Keywords
Chemosensing, diabetes, enteroendocrine, GLP-1, GPCR, intestine

Abstract
Gastrointestinal (GI) polypeptides are secreted from enteroendocrine cells (EECs). Recent technical advances and the identification of endogenous and synthetic ligands have enabled exploration of the pharmacology and physiology of EECs. Enteroendocrine signaling pathways stimulating hormone secretion involve multiple nutrient transporters and G protein-coupled receptors (GPCRs), which are activated simultaneously under prevailing nutrient conditions in the intestine following a meal. The majority of studies investigate hormone secretion from EECs in response to single ligands and although the mechanisms behind how individual signaling pathways generate a hormonal output have been well characterized, our understanding of how these signaling pathways converge to generate a single hormone secretory response is still in its infancy. However, a picture is beginning to emerge of how nutrients and full, partial, or allosteric GPCR ligands differentially regulate the enteroendocrine system and its interaction with the enteric and central nervous system. So far, activation of multiple pathways underlies drug discovery efforts to harness the therapeutic potential of the enteroendocrine system to mimic the phenotypic changes observed in patients who have undergone Roux-en-Y gastric surgery. Typically obese patients exhibit ~30% weight loss and greater than 80% of obese diabetics show remission of diabetes. Targeting combinations of enteroendocrine signaling pathways that work synergistically may manifest with significant, differentiated EEC secretory efficacy. Furthermore, allosteric modulators with their increased selectivity, self-limiting activity, and structural novelty may translate into more promising enteroendocrine drugs. Together with the potential to bias enteroendocrine GPCR signaling and/or to activate multiple divergent signaling pathways highlights the considerable range of therapeutic possibilities available. Here, we review the pharmacology and physiology of the EEC system.

Abbreviations
[Ca^{2+}]_{i}, intracellular Ca^{2+}; 5-HEPE, 5-hydroxy-eicosapentaenoic acid; BBR, bombesin receptors; cAMP, cyclic adenylyl monophosphate; CaSR, calcium sensing receptor; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; CNS, central nervous system; DAG, diacylglycerol; EECs, enteroendocrine cells; ENS, enteric nervous system; ERK, extracellular signal regulated kinase; GALT, galanin receptor; GI, gastrointestinal; GIP, glucoinsulinotropic peptide; GLP-1, glucagon-like peptide 1; GPCR, G protein-coupled receptor; GRP, gastrin-releasing peptide; IMP, inosine-5-monophosphate; IP_{3}, inositol triphosphate; NAM, negative allosteric modulator; NMB, neuromedin B; OEA, oleoylthanolamide; OLDA, N-oleoyldopamine; PACAP, pituitary adenylate cyclase-activating protein; PAM, positive allosteric modulator; PepT1, peptide transporter; PI3K, phosphoinositil 3-kinase; PKA, protein kinase A.
The Enteroendocrine System

The traditional role of the intestine as a semipermeable membrane, containing transporters for nutrient uptake has largely been superseded. In addition to performing nutrient uptake vital for life, it also comprises the largest endocrine system in the body. The role of the enteroendocrine system is to detect the components of the intestinal lumen, monitor the prevailing energy status of the body, and elicit appropriate physiological responses to hormones arriving in the intestine, hormones circulating in the blood supply, and the metabolic status of the organism. Nutrients are detected by a large number of proteins including nutrient transporters and G protein-coupled receptor (GPCRs, Reimann et al. 2008; Mace and Marshall 2013). Other components of the intestinal chyme including inflammatory cytokines (Franckhauser et al. 2008; Holmes et al. 2008), progesterone (Flock et al. 2013), bile acids, gut hormones, and neurotransmitters are also detected by EECs. For example, circulating levels of the inflammatory cytokine interleukin-6 (IL-6) are increased during exercise and associated with increased GLP-1 levels (Holmes et al. 2008). The receptor for IL-6 is expressed in the enteroendocrine GLUTag cell line and exposure to IL-6 stimulates GLP-1 generation and secretion (Ellingsgaard et al. 2011). Bile acids that facilitate the emulsification and digestion of lipids also stimulate GLP-1 secretion in humans through activation of the bile acid receptor, GPBAR5 (TGR5) (Adrian et al. 2012; Wu et al. 2013). Hormone output from the EEC is also regulated by other EEC subtypes including somatostatin released from D cells which inhibits GLP-1 secretion from L-cells in the intestine presumably through SSTR5 (Moss et al. 2012).

Enteroendocrine cells (EEC) are morphologically and biochemically similar to taste cells of the lingual epithelia, expressing a similar array of nutrient sensing proteins and are deliberately distributed solitarily along the mucosa of the GI tract. In terms of physiology, open EECs such as intestinal GIP-producing K cells or GLP-1-secreting L-cells, stretch from the lumen of the intestine, where they possess extended microvilli ideally positioned to contact apical stimuli and span the width of the mucosa to reach the serosal blood supply (Shakhlamov and Makar’ 1985). These cells “sense” the contents of the intestinal lumen. More generally, GIP-secreting EECs are located in the proximal duodenal region, CCK-secreting EECs in the duodenal and jejunal regions, GLP-1-secreting EECs in the jejunal, ileal, and colonic regions, and PYY-secreting EECs appear more restricted to the ileal and colonic regions (Sjolund et al. 1983). Enterochromaffin cells (ECs) are a type of EEC that resides in the epithelia of the GI tract that secretes serotonin and regulates secretory and peristaltic reflexes, and activates vagal afferents through 5-HT3 receptors to signal to the CNS.

In contrast, closed EECs such as the appetite-stimulatory ghrelin-secreting A cells in the stomach appear buried within the epithelial mucosa and make contact with the serosal blood supply only (Shakhlamov and Makar’ 1985). These cells are presumed to detect mechanical, neuronal, and paracrine stimuli since they do not directly contact the luminal cavity. In addition to EECs-secreting ghrelin that reside in the stomach, D cells that secrete somatostatin, G cells that secrete the acid-releasing hormone gastrin, and P cells that secrete the satiety hormone, leptin, also reside in the stomach. A second population of chromaffin cells also reside in the gastric mucosa, and appear like ECs but do not contain 5-HT. These cells respond to gastrin secreted from G-cells to release histamine that stimulates the secretion of gastric acid from parietal cells.

Enteroendocrine cells integrate signals from digestion products arriving in the intestine, hormones circulating in the blood supply, and the metabolic status of the organism. Nutrients are detected by a large number of proteins including nutrient transporters and G protein-coupled receptor (GPCRs, Reimann et al. 2008; Mace and Marshall 2013). Other components of the intestinal chyme including inflammatory cytokines (Franckhauser et al. 2008; Holmes et al. 2008), progesterone (Flock et al. 2013), bile acids, gut hormones, and neurotransmitters are also detected by EECs. For example, circulating levels of the inflammatory cytokine interleukin-6 (IL-6) are increased during exercise and associated with increased GLP-1 levels (Holmes et al. 2008). The receptor for IL-6 is expressed in the enteroendocrine GLUTag cell line and exposure to IL-6 stimulates GLP-1 generation and secretion (Ellingsgaard et al. 2011). Bile acids that facilitate the emulsification and digestion of lipids also stimulate GLP-1 secretion in humans through activation of the bile acid receptor, GPBAR5 (TGR5) (Adrian et al. 2012; Wu et al. 2013). Hormone output from the EEC is also regulated by other EEC subtypes including somatostatin released from D cells which inhibits GLP-1 secretion from L-cells in the intestine presumably through SSTR5 (Moss et al. 2012).
Activation of the Enteroendocrine System by Nutrient Transporters

Physiologically, the delivery of dietary nutrients including carbohydrates, proteins, and fat activates EEC hormone secretion. Activation of the enteroendocrine system by nutrient triggers physiological processes to assimilate nutrient uptake, distribution, and disposal to maintain whole body metabolic homeostasis. The delivery of glucose into the small intestine triggers GIP and GLP-1 release, and the release of CCK to a lesser extent (Hasegawa et al. 1996; Chaikomin et al. 2008; Kuo et al. 2008). In humans, GLP-1 secretory responses have also been observed in response to fructose, albeit smaller in magnitude than that seen with glucose (Kuhre et al. 2014). Protein ingestion potently stimulates CCK secretion, as well as GIP, GLP-1, and PYY. The latter hormones associated with the increased feeling of fullness and satiety following ingestion of high protein meals (Batterham et al. 2006). Dietary lipids also potently stimulate hormone secretion from EECs (Liddle et al. 1985; Spiller et al. 1988; Elliott et al. 1993).

Nutrient uptake across the apical brush border membrane elicits membrane depolarization (Fig. 1A). Depolarization of the plasma membrane (PM) regulates the opening of voltage-gated Ca\(^{2+}\) channels (VGCCs), including \(\mathrm{L}\)-type Ca\(^{2+}\) channels, which control EEC secretory activity (Fig. 1). The traditional dogma has been that functional \(\mathrm{L}\)-type Ca\(^{2+}\) channels are not expressed in intestine, however, this has been challenged in more recent years (Morgan et al. 2003, 2007; Mace et al. 2007; Kellett et al. 2008; Kellett 2011). During the prandial period, the PM of the intestine is hyperpolarized; VGCCs are closed, ATP-sensitive K\(^+\) channels are open, and EECs are silent. The PM becomes depolarized as a result of electrogenic or facilitative nutrient transport following nutrient ingestion. Glucose transport via the sodium-coupled glucose transporter (SGLT1), peptide transport via the proton-coupled peptide transporter (PepT1), or amino acid transport via their electrogenic transporters (e.g., glutamine or asparagine) depolarize the PM. Numerous studies have shown through both pharmacological and genetic methods that SGLT1 transport plays a vital role in GIP, GLP-1, and PYY secretion (Sykes et al. 1980; Table 1.

| EEC Hormone | GPCR(s) expressed | Location(s) | Target | Physiological function(s) |
|-------------|-------------------|-------------|--------|--------------------------|
| Ghrelin     | T1R1 + T1R3, T2Rs | Stomach     | Appetite control, food intake, growth hormone release |
| Somatostatin| GPBAR1, GPRC6A   | Stomach, small intestine | Neuroendocrine cells of the gastric gland (enterochromaffin-like cells, parietal cells) |
| Gastrin     | LPAR5, GPRC6A    | Stomach (pyloric antral) | Gastrin release (stomach) |
| CCK         | T2Rs, FFA1, GPR120, GPBAR-1, CaSR | Proximal small intestine | Gall bladder, pancreas, gastric smooth muscle |
| GIP         | GPR119, GPR120, GPR40 | Proximal small intestine | Pancreatic \(\beta\)-cells |
| GLP-1, GLP-2, PYY, oxyntomodulin | T2Rs, T1R2 + T1R3, GPR40, GPR119, GPBAR-1, GPR120, CaSR, GPRC6A, SSTR5 | Distal small intestine, colon | Endocrine pancreas, gallbladder contraction, inhibits stomach emptying, pancreatic enzyme secretion and food intake, stimulates pancreatic enzyme and \(\mathrm{HCO}_3^-\) secretion |
| Motilin     | GPBAR-1          | Small intestine | Smooth muscle of stomach and duodenum |
| Neuropeptide | GPR40, GPR41, GPR43, GPR120 | Small (distal) and large intestine | Gastric acid secretion, biliary secretion, intestinal mucosal growth, intestinal peristalsis |
| Leptin      | Nutrient receptors | Stomach | Appetite regulation; food intake |
| Secretin    | Potential acid receptor | Proximal small intestine | Pancreas, stomach |

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There is no doubt that the stimulation of GIP and GLP-1 by luminal glucose is diminished by pharmacological inhibitors of electrogenic glucose uptake (Sykes et al. 1980; Ritzel et al. 1997; Mace et al. 2012). Facilitative transport can also depolarize the PM by virtue of intracellular sugar metabolism, altering the ADP:ATP ratio and closure of ATP-sensitive K+ channels. The protein components required for the stimulation of hormone secretion from EECs by intracellular metabolism, including glucokinase and ATP-sensitive K+ channels, are expressed by L and K cells, and studies using GLUTag cells indicate that intracellular sugar metabolism may stimulate secretory activity (Parker et al. 2012a); the facilitative glucose (GLUT2) and fructose (GLUT5) transporters are also expressed. Additional physiological evidence, in addition to that showing oral fructose is able to stimulate GLP-1 secretion in mice, rats, and humans (Kong...
et al. 1999; Kuhre et al. 2014), for a role of facilitative transport in hormone secretion derives from genetic studies in which the GLP-1 secretory response to oral glucose in GLUT2<sup>−/−</sup> mice was diminished (Cani et al. 2007) and from isolated perfused rat intestine preparations where fructose stimulated GLP-1 secretion (Ritzel et al. 1997) and GIP, GLP-1, and PYY secretion could be blocked using pharmacological inhibitors (Mace et al. 2012). More recently, apical glucose transport has also been demonstrated to control the secretion of the neurohormone, neurotensin, from enteroendocrine N-cells (Table 1) using preparations of isolated rat small intestine (Kuhre et al. 2015). Pharmacological inhibition of SGLT1 or GLUT2 blocked neurotensin release in response to luminal glucose; the facilitative glucose transporter involved a molecular pathway causing closure of ATP-sensitive K<sup>+</sup> channels (Kuhre et al. 2015).

Enteroendocrine cells also sense systemic glucose levels affording them the capability to monitor energy status and modulate hormone secretion appropriately. For example, the activity of the lipid amide GPCR GPR119 and the melanocortin GPCR MC4R that stimulate hormone secretion from EECs have been shown to be sensitive to systemic glucose levels (Panaro et al. 2014). In Ussing chamber preparations of mouse colonic mucosa, removal of glucose from the basolateral side inhibited the ability of GPR119 and MC4 agonists to stimulate PYY secretion and mediate antisecretory effects (Cox et al. 2010; Panaro et al. 2014; Patel et al. 2014) (Fig. 1). Mice lacking GLUT2 exhibit lower GLP-1 content and reduced GLP-1 secretion following an oral glucose tolerance test (Cani et al. 2007), and the localization of GLUT2 on the basolateral surface may afford it with a plasma glucose-sensing capacity. Although not released by intravenous glucose administration in fasting humans, GLP-1 secretion was altered by plasma glucose concentration in preparations of porcine ileum (Hansen et al. 2013)).

**Activation of the Enteroendocrine System by G Protein-Coupled Receptors**

G protein-coupled receptors play a key role in the regulation of EEC secretory output (Fig. 1B). Physiologically, the products of digestion (including glucose and fructose, amino acids and oligopeptides, and medium- and long-chain fatty acids) (Tolhurst et al. 2009), microbial fermentation products and metabolites (including short chain fatty acids and indoles) (Tolhurst et al. 2012), triglycerides derivatives (including oleoylthanolamide and 2-monoacylglycerols) (Patel et al. 2014), inflammatory cytokines (including IL-6) (Ellingsgaard et al. 2011), as well as toxins (including bitter tasting compounds (Wu et al. 2002) and bacterial toxins (Bogunovic et al. 2007)), systemic hormones (including progesterone (Flock et al. 2013)) and neurotransmitters (Plaisancie et al. 1994) are detected by GPCRs expressed by EECs to regulate hormone secretion. Activation of the enteroendocrine system by GPCRs triggers physiological processes to assimilate or expel ingested substances, regulate gut secretions, control gastric emptying and GI motility, and influence food intake and appetite to maintain whole body metabolic homeostasis.

Those GPCRs expressed exclusively in EECs have not been easily detected because of their sparse distribution. Enteroendocrine cells comprise 1% of the total intestinal epithelial cell population and expression of GPCRs restricted to EECs appear very low in mRNA expression analyses using homogenates of intestinal mucosal tissue. Recent advances enriching EECs from heterogeneous intestinal epithelial cell populations, in combination with immunohisto- and cytological methods have revealed multiple GPCRs whose expression is restricted to EECs that respond to luminal or basolateral stimuli.

GPCRs consist of seven transmembrane (TM) spanning domains (Fig. 2), and transduce ligand binding events into intracellular signals. Of those GPCRs expressed in EECs, the majority belong to class A including those that detect short chain fatty acids (SCFAs). Enteroendocrine cells also express members of the secretin-like class B GPCRs and include those which respond to hormones such as GIP and GLP-1. Finally, class C GPCRs expressed in EECs are distinguished by a large extracellular venus flytrap (VFT) ligand-binding domain and includes GPCRs which detect nutrients including taste receptors (T1R), the calcium sensing receptor (CaSR) and GPRC6A. Class C GPCRs are notable in that they function as constitutive receptor dimers or higher order oligomers (Venkatakrishnan et al. 2013). The crystal structures of members of Class A, B, and C have recently been solved allowing a deeper understanding of the GPCR movements that elicit signaling events. Figure 2 shows three generic representations depicting proposed structures of GPCRs from Class A, B, and C. The N-terminal domains probably do not sit directly above the 7TM region, and are also likely to interact with the phospholipid bilayer of the PM.

GPCRs undergo conformational changes upon ligand binding. This movement alters the interaction with membrane bound heterotrimeric guanine nucleotide-binding G proteins. GPCRs can adopt multiple conformational states, and can couple to more than one type of G protein. The ligand chemotype (Hudson et al. 2014b), engagement of the orthosteric or allosteric binding site (Edfalk et al. 2008; Luo et al. 2012), oligomerization (Ferre et al. 2014), and the composition of the lipid bilayer (Oates et al. 2012) also
determine which downstream signaling pathway(s) are deployed. Multiple G protein subunits control the intracellular signaling pathways employed (Fig. 1). Ga₃ couples to adenylate cyclase (AC), and generates cyclic adenosine monophosphate (cAMP) production, while Gaᵢ inhibits AC to diminish cAMP levels. Ga₉ generates diacylglycerol (DAG) and inositol triphosphate (IP₃) via activation of phosphoinositol 3-kinase (PI3K) to mobilize protein kinase C (PKC) and raise intracellular Ca²⁺ levels ([Ca²⁺]ᵢ). Ga₁₂/₁₃ activates the small G protein, Rho. The βγ subunits also regulate intracellular signaling involving AC, phospholipase C (PLC), PI3K and G protein-regulated inwardly rectifying K⁺ channels. Gβγ subunits are also capable of modulating other receptors.

Adding to the complexity of GPCR signaling, GPCRs also signal independently from G proteins. For example, coupling to β-arrestin has been shown to mediate an extensive range of downstream signaling from GPCRs (Shenoy and Lefkowitz 2011). G protein-coupled kinases and interactions with scaffolding proteins are also involved in GPCR

Figure 2. Representative structures of G protein-coupled receptors from Class A, B, and C. As there are no actual structures that exist with the N-terminal domain and 7 TM domain connected together, the ribbon diagrams show the size of the N-terminal domain relative to the 7 TM region. The 7 TM of the Class A example is Orexin. Generally, amine receptor ligands of Class A bind between the TM domains of the receptor while peptide and glycoprotein hormone receptors of Class A bind between the N-terminal domain, extracellular loops, and upper part of the TM domain. The N-terminal domain of the Class B example is from the GLP-1 receptor and the 7 TM section from the glucagon receptor. The peptide ligands of Class B generally bind to the extracellular region and reach into the lower part of the TM domain. The N-terminal domain of the Class C example is from mGluR1 and the 7 TM section from mGluR5. For clarity, the Cystein-rich region of Class C is missing from between the N-terminal and 7 TM domain. Class C GPCRs exhibit a large amino-terminal domain, which binds orthosteric agonists, while allosteric modulators generally bind to the 7 TM domain.

Figure 3. The pharmacology of known G protein-coupled receptors that regulate gut hormone secretion from EECs. The in vitro properties (pEC₅₀) of some ligands from Class A (3a) B (3b) and C (3c) are shown from functional (readouts being cAMP, CRE, IP₃, Ca²⁺, GLP-1 secretion) cell-based assays (cells lines used include HEK, GLUTag, NCI-H716, STC-1) and the effects of these on gut hormone secretion in ex vivo intestinal tissue, in vivo rodent models or in humans are summarized.
signaling and promote receptor internalization which may maintain or terminate signaling depending on the receptor.

**Class A**

Ligands that activate Class A GPCRs include cations, bile acids, fatty acids, peptones, and bitter ligands; a number of these stimulate the activation of EECs. Briefly, a disulphide bridge between the E2 loop and the upper part of the third transmembrane spanning domain (TM3), and palmitoylated Cysteine residues in the C-terminus are typical for most of Class A (Fig. 2). In addition, there are several highly conserved residues and motifs, including the DRY motif in TM3 which contributes to the so called “ionic lock” stabilising the inactive state of the receptor. The small amine receptor ligands bind primarily within the 7TM domains of the receptor. In contrast, the peptide and glycoprotein hormone receptors bind primarily to extracellular domains including the N-terminus, extracellular loops, and upper part of the TM domain with only part of the ligand accessing the 7TM domain bundle. For a review of GPCR crystal structures, the reader is referred to (Shonberg et al. 2014).

**Peptone sensing by LPAR5 (GPR92/93)**

In addition to PepT1 that has been shown to stimulate hormone secretion from EECs in response to di- and tripeptides, another receptor has also been reported to regulate hormone EEC hormone secretion in response to protein. The recently deorphaned LPAR5 (also called GPR92/93) responds to the bioactive phospholipid, lyso-phosphatidic acid (Kotarsky et al. 2006). Activation of LPAR5 by LPA stimulates phosphoinositide hydrolysis and cAMP production. Although its expression is not enriched in primary I cells (Liou et al. 2011b) or L cells (Diakogiannaki et al. 2013), it is expressed in EECs. Protein hydrolysate was able to trigger LPAR5-dependent CCK release from the enteroendocrine STC-1 cell line (Choi et al. 2007b). Furthermore, the secretion of CCK from STC-1 cells was pertussis toxin sensitive, indicative of G\(_{q}\)- and G\(_{i}\)-coupled pathways (Choi et al. 2007b). Protein hydrolysate also stimulates the expression and transcription of CCK (Cordier-Bussat et al. 1997; Liddle 1997; Nemoz-Gaillard et al. 1998). Choi et al. (2007a,b) demonstrated that protein hydrolysates consisting of a mixture of proteolytic degradation products activated LPAR5 and stimulated [Ca\(^{2+}\)]\(_{i}\) signaling with an EC\(_{50}\) of 10.6 mg/mL using hBRIE 380i cells, that do not express PepT1. In contrast to the Ca\(^{2+}\) response to protein hydrolysate by PepT1 mediated by extracellular Ca\(^{2+}\) influx through VGCCs, the Ca\(^{2+}\) signal from LPAR5 activation was intracellular-store derived, blocked by thapsigargin and not blocked by nifedipine (Choi et al. 2007a). In combination, LPA and protein hydrolysate operate...
### Class C

| TIR2+TIR3 | GPRC6A |
|-----------|--------|
| ![Molecule 1](image1) | ![Molecule 2](image2) |
| Sucralose | Saccharin | Acesulfame-K | Glycyrrhizin |
| *in vitro*, stimulates cAMP and Ca\(^{2+}\) and GLP-1 secretion in HuTu-80, STC-1 and HCl-H716 cells | *in vitro*, stimulates cAMP and Ca\(^{2+}\) and GLP-1 secretion in HuTu-80 cells | *in vitro*, stimulates cAMP and GLP-1 secretion in HuTu-80 cells | *in vitro*, stimulates intracellular Ca\(^{2+}\) and GLP-1 secretion from GLUTag cells |
| ex vivo stimulates GIP, GLP-1 and PYY secretion in preparations of rat intestine |
| *in vivo*, reported to stimulate total GLP-1 secretion in mouse |
| Jang et al., 2007; Mace et al., 2012 | Ohtsu et al., 2014 | Ohtsu et al., 2014 | Oya et al., 2013 |

| CasR | TIR1+TIR3 |
|------|----------|
| ![Molecule 3](image3) | ![Molecule 4](image4) |
| Phe, Gin, Arg, Asn, and Trp | Peptides |
| Peptides | NPS-R568 |
| *in vivo*, stimulates CCK secretion from isolated mouse CCK cells | *in vivo*, stimulates CCK secretion from STC-1 and intracellular Ca\(^{2+}\) and GLP-1 secretion in primary cultures of mouse intestinal epithelial cells |
| *in vivo*, stimulates CCK secretion from STC-1 cells |
| *in vivo*, stimulates CCK secretion from STC-1 cells |

**Figure 3.** continued
synergistically; activation of LPAR5 by both elicited a Ca^{2+} response that was more than additive for the two agents alone suggesting that LPA and protein hydrolysates act upon LPAR5 at different sites. Physiologically, the synergy of LPA and protein hydrolysate on LPAR5 activation suggests that the sensitivity of LPAR5 to endogenous agonist stimulation is likely to be modulated by the prevailing nutrient constituents of the luminal chyme.

### Short-chain free fatty acid sensing by FFAR2 and FFAR3

Several GPCRs are involved in sensing lipids in the intestine. Physiologically, lipid arrival follows the hydrolyzation of dietary fat and its emulsification with bile acids in the duodenum. Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, accumulate in the lower intestine as a result of the microbial fermentation of nondigestible carbohydrates. The enteroendocrine system is also adjusted for the microbial population of the large intestine, that plays a central role in whole body metabolic status (Backhed et al. 2004; Ley et al. 2006; Turnbaugh et al. 2006; Samuel et al. 2008; Cani et al. 2009; Ridaura et al. 2013). Microbial metabolites accumulate to detectable levels in the large intestine including the SCFAs that activate FFAR2 (GPR43) and FFAR3 (GPR42) (Brown et al. 2003; Le et al. 2003; Karaki et al. 2006, 2008; Tazoe et al. 2009). Other metabolites including a metabolite-derived indole has also shown to modulate GLP-1 secretion (Chimerel et al. 2014).

In cultures of primary intestinal epithelial cells, SCFAs increase [Ca^{2+}]I levels (Tolhurst et al. 2012). FFAR2 and FFAR3 are expressed in rodent and human colonic epithelia (Karaki et al. 2006, 2008; Tazoe et al. 2009) and are sensitive to SCFAs (Brown et al. 2003; Le et al. 2003). FFAR2 and FFAR3 are expressed in L-cells of the colon and are activated by bacterial fermentation products, as well as SCFAs, which stimulate GLP-1 secretion (Nohr et al. 2013).

Further evidence for the involvement of FFAR2 in GI hormone secretion was derived from knockout experiments, where silencing FFAR2 diminished SCFA-mediated GLP-1 release. While FFAR2 can couple to both G_{s} and G_{i}, FFAR3 is predominantly coupled to the G_{i}-signaling pathway (Brown et al. 2003; Le et al. 2003). Genetic FFAR3^{−/−} mice show diminished plasma GLP-1 (Samuel et al. 2008; Tolhurst et al. 2012; Psichas et al. 2015). Transcripts for both receptors are enriched in intestinal I (Sykaras et al. 2012) and gastric A cells (Engelstoft et al. 2013). Interestingly, while activation of FFAR2 in L-cells appears to increase GLP-1 secretion, FFAR2 agonism appears to inhibit ghrelin secretion (Engelstoft et al. 2013) possibly reflecting differential levels of G_{i} protein expression in gastric A cells compared with intestinal L-cells.

A series of SCFAs showed varying degrees of selectivity for FFAR2 over FFAR3 (Schmidt et al. 2011). To date, two chemical series have been described as FFAR2 antagonists (Hudson et al. 2013; Pizzonero et al. 2014). Positive allosteric modulators also exist for FFAR2, including AMG-7703, which requires extracellular loop 2 of hFFAR2 for transduction of cooperative signaling between orthosteric and allosteric binding sites (Smith et al. 2011). There are also a series of SCFAs that show varying degrees of selectivity for FFAR3 over FFAR2 and these have recently been reported as allosteric modulators, both positive and negative, of FFAR3 (Hudson et al. 2014a). For a review of fatty acid receptor agonists for the treatment of type 2 diabetes, the reader is referred to (Watterson et al. 2014).

### Medium- and long-chain fatty acid sensing by FFAR1 and FFAR4

FFAR1 (GPR40) and FFAR4 (GPR120) detect medium and long-chain fatty acids (LCFAs), and both couple predominantly through G_{s} (Hirasawa et al. 2005; Hara et al. 2011). For a review of GPCRs sensitive to LCFAs, the reader is referred to (Holliday et al. 2011; Milligan et al. 2014). Their expression is enriched in I- L- and K-cells (Reimann et al. 2008; Parker et al. 2009; Liou et al. 2011a). It is also difficult to distinguish between FFAR1 and FFAR4 in EECs, given their coexpression and similar ligand pharmacology. They have been shown to be functional in both in vitro cultures of mouse and human colonic epithelium and in physiological in vivo studies (Hirasawa et al. 2005; Habib et al. 2013). Rodent data from FFAR1^{−/−} mice show diminished GIP and GLP-1 secretion (Edfalk et al. 2008), and reduced LCFA-mediated CCK secretion (Liou et al. 2011a). Interestingly, ghrelin cells which also express FFAR4 exhibit reduced hormone secretion in response to FFAR4 agonism (Engelstoft et al. 2013). One selective FFAR1 agonist has made it into the clinic for the treatment of type 2 diabetes. TAK-875 reached phase III clinical trials for the treatment of type 2 diabetes (Srivastava et al. 2014), however its progress was discontinued recently due to hepatic toxicity issues.

Pharmacologically, there are numerous small molecule agonists for FFAR1, including AMG-837 (Lin et al. 2011; Houze et al. 2012), TUG-770 (Christiansen et al. 2010, 2013), GW-9508 (Ou et al. 2013), and TAK-875 (Negoro et al. 2010) as well as one antagonist, GW1100 (Zhao et al. 2011). Agonists of FFAR1 have been used to explore the molecular mechanisms of FFAR1 activation in EECs. ω-linolenic acid triggers CCK release by FFAR1 activation...
via a PKA and 1-type VGCC-dependent mechanism, while oleic acid triggers GLP-1 secretion through PKCζ (Iakoubov et al. 2007, 2011) suggesting they can couple to alternative G proteins, presumably dependent on the nature of the ligand, the binding site occupied and the conformation of the receptor that is stabilized (Liu et al. 2011a). Partial agonists and full agonists appear to bind to different sites. AM-6331 behaves as a partial agonist, while AM-8182 acts as a full agonist of FFAR1 (Luo et al. 2012). In combination, they exert a positive allosteric effect indicating that the agonists do not bind at the same site. Moreover, GLP-1 secretion in mice was stimulated by full agonists of FFAR1, while partial agonists failed to raise plasma GLP-1 significantly; only the full agonist-binding site of FFAR1 appears to be capable of generating a conformation that can mobilize the intracellular signaling pathway necessary to evoke hormone secretion (Luo et al. 2012). In combination, binding of the partial and the full agonist enhanced GLP-1 secretion in a synergistic manner, confirming positive co-operativity of the sites. It, therefore, appears that FFAR1 ligands can act at orthosteric and allosteric sites, similar to the other nutrient sensing GPCRs where allosteric modulators and allosteric agonists have been identified including FFAR3 (Hudson et al. 2014a), FFAR2 (Smith et al. 2015), T1R (Zhang et al. 2008, 2010), and T2R (Zhang et al. 2011), FFAR2 (Smith et al. 2011), CaSR (Chen et al. 2015), T1R (Zhang et al. 2008, 2010), and T2R (Mooser 1980). The recently reported high-resolution co-crystal structure of hFFAR1 with the potent and selective partial FFAR1 agonist, TAK-875, reveals a unique binding mode and suggests entry via the lipid bilayer (Srivastava et al. 2014). Hauge et al. (2014) recently reported that FFAR1 can signal through Gzq and Gz3 signaling pathways when stimulated with AM-1638 and AM-5262, in contrast to endogenous ligands, TAK-875 and AM-837 which only signal through Gzq. In a primary cell model, the Gzq only agonists weakly stimulated GLP-1 secretion, whereas agonists that stimulated both Gzq and Gz3 pathways triggered higher levels of GLP-1 output.

FFAR4 responds to medium and long-chain fatty acids including α-linolenic acid, palmitoleic acid and docosahexaenoic acid, and also preferentially couples to Gzq (Hirasawa et al. 2008). FFAR4 is activated by saturated free fatty acids (C14–C18), and mono- and poly-unsaturated free fatty acids (C16–C22) (Hirasawa et al. 2008). Generally, FFAR4 is regarded as a receptor for unsaturated fatty acids, and not for saturated fatty acids (particularly the C16-C18 chain length fatty acids which do not typically appear to show activity at FFAR4. FFAR4 colocalizes with GLP-1 in L-cells. Hara et al. (2011) also showed that a partial agonist derived from A. ovina was capable of activating FFAR4 in STC-1 cells; the [Ca2+]i response and secretion of GLP-1 were both abolished using siRNA against FFAR4. A close analogue, 4-[(2-(phenyl-2-pyridinylamino)ethoxy)phenyl]butyric acid, 3-(4-[(2-[phenyl(pyridin-2-yl)amino]ethoxy)phenyl)propionic acid (compound 10), and a synthetic compound, NCG120, have also been shown to act as agonists of FFAR4 (Suzuki et al. 2008; Sun et al. 2010; Hara et al. 2011). Agonism of FFAR4 decreases ghrelin secretion (Gong et al. 2014), while in L-cells stimulates GLP-1 secretion (Hirasawa et al. 2005). Stimulation of FFAR4 by α-linolenic acid has also been reported to trigger CCK release (Tanaka et al. 2008).

**Fatty acid amide sensing by GPR119**

Together with FFAR1 – 4, GPR119 is another lipid-sensing receptor that is also expressed in EECs (Chu et al. 2008; Reimann et al. 2008; Parker et al. 2009) and endogenous agonists include the fatty acid amide oleoylthanolamide (OEA), the endovanilloid N-oleoyldopamine (OLDA) and 5-hydroxy-eicosapentaenoic acid (5-HEPE), which displays agonist potencies of 0.003–3 μmol/L against human and mouse GPR119 and have been reported as the most potent natural agonists (Kogure et al. 2011; Hansen et al. 2012). GPR119 couples to Gz3 and activation increases intracellular cAMP levels (Overton et al. 2006; Chu et al. 2008; Cox et al. 2010), well established to stimulate hormone secretion from EECs. More recently, GPR119 has also been shown to signal with a high degree of constitutive activity (Engelstoft et al. 2014). In GPR119-expressing human COS-7 cells, 2-oleoylglycerol stimulated cAMP production and physiologically, administration to humans significantly increased plasma GIP and GLP-1 levels (Hansen et al. 2011). Application of GPR119 agonists to both rodent and human intestinal mucosa stimulates GPR119-specific PYY-dependent antisecreatory responses, demonstrating the existence of local paracrine networks that inhibit electrolyte secretion (Cox et al. 2010). Patel et al. (2014) compared the efficacy of GPR119 agonism in rodent models of diabetes showing that GPR119 stimulation causes glucose lowering in both lean and diabetic rodent models similarly through the release of GIP, GLP-1 and PYY. Furthermore, an oral GPR119 agonist stimulated postprandial GIP and GLP-1 secretion in recent clinical trials, however, the glucose-lowering efficacy of JNJ-38431055 in patients with type 2 diabetes was not deemed sufficient for it to progress further (Katz et al. 2012).

In addition, lower affinity synthetic agonists similar to AR231453 and 2,5-disubstituted pyridines have been discovered for GPR119 (Semple et al. 2008; Wu et al. 2010). In the enteroendocrine GLUTag cell line, AR231453 stimulated Ca2+ influx via VGCCs and GLP-1 secretion showing that in vitro agonism of GPR119 can stimulate gut hormone secretion through signaling pathways involving
extracellular Ca\(^{2+}\) entry (Lan et al. 2012). Lan et al. (2012) also demonstrated that GPR119 agonists can stimulate GLP-1 secretion in a glucose-independent manner from cultures of GLUTag and primary intestinal epithelial cells, as well as in vivo showing GLP-1 secretion was not glucose-dependent. Studies on the conformation of compounds from a series of potent bridged piperidine agonists and antagonists by McClure et al. (2011) showed that the conformation of the molecule in either equatorial or axial form determined its property to act as either an agonist or antagonist at GPR119).

**Bile acid sensing by GPBAR1 (TGR5)**

Enteroendocrine cells also respond to luminal stimuli that are not nutrients, for example, bile acids. Absorption of lipids requires emulsification with bile acids that are released from the gall bladder. Transcripts for GPBAR1 (TGR5), the bile acid receptor, have been detected in the EEC line, STC-1 as well as in L-cells of the lower intestine (Thomas et al. 2009; Parker et al. 2012b). Physiologically, bile acids have also been shown to dose-dependently stimulate GLP-1 secretion, in a cAMP dependent manner, in both rodent and human models (Patti et al. 2009; Adrian et al. 2012). Furthermore, increased bile acid accumulation in the lower intestine may provide one explanation for the increased GLP-1 and PYY levels observed following Roux-en-Y surgery (Patti et al. 2009; Pournaras et al. 2012). Overexpression of GPBAR1 induces GLP-1 secretion in cultured mouse enteroendocrine STC-1 cells, while knockdown using siRNA impairs secretion (Katsuma et al. 2005). The activity of bile acids via GPBAR1 can be distinguished from nuclear receptor activity by ligand specificity and cAMP responses. Selective agonists have been reported with selectivity for GPBAR1 (e.g., 23-alkl-substituted and 6,23-alkyl-disubstituted derivatives of chenodeoxycholic acid) over farnesoid X nuclear hormone receptor (Pellicciari et al. 2009). A series of 3-aryl-4-isoxazole-rings bile acids and derivatives; INT-777 is a potent and selective GPBAR1 agonist, and induces GLP-1 secretion from mouse STC-1 and human intestinal NCI-H716 cell lines (Pellicciari et al. 2009). A series of 3-aryl-4-isoxazole-carboxamides, also discovered by Intercept Pharma, were shown to increase GLP-1 secretion in vivo (Evans et al. 2009; Budzik et al. 2010).

Whether GPCR signals to stimulate EECs to secrete their hormones originate from the luminal or systemic surface has been partly addressed for GPBAR1. Restricting GPBAR1 agonists to the lumen of the intestine may circumvent potential systemic side effects that have been observed for GPBAR1 agonists in the past. However, peptide secretion from the EEC by GPBAR1 appears to be dependent on systemic and not luminal activation. Using the bioavailable and poorly bioavailable GPBAR1 agonists RO5527239 and taurine-RO5527239, respectively, Ullmer et al. (2013) showed that administration of 10 mg/kg RO5527239 in mice via po or iv routes generated comparable levels of PYY secretion. However, only an iv delivery of taurine-RO5527239 was able to elevate PYY secretion showing that systemic exposure was necessary to activate GPBAR1-mediated PYY secretion.

**Cation sensing by GPR39**

GPR39 is a Zn\(^{2+}\)-sensing receptor expressed throughout the intestinal epithelial cell population (Depoortere 2012). At present, although GPR39 expression has not been reported in native EECs, it is endogenously expressed in a mouse GLP-1 secreting enteroendocrine cell line (STC-1) (Peukert et al. 2014). Zn\(^{2+}\) is the only known physiologically active stimulator of GPR39 activity, which couples through Gz, Gq, and G\(_{12/13}\), with an EC\(_{50}\) value in the μmol/L range (Yasuda et al. 2007; Storjohann et al. 2008; Popovics and Stewart 2011). On the basis of mutagenesis studies, Zn\(^{2+}\) has been shown to act as an agonist by coordinating the His-17 and His-19 residues on the extracellular domain (Storjohann et al. 2008).

Recently, Novartis reported on the development of synthetic agonists for GPR39 (Zeng et al. 2012; Bassilana et al. 2014). They reported the development of 2-pyridyl-pyrimidines as the first orally bioavailable GPR39 agonists (Peukert et al. 2014). In a recombinant HEK293 cell-based assay expressing rGPR39, the potency of the Novartis compound, reported as compound 3, in a G\(_{q}\)-coupled readout was EC\(_{50}\) 0.4 nmol/L and using a recombinant hGPR39 system, the potency of compound 3 in a G\(_{q}\)-coupled readout was reported as EC\(_{50}\) 0.06 nmol/L. In studies with sub EC\(_{50}\) concentrations of Zn\(^{2+}\), the potent 2-pyridylpyrimidine GPR39 agonist stimulated a G\(_{q}\) response that was mediated by either substance alone, suggesting that the Novartis compound and Zn\(^{2+}\) do not compete for binding at the receptor, suggesting separate binding sites resulting in a receptor response; the 2-pyridylpyrimidines acting as an allosteric agonist (Peukert et al. 2014). In the GLP-1-secreting mouse STC-1 EEC line, compound 3 directly stimulated GLP-1 secretion with an EC\(_{50}\) of 0.06 nmol/L, and an Emax of 3.5-fold above basal suggesting GPR39 activation could directly stimulate hormone secretion. In an in vivo mouse model, compound 3 increased active GLP-1 secretion sixfold.
above vehicle following an oral glucose tolerance test (Peukert et al. 2014). There remains the possibility that GPR39 agonism may indirectly stimulate GLP-1 secretion from L-cells through local paracrine mechanisms. Pfizer has also recently reported on a piperazine GPR39 agonist that stimulates predominantly the Gz₁₆-signaling pathway over Gz₁ (Boehm et al. 2013). These observations support the model that GPR39 can adopt multiple active conformations, stabilized by different molecules, which affect intracellular signaling distinctly.

**Bitter sensing by the taste receptor, T2R**

In contrast to the mechanisms above which sense components of the lumen with beneficial value, the existence of bitter taste receptors in the gut epithelium suggests that they play a role to determine whether a protective response including vomiting (if detected prior to exiting the stomach) or epithelial mucosal defence mechanisms (if detected in the intestine) are required. Activation of T2Rs by bitter molecules triggers increased Ca²⁺ signals in EECs expressing bitter taste receptors demonstrates that bitter taste receptors are functionally active outside the lingual epithelium and suggests they operate in EECs (Wu et al. 2002; Chen et al. 2006; Rozengurt et al. 2006). The release of PYY, GLP-1, and CCK in response to bitter tasteants enter the circulation and activate neuronal pathways including extrinsic afferent neurones to send messages to the central nervous system (CNS) and intrinsic afferent neurones in the enteric nervous system (ENS) (Chen et al. 2006). Physiologically, when T2R agonists are administered to rats via the oral route, extrinsic afferent neurones are activated and the number of c-Fos (the immediate-early gene product) positive neurones, a marker of neuronal activity, is increased in the nucleus of the solitary tract (Yamamoto and Sawa 2000).

Activation of T2Rs results in Gzguantducin- and Gz₁₄-mediated signaling, and involves downstream PLC β2, TRPM5, and IP₃ signaling pathways (Zhang et al. 2003; Hisatsune et al. 2007). Although predominantly expressed in the lingual epithelium, they are also expressed in the GI tract. RNA for members of the T2R family were initially detected in the EEC line, STC-1, implying they may be functionally active in EECs. Activation by denatonium benzoate and phenylthiocarbamide stimulated CCK secretion via extracellular Ca²⁺ influx through L-type VGCCs, at least in STC-1 cells.

**Energy sensing by MC4R**

The melanocortin 4 receptor (MC4R) belongs to Class A and has been largely investigated based on the ability of agonists to decrease food intake and body weight in rodent models. Central MC4R pathways regulate GI activity. However, the receptor is also selectively expressed in EECs along the length of the GI tract and was found to be the second most highly expressed GPCR in enteroendocrine L-cells (Panaro et al. 2014). It is presumed to reside on the basolateral membrane where it can respond to its circulating peptide hormone, α-melanocyte-stimulating hormone (α-MSH). In contrast to those receptors above that detect luminal constituents, MC4R regulates EEC secretory activity from the basolateral surface. Physiologically, intraperitoneal (i.p.) administration of melanocortin peptide to mice stimulated GLP-1 and PYY secretion acutely (Panaro et al. 2014). Recently, intraperitoneal (i.p.) administration in mice of the α-MSH analog, NN2-0453, developed by Novo Nordisk failed to stimulate PYY secretion from L cells, whereas the peptide agonist, LY212688, developed by Eli Lilly stimulated PYY secretion approximately threefold above the saline control (Ghamari-Langroudi et al. 2015). Interestingly, NN2-0453 is a partial agonist of MC4R in a mouse colonic mucosal Ussing chamber assay of MC4R activity suggesting full agonism is required to bring about MC4R-dependent hormone secretion from EECs (Ghamari-Langroudi et al. 2015). Administration of α-MSH (i.p.) evokes MC4R specific PYY-dependent antisercretoy responses consistent with a role for MC4R in paracrine inhibition of electrolyte secretion, the same PYY-dependent antisercretoy responses are also observed for GPR119 agonists (Cox et al. 2010; Panaro et al. 2014; Patel et al. 2014). Responses to α-MSH were diminished when glucose was removed from the basolateral surface of Ussing chamber preparations of mouse colonic mucosa, in contrast to NPY1R responses that were not sensitive to glucose (Panaro et al. 2014). This observation is similar to the glucose sensitvity of GPR119 in L-cells (Cox et al. 2010; Patel et al. 2014). Pharmacologically targeting glucose-sensitive agonism of MC4R and GPR119 should show reduced risk of hypoglycaemia from hormones secreted in the absence of glucose. The ability of MC4R to regulate the secretion of gut peptides highlights a peripheral mechanism of action underlying observations regarding the effect of agonists on food intake, in addition to vagal central functions.
Energy sensing by bombesin receptors

The bombesin-related peptides, neuropeptide B, and gastrin-releasing peptide (GRP), have also been reported to regulate gut hormone secretion. In addition to GRP that stimulates gastrin release from G cells, physiologically, both CCK produced by enteroendocrine I cells, and GIP secretion is enhanced in humans in response to stimulation with GRP (Ghatei et al. 1982). The bombesin receptor 1 and 2 (BB1 and BB2) are Gαq-coupled receptors that bind NMB and GPR, respectively (Jensen et al. 2008). The role of BB2 in GLP-1 secretion was uncovered using BB2−/− mice, in which GLP-1 secretion was diminished when challenged with an oral glucose tolerance test (Jensen et al. 2008). The bombesin-related peptides are strong stimulants of GLP-1 secretion in a perfused pig intestinal model (Plaisancie et al. 1994). In contrast to bombesin-related peptides, tachykinins, enkephalins, dynorphin, TRH and members of the secretin family, vasoactive intestinal peptide, peptide histidine isoleucine and neuropeptide Y, were less effective (Plaisancie et al. 1994). Together with CCK, the bombesin-related receptors provide a further source of negative feedback signals to depress eating (Yamada et al. 2002).

Inhibitory G protein-coupled receptors

Hormone secretion from EECs is also regulated by inhibitory-signaling pathways. In contrast to the above receptors which activate stimulatory Gαs- and Gαq-signaling pathways, Gαq-coupled receptors from Class A are expressed in EECs which mediate inhibitory-signaling pathways to inhibit gut hormone secretion (Herrmann-Rinke et al. 1996; Saifia et al. 1998).

G protein-coupled receptors that mediate inhibitory signaling include the cannabinoid receptors, which are expressed in the GI tract and ENS (Lopez-Redondo et al. 1997; Coutts et al. 2002). Expression is highest in I (Sykaras et al. 2012) and K cells. Cannabinoid receptor 1 (CNR1, or CB1) couples to Gαi and it inhibits the secretion of GIP from K-cells via inhibition of cAMP production (Moss et al. 2012).

There are three galanin receptors (GAL1-3) which are activated by the peptides galanin and galanin-like peptide. Galanin is expressed throughout the ENS and inhibits the secretion of enteroendocrine peptides, such as GIP, GLP-1 and CCK (Chang et al. 1995; Saifia et al. 1998). Although GALs 1 and 3 were found to be highly expressed in ghrelin cells, there was no effect of galanin on ghrelin secretion from primary cultures (Engelstoft et al. 2013). Physiologically, galanin-inhibited GLP-1 secretion that was prestimulated with GIP in preparations of perfused rat intestine (Herrmann-Rinke et al. 1996). Similar effects have also been observed in the clinic where galanin inhibits gastric transit and inhibits GLP-1 and PYY secretion (Bauer et al. 1989). Of particular interest is the recent observations that galanin levels are abnormally elevated in patients with diabetes and obesity (Fang et al. 2013). The GAL(s) responsible remains unknown, although it appears to act via a Gαq-coupled mechanism since it is sensitive to pertussis toxin (Saifia et al. 1998).

In addition to galanin, somatostatin is another inhibitor of EEC secretion. Somatostatin-producing D cells tightly modulate gastrin release from G cells in the stomach, and in the intestine act as an inhibitory feedback network (Zaki et al. 1996; Schubert 2014). There are five somatostatin receptors (SSTR1-5), which couple to Gαi. SSSTR5 inhibits GLP-1 and PYY release from L-cells, presumably due to depression of intracellular cAMP (Chisholm and Greenberg 2002), and GIP from K-cells (Moss et al. 2012). In both GLUTag and primary L cells, this occurs through SSSTR5 (Moss et al. 2012). Somatostatin may operate through SSSTR1, 2 or 3 accounting for its inhibitory effect on ghrelin secretion from gastric EECs (Engelstoft et al. 2013).

The GIP and GLP-1 peptides have also been reported to display inhibitory functions. The GIP receptor is expressed on ghrelin cells and predominantly couples to Gαia, suggesting alternative pathways (Engelstoft et al. 2013). GLP-1 has also been suggested to act as an autoinhibitory feedback mechanism on GLP-1 secretion from the L-cell. This hypothesis is based on observations that the GLP-1 agonist, Exendin-4, depressed GLP-1 secretion in humans. GLP-1 has also been shown to stimulate the secretion of somatostatin in perfused pig intestine, suggesting an alternative indirect inhibitory pathway (Hansen et al. 2000).

In addition to luminal stimuli, cholinergic impulses control gut hormone secretion which predominantly involves GPCRs. In pig intestine, α-adrenergic signals exert an inhibitory tone on GLP-1 secretion. When this was inhibited with phentolamine, an excitatory cholinergic stimulus was revealed in keeping with a role for Muscarinic M1 and M2 receptors in GLP-1 release (Plaisancie et al. 1994; Anini and Brubaker 2003). M3 which couples to Gαq, has also been shown to regulate gastrin release from G-cells (Yokotani et al. 1995). Using a rat arterially perfused intestinal model, infusion of cholinergic agonists strongly enhanced GLP-1 secretion. This was counteracted by the addition of atropine, while histamine, dopamine, 5-hydroxytryptamine, γ-aminobutyric acid, and norepinephrine had no effect (Plaisancie et al. 1994).

Class B

Class B is a relatively small family that includes receptors for peptide hormones from the glucagon hormone family. They consist of a 7TM region which lacks significant
sequence identity to Class A and therefore has its own characteristic 7TM signature (Fig. 2). Few receptors belonging to this class have been found to modulate gut peptide secretion from EECs. Those that do include vasoactive intestinal peptide (VIP) receptors (VPAC1 and VPAC2), pituitary adenylate cyclase-activating protein (PACAP) receptor (PAC1), and calcitonin gene-related peptide (CGRP) receptors (CTR and CLR).

PACAP, VIP, and CGRP are colocalized in the gut, and whilst neural activation by CGRP stimulates GLP-1 secretion (Herrmann-Rinke et al. 2000), PACAP has been reported to stimulate CCK (Deavall et al. 2000), GLP-1, and PYY secretion. Using cultures of foetal rat intestinal cells, Brubaker (1991) demonstrated that CGRP, bombesin, and bombesin-related GRP stimulate GLP-1 secretion. Using preparations of rat ileum, CGRP has also been shown to stimulate GLP-1 secretion (Herrmann-Rinke et al. 2000). Consistent with the enrichment of receptor expression for CGRP in ghrelin cells, its activation stimulates ghrelin secretion (Engelstoft et al. 2013).

Class C

The members of Class C that have been identified to regulate gut hormone secretion include the sweet taste receptor (T1R), that detects carbohydrates, the calcium-sensing receptor (CaSR) and a promiscuous l-α-amino acid receptor (GPRC6A) that respond to l-amino acids and peptides. Class C are characterized by a large amino-terminal domain, which binds the endogenous orthosteric agonist (Fig. 2). A number of allosteric modulators have also been identified which bind to the 7TM domain of Class C GPCRs.

Carbohydrate sensing by the sweet taste receptor, T1R2 + T1R3

 Activation of EECs by glucose stimulates GIP and GLP-1 secretion from K- and L-cells, respectively. Preclinically, there are several independent reports that activation of the sweet taste receptor, T1R2 + T1R3, stimulates secretion of GLP-1 (reviewed in Mace and Marshall (2013)). Clinically, the picture is less clear. Rodent and human cell lines secrete GLP-1 in response to activation of T1R2 + T1R3 by artificial sweeteners (Mace et al. 2012; Ohtsu et al. 2014), and there are independent reports that demonstrate that activation of the sweet taste receptor stimulates GLP-1 secretion in humans (Steinert et al. 2011; Temizkan et al. 2014). The T1R2 + T1R3 receptor couples to the G protein, Gz\textsubscript{gustducin} which stimulates phosphodiesterase activity, while the β\textsubscript{2} subunit activates PLC β2. In rodents, knockout of Gz\textsubscript{gustducin} significantly diminishes GLP-1 release in response to glucose (Jang et al. 2007).

The receptors possess a large extracellular N-terminal domain (NTD), known as the VFT domain linked to the 7TM by a shorter Cys-rich region. Currently, there are not enough structural data to define the exact binding site for their ligands, and each domain can be involved in agonist activation, explaining the diversity of chemically distinct agonists. Sucralose and noncaloric sweeteners such as aspartame and neotame bind to the VFT domain of T1R2 (Cui et al. 2006). Other artificial sweeteners such as cyclamate and neohesperidin dihydrochalcone interact within the TMD of T1R3 (Winnig et al. 2007) and can be considered allosteric modulators. While S819, a synthetic sweet agonist interacts with the TMD of T1R2, the sweet-tasting protein brazzein requires the cys-rich domain of hT1R3 to activate the receptor (Cui et al. 2006).

Positive allosteric modulators (PAMs) of Class C appear to show little or no agonist activity on their own right but significantly enhance the activity of the agonist of the receptor and, in functional assays, this behavior is depicted by a leftward shift of the agonist dose-response in the presence of the PAM. Synomyx Inc., has identified PAMs of the sweet taste receptor, that considerably increase the sucralose and sucrose potencies of the sweet taste receptor in cell-based assays, and yet are not sweet on their own (Servant et al. 2010, 2011). These PAMs bind within the VFT domain (Zhang et al. 2010).

There are clearly different EEC populations that have been isolated by different laboratories since Parker et al. (2009) fail to detect T1R2 + T1R3 enrichment in purified mouse EEC preparations and their cultures of mouse primary intestinal epithelial cells failed to respond to artificial sweeteners. In contrast the human EEC line, Htu-80, responds to artificial sweeteners. Activation of T1R2 + T1R3 by sucralose, saccharin, acesulfame K, and glycyrrhizin (a natural sweetener derived from licorice root) increased intracellular cAMP levels (Ohtsu et al. 2014). However, the effects of sweetener on [Ca\textsuperscript{2+}]\textsubscript{i} levels were diverse. Activation of T1R2 + T1R3 by sucralose and saccharin stimulated extracellular Ca\textsuperscript{2+} influx via a nifedipine-sensitive l-type VGCC which was abolished by the Gzq inhibitor, YM254890 (Ohtsu et al. 2014). Activation by acesulfame K, however, reciprocally regulated intracellular cAMP and Ca\textsuperscript{2+} levels. [Ca\textsuperscript{2+}]\textsubscript{i} levels were reduced by acesulfame K via a calmodulin-dependent Ca\textsuperscript{2+} pump, while intracellular cAMP levels were raised (Ohtsu et al. 2014). Glycyrrhizin caused a biphasic Ca\textsuperscript{2+} response with an initial decrease in [Ca\textsuperscript{2+}]\textsubscript{i} followed by a sustained increase (Ohtsu et al. 2014). Clearly, coupling of T1R2 + T1R3 in EECs and the recruitment of downstream signaling pathways is agonist dependent.
These artificial sweeteners are structurally unrelated. T1R2 + T1R3 contains more than four potential binding sites for sweet molecules, stabilizing multiple conformational states, enabling T1R2 + T1R3 to switch between activation of differential signaling pathways depending on the agonist bound. In this respect, agonists at T1R2 + T1R3 appear as biased. Additionally, the T1R2 + T1R3 antagonist lactisole specifically inhibited the Ca$^{2+}$ signal without affecting the cAMP signal, behavior consistent with a biased antagonist (Ohtsu et al. 2014). In EECs, T1R2 + T1R3 appears as a multifunctional GPCR, capable of activating several intracellular signaling pathways in various combinations.

**l-amino acid sensing by the umami receptor, T1R1 + T1R3**

Although not enriched mRNA transcripts for the umami receptor, T1R1 + T1R3, have been detected in I-, K- and L-cells (Bezencon et al. 2007; Daly et al. 2013). T1R1 + T1R3 has also been shown to act as a sensor for CCK secretion in the EEC line, STC-1, and in primary mouse small intestinal tissue explants (Daly et al. 2013). T1R1 + T1R3 is activated by glutamate and sensitive to aliphatic l-amino acids. It is allosterically enhanced by inosine-5-monophosphate (IMP) and guanosine 5-monophosphate which stabilize the active conformation (Li et al. 2002). The glutamate- and IMP-binding sites appear to lie in the VFT domain of T1R1 and molecular modeling appears to suggest that IMP exhibits its allosteric effect by binding adjacent to glutamate, stabilizing the closed conformation of the VFT by coordinating the positively charged residues of the pincer (Toda et al. 2013).

**l-amino acid, Ca$^{2+}$, and peptide sensing by CaSR**

The CaSR is expressed in a number of EECs, and activation by l-amino acids stimulates secretion of CCK from I-cells (Nakajima et al. 2010, 2012), gastrin from G-cells (Feng et al. 2010), GIP from K-cells and GLP-1 from L-cells (Mace et al. 2012; Diakogiannaki et al. 2013). The CaSR responds to aromatic l-amino acids which allosterically increase the potency of Ca$^{2+}$ for the receptor; the rank order of amino acid potency being L-Phe, L-Trp, L-His > L-Ala > L-Ser, L-Pro, L-glutamic acid > L-aspartic acid (Conigrave et al. 2007; Conigrave and Ward 2013). In the absence of Ca$^{2+}$, aromatic amino acids and calcimimetic agonists are ineffective.

Similar to other members of the family, the CaSR is a multifunctional GPCR, and couples to Gz3, Gz5, Gz6, and Gz12,13 depending on the agonist bound and the conformational state stabilized. Biased signaling was recently demonstrated for cinacalcet and NPS 2143, and for a variety of Type I calcimimetics using rat or human CaSR (Davey et al. 2012). Such biased signaling indicates that the CaSR can adopt multiple conformational states, stabilized by different ligands or differential cooperativity between allosteric and orthosteric ligands that affect one or more signaling pathways.

Multiple PAMs have been identified for the CaSR, which all bind to the TMD (Hu 2008). Chimeric CaSR-mGlutR1 was used to show that phenylalkylamine calcimimetics like NPS R-568 bind in the TMD, whereas Ca$^{2+}$ binds in the extracellular domain of the CaSR (Brauner-Osborne et al. 1999a,b). All of the calcilytics studied to date bind in the TMD. In contrast, naturally occurring Type II calcimimetics such as aromatic amino acids and glutathione bind in the extracellular domain, whereas synthetic Type II calcimimetics that bind the TMD of the CaSR exert allosteric effects in response to Ca$^{2+}$. In EECs, the CaSR appears sensitive to external Ca$^{2+}$ because it requires Ca$^{2+}$ binding to the VFT to elicit opening of VGCCs (Diakogiannaki et al. 2013). In addition to amino acids, the CaSR has also been implicated in di-, tri-, and oligopeptide-mediated secretion of gut hormones, including gastrin, CCK, GIP, GLP-1, and PYY (Dufner et al. 2005; Hira et al. 2008; Ceglia et al. 2009; Feng et al. 2010; Nakajima et al. 2012; Diakogiannaki et al. 2013); L-cell hormone secretion was also sensitive to the CaSR antagonists, NPS2143, and Calhex231 (Mace et al. 2012; Diakogiannaki et al. 2013; Joshi et al. 2013). There are few studies evaluating whether these are competitive or non-competitive. However, the binding sites for polycationic agonists are presumed to lie in a different location to these antagonists. Ca$^{2+}$ is believed to bind in the extracellular domain and Gd$^{3+}$ in the TM domain (Miedlich et al. 2004; Silve et al. 2005), while the binding site for the calcimimetic agonists appears to overlap with that of the antagonists (Brauner-Osborne et al. 1999a,b).

**L-Amino acid sensing by GPRC6A**

GPRC6A responds to multiple l-amino acids and is allosterically modulated by physiological concentrations of Ca$^{2+}$ and Mg$^{2+}$. It is involved in amino acid-induced GLP-1 secretion from the EEC line, GLUTag (Oya et al. 2013). The GPRC6A agonist, L-ornithine stimulated GLP-1 secretion from GLUTag cells via a rise in [Ca$^{2+}$]I and was abolished with the GPRC6A antagonist, calindol (Oya et al. 2013). This was also supported by siRNA knock-down of GPRC6A and inhibition of L-ornithine induced Ca$^{2+}$ increase and GLP-1 secretion (Oya et al. 2013). Immunohistochemical studies have also shown GPRC6A to colocalize with gastrin and somatostatin in the stomach mucosa, implying that it may also control the release of these peptides (Haid et al. 2011).
To date, no competitive antagonists have been identified at GPRC6A, however, negative allosteric modulators (NAMs) have been published. Two antagonists identified for GPRC6A, the calcimimetic calindol and the calcyltic, NPS 2143 suffer from a lack of selectivity as they possess ~30-fold higher potency at the CaSR, and only show partial inhibition of GPRC6A responses (Faure et al. 2009). Both compounds bind in overlapping (allosteric) binding sites in the CaSR TM domain, and display opposing activity at CaSR: NAM and PAM modes for NPS 2143 and calindol, respectively (Petrel et al. 2004). However, GPRC6A antagonists developed by Gloriam et al. (2011) have a distinct site of action from calindol, and are proposed to bind at the top of TM helix 6 and 7 at the extracellular interface.

**Interpreting Enteroendocrine Communication and the ENS**

Hormones secreted from EECs also enter the systemic circulation where they may act in a more conventional endocrine manner (Fig. 1C). Hormones released from EECs may communicate through paracrine mechanisms on the neighboring cell population (Fig. 1D and E). Cox et al. (2010) demonstrated that the secretion of PYY from EECs can inhibit epithelial Cl⁻ secretion through activation of basolateral Y1 receptors (Panaro et al. 2014) showing that hormones secreted from EECs can also act in a paracrine fashion on the adjacent neighboring epithelial cell population (Fig. 1D). In addition, enteroendocrine hormones released into the serosa may act in a neurocrine fashion to activate neuronal pathways including extrinsic afferent neurones to send messages to the CNS (Fig. 1F) and intrinsic afferent neurones in the ENS (Fig. 1G) (Chen et al. 2006; Bohorquez and Liddle 2011, 2015; Bohorquez et al. 2011, 2014, 2015). Enteroendocrine cell lines that secrete CCK are also activated by LPS, a TLR agonist, to evoke CCK secretion and pro-inflammatory programs (Palazzo et al. 2007; Selleri et al. 2008). Activation of TLRs evokes NF-kB and inflammatory cytokine cascades (Akhtar et al. 2003; Suzuki et al. 2003). Enteroendocrine cell lines that secrete CCK are also activated by LPS, a TLR agonist, to evoke CCK secretion and pro-inflammatory programs (Palazzo et al. 2007; Selleri et al. 2008).

Since the disregulation of any one of these processes can be fundamental in several metabolic and GI pathologies including diabetes, obesity, gastroparesis, irritable bowel syndrome and possibly inflammatory bowel disease, harnessing the therapeutic potential of the endogenous EEC population is of immense importance.

**Perspectives**

Attempts to mimic the secretory hormone profile that occurs following Roux-en-Y gastric surgery to obtain the beneficial metabolic changes that have been observed in obese and obese diabetic patients are still to be achieved (Papamargaritis et al. 2012). “Roux-en-Y” in a pill remains the holy grail of harnessing the enteroendocrine system for the treatment of metabolic disease. However, the plethora of signaling events that occur at both the apical and basolateral PM of EECs to regulate secretory hormone output have made this achievement very challenging; even more so, given the nature of the sensor proteins involved. The activation of additive secretory enteroendocrine signaling pathways to secrete larger quantities of hormone secretion may provide further therapeutic benefit beyond individual gut peptide secretagogues alone. This may be achieved through coactivation of different Gx₄- and Gx₉-,coupled receptors. For example, endogenous ligands of GPR40 such as long-chain fatty acids couple to Gx₉ and provide a small incretin response (Luo et al. 2012). It is feasible that together with activation of Gx₄- coupled receptors such as GPR119 or GPBAR-1, may elicit a heightened incretin response. Alternatively, a larger incretin response may be achieved pharmacologically through activation of a single GPCR coupling to both Gx₉ and Gx₄ pathways, for example, AM-5262 which couples GPR40 to both Gx₉ and Gx₄ pathways (Luo et al. 2012). Stimulation of Gx₉ and Gx₄
signaling and robust incretin responses by ago-allosteric agonists could make these targets extremely interesting. Allosteric modulators of GPCRs have also emerged which may subtly modulate and fine-tune EEC activity. However, physiologically meaningful effects are often achieved with small changes in receptor activity, so the subtle effects of allosteric compounds do not mean that they will be less efficacious than orthosteric modulators. The interaction between GPCR and nutrient transporter signaling pathways is also complex. Nutrients may well modulate GPCRs allosterically, as well as drive EEC signaling through transporter-mediated mechanisms for example, amino acids behaving as PAMs of the CaSR, as well as substrates for Na⁺- or H⁺-coupled amino acid transporters (Mace et al. 2012; Conigrave and Ward 2013). Furthermore, the neuronal circuits formed between cells of the enteroendocrine system and the ENS that communicate bidirectionally with the CNS enable the GI tract to control mood and behaviors associated with food intake peripherally. Modulation of feeding behavior may not necessarily require central penetration (Panaro et al. 2014), and could be harnessed through the enteroendocrine system. As the complexity of the enteroendocrine system is revealed, opportunities to harness the therapeutic potential of the enteroendocrine system become teasingly closer.

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None declared.

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