



REVIEW ARTICLE

Classification and functions of enteroendocrine cells of the lower gastrointestinal tract

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Summary

With over thirty different hormones identified as being produced in the gastrointestinal (GI) tract, the gut has been described as ‘the largest endocrine organ in the body’ (*Ann. Oncol.*, 12, 2003, S63). The classification of these hormones and the cells that produce them, the enteroendocrine cells (EECs), has provided the foundation for digestive physiology. Furthermore, alterations in the composition and function of EEC may influence digestive physiology and thereby associate with GI pathologies. Whilst there is a rapidly increasing body of data on the role and function of EEC in the upper GI tract, there is a less clear-cut understanding of the function of EEC in the lower GI. Nonetheless, their presence and diversity are indicative of a role. This review focuses on the EECs of the lower GI where new evidence also suggests a possible relationship with the development and progression of primary adenocarcinoma.

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Development of enteroendocrine cells of gastrointestinal tract

The alternative terms, ‘neuroendocrine’ and ‘APUD’ cells, used to describe EECs reflect how these cells were once thought to originate from the neural crest. Although this was originally suggested by similarities the cells share with neurons, Andrew *et al.* (1998) chronicle a robust body of evidence demonstrating that EECs of the gut are derived from the endoderm and not the neural crest. Indeed, enteroendocrine cells (EECs) have been shown to develop from the same pluripotent stem cells as the other three cell lineages of the intestinal epithelium: absorptive enterocytes, goblet cells and paneth cells (Gordon 1993).

The process of differentiation of EECs begins as proliferating pluripotent stem cells at the base of the intestinal crypt and progresses as daughter cells migrate, in an upward linear fashion, towards the epithelial cuff at the luminal surface

(Figure 1). It is thought that pluripotent cells commit to one of the four cellular lineages in the transitional area, located in the lower and middle thirds of the crypt (Gordon & Hermiston 1994). The same, upward, direction of migration is shared by differentiating cells of the enteroendocrine, absorptive and goblet cell lineages albeit it more rapidly in the latter two. Conversely, differentiating cells of the Paneth cell lineage mature as they migrate along the same axis but in the opposite direction (Hocker & Wiedenmann 1998).

In a study by Roth *et al.* on the proximal colon of mice, colocalization patterns of peptide markers for different EECs were examined. It was found that serotonin and substance P failed to colocalize with peptide YY, GLP-1, cholecystokinin or neurotensin and that the latter four peptide markers often colocalized with each other. The investigators presented these findings as evidence of two branches of cellular differentiation once cells have committed to the EEC lineage (Roth *et al.* 1992). In their review, Schonhoff *et al.* present

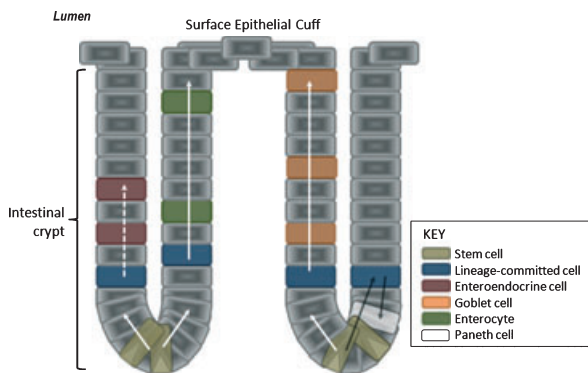


Figure 1 Development of enteroendocrine cells of the gastrointestinal tract. As daughter cells of the pluripotent stem cells migrate from the base of the crypt towards the surface epithelial cuff, they commit to one of the four cellular lineages. Enteroendocrine cell-committed differentiating cells migrate slowly in comparison with differentiating cells of the goblet and enterocyte lineages. Paneth cell-committed differentiating cells migrate towards the base of the crypts as they mature.

evidence from gain and loss of function studies in transgenic mice for a cascade of basic helix-loop-helix (bHLH) factors that are expressed sequentially during EEC differentiation. Neurogenin 3, a downstream factor of Math1, is thought to be necessary for commitment to the EEC lineage, and expression of further downstream factors including Pax 4 and Pax 6 results in specific hormone-producing EECs (Schonhoff *et al.* 2004).

Roth *et al.* also demonstrated characteristic distributions along the crypt-to-surface epithelial cuff axis for different EEC subtypes. The location of EECs and their terminal differentiation produces replicable patterns not only in the crypt-surface epithelial cuff axis, but also in the proximal-distal axis of the gastrointestinal (GI) tract. Holle *et al.* (2003) examined how these patterns come about and demonstrated that denervation of small intestine in Wistar rats by myenteric ablation produced alterations in the distribution of EECs within the targeted intestinal mucosa. This suggests that intramural innervation of the small intestine has some influence on the differentiation of EECs, although similar findings have yet to be reported in the large intestine.

General features of enteroendocrine cells

Intestinal EECs are restricted to the mucosa, predominately located within its deeper half, and comprise only a small minority (<1%) of the overall epithelial cell population, often lying isolated from one another interspersed by non-endocrine epithelial cells (Buffa *et al.* 1978; Sternini *et al.* 2008). A mechanism underlying this scattered distribution is described by Schonhoff *et al.* (2004) whereby the signaling pathway of cell surface protein 'Notch' prevents adjacent cells from differentiating into EECs by lateral inhibition.

The EEC population of the large bowel is generally less diverse than in the small intestine (Buffa *et al.* 1978). For instance, cholecystokinin-secreting cells, secretin-secreting S cells, gastric inhibitory polypeptide-secreting cells, motilin-secreting M cells and neurotensin-secreting N cells are found in the small intestine but are absent from the large (Rindi *et al.* 2004). From duodenum to rectum, the frequency of EECs is highest proximally and falls steadily to reach a trough at the colon before rising again within the rectum. After proximal small bowel, the rectum is the location with the next greatest frequency of EECs and the only location in the GI tract where EECs are occasionally seen adjacent to each other or in clusters (Cristina *et al.* 1978; Shamsuddin *et al.* 1982; Sjolund *et al.* 1983).

Although cellular morphology has been demonstrated to vary with EEC cell subtype, there are some general features common to most of them. For instance, EECs often have an apical cytoplasmic process with microvilli that extend towards the luminal surface, and a unique morphological feature of EECs of the large intestine is the presence of basal processes that extend towards adjacent epithelial cells, which is not seen in small intestinal EECs (Capella *et al.* 1976; Sjolund *et al.* 1983).

Enteroendocrine cells are characterized by the presence of secretory vesicles, which are either large dense-core vesicles (LDCVs) or the smaller synaptic-like microvesicles (SLMVs) similar to those found in neurones. Components of these vesicles can be exploited as general markers for EECs using immunohistochemistry (IHC) and include chromogranin A, which is a matrix-soluble glycoprotein commonly found in LDCVs, and synaptophysin, a membrane glycoprotein of SLMVs (Varndell *et al.* 1985; Weidenmann *et al.* 1986). Other general markers for EECs include neuron-specific enolase and protein gene product 9.5, both of which are found within the cellular cytoplasm (Rindi *et al.* 2004).

Biogenic amine transporters allow the passage of amines across the vesicular membrane against their concentration gradient acting to compartmentalize them within the secretory vesicles. The vesicular mono-amine transporter, VMAT1, carries out this process for serotonin (5-HT) in 5-HT-secreting EECs (Weihe & Eiden 2000). In a recent review by Borges *et al.* (2010) based on a series of experiments with knockout mice, chromogranin A has been implicated as a transporter of catecholamines in adrenal chromaffin cell LDCVs and may serve a similar function, as a biogenic amine transporter, in EECs.

In a striking similarity with neuronal postsynaptic vesicles, it has been shown that the contents of these SLMVs are exocytosed in response to cellular plasma membrane depolarization, and by *in vitro* study of mouse cells, Uehara *et al.* have specifically demonstrated this in intestinal EECs (Ashcroft *et al.* 1994; Franklin & Wollheim 2004; Uehara *et al.* 2006). This offers a mechanism by which EEC secretion may be under neural control, a hypothesis strengthened by the finding of true synapses between neurones and EECs in rat ileal mucosa at what have since been termed 'neuroendo-

crine complexes' (Ahlman & Dahlstrom 1983; Ahlman & Nilsson 2003).

Neuroendocrine tumours (NETs) have been classified by the World Health Organisation into well-differentiated NET, well-differentiated neuroendocrine carcinoma and poorly differentiated neuroendocrine carcinoma (Kloppel *et al.* 2004). The incidence of these rare tumours has been cited as two per 100,000 and is relatively more common in the rectum than in the colon, rectal NETs comprising 27% of all GI NETs (Kloppel & Anlauf 2005; Lowell *et al.* 2010). Whilst poorly differentiated tumours can be identified by the expression of general enteroendocrine markers such as synaptophysin and chromogranin A, more differentiated tumours also express the peptide products of their constituent EEC subtype. In the most recent WHO classification of NETs, released in 2004, NETs are further subclassified according to size, functionality, site and invasion. Of note, EEC cell subtype is not included in the classification and the College of American Pathologists does not recommend routine staining of NETs to identify the specific EEC cell subtype because of a lack of evidence of its prognostic significance (Washington *et al.* 2010). The ontology around NETs is in flux: in the WHO classification of NETs, they are still referred to as 'NETs', although in a consensus report of the TNM staging of NETs they use the terms 'NETs', '(neuro)endocrine tumours' and 'endocrine tumours' interchangeably (Rindi *et al.* 2007).

As the most specific feature of EEC subtypes, the peptide/amine(s) contained within a cell's secretory vesicles has formed the basis for the classification of EECs (Solcia *et al.* 1978); prior to this, EEC subtypes were distinguished by differences in the appearance of their secretory vesicles on electron microscopy. Investigations utilizing a combination of immunohistochemical and ultrastructural techniques have demonstrated three main cell types prevalent within the lower GI tract. These cell types are summarized in Table 1.

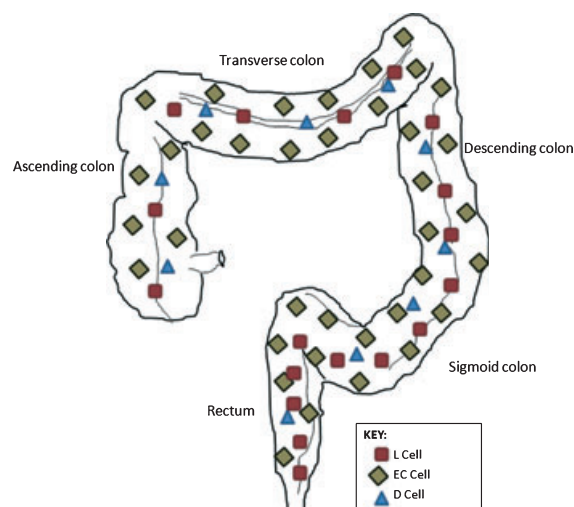


Figure 2 Distribution of enteroendocrine cell subtypes. Enterochromaffin cells are the most abundant enteroendocrine cell subtype of the colon and rectum. D cells are uncommon although scattered evenly throughout the gastrointestinal tract. L cells are uncommon proximal to the terminal ileum, and their frequency increases from proximal to distal being most concentrated in the rectum.

Enterochromaffin cells

Enterochromaffin (EC) cells are the most abundant EEC of the GI tract and are distributed widely, populating the gastric antrum, duodenum, jejunum, ileum and appendix as well as the colon and rectum (see Figure 2). EC cells have been shown to make up over 70% of the EEC population in the proximal large bowel, which falls to around 40% in the rectum. As the absolute numbers of EC cells are fairly constant throughout the large intestine, this fall in proportion reflects an increase in the incidence of other EEC cell types

Table 1 Features of EECs of colon and rectum

Cell type	Distribution	Ultrastructural features	Secretory granules	IHC	Secretory products
EC cell	Most common EEC type; throughout GIT	Pyramid shaped; often have slender apical process reaching luminal surface	150–500 nm; pleomorphic	CgA, Syn, 5-HT, TPH	5-HT
D cell	Throughout GIT. Least common EEC type in colon and rectum	Spindle shaped; often have slender apical process and one shorter wider basal extension	150–300 nm; round	(Syn), somatostatin	Somatostatin
L cell	Found in duodenum to rectum; rare before terminal ileum; greatest frequency in rectum	Bottle shaped; often have apical process reaching luminal surface; sometimes have basal process along basement membrane	200–400 nm; round	(CgA), (Syn), PYY, GLP-1, GLP-2, oxyntomodulin, glicentin	Peptide YY, GLP-1, GLP-2, glicentin, oxyntomodulin

IHC, immunohistochemistry; EEC, enteroendocrine cell; GIT, gastrointestinal tract; CgA, chromogranin A; Syn, synaptophysin; 5-HT, serotonin; TPH, tryptophan 5-hydroxylase; PYY, peptide YY; GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2.

Table 2 Secretory products of enteroendocrine cells of the colon and rectum and their actions

Peptide	Actions
5-HT	Intestinal motility; intestinal secretion; visceral sensation; appetite
Somatostatin	Major inhibitory hormone for digestive endocrine and exocrine function; stimulates colonic peristalsis
PYY	Inhibits gastric emptying and intestinal motility; inhibits gastric acid secretion and pancreatic exocrine function; suppresses appetite; stimulates mucosal enterocyte proliferation
GLP-1	Incretin effect; delays gastric emptying; postprandial satiety
GLP-2	Stimulates mucosal enterocyte proliferation
Glicentin	Stimulates mucosal enterocyte proliferation; inhibits gastric emptying
Oxyntomodulin	Inhibits gastric emptying

PYY, peptide YY; GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2.

in the distal colon and, especially, rectum (Cristina *et al.* 1978; Sjolund *et al.* 1983).

Originally named because of their affinity to bind chromium salts, EC cells' main secretory product is serotonin (5-HT, Table 2), which is synthesized by the hydroxylation and decarboxylation of tryptophan (Ahlman & Nilsson 2003). EC cells are approximately 8 micrometres in size and triangular or pyramidal in shape, displaying an apical process that often extends to the luminal surface (Buffa *et al.* 1978; Modlin *et al.* 2006). LDCVs are found apically and basally (Spiller 2008), and the diameter of the pleomorphic EC secretory granules has been reported to be approximately 150–500 nm (Solcia *et al.* 1998). Some regional differences in EC cell morphology have also been noted. For instance, occasional EC cells with basal extensions containing secretory vesicles have been observed in the colon or rectum (but not in the small intestine), which Gustafsson *et al.* (2006) postulate may communicate with neurones. Gastric ECs also differ slightly in that their secretory vesicles are often smaller, approximately 100–150 nm (Cristina *et al.* 1978).

Immunohistochemical studies demonstrate EC cells stain positively for chromogranin A in all regions of the GI tract (Schmid *et al.* 1989; Portela-Gomes *et al.* 1997). That staining intensity is strong in all regions except the gastric antrum explains the popular use of chromogranin A as a general marker for EECs. A series of colocalization studies of chromogranins and various hormones of EEC origin in colonic mucosa demonstrated that whilst chromogranin A colocalizes with 5-HT, it also colocalizes to some extent with peptide YY and enteroglucagon that are not EC cell products (Portela-Gomes *et al.* 1997), limiting its use as a specific marker of EC cells.

Antibodies directed against tryptophan 5-hydroxylase (TPH), the initial enzyme in 5-HT biosynthesis, have recently shown promise as specific markers for EC cells (Modlin *et al.* 2006). Whereas 5-HT is present in neurones

within the intestinal mucosa as well as with EC cells, differences have been reported between neural and non-neural TPH isotypes (Kuhn *et al.* 1980; Hasegawa *et al.* 1987). Difficulties in developing antibodies specific to non-neural TPH have been overcome by the use of dual staining immunofluorescence techniques for acridine orange (AO) and TPH, where EC cell morphology can be confirmed ultrastructurally by the localization of AO within the vesicles and TPH within the cytoplasm (Yu *et al.* 1999; Modlin *et al.* 2006). It is noteworthy that 5-HT-containing neurones of the gut are likely to be intrinsic as they are present after 3 weeks in tissue culture, by which time extrinsic neurones have degenerated (Dreyfus *et al.* 1977).

Ninety-five per cent of the body's 5-HT content is within the GI tract, the majority of which is contained within the secretory granules of EC cells. Furthermore, 5-HT content is said to be highest in the rectum (Spiller 2008). In a study on EC cells isolated from human ileal mucosa, Modlin *et al.* profiled the receptors that are expressed by EC cells and assessed the receptors' downstream effects by applying specific agonists. The results implicated β -adrenergic and PACAP receptors in the secretory stimulation pathway and GABA_A and cholinergic receptors in the secretory inhibition pathway (Modlin *et al.* 2006). These findings suggest that 5-HT release by EC cells is, at least in part, neurally regulated, a hypothesis supported by experimental evidence from animal studies (Ferrara *et al.* 1987).

Mucosal stroking, an *ex vivo* technique that simulates the passage of local luminal content matter, was utilized by Kellum *et al.* (1999) to demonstrate its potent effect on 5-HT secretion in human jejunum. Mechanisms that have been suggested for this effect include direct mechanically evoked 5-HT release from EC cells and indirect mechanisms such as neural reflexes.

The possibility that chemical stimulants within the bowel lumen act as regulators of 5-HT release from EC cells has been raised by studies that have demonstrated the expression of a variety of tastant and olfactant receptors by EC cells (Modlin *et al.* 2006; Braun *et al.* 2007). This hypothesis is further strengthened by the observation that 5-HT release is observed in response to adding various tastants to cell cultures (Kidd *et al.* 2008). Pertinent to the lower GI tract, this effect has been shown for short-chain fatty acids (SCFAs), which are the products of fermentation of undigested carbohydrates by anaerobic bacteria in the large bowel (Fukumoto *et al.* 2003). Animal studies demonstrate an interaction between T lymphocytes and EC cells, with a reduction in EC cell number in mice lacking the T-cell receptor and reduced 5-HT in severe combined immunodeficiency (SCID) mice (Rubin *et al.* 2005; Wang *et al.* 2007; Spiller 2008). These findings may suggest EC cells play a role in mediating effects as part of the immune response.

Seven families of receptor subtypes of 5-HT have been identified and are expressed by a variety of cell types within the GI tract including enteric neurones, EC cells, smooth muscle myocytes, absorptive enterocytes and interstitial cells (Tonini 2005; Van Lelyveld *et al.* 2007; Hasler 2009; Hol-

brook *et al.* 2009). As well as giving us an idea of the range of target effects of 5-HT within the GI tract, this suggests that EC-derived 5-HT acts not only in an endocrine manner but by autocrine, paracrine and neurocrine means also.

Information regarding the functions of 5-HT can be drawn from both experimental data and lessons learned from pathological conditions affecting EC cells (Gershon & Tack 2007; Hasler 2009). Animal and human studies have demonstrated that 5-HT causes increased gut motility and accelerated intestinal transit (Lordal *et al.* 1998; Chen *et al.* 2001; Coleman *et al.* 2003). It has been suggested that bidirectional interaction between EC cells and the enteric nervous system is partly responsible for this effect whereby 5-HT released from EC cells binds to 5-HT₄ receptors on the nerve terminals of intrinsic primary afferent neurones (IPANs), which in turn results in the activation of excitatory cholinergic neurones that innervate smooth muscle (Costedio *et al.* 2007; Gershon & Tack 2007). 5-HT also contributes to gut motility by its direct effect on smooth muscle 5-HT receptor subtypes, although stimulation of these receptors mainly produces relaxation (Bjornssen *et al.* 2002). The simultaneous relaxation of local smooth muscle by direct action of EC cell-derived 5-HT and contraction of neighbouring smooth muscle via the neural pathway may explain a mechanism by which EC cells produce a pattern of smooth muscle contraction compatible with the peristaltic waveform.

Borman and Burleigh's experiments on human intestinal mucosa demonstrated that local application of 5-HT resulted in the secretion of an electrogenic fluid. The greatest secretory response was found in the mucosa of terminal ileum, followed by that of the sigmoid colon and finally by that of the ascending colon. Symptoms relating to colonic hypersecretion and increased colonic motility commonly seen in inflammatory bowel disease (IBD) may at least partly be attributable to a raised 5-HT release. A mechanism by which this occurs is offered by Wang *et al.* who demonstrated an increase in EC cell number and 5-HT production in SCID mice when reconstituted with helper T cells (Rubin *et al.* 2005; Wang *et al.* 2007). A similar effect of helper T cells may occur in IBD. Additionally, Dunlop *et al.* (2005) observed a reduced number of EC cells in constipation-predominant irritable bowel syndrome (C-IBS), suggesting that EC cells may have a role in the pathophysiology of this condition also. Through the use of specific 5-HT receptor antagonists, the 5-HT₄ and 5-HT_{2A} receptor subtypes have been implicated in mediating ileal and colonic secretion, respectively, and may represent avenues of future research exploring the symptomatic treatment of the these conditions (Borman & Burleigh 1997; Spiller 2008).

5-HT is involved in the perception of colorectal distension as evidenced by the presence of 5-HT receptors on ascending neurones and the efficacy of 5-HT₃ antagonists (Gershon & Tack 2007) in relieving visceral pain. Unlike colonic motility and intestinal secretion, where effects have been shown to persist after *in vitro* application of neurotoxin, it is much more difficult to demonstrate that visceral sensation is medi-

ated by EC cell-derived 5-HT rather than neurally derived 5-HT (Kuemmerle *et al.* 1987). In their review, Garfield and Heisler (2009) summarize the evidence from experimental studies on rodents of 5-HT causing a reduction in appetite and evaluate the therapeutic effect of 5-HT receptor agonists in the treatment of obesity in humans. This is thought to occur by the action of 5-HT in the hypothalamus, particularly the arcuate, paraventricular and ventromedial nuclei. Again, whether this is an effect mediated primarily by neurally derived 5-HT or EC cell-derived 5-HT that reaches the hypothalamus via the bloodstream has yet to be determined.

D cells

D cells, like EC cells, are found throughout the GI tract but contrastingly are found in much lower numbers, making up around 3–5% of the EEC population in the lower GI tract (Buffa *et al.* 1978; Cristina *et al.* 1978; Sjolund *et al.* 1983). Penman *et al.* (1987) report the highest frequency of D cells in the duodenum and pancreas, with lower levels in the ileum and colon compared with the upper GI tract (see Figure 2). Within the mucosa, D cells have a propensity towards the lower third of the crypt and their main secretory product is somatostatin (Low 2004).

Hauso *et al.* describe the ultrastructure of D cells as being relatively homogenous in the large intestine with one elongated apical extension and one shorter, wider basal extension. This differs from the overall appearance of D cells in the stomach which were described as being much more varied, often with two or three cytoplasmic extensions that appeared to end in bulbous swellings connected to other epithelial cells (Hauso *et al.* 2007). Studies have demonstrated the secretory vesicles of D cells to be approximately 200–400 nm in diameter (Solcia *et al.* 1998; Seretis *et al.* 2004).

D cells are identified by IHC as being somatostatin immunoreactive. Somatostatin (known as SST14 in its tetradecapeptide form) is located in the hypothalamus (where it is involved in the regulation of pituitary hormones), as well as in the pancreas and GI tract where it is also produced. Within the GI tract, somatostatin immunoreactivity occurs within the muscular layer, where it has been localized to neurones of the myenteric plexus, and within the mucosa, where it localizes to D cells (Penman *et al.* 1987). Patel *et al.* (1981) discuss a number of high molecular forms of SST-14 including the 28 amino acid form, SST-28, which they found also to be immunoreactive for antibodies directed against SST-14. Through the use of gel chromatography, whereby the difference in molecular weights between SST-14 and SST-28 were taken advantage of, SST-14 was found to be the predominant form of somatostatin in neural tissue and gastric D cells, whereas SST-28 was the predominant form in D cells throughout mucosa of the GI tract distal to the stomach (Patel *et al.* 1981; Penman *et al.* 1987).

In contrast to the postprandial rise in the circulating levels of somatostatin which remains raised for up to 4 h and is likely to be released by D cells of the upper GI tract, Kido *et al.* found that somatostatin concentration and mRNA

expression within the rat colon increased after 48 h of fasting (Ensinck *et al.* 1990; Ensinck *et al.* 2002, 2003; Kido *et al.* 2003). Studies on patients with *H. pylori*-related chronic gastritis and on patients with IBD have demonstrated a decrease in both the number of D cells and circulating somatostatin levels in the stomach and colon, respectively, during these chronic inflammatory processes, providing further evidence of a role of the immune system in EEC regulation (Watanabe *et al.* 1992; Milutinovic *et al.* 2003). Stanisz *et al.* (1986) found that somatostatin has inhibitory effects on the immune system including the proliferation of T lymphocytes and production of immunoglobulins. D cell-derived SST has also been demonstrated to inhibit the secretion of pro-inflammatory cytokines from both T lymphocytes and intestinal epithelial cells, indicating that the regulatory influence between D cells and immunity is two way (Blum *et al.* 1992; Chowers *et al.* 2000).

Somatostatin is the major inhibitory hormone of the digestive system, acting to decrease the release of all known GI hormones including itself, as well as exocrine functions of the GI tract and pancreas (Reichlin 1987; Table 2). Saras *et al.* (2007) demonstrated that within 2 min, somatostatin causes the contraction of EECs through rearrangement of the actin filament system and a translocation of their secretory vesicles from the cell periphery to the perinuclear region.

Five somatostatin receptor (SSTR) subtypes have been identified, of which SSTR2 is especially relevant to the effects of somatostatin within the large intestine, where it is the most prevalent of the receptor subtypes, having been demonstrated to stimulate peristalsis and ion secretion in the colon upon activation (Grider 2003; Abdu *et al.* 2002; Hope *et al.* 2001). In the colon, somatostatin also acts on SSTRs of myenteric neurones to mediate descending relaxation of the smooth muscle (Low 2004).

L cells

L cells occur from the duodenum to the rectum although are rare proximal to the terminal ileum. Within the large intestine however, they constitute the second largest population of EECs, their frequency rising from proximal to distal and make up approximately 14% of the EEC population in the rectum (Figure 2; Sjolund *et al.* 1983; Cristina *et al.* 1978). L cells have been observed to occur in all parts of the crypts but with some predominance in the basal portion and their secretory products are proglucagon-derived peptides (PGDPs, see Figure 3) and peptide YY (Bottcher *et al.* 1984; Table 2).

L cells have been described by Buffa *et al.* (1978) as being bottle or flask shaped. They often display an open morphology whereby an apical protrusion of cytoplasm extends to the luminal surface and exhibits microvilli. Bottcher *et al.* also report basal processes that run along the basement membrane, and their secretory vesicles have been observed to occur adjacent to the basolateral membranes and are approximately 150–300 nm in diameter (Bottcher *et al.* 1984; Solcia *et al.* 1998).

A series of immunohistochemical investigations have demonstrated the localization of peptide YY and PGDPs to intestinal L cells (Bottcher *et al.* 1984; Fiocca *et al.* 1987; Nilsson *et al.* 1991). Pro-pancreatic polypeptide (Pro-PP), the precursor molecule of PYY, and proglucagon, precursor to the PGDP, have been shown to be expressed both in pancreatic alpha cells and in intestinal L cells. The profile of precursor-derived peptides, however, differs between the two cell types. For instance, pancreatic glucagon, a product of proglucagon processing, is found in the pancreas but not in the gut, and the reverse is true for GLP-1, which is not present in pancreatic alpha cells (Figure 3). These findings demonstrate that alternative post-transcriptional splicing of the

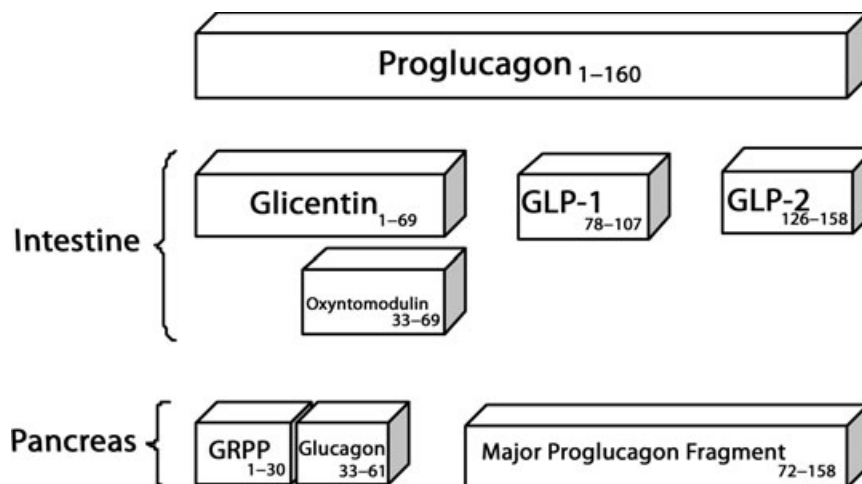


Figure 3 Processing of proglucagon. Alternative post-transcriptional splicing of the proglucagon precursor molecule is into glicentin, GLP-1, GLP-2 and oxyntomodulin in intestinal L cells and into pancreatic glucagon in pancreatic alpha cells. GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2. Adapted from Holst (2007).

same precursor molecules occurs in distinct patterns between pancreatic alpha cells and intestinal L cells to yield different bioactive peptides (Rehfeld 1998).

A study on rectal carcinoid tumours and the unaffected adjacent rectal mucosa in humans showed immunoreactivity of intestinal L cells to a range of PGDPs including glicentin (1–69), GLP-1 (72–108), GLP-2 (126–159) and oxyntomodulin (33–69) – so-called glucagon-like immunoreactivity – in addition to PYY (Fiocca *et al.* 1987). In a study by Nilsson *et al.* (1991) utilizing double immunogold labelling in rabbit colon, PYY and PGDP were both found in all L cells examined, colocalizing to 85% of secretory vesicles, the remaining vesicles containing PYY only. Despite colocalization of these two hormones to the same cells, a number of studies have demonstrated the selective secretion of one hormone, suggesting discrete intracellular mechanisms underlying their release (Pironi *et al.* 1993; Plaisancie *et al.* 1995; Anini *et al.* 1999).

In a study on 151 healthy human volunteers, circulating PYY and GLP-1 levels peaked 20 min after having an oral glucose load and remained elevated for 2 h (Kim *et al.* 2005). Although this could represent direct stimulation of duodenal L cells, the majority of L cells are found in the distal colon and rectum, and so Anini *et al.* (1999) hypothesized this early response to be neurally regulated. They tested their hypothesis on Wistar rats and demonstrated that PYY and GLP-1 release was suppressed or inhibited by techniques including bilateral vagotomy and administration of hexamethonium (an anticholinergic agent), supporting their hypothesis. They also demonstrated a later phase of PYY release in response to intraluminal SCFAs that was replicated by direct intracolonic infusion and unaffected by hexamethonium, making this later response much more likely to be as a direct effect of intraluminal nutrients acting directly on L cells (Anini *et al.* 1999). In addition to glucose and SCFAs, studies have also demonstrated the release of PYY in response to intraluminal bile salts and amino acids (Adrian *et al.* 1993; Zhang *et al.* 1993). PYY release has been shown to be influenced by regulatory peptides including cholecystokinin, vasoactive intestinal peptide and GLP-1, and Chisholm and Greenberg demonstrated the inhibitory effect of somatostatin, particularly in its SST-28 form, on PYY release from foetal rat intestinal cell cultures (Greeley *et al.* 1989; Rudnicki *et al.* 1992; Näslund *et al.* 1999; Chisholm & Greenberg 2000).

Studies involving intravenous infusion of PYY in healthy human volunteers have demonstrated reduced intestinal and colonic transit, reduced gastric emptying and reduced gastric acid and pancreatic exocrine secretion (Ballantyne 2006). This illustrates PYY as an important mediator of the 'ileo-colonic brake' whereby unabsorbed nutrients in the distal intestine and colon trigger the inhibition of motility and secretion upstream. A recent review by Karaki and Kuwahara (2010) outlines the evidence linking SCFAs within the colonic lumen to the inhibition of proximal intestinal motility, which is thought to be through the release of PYY from intestinal L cells. In their review, El-Salhy *et al.* (2002) discuss the evi-

dence of reduced tissue and circulating levels of peptide YY in patients with IBD, which may contribute to diarrhoea in these patients owing to increased gastric emptying, intestinal motility and intestinal secretions. A further inhibitory action of PYY is outlined in a review by Dietrich and Horvath who discuss the role of PYY in the anorexigenic response and present evidence for the peptide reducing food intake when administered peripherally. This suggests an interaction between L cells and the central nervous system in producing postprandial satiety (Dietrich & Horvath 2009). Additionally, following observations in animal studies whereby raised circulating levels of PYY were seen following intestinal resection suggesting an adaptive response, *in vitro* investigations provide evidence that PYY acts on Y1 receptors in the human colon epithelium to stimulate cell proliferation, suggesting a role of L cells in maintaining intestinal mucosal integrity (Mannon 2002).

Elliott *et al.* (1993) found that in healthy human subjects, GLP-1 release increases after ingestion of carbohydrate, fat and protein meals. This effect has been demonstrated on the GLUTag cell line, a cellular model for intestinal L cells derived from the colonic tumour of transgenic mice, and further studies on the GLUTag cell model have implicated GABA, a neurotransmitter of the enteric nervous system, in the regulatory mechanism of GLP-1 release (Gameiro *et al.* 2005; Reimann *et al.* 2006).

One of the most important functions of GLP-1 is its major contributory role in the incretin effect. This is whereby gut hormones stimulate pancreatic insulin release in response to ingested glucose in a dose-dependent manner. Evidence of the effect comes from studies demonstrating a much greater insulin release observed after an oral glucose load in comparison with intravenous administration (Mari *et al.* 2002). By interaction between GLP-1 and its receptor on beta cells of the pancreatic islets, insulin transcription, biosynthesis and release are stimulated as is beta cell differentiation and proliferation. This has attracted interest in the role of GLP-1 in the pathogenesis of type 2 diabetes mellitus, as studies show a reduced GLP-1 response in sufferers of this condition (Holst & Gromada 2004). Glucose-dependent insuliotropic peptide (GIP) is secreted by K cells in the stomach and proximal small intestine and also potentiates insulin release by pancreatic beta cells. Mortensen *et al.* demonstrated that GIP and GLP-1 colocalize in the mid-small intestine of human, rats and pigs and these cells have been called K/L cells (Wang *et al.* 2002; Mortensen *et al.* 2003; Cho & Kieffer 2010).

The study by Wettergren *et al.* (1993) on humans, whereby synthetic GLP-1 was administered intravenously to physiological levels, found an inhibitory effect on gastric acid secretion and exocrine function of the pancreas. The feedback mechanism between the distal large bowel and proximal stomach is directly demonstrated by Jian *et al.* (1981) who carried out a study involving 16 healthy volunteers in whom gastric acid secretion was reduced in response to intracolonic glucose infusion. Given that in further studies, truncal vagotomy abolishes the inhibitory effect of GLP-1

on gastric acid secretion, and that the half-life of circulating GLP-1 is very short, it has been proposed that this mechanism is neurally mediated (Holst 2007). GLP-1 has been shown to have an additive inhibitory effect on gastric acid secretion with PYY (Wettergren *et al.* 1997). Furthermore, animal models have demonstrated the inhibitory effect of GLP-1, oxynomodulin and glicentin on gastric outflow (Schjoldager *et al.* 1989; Otani *et al.* 1994; Tolessa *et al.* 1998).

Verdich *et al.*'s (2001) meta-analysis of nine randomized cross-over trials examining the effect of intravenous GLP-1 on energy intake demonstrated the effect was found to be dose dependent and inhibitory. The mechanism for this is likely to involve the arcuate nucleus of the hypothalamus (Tang-Christensen *et al.* 1998). Given that some studies demonstrate postprandial secretion of GLP-1 to be impaired in morbidly obese patients, but that the satiety response to intravenously administered GLP-1 remains intact, this PGDP may offer potential in the future treatment of obesity (Naslund *et al.* 1998).

GLP-2 is cosecreted into the bloodstream with GLP-1 shortly following a meal but has been shown to have no effect on the regulation of glucose homeostasis. Its main action is as a potent stimulator of mucosal epithelial proliferation (Drucker 2002). Animal studies have demonstrated an increase in both large and small bowel mucosa following GLP-2 injection, although the small bowel seems to be more sensitive to the effects of GLP-2 (Tsai *et al.* 1997; Ghatei *et al.* 2002). Glicentin has been shown to exhibit a similar but less potent effect on intestinal epithelial cell proliferation and, together with GLP-2, has been shown to be part of the adaptive response in short bowel syndrome, offering a promising therapeutic avenue in this condition as well as TPN-associated gut atrophy (Drucker 2002; Chiba *et al.* 2007). Independent of its effect in epithelial cell proliferation, glicentin has also been implicated as a protective mucosal agent, preventing bacterial translocation of enteric bacteria when added to cell lines *in vitro* (Chiba *et al.* 2007).

Enteroendocrine cells and colorectal carcinoma

Differentiated EECs have been detected in approximately 35% of colorectal carcinomas (Swatek & Chibowski 2000; Gulubova & Vlaykova 2008). Given that EEC products serotonin, PYY, glicentin and GLP-2 have been proposed to enhance cellular proliferation within the colonic epithelium, the hypothesis arises that colorectal carcinomas with an enteroendocrine component are endowed with a proliferative advantage. In addition to this, some EECs in colorectal adenocarcinomas have been demonstrated to express pro-angiogenic factors including vascular endothelial growth factor (VEGF), which is known to be an important factor in the development and progression of colorectal adenocarcinoma (La Rosa *et al.* 1998; Gulubova & Vlaykova 2008). This being the case, this subgroup of carcinomas would be expected to be associated with a worse prognosis as is seen in primary colorectal neuroendocrine carcinomas compared with pure adenocarcinoma (Saclarides *et al.* 1994).

Studies utilizing both general and specific markers for EECs have examined the possible relationship between the presence of EECs within colorectal carcinomas and prognosis. Overall, the findings of these studies have been equivocal as some studies have identified a significant relationship between the presence of EECs within the carcinoma and prognosis (Hamada *et al.* 1991; De Bruine *et al.* 1993; Grabowski *et al.* 2006; Gulubova & Vlaykova 2008), whereas others have not (Smith & Haggitt 1984; Mori *et al.* 1995; Swatek & Chibowski 2000). Of note, a study by Lloyd *et al.* examining 289 cases of moderately differentiated (grades II and III) colorectal carcinomas found that EECs identified by IHC for chromogranin A and by *in situ hybridization* analysis for chromogranin A and B were present in 26% of cases. With a mean follow-up period of 7 years, it was concluded that the number of chromogranin-positive cells did not correlate significantly with a number of factors including histological grade and survival (Lloyd *et al.* 1998).

It is well established that the incidence of colorectal cancer is lower in populations with a high dietary fibre intake. Given their location within the intestinal crypts and their ability to respond to chemical stimulants within the lumen, EECs are a potential candidate for being contributors to this effect (Bingham *et al.* 2003). Indeed, free fatty acid receptors FFA 2 and 3 have been demonstrated to be expressed by L cells and act as receptors to SCFAs (Karaki & Kuwahara 2010). Cani *et al.* fed rats a diet supplemented with non-digestible carbohydrate oligofructose for 4 weeks and observed an increased number of L cells in the ascending colon compared with rats fed with a standard diet. EEC differentiation factors NGN3 and NeuroD were also found to be increased, suggesting a relationship between dietary carbohydrates and enteroendocrine differentiation (Cani *et al.* 2007).

In a study on 42 cases of primary colorectal carcinoma whereby tissues were examined with immunohistochemical markers for the cellular lineages of colonic epithelial stem cells, 9% were found to be pluripotent, expressing markers for all four cell lineages: secretory component for absorptive enterocytes, mucin for goblet cells, chromogranin A for EECs and lysozyme for paneth cells (Ho *et al.* 1989). In accordance with the shared ancestry model of colonic epithelial cell lineages, this finding can be attributed to neoplastic transformation occurring at an earlier stage of stem cell differentiation. This hypothesis is strengthened by the work of Yeung *et al.* (2010) who have demonstrated, with colorectal cancer cell lines, that cancer stem cells continue to differentiate in a similar pattern to that observed in normal stem cells. With this in mind, adenocarcinomas and NETs may be viewed as opposite ends of a spectrum, adenocarcinomas with an enteroendocrine component being an intermediary. Lessons regarding therapeutic intervention may therefore be transferrable from gastroenteropancreatic NETs. This hypothesis could be tested by directly comparing the survival outcomes of colorectal adenocarcinomas with an endocrine component with poorly differentiated NETs of the same site.

One particularly promising field in this regard is the use of somatostatin analogues. Although the mainstay of symptomatic treatment of hypersecretion syndromes is associated with NETs, recent data also provide substantial evidence that administration of long-acting somatostatin analogues inhibits tumour growth and increased time to tumour progression; however, this effect was most pronounced in the more differentiated NETs (Rinke *et al.* 2009). The mechanism underlying the antiproliferative effects of somatostatin and related analogues on tumour cells is incompletely understood. It has been proposed that the effect is because of an inhibition of release of various growth factors including VEGF. This hypothesis is supported by the finding that VEGF release is inhibited by SSTR-1 agonists in human dermal microvascular endothelial cells (HMEC) (Bocci *et al.* 2007). As high levels of SSTR subtypes have been found to be expressed in colorectal adenocarcinoma as well as in NETs, there may be potential for the therapeutic application of somatostatin analogues in the treatment of SSTR-positive colorectal adenocarcinoma (Reubi 2004).

Conclusion

Normal GI physiology involves complex interactions between the central nervous system, the enteric nervous system and the endocrine system whereby information is transferred by feedback and feedforward mechanisms to regulate GI function. EECs of the lower GI tract play an important role in this complex process by acting as sensors to luminal contents and mechanical distension as well as mediators of function. Their peptide secretory products act both in a paracrine fashion to exert local effects such as on colonic motility and secretion and in an endocrine fashion to exert effects at distant sites in the GI tract, such as gastric emptying.

As yet data exploring the role of EECs in pathological conditions affecting the GI tract are preliminary, yet suggest that EEC perturbation may have a role in the pathology of conditions including irritable bowel syndrome and colorectal adenocarcinoma. Even more preliminary data suggest that colorectal tumours themselves cause perturbations in the balance of EEC (Nitta *et al.* 2001) and by extension in GI function. This may explain why lesions of modest size, such as small adenoma, may lead to altered bowel function as an indicator of early colorectal carcinogenesis. Future investigations should address the two-way interaction between EEC and lesion as a route to new chemopreventive and diagnostic targets.

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