The wall of the gut responds to an impressive array of signals originating in the lumen, including nutrient and non-nutrient chemicals, mechanical factors, and micro-organisms. The idea that the gut wall exhibits luminal chemo-sensitivity is implied in the original discovery of secretin by Bayliss and Starling, and has become an integral part of models of neurohumoral control of gastrointestinal function. Entero-endocrine cells are specialised for luminal nutrient sensing but sub-epithelial nerve fibres may also respond to luminal chemicals that freely diffuse across the epithelium eg short chain fatty acids. The molecular recognition mechanisms include G-protein coupled receptors (GPCRs) eg the extracellular Ca\(^{2+}\) sensing receptor which also responds to aromatic amino acids. There are also GPCRs sensing fatty acids, as well as bitter or noxious compounds. In addition, though, gating of ion channels including events secondary to energy availability eg ATP, may be involved in sensing some luminal chemicals. There is likely to be integration of luminal signals at several levels including at the level of entero-endocrine cells and at sub-epithelial nerve fibers. For example, the intestinal hormone CCK acts on primary afferent nerve fibers of the vagal trunk. The same fibers also express leptin receptors that are thought to respond to leptin released from gastric chief cells, orexin receptors (activation of which inhibits CCK) and possibly ghrelin receptors. Multiple signalling mechanisms allow specific responses to be matched to meals of differing content.

**Key words:** Enteroendocrine cells; GPCRs, CCK, ghrelin, orexin, vagus

**INTRODUCTION**

The idea that the gut wall exhibits luminal chemo-sensitivity is implied in the original discovery by Bayliss and Starling of the first gut hormone, secretin, which they showed to be released by luminal acid (1). The concept has become an integral part of all models of neurohumoral control of gastrointestinal
function. It is clear now that the gut responds to an impressive array of signals originating in the lumen, including nutrient and non-nutrient chemicals, mechanical factors, and micro-organisms (2). There are specialised classes of cell responding to luminal signals relevant to the control of digestion, and to immune mechanisms (Fig 1). However, it seems possible that a wide variety of epithelial cells possess at least some capacity to respond to luminal factors. The enteroendocrine cells are specialised transducers of luminal factors that respond by release of signalling molecules (hormones and paracrine factors) at the basolateral side. They are particularly important for the control of digestion and food intake. In addition, these cells are integrators, since they also respond to many different neurohumoral factors, growth factors and cytokines delivered to their basolateral membrane. They are the first level of integration of information from the gut lumen; a second level is provided by sub-epithelial neurones, either intrinsic to the gut or extrinsic eg primary afferent neurones. While enteroendocrine cells are specialised for luminal nutrient sensing, sub-epithelial nerve fibers may also respond to luminal chemicals that freely diffuse across the epithelium eg short chain fatty acids (Fig. 1) (3).

The enteroendocrine system

The enteroendocrine system of the gut can be conveniently viewed as consisting of three broad domains: the gastric, upper small intestinal and

![Diagram](diagram.png)

*Fig. 1. There are sensing mechanisms for chemicals (both nutrient and non-nutrient), mechanical factors and micro-organisms in the gut lumen. Enteroendocrine cells are specialised for luminal sensing and release humoral (hormonal or paracrine) factors at their basolateral membrane. These cells are also integrators that respond to neurohumoral factors, growth factors and cytokines. Some luminal factors may penetrate the epithelium and act directly on primary afferent neurones; the latter also respond to enteroendocrine cell products.*
ileum/colon. There is a characteristic spectrum of enteroendocrine cell types in each domain. In many cases, enteroendocrine cells act to control digestion within their region of the gut, or to regulate delivery of nutrient to it. The actions may be exerted on a wide variety of targets including secretory cells, smooth muscle, proliferating cells and signalling to the brain to determine food intake. Traditionally, it has been thought that hormone secretion from enteroendocrine cells is triggered by delivery of nutrient. However, one of the new ideas in the field is that some hormones can be released during the interdigestive period i.e. signalling the absence of food in the gut. Thus, while the phasic release of motilin in the interdigestive period has long been known, this is no longer alone example since the motilin-like hormone ghrelin is now known to be released in fasting, and perhaps also another orexigenic peptide, orexin.

**Luminal sensing**

The sensing of nutrient, notably glucose, by pancreatic endocrine cells has been intensively studied for many years. It is recognised that in islet β-cells the secretion of insulin depends on changes in intracellular ATP that gate ATP-sensitive potassium channels leading to depolarisation and opening of voltage gated calcium channels (4). The rise in intracellular calcium then activates mechanisms of exocytosis (Fig. 2). Direct gating of ion channels is also known to mediate the sensations of salt and sour tastes in the tongue (5). In both cases, similar mechanisms may operate in enteroendocrine cells. However in recent years there has been growing interest in the possible function of G-protein coupled receptors (GPCRs) as sensors of the luminal contents in these cells.

Signalling via GPCRs mediates sensations of sweet and bitter taste (5). At least some of the relevant receptors have also been shown to be expressed by gut endocrine cells (6). Moreover G-proteins such as Go<sub>gustducin</sub> that work in tongue taste cells have also been found in gut cells (6, 7). The physiological importance of GPCR signalling in gut endocrine cells, and in particular the relevance for nutrient sensing, is still unclear. There is, however, evidence that the extracellular Ca<sup>2+</sup> sensing receptor, which is a GPCR, also functions as a receptor for aromatic amino acids and may account for sensing mechanisms in some parts of the gut (8 - 10). In an interesting extension to the idea of functional links between taste and enteroendocrine cells, recent work suggests that the intestinal hormone CCK is expressed in taste cells and may work as a mediator or regulator of transmission (11).

**CCK - a primary integrator of brain and gut**

The upper small intestinal hormone cholecystokinin (CCK) is one of the primary endocrine regulators of small intestinal digestion. The CCK system has served as a paradigm over many years for the study of integrative mechanisms in nutrient sensing and signalling to the brain. CCK acts by balancing the capacity
for digestion in the small intestine with the delivery to the duodenum of substrate to be digested. This is achieved by, on the one hand stimulating pancreatic exocrine secretion and gall bladder contraction to promote delivery of the enzymes and bile salt required for digestion, while on the other hand there is inhibition of gastric emptying and food intake (thereby limiting the delivery of further substrate to the duodenum). It is exactly 30 years ago that CCK was first described as a satiety hormone (12).

**The specificity of fatty acid effects on CCK release**

It has long been appreciated that CCK is released by fat and protein. One of the key observations underlying recent work in this area is that in normal human subjects there is a sharp cut-off in fatty acid chain length required for CCK release. Saturated fatty acids with a chain length >C12 are effective releasers (and chain length has little further effect), while C11 or shorter fatty acids are no different to vehicle (13). Essentially the same specificity is found in a CCK-producing cell line, STC-1 cells, where it has been shown that fatty acids with the critical chain length for CCK release also produce a reproducible rise in intracellular calcium that is inhibited by calcium channel blockers like...
nicardepine (14). This suggests that the relevant molecular recognition events reside at the level of the enteroendocrine cell.

In view of the growing evidence for a role of GPCRs in nutrient sensing, it is of interest that fatty acids appear to activate one such receptor, GPR40 (15, 16). Considerably more work needs to be done in this area to determine the role of GPR40 in CCK cell responses to fatty acid. In this context it is worth noting, though, that fatty acids may also exert other relevant effects. For example, some fatty acids like oleylethanolamide (OEA) which function as satiety agents apparently act directly on transcription factors like PPARα (17, 18).

**CCK effects on the CNS: modulation of CCK-vagal signalling**

In the last 30 years a substantial body of experimental evidence has been gathered (mainly in the rat) to indicate that CCK released from the small intestine acts directly at CCK-A (or CCK-1) receptors which are expressed by small diameter vagal afferent neurones serving the stomach and the jejunum (12, 19 - 23). This leads to inhibition of food intake, relaxation of the stomach and inhibition of gastric emptying. The picture emerging from recent work is that CCK effects on the vagus can be modulated by both potentiating and inhibitory factors (Fig. 3).

![Fig. 3. Vagal dependent mechanisms regulating food intake. CCK is a primary inhibitor of food intake acting via vagal afferent nerve fibres. Similar effects may also be produced by gastric distension, oleylethanolamide (OEA) and possibly cytokines. Leptin potentiates the effect of CCK on vagal afferent neurones. There are also vagal pathways associated with stimulation of food intake potentially mediated by peptides such as ghrelin and orexin: these may work by inhibiting responses to CCK.](image-url)
Potentiation. The vagal afferent neurones that express CCK-A receptors can also express the leptin receptor (24-25). Interestingly, leptin is expressed by gastric chief cells (26), as well as by adipocytes. One possibility is that locally high concentrations of leptin released in the vicinity of vagal afferent nerve terminals provide a background potentiation to augment CCK effects. In this context it is therefore notable that there is functional evidence for potentiating interactions between leptin and CCK both in stimulating the discharge of vagal afferent neurones and in inhibiting food intake (27, 28).

Inhibition. Quite recently the idea has emerged that there might be mechanisms inhibiting or restraining the action of CCK on vagal afferent neurones. An important example is provided by the orexigenic peptide orexin. This was first identified in hypothalamus, but it has since been identified in gut endocrine cells (29). There are two peptides, orexin-A and -B, which exhibit similar affinities for orexin-R2 receptors, while orexin-B has somewhat lower affinity for orexin-R1 receptors. Orexin receptors are widely distributed within the CNS. Recent work has shown the orexin-R1 receptor is also expressed by rat and human vagal afferent neurones (30). The same neurones express CCK-A and leptin receptors. There are, however, important differences in the neurochemical organisation of the rat and human nodose ganglia (30). In human nodose ganglia orexin-R1 receptors are expressed by large neuronal cell soma and by satellite cells that surround them. Neither positive satellite cells, nor for that matter large neurones surround by satellite cells, are found in the rat. This suggests there may be some species differences in the neurochemistry of the nodose ganglion in rat and man, although this remains largely unexplored.

Importantly, orexin-A inhibits the action of CCK on vagal afferent nerve discharge (30). In particular, pre-treatment with orexin A inhibits the subsequent response of rat jejunal afferent nerve fibres to CCK. This is of course compatible with the orexogenic effects of orexin - although the effects would clearly appear to be pre-hypothalamic.

Inhibitory effects of orexigenic peptides on the vagus nerve are not limited to orexin. A second candidate is ghrelin which is produced in gastric X-cells and secreted in the interdigestive period (31). A growing body of evidence indicates that while ghrelin may have some actions directly on hypothalamic neurones it also acts via the vagus nerve. Thus ghrelin receptors are expressed by the nodose neurones and in electrophysiological experiments ghrelin is reported to inhibit the basal discharge of vagal afferent neurones (32, 33).

Finally it should be noted that some fatty acids such as OEA inhibit food intake at least in part via the vagus (18). There appear to be mechanisms that inversely regulate the abundance of OEA and the related compound anandamide in the intestine in feeding and fasting. Anandamide is an endogenous cannabinoid receptor agonist which is associated with stimulation of food intake by a vagal mechanism (34). Thus in addition to peptide hormones that act via the vagus to both inhibit or stimulate food intake there appear to be endogenous fatty acids...
that either inhibit or stimulate food intake via the vagus too. Enough is known to suggest there are interactions between these signalling systems (34), but their full extent and significance for the normal control of food intake is still uncertain.

Overview

Multiple mechanisms mediate nutrient sensing, and there is likely to be integration of luminal signals at several levels. Thus, at the epithelium apical and basolateral stimuli regulate the secretion of enteroendocrine cells. In the case of gut-brain signalling, there is also some integration of stimuli at the level of afferent neurones. For example, CCK acts on primary afferent nerve fibres of the vagal trunk. The same fibres also express leptin receptors that are thought to respond to leptin released from gastric chief cells, orexin receptors (activation of which inhibits CCK) and possibly ghrelin receptors. Together these mechanisms presumably allow specific responses to be matched to meals of differing content. The way that this occurs must now be determined.

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Author's address: Graham Dockray, Physiological Laboratory, Crown St, Liverpool, L69 3BX, UK, Tel: (44)(0)151 794 5324; Fax: (44)(0)151 794 5315
Email: g.j.dockray@liverpool.ac.uk