Taste Receptors in the Gastrointestinal Tract.

I. Bitter taste receptors and α-gustducin in the mammalian gut

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Rozengurt, Enrique. Taste Receptors in the Gastrointestinal Tract. I. Bitter taste receptors and α-gustducin in the mammalian gut. Am J Physiol Gastrointest Liver Physiol 291: G171–G177, 2006; doi:10.1152/ajpgi.00073.2006.—Molecular sensing by gastrointestinal (GI) cells plays a critical role in the control of multiple fundamental functions in digestion and also initiates hormonal and/or neural pathways leading to the regulation of caloric intake, pancreatic insulin secretion, and metabolism. Molecular sensing in the GI tract is also responsible for the detection of ingested harmful drugs and toxins, thereby initiating responses critical for survival. The initial recognition events and mechanisms involved remain incompletely understood. The notion to be discussed in this article is that there are important similarities between the chemosensory machinery elucidated in specialized neuroepithelial taste receptor cells of the lingual epithelium and the molecular transducers localized recently in enteroendocrine open GI cells that sense the chemical composition of the luminal contents of the gut.

T2R family; transducin; brush cells; gastrointestinal peptides; enteroendocrine cells

The gastrointestinal (GI) tract is a sensory organ that responds to a large array of signals originating in the lumen, including nutrient and nonnutrient chemicals, mechanical factors, and microorganisms. Molecular sensing by GI cells plays a critical role in the control of multiple fundamental functions in digestion, including secretory activity of GI glands, absorptive activity, motility, and blood supply of the intestinal tract. Furthermore, molecular sensing of luminal contents also initiates hormonal and/or neural pathways leading to the regulation of caloric intake, pancreatic insulin secretion, and metabolism. Molecular sensing in the GI tract is also responsible for the detection of ingested harmful drugs and toxins, thereby initiating responses critical for survival. Although these fundamental control systems have been known for a considerable amount of time, the initial molecular recognition events that sense the chemical composition of the luminal contents of the GI tract have remained elusive. Indeed, the receptors and signaling pathways that transduce the effects of many luminal molecules have not been identified, and the cellular and neural pathways that mediate biological responses to luminal stimuli in general, and bitter stimuli in particular, remain poorly characterized.

The gustatory system has been selected during evolution to detect nonvolatile nutritive and beneficial (sweet) compounds as well as potentially harmful (bitter) substances. In particular, bitter taste has evolved as a central warning signal against the ingestion of potentially toxic substances, including plant alkaloids and other environmental toxins (27). Recently, a family of bitter taste receptors (referred as T2Rs) expressed in specialized neuroepithelial taste receptor cells organized within taste buds in the lingual epithelium has been identified in humans and rodents (1, 7, 18). These putative taste receptors belong to the guanine nucleotide-binding regulatory protein (G protein)-coupled receptor (GPCR) superfamily, which are characterized by a short extracellular NH2-terminal segment, seven transmembrane α-helices, three extracellular loops (ex-loops), three cytoplasmic loops (cytoloops), and a COOH-terminal segment. Similarly, the GPCRs of the T1R family, namely T1R1, T1R2, and T1R3, characterized by a long extracellular NH2-terminal segment, have been identified as the receptors that perceive sweet substances, including amino acids (14, 20). Interestingly, the members of the T1R family of taste receptors function as molecular complexes (27). For instance, the heterodimeric T1R2/T1R3 sweet taste receptor binds sweet stimuli, whereas T1R1/T1R3 recognizes amino acids.

Heterotrimeric G proteins, composed of α-, β-, and γ-subunits, transduce external signals from heptahelical receptors to intracellular effectors. Genetic and biochemical evidence indicates that specific G proteins, gustducin (Ggust) and transducin (Gt), mediate bitter and sweet gustatory signals in the taste buds of the lingual epithelium (16, 27). More recently, a member of the transient receptor potential (TRP) family, TRPM5, has been specifically linked to bitter and sweet signal transduction (21, 29, 33). TRPM5 has been identified as a voltage-modulated, Ca2+-activated, monovalent cation channel that activates and deactivates rapidly, thereby inducing transient membrane depolarization (15, 22).

The notion to be discussed in this article is that there are important similarities between the chemosensory machinery elucidated in specialized neuroepithelial taste receptor cells of the lingual epithelium and the molecular transducers localized in individual GI cells that sense the chemical composition of the luminal contents of the gut. Consequently, the purpose of the present article is to discuss recent developments demonstrating the expression and function of molecular elements of taste signal transduction in individual cells interspersed among the mucosal epithelial cells in the lining of the GI tract and in enteroendocrine cell lines.

Expression of the α-Subunits of Gustducin and Transducin in GI Tissues

It is well established that gustducin plays an important role in mediating bitter and sweet gustatory signals in the taste buds of the lingual epithelium. Outside the lingual epithelium, expression of the α-subunit of gustducin (Gαgust) has been localized to gastric (13, 32) and pancreatic (12) cells, suggesting that a taste-sensing mechanism may also exist in the GI tract (31, 32). Furthermore, expression of transcripts encoding

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Go$_{gust}$ was demonstrated using RT-PCR performed on mRNA isolated from mucosal scrapings of the GI tract of mice and rats (32). Sequence analysis verified that a major PCR product of the predicted size generated in these reactions corresponded to amplified Go$_{gust}$.

The $\alpha$-subunits of transducins 1 (Go$_{t-1}$) and 2 (Go$_{t-2}$), originally thought to be expressed only in photoreceptor cells of the retina, are also present in taste cells of the lingual epithelium where they are implicated in intracellular taste signal transduction (16). Using RT-PCR, we detected Go$_{t-2}$ transcripts in the GI mucosa (31, 32). Collectively, these results indicate that Go$_{gust}$ and Go$_{t-2}$ are expressed in the mucosa of the GI tract of mice and rats and supported the notion that taste-specific signal transduction pathways may operate in the molecular sensing of the luminal contents of the GI tract (31, 32).

Are Go$_{gust}$ and Go$_{t-2}$ localized to the same GI cells? To answer this question, the expression of Go$_{gust}$ and Go$_{t-2}$ in rodent GI cells was examined by immunohistochemistry, using specific antibodies directed against unique amino acid sequences of Go$_{gust}$ and Go$_{t-2}$. As revealed by the examination of consecutive sections, the distribution and morphology of Go$_{t-2}$-positive cells were different from those of Go$_{gust}$-positive cells (32). Specifically, Go$_{t-2}$ was localized to cells present in the base rather than the apical region of the glands in sections of mouse fundic mucosa. Conversely, most Go$_{gust}$-positive cells of the fundus were located in the upper (neck) region of the glands, the isthmus, or the surface epithelium but not in the basal portion. Go$_{t-2}$-positive cells were found rarely in the antral mucosa, whereas Go$_{gust}$-positive cells were abundant in that zone of the stomach. Thus Go$_{gust}$ and Go$_{t-2}$ are expressed by different epithelial cell types in the gastric mucosa.

Recent studies demonstrated substantial expression of Go$_{gust}$ in the mouse colon mucosa, as judged by RT-PCR and immunohistochemistry (N. Rozengurt, S. V. Wu, M. Chen, C. Huang, C. Sternini, and E. Rozengurt, unpublished results). Go$_{gust}$ was localized to elongated cells present in the surface epithelium as well as in round cells located in the glandular epithelium of the colonic mucosa.

The precise identity of the GI cells involved in Go$_{gust}$-dependent signaling remains incompletely understood. A previous report indicated that in rats, Go$_{gust}$ is expressed by a distinct population of GI epithelial cells called brush or caveolated cells (13). These cells are characterized by apical and basolateral expression of the actin-bundling protein villin and lack of intracellular secretory vesicles typical of enteroendocrine cells. These cells contain microvilli with filaments that stretch into the cytoplasm, often forming rootlike structures. We confirmed that solitary Go$_{gust}$-positive cells contain villin immunoreactivity in the epithelium of the fundic region of the gastric mucosa. A typical villin-positive brush cell containing Go$_{gust}$ immunoreactivity in the fundic region of the mouse stomach is shown in Fig. 1 (top). In contrast to previous results (13), we also identified Go$_{gust}$-Positive cells that do not contain villin, which are especially evident in the antrum rather than in the fundus of the mouse stomach. As an example, Fig. 1 (insets and bottom) shows Go$_{gust}$-positive cells in the antral portion of the stomach that either contain or do not contain immunoreactive villin (N. Rozengurt, C. Sternini, and E. Rozengurt, unpublished results). These results support the notion that there are distinct subpopulations of Go$_{gust}$-positive cells in the mouse gastric epithelium. Interestingly, most Go$_{gust}$-positive cells in the mouse colon do not coexpress basolateral villin, further reinforcing the notion that Go$_{gust}$-dependent signaling operates in a variety of rodent GI cell types.

Expression of GA$_{gust}$ in Human GI Tissues

It is generally accepted that the brush or caveolated cells of the respiratory and digestive apparatus are the same cell type. Interestingly, it appears that this cell type is much less abundant in the GI and respiratory tract of normal humans than in animals (25). It is conceivable that Go$_{gust}$-signaling does not operate in the GI tract of humans or, alternatively, that Go$_{gust}$ might be expressed by a different population of specialized GI cells. Therefore, it is important to determine whether Go$_{gust}$ and receptors that mediate taste signaling are expressed by cells implicated in molecular sensing in the human GI tract.

Recently, we demonstrated the expression of Go$_{gust}$ in individual human cells of the GI tract and observed that these cells are prominently localized in the colonic mucosa (N. Rozengurt, S. V. Wu, M. Chen, C. Huang, C. Sternini, and E. Rozengurt, unpublished data). Using double-labeling immunofluorescence, we demonstrated that most Go$_{gust}$-positive cells in the surface and glandular epithelium of human colonic mucosa coexpress chomogranin A immunoreactivity, an established marker of endocrine cells in the GI tract. These results provide the morphological basis for the notion that Go$_{gust}$ signaling functions in endocrine cells of the human colon.

The endocrine cells of the GI tract represent <1% of the intestinal epithelium, but as a whole, they constitute the largest endocrine organ of the body, producing and releasing multiple hormones (24). In the context of molecular sensing, the open enteroendocrine cells have been regarded as specialized transducers of luminal factors that respond by releasing GI peptides at the basolateral side. In view of our recent results identifying a subpopulation of enteroendocrine cells that express Go$_{gust}$, it is reasonable to conclude that Go$_{gust}$-dependent signaling pathways may play a role in molecular sensing of the luminal contents by these cells. In view of these results, it is important to determine whether transcripts encoding members of the taste receptor families coupled to Go$_{gust}$, namely T1R and T2R, are expressed in the GI tract of mice, rats, and humans.

Molecular Organization of the T2R Family in the Mouse, Rat, and Human Genomes

To complete the identification of all members of the T2R family in the mouse and rat genomes, we undertook a bioinformatics homology-based screen of the mouse and rat genome for sequences related to the T2R family of bitter taste receptors. We searched the mouse and rat genome database at the National Center for Biotechnology Information using published sequences of taste receptors as well as a commercial database (Celera Discovery System). Potential T2R gene candidates were compared with known T2R sequences from the databases using the Blast program at both nucleic acid and protein level.

After we filtered through the criteria that define T2R family members (e.g., sequence homology, protein motif, site of expression, and ligand specificity), we determined a total of 36 true T2R genes in each species with small variations in pseudogene numbers and locations. Rodent genes are unevenly
distributed amongst three chromosomes. Two chromosomes each contain a single T2R gene (mT2R119 and mT2R134 and their respective rat orthologs), whereas the third chromosome harbors the rest of the T2R genes and pseudogenes in multiple clusters. Specifically, the genes in the mouse genome can be divided into three subgroups, based on their homology and location in chromosomes 15, 2, and 6 (3 clusters). Similarly, a total of 36 rat T2R genes is distributed in chromosomes 2, 3, and 4, respectively. Overall, mouse and rat T2R gene clusters are organized almost identically on mouse chromosome 6 and rat chromosome 4 based on our analysis of all available genomic assembly.

Using a similar analysis, we identified 25 T2Rs in the human genome. One hT2R is located on chromosome 5, and nine hT2Rs (plus 3 pseudogenes) are located in an extended cluster on chromosome 7. The remaining 15 hT2Rs (and 9 pseudogenes) are located in a dense cluster on chromosome 12. Analysis by others produced a similar number of intact human hT2R genes (5, 8, 23). A comparison between human and rodent T2R genes indicates that significantly higher numbers of rodent T2R genes exist as a result of higher frequency of gene duplication. Thus rodents possess ~30% more bitter taste receptors and probably a wider spectrum of bitter sense detection.

The most conserved T2Rs (e.g., T2R1, T2R4, and T2R38) between human and rodents have known bitter trait or ligands [6-n-propylthiouracil/sucrose octaacetate/phenylthiocarbamide (PTC)]. Denatonium benzoate (DB) is regarded as the most bitter substance perceived by humans and is widely used in taste warning. Potential T2Rs that recognize DB include hT2R4 and mT2R108 and hT2R44 and mT2R120, as demonstrated by their sensitivity to DB in a heterologous expression system. On the other hand, the ability to taste PTC is a well-documented Mendelian trait (4). PTC is bitter tasting to most people but not to some (nontasters). Recently, with the use of a positional cloning approach, the PTC receptor has been identified as a member of the T2R family (hT2R38) from the bitter locus on human chromosome 7q34–35. Three residues account for the genetic variation of PTC receptors in humans: the dominant P88, A262, and V297 as the taster phenotype versus the recessive A, V, and I at the same positions as the nontaster phenotype (4).

It is interesting that ortholog T2R receptors show considerable interspecies sequence variability and that the T2R reper-
Expression of Transcripts Encoding Members of the T2R Family of GPCRs in the GI Tract

Because gustducin and transducins are expressed in specific cells interspersed in the epithelium lining the GI tract, it was important to determine whether any member of the taste receptor families identified in taste cells of the lingual epithelium are also expressed in the GI tract. RT-PCR using subtype-specific primers detected multiple T2R transcripts in rat antrum, fundus, and duodenum (32). Amplified products were cloned and sequenced, confirming that they are identical to known taste receptor sequences. In contrast, RT-PCR using RNA isolated from a variety of other tissues and organs, including liver, heart, kidney, and brain as well as from the undifferentiated intestinal epithelial cell lines IEC-6 and IEC-18 did not detect any of these transcripts.

We also determined whether mT2Rs are expressed in the mouse GI tract. Initially, RT-PCR and sequencing analysis indicated that transcripts corresponding to mT2R119 were present in mouse gastric and intestinal tissues as well as in the tongue but not in other tissues, including liver, heart, and kidney (32). To verify that the taste receptors expressed in the GI mucosa are identical to those identified in taste cells from the lingual epithelium, we isolated the full-length cDNA of mT2R119 and mT2R108 from the gastric tissues and confirmed that their sequences were identical to those from the tongue.

More recently, we determined whether T2Rs with known ligands are expressed in GI tissues and cells (31). Specifically, we performed RT-PCR to detect transcripts encoding for mT2R108 and mT2R138, whose putative ligands are DB and PTC, respectively. In addition, we also examined the expression of the unique rodent-specific mT2R134 in GI tissues and in the tongue, which is the primary site of expression for all functional taste receptors. We detected mT2R138 and mT2R108 in the fundus, antrum, duodenum, and tongue but not in the liver. In addition, the rodent-specific mT2R134 was similarly expressed in gastric and duodenal tissues as well as in the tongue (31). The rat orthologs of putative PTC (rT2R38) and DB (rT2R16) receptors were also expressed in GI tissues but not in the liver. The expression of rodent-specific rT2R34 was also observed, and its full-length coding sequence was confirmed from both cDNA and genomic DNA clones. Collectively, these results demonstrated the expression of transcripts encoding for taste receptors of the T2R family in the rat and mouse gastric and intestinal mucosa.

Expression of Taste-Specific Marker, Including Go$_{\text{gust}}$ and Members of the T2R Family, in Enteroendocrine STC-1 Cells and Amphicrine AR42J Cells

Cultured cell lines have been used extensively to investigate basic questions related to the organization, signaling pathways, and function of intestinal cells. In this context, mouse enteroendocrine STC-1 cells have provided a model system for studying the regulation of GI hormone release in response to multiple stimuli, including bombesin/gastrin-releasing peptide (GRP), pituitary adenylate cyclase-activating peptide (PACAP), leptin, orexin, fatty acids, amino acids, and peptidomimetic compounds. STC-1 cells produce and release CCK, secretin, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) and have also been used as a model for studies of enteroendocrine cell differentiation. This cell line was derived from an endocrine tumor that developed in the small intestine of a double transgenic mouse expressing the rat insulin promoters linked to the SV40 large T antigen and to the polyomavirus small T antigen.

Several lines of experimental evidence indicate that STC-1 cells express signaling pathways that respond to bitter tastants. 1) Using RT-PCR and sequencing analysis, we demonstrated that STC-1 cells express multiple T2Rs, including mT2R102, mT2R105, mT2R107, mT2R108, mT2R110, mT2R119, mT2R123, mT2R118, mT2R130, mT2R126, mT2R134, mT2R137, and mT2R 138. Real-time PCR analysis further confirmed high levels of mT2R108 expression in STC-1 cells (31, 32; also unpublished results). 2) RT-PCR and sequencing also revealed the presence of transcripts for Go$_{\text{gust}}$ and Go$_{\text{PTC}}$ and PACAP is in line with the notion that enteroendocrine cells function as integrators of signals from the lumen and neurohumoral signals that act on basolateral receptors. Collectively, these results indicate that STC-1 cells provide a model to elucidate signaling pathways and secretory responses elicited by bitter stimuli in a GI cell system.

The rat pancreatic acinar tumor cell line AR42J is characterized by amphicrine properties, that is, AR42J cells possess both neuroendocrine and exocrine cell actions. The neuroendocrine properties include the presence of voltage-activated Ca$^{2+}$ channels and synaptic-like microvesicles containing neurotransmitters. These cells also possess zymogen granules containing digestive enzymes and can secrete them in response to receptor stimulation. RT-PCR of mRNA prepared from AR42J cells demonstrated the expression of rodent orthologs of mT2R108, mT2R138, and rodent-specific mT2R134 (full-length sequence for rT2R16 and rT2R34 and partial sequence for rT2R38). Transcripts encoding Go$_{\text{gust}}$ and Go$_{\text{PTC}}$ (full length) were also found in these cells (31). Thus rat AR42J cells provide another model to elucidate signaling pathways and secretory responses elicited by bitter stimuli in a GI cell system.

Signal-Transduction Pathways Activated By Bitter Stimuli in GI Cells

The complex pathways that mediate taste signaling in taste receptor cells of the lingual epithelium are becoming increasingly understood (16, 27). Activation of bitter taste receptors has been shown to promote rapid changes in the level of second messengers through a bifurcating signaling pathway involving the G protein gustducin (Fig. 2). The βγ-subunits of this heterotrimeric G protein stimulate PLCβ2-mediated synthesis of inositol 1,4,5-trisphosphate leading to the release of Ca$^{2+}$ from intracellular stores, whereas the α-subunit reduces the intracellular level of cAMP through activation of phosphodiesterases. Transducin is thought to elicit a similar signaling
cascade in taste cells (11). In addition, bitter tastants can also modulate the gating of voltage-sensitive ion channels that mediate Ca\(^{2+}\) entry into the cell. Recent evidence has demonstrated that the monovalent cation channel TRPM5 is necessary for both bitter and sweet taste signaling in the lingual epithelium (21, 29, 33). The precise coupling of T2Rs, gustducin and PLC\(\beta2\), to TRPM5 remains incompletely understood, although it is plausible that a release of Ca\(^{2+}\) from internal stores triggers the activation of TRPM5, which in turn promotes membrane depolarization. The increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) induced by tastants in cells of the lingual epithelium elicits the release of ATP onto purinergic receptors of afferent nerve endings (9), which conduct the information to the gustatory centers where the sensory information is coded and integrated (27).

In view of the results discussed demonstrating the expression of taste transducers, including receptors (e.g., multiple T2Rs), G proteins (G\(_{\alpha}\)gust and G\(_{\alpha}\)t2), and effectors (PLC\(\beta2\) and TRPM5) in GI cells, it is logical to hypothesize that bitter and sweet stimuli initiate cellular and molecular responses in endocrine cells of the GI tract. Although very little is known about the signal-transduction cascades initiated by bitter stimuli in endocrine cells from the GI tract, some initial results suggest that some elements of taste-specific signaling are operative in enteroendocrine cells (illustrated in Fig. 2). Recent results demonstrated that STC-1 cells show a marked increase in [Ca\(^{2+}\)]\(_i\) in response to the addition of bitter stimuli, including DB, PTC, 6-n-propil-2-thiouracil, caffeine, nicotine, and cycloheximide (32). More recently, Masuho and colleagues (17) also demonstrated that bitter stimuli induce Ca\(^{2+}\) signaling in STC-1 cells. Interestingly, some of the bitter stimuli induce oscillatory changes in [Ca\(^{2+}\)]\(_i\), as revealed by microscopic Ca\(^{2+}\) imaging (32). In agreement with the notion that PTC and DB act through different receptors, the addition of PTC did not prevent the increase in [Ca\(^{2+}\)]\(_i\) elicited by a subsequent addition of DB (M. Chen, S. V. Wu, J. Reeve, and E. Rozengurt, unpublished data).

Several lines of evidence indicate that the increase in [Ca\(^{2+}\)]\(_i\), induced by DB or PTC in STC-1 cells is mediated primarily by an increase in Ca\(^{2+}\) influx from the extracellular medium. Specifically, inhibition of Ca\(^{2+}\) influx by chelating extracellular Ca\(^{2+}\) with EGTA blocked the increase in [Ca\(^{2+}\)]\(_i\), induced by either DB or PTC but, in contrast, did not prevent the peak increase in [Ca\(^{2+}\)]\(_i\), induced by bombesin, which is caused by mobilization of Ca\(^{2+}\) from intracellular stores. These results indicate that Ca\(^{2+}\) influx is responsible for a major component of the increase in [Ca\(^{2+}\)]\(_i\), induced by DB or PTC in STC-1 cells. Furthermore, treatment of STC-1 cells with thapsigargin, an agent that selectively inhibits endoplasmic reticulum Ca\(^{2+}\) ATPase and thereby depletes intracellular Ca\(^{2+}\) stores, did not prevent the increase in [Ca\(^{2+}\)]\(_i\), induced by either PTC or DB in STC-1 cells but completely prevented the increase in [Ca\(^{2+}\)]\(_i\), induced by bombesin (M. Chen, S. V. Wu, J. Reeve, and E. Rozengurt, unpublished data). Collectively, these results indicate that DB and PTC elicit an increase in [Ca\(^{2+}\)]\(_i\), by stimulating Ca\(^{2+}\) influx into STC-1 cells.

Subsequent experiments were designed to identify the Ca\(^{2+}\) permeability pathways activated by bitter stimuli in STC-1 cells. L-type voltage-sensitive Ca\(^{2+}\) channels (VSCCs) mediate the influx of extracellular Ca\(^{2+}\) into neuronal and neuroendocrine cells in response to membrane depolarization (6). In contrast, very little is known about the role of VSCCs in mediating responses initiated by bitter stimuli in taste cells of the lingual epithelium. The common pharmacological feature of all isoforms of L-type VSCCs is their sensitivity to dihydropyridines (e.g., nitrendipine, diltiazem) inhibited the influx from the extracellular medium. Consequently, we examined the effects of these compounds also stimulate the release of CCK from these cells. Thus bitter tastants increase [Ca\(^{2+}\)]\(_i\)_ induced by bombesin (M. Chen, S. V. Wu, J. Reeve, and E. Rozengurt, unpublished data). Collectively, these results indicate that L-type VSCCs play a major role in mediating the increase in [Ca\(^{2+}\)]\(_i\)_ induced by bitter tastants in STC-1 cells. Thus bitter tastants increase [Ca\(^{2+}\)]\(_i\)_ through Ca\(^{2+}\) influx mediated by the opening of L-type VSCCs in enteroendocrine STC-1 cells.

Having demonstrated robust increases in [Ca\(^{2+}\)]\(_i\)_ in STC-1 cells challenged with bitter tastants, we assessed whether these compounds also stimulate the release of CCK from these cells. Our results show that DB is a potent stimulant of CCK release from enteroendocrine STC-1 cells and that treatment with either EGTA or nitrendipine prevented this effect. We conclude that taste-elicted CCK release is also mediated by an increase in [Ca\(^{2+}\)]\(_i\)_ produced by the opening of L-type VSCCs.

As indicated before, we demonstrated that rat pancreatic AR4-23 cells, which display neuroendocrine and exocrine cell actions and thus are considered as amphiregulin cells, also express bitter taste receptors and signaling molecules implicated in taste reception (31). Our recent results demonstrate...
that AR42J cells, similar to STC-1 cells, show a marked increase in [Ca\(^{2+}\)]\(_i\), in response to the addition of PTC, DB, or the GI peptide bombesin.

To substantiate further the specificity of the [Ca\(^{2+}\)]\(_i\) signals induced by bitter compounds in GI cells, the effect of DB and PTC was assessed in other cell types that neither exhibit neuroendocrine properties nor express receptors or G proteins required for the reception of taste stimuli (31). The addition of either PTC or DB to undifferentiated rat intestinal epithelial IEC-6 cells neither induced a detectable Ca\(^{2+}\) response nor prevented the marked increase in [Ca\(^{2+}\)]\(_i\), induced by vasopressin in these cells. In addition, neither PTC nor DB induced any increase in Ca\(^{2+}\) signaling in pancreatic adenocarcinoma BxPc3, whereas these cells responded to bradykinin, used as a positive control. Similarly, PTC did not increase [Ca\(^{2+}\)]\(_i\) in IEC-18 cells, Swiss 3T3 fibroblasts, or PAN-1 cells, another line of ductal adenocarcinoma cells.

Cycloheximide (CYX) is another compound extensively used in bitter taste studies. Previously, we have demonstrated that the CYX receptor (mT2R105) and its rat ortholog (rT2R9) are expressed in STC-1 cells and rat GI tissues, respectively. The addition of CYX to AR42J cells induced an increase in [Ca\(^{2+}\)]\(_i\). In contrast, Rat-1 cells, which do not express T2Rs, did not respond to either CYX or DB (31). The putative CYX receptor rT2R9 was also detected in AR42J cells by RT-PCR and might confer the CYX-stimulated calcium response observed in our study. Furthermore, the addition of DB, PTC, or CYX did not induce any detectable change in [Ca\(^{2+}\)]\(_i\) in human HEK-293 cells, a cell line that does not express endogenous receptors involved in taste signaling (M. Chen, S. V. Wu, J. Reeve, and E. Rozengurt, unpublished data).

The fact that bitter stimuli, including DB, PTC, and CYX, did not induce any effect on Ca\(^{2+}\) signaling in a variety of cell lines that do not express T2Rs and G proteins implicated in bitter taste reception, including rat (IEC-18, IEC-6, and Rat-1), mouse (Swiss 3T3), or human (PANC-1, BxPC3, and HEK-293) cells, reinforces the notion that the effects of bitter compounds on second-messenger production in STC-1 and AR42J cells are mediated by specific receptors and signal transducers that are expressed in these GI cells.

In recent years, remarkable progress has been made in demonstrating the expression of molecular transducers implicated in taste signaling in cells of the GI tract, yet it is also clear that the field is in its infancy. As a first step for elucidating the physiological significance of these findings, it is important to map the localization of taste receptors and downstream signal-transduction elements to specific enterodocrine cells distributed in the lining of the rodent and human GI tract. In this context, our recent result demonstrating that Go\(_{gust}\) is expressed in peptide YY (PYY)-positive cells (enterodocrine L cells) in both human and rodent colonic mucosa (N. Rozengurt, S. V. Wu, M. Chen, C. Huang, C. Sternini, and E. Rozengurt, unpublished data) is a first step in this direction. PYY is attracting intense interest because the peripheral administration of the PYY\(_{3-36}\) form of the peptide reduced food intake in mice, rats, and humans (2, 3) and, similar to bitter stimuli, evoked an aversive food response in mice (10). As implied by Fig. 2, it is tempting to speculate that the increase in [Ca\(^{2+}\)]\(_i\) induced in enterodocrine cells by bitter and/or sweet tastants triggers the release of GI peptides, including PYY and GLP-1, that activate neural reflexes and/or modulate the activity of adjacent or distant target cells.

In view of these considerations, it will be of interest to determine whether bitter stimuli promote the release of PYY into the bloodstream and whether the effects of this peptide are potentiated by other GI peptides cosecreted with PYY from the same L cells or released from other enterodocrine cells that also participate in chemosensing. In this regard, recent results indicate that the other peptide released from L enterodocrine cells, GLP-1, cooperates with PYY in reducing food intake in mice (30) and humans (19), and, similar to bitter stimuli, both peptides mediate an aversive food response in mice (10, 26). Thus PYY and GLP-1 are implicated in fundamental mechanisms of regulation in response to caloric intake and may participate in the pathogenesis of the most common metabolic disorders, namely, obesity and Type 2 diabetes. Recent colocalization studies suggest that Go\(_{gust}\)-dependent signaling mechanisms may participate in the regulation of the release of PYY and GLP-1 from open enterodocrine L cells of the human colon (N. Rozengurt, S. V. Wu, M. Chen, C. Huang, C. Sternini, and E. Rozengurt, unpublished data). These results raise the attractive possibility of exploiting the stimulation of the endogenous release of these GI peptides elicited by non-permeable tastants as a novel approach for therapeutic intervention in obesity and Type 2 diabetes.

Drugs and toxins stimulate vomiting reflexes or promote aversive behavior in rodents; several food components regulate appetite and satiety, alter motility of the stomach and intestine, and initiate neural and hormonal pathways necessary for normal digestive function. The identification of chemosensory receptors in the stomach and intestine that perceive chemical components of ingested substances including drugs and toxins has a number of important implications, including the design of novel molecules that modify responses elicited by the activation of these receptors. The expression and function of taste receptors in specific cells of the lining of the GI mucosa and the unraveling of the signal-transduction pathways that mediate their biological effects in these cells open new avenues for understanding molecular sensing in the GI tract. An attempt to

### Chemical Composition of Ingested Food

![Chemical Composition of Ingested Food](http://ajpgi.physiology.org/)

Fig. 3. A scheme showing the potential biological effects elicited by taste receptor activation in enterodocrine cells. GLP-1, glucagon-like protein.
organize the emerging questions and potential biological responses is presented in Fig. 3. In view of the fact that GPCRs are among the most common targets for currently used therapeutic drugs, the identification of chemosensory GPCRs in GI cells could pave the way for developing novel therapeutic compounds that modify their function in the gut.

REFERENCES


