NUTRIENT SENSING MECHANISMS IN THE SMALL INTESTINE:

Localisation of taste molecules in mice and humans with and without diabetes

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1. INTRODUCTION

1.1 General overview

The absorption of dietary nutrients from ingested meals into the body occurs via highly coordinated and tightly controlled digestive processes within the gastrointestinal tract. The digestion and absorption of nutrients by the small intestine is dependent on the timely initiation of facilitative processes such as digestive enzyme secretion, increased mucosal blood flow and synchronization of gastrointestinal motility. It is therefore crucial for the small intestine to possess the ability to monitor and respond to the contents of the lumen via a feedback system that can be continually updated and adapted to changing needs and cues.

Archetypal examples of feedback responses to luminal nutrients include the slowing of gastric emptying and pre-absorptive satiation. Both these reflexes function to optimise digestion and absorption by preventing nutrient influx beyond the intestinal processing capacity by limiting the rate of nutrient entry from the stomach and reducing further food intake respectively. The importance of the small intestine as a chemosensory organ is exemplified by its extensive innervation by sensory neurons of enteric, spinal and vagal origin and multiple endocrine cell types, allowing regulatory control via multiple detection systems. Although these neural and endocrine components are clearly important in mediating intestinal feedback in response to the luminal contents, the cellular and molecular basis of gut chemoreception is not well defined.

1.2 Nutrient feedback from the small intestine

Solid or liquid nutrient meals have been unequivocally shown to empty from the stomach at a slower rate than equivalent non-nutrient meals (8, 189, 218, 224, 354). This slowing of gastric emptying occurs due to reflex feedback inhibition, which is initiated by activation of chemoreceptors in the small intestine. Signals arising from products of digestion and chemicals in the lumen are monitored by multiple detection systems within the gastrointestinal mucosa (101). These detection systems activate sensory neural reflexes involving both intrinsic and extrinsic circuits. Intrinsic primary afferent neurons, with cell bodies in nerve plexuses within gastrointestinal muscle layers, form connections and initiate reflexes entirely within the enteric nervous system and function independent of the central nervous system (CNS) (21, 101, 141). In contrast extrinsic nerve reflexes are conveyed to the CNS by vagal afferents, which have cell bodies in nodose and jugular ganglia and project to the brainstem and to a lesser extent by spinal afferent neurons, which have cell bodies in dorsal root ganglia and project to the spinal cord (21, 101, 141). These pathways serve to modulate gastrointestinal functions via actions through the CNS.

The vagus nerve plays an eminent role in many digestive processes and exerts this influence via extrinsic vago-vagal reflexes. Gastrointestinal vago-vagal reflexes are characterised by an initial stimulus activation of vagal sensory afferent fibres within the gut, followed by transfer of the signal along fibres in the vagus nerve to their termination in the nucleus of the solitary tract (NTS). Projections from the NTS to the dorsal motor nucleus of the vagus (DMV) integrate sensory inputs and trigger outflow via vagal efferents to the viscera which modify secretory and/or motor responses of the gastrointestinal tract (285) (see Figure 1.2.1). The importance of the vagus in modifying gastric motility in response to luminal nutrients has been demonstrated in studies performed in rat, in which vagal pathways were ablated either by subdiaphragmatic vagotomy or application of sensory neurotoxin capsaicin (270, 292, 314). The absence of functional vagal

connections to the gut largely or completely suppressed changes in gastric emptying in response to intraduodenal infusions of glucose, lipid or protein.

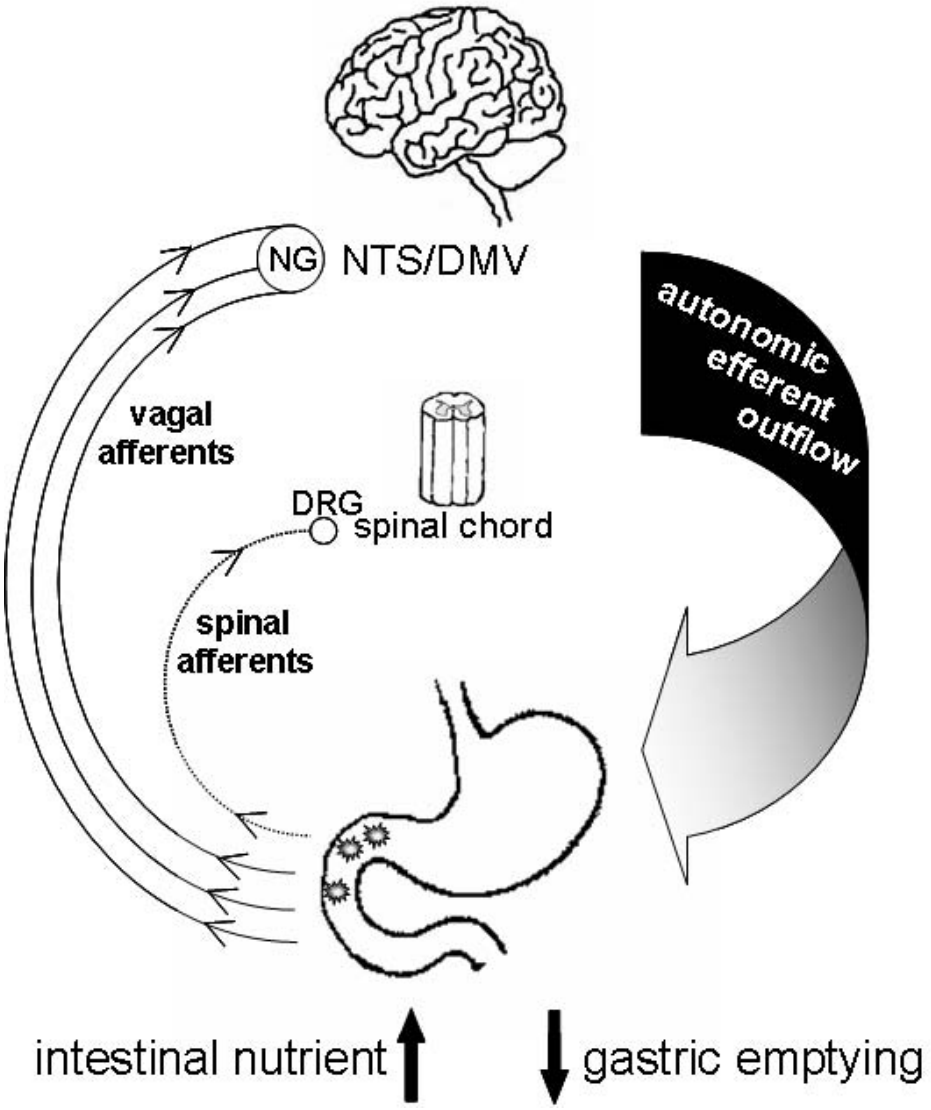

Figure 1.2.1 Extrinsic neural reflexes in nutrient-induced feedback control of gastric emptying.
The presence of specific nutrients within the intestinal lumen leads to activation of extrinsic sensory afferent fibres. Transmission of nutrient specific information through vagal, and to a lesser extent spinal, afferent pathways feeds into autonomic efferent pathways and results in alterations of gastrointestinal motor functions and delays gastric emptying. NG; nodose ganglion, NTS; nucleus of the solitary tract, DMV; dorsal motor nucleus of the vagus, DRG; dorsal root ganglia.

1.2.1 Intestinal carbohydrate and feedback inhibitions of gastric emptying

There is unequivocal evidence that the presence of carbohydrate in the proximal small intestine slows gastric emptying in a response that is nutrient-specific, and not secondary to an increase in osmolality detected by intestinal osmoreceptors (189). Furthermore the rate of slowing of gastric emptying differs according to the monosaccharide present, with glucose having the most pronounced effect (86, 226). Electrophysiological studies in a number of species have shown that small intestinal vagal afferents are activated in the presence of luminal glucose (24, 209, 315, 381). In the cat, vagal fibres that increase their afferent discharge in response to glucose but not other chemical or mechanical stimuli have been described (209) suggesting the existence of specific vagal glucoreceptors. In other species small intestinal vagal afferents responsive to glucose are 'polymodal' and equally respond to other chemical and mechanical stimuli (24, 209, 315, 381). Intraluminal application of capsaicin has confirmed that the vagal regulation of gastric motor function is conveyed largely through afferents located in the small intestinal mucosa (382) and not at post-absorptive sites, such as the portal vein, where vagal afferents responsive to glucose have also been reported (240, 241). Anterograde nerve tracing studies in rats have further shown that vagal sensory endings are present within the parenchyma of mucosal villi and terminate in the lamina propria, below the epithelial surface (16). This largely precludes a direct action of luminal nutrient on afferent neurons and suggests that an intermediary transduction event is required within the epithelium to signal the presence of nutrients to vagal sensory endings.

1.2.2 Regional specificity of intestinal carbohydrate-feedback inhibition

Nutrient detection at various sites along the length of the small intestine is critical for the coordination and optimisation of gastrointestinal transit. As such the three regions of the small intestine, duodenum, jejunum and ileum, are variously implicated in nutrient feedback.

Carbohydrate absorption from the small intestine is a rapid process, with essentially all glucose absorbed within the proximal small intestine (126). It has been reported that around 75% of sugars entering from the stomach are absorbed in the proximal small intestine in humans (162). However the most proximal segment of the small intestine does not initiate reflex inhibition of gastric emptying in response to carbohydrate. Restricted infusion of glucose into the first 5 cm (53) or 15 cm (188) of the small intestine does not slow gastric emptying in dogs. In contrast, occlusion of the proximal gut and carbohydrate infusion into the jejunum in healthy human subjects potently inhibits gastric emptying (218, 354). This may reflect an absence of nutrient receptive mechanisms in the proximal duodenum or nutrient receptors do not connect to vagal pathways, or that the number of sensors activated may be below threshold for reflex activation. It should be noted that most dietary sugars presented to the small intestine are broken down by enzymes into the absorbable units of glucose and galactose (365), therefore a steady supply of glucose becomes available in contrast to the single glucose bolus used in experimental situations. Evidence that the total length of the intestine exposed to glucose is important has been shown in canine studies (188). Here, reflex slowing of the rate of gastric emptying was observed only when glucose was infused beyond 15 cm of small intestinal while 50% of maximum slowing was observed at 65cm exposure and maximal inhibition reached at 150cm of exposure with no further increase with glucose exposure to the whole small intestine. Importantly exposure to only the distal small intestine did not slow gastric emptying as potently. This suggests length of intestine exposed to nutrient is critical for triggering nutrient reflexes, with exposure to the proximal half of the intestine most effective.

In general terms, any significant amounts of glucose remaining in the distal small intestine may reflect malabsorption due to various gastrointestinal disorders, or result from rapid gastrointestinal transit which is often accompanied by symptoms of diarrhoea and abdominal pain (365). However the presence of nutrient in the distal intestine is known to alter the motility of proximal gastrointestinal segments in a mechanism known as the ‘ileal-brake’. This serves to slow the transit of further nutrients into the distal gut (352).

Peptides such as neurotensin, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are released in response to nutrient and are important in mediating this response (244, 329). This mechanism, however, is primarily tuned to respond to digestion products of fat, as protein and carbohydrates in the ileum do not alter proximal intestinal motility (329). Glucose infusion (50mM) into the ileum in humans has no effect on the rate of gastric emptying (360). Carbohydrate infusion into the ileum, but not proximal intestine, in dogs however significantly reduces gastric tone, although gastric emptying itself was not measured in this study (7). Collectively these data further support the fact that carbohydrate induced reflexes are initiated primarily from the proximal gut under normal circumstances. However different intestinal regions are implicated in feedback control under pathological conditions.

Nutrient sensors are therefore likely to be distributed uniquely along the oral-aboral axis of the intestinal tract. Enteroendocrine cells and their corresponding endocrine products also show regional expression along the gastrointestinal tract (282), however the regional expression profile of specific nutrient receptors in the small intestine and their relationship to enteroendocrine cells are not well defined. Intestinal glucose sensors which connect to vagal feedback pathways governing gastrointestinal motility and ingestive behaviour are likely to display regional specificity reflecting their roles in initiation of feedback along the length of the small intestine.

1.2.3 Transport and signalling of glucose in the small intestine

One of the primary roles of the small intestine is to absorb ingested nutrients from the lumen across the epithelial layer into the circulation for transport to and use by target cells. As the epithelial cell layer of the small intestine forms a barrier between the lumen and the underlying mucosa, distinct transport mechanisms exist for the absorbable units of each macronutrient class allowing transepithelial passage. Enterocytes are polarized epithelial cells and the absorptive cells of the intestine, able to transport

molecules in a vectorial fashion from the apical membrane to across the basolateral membrane (136). For carbohydrates, the monosaccharides glucose and galactose are transported from the lumen into the enterocyte by glucose transporters, notably the active transporter sodium-glucose co-transporter (SGLT1) and also the facilitative transporter, GLUT2, which is rapidly inserted into the apical brush-border membrane in response to the presence of sugars in the lumen (110, 256). Accumulation of sugar molecules within the enterocyte promotes their exit down their concentration gradient via basolaterally located GLUT2 transporters, subsequently allowing movement into villous capillaries (43).

Monosaccharides, such as glucose, also interact with other types of cells in the gastrointestinal epithelium. Enteroendocrine cell populations in the gastrointestinal tract release a wide array of hormones, and together constitute the largest endocrine organ in the body (199). More than 20 unique signalling molecules including peptide hormones and the biogenic amine 5-HT have been identified within, and are used to define enteroendocrine cell subpopulations. These hormones and signalling molecules are typically enclosed within granules in the basolateral cytoplasm of the cells and are released in response to various stimuli. Although there are multiple phenotypes within the enteroendocrine cell population many are of the 'open cell' type, possessing apical microvilli which penetrate the brush border to have direct access to the lumen. A number of these hormones and signalling molecules are released by enteroendocrine cells in the presence of luminal nutrient, with specific mediators released according to the macronutrient present (31). In the case of carbohydrates, glucose has been shown to induce release of GLP-1 (283, 324), gastrin inhibitory polypeptide (GIP) (335) and 5-HT (263).

The signalling potential of intestinal enteroendocrine cells renders them attractive candidates as primary chemosensors of the gastrointestinal tract. Gut hormone release allows digestive signals to be conveyed not only to local digestive organs, such as the gall bladder and pancreas, but also to the CNS through the blood-brain barrier. There is also clear evidence that signals from intestinal enteroendocrine cells can be

relayed to the CNS via neural mechanisms, this is exemplified in the fact that receptors for many neurotransmitters and peptides released from intestinal enteroendocrine cells are expressed by sensory afferents (174). Intestinal enteroendocrine cells, however, do not appear to form synaptic connections with vagal afferents and vagal endings identified by nerve tracing from the nodose ganglion are shown to terminate tens to hundreds of microns from these specialised epithelial cells (18) indeed as epithelial cells are constantly migrating from the crypt to villus any close connections with afferent terminals would soon be lost. Paracrine-signalling mechanisms are therefore likely to underlie activation of afferent fibres upon release of neuropeptides from the epithelium (Figure 1.2.1). Perhaps the best studied example of such neuroendocrine communication in relation to nutrient feedback is that of cholecystokinin (CCK), a regulatory peptide hormone released from proximal intestinal enteroendocrine cells by luminal nutrient, most potently by the presence of digestion products of fat and protein. Vagal afferents express CCK receptors (227) and the afferent discharge of intestinal vagal mucosal fibres is increased by CCK (24). Furthermore, afferent discharge evoked by luminal lipid is blocked by CCK_1 receptor antagonists (181, 264). Inhibition of gastric emptying in response to duodenal infusion of lipid is dependent on both CCK and a vagal afferent pathway (140) and is severely impaired in CCK_1 receptor knock out mice (362). This anatomical and functional evidence support a model of nutrient feedback via activation of vagal afferent pathways secondary to nutrient detection by intestinal 'sense' cells, in this case CCK-containing I cells. There is some evidence to suggest a similar mechanism of activation of vagal afferents in response to glucose in gastric emptying pathways via 5-HT in that $5\text{-}HT_3$ receptor antagonists also suppress the glucose-induced delay in emptying time in rats (269). A primary role for at least one enteroendocrine cell subtype as a 'sense' cell in glucose-stimulated feedback pathways is therefore supported. *However, the receptors and transduction molecules within enteroendocrine cells which allow detection of luminal nutrient have not been identified and therefore the precise mechanisms by which enteroendocrine cells recognise luminal nutrients are not known.*

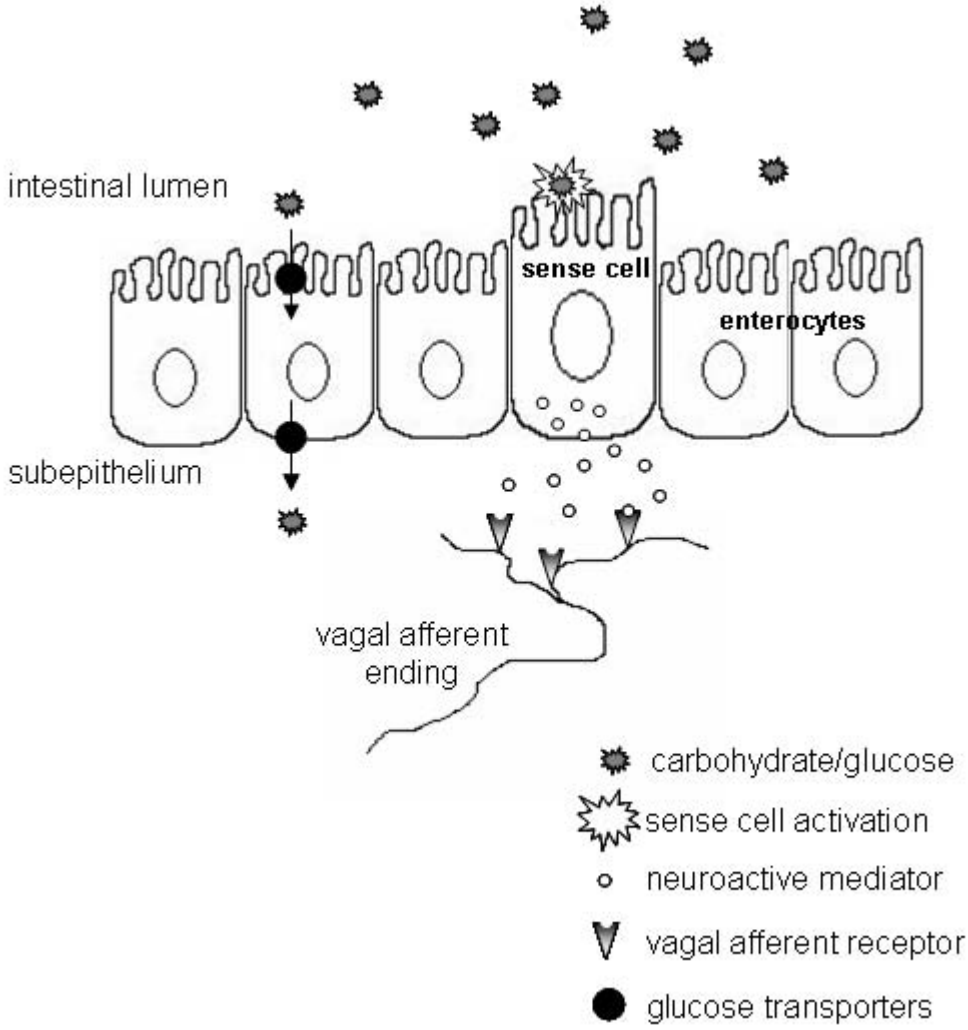

Figure 1.2.2 Hypothesised pathway for activation of subepithelial vagal afferents by luminal carbohydrates.

Intestinal carbohydrates cannot directly activate subepithelial vagal afferent endings from the lumen. Although glucose is transported across the epithelial barrier into the mucosa via glucose transporters, a popular concept of vagal afferent activation is via a specialised secondary 'sense' cell within the epithelium. Specialised epithelial cells, such as the enteroendocrine cells, are equipped with apical molecular machinery to sense luminal glucose and respond by the basolateral release of neuroactive mediators. These mediators in turn initiate vagal activation via specific receptors expressed on nerve terminals.

1.3 Adaptation of nutrient feedback from the small intestine in diet and disease

Feedback from the small intestine in response to nutrient shows remarkable plasticity in response to changes in dietary intake or in disease states. Nutrient signalling is altered by prior ingestive patterns, such that prior high intake of an individual macronutrient (eg carbohydrates) can reduce feedback to that nutrient in subsequently ingested meals (143). Moreover disordered upper gastrointestinal motor function and sensation is common in patients with longstanding diabetes (149) and may reflect altered feedback responses arising from the small intestine.

1.3.1 Acute modification of intestinal function by dietary influences

The intestinal mucosa rapidly adapts to the increased nutrient processing demands after feeding as evidenced by the ability to up-regulate epithelial glucose transporters. The intestinal glucose transport capacity is a determinant of the rate and completion of glucose absorption and therefore tightly matched to momentary demands. Increased absorption is due to a glucose-stimulated increase in the number of active sodium-dependent glucose cotransporters (SGLT-1) present at the apical brush border membrane of enterocytes (91, 131). SGLT-1 transporters can be rapidly inserted into the brush border within minutes in response to intestinal glucose infusions (82, 131, 320), due to an increase in number of transporter proteins inserted into the membrane from the intracellular pool, without change in mRNA levels (131). GLUT2 transporters are expressed on the enterocyte basolateral membrane and allow passive exit of glucose from the cell and are absent from the apical brush border membrane during fasting (2), however, in response to luminal glucose, GLUT2 is rapidly trafficked and inserted into the apical membrane of the enterocyte to increase glucose absorption beyond the saturation kinetics of the SGLT-1 transporter (110, 166, 167). The presence of dietary carbohydrates in the small intestine, therefore, initiates short term adaptive responses within the mucosa to increase the absorptive capacity for glucose, and as a consequence, to elevate

plasma glucose concentration. Although rapid and reversible adaptations occur in the intestinal epithelium in response to luminal sugars the mechanisms governing this regulation are not well understood. However there is emerging evidence that SGLT-1 induction occurs though a G-protein coupled receptor at the luminal surface (82).

It is well accepted that acute changes in blood glucose levels have substantial and reversible effects on motor and sensory functions of the gastrointestinal tract (123). Although several factors may contribute to postprandial glycaemia, the rate of gastric emptying has a major influence, accounting for around 35% of the variation in blood glucose levels after ingestion of glucose (145). This relationship between blood glucose levels and gastric emptying rate is reciprocal - gastric emptying is slowed during physiological hyperglycemia (202, 312) and accelerated during hypoglycaemia (311). This provides a physiological advantage, as reducing the transit time of nutrient from the stomach to the absorptive surface of the small intestine in hyperglycemia acts to attenuate further rises in blood glucose. The mechanisms governing glycaemic control of gastric emptying are not clear but there is evidence that glucose can decrease gastric motility by acting within the CNS (299, 323).

The glycaemic status of an individual is known to alter the effects of nutrient feedback on upper gastrointestinal sensation and motility (121, 122, 192). The sensation of appetite (hunger) in fasted, healthy humans does not differ if blood glucose levels are held at physiologically high or low levels (4, 183) but hunger is decreased at high blood glucose levels in the presence of intestinal nutrient infusion. This decrease in hunger during hyperglycaemia in response to intraduodenal infusions was mirrored also by decreases in gastric motility (4). Intestinal nutrient is therefore an important modulator of the effects of glycaemia on gastrointestinal function and sensation, suggesting a synergy between these signals. This illustrates that nutrient feedback signals may be modulated on a short time scale to adapt to nutritional status.

The mechanism(s) behind the effect of hyperglycaemia on nutrient feedback signalling are not clear and several sites of action or integration may be possible. Glucose can act directly within the CNS (299, 323) and the influence of intestinal nutrient stimuli may reflect a central integration of these two peripheral signals. Vagal nerve function is reversibly altered during hyperglycemia (340, 371) and an attenuation of nutrient feedback signals in high glucose concentrations may be another potential mechanism. In addition, the bioactivity of released gut peptides, which mediate many of these regulatory gastrointestinal processes, may be altered in hyperglycemia. For example CCK secretion is not changed in response to blood glucose levels (70) but effects of CCK on pyloric pressure are increased at blood glucose levels of 8 mmol/l compared to 4 mmol/l (273). The mechanism of this effect is not clear, however these data underscore the ability of glycaemic status to acutely modify signalling of gastrointestinal nutrients.

1.3.2 Chronic modification of intestinal function by dietary influences

Chronic modification of diet also leads to changes in intestinal functions. For example, the brush border glucose transporters SGLT-1 and GLUT2 which are acutely up-regulated in response to luminal carbohydrate are regulated chronically in response to a high carbohydrate diet (89). A carbohydrate rich diet fed over 1-2 days to mice leads to an increase in intestinal SGLT-1 mRNA and protein and subsequent changes in glucose absorption (75, 90). The mechanism by which the intestinal mucosa senses changes in luminal glucose and adapts over the longer term has not been elucidated.

Nutrient signalling in the intestinal is also altered by prior dietary intake of macronutrients. Prior high level consumption of carbohydrate or fat will accelerate the rate of gastric emptying of that macronutrient in subsequent meals (62, 63, 143, 271), but responses to other macronutrient types remain unchanged (63). Conversely after short-term starvation (57) or in anorexia nervosa (79) gastric emptying of a mixed meal is

profoundly slowed (139) and can be normalised following a short-term refeeding programme (281). This indicates that feedback mechanisms in the intestinal mucosa are subject to reversible sensitisation or desensitisation in response to prior nutrient intake. These adaptive mechanisms could potentially reflect alterations in the numbers of intestinal detectors for nutrient and/or altered sensitivity of vagal afferents to mediators released from epithelial 'taste' cells.

1.3.3 Adaptation of nutrient feedback in diabetes

Altered gastric motility in diabetes mellitus

Disordered gastrointestinal function is common in patients with diabetes mellitus and delayed gastric emptying is frequent in those with longstanding Type 1 (97, 168, 300) and Type 2 (6, 58, 146) diabetes. Approximately 30-50% of these patients are affected and in severe cases (diabetic gastroparesis) they experience symptoms such as postprandial abdominal pain, nausea, vomiting and bloating which are partly attributable to gastric stasis, and can be incapacitating (176). Gastric emptying is slower in patients with diabetes during hyperglycemia (312), as in the normal population, although in diabetics this has the further implication of compromising glycaemic control (275) and delivery of oral medications. Upper gastrointestinal symptoms such as early satiety and post-prandial fullness are also common (39, 313) although are not necessarily predictive of delayed gastric emptying, as the relationship between the two is relatively weak (147, 163, 300).

Pathogenesis of upper gastrointestinal dysfunction in diabetes

Despite the prevalence of abnormal upper gastrointestinal sensation and delayed gastric emptying in the diabetic population *there is currently no satisfactory pharmacological treatment for diabetic gastroparesis*, *due to a lack of understanding of the underlying molecular pathophysiology*.

Gastrointestinal myopathy may, in part, contribute to delayed gastric emptying, as gastric muscle strips from diabetic rats show impaired contractile responses to myogenic stimuli (339). But gastric smooth muscle degeneration is found in only a limited number of patients with gastroparesis (87) while larger studies have reported no abnormalities in the gastric wall (373). Reduced axon density and changes in dendrite structure of extrinsic autonomic (309) and enteric neurones (99) that innervate the gastrointestinal tract have been described in a number of morphological studies in animal models of diabetes, and suggest an underlying impairment of autonomic function. Deficiencies in 5-HT, substance P and calcitonin gene-related peptide (CGRP) neurotransmitters have also been shown (14, 219). The ability of a 5-HT_3 receptor antagonist to reduce duodenal motor activity is impaired in diabetic rats (338) and nitric oxide synthase (NOS) immunoreactive cells are reduced in the gastric myenteric plexus (341). The latter is a potentially significant finding as nitric oxide is an important transmitter in intestinal feedback control of gastric emptying (247). A significant reduction in the number of interstitial cells of Cajal (ICC), the pacemaker cells of the myenteric plexus, and their associations with enteric nerve terminals have also been demonstrated in diabetic mice (246).

How well these changes reflect the situation in human diabetes is not yet established. Diabetic gastroparesis is only weakly correlated with autonomic dysfunction, so neuropathy is unlikely to be the major underlying cause (149). Patients with diabetic gastroparesis do not differ from control subjects in the number of axons in the abdominal vagus nerve (373). A reduced number of ICC have been described in

human gastric biopsies from patients with type 2 diabetes and intestinal biopsies from a patient with type 1 diabetes (118, 158). A reduction in neurotransmitter content for NO and substance P have also been described in these biopsies.

Within the epithelium, the number of duodenal enteroendocrine cells containing gastric inhibitory polypeptide (GIP) or CCK are decreased and the number of secretin-containing cells are increased in diabetic mice (85, 328, 338). However there has been limited investigation of changes in the regulation and release of gastrointestinal hormones and neurotransmitters in animal models of diabetes or patients, as most focus has been directed toward investigation of potential neural dysfunction.

Blood glucose concentration and the rate of gastric emptying rate share an inverse relationship in both health and diabetes, although the mechanisms governing this relationship are unknown (272, 275). It is also unknown whether prolonged hyperglycaemia is directly responsible for morphological changes in the gut.

Altered intestinal feedback in diabetes

Altered responsiveness of nutrient feedback mechanisms in the small intestine may lead to abnormal gastrointestinal motor and sensory function in patients with diabetes, particularly if nutrient signals are inappropriately intense (187). Such hypersensitivity could markedly prolong gastric emptying time, however, this possibility has not been well studied (277). In some studies of patients with functional dyspepsia it has been shown that heightened perception of intestinal nutrients contributes to altered sensory and motor functions, and that these responses are nutrient specific (12, 316). These data suggest that alteration in mucosal sensors and/or their connected neural circuitry are critical to heightened nutrient sensitivity.

Equivalent studies in patients with diabetes have not been undertaken and will be important to undertake during hyperglycemia as well as euglycemia (276, 277) due to the significant affects of blood glucose concentration on gut sensory and motor function. A study in Type 1 patients during euglycemia has demonstrated increased perception of proximal gastric distension compared to control subjects (278), providing evidence that visceral sensation is altered in diabetes. Evidence has also shown that intraduodenal nutrient infusions significantly increase duodenal motility in type 1 patients during euglycaemia compared to healthy controls (276). But direct studies assessing responsiveness of intestinal nutrient-induced sensory and motor functions during hyperglycemia have not been undertaken.

Intestinal nutrient detection systems are not well understood and therefore mechanisms of their plasticity in response to dietary modifications and disease states are unknown. Altered nutrient detection in the intestinal mucosa may occur via changes at the cellular or molecular level and experiments are required to resolve the relative contribution of these elements.

1.4 Peripheral glucose-sensing mechanisms

A range of mechanisms for nutrient-sensing across the plasma membrane of eukaryote cells are known, including nutrient detection by G-protein-coupled receptors (GPCRs), non-transporting nutrient carrier homologue receptors and nutrient transporters themselves (138, 286). In mammals, the best-characterised peripheral glucose sensing mechanism is that of the pancreatic β-cells which respond to increases in blood glucose by the release of insulin. The glucosensitivity of β-cells is achieved by the entry of glucose down its concentration gradient through GLUT2 (346). Intracellular glucose metabolism producing ATP is then the initiating step for membrane depolarisation via ATP-sensitive potassium channels (K_{ATP}) (217, 319). This coupling of glucose metabolism to the electrical excitability of the cell also operates in glucose-sensing neurons in the hypothalamus (310, 370). However the presence of non-metabolisable sugars in the small

intestine also initiates nerve reflexes that slow gastric emptying (269), supporting the view of a glucose sensing mechanism that does not operate through cellular metabolism, such as a GPCR signalling cascade. Recently, evidence has emerged showing GPCRs that act as nutrient sensors in the gastrointestinal tract. The extracellular calcium-sensing receptor, CaR was shown to act as an amino acid sensor (51, 52) and the previously orphan GPR40 receptor, which is abundantly expressed in the pancreas, was found to be activated by free fatty acids and to facilitate insulin secretion (157).

1.4.1 Taste transduction on the tongue

Anatomy and innervation of lingual taste cells

Nutrients are first detected on the tongue, where taste discrimination occurs between different compounds. This perception is vital for health and wellbeing of the individual, with sweet and savoury sensations promoting the intake of high caloric compounds of nutritional benefit, while bitterness leads to avoidance of noxious or toxic substances.

'Taste' in its true sense refers strictly to the conscious perception of sapid compounds signalled through activation of taste receptor cells in the oral cavity. This process involves two steps; initial detection by sensor 'taste' cells followed subsequently by activation of sensory nerve fibres, allowing transmission of gustatory signal to the CNS.

The sensory nerve projections to the tongue are classified either as perigemmal fibres, those that do not directly innervate taste buds but surround them and relay supplementary gustatory information such as from thermal and tactile stimuli, or intragemmal fibres which innervate taste buds to receive input arising from taste stimuli (127). The sensory innervation of the oral cavity is derived from one of three main nerve

branches which each project to specific regions. Sensory fibres in the anterior two-thirds of the tongue originate from one branch of cranial nerve VII, the chorda tympani, while another branch, the greater superficial petrosal, innervates the palate. The glossopharyngeal nerve (cranial nerve IX) sends projections to the posterior tongue while the superior laryngeal nerve (vagus, cranial nerve X) innervates the larynx and epiglottis (127, 318).

The surface of the tongue possesses specialised structures known as papillae, comprising four distinct forms, each with specific topographical locations. Filiform papillae are most common and distributed across the surface of the tongue; these are not involved in chemoreception but play a role in tactile sensation (327). The sensory units of the gustatory system, taste buds, are embedded in the epithelium of the other three papillae types; the large circumvallate papillae located on the posterior tongue, foliate papillae which form small trenches on the posterior lateral edges and the smallest protrusions, the fungiform papillae which are scattered throughout the anterior two-thirds of the tongue (Figure 1.4.1) (41, 318, 327). The density of taste buds in each papillae type differs, with hundreds located in individual circumvallate and foliate papillae, while few (1-2 taste buds) are found within fungiform papillae.

Taste buds are comprised of ovoid clusters of 50-100 cells, containing individual cells dedicated to taste reception and supporting, precursor and basal cells (127, 191). Taste cells themselves are spindle-shaped, bipolar cells which exhibit morphological specialisations including apical microvilli that project to the open ‘taste pore’ of the taste bud, and a comparatively large basolateral membrane allowing synaptic connections with sensory afferent fibres. Taste cells are considered specialised neuroepithelial cells and share many neuronal properties, such as the ability to generate action potentials and subsequently release neurotransmitters. Taste cells are also distributed within the oral cavity, in the epithelium of the soft palate and epiglottis.

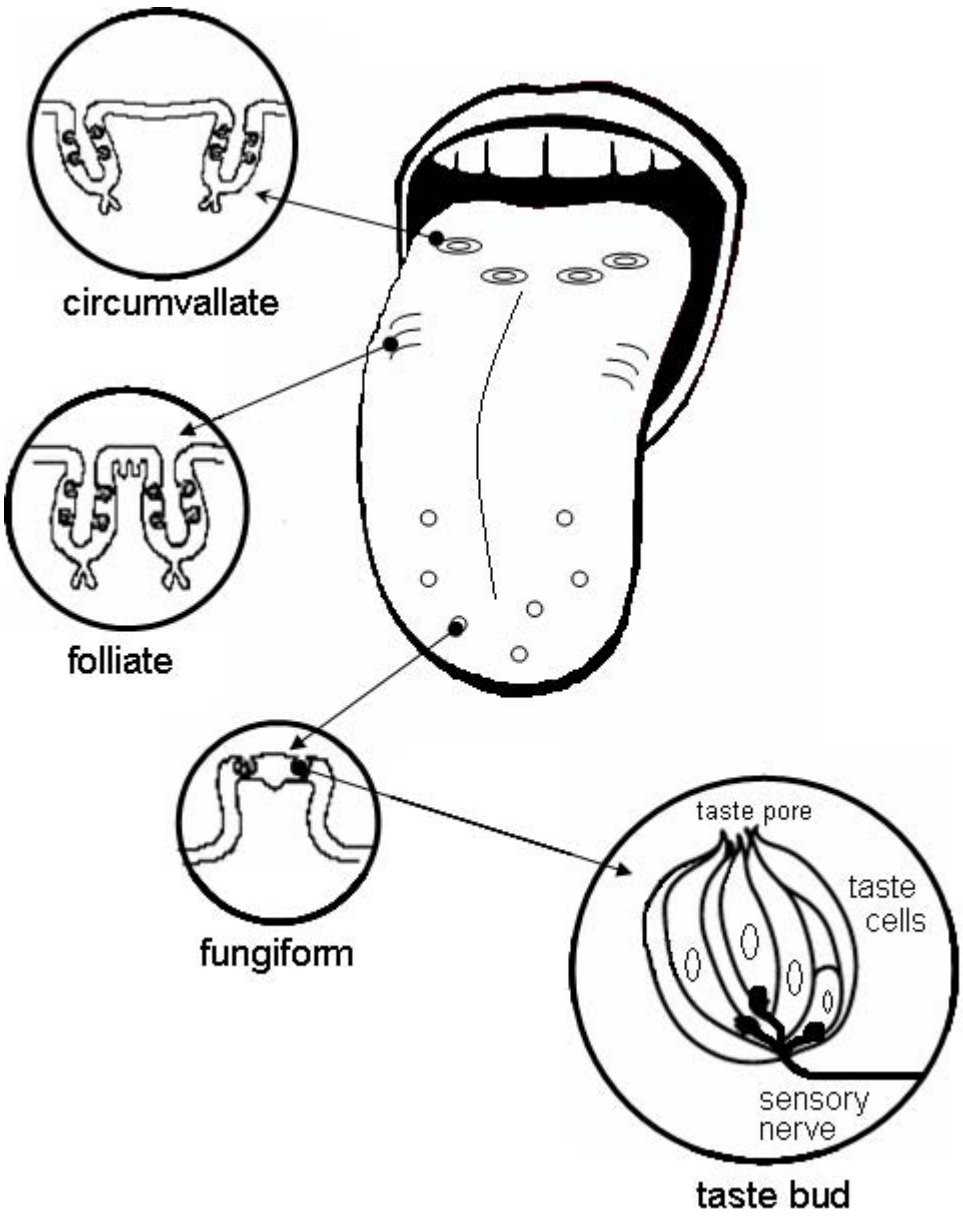

Figure 1.4.1 Anatomical arrangement of taste cells on the tongue.
Taste receptor cells are clustered into groups of cells know as taste buds. In this arrangement taste cells have access to sapid molecules in the oral cavity via their apical membrane located at the taste pore while sensory nerves innervate the basalolateral portion of the taste bud. Taste buds are located within three papillae types on the surface of the tongue; circumvallate, foliate and fungiform.

Taste cells within the taste bud have been classified into three distinct morphological types of cells. Type I cells are the most common cell type in the taste bud, they contain dense granules in their apical region and a supportive or glial-like role in the taste bud has been suggested for them (191, 261). Type II cells are taste receptor cells as they specifically express taste transduction molecules (47, 369). Although Type II cells are closely associated with afferent fibres, they do not form direct synapses (47). Type III or intermediate cells on the other hand are not thought to be directly receptive through these same transduction pathways but do directly synapse with intragemmel nerve fibres (191, 372).

Molecular mechanisms of taste transduction

Taste sensations are classified into five distinct modalities: sweet, bitter, salty, sour and the most recently recognised umami, the taste of L-amino acids (41, 127, 173, 190, 318), although other taste modalities such, as that of fatty acids, are now being recognised (107, 205, 221). The division of taste into these five categories have been corroborated with the discovery of dedicated molecular receptive mechanisms for each.

Salty and sour tastes are evoked by the direct interaction of tastants with ion channels in the membrane of the taste receptor cell (TRC) resulting in cellular depolarisation (41, 173, 190, 318). In contrast, the molecular mechanisms of bitter, sweet and umami (L-amino acid) taste transduction have been revealed by the identification of two distinct mammalian taste GPCR families (1, 142, 380). The T1R family consist of three receptor members which heterodimerise in unique combination to form umami receptors (T1R1 + T1R3) or a broadly tuned sweet taste receptor, responsive to a wide range of natural and artificial sugars (T1R2 + T1R3) (236, 237, 380). In contrast, bitter taste is mediated by the TR2 family of receptors which comprise ~30 divergent members which respond to a vast repertoire of bitter stimuli (1).

The G-protein alpha subunit of gustducin ($G\alpha_{gust}$), is uniquely expressed in taste cells of the tongue and is specific for taste transduction (208). This form of $G\alpha$ specialisation is also found in other sensory systems such as vision, where photoexcitation of receptors in the retina is signalled specifically through $G\alpha$-transducin (208). $G\alpha_{gust}$ is common to transduction of taste modalities that are signalled through T1R and T2R GPCRs as is able to couple equally to both types of receptors which for the most part are located in distinct taste cell populations (119, 120, 364). Along with the $\beta\gamma$ subunits of gustducin, $G\alpha_{gust}$ initiates secondary messenger systems in taste cells including activation of phosphodiesterase, phospholipase Cβ2 (PLCβ2) and adenylate cyclase (108, 173), leading to production of intracellular messenger inositol-1,4,5-trisphosphate (IP_3) and gating of the taste specific transient receptor (TRP) channel TRPM5 (377). These transduction events ultimately depolarise the cell to stimulate transmitter release and lead to sensory nerve activation (Figure 1.4.2). These key taste molecules involved in the detection of sweet are discussed individually in detail below.

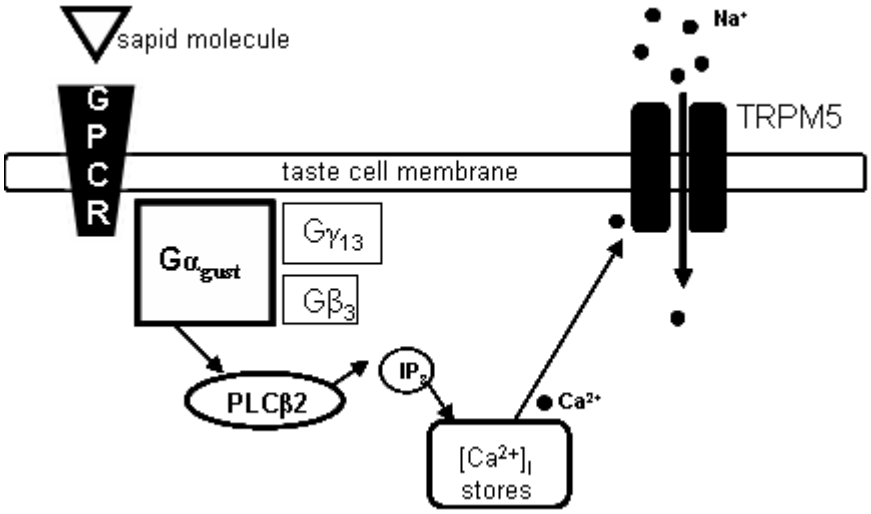

Figure 1.4.2 Molecular mechanisms of taste transduction.
The molecular mechanisms of sweet, bitter and umami taste reception are initiated via G-protein coupled receptors. Sapid molecules in the oral cavity bind to these receptors leading to the activation of the taste cell specific G-protein gustducin, consisting of $G\alpha_{gust}$, $G\gamma_{13}$ and $G\beta_3$ subunits. This leads to activation of PLCβ2, IP_3 and release of Ca^{2+} from intracellular stores, which results in gating of taste ion channel TRPM5. Cation influx through TRPM5 leads to membrane depolarisation, neurotransmitter release and activation of sensory nerve fibres allowing transmission of the signal to taste centres in the brain.

1.4.2 Key molecules in sweet taste transduction

Sweet taste receptors T1R2 and T1R3

T1R2 and T1R3 are members of the type C family of seven transmembrane GPCR which exhibit large extracellular protein domains thought to be involved in ligand binding (318). T1R2 and T1R3 were originally identified by searching genetic loci in mouse implicated in sweet taste sensitivity, the *Sac* locus on chromosome 4, for genes that encode GPCR which may be putative sweet taste receptors (142, 222). These subsequently identified candidates were found to be highly and specifically expressed in taste cells of the tongue but not expressed in non-taste lingual tissue or in other sensory or non-sensory tissues (142, 175, 206, 222). Each receptor showns specific topographic distribution on the lingual epithelium, with T1R3 expression found in all regions, while T1R1 expression was mostly confined to fungiform papillae and T1R2 most prominent in the circumvallate papillae (142, 175, 206, 222). The non-overlapping expression of T1R1 and T1R2 each with T1R3 expression in taste buds was suggestive of separate coexpression patterns of T1R1 + T1R3 and T1R2 + T1R3 receptor types, particularly when it was shown that very nearly all T1R2 expressing cells coexpressed T1R3 (172, 222, 237).

The observation that T1Rs encode taste receptors and specifically, that T1R2 and T1R3 combine to form a functional sweet taste receptor has been demonstrated in a heterologous expression system (237). Rat genes for T1Rs were expressed in HEK-293 cells individually and in combination with an array of Gα proteins which linked receptors to phospholipase Cβ. In this single cell reporter system any rise in intracellular calcium as a result of receptor activation was detected by fluorimetry with FURA-2 calcium indicator dye. Data showed that cells expressing T1R2 and T1R3 were responsive to range of sweet compounds including sucrose, fructose, saccharin, acesulfame-K and dulcin, an effect that was dependent on Gα [147]. Although these results functionally validated heterodimeric T1R2 and T1R3 as a receptor for a

wide range of sweet compounds no receptor activation was observed to a number of natural and artificial sugars, including glucose, galactose, maltose and aspartame.

Subsequent studies tested human T1R receptors in a similar cell based expression system to monitor activation via fluorimetry (185). In individual cells transfected with these human T1R2 and T1R3 receptors activation was apparent to natural sugars (uncluding fructose, galactose, glucose, lactose and maltose), artificial sugars (acesulfame-K, aspartame, cyclamate, dulcin, neotame, saccharin and sucralose), amino acids glycine and D-tryptophan and the sweet proteins monellin and thaumatin perhaps suggesting a species difference in receptor activation in response to various sweet stimuli. However, after expressing alternate Gα species in HEK-293 cells, resulting in improving receptor to G-protein coupling, this time cells transfected with rat T1R2 and T1R3 responded to all stimuli apart from aspartame and monellin (which are not sweet to rodents). Thus suggesting that lack of responses to sweet substances in the first study may purely be due to inferior binding interactions of particular Gα types expressed in their *in vitro* system. This study therefore provides stong support for T1R2 + T1R3 as a broadly tuned sweet taste receptor across species responsive to a range of sweet substances which are both biologically relevant and molecularly diverse.

Functional evidence for T1R2 + T1R3 as a broadly tuned sweet taste receptor has been provided by behavioural and physiological studies in KO mice deficient in expression of T1R2, T1R3 or both (double KOs). Taste preferences measured by short-term lick responses and long-term (48 hour) consumption volumes in two-bottle taste preference tests were altered in T1R2, T1R3 and double KO mice, which showed no distinguishable attraction to sugars (65, 380). Furthermore electrophysiological responses of lingual afferents (chorda tympani) to sugars were greatly diminished in these mice (65, 380). As expected, T1R KO mice displayed normal responses to sour, salty or bitter tastants (380). Interestingly concentrations of natural sugars exceeding 300 mM, but not artificial sugars, evoked a small response in lingual afferents

in T1R2 or T1R3 KO mice; this was absent in double KO mice (380). In a similar manner, T1R3 alone elicited responses to high concentrations of natural sugars in cell-based reporter systems (380). It is likely therefore that T1R3 may act individually as low affinity receptors for natural sugars. This is physiologically relevant as a population of lingual taste cells have been identified that express only T1R3 (237).

Given the wide array of potentially harmful bitter substances available within the environment, and the corresponding diversity of T2R bitter receptors, the evidence that T1R3 KO mice responded to sugars raised questions as to whether T1R3-independent mechanisms existed for sweet taste (65). While no additional members of the T1R GPCR family have been identified, the possibility of an unrelated receptor with a role in sweet taste detection cannot be excluded. However functional results to date showing that T1R in both humans and rats are broadly activated by a sugars and that double KO mice lack discernable responses to sugars provide strong evidence that the T1R2/T1R3 heterodimer is the principal, if not sole mammalian sweet taste receptor.

$G\alpha_{gust}$ and other transduction mediators

Electrophysiological and behavioural studies in KO mice have established that the taste G-protein, $G\alpha_{gust}$, is directly involved in sweet taste transduction. In these studies, mice lacking $G\alpha_{gust}$ showed no altered preference for salty or sour solutions in 48-hour two-bottle preference tests in comparison to wild-type mice; however, their aversion to bitter substances and preference for sucrose and sweetener SC45647 was severely impaired (364). These studies were the first to demonstrate a common role for $G\alpha_{gust}$ in the transduction of sweet and bitter taste signals. This behavioural preference test has been since expanded upon in a further study to show that mice deficient in $G\alpha_{gust}$ display significantly reduced, or lack responses to a large range of sweet tastants, including carbohydrates (sucrose and fructose), artificial sweeteners (dulcin, saccarin, NC00174, SC45647) and sweet amino acids (D-phenylalanine, L-proline, D-tryptophan) (66). The preference of $G\alpha_{gust}$ KO mice for sweet substances has also been studied in short-term studies to

avoid artefacts arising from post-ingestive feedback, other sensory inputs or learning effects. Quantification of the immediate lick response to small volumes of sweet test solutions compared to water in a 5s period show that deletion of $G\alpha_{gust}$ significantly reduced the lick responsiveness to the tested sweet substances (sucrose, maltose and SC45647) compared to responses in wild type mice (109).
Electrophysiological responses of lingual afferents in $G\alpha_{gust}$ KO mice also show a lack of responsiveness to sucrose or SC45647, even at concentrations that would normally be intensely sweet (364). It is known that lingual afferent nerves of both chorda tympani and glossopharyngeal origin show reduced responses to sweet tastants in $G\alpha_{gust}$ KO mice in comparison to wild-type mice (66). However these differences are more pronounced in chorda tympani recordings (66, 119), and in general, it appears that the chorda tympani is more responsive to sweet stimuli, while the glossopharyngeal nerves preferentially convey responses to bitter stimili (67). It appears therefore that the chorda tympani responds to sweet tastants in mice by signalling through $G\alpha_{gust}$.

$G\alpha_{gust}$ has been unequivocally shown to interact directly with T1R2 + T1R3 receptors in mediating behavioural responses to sweet tastants in mice (296). In this study a dominant-negative form of $G\alpha_{gust}$ was produced from a $G\alpha_{gust}$ transgene carrying a mutation in the C-terminus where interaction with GPCR occurs. The mutant $G\alpha_{gust}$ gene product acted as a dominant-negative in wild type mice, significantly reducing preference for sucrose solutions in 2-bottle preference tests.

This, and other studies have also shown that $G\alpha_{gust}$ null mice retain residual responses to higher concentrations of bitter and sweet tastants, suggesting that $G\alpha_{gust}$ independent pathways exist in sweet taste signalling (66, 296, 364). In $G\alpha_{gust}$ null mice insertion of a transgene encoding normal $G\alpha_{gust}$ restored sweet preference to wild-type levels, however, insertion of the mutant $G\alpha_{gust}$ transgene further decreased sweet preference (296). As the mutant α-subunit would likely bind all available $\beta\gamma$ gustducin subunits, it was proposed that residual taste responses in $G\alpha_{gust}$ null mice were mediated through other $G\alpha$-subunit species.

Indeed, a number of Gα proteins are highly expressed in taste-bud tissue, such as $G\alpha_{i2,}$ which directly couples to taste GPCR and is expressed in circumvallate taste cells at higher levels than $G\alpha_{gust}$ (180). A further α-subunit candidate for taste signalling is the structurally related rod α-transducin which is expressed in taste cells at lower levels than $G\alpha_{gust}$ (295). Expression of an α-transducin transgene in $G\alpha_{gust}$ knock out mice partially restored behavioural preference for consumption of sucrose solution (119), suggesting that α-transducin may be able to partially substitute for $G\alpha_{gust}$ in sweet taste transduction pathways. However, α-transducin KO mice themselves do not show reduced neural or behavioural responses to sucrose or SC45647 (120). Collectively these data show that although $G\alpha_{gust}$ independent mechanisms of sweet transduction exist in taste cells, $G\alpha_{gust}$ plays the predominant role in mediating behavioural and functional responses to sweet tastants.

Despite functional and behavioural evidence for $G\alpha_{gust}$ in sweet taste signalling, anatomical reports differ on the relative coexpression of $G\alpha_{gust}$ with T1R2+T1R3 in taste cells. T1R3 and $G\alpha_{gust}$ have been shown to be expressed in largely distinct cell populations in the circumvallate papillae of mice, with only 10% of cells identified by in situ hybridisation as expressing both molecules (172, 222). In contrast, bitter T2R receptors do colocalise with $G\alpha_{gust}$ expression. A low level of overlap has also been reported for T1R1 and T1R2 in rat foliate papillae with approximately 20% of T1R1 and 10% of T1R2 positive cells shown to also express $G\alpha_{gust}$ (142). Using a southern blot approach to investigate expression in individual mouse circumvallate taste cells it was shown that the majority $G\alpha_{gust}$ expressing cells coexpressed T1R3, while a subset of these also expressed T1R2 (206). This indicated that $G\alpha_{gust}$ was expressed in additional circumvallate taste cells to those expressing the T1R2 sweet taste receptor in mice. This study further demonstrated that immunolabelling for T1R3 was present in a subset of human circumvallate taste cells that immunolabelled for $G\alpha_{gust}$ (206) with the total number of $G\alpha_{gust}$ cells exceeding those containing T1R3, in direct support of findings from expression profiling.

Previous studies have shown that expression of sweet taste T1R2 is undetectable, or low, in fungiform papillae (142, 175, 222), in contrast to levels in circumvallate papillae. However, a recent study using a multi-labelling approach with riboprobes has established that approximately half of fungiform T1R3 expressing taste cells coexpress T1R2 and that this subpopulation also coexpresses $G\alpha_{gust}$ (172). This pattern of gene expression is functionally relevant as $G\alpha_{gust}$ could directly mediate sweet transduction in fungiform papillae located in anterior portions of the tongue, in accord with electrophysiological data indicating that nerve responses to sweet stimuli are strongest in the chorda tympani which innervates the anterior tongue (67). The different expression patterns reported for $G\alpha_{gust}$ may be due to differences in species and in relative sensitivities of the assays used, however, it is clear that significant populations of taste cells on the tongue coexpress T1R2, T1R3 and $G\alpha_{gust}$.

Heterotrimeric G proteins are comprised of α, β and γ subunits. $G\gamma$ subunits are diverse and can be expressed in a tissue-specific manner. In particular, the G protein γ subunit, $G\gamma_{13}$, has been shown to be specifically expressed in taste tissue (152). Southern hybridisation to single-cell RT-PCR product and immunolabelling in sections of taste bud tissue show that expression of $G\alpha_{gust}$ and $G\gamma_{13}$ is fully coincident (152, 206). Trypsin sensitivity assays show that $G\alpha_{gust}$ can interact directly with $G\gamma_{13}$, and $G\gamma_{13}$ has been shown to play a functional role in taste transduction as IP_3 generation in response to denatonium (a bitter tastant) is attenuated when the taste tissue is preincubated with antibodies against $G\gamma_{13}$ (152). $G\gamma_{13}$ can form stable dimers with $G\beta_1$ or $G\beta_3$ (25) and expression of these dimers in taste cells overlaps with $G\alpha_{gust}$ suggesting the $G\alpha_{gust}\beta_{1/3}\gamma_{13}$ heterotrimeric complex is responsible for the transduction of taste responses (152). Support for this role in the transduction of sweet as well as bitter has been demonstrated by evidence that the seven transmembrane domain of the human T1R2 receptor directly couples to $G\beta_1\gamma_{13}$ (297).

A novel subtype of PLC, PLCβ2, has been specifically identified in taste cells (289) where it coincides with expression of $G\alpha_{gust}$, $G\gamma_{13}$ and T1Rs (206, 377). Functional and behavioural studies in mice lacking PLCβ2

have demonstrated an essential role for this PLC isoform in the transduction of sweet taste. Lingual nerve responses to sweet stimuli are lost in PLCβ2 knockout mice and their preference for sweet substances in behavioural taste tests is profoundly reduced (377). PLC activation leads to generation of IP_3 which activates intracellular IP_3 receptors, leading to release of Ca^{2+} from internal stores and a direct increase in intracellular Ca^{2+} concentration. The IP_3 receptor isoform 3 (IP_3R3) is the only IP_3 receptor subtype expressed in taste cells at significant levels, and is found coexpressed with PLCβ2, T1R2, $G\alpha_{gust}$ and $G\gamma_{13}$ (47, 220). Depolarisation of taste receptor cells in response to sweet tastants is therefore likely to occur through release of intracellular Ca^{2+} via this pathway (15), however PLC activation also signals via diacylglycerol (DAG). Support for the IP_3 pathway as the major signalling pathway involved in taste transduction has been provided in a recent study in IP_3R3 knockout mice, which showed markedly reduced behavioural taste preference and lingual afferent responses to natural and artificial sugars compared to wild type mice (132).

TRPM5

The taste-specific transient receptor potential ion channel member M5 (TRPM5) provides the link between intracellular Ca^{2+} release (via IP_3R3) and membrane depolarisation in taste cells (257). TRPM5 is the taste-specific member of a large family of ion channels which are gated by intracellular Ca^{2+} release and which facilitate influx of extracellular ions leading to cellular depolarisation (262). The expression of TRPM5 in taste cells has been shown to coincide with expression of PLCβ2, IP_3R3, $G\gamma_{13}$ and $G\alpha_{gust}$ (257).

TRPM5 is a non-selective cation channel equally permeable to monovalant ions such as Na^+, K^+ and Cs^+ but impermeable to divalent ions such as Ca^{2+} (186, 260). The activation of TRPM5 channels in cellular expression systems occurs in direct response to a rise in intracellular Ca^{2+}, as shown in patch clamp experiments (194, 260) and not through depletion of intracellular stores (260). In intact mouse taste cells

TRPM5 activation has been shown to occur through both a rise in intracellular Ca^{2+} or an increase in intracellular IP_3 levels (378).

TRPM5-null mice completely lack functional and behavioural responses to a wide range of sweet and bitter tastants (377). These mice failed to elicit a lingual afferent response to natural or artificial sugars while robust increases in activity were observed upon application of these stimuli to wild-type mice. In contrast, taste responses to salty or sour tastants were unaffected, Two-bottle preference tests using sweet solutions versus water also supported electrophysiological results, with TRPM5-null mice failing to show normal preference for sweet solution over water.

1.4.3 Taste coding in taste cells at the periphery

A longstanding question of chemoreception in the tongue is how different taste qualities are encoded by taste buds. Specifically, whether individual taste cells recognise a single taste modality and convey this information through a single nerve fiber (the “labelled lines” model) or whether individual taste cells and/or fibres express receptors for, and respond to multiple taste qualities (the “across-fiber” model) (41). Type II cells in the taste bud are the primary receptor cells for taste modalities that are detected via GPCR (bitter, sweet and umami) (47). Double labelling experiments show that expression of T2R and T1R receptors do not overlap, with individual taste cells expressing either bitter or sweet/umami receptors (1, 237). Furthermore within the T1R expressing taste cell population T1R2 and T1R3 are coexpressed in all circumvallate, foliate and palate taste buds, while T1R1 and T1R3 are expressed together in fungiform and palate taste buds with an additional small population of cells showing non-overlapping expression of T1R3 (237). This expression profile would suggest individual taste cells are equipped to detect single bitter, sweet or umami stimuli. Indeed, evidence in PLCβ2-null mice has shown that sweet, bitter and umami tastes are encoded by different taste cell types despite signalling via common signalling cascades (377). Selective

engineering of PLCβ2-null mice (which lack responses to sweet, umami and bitter tastants) to express PLCβ2 only in cells that expressed T2R receptors restored normal taste responses to bitter stimuli only, while responses to sweet and umami remained severely impaired. Calcium imaging in lingual slice preparations in vallate papillae from mouse support this by showing that 82% of type II receptor cells elicit responses to only one type of taste stimulus (347). Indeed as it is well recognised now that the receptors for sweet, bitter, umami, sour and by extrapolation salty (41) are localised within mutually exclusive taste cell populations, a 'labelled lines' model of taste coding with dedicated primary taste receptor cells are tuned to detect a single taste modality. How taste information is then coded at the level of the neral circuitary is less clear and may not be restricted to a simple 'labelled lines' arrangement where a single taste cell has input into a single nerve fiber. Indeed something more akin to an 'across-fiber' model with signals from multiple sensors converging on single pathways may be more likely. However the complex picture of neurotransmission within and beyond the taste bud is only just immerging.

1.4.4 Neurotransmitters in taste: cell-to-cell communication and activation of gustatory afferents

Knowledge of the mechanism that taste receptor cells use to activate sensory afferents which carry gustatory information to the CNS is limited but insights into the process have emerged in recent years. This process is turning out to be more complex then the original concept that taste receptor cells released a single neurotransmitter and activate a single afferent via synaptic connections.

Type II taste cells are the primary sensor cells which express taste GPCR and signal transduction molecules, and although frequently associated with gustatory afferent fibres, possess no morphologically identifiable synaptic connections (47). Immunolabelling indicates that Type II cells also do not express a presynaptic SNARE protein (SNAP-25) which is associated with classical synapses in taste cells (46). On

the other hand, Type III taste cells do form classical synaptic connections with afferent fibres within the taste bud and express a number of presynaptic marker proteins, including SNAP-25 and a voltage-gated calcium channel (47, 73). This anatomical arrangement has lead to the hypothesis that communication occurs within the taste bud between Type II taste transduction cells and Type III synaptic cells.

Neurotransmitters known to be expressed in the mammalian taste bud include noradrenalin, acetyl choline, GABA, glutamate and serotonin (128). A number of neuropeptides have also been identified in taste buds including vasoactive intestinal peptide, cholecystokinin (CCK) and neuropeptide Y (129, 321, 379). Recently ATP has been implicated as an important taste neurotransmitter as gustatory nerves have been shown to be immunoreactive for ionotropic purinergic receptors $P2X_2$ and $P2X_3$ (26) and their nerve endings form close associations with $G\alpha_{gust}$ immunopositive cells (165). A functional role for ATP has been demonstrated in electrophysiological experiments in $P2X_2/P2X_3$ double knockout mice, where sweet, umami and bitter taste responses were abolished (93). Although ATP and other transmitters are critical in transmission of taste signals to afferent fibres their exact cellular targets and pathways of communication within the taste bud are not yet known; potential roles as synaptic transmitters, paracrine mediators or in cell to cell communication are all possible (287).

Type II taste cells do not contain voltage-gated Ca^{2+} channels (46), the channels through which Ca^{2+} influx occurs in conventional synapses and which facilitate vesicular release from the presynaptic membrane. Although TRPM5 is clearly involved in membrane depolarisation and transduction in taste cells (377), the transduction events which lead to neurotransmitter release downstream of TRPM5 are unknown. Release of transmitter may occur in type II taste cells through mechanisms independent of Ca^{2+} influx, or that depend on Ca^{2+} release from intracellular stores (287). Non-synaptic routes of transmission have also been considered, and include mechanisms such as paracrine secretion or gap junction communication between taste cells that allow transmission of membrane potentials or second messengers (such as IP_3) (287). In support of non-synaptic transmitter release, recent studies have shown that ATP is secreted from taste

receptor cells through multiple connexion and pannexin hemichannels in response to taste stimuli, via a mechanism that does not involve vesicular exocytosis (153).

1.5 Evidence for taste molecules in the gastrointestinal tract

Chemoreceptive mechanisms in the intestinal mucosa may closely mirror those of the tongue, with chemoreception by specialised epithelial cells which then transmit via sensory afferents. Indeed the concept of intestinal "taste cells" linked to sensory afferents were first hypothesised in 1982, based on observations in rat ileal crypts (239). Analogies have since been drawn with the gustatory taste system (100, 113). The taste modalities distinguished by the gustatory afferent system have equivalence in the gastrointestinal tract with sour detected as acid, salty as sodium chloride, sweet as carbohydrate, umami as protein and bitter indicating toxins (113). This view of an intestinal 'taste' mechanism is now gathering acceptance, based on recent evidence that expression of taste molecules are common to both the lingual and intestinal epithelium. Soon after the identification of $G\alpha_{gust}$ in lingual taste transduction, its restricted expression was shown in solitary epithelial cells within the gastric cardia and duodenum of the rat by immunohistochemistry (137). Double-labelling assays revealed that $G\alpha_{gust}$ immunopositive cells colabelled for the cytoskeletal markers villin and cytokeratin 18, typical of brush cells, which have morphological similarities to taste cells. In contrast, enteroendocrine cells immunopositive for chromogranin A and serotonin did not express $G\alpha_{gust}$ suggesting that these gut enteroendocrine cells did not have major roles in taste transduction. However chromogranin A is not a marker of all enteroendocrine subpopulations and *it is not known in other enteroendocrine cell types express taste transduction molecules*.

This expression of $G\alpha_{gust}$ in the gastrointestinal mucosa of both rats and mice has since been confirmed in immunoblots and by RT-PCR (137, 366). This approach has also been used to demonstrate expression of

$G\alpha_{gust}$ transcript and protein in STC-1 cells, an enteroendocrine cell line of mixed populations of gut endocrine cells (81, 366). Although not direct evidence of $G\alpha_{gust}$ in enteroendocrine cells *in vivo*, this provided the first indication that $G\alpha_{gust}$ expression may occur, at least in part, in populations of enteroendocrine cells.

Additional G protein subunits involved in lingual taste transduction are also expressed in the gastrointestinal mucosa. Transducin $G\alpha$ subunits are expressed in STC-1 cells and gastric and duodenal mucosa of the rat and mice (366), although immunohistochemistry performed in serial sections of gastric mucosa revealed transducin and $G\alpha_{gust}$ are expressed in separate cells suggesting that perhaps there are different subtypes of chemosenory enteroendocrine cells in the gut. Northern analysis of expression of $G\gamma_{13}$ in human and mouse tissues show that in addition to taste tissue, $G\gamma_{13}$ transcript was detected in retina and olfactory epithelium and brain, with lower levels positively identified in stomach and small intestine (152). Collectively these data show that subunits of the heterotrimeric G protein complex involved in taste have specific expression in gastrointestinal tissues.

T1R and T2R taste receptors are also expressed in gastrointestinal tissue. RNA transcripts for a number of bitter T2R receptor have been detected in rat and mouse stomach and duodenum as well as in the STC-1 cell line (366). However this study failed to detect transcripts for T1R1 and T1R2 using the same methods. Once the T1R3 was identified and classified as a sweet taste receptor (see 1.4.2), a survey of mouse tissue expression of this receptor was undertaken and northern blot analyses confirmed that T1R3 was expressed in taste tissue but failed to show expression in any other tissues examined, included stomach and small intestine (206). Although this documented highly restricted expression of T1R3 as a lingual taste receptor, standard northern procedures may have failed to detect lower level expression of T1R3 due to expression in solitary and dispersed cells in the gastrointestinal tract, compared to the high density of expressing taste cells in the tongue. Indeed, a later study using quantitative real-time PCR (which is more sensitive than both

conventional PCR and northern blotting) revealed transcript expression of both T1R2 and T1R3 in the mouse small intestine and STC-1 cells. This same study reported also reported protein expression assessed by western blot however the published results did not show a strong signal (81). The identification of T1R transcripts in STC-1 cells indicated that at least some subtypes of native enteroendocrine cells were likely to express T1R receptors, whereas absorptive enterocyte cell lines showed no evidence of T1R transcript expression (81).

Northern blot approaches have also been used to show that the taste transduction channel TRPM5 is expressed in lingual taste tissue and in the stomach and small intestine in mice (257). This provides direct evidence that TRPM5 is expressed in known $G\alpha_{gust}$ expressing tissues, which have chemosensory abilities.

In summary, there is clear evidence that RNA and protein for key molecules involved in sweet taste are expressed in the gastrointestinal tract. However definitive information about their expression, location and functions has not yet been fully documented.

1.6 Primary chemosensory cell types for carbohydrate detection in the small intestine

A wide array of candidate cell types exists in the intestinal mucosa that may serve as primary chemosensors of carbohydrates in the small intestine, triggering reflex feedback via vagal pathways.

1.6.1 Enterochromaffin cells

5-HT is an important signalling molecule in the gut where it acts both as a paracrine messenger and neurotransmitter (105). It is synthesised in serotonergic neurons, however the primary source is

enterochromaffin (EC) cells in the intestinal mucosa. 5-HT is strongly implicated in carbohydrate-induced inhibition of gastric emptying (266, 268). Luminal glucose has been shown to increase vagal afferent discharge in electrophysiological experiments and this response is attenuated when 5-HT stores in enterochromaffin cells (but not serotonergic neurons) are depleted (381). Vagal afferents innervating the duodenum in rats are also known to express 5-HT_3 receptors and pretreatment of the intestinal lumen with 5-HT_3 receptor antagonists functionally abolishes glucose-induced inhibition of gastric emptying (269). However there is some controversy as to whether 5-HT_3 receptor antagonists result in an acceleration of gastric emptying in humans. There is no evidence that one particular antagonist, ondansetron, has any effect on gastric motility after a solid meal or intestinal nutrient exposure (88, 238). Administration of an alternate 5-HT_3 receptor antagonist, tropisetron, has however been shown to result in accelerated emptying times of a solid meal (3, 207) although this result was not replicated in a study of females with anorexia nervosa (331). Although there is some evidence of the involvement of 5-HT_3 receptors in gastric emptying in humans, overall findings have been inconsistent and species differences in the 5-HT receptor subtypes involved may exist.

5-HT release from enterochromaffin cells occurs in response to mechanical stimulation of the mucosa (33, 171) and there is some additional evidence that luminal carbohydrates may be a direct stimulus for its release. Glucose perfusion of the isolated rabbit duodenum results in a 5-HT response in the venous drainage (204). A direct action of glucose on enterochromaffin cells leading to 5-HT release has been demonstrated using BON cells (a human carcinoid endocrine cell line) (170). However some caution in interpreting these findings as unequivocal evidence for direct stimulation of native enterochromaffin cells by glucose must be observed. These cultured cells differ to gastrointestinal enterochromaffin cells in that they secrete various peptides in addition to 5-HT (348). These cells are also not polarised in culture (31) making direct investigations of the sensory function of the apical pole difficult, as it is impossible to tell whether stimulation was via the apical or basolateral surface.

Various lines of evidence therefore exist to support a potential role for enterochromaffin cells as primary sensors capable of detecting glucose and mediating vagal afferent activation via release of 5-HT. *However the exact molecular mechanism by which glucose is able to trigger 5-HT release from enterochromaffin cells is not known.*

1.6.2 L cells

Glucagon-like peptide-1 (GLP-1) is a product of the proglucagon gene expressed in enteroendocrine L-cells distributed throughout the gastrointestinal mucosa, but most frequently in the distal small intestine and colon (84). L cells express other products of the proglucagon gene glicentin, oxyntomodulin and glucagon-like peptide 2 as well as peptide YY (229, 345). GLP-1 is an 'incretin' hormone in that it is released from the intestinal mucosa in response to luminal carbohydrate (283) and stimulates insulin release via GLP-1 receptors on pancreatic β-cells (9, 78, 211, 306). However the primary physiological role of GLP-1 appears to be to slow gastric emptying following a meal, leading to reduced food intake and appetite (94, 210). GLP-1 may exert these effects via actions at a peripheral or central site - circulating GLP-1 has direct access to the CNS through the blood brain barrier (248) and intracerebroventricular injection in rats reduces food intake (305). Equally, inhibition of gastric emptying by peripherally administered GLP-1 is abolished by dennervation of vagal afferents (155) and vagal neurons in the nodose ganglion have been shown to express GLP-1 receptors (234). However, due to the rapid breakdown of circulating GLP-1 by the catabolic enzyme dipeptidyl peptidase IV, the primary pathway for slowing of gastric emptying is likely to be via vagal afferent activation in the periphery following mucosal GLP-1 release (212, 213, 234). *The exact molecular mechanisms for sensing luminal glucose in L-cells and subsequent GLP-1 release, however, are unknown.*

1.6.3 Brush cells

Brush cells (also known as tufted or caveolated cells) are specialised epithelial cells found within the epithelium of the respiratory and gastrointestinal tracts (133, 134). Brush cells have a pear or bottle shape, an apical tuft of microvilli and long rootlets (304) and appear to have strong immunoreactivity for antibodies to villin and Cytokeratin 18 (134). The function of brush cells has remained elusive, however, based on their morphological similarities to the taste receptor cells of the tongue a chemosensory role has been proposed. Indeed, such speculation has increased with findings that brush cells in the stomach and intestine of the rat are immunoreactive for $G\alpha_{gust}$ (137). In the same manner as enteroendocrine cells, brush cells do not possess synaptic contacts with mucosal nerve fibres (136), however unlike enteroendocrine cells they do not contain secretory granules or transmitter vesicles. If brush cells play a role in chemosensation alternate signalling molecules would have to be utilised. It has been observed that brush cells in the rat stomach are strongly immunoreactive for nitric oxide synthase (179) so it is conceivable that brush cells may use at least nitric oxide as a gaseous messenger but functional studies are yet to be undertaken. Brush cells could be one of the primary taste cell types of the gut which transduce signals for direct activation of vagal nerve endings or alternatively they may play a paracrine signalling role to adjacent enteroendocrine cells. However the precise functions of this rare cell type is still largely mysterious.

1.6.4 Enterocytes

The primary absorptive cell type of the intestine, enterocytes, may themselves possess chemosensory ability. It is well established that enterocytes up-regulate their glucose transporter expression in response to a high carbohydrate luminal environment (89), a process mediated via activation of GPCR at the luminal surface (82). Enterocytes are also strongly immunoreactive for $G\alpha_{transducin}$ (the rod α-transducin which is also highly expressed in taste cells) at their apical border (133). Like brush cells, enterocytes are devoid of

secretory vesicles, and may use alternative signalling molecules such as gaseous mediators. It is possible that enterocytes are able to release ATP (317) which may act on near by enteroendocrine and/or nerve cells however, details of their role in nutrient signalling is limited.

1.6.5 Afferent neurons

Afferent endings themselves may be directly chemosensitive to nutrients such as glucose. The observation that vagal afferents terminate below the epithelial surface and do not have direct access to luminal nutrients (16) is integrated into the paracrine model of vagal afferent activation by mediators released by epithelial sense cells (112, 265, 266). However an alternate mode of vagal activation may occur directly at afferent endings within the lamina propria following transepithelial transport of glucose (113). Interestingly inhibition of gastric motility is abolished by application of phloridzin which blocks SGLT1 glucose transport (271) and afferent nerve discharge in rat ileum has been shown to to be evoked only by transportable hexoses (116), suggesting that sugar transport is a key event in nutrient signalling. Although afferents express many receptor types (174), the possibility of direct activation of vagal afferent endings by glucose, and putative vagal glucose receptors has not been explored.

1.7 Research objectives

Homeostasis of energy intake and control of digestive processes in response to dietary intake and disease are critical to survival of the organism. Feedback arising from the small intestine in response to the presence of nutrient is therefore central to this surveillance. The precise molecular mechanisms of intestinal chemosensation, however, are not known although evidence is gathering for taste transduction within the

gastrointestinal tract. The localisation of specific taste transduction molecules in the small intestine and comparisons of their expression under different dietary and disease states will reveal chemosensory roles taste receptors play within the gut, and their suitability for therapeutic targeting in human disease.

1.8 Aims and hypotheses

1.8.1 Aims

1. To localise and quantify expression of taste molecules throughout the small intestine in mice and humans.
2. To identify the phenotype(s) of intestinal epithelial cells expressing taste molecules in mice.
3. To assess whether gastrointestinal vagal afferents in mice are directly activated by exposure to GLP-1.
4. To identify altered expression of taste molecules in fed and fasted mice and in humans with type 2 diabetes.

1.8.2 Hypotheses

1. Taste molecules T1R2, T1R3, $G\alpha_{gust}$ and TRPM5 are expressed in the small intestinal mucosa in mice and humans.
2. Taste molecules are expressed in regions of the intestinal epithelium that detect carbohydrate, and in cells equipped to release 5-HT and GLP-1 activators of vagal afferents in mice and humans.
3. Expression of intestinal taste molecules is altered by nutritional status in mice, and increased in humans with type 2 diabetes thereby explaining the hypersensitive response to intestinal nutrient seen in some diabetics.