Taste Receptors in the Gastrointestinal Tract.

IV. Functional implications of bitter taste receptors in gastrointestinal chemosensing

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Sternini C. Taste receptors in the gastrointestinal tract. IV. Functional implications of bitter taste receptors in gastrointestinal chemosensing. Am J Physiol Gastrointest Liver Physiol 292: G457–G461, 2007. First published November 9, 2006; doi:10.1152/ajpgi.00411.2006—Changes in the luminal contents of the gastrointestinal tract modulate gastrointestinal functions, including absorption of nutrients, food intake, and protection against harmful substances. The current notion is that mucosal enteroendocrine cells act as primary chemoreceptors by releasing signaling molecules in response to changes in the luminal environment, which in turn activate nerve terminals. The recent discovery that taste receptors and G protein subunits α-gustducin and α-transducin, involved in gustatory signal transduction, are expressed in the gastrointestinal mucosa supports the concept of a chemosensory machinery in the gastrointestinal tract. An understanding of luminal sensing processes responsible for the generation of the appropriate functional responses to specific nutrients and nonnutrients is of clinical importance since aberrant or unstable responses to changes in luminal contents might result in disease states ranging from intoxication to feeding disorders and inflammation. The purpose of this theme article is to discuss the functional implications of bitter taste signaling molecules in the gastrointestinal tract deduced by their localization in selected populations of epithelial cells and their relationship with neural pathways responsible for the generation of specific responses to luminal contents.

α-gustducin; α-transducin; enteroendocrine cells; intrinsic afferent neurons; visceral afferent neurons; chemoreception

The Gastrointestinal Mucosa comes in direct contact with a vast array of potentially beneficial or harmful substances in the lumen and acts as a sensory organ by detecting luminal components and sending messages to the nervous system to initiate the appropriate response of digestion and absorption of nutrients or neutralization and expulsion of drugs, toxins, and microorganisms. Sensing of luminal content is also important for food intake control via gut-to-brain signaling pathways. This complex process of chemosensory perception is regulated by different sensors, including enteroendocrine cells and neural pathways (3, 7, 9, 15). Since nerve terminals do not reach the lumen and do not enter the mucosal lining, enteroendocrine cells serve as the first level of integration of information from the gut lumen acting as specialized transducers of luminal factors by releasing signaling molecules that in turn activate nerve fibers, which provide a second level of integration of gastrointestinal chemosensing (9, 15). The cellular and neural pathways that mediate the biological responses to luminal molecules are still elusive. The recent finding that receptors and G proteins mediating gustatory signals in the taste buds of the tongue are expressed in the gastrointestinal mucosa lining (16, 17, 27) has opened a new horizon of investigations for understanding the mechanisms underlying the functional responses induced by luminal content including harmful substances. The purpose of this theme article is to discuss the current knowledge on different detector systems responsible for the generation of specific responses to luminal contents in relationship to taste signaling molecules and their functional implications in chemosensing.

The Gastrointestinal Endocrine System

The enteroendocrine cells of the gastrointestinal tract are specialized cells intermingled with other epithelial cells. They secrete a variety of signaling molecules in response to changes in the gastric and intestinal luminal composition (3). They comprise the “open” endocrine cells with microvilli that project into the gastrointestinal lumen and sense luminal contents directly, which have been regarded as specialized transducers. Examples are the I cells of the duodenum and jejunum producing cholecystokinin (CCK), and the L cells of the ileum and colon either secreting glucagon-like peptide 1 (GLP-1) or peptide YY (PYY). Enteroendocrine cells also include the “closed” cells without microvillar luminal projections, which are mainly located toward the basement membrane, like the histamine-producing enterochromaffin-like cells. The secretory products of enteroendocrine cells, most of which are peptides, can either enter the bloodstream, hence acting like classic hormones and targeting other parts of the digestive system, or diffuse through the extracellular fluid, thus acting in a paracrine fashion on structures located in their vicinity, including nerve fibers.

Luminal chemical signals activate enterodendocrine cells with a certain level of specificity. For instance, the products of the breakdown of fats and of proteins like di- and tripeptides induce CCK release from the duodenum (19). CCK triggers the release of digestive enzymes from the pancreas and the emptying of bile salts from the gallbladder into the duodenum, which then induce protein and fat digestion. CCK release also regulates gastrointestinal motility, gastric emptying, and gastric acid secretion, perhaps through a local effect on nerve terminals. Indeed, it is now well established that CCK induces reflex inhibition of gastric emptying and satiation by stimulating vagal afferent endings by activating CCK-1 or CCK-A receptors (11). The primary nutrients that induce release of
PYY (2) from L cells of the distal ileum and colon are lipids and carbohydrates that can either act directly on PYY-producing cells or indirectly by neurohumoral signals deriving from the proximal gut. PYY plays an important role in the inhibitory feedback mechanism regulating nutrient transit, known as the ileal brake, by inhibiting gastrointestinal motility and secretion and reducing gastric emptying.

Neuronal Detector System

The neurons that detect luminal contents as well as muscle distension and mucosal mechanical distortion are referred to as sensory or afferent neurons in that they perceive changes and transmit information through a complex network, the brain-gut axis. These comprise extrinsic primary afferent neurons, whose cell bodies are in the vagal and spinal dorsal root ganglia, and intrinsic primary afferent neurons (IPANs), whose cell bodies are located in the gut wall and are a component of the enteric nervous system (ENS) (8, 12).

The vagus nerve is a key component of the extrinsic, afferent pathway between the gut and the brain that transmits meal-related signals. Vagal afferents are widely distributed to the gut wall where they arborize extensively, including the mucosa, covering wide areas with endings in the lamina propia. Vagal afferent discharge is activated by the macronutrient content of the gut lumen resulting in reflex changes in gastrointestinal function and inhibition of food intake (23). In addition, vagal afferents are involved in reflexes that could be associated with vomiting, nausea, and satiety (1). Vagal afferent terminals are found in close vicinity to enteroendocrine cells, providing the anatomical substrate for sensory transmission from these cells, which are likely to be the sensory transducers of luminal contents, and the nervous system. For instance, a close relationship between duodenal vagal afferents and CCK immunoreactive cells has been described in the duodenal villi, supporting the concept that CCK released by nutrients, particularly fats, in the gut lumen activates vagal afferents by interacting with CCK 1 receptors.

The IPANs, which are also known as Dogiel type II or AH neurons on the basis of their shape and electrophysiological properties, are the first neurons in the ENS that perceive changes and send information through a complex network, the brain-gut axis. These IPANs are responsible for generating reflex responses to changes in the intestinal contents by activating secretomotor, vasodilator, and motoneurons through neural circuits within and between the submucosal and myenteric plexuses, inducing changes in motility that in turn result in mixing and propulsive movements and changes in secretion and blood flow. Peristaltic and secretory reflexes originating from the mucosa are dependent on submucosal intrinsic sensory neurons, whereas reflexes generated by muscle stretching are mediated by intrinsic primary neurons located in the myenteric plexus. As for the extrinsic afferent neurons, the detection of the luminal contents by the intrinsic sensory neurons is likely to occur indirectly via the release of signaling molecules from the enteroendocrine cells, since their terminals do not reach the lumen itself.

Neuronal activation of either intrinsic or extrinsic afferent neurons could also occur by postabsorptive mechanisms, in which case substances absorbed from the lumen could serve as the intermediary step between the luminal contents and the neurons. This pathway seems less likely than the preabsorptive pathway involving release of signaling molecules given the level of selectivity in response to nutrients. In addition to nutrient signals, other lumen contents, including nonnutrient chemicals, microorganisms, drugs, and toxins, are important in signaling across the gastrointestinal mucosa and they also initiate changes in digestive function. The initial molecular recognition events that sense the chemical composition of the luminal contents are poorly understood. However, there is now evidence for the expression of a number of different membrane and intracellular proteins by specific endocrine cells that might be involved in gastrointestinal chemosensing and mediate the biological responses to luminal molecules. These include taste receptors and Gα proteins mediating taste signaling in the lingual epithelium, which might serve as chemosensory receptors in the gut.

Taste Receptors: From the Tongue to the Gut

The body encounters tastants in the oral cavity, where they act on taste buds, which are sensory structures located in the lingual epithelium. Taste buds have specialized cells, taste receptor cells, that detect putative nutritive substances (sugars, proteins, fat, salt) at submolar concentrations and potentially harmful compounds (toxins, drugs) at submicromolar concentrations, thus preparing the gastrointestinal tract for digestion or for rejection and expulsion of the oral content compounds (14, 18). The distinction among tastants is important for survival and nutrition. For instance, bitter taste might serve as a warning signal protecting against the ingestion of toxic substances, like toxins (26), by inducing nausea and vomiting or by impairing gastric emptying resulting in delayed delivery of toxins to the gut. By contrast, sweet or palatable taste might stimulate saliva as well as gastric and pancreatic secretions, thus preparing for digestion and absorption. Taste receptor cells contain an array of proteins including ion channels, ligand-gated channels, transporters, and G protein-coupled receptors (GPCRs) that serve as receptors for different tastants. Activation of taste receptor cells by a vast array of stimuli ranging from ions to complex structures like proteins induces membrane depolarization with the release of signaling molecules activating nerve reflexes and adjacent cells (18) through different mechanisms. For instance, ionic stimuli like salt and some amino acids activate taste receptor cells by direct interaction with ion channels, resulting in cell membrane depolarization and activation of voltage-dependent Ca2+ channels with consequent Ca2+ entry into the cell. By contrast, complex stimuli like sweet and bitter-tasting components and some amino acids activate GPCRs, inducing the synthesis of second messengers, including cAMP and IP3, leading to the release of Ca2+ from intracellular stores (14).

A large family of taste receptors that mediate bitter gustatory signals, named T2Rs (about 30 members), and a small family of taste receptors that mediate sweet signals, including t-amino acids, (T1R1, T1R2, and T1R3) have been discovered and cloned (5, 22). Taste receptors belong to GPCR superfamily, a large and versatile class of cell surface signal transducing proteins, which activate different effector systems to induce a variety of biological functions. Taste receptors are expressed in the tongue taste buds in humans and rodents and interact with specific Gs subunits, α-gustducin and α-transducin, that mediate gustatory signaling in the taste buds of the lingual
epithelium (4, 21). The involvement of α-gustducin in bitter taste transmission has been confirmed by in vitro and in vivo studies in animals with deletion of the α-gustducin gene. Functional studies using Ca²⁺ imaging to measure the response of taste receptor cells to bitter stimuli combined with immunohistochemical analysis of α-gustducin distribution showed that many but not all bitter-sensitive taste cells contain α-gustducin and that deletion of α-gustducin resulted in reduced aversion to bitter compounds and significant reduction but not elimination of Ca²⁺ response to bitter stimuli (4). These observations support the existence of G protein α-subunits other than α-gustducin in bitter taste transduction, including α-transducin (20). Indeed, the α-subunits of transducin that are expressed by the photoreceptor cells of the retina are also localized to vertebrate taste cells, where they are implicated in intracellular taste signaling (21). Taste receptor stimulation results in the activation of Gα proteins like α-gustducin and α-transducin, which in turn activate second messengers like cAMP and phospholipase C to elevate intracellular Ca²⁺ to induce transmitter release (20).

In addition to the oral epithelia, the expression of taste signal transduction elements, including α-gustducin, α-transducin, and members of the T2R family of bitter taste receptors, has been reported in the gastric and intestinal mucosa and in the pancreas (16, 17, 25, 27) in humans and rodents as well as in enteroendocrine cell lines in culture. Transcripts for α-gustducin and α-transducin (the latter being mostly the Gα-transducin 2) are expressed in the mucosa of rodent stomach and duodenum. Similarly, immunoreactivities for α-gustducin and α-transducin are localized to epithelial cells in the corpus, antrum, and intestine of the rat (17, 27), mouse (Fig. 1A), and humans where it is prominent in the colonic mucosa (25) as well as in the pancreatic duct (16).

The identity of epithelial cells expressing taste receptors and the α-subunits of the heterotrimeric G proteins gustducin and transducin is still largely unknown. It is likely that these signaling molecules are expressed by distinct types of epithelial cells that might differ in different species and regions. For instance, α-gustducin immunoreactivity has been described in a distinct population of epithelial cells in the rat gastric and intestinal mucosa and in the rat pancreatic duct system (16, 17) as well as in the mouse stomach (24) (C. Sternini, unpublished observations) that are known as brush or caveolated cells, which are characterized by the apical and basolateral expression of villin and the lack of intracellular secretory vesicles. Brush cells are a population of specialized epithelial cells that are scattered within the epithelial lining of the respiratory and gastrointestinal tract including the gallbladder and biliary and pancreatic ducts. In the stomach, brush cells are particularly

Fig. 1. Expression of α-gustducin immunoreactivity (IR) (A) in the mouse antrum. Colocalization of α-gustducin IR (B) and chromogranin A IR (C) is shown; D is an overlay of both stainings. Arrows in C and D show a cell with chromogranin A IR lacking α-gustducin IR. Bars = 5 μm. Tissue was fixed and processed for immunohistochemistry as previously described (25).

Fig. 2. Diagram illustrating levels of detection and integration of information from the gastrointestinal lumen and different pathways that are likely to be activated. Chemicals in the lumen (nutrients, drugs, toxins) are detected by the mucosal epithelium containing specialized cells, the enteroendocrine cells, that release hormones (endocrine message) that enter the circulation or signaling molecules that activate extrinsic afferent neurons (predominantly vagal), thus sending neuronal messages to the central nervous system (CNS), and intrinsic primary afferent neurons (IPAN) in the enteric nervous system (ENS), thus inducing intrinsic reflexes involving enteric neurons (interneurons and motoneurons).
abundant in the cardiac region. They have a pear or bottle shape with microvilli projecting to the lumen and a thin process reaching the lamina propria (15). The function of these cells is not clearly understood, but several roles have been proposed, including resorption, secretion, and reception, mostly on the basis of their morphology (15). These cells resemble taste receptor cells of the tongue because of the presence of a prominent tuft of microvilli and the high content of cytokeratin. These ultrastructural similarities together with the presence of α-gustducin have lead to the hypothesis that brush cells might play a role in chemoreceptive signaling. However, immunoreactivity for α-gustducin and α-transducin in the mouse gut is also found in subpopulations of endocrine cells, as indicated by colocalization with markers for enteroendocrine cells, including chromogranin A, a marker for large dense core vesicles (Fig. 1, B–D) or synaptophysin, a marker for synaptic-like microvesicles. In human colon, α-gustducin appears to be confined to endocrine cells since most of them contain chromogranin A, but not cytoplasmic villin (25). A subpopulation of α-gustducin immunoreactive cells contains immunoreactivity for PYY and GLP-1 (25). PYY is synthesized by L open cells of the ileum and colon, it is released in response to fatty acids and other nutrients, and it acts as a major regulator of food intake (2). PYY affects a variety of functions in the gastrointestinal tract and reduces food intake in mammals, including humans; furthermore, like bitter stimuli, it evokes an averse food response in mice (13). GLP-1 also plays an important role in nutrient response, food intake, and satiety, as well as glucose homeostasis when released in response to luminal chemicals (10). It is likely that α-gustducin is expressed in other types of endocrine cells. Likely candidates are the CCK-containing cells of the small bowel, which would be consonant with the finding that bitter tastants induce CCK release from cell lines that express bitter taste signaling molecules (6). By contrast, the distribution of α-gustducin cells appears to be distinct from those containing serotonin, which are open cells (25). Furthermore, which types of endocrine cells contain α-transducin still remains to be elucidated.

**Functional Implications of Bitter Taste Receptors and G Proteins in the Gastrointestinal Tract**

The presence of multiple transcripts for bitter taste receptors in the gut mucosa and the localization of Gα proteins involved in taste transduction in specialized cells (enteroendocrine and brush cells) of the mucosa lining suggest that the gastrointestinal tract participates to the complex chemosensory processes that determine whether ingested substances are beneficial thus initiating digestion and absorption or harmful thus inducing a protective response including vomiting and aversive behavior. Whether taste receptors in the gut mucosa are functional and how they convey a message across the epithelium to induce a functional response appropriate to the luminal stimuli are still open questions that need to be addressed. The findings that bitter tastants induce an increase in intracellular Ca²⁺ in enteroendocrine cell lines expressing bitter taste receptors and Gα proteins (6, 25, 27) provide indirect evidence for the functionality of bitter taste receptors outside the tongue and suggest that the same signaling pathways mediating taste signaling in lingual taste cells could be operative in enteroendocrine cells. It is reasonable to postulate that activation of bitter taste receptor signaling molecules expressed in enteroendocrine cells by luminal content (nutrients, drugs, and toxins) induces increase in intracellular Ca²⁺ that triggers release of peptides, like PYY and GLP-1, as suggested by their colocalization with α-gustducin (25) or CCK as suggested by its release by intestinal endocrine cell lines in response to bitter agonists (6). Once released, signaling molecules either enter the circulation to reach peripheral targets thus acting as classical hormones or activate neuronal pathways, including extrinsic afferent neurons (predominantly vagal), which send neural messages to the central nervous system, and intrinsic afferent neurons in the ENS, which induce intrinsic reflexes (Fig. 2). Indeed, bitter tastants, when administered into the stomach by oral gavage, induce increased expression of the immediate-early gene product, c-Fos, a neural activity marker, and increased number of c-Fos-positive neurons in the nucleus of the solitary tract, which appears to be in part vagally mediated (H. Raybould and C. Sternini, unpublished observations), indicating activation of extrinsic afferent neurons. This is in agreement with the observation that intraoral and intra-gastric stimulation with different chemical solutions induces c-Fos expression in the nucleus of the solitary tract (28) at different levels depending on the site of stimulation. Ingested substances could also be sensed by other intestinal epithelial cells, like the brush cells that have been shown to express Go proteins (17). Sensing of luminal content by these cells could trigger release of nitric oxide, since brush cells contain high levels of nitric oxide synthase immunoreactivity (15), which in turn could activate adjacent enteroendocrine cells or neuronal processes innervating the villi (15).

In summary, it is becoming apparent that the gastrointestinal mucosal lining is equipped with a chemosensory machinery responsible of triggering the appropriate response to specific nutrients or harmful substances, thus preparing the gut to either absorb them or initiate a protective response. Additional studies are required to unravel the pathways by which luminal contents activate detector systems (endoctrine cells and neurons) that generate integrated responses ranging from secretion, motility, absorption, or aversion to more general behavior like food intake. An understanding of chemical sensing processes responsible for the generation of the appropriate functional response to specific nutrients and nonnutrients is important for developing therapeutic agents to treat conditions like feeding disorders, intoxication, or inflammation that could result from altered or uncontrolled response to changes in luminal contents.

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