

Signalling in the Gut Endocrine Axis

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Abstract

Enteroendocrine cells are sensory cells located in the intestinal epithelium that produce a variety of hormonal regulators of gastrointestinal physiology, metabolism and food intake. They detect the absorption of a wide range of ingested nutrients via pathways that include transporter-mediated uptake, G-protein coupled receptors and ion channels. This review will describe mechanisms by which the L-cells that produce Glucagon Like Peptide-1 (GLP-1) respond to different components of ingested food, and the importance of these pathways not only for understanding physiology, but also for developing new treatments for type 2 diabetes and obesity.

Introduction

Enteroendocrine cells comprise 0.1 to 1% of the gut epithelium, and produce hormones that orchestrate the body's response to food ingestion. Although originally identified as regulators of gastrointestinal processes such as gall bladder contraction and pancreatic enzyme secretion, gut hormones also play important roles in the control of metabolism, being the first step in a gut-brain-pancreatic axis. As enteroendocrine cells directly face into the intestinal lumen, they are ideally placed to detect the composition of ingested food, the rate of nutrient absorption and the products of intestinal microbial metabolism. This review will discuss mechanisms by which enteroendocrine cells sense the luminal contents, and the importance of gut hormones for metabolic physiology and disease.

Enteroendocrine cells and their metabolic importance

Enteroendocrine cells are located along the length of the intestinal epithelium from the stomach to the rectum. They have classically been divided into sub-populations, each classified according to their production of one or a few distinct hormonal products. K-cells, for example, are predominantly located in the duodenum and produce glucose-dependent insulinotropic polypeptide (GIP), whereas L-cells are located in higher numbers in the distal small intestine and colon and primarily produce glucagon-like peptide-1 (GLP-1) and peptideYY (PYY) [1]. Enteroendocrine cells are formed together with the other epithelial cell types by continuous stem cell division in the crypts, and have a life span of 3-5 days in the small intestine [2]. Recent literature suggests that there is a high degree of transcriptional overlap between different enteroendocrine cell populations [3, 4], and it remains uncertain whether the hormonal signatures of individual cells are determined predominantly by their location along the gastrointestinal axis, or whether they are also significantly influenced by diet and the metabolic state.

The most studied of the metabolically active gut hormones is GLP-1, which was identified alongside GIP as a physiological incretin hormone [5]. The action of incretin hormones underlies 50-70% of insulin secretion after a meal and arises because GLP-1 and GIP are secreted as glucose is absorbed in the gastrointestinal (GI) tract, and amplify glucose-stimulated insulin secretion from pancreatic beta cells [6]. In the early 1990's it was shown that GLP-1 is an effective stimulus of insulin secretion even in patients with type 2 diabetes [7], a finding that led to the development of GLP-1 analogues and inhibitors of GLP-1 degradation (DPP4 inhibitors) for the treatment of 2 diabetes and obesity. As a treatment, GLP-1 analogues have many desirable effects, including stimulation of insulin release, appetite suppression, inhibition of glucagon secretion and slowing of gastric emptying [8]. Some GLP-1 mimetics have also recently been shown to reduce the frequency of cardiovascular events in high risk patients with type 2 diabetes [9].

As well as enhancing insulin secretion, another major role of the gut endocrine axis is to control food intake. Whereas the most proximally located hormone affecting appetite is Ghrelin [10] – an orexigenic hormone released from the stomach epithelium – the majority of gut hormones are anorexigenic. In the proximal gut CCK has profound inhibitory effects on food intake, likely mediated by signalling through the afferent vagus [11]. GLP-1 and PYY are released when nutrients penetrate more distal regions of the small intestine, and further suppress appetite. The exact sites of physiological GLP-1 and PYY detection remain controversial, but receptors for PYY detection have been located in the hypothalamus [12], and GLP-1 detection has been observed at a variety of stages of the afferent vagus/brainstem/hypothalamus circuitry [13, 14].

Methods for studying signalling in EECs with focus on L-cells

Early studies investigating signalling pathways in EECs used the murine cell lines GLUTag and STC-1. GLUTag cells are a colonic tumour cell line producing the hormones GLP-1 and CCK, but not PYY [15, 16]. This pattern doesn't exactly match that of colonic L-cells which produce GLP-1 and PYY but only little CCK [3], but a large body of evidence suggests that they are a good model of mouse L-cells. STC-1 cells were derived from a small intestinal tumour, and produce a number of hormones, including GLP-1, CCK, secretin and GIP [17]. The plurihormonal nature of STC-1 cells originally raised concerns

that this cell line might represent immature and incompletely differentiated EECs. Recent data, however, highlight that native small intestinal EECs are also plurihormonal [3, 4], so STC-1 might, after all, be a valid model of small intestinal EECs.

To circumvent the drawbacks of working with cell lines, we and a number of groups made transgenic reporter mice in which different EEC populations are labelled with fluorescent reporters and sensors, driven by cell-specific hormonal promoters [18, 19]. These mouse lines enable the purification and characterisation by single cell imaging of native enteroendocrine cells, and have led to a much deeper understanding of how EECs respond to different stimuli. Transcriptomic comparisons between cell populations labelled using different hormonal promoters have highlighted the similarities between enteroendocrine cell types that were classically supposed to be distinct, revealing that EEC sub-populations are highly overlapping and potentially exhibit plasticity in their hormonal signatures [3, 4, 20, 21]. CCK, for example, is traditionally viewed as a marker of I-cells, but was also detected at high levels in K-cells and L-cells [18, 19], suggesting that stimuli that increase secretion of GLP-1 and GIP will also enhance CCK release. Despite the overlapping expression profiles of EECs producing different hormones, each hormone exhibits a distinct post-prandial plasma profile that varies according to the meal composition. GLP-1 secretion is often separated into early and late phases, and some evidence supports the idea that the early post-prandial rise in GLP-1 and PYY levels reflects a neural circuit that activates L-cells located in the distal gut [22]. However, it has more recently been established that enough GLP-1-producing cells are located in the proximal human gut to account for the observed early peak of secretion [23], and the temporal changes in the plasma concentration of each gut hormone after a meal are likely dominated by a complex interplay between the location of enteroendocrine cells producing that hormone, the sensory machinery they express, and the sites of nutrient absorption.

Basic properties of Enteroendocrine cells

Enteroendocrine cell lines and primary colonic L-cells are electrically active, firing action potentials carried by voltage gated sodium and calcium channels [24-27]. A number of nutrients, including glucose and amino acids, trigger membrane depolarisation and increased action potential firing [28, 29]. This is associated with the influx of calcium through voltage gated Ca^{2+} currents, elevation of intracellular Ca^{2+} concentrations, and consequent stimulation of vesicle exocytosis [30]. Studies in GLUTag cells suggested that inhibition of voltage gated Na^+ channels using tetrodotoxin blocked action potential firing but did not impair glucose-triggered GLP-1 secretion, suggesting that action potentials might not be critical for hormone release [24]. In primary colonic L-cells, however, TTX both impaired glutamine-triggered GLP-1 release and slowed action potential firing [25]. As action potentials in primary L-cells were not completely abolished by TTX, the remaining electrical activity is likely dependent on voltage gated Ca^{2+} currents. Further experiments including inhibitors of P/Q, L, N and T-type Ca^{2+} channels will be required before conclusions can be drawn regarding the importance of action potentials for enteroendocrine cell secretion.

Enteroendocrine cells also express a variety of G-protein coupled receptors that are important for linking hormone secretion to a variety of nutritional and hormonal stimuli. Receptors that activate $\text{G}_{\alpha\text{q}}$ proteins elevate intracellular Ca^{2+} by IP3-dependent release from intracellular stores [31]. In addition, however, our data suggest that some $\text{G}_{\alpha\text{q}}$ -coupled receptors, such as the calcium sensing receptor (CASR) and free fatty acid receptor-1 (FFAR1), recruit voltage gated Ca^{2+} entry in

enteroendocrine cells through the activation of transient receptor potential (TRP) channels [32, 33]. In the case of FFAR1, Ca^{2+} responses to the ligand GW9508 in L-cells were abolished by the specific TRPC3 channel blocker Pyr3, suggesting that activation of this $G_{\alpha q}$ coupled receptor can result in membrane depolarisation via TRPC3 opening, which in turn leads to voltage gated Ca^{2+} channel entry [33].

Activation of $G_{\alpha s}$ coupled receptors in L-cells can also trigger small Ca^{2+} responses in L-cells. In the GLUTag cell model, cAMP was found to increase the activity of hyperpolarisation activated currents and to close a background potassium conductance, both of which effects resulted in enhanced membrane excitability and increased responsiveness to glucose [34]. The relative importance of these mechanisms in primary enteroendocrine cells remains to be elucidated.

Mechanisms of intestinal chemosensation:

Glucose sensing:

In perfused intestine experiments, lumenally-applied glucose or non-metabolisable glucose analogues were found to trigger GLP-1 secretion as long as the sugars were substrates of the brush border glucose uptake pathway and applied in the presence of Na^+ ions [35, 36]. Both glucose uptake and GLP-1 secretion are blocked by phloridzin, an inhibitor of sodium-coupled glucose transporters, SGLT1, that are responsible for active glucose absorption across the apical epithelial surface. These findings suggest that glucose must be absorbed across the brush border in order to trigger GLP-1 secretion, but do not identify the reason why. Several potential explanations are possible: a) Glucose might be sensed on the basolateral membrane of L-cells, and only therefore be effective after absorption; b) Glucose might be detected by an intracellular sensor and thus require entry across the apical membrane; and c) The process of glucose absorption might itself act as a sensory pathway.

A number of experiments argue against the idea that glucose is sensed on the basolateral membrane of L-cells. In classical assessments of the incretin effect in humans, responses to an oral glucose bolus have been compared with those produced by an intravenous infusion designed to produce a matched plasma glucose profile. In these studies, plasma GLP-1 and GIP concentrations were elevated after oral but not intravenous glucose, suggesting that the enteroendocrine cell glucose sensor is located on the apical rather than the basolateral membrane surface [37]. Glucose-triggered GLP-1 secretion has also been demonstrated in primary intestinal cultures, where glucose had unrestricted access to basolateral as well as apical cell membranes [19]. As in the perfused intestine, GLP-1 secretion from primary cultures was blocked by phloridzin or global SGLT1 knockout [38, 39], even though these interventions would not influence glucose concentrations at the basolateral membrane in cell cultures, again arguing against the concept of a critical basolaterally located receptor. The possibility has been raised that glucose might be detected by L-cell sweet taste receptors, but we have been unable to detect significant expression of components of the sweet taste receptor pathway in L-cells [19], and most physiological studies have found very little response to sweet tastants in humans and animals [40, 41]. Taken together, these findings do not support the existence of an L-cell glucose receptor pathway located basolaterally in the intestinal epithelium.

To investigate whether SGLT1 acts by concentrating glucose inside the L-cell where it might be sensed by an intracellular signalling pathway, we imaged cytoplasmic glucose concentrations using a

FRET-based glucose sensor. In GLUTag and primary L-cells, phloridzin blocked GLP-1 secretion, but did not impair the cytoplasmic glucose signal observed after increasing extracellular glucose concentrations. By contrast, phloretin, a general inhibitor of facilitative glucose transporters (GLUTs), prevented the cytoplasmic glucose rise triggered by extracellular glucose, but did not impair glucose-dependent GLP-1 release [39]. These findings suggest that the L-cell glucose sensor is largely dissociated from glucose concentrations inside the cell, making the concept of a cytoplasmically located glucose sensor unlikely.

The third idea, that the apical SGLT1 transporter is directly linked to GLP-1 secretion, is supported by a large body of evidence, including that SGLT1 knockout and SGLT1 inhibitors block GLP-1 and GIP secretion in both in vitro and in vivo systems [38, 39]. Whilst we cannot be certain exactly how the interaction of glucose with SGLT1 stimulates gut hormone secretion, glucose triggered a small but measurable sodium-dependent inward current and corresponding membrane depolarisation in GLUTag cells, likely due to the coupled uptake of 2Na^+ ions with each glucose molecule [27]. In our working model of the L-cell, we proposed that the depolarising effect of the Na^+ ions that accompany glucose influx are responsible for membrane depolarisation, voltage gated Ca^{2+} entry and triggering of vesicular exocytosis. This idea is supported by mathematical modelling that takes into account the measured magnitudes of SGLT1 and voltage-dependent currents in GLUTag and L-cells [42].

Fat sensing:

Ingested fat is emulsified with bile acids and enzymatically hydrolysed to release free fatty acids and monoacylglycerols. Each of these components - long chain fatty acids, monoacylglycerols and bile acids – activates distinct sensory pathways in enteroendocrine cells. Unlike glucose sensing, the majority of fat sensing likely involves the activation of G-protein coupled receptors (GPCRs).

Long chain fatty acid sensing in L-cells has been most strongly linked to the activation of GPR40 (FFAR1), with a possible contribution from GPR120 (FFAR4) [33]. Both are predominantly G_{α_q} -coupled receptors, although there have been some reports of G_{α_i} pathways being activated downstream of GPR120 [43], and of some GPR40 ligands activating G_{α_s} [44]. GPR40 ligands elevate Ca^{2+} concentrations in I-cells and L-cells [33, 45], but this does not seem entirely dependent on Ca^{2+} release from intracellular stores, as there are many reports of fatty acid triggered Ca^{2+} signals being blocked by inhibitors of voltage gated Ca^{2+} channels [33]. In L-cells we demonstrated the GPR40-dependent activation of TRPC3, as has also been reported in pancreatic beta cells [33, 46]. Interestingly, as described for glucose above, fatty acid absorption across the epithelial layer seems critical for GLP-1 secretion by ingested fat, as small molecule GPR40 ligands were found to be effective when applied via the vasculature but not via the lumen when applied in the perfused intestinal model [47].

Mono-oleoylglycerols are detected by GPR119 – another receptor found highly expressed and enriched in the enteroendocrine cell population [18, 19, 48]. GPR119 is predominantly G_{α_s} coupled, linked to the elevation of cytoplasmic cAMP concentrations in L-cells [49]. GPR119 seems particularly important for GLP-1 release, as L-cell specific Gpr119 knockout in mice almost completely abolished the plasma GLP-1 rise triggered by oral triglycerides [49]. Although agonists of GPR119 elevated GLP-1 levels in mice, they were less effective in humans and the metabolic effects

of activating this receptor were pronounced only in the rodent models [50-52]. The reason for their low effectiveness in humans is unclear, but studies on primary intestinal cultures suggested that even in mice, GPR119 ligands only increased cAMP concentrations in ~50% of L-cells [49]. Whether this percentage is similar or lower in human compared with murine L-cells remains to be established. Studies to examine whether GPR119 is located on the apical or basolateral membrane have concluded that it is accessible from both directions [53, 54].

Bile acids activate an L-cell enriched G-protein coupled receptor known as TGR5, or GPBAR1. GPBAR1 is a $G_{\alpha s}$ coupled receptor linked to robust elevation of cAMP and a strong GLP-1 secretory response in primary L-cells from mouse and human [55]. Like GPR40, ligands of GPBAR1 seem to have to cross the epithelial layer before they are able to trigger GLP-1 secretion. Application of apical bile acids in Ussing chambers, for example, triggered GLP-1 secretion that was prevented when bile acid absorption was inhibited by blockade of the apical sodium coupled bile acid transporter, ASBT, yet ASBT inhibition did not affect bile acid triggered GLP-1 secretion from primary cultures with free access to both membrane compartments. In agreement with this finding, a specific GPBAR1 ligand triggered GLP-1 secretion when applied to the basolateral but not the apical compartment in Ussing chambers [55].

Protein sensing:

Enteroendocrine cell sensing of ingested protein seems to involve a variety of transporters and receptors, and resembles aspects of both glucose and fat sensing. Amino acid uptake across the brush border involves a variety of transporters, many of which take advantage of the inwardly directed Na^+ gradient to drive amino acid absorption against a concentration gradient. As found for SGLT1, we detected small glutamine and asparagine-dependent inward currents in GLUTag cells, that were abolished in the absence of Na^+ ions and likely reflected the activity of a sodium coupled transporter such as ATA2 (Slc38a2) [28]. Another transporter, PEPT1, harnesses the inwardly-directed proton gradient to drive uptake of di and tri-peptides. Mice lacking Pept1 exhibited impaired GLP-1 release in primary cultures [56]. It therefore appears that a number of amino acids and di/tri peptides are likely capable of triggering gut hormone release as a consequence of ion-coupled uptake across the apical membrane, which in turn leads to membrane depolarisation and voltage gated Ca^{2+} entry.

In addition to these depolarising uptake pathways, amino acids and oligopeptides have been found to activate calcium sensitive receptors (CASR) and GPR142 in primary L-cells [56-58]. CASR is a predominantly $G_{\alpha q}$ -coupled receptor that in L-cells has been linked to the detection of phenylalanine, glutamine and peptones [32, 56, 57]. Its activation in primary L-cells triggers a robust Ca^{2+} signal that is dependent on TRP-channel mediated membrane depolarisation [32]. A related receptor, GPRC6A, has been implicated in the detection of ornithine by GLUTag cells, but is only poorly expressed in mouse primary L-cells [59]. GPR142 has been implicated in tryptophan and phenylalanine sensing by enteroendocrine cells [58]. The localisation of these amino acid sensing receptors on enteroendocrine cells remains to be established.

Although Pept1 knockout resulted in minor impairment of secretion in vitro, the effects of CASR on GLP-1 release appear larger and have been observed using a variety of experimental approaches [56, 57]. It is interesting that CASR and GPR142 are more strongly activated by the essential aromatic amino acids and the conditionally essential amino acid glutamine. The utilisation by L-cells of these

receptors for amino acid sensing suggests that the gut endocrine system might be particularly tuned to detecting the ingestion of these essential and conditionally essential amino acids.

L-cell cross talk with the colonic microbiome

Under most circumstances, the digestion and absorption of ingested nutrients is completed within the proximal small intestine, and the colon should not receive much digestible food from the diet. Colonic microbiota will, however, metabolise a variety of substrates, including dietary fibre and bile acids, and even if ingested nutrients are efficiently absorbed higher up the intestinal tract, epithelial cell turnover results in persistent loss of dead cells into the gut lumen, which can act as an additional food source for colonic microbiota. A number of metabolites produced by gut microbiota have been linked to changes in GLP-1 secretion, including short chain fatty acids, indole and secondary bile acids [55, 60, 61].

Short chain fatty acids can influence cellular activity through a variety of signalling pathways, but the receptor most strongly linked to GLP-1 secretion is GPR43 (FFAR2) [60, 62]. This is a $G_{\alpha i/q}$ coupled receptor that is highly expressed in enteroendocrine cells, where its activation results in elevated intracellular Ca^{2+} concentrations and enhanced GLP-1 release [60]. Whilst an acute stimulation of GLP-1 secretion by SCFA is observed in primary colonic cultures, however, the potential importance of GPR43 or GPR41 dependent $G_{\alpha i}$ activation remains uncertain and the effects of locally produced SCFA on enteroendocrine secretion in vivo are unclear. In intestinal organoids, SCFA also led to an increase in L-cell number [63], although the underlying pathway has not been established. Thus, although high fibre diets have been linked to elevated GLP-1 and PYY levels, the precise underlying pathways remain to be established.

Indole is a microbial product of tryptophan metabolism that can rise to low millimolar concentrations in the colonic lumen, and has opposing short and longer term effects on GLP-1 release [61]. Its acute effect is to raise intracellular Ca^{2+} and enhance GLP-1 secretion. This is brought about not via a specific indole receptor, but by the inhibitory action of indole on voltage gated potassium channels. Blocking K^+ channels results in a widening of action potentials and consequent increased voltage gated Ca^{2+} entry. When indole is raised for an hour or more, however, we observed suppression of GLP-1 release, coinciding with impaired mitochondrial ATP generation. As for SCFA, the relative importance of the stimulatory and inhibitory effects of indole in vivo is not yet clear.

Indole and SCFA contribute to a soup of microbially produced enteroendocrine cell modulators found in the colonic lumen. Together with bile acids, nutrients that have escaped absorption, and other products of microbial metabolism, they likely exert a complex mixture of effects on enteroendocrine cells in the local vicinity. The overall importance of these microbial products for gut hormone secretion is uncertain, but it is possible that they contribute to fasting gut hormone concentrations when nutrient stimuli are no longer dominant. There are a number of important questions still to be addressed, including which of the microbially-produced regulators wins out in vivo, whether the balance of activity is changed when the microbiome changes, and how the interplay between microbial metabolites and gut hormone secretion is modified by changes in dietary habits.

Translational aspects – influence of diet, weight loss and surgery

An important unanswered question is whether the responsiveness of the gut endocrine axis is significantly altered by longer term dietary changes. Data from human studies suggest that people with obesity have raised fasting GLP-1 and lower fasting PYY plasma concentrations [64, 65], but whether these changes reflect the influence of different diets or body weight/adiposity is not clear. We evaluated whether L-cell function was altered by high fat diet feeding in mice, and observed higher baseline GLP-1 secretion in primary intestinal cultures that mirrored the increase in plasma GLP-1 concentrations found in obese humans [66]. Additionally we observed a reduction in the expression of enteroendocrine markers in purified L-cells from the high fat fed group, suggesting that western-style diets might impair function of the GLP-1 axis.

Complementing these findings, a recent study of people who had lost weight and then maintained a lower body weight for a year, interestingly found that post-prandial GLP-1 concentrations were elevated after maintained weight loss, suggesting that enteroendocrine cell function is restored by prolonged exposure to a healthy diet or healthy weight [67]. The idea that L-cell function can be impaired and repaired is supported by the rapid turnover of enteroendocrine cells, because any L-cells damaged by diet or obesity will be fully replaced after a few days by a new population generated from the crypt stem cells. Unless the stem cell population has itself suffered any epigenetic or other modification, newly generated enteroendocrine cells should not carry any memory of historic dietary exposure.

Activity of the gut endocrine axis is dramatically altered by bariatric surgery, and likely contributes to the beneficial effects of these procedures on appetite and type 2 diabetes [68]. Post prandial secretion of hormones that predominate in the distal gut, particularly GLP-1 and PYY, are elevated approximately 10-fold after surgery, whereas hormones from the proximal gut are less affected [69]. Evidence suggests that, at least in humans, the elevated GLP-1 concentrations enhance pancreatic insulin release, and that both GLP-1 and PYY suppress appetite [70, 71]. Findings from mouse models, however, suggest that additional factors, such as bile acids, contribute to the body weight reduction observed after bariatric surgery [72].

GLP-1 based medications are already used clinically for the treatment of type 2 diabetes and obesity, and there are a number of ongoing initiatives to try to improve on the effectiveness of GLP-1 receptor agonism by the co-activation of other hormone receptors, such as those for GIP, glucagon and PYY. Results from a number of preclinical trials suggest that co-targeting more than one gut hormone receptor can lead to enhanced metabolic and body weight responses.

Conclusions

The gut endocrine axis provides an interface with the outside world, enabling the body to develop coordinated metabolic responses to nutrient intake. Enteroendocrine cells have developed a variety of sensory machineries capable of detecting multiple different components of ingested food. Interestingly, however, enteroendocrine cells seem particularly tuned to respond to nutrient absorption, rather than the mere presence of nutrients in the gut lumen. This solution enables the body to mount responses to nutrients at the time when they arrive in the bloodstream.

There is widespread hope that an increased understanding of the sensory mechanisms in gut endocrine cells will lead to new dietary or medicinal strategies to harness the body's endogenous reserve of gut hormones, and lead to new strategies to mimic bypass surgery. Therapies that harness

the enteroendocrine axis for the treatment of obesity and type 2 diabetes are already in widespread use, and the success of bariatric surgery has spurred the ongoing development of many more.

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