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Interactions of Gut Endocrine Cells with Epithelium and Neurons

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Abstract

Even the simplest animals possess sophisticated systems for sensing and securing nutrients. After all, ensuring adequate nutrition is essential for sustaining life. Once multicellular animals grew too large to be nourished by simple diffusion of nutrients from their environment, they required a digestive system for the absorption and digestion of food. The majority of cells in the digestive tract are enterocytes that are designed to absorb nutrients. However, the digestive tracts of animals ranging from worms to humans contain specialized cells that discriminate between nutrients and nondigestible ingestants. These cells “sense” both the environment within the gut lumen and nutrients as they cross the gut epithelium. This dual sensing is then translated into local signals that regulate the gut epithelium or distant signals through hormones or nerves. This review will discuss how sensors of the gut interact with cells of the epithelium and neurons to regulate epithelial integrity and initiate neural transmission from the gut lumen.

Introduction

Sensory cells of the gut possess several unique features that distinguish them from surrounding enterocytes. When gut epithelium was examined by light microscopy using special staining techniques, a small minority of cells were argyrophilic providing the first evidence that they contained chemical messengers (65). Electron microscopy demonstrated secretory vesicles distributed at the base of argyrophilic cells (64). It was not difficult to imagine that these cells secreted their contents from their basal surface into the paracellular space. From this location, chemical messengers could act on nearby cells or be taken up into the bloodstream and affect distant organs. This latter possibility became a reality when (or seemed plausible because) extracts of intestine injected into the blood stream of dogs were found to stimulate pancreatic secretion, thereby establishing the concept of hormones (4).

Since the discovery of the first hormone, secretin, other proteins, peptides, and small bioactive molecules have been found in cells of the gut epithelium and these argyrophilic cells have since become known as gut endocrine or enteroendocrine cells (EECs), a broad concept that includes serotonin-containing cells named enterochromaffin cells.

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Anatomy of EECs

Most gut hormones are released in response to eating food indicating that EECs can “sense” nutrients. Microscopic analysis revealed that most EECs possess microvilli on a narrow apical extension that reaches into the gut lumen. With their basal surface abutting the basal lamina, EECs are in a position to receive signals from the gut lumen and relay those signals through the release of hormones stored along their basal surface. Thus, it has been assumed that EECs, through their microvilli, sense nutrients in the gut lumen. Although this may be true for some nutrients, the regulation of EECs appears to be more complex (see later).

Typically, EECs are elliptical or flask-shaped with a broad base (Fig. 1). Invariably, secretory vesicles are distributed along the basal region. Unlike other endocrine organs in which hormone-containing cells are clustered together, EECs are rarely seen adjacent to one another. Instead, EECs are scattered throughout the mucosa and individual EECs are surrounded by absorptive enterocytes.

All of the aforementioned anatomical features of EECs were recognized using traditional microscopy. With the advent of genetically modified mice, which express green fluorescent proteins under the guidance of specific gastrointestinal hormone gene promoters, it was possible to characterize EECs in thick tissue sections using fluorescence confocal microscopy. Microscopy was no longer limited to analyzing tissues in a single plane and EECs could be reconstructed in three dimensions. A striking feature of both cholecystikinin (CCK)- and peptide YY (PYY)-containing cells was the appearance of extensions from the basal surface (7, 15). Thirty to fifty percent of EECs appeared to possess extensions that were of various shapes and sizes (Fig. 2). In the proximal small intestine, extensions were short, would extend in any direction, and in some cells they were multiple (8). By contrast, in the distal small intestine and colon, EECs possessed only single extensions, that ran along the basal lamina, and were much longer than those in the proximal intestine with some extending up to 70 μm in length (9). What these extensions did was unclear.

In hopes of gaining a better understanding of the nature of the basal extension, a combination of confocal microscopy and three-dimensional electron microscopy was used to characterize the ultrastructure of an EEC (Fig. 3). Several unique features were identified. First, the basal extension was comprised of neurofilaments, the typical structural proteins of neuronal axons. Second, it contained over 70% of the secretory vesicles of the cell. And third, it was intimately connected to glia. Accordingly, based on their many neuron-like features the basal extensions were named neuropods. Since their discovery, it has been noted that neuropods are regulated by neurotrophins, most likely produced by surrounding glia. EECs express the neurotrophin receptors such as TrkA and glial-derived neurotrophic factor 3 (Gfra3) through which nerve growth factor increases neuropod length and artemin stimulates branching or growth of multiple neuropods, respectively (9).

Function of Neuropods

Electron microscopy demonstrated that EECs possess two types of secretory vesicles—large dense vesicles typical of hormone-containing granules, and small clear vesicles resembling

neurotransmitter vesicles. These findings led us to ask if EECs contained synaptic proteins and if so, did they communicate with nerves? With the development of mice expressing green fluorescent protein in EECs, it was possible to purify EECs by fluorescence-activated cell sorting (FACS). Analysis of RNA from purified CCK and PYY cells revealed that EECs expressed both pre- and postsynaptic proteins (10). It seemed plausible that should EECs receive signals from the gut, they could transmit those signals to nerves, thus their expression of presynaptic proteins seemed understandable. However, it was a surprise to discover that EECs also expressed postsynaptic proteins, implying that EECs *receive* signals from the nervous system.

To prove that EECs connect with nerves, neuronal tracing was employed using a modified rabies virus (10). Fluorescence-labeled rabies virus lacking its viral coat, necessary for packaging rabies allowing it to travel trans-synaptically, was introduced into the lumen of the colon of mice expressing the glycoprotein coat exclusively in EECs. Detection of virus in enteric nerves adjacent to EECs indicated that rabies virus (1) infected EECs, (2) was packaged with a glycoprotein coat, and (3) traveled trans-synaptically into a nerve. It was originally thought that transmission of rabies virus from EECs was similar to that in the central nervous system, where virus travels in a retrograde direction, however, this may not hold true in peripheral nerves therefore, these data alone do not tell us whether EECs receive efferent signals from the nervous system. Although expression of pre- and postsynaptic proteins in EECs imply that they both send and receive neural signals, we anxiously await functional studies demonstrating the directional flow of communication between EECs and nerves.

It was recently shown that enterochromaffin cells (ECs), a subtype of EECs that produce and secrete serotonin, express specific chemosensory receptors, and are electrically excitable (5). ECs express presynaptic proteins that are located along their basal surface and appear to modulate serotonin-sensitive primary afferent nerve fibers via synaptic connections (5). Although our work has focused on two types of EECs (CCK and PYY/GLP cells), we believe this model applies to other types of EECs including enterochromaffin cells. A model for the EEC-neural circuit is presented in Figure 4.

Several features suggest that neuropods are involved with hormone release and neurotransmission. In addition to a high density of secretory vesicles, neuropods contain abundant mitochondria that may influence neurotransmitter release (53). Synaptic proteins are also concentrated in neuropods indicating that they are a point of contact with nerves and a site of neurotransmission.

For many years, it was assumed EECs were short-lived like enterocytes, which arise from intestinal stem cells and over the ensuing 3 to 5 days migrate from the crypt to the villus tip where they undergo apoptosis and are sloughed into the intestinal lumen. Such rapid migration would pose a challenge to the formation and maintenance of connections between EECs and nerves. The sustainability of the EEC-neuronal circuit seemed more plausible when it was shown that EECs live longer than previously expected with some EECs living at least 60 days (10). The long-lived EECs appeared to settle in the mid-portion of the crypt-villus architecture. Regional differences in EEC survival have been reported although direct

comparisons have not been rigorously studied. In the proximal small intestine EECs survive up to ten days while EECs in the stomach may live up to four months (48). It is not known if the contact with nerves contributes to the longevity of EECs possibly by providing prosurvival signals or an anchor for maintaining cells in the intestine.

The Dynamic Nature of EEC Connections

A notable feature of the intestine is the migration of cells from the crypt base to the villus over the span of several days. This process is even more remarkable given that some cells live longer than others. When EECs set up residence at the crypt-villus junction, by necessity, other migrating cells move around them on their journey to the villus tip. Staying in constant contact with surrounding cells, EECs and enterocytes must constantly break and reform tight junctions.

A glimpse into the dynamic nature of EECs was gleaned by an *in vitro* study when EECs were placed in culture with sensory nerves (10). As shown in Figure 5, a motile EEC approached a neuron, which in turn sent out a cellular process toward the EEC. The EEC then moved to contact the nerve and then withdrew exuding an axon-like connection between the two cells. The EEC-neuronal connection remained stable for days. In another study, an EEC made a connection with a distant neuron and then traveled along the neuron's axonlike process. Although demonstrating EEC motility *in vivo* is challenging, it is evident that EECs have an inherent ability to travel.

These studies illustrate a dynamic interaction between EECs and neurons and reveal several novel EEC characteristics. First, EECs are mobile. Second, they appear to seek neurons, probably in response to a chemical signal emitted by the nerve. And third, EECs and neurons themselves appear to possess all of the machinery necessary to establish and maintain the EEC-neuronal connection. Moreover, these observations indicate that EECs and neurons communicate even before they establish physical connections. This communication between EECs and neurons together with the previously described observation that glial-derived neurotrophins stimulate neuropod elongation and branching indicate that EECs receive a number of signals from the nervous system that regulate their structure and function.

It seems likely that a connection between an EEC and a neuron is stable. However, drawing from other observations in the nervous system, where synapse formation and pruning are ongoing processes, it is possible that the interactions between EECs and enteric nerves are dynamic and synaptic connections form, disassemble, and reform.

Stimulation of EECs by Nutrients

Most gastrointestinal hormones are released into the blood in response to eating. Passing through the intestine, food comes into contact with cells in the epithelium so it was naturally assumed that food interacting with the apical surface of EECs stimulated their secretion. For some types of food, this may be true. However, for other nutrients it is not as obvious (Fig. 6).

Apical exposure of nutrients leading to gut hormone secretion

Tastants such as sugars, artificial sweeteners, and bitter molecules convey stimuli that are perceived through taste receptors on sensory taste cells (1, 17, 58). Since their discovery, taste receptors for sweet (T1R) and bitter (T2R) molecules have also been identified on EECs (30, 67, 81). Taste receptors couple through G proteins α -gustducin and transducin to activate phospholipase C. Some taste receptors have been identified on the apical surface of enterocytes and EECs that are open to the gut lumen (27,54). Therefore, taste receptors appear to have the ability to sense stimuli within the lumen of the gut. However, the bitter taste receptor T2R38 has also been found on EECs that are closed to the lumen and do not possess an apical surface (45). It is not clear if tastant molecules may also activate receptors on EECs after they are absorbed.

Some nutrients like glucose, amino acids, and small peptides are transported across the apical surface of EECs (66). The Na^+ -coupled glucose transporter, SGLT1, mediates glucose stimulated incretin release, and has been localized to the luminal surface of GIP and GLP-1 cells in the intestine (36). Several pathways have been demonstrated for amino acid-stimulated hormone secretion. Dietary protein is a potent stimulus of several gut hormones including CCK, GLP-1 and GIP and these effects are mediated in part by protein breakdown to di- and tri-peptides which are taken up by EECs via the H^+ -coupled brush border peptide transporter 1 (PepT1) (49,57). In addition, amino acid transporters including Na^+ -dependent transport of L-glutamine cause depolarization of the plasma membrane as amino acids cross the apical surface of EECs and stimulate hormone secretion (74). It appears therefore, that EECs may respond to some small molecule nutrients in the lumen of the gut.

Basolateral stimulation of EECs leading to gut hormone secretion

However, there is accumulating evidence that some nutrients stimulate EECs only after they are absorbed. For example, glucose may be transported across the basolateral surface of EECs by glucose transporter 2 (Glut2) where upon reaching the cell interior it is metabolized by glucokinase leading to K_{ATP} channel closure and membrane depolarization (13). Additionally, aromatic amino acids such as tryptophan and phenylalanine appear to stimulate CCK, ghrelin, GIP, and GLP-1 release through the Gq-coupled calcium-sensing receptor (CaSR) on the basolateral cell surface (50, 55, 77).

Evidence is accumulating that postabsorption stimulation of EECs is a primary mechanism for fat-stimulated hormone secretion. Fatty acids liberated through the breakdown of dietary lipids stimulate gut hormone secretion through several receptor-mediated mechanisms. Free fatty acid receptor 1 (FFAR1, previously known as GPR40) and FFAR4 (previously known as GPR120) respond to medium and long chain fatty acids, couple to Gq and activate phospholipase C and IP_3 to increase intracellular calcium and stimulate hormone secretion (31,39). FFAR1 ligands stimulated GLP-1 secretion when administered intravascularly but not into the gut lumen suggesting that FFAR1 is located on the basolateral surface of L cells (20).

GPR119 is activated by oleoylethanolamide and 2-monoacyl-glycerols derived from triglycerides (38) which activate intracellular cyclic AMP, a well-established mediator of gut

hormone secretion. FFAR2 (GPR43) and FFAR3 (GPR41) are receptors for short chain fatty acids such as acetate, propionate, and butyrate that are produced by bacterial fermentation (59). Each of these fatty acid receptors appears to be expressed on the basolateral surface of EECs.

In addition to single ligand receptors, more complicated fat sensing mechanisms have been identified in EECs that require chylomicron formation or simultaneous stimulation by co-ligands. Inhibition of chylomicron formation with Pluronic-81 reduced release of several gut hormones including CCK, GLP-1, and GIP (52, 68). It has also been observed in mice that enzymes involved in triglyceride synthesis and incorporation into chylomicrons, such as diacylglycerol acyltransferase 1 (DGAT1) and monoacyl-glycerol acyltransferase 2 (MGAT2), are necessary for GIP secretion (60). In addition, in mice, the ability of fatty acids to stimulate CCK release required the simultaneous exposure to high density lipoproteins (HDL) which together with medium and long chain fatty acids activated immunoglobulin-like domain containing receptor 1 (ILDR1) (16). It is important to note that EECs reside in the mucosa as single cells surrounded by enterocytes that synthesize and release HDL from their basal surface. Therefore, it appears that absorbed fatty acids together with locally synthesized HDL stimulate CCK secretion. These findings indicate that absorbed lipids stimulate gut hormone secretion and suggest that many lipid-sensing receptors are located on the basolateral surface of EECs.

Nevertheless, these findings do not exclude the possibility that other lipid sensing receptors could also be expressed on the apical surface of EECs. GPR119, the receptor for oleoylethanolamide, is one notable example since it appears to be coupled to GLP-1 secretion following agonist administration via either an intraluminal or intravascular route (46). This finding was supported by the demonstration that GLP-1 containing cells could be activated by both apical and basolateral application of a GPR119 agonist (63).

Gut Microbiota and EECs

Gut microbiota have profound effects on nutrient sensing, food intake, energy expenditure and metabolism (34, 40, 75). The mechanisms of these actions are only partially understood but may be mediated at least in part by EECs. It appears that microbiota interact with EECs in three principal ways. First, microbiota may directly contact the apical surface of EECs. Second, microbiota may release bacterial factors such as lipopolysaccharide (LPS) that bind to specific receptors on EECs (e.g., toll-like receptors) (6). And third, gut microbiota may ferment food or undigested ingredients to generate chemical messengers such as short chain fatty acids that activate EEC receptors (e.g., FFAR2 and FFAR3) (59, 69, 72).

Both murine and human EECs express toll-like receptors and studies in cell lines demonstrated that activation by LPS or bacterial lipoprotein activated NF- κ B and MAP kinase, stimulated hormone release, and induced secretion of the cytokine tumor necrosis factor (TNF α) (6). Interestingly, in PYY- and GLP-producing cells, TLR activation increased gene expression of *Tlr4* and *Pyy* but not proglucagon (43). These effects were distinct from those of butyrate indicating that microbiota effects were mediated by both TLR and fatty acid sensing pathways. Thus, it appears that EECs may respond to pathogen

associated molecular patterns (PAMPs) and signal through the release of hormones or cytokines to nerves and inflammatory cells (80).

Gut microbiota produce other potentially bioactive substances that could affect EEC function. These products range from short chain fatty acids to secondary bile acids, phenols, indoles, neurotransmitters, and other bioactive lipids that could affect EECs (18). This panoply of actions was recently illustrated by the discovery that commensal bacteria produce GPR119 agonists that regulate GLP-1 secretion and modulate glucose homeostasis (22). These examples illustrate the immense complexity between gut microbiota and EEC function yet raise the possibility that bacteria could be harnessed to regulate gut endocrine signaling.

EECs and their Neighbors

Paracrine signaling was initially demonstrated in the enteroendocrine system with the discovery of dendrite-like extensions on somatostatin cells in the stomach (44). These cellular extensions allowed the release of somatostatin onto nearby cells to control gastrin release or acid secretion from parietal cells. The local release of bioactive transmitters is both precise and economical since hormones are not diluted in the systemic circulation and seems ideally suited for the gastrointestinal tract where EECs are scattered throughout gut mucosa. Although prototypical EECs are oblong or flask-shaped, occasional cell processes have been described in other EECs (37) but outside of the stomach, their function has not been clearly delineated. In the distal intestine, it was thought that cell processes were the result of EECs migrating away from their birthplace in the crypt on their way to the villus tip. However, similar processes were not observed in other migrating cells such as enterocytes and goblet cells, making this explanation unlikely. The discovery that these extensions possessed neuron-like features including secretory vesicles, synaptic proteins, and neurofilaments, introduced other possible functions such as communicating with the nervous system (9). This possibility was confirmed with the demonstration of a synaptic connection between EECs and enteric nerves (9). The cell extensions, now known as neuropods, possess abundant secretory vesicles including both large, dense core granules typical of those that contain hormones and small, clear neurotransmitter type vesicles. These findings suggest neuropods are capable of both releasing hormones and communicating through synapses.

In addition to connecting EECs with nerves, neuropods may also facilitate EEC paracrine communication. Two morphological features lend themselves to this possibility. First, EECs reside as isolated hormonal cells surrounded by enterocytes but in the absence of cellular extensions, they are in contact with only a few cells. Neuropods provide a way for the EEC to reach a larger number of cells in the mucosal lining of the gut. In the proximal small intestine, neuropods are generally short, on the order of 1 to 5 μm , however in the distal intestine, neuropods as long as 70 μm have been identified (7, 15). Such long extensions enable EECs to contact many more cells than otherwise would be possible.

Second, most neuropods run along the basal surface of enterocytes (8). Using genetic tools and radioligand binding, receptors for various hormones have been identified on enterocytes.

For example, Y1 and Y4 receptor subtypes have are expressed in mouse and human colon where antisecretory effects have been observed following systemic administration (Fig. 7). Y1 has been localized to the basal surface of human colonocytes where they would be in contact with PYY cells or their neuropods (23, 24, 56). Therefore, it seems likely that PYY cells regulate colonocyte secretory activity through local release of hormone. A low level of PYY-like activity is responsible for a basal tone of antisecretory activity that is evident when the Y1 receptor is blocked or its gene is ablated (41).

Although pharmacological effects can be demonstrated by systemic administration of glucagon-like peptide-2, it is likely that GLP-2-producing EECs maintain intestinal mucosal integrity through paracrine actions (29). If GLP-2 is eliminated, the gut is more susceptible to injury and augmentation of GLP-2 signaling increases intestinal mucosal growth and enhances nutrient absorption (28).

Paracrine Signaling in Reverse

Transcriptomic profiling of EECs unveiled the surprising discovery that EECs not only express genes for presynaptic proteins but also express genes encoding postsynaptic proteins. A functional connection between EECs and efferent nerves was suggested with the demonstration that a rabies virus construct could pass trans-synaptically from PYY cells to nerves possibly in a retrograde manner (10) (Fig. 8). It is easier to envision EECs transmitting signals from the gut to the nervous system, but it is equally intriguing to imagine that the nervous system sends signals to EECs in the gut mucosa. The functional significance of efferent signaling to EECs is currently unknown but one may speculate that EECs are “finely tuned” to respond to signals as they arrive or to adjust local signals to neighboring cells to regulate such functions as intestinal secretion.

Our traditional view has been that EECs respond to signals from the lumen of the gut. However, as illustrated by stimulation of ILDR1 on CCK cells by the enterocyte products HDL and fatty acids (16), it now appears that EECs also receive signals from enterocytes which may be thought of as a “reverse” type of paracrine signaling. This concept expands the role of EECs as they integrate signals from the lumen of the gut, neighboring cells and the nervous system and transmit signals throughout the body via endocrine, paracrine, and neuronal pathways.

Implications of the EEC-Neural Circuit

Historically, the function of EECs has been determined by actions of the hormones they produce. These actions can be either systemic, by hormones released into the blood, or paracrine, by local effects of these transmitters on adjacent cells. However, by virtue of their neural connections, EECs can transmit signals directly to the nervous system. This neurotransmission may involve small molecule neurotransmitters or peptides and may not necessarily be accompanied by hormonal signaling. Importantly, however, a neural circuit connecting the lumen of the gut to the brain provides a route for luminal contents such as food or microbes to transmit signals to the brain.

It is well known that several gastrointestinal peptides modulate appetite. For example, CCK and PYY are potent satiety agents and can exert their anorectic effects through the systemic circulation or by acting on the vagus nerve in a paracrine fashion (35, 42, 70). The recently identified EEC-neural circuit raises the possibility that these peptides may exert satiety effects through a direct neural connection to regions of the brain involved in regulating food intake (Bohórquez and Liddle unpublished observation).

L cells of the ileum and colon produce PYY as well as glucagon-like peptides (GLP) 1 and 2. When administered systemically, GLP-1 potentiates insulin secretion and along with glucose-dependent insulinotropic peptide (GIP) is one of the two major incretin hormones. However, GLP-1 receptor mediated effects are observed without a significant increase in circulating GLP-1 levels suggesting that GLP-1 may exert its physiological incretin effects through a neural or paracrine mechanism (25). Whether defects in EEC-neural signaling exacerbate glucose tolerance or diabetes mellitus is unknown.

Enteric nerves are not exposed to the intestinal lumen; therefore, they are not directly exposed to luminal contents. However, EECs possess many neuron-like properties and express neurotrophin receptors, which are a target of rabies virus and a mode for rabies infection. The demonstration that rabies can infect EECs and transfer onto enteric nerves raises the possibility that EECs can be a route for disease transmission from the gut to the nervous system and even the brain. It is possible that the EEC-neural circuit could provide a route for transmission of other neurotropic infections from the gut.

Mad cow disease is a neurodegenerative disease that is transmitted by the ingestion of prion-infected meat. However, how prions, the infectious agents, gain access to the nervous system remains uncertain. Although prions can be taken up by M cells and found in Peyer's patches, neuroinvasion is observed even in the absence of follicular dendritic cells, indicating other cells are likely to be involved in the transmission of prions to the vagus nerve or sympathetic nervous system (78). It is possible that prions gain access to the nervous system through EECs, which then provide a direct route to the vagus nerve.

There is epidemiological, experimental, and clinical evidence that Parkinson's disease arises in the gut (11, 76). The pathological hallmark of Parkinson's disease is Lewy bodies, which are found in enteric nerves and brain of infected individuals (2). The major constituent of Lewy bodies is α -synuclein, a neuronal protein involved in neurotransmission. It is believed that Parkinson's disease is initiated by the misfolding of α -synuclein, which leads to protein aggregation and ultimately Lewy body formation (26). Misfolded α -synuclein also has the ability to transfer from nerve to nerve in a prion-like cascade and serves as a template for misfolding of α -synuclein in recipient cells (3). Vagotomy reduces the risk of developing Parkinson's disease raising the possibility that α -synuclein travels from the gut to the brain via the vagus nerve (51, 71). How Parkinson's disease originates in the gut is unknown. However, we have recently demonstrated that EECs express α -synuclein (14). Therefore, should α -synuclein become misfolded in EECs, it is conceivable that by virtue of its prion-like behavior, it could spread into the nervous system including the vagus nerve and ultimately the brain.

Exposure to certain toxins, including pesticides such as rotenone has been associated with Parkinson's disease (73). Moreover, rotenone causes α -synuclein misfolding in neuronal cell lines and feeding rotenone in experimental animals causes α -synuclein aggregation in the vagus nerve and Parkinson's disease like behavioral changes (19, 47, 61). It is conceivable that ingested toxins affect α -synuclein in EECs, which could be the initiating event in Parkinson's disease progression.

Irritable bowel syndrome (IBS) is characterized by abdominal pain and disturbances in bowel motility without a defined anatomical abnormality. It has been proposed that affected individuals have heightened sensitivity to intestinal stimuli raising the possibility that chemosensory cells in the intestine are involved. A number of studies have reported alterations in the density of EECs in patients with IBS (12, 21, 33). Reduction in serotonin cell abundance in the small intestine of patients with IBS (32) together with increased activity of the serotonin transporter SERT would be expected to reduce serotonin signaling (79). However, increased sensitivity to intraluminal pressure correlated with increased EC abundance in the rectum (62) of patients with IBS indicating that serotonin signaling may be important in pain perception in these patients. In contrast, an increase in PYY cells has been reported in patients with constipation and IBS (32). Despite these provocative insights, to date, the relationship between EEC abundance and function and their contribution to disease pathogenesis remain unclear and future studies are needed to address how EECs are regulated in IBS and their role in sensory perception and disease progression.

Conclusion

As sensory cells of the gastrointestinal tract, EECs detect ingested nutrients and microbes within the lumen of the gut and respond by sending signals to the epithelium through paracrine actions, into the blood stream through hormones, and to the nervous system through direct neural connections. Through these actions, EECs coordinate the ingestion, absorption, and digestion of food. In addition, EECs sense danger signals, enhance the protective barrier function of the gastrointestinal epithelium, and integrate sensations from the gut to the brain.

A neural circuit connecting the gut lumen with the nervous system provides a route for nutrients or microbes to communicate with the host in a manner not previously appreciated. Although yet unexplored, this pathway has the potential for microbes and toxins to influence the nervous system.

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Didactic Synopsis**Major teaching points**

- Enteroendocrine cells (EECs) are sensory cells of the gastrointestinal tract.
- EECs synthesize and secrete gut hormones in response to ingested nutrients through nutrient transporters and receptors.
- EECs connect to enteric neurons and sensory afferent nerves and have the tools to send and receive signals with the nervous system.
- EECs act locally in a paracrine manner to influence intestinal secretion and mucosal integrity.

Figure 1.

Anatomy of an EEC. EECs are single cells dispersed among enterocytes and other cells of the intestinal mucosa. The apical surface of most EECs is covered with microvilli and is open to the gut lumen. The basal region contains secretory vesicles and rests on the lamina propria. Secreted hormones are taken up into the blood. Some EECs (not pictured here) like ghrelin cells of the stomach are not exposed to the gut lumen.

Figure 2.

Morphology of EECs throughout the GI tract. Many EECs in the proximal intestine have short and often multiple neuropods while EECs in the ileum and colon characteristically possess single neuropods that extend along the base of adjacent enterocytes. Modified, with permission, from (8).

Figure 3.

3D image of an EEC with a prominent neuropod and other neuron-like features. (A) Serial block face scanning electron microscopy (SBEM) of an image with a tissue volume of 52327 μm³. (B) All nuclei from 145 epithelial cells, including 129 enterocytes, 11 goblet cells, 4 cells of various types, and 1 EEC. (C) The EEC was traced on each slice to reveal its entire ultrastructure. On the left, the cell has a tuft of microvilli exposed to the gut lumen, and on the right, there is a prominent neuropod that extends toward the basal lamina propria. (D) This neuropod is populated by mitochondria, in particular at the tip (blue), secretory vesicles (yellow), and filament-like structures (orange). Top panels show the reconstructions

of the cells, and bottom panels show a representative SBEM image of each feature. Structures of interest in the bottom panel have been pseudocolored to facilitate their visualization. Bars = 1 μ m. From (9) with permission.

Figure 4. EEC-neural connection. Schematic diagram illustrating the recently described connection between EECs and enteric nerves. It is believed that both enteric and vagal nerves innervate EECs.

Figure 5. EEC-neural connection forms *in vitro*. (A) Coculture scheme of an enteroendocrine cell and a primary sensory neuron. EEC, enteroendocrine cell; TG, trigeminal neuron. (B) Time-lapse sequence showing how a single *Cck-GFP* EEC (green) connects to a sensory neuron (DiI-labeled, red) *in vitro*. (C) The EEC–neuron connection is stable at 23 min, 14 s, and cells remained connected for 88 h until the end of the experiment. Time, hours:minutes. Scale bars: 10 μ m. Modified, with permission, from (10).

Figure 6.

Nutrients stimulate EECs through apical and basolateral receptors and transporters. Transporters for glucose (SGLT1), amino acids, and di- and tri-peptides are expressed on the apical surface of EECs. Other transporters (e.g., GLUT2) and receptors for long (FFAR1 and FFAR4) and short (FFAR2 and FFAR3) chain fatty acids, fatty acids and lipoproteins (ILDR1), and amino acids (CaSR) are located on the basolateral surface of EECs and respond to absorbed nutrients.

Figure 7. Paracrine signaling in the intestine. Paracrine regulation of gastrin and gastric acid secretion by somatostatin-producing cells in the stomach unveiled a route by which EECs may regulate neighboring cells. In the intestine, local release of PYY controls enterocyte fluid secretion and growth.

Figure 8.

Fine tuning of EECs. It is likely that EECs respond to signals from neighboring cells such as enterocytes, glia, and efferent nerves. Absorbed fatty acids and locally released chylomicrons and lipoproteins stimulate receptors such as ILDR1 (red). Glia produce neurotrophins, which induce neuropod growth and some EECs, are also innervated by efferent nerves. An example illustrates nerve growth factor binding to the receptor TrkA (gray).