved in the control of epithelial renewal in the gut namely, a (local?) negative feedback system from the functional (villus) to the reproductive zone (crypt), the direct or indirect effects of food (luminal nutrition and/or intestinal workload) and the effects of humoral factors[103].

Parabiotic studies in which the blood systems of two animals are linked have indicated that a hormonal factor may cross-circulate from a stimulated animal to its partner[52,100]. A similar response has also been noted in less extreme models where isolated loops of small intestine still respond to altered food intake[20], and after intestinal resection [7,37].

The study of cell renewal and epithelial growth control necessitates the use of suitable methods, and unfortunately many studies in this field have been bedeviled by the use of totally inappropriate methods. The problems involved have been spelt out in detail elsewhere[5,19,35,66,102,103], and are as follows; 1) The intestine contains a large proportion of non-epithelial cells (muscle, submucosa lymphoid aggregates); thus any gross measure may give a misleading result. Even the mucosa itself is approximately 20% non epithelial[19]. 2) The choice of a suitable denominator is of vital importance, as many measures, such as labelling index and mitotic index will not detect a general increase in compartment size. These measures also suffer from being 'state' measures, and as such can be misleading if the duration of the DNA synthesis phase or mitosis is altered. 3) Measures based on the gross uptake of tritiated thymidine can be especially misleading, as although usually equated with growth, triatiated thymidine uptake can be affected by a variety of stimuli. Thymidine itself is not a precursor in the de novo synthesis of DNA, but is incorporated by a salvage pathway which depends on the activity of several enzymes and transport mechanisms plus the size of the endogenous thymidine pool. All of these factors can be influenced by hormones or growth factors. Thymidine can also be stored and recycled, and it can also be taken up by bacteria[66].

Most of these pitfalls can be avoided if the accumulation of arrested metaphases in microdissected crypts is determined. This 'rate' measure also avoids the several problems involved in the quantification of sectioned material, and expressing the results on a per crypt basis can account for all the factors that may influence epithelial cell production (cell cycle time, size of the growth fraction and size of the crypt itself)[5,19,35,66,102,103].

Gastrin as a trophic hormone in the gastrointestinal tract

There is a considerable body of evidence for a powerful pharmacological and possibly physiological modulation of cell proliferation by gastrin in the stomach[21,63,99]. There is also evidence, unfortunately mainly based on the gross uptake of tritiated thymidine, that this trophism extends into the small intestine and colon[45,46,48,58]. Claims by Johnson[43,44] for a major trophic role for gastrin were also supported by a study of the effects of gastrin on primary duodenal explants in short-term culture[58]; but this study is especially open to criticism[101].

On the other hand several groups of workers have failed to show any structural or functional changes in the small bowel after a variety of manœuvers. No proliferative effects were noted after pentagastrin infusion[65], and a large series of experiments designed to give a wide range of gastrin levels failed to show any relationship between plasma gastrin levels and cell proliferation[69,74,75]. The previously observed increases in tritiated thymidine uptake could alternatively be due to gastrin increasing cellular permeability and transport[75,84]. Thymidine kinase activity increases after refeeding, but before the increase in gastrin[61]. The reported increase in tritiated thymidine uptake without any increase in tissue mass or protein or DNA content[88] also argues for alterations in cellular permeability confounding gross thymidine measures.

The use of more robust cell kinetic measures to quantify crypt cell production have failed to show any correlation between gastrin and proliferation in starved and refed rats[36], after intestinal resection[6,81,82] and after a variety of dietary manipulations[31].

Thus although there is good evidence for a trophic effect of gastrin in the stomach (but only in the fundus, not in the antrum[18]), a general trophic role for gastrin is not proven.

Enteroglucagon

Enteroglucagon is considered by many to be the prime candidate for the title of 'enterotrophin' and there is a considerable body of evidence to support this.

A enteroglucagon secreting renal tumour was associated with marked mucosal hypertrophy which was reversed on removal of the tumour[9,30]. Plasma enteroglucagon levels rise in a variety of hyperproliferative models such as after intestinal resection[6,11,41,81,82], in lactating and in hypothermic-hyperphagic rats[25,42], and elevated plasma levels are seen in several human pathological conditions associated with intestinal hyperplasia[13].

There is also an excellent correlation between plasma enteroglucagon and crypt cell production rate in a wide range of hypo- and hyperproliferative models of intestinal adaptation. These include starvation and refeeding[36], intestinal resection[81,82], pancreatico-bilary diversion and resection[6] and dietary manipulation[31]. Enteroglucagon cells are located throughout the gut, but most are localized in the dital gut, especially the terminal ileum[13], which is the strategic position for monitoring the efficiency of digestion and either delaying intestinal transit or increasing absorptive function (via increased crypt cell output). The enteroglucagon hypothesis is thus very attractive, but is entirely based on circumstantial evidence, so that until pure enteroglucagon is purifed or isolated the definitive test of the hypothesis cannot be performed.

Peptide YY (PYY)

PYY is a novel (36 amino acid) candidate hormone which may to be co-localized with enteroglucagon[3,26,27] and like enteroglucagon it can also inhibit gastric acid secretion and emptying[4,90]. It also has a high degree of sequence homology with pancreatic polypeptide (PP) and neuropeptide Y (NPY). Like enteroglucagon it is found throughout the gastrointestinal tract, with most cells in the distal gut, but the majority of PYY cells are located in the colon[1]. PYY receptors have been located in the small intestine[51]. PYY receptors have been located in the small intestine[51]. PYY abnormalities have been reported in various disease states associated with malabsorption[2]. PYY levels rise after intestinal resection and correlate well with intestinal crypt cell production rates, but the direct infusion of PYY via osmotic mini-pumps appeared to have little effect on intestinal crypt cell production[80]: Nonetheless plasma PYY levels also correlate quite well with intestinal cell proliferation after dietary manipulations, and correlate very well with plasma enteroglucagon levels[31]. PYY receptors are mainly found in the small intestine while most PYY containing cells are localized in the colon, thus the possibility of a feedback loop from the hind gut to the small intestine seems attractive.

Bombesin

Bombesin is a tetradecapeptide first isolated from amphibian skin, and bombesin-like immunoreactivity is present along the digestive tract of mammals[77,98]. Bombesin can stimulate gastrin release and gastric acid secretion[95]. It can also effect the release of several gut hormones in man[12]. The mammalian equivalent of bombesin is thought to be gastrin releasing peptide (GRP) a 27 amino acid peptide first isolated from porcine gut[67,68]. In vitro administration of bombesin stimulates proliferation of the 3T3 mouse fibroblast line[79]. Bombesin also acts as an autocrine growth factor for some lung tumours[22]. In the suckling rat bombesin stimulates growth of the entire gastrointestinal tract and pancreas[56] while in the adult it has been reported that it can stimulate antral gastrin cell proliferation[54]. Another study has shown that bombesin can also stimulate intestinal crypt cell production in transected rats, but it could not stimulate the already elevated rates of proliferation in animals with intestinal resection[83]. Thus bombesin is another peptide whose role in the control of intestinal epithelial cell proliferation warrants further investigation.

Epidermal growth factor (EGF)

The location of the main sites of production of EGF in the salivary glands and Brunners glands of the duodenum of man[38] and the rat[71] would imply that EGF may have a role in the maintenance of gastrointestinal homeostasis.

While the growth promoting actions of EGF in vitro are well characterized[17], its role in vivo is uncertain: EGF stimulates the proliferation and differentiation of the epidermis, maturation of the pulmonary epithelium and accelerates the healing of corneal epithelium in the foetus and newborn[17]. EGF also stimulates the proliferation and maturation of the neonatal intestine[15,60,70], where it increases the activity of ornithine decarboxylase[28], an enzyme associated with the initiation of cell proliferation[59]. The presence of EGF in a variety of body fluids, including saliva, plasma[17] and milk[16], its production by the salivary and Brunners glands[38,71], the reports of a trophic action of saliva on the intestine[57,72] the demonstration of EGF receptors in intestinal epithelial cells[29,89]and its reported cytoprotective effects on the duodenal mucosa[50] all suggest that it has a role in the control of gastrointestinal homeostasis other than the inhibition of gastric acid secretion.

The injection of EGF into rodents has produced conflicting reports some finding that it can increase the incorporation of tritiated thymidine into DNA throughout the gastrointestinal tract[85,86], others only observing this in the stomach[47] or only in starved animals[23]. EGF may also aid gastrointestinal growth in undernourished young rats[62]. A study of the short-term effects of EGF administration using the crypt cell production method[8], showed a trophic effect in some sites of the intestine.

The ideal model of the hypoplastic intestine is provided by maintaining animals on isocaloric total parenteral nutrition (TPN), which is generally agreed to be the pertinent system for the study of effects of humoral factors on the intestine[78]; since the intestine of the TPN rat is in a steady state and basal level of proliferation.

The TPN model was used to investigate the effects of recombinant EGF (human B-urogastrone) on cell proliferation. EGF-urogastrone infusion increased intestinal crypt cell production throughout the gut[33], especially in the colon. It also progressively increased proliferation with increasing dose, and was equally effective whether given continuously or when given after hypoplasia had become established[34]. A proliferative effect on the intestine has also been seen in a human infant maintained on intravenous infusion[97]. EGF was not effective when given intragastrically[34,73]. The continuous intra-ileal infusion of EGF has nonetheless been reported as increasing intestinal cell proliferation both in the perfused section and in the jejunum (which did not receive any luminal EGF[94]). EGF can be absorbed from the intestine, at least in the young animal[93], but may be partially degraded as it passes through.

It is thus likely that EGF may have both local and systemic effects on the gut. The evidence for a systemic role for EGF in the control of gastrointestinal epithelial cell proliferation is far stronger than that obtained for any other peptide, but the question of whether this is a physiological or a pharmacological effect remains to be seen. A final twist to the EGF story is provided by the discovery that transforming growth factor is both structurally and functionally very similar to EGF[64,91].

Other peptides

Cholecystokinin (CCK) is a peptide closely related to gastrin and while there is some evidence for it having a trophic effect on the gut[39,40], the direct infusion of low and high doses of CCK had no effect on intestinal structure and function (but did markedly stimulate the pancreas)[24]. The levels of gastrointestinal somatostatin increase on starvation[96] and somatostatin can inhibit cell proliferation in the stomach[55], in the duodenum[14], and it can also inhibit the rise in crypt cell proliferation and enteroglucagon normally seen after resection[83]. Somatostatin also inhibits EGF secretion from Brunner's glands[49].

The above list of possible trophic agents cannot be regarded as final, as there are still more peptides to be discovered let alone investigated, for example growth hormone releasing factor (somatocrinin) has recently been reported to stimulate intestinal epithelial cell proliferation in the stomach and duodenum[53].

Conclusion

The control of gastrointestinal epithelial cell proliferation is undoubtedly a multifactorial affair, involving local negative feedback, the direct and indirect effects of food (food intake is one of the best predictors of intestinal cell production and intestinal function[32]) and the local and systemic effects of humoral factors.

The data presently available suggests that several peptides may play a role in the control of epithelial cell renewal. The relative importance of these peptides has yet to be established, and the further investigation of these important factors demands the use of valid techniques, which should be applied to the entire gastrointestinal tract, as the response of the stomach, colon and small intestine have been seen to vary.

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Precursors to regulatory peptides: their proteolytic processing

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Summary. Precursors to regulatory peptides undergo maturation processes which include proteolytic processing. The enzymes involved in this process remove the hydrophobic peptide located at the amino-terminus of the precursor. Endoprotease cleavage also occurs at single and two adjacent basic residues, this is followed by a removal of basic residues located at the C-terminus of the peptides by a carboxypeptidase-like enzyme.
Key words. Prohormone processing; regulatory peptides; precursors; proteolytic enzymes.

Regulatory peptides are diverse in their function and localization; however, they share a common property in that they all are initially synthesized as larger precursors which are processed proteolytically to form biologically active products[15,45]. Figure 1 is a schematic representation of several regulatory peptide precursors showing their processing sites and indicating that these precursors can have a molecular weight greater than 10 times that of the biologically active peptides[29], or, they may lose only a few amino acids during their maturation process[63].

Precursors to regulatory peptides have in common 1) a similar 'route' from their site of synthesis to the ultimate export of their products from the cell and 2) they all undergo proteolytic processing events. The proteolytic processing events include removal of the signal sequence, which is necessary for sequestration of the protein into the endoplasmic reticulum as well as subsequent endoproteolytic and exoproteolytic cleavages. Specific regulatory peptides can also undergo other post-translational modifications which include disulphide bond formation, carbohydrate addition, sulphation, phosphorylation, acetylation, and amidation to mention only a few of the numerous modifications which have been described[87].

In this brief review, it is not possible to examine throughly all