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Taste-Signaling Proteins Are Coexpressed in Solitary Intestinal Epithelial Cells

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Abstract

The taste system, made up of taste receptor cells clustered in taste buds at the surface of the tongue and the soft palate, plays a key role in the decision to ingest or reject food and thereby is essential in protecting organisms against harmful toxins and in selecting the most appropriate nutrients. To determine if a similar chemosensory system exists in the gastrointestinal tract, we used immunohistochemistry and real-time polymerase chain reaction (PCR) to investigate which taste-signaling molecules are expressed in the intestinal mucosa. The PCR data showed that T1r1, T1r2, T1r3, α-gustducin, phospholipase Cβ2 (PLCβ2), and Trpm5 are expressed in the stomach, small intestine, and colon of mice and humans, with the exception of T1r2, which was not detected in the mouse and human stomach or in the mouse colon. Using transgenic mice expressing enhanced green fluorescent protein under the control of the Trpm5 promoter, we found colocalization of Trpm5 and α-gustducin in tufted cells at the surface epithelium of the colon, but these cells did not express T1r3 or PLCβ2. In the duodenal glands, 43%, 33%, and 38% of Trpm5-expressing cells also express PLCβ2, T1r3, or α-gustducin, respectively. The duodenal gland cells that coexpress PLCβ2 and Trpm5 morphologically resemble enteroendocrine cells. We found a large degree of colocalization of Trpm5, α-gustducin, T1r1, and T1r3 in tufted cells of the duodenal villi, but these cells rarely expressed PLCβ2. The data suggest that these duodenal cells are possibly involved in sensing amino acids.

Key words: chemoreceptor cell, gustducin, gut, T1rs, transgenic mice, Trpm5

Introduction

Taste sensation is initiated in primary taste sensory cells located in papillae at the surface of the tongue and in the soft palate. The papillae contain one to several taste buds, each composed of 40–140 cells including taste receptor cells (TRCs), precursor, and support cells (Lindemann 2001). TRCs are specialized epithelial cells that typically respond to certain tastants by undergoing depolarization, elevating intracellular calcium, releasing a neurotransmitter, and activating the afferent taste neurons (Gilbertson et al. 2000).

For bitter, sweet, or umami taste, the signal transduction cascade is initiated by tastants binding to G-protein–coupled receptors (GPCRs). Several tastant-responsive GPCRs have been identified, including the sweet-responsive T1r3/T1r2 heterodimers, the amino acid/umami-responsive T1r3/T1r1 heterodimers, a truncated form of mGluR4 that responds to glutamate in vitro, and the T2rs, a family of bitter-responsive receptors (Chandrashekar et al. 2000; Chaudhari et al. 2000; Nelson et al. 2001, 2002; Li et al. 2002). The G-proteins that couple these receptors to second messenger-modulating enzymes include gustducin, transducin, and possibly Gαi2 (McLaughlin et al. 1992; Ruiz-Avila et al. 1995; Wong et al. 1996). Gustducin is a heterotrimeric G-protein made of α-gustducin, Gβ3, and Gγ13 (Huang et al. 1999). Upon activation, heterotrimeric gustducin separates into α and βγ subunits. In a pathway common to bitter, sweet, and umami transduction, the βγ subunit of gustducin activates phospholipase Cβ2 (PLCβ2), which catalyzes the formation of IP3, leading to release of calcium from intracellular stores. Increasing cytoplasmic calcium concentration activates Trpm5, resulting in the entry of monovalent cations (Liu and Liman 2003; Prawitt et al. 2003). A second modality-specific pathway also exists in TRCs. Upon activation by sugars, the α-subunit of gustducin activates adenylyl cyclase leading to an increase in cyclic adenosine 3′,5′-monophosphate (cAMP), whereas simulation by bitter molecules leads to activation of phosphodiesterase resulting in a drop of cAMP and cyclic guanosine monophosphate (Gilbertson et al. 2000). For umami taste, both a drop and a rise in cAMP appear to play a role, depending on the location at the front or the back of the tongue of the TRC that is activated (Ninomiya et al. 2000; Abaffy et al. 2003).

Taste plays an important role in the decision to ingest or reject food. It helps protect against harmful toxins (bitter) and spoiled food (sour) and favors the ingestion of calorie-rich (sweet), sodium-rich (salty), or protein-rich (umami) food. Whereas bitter taste constitutes a first line of defense against toxins, other mechanisms such as emesis exist to rid the body of harmful substances if they are accidentally ingested. Once ingested, nutrients induce several physiological changes in the body, for example, secretion of digestive hormones, absorption of nutrients, reduction of appetite, and modulation of intestinal motility and gastric emptying. These changes may be initiated by mechanical events (e.g., stretching of the stomach), metabolic effects (e.g., rise in blood glucose), or chemical events that are nutrient specific. It is believed that the gastrointestinal tract (GI) has the ability to analyze the chemical composition of its content in order for the body to adequately and specifically respond to the ingestion of food, implying the existence of gastrointestinal chemoreceptor cells. However, the molecular nature of the chemoreceptors is unknown.

Solitary cells expressing taste cell signal transduction proteins have been described in several hollow organs including the airways, the pancreatic duct, the nasal epithelium, the larynx, and the GI. It has previously been shown that α-gustducin, α-transducin, the T1rs, and several T2rs are expressed in the GI (Hofer et al. 1996; Wu et al. 2002; Dyer et al. 2005). It was proposed that the cells expressing these G-proteins might be the elusive gut chemoreceptor cells. We set out to determine if other taste signal transduction proteins were also expressed and colocalized in the GI and to get insight into the role of the cells expressing these proteins.

Materials and methods

Mice

All animal procedures were approved by the Veterinary Office of the Canton de Vaud. The construct used to generate Trp-eGFP transgenic mice contained (5′ to 3′) 11 kb of murine Trpm5 5′ flanking sequence, Trpm5 Exon 1 (untranslated), Intron 1, and the untranslated part of Exon 2; the enhanced green fluorescent protein (eGFP) coding sequence, the encephalomyocarditis virus internal ribosome entry site; and a truncated human CD4 lacking the 35-residue C terminal intracellular region (Figure 2a). The truncated CD4 was included in the construct to provide a surface marker for isolation of the cells expressing the transgene. Because it is lacking the intracellular region, the truncated CD4 is inactive. The construct was freed from plasmid sequence and microinjected into CB6 mouse zygotes according to standard methods (Hogan et al. 1994). Founder transgenic mice were bred to wild type C57BL6/J mice. Transgenic animals were identified by polymerase chain reaction (PCR) amplification of tail DNA with eGFP-specific primers.

Cell sorting

The mouse small intestine was dissected from the pylorus to the ileocaecal junction, opened longitudinally, washed twice in Hanks balanced salts solution without calcium supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HBSS), and then incubated for 10 min at 37 °C in HBSS containing 2 mg/ml collagenase A and 1 mg/ml dispase II. The intestinal epithelium was gently scrapped using bent forceps and incubated for 30 min at 37 °C in the enzyme solution with pipetting up and down every 5 min to dissociate the cells. The cells were then passed twice through a 40-μm cell strainer, centrifuged at 860 × g for 3 min, and resuspended in HBSS with calcium supplemented with 10% fetal bovine serum and 100 units/ml DNAse. The cells were passed again through a 40-μm cell strainer, and propidium iodide was added to a final concentration of 2.5 μg/ml. The cells were then sorted on a FACSAria cell sorter (BD Biosciences, San Jose, CA) according to propidium iodide signals and green fluorescent protein (GFP) signals and collected in the cell lysis buffer of the RNA isolation kit.

Real time-PCR

The mRNA from human stomach, duodenum, jejunum, ileum, and colon was purchased from Clontech (Mountain View, CA). Total mouse RNA was extracted from fluorescence-activated cell sorting (FACS) sorted cells and from antrum, duodenum, jejunum, ileum, and colon using the Nucleospin RNA II kit (Macherey-Nagel, Oensingen, Switzerland). Mouse and human RNAs were reverse transcribed into cDNA using SuperScript III (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The cDNA (equivalent to 50 ng RNA) was amplified by real time (RT)–PCR using an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Taqman primers and probes were purchased from Applied Biosystems (Hs00190117_m1 for human PLCβ2, Hs00371023_m1 for human T1R1, Hs00541095_m1 for human T1R2, Hs01026531_g1 for human T1R3, Hs00175822_m1 for human TrpM5, custom made primers GCAAGAACCGTAAAGCT GCTACT and TCCATGCATTCTTGCTCACTGTAA and probe CCATTCTTATGGATGATCTTC for α-gustducin, Mm00473433_m1 for mouse T1r1, Mm00499716_m1 for mouse T1r2, Mm00473459_g1 for mouse T1r3, Mm00498453_m1 for mouse Trpm5). Detection of amplification relied on monitoring a reporter dye (6-FAM) linked to the 5′ end of a probe complementary to the sequence amplified by the primers. The cycling conditions were 1 cycle at 50 °C for 2 min, 1 cycle at 95 °C for 10 min, and then 60 cycles of 95 °C for 15 s and 60 °C for 1 min. The β2 microglobulin primers were used as positive control and to normalize the results obtained with the taste gene–specific primers. Negative controls were RNA samples where the reverse transcriptase was omitted from the reverse transcription mix. All primer pairs span an intron.

Immunohistochemistry

Transgenic mice were anesthetized with a cocktail of ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight). Heart perfusion was performed with phosphate-buffered saline (PBS) then with 4% paraformaldehyde in PBS. Biopsies were taken from the tongue and the GI and fixed in 4% paraformaldehyde in PBS for 1 h and 30 min at 4 °C then quickly frozen in Tissue-Tek optimal cutting temperature compound (Sakura, Tokyo, Japan). Twelve-micron sections were taken from the frozen tissues using a cryostat. Immunostaining of the sections was as described (He et al. 2002). Briefly, the sections were washed with blocking buffer, the primary antiserum was applied for 24 h at 4 °C, the sections were washed again with blocking buffer, and then a Cy3-conjugated secondary antibody was applied (Jackson Immunoresearch Laboratories, West Grove, PA). In the negative controls, the primary antiserum was omitted. The sections were photographed under confocal microscopy. Primary antibodies were purchased from Alpha Diagnostics, San Antonio, TX, catalog number TR11-A for T1r1; Santa Cruz Biotechnology, Santa Cruz, CA, catalog numbers sc-22456 for T1r2, sc-206 for PLCβ2, sc-26781 for Gγ13, and sc-395 for α-gustducin; abcam, Cambridge, UK, catalog number ab12677 for T1r3; and Phoenix Pharmaceuticals, Belmont, CA, catalog number H-059-03 for PYY. The dilutions of the primary antibodies were 1:500 for all antibodies, except for α-gustducin and PYY (1:1000). For the colocalization studies, biopsies were taken from the duodenum and colon of 3 mice, and 3 sections from each biopsy were immunostained. All cells from each section were counted and scored as eGFP only, immunostain only, or eGFP and immunostain.

Results

Which taste signal transduction proteins are expressed in the mouse and human GIs?

We performed RT-PCR on polyA enriched RNA from human stomach, duodenum, jejunum, ileum, and colon. We found that T1R1, T1R2, T1R3, PLCβ2, α-gustducin, and TrpM5 were expressed in every GI tissue tested, with the exception of T1R2, which was not detected in the stomach and was very weakly expressed in the other tissues (Figure 1a). In general, the expression levels of each gene are in the same order of magnitude throughout the GI. There are, however, marked differences from gene to gene, with 4 orders of magnitude difference between PLCβ2 and T1R2 (Figure 1b). RT-PCR with mouse GI total RNA showed that T1r3 and Trpm5 are expressed in all the tested compartments of the GI, T1r1 is expressed in the antrum, duodenum, ileum, and colon, but not in the jejunum, and T1r2 is expressed only in the ileum (not shown). The relative levels of expression between genes were consistent with those of the human genes.

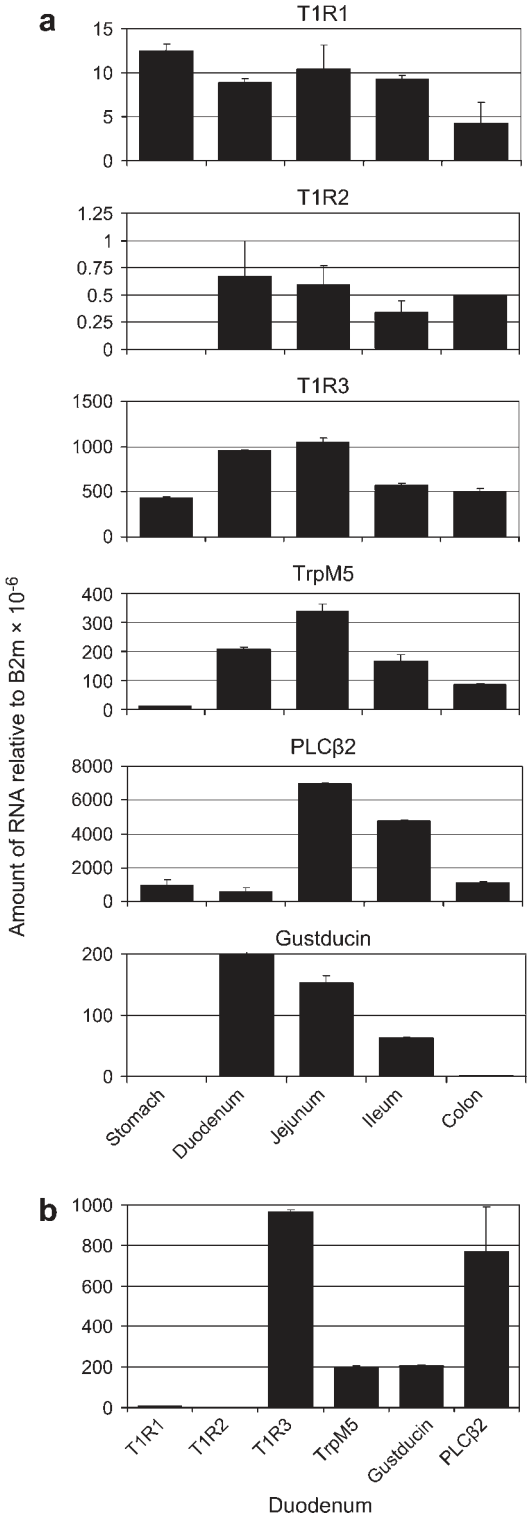

Figure 1 RT-PCR of reverse transcribed mRNA from several parts of the human GI. The values shown are amount of RNA relative to β2 microglubulin (B2m) RNA. Note that the scales of the *x* axes in **(a)** are different. To give a better visual comparison of the differences of expression levels between genes, the expression levels of the 6 genes in the duodenum were plotted in the same graph in **(b)**. Error bars represent standard error of the mean.

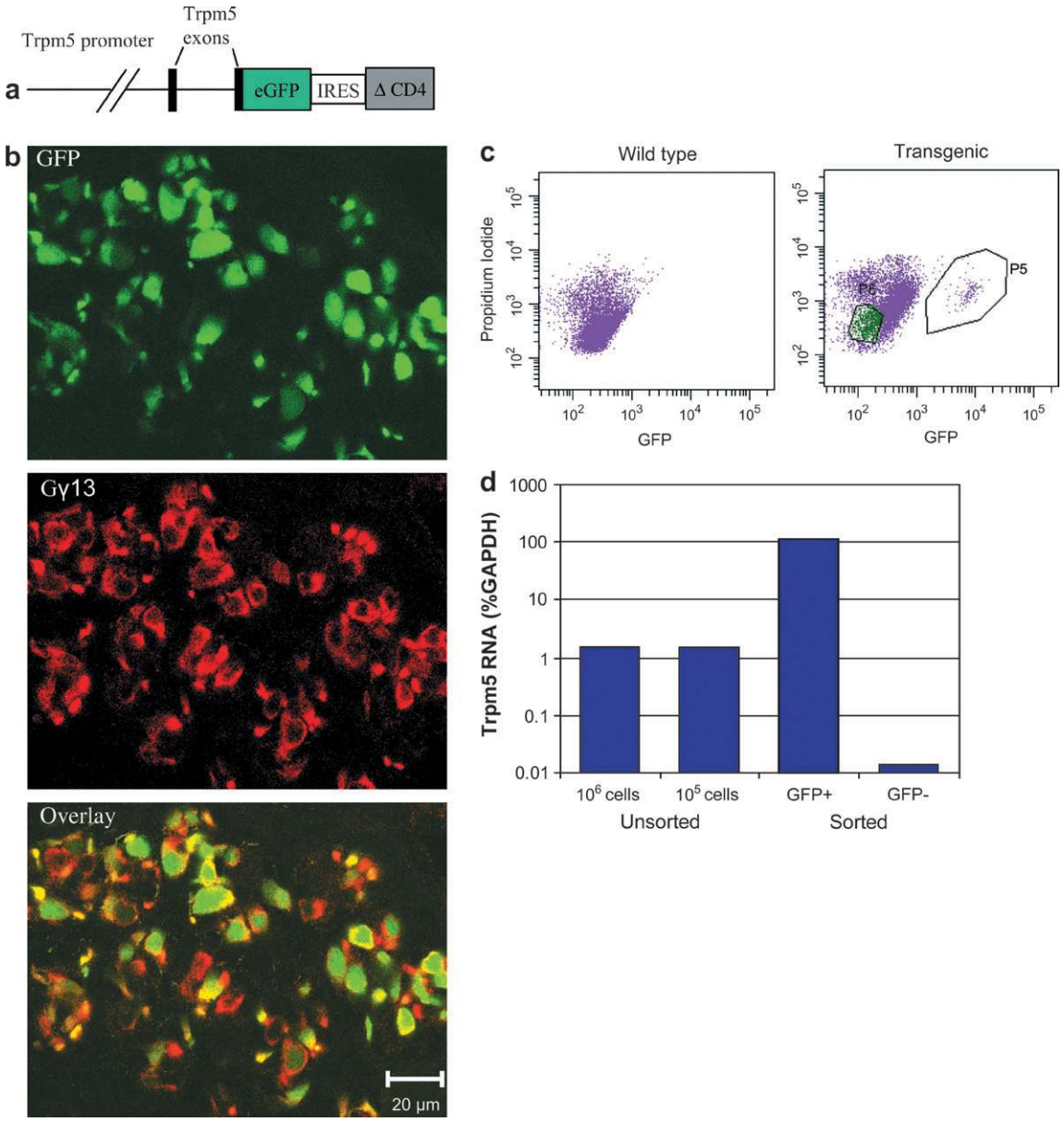

Figure 2 **(a)** Diagram of the DNA construct used to produce Trp-eGFP transgenic mice. **(b)** Transverse sections from the circumvallate papilla of a Trp-eGFP transgenic mouse immunostained with a Gγ13-specific antibody. Gγ13 and Trpm5 are expressed in the same cells in the taste buds. The secondary antibody is a Cy3-conjugated anti-rabbit IgG. There is >90% colocalization of the eGFP (green) and Gγ13 (red) fluorescence. **(c)** Scatter plots of sorted dissociated intestinal cells from a wild type and a transgenic mouse. The cell population from the transgenic mouse labeled P5 shows more intense GFP fluorescence than propidium iodide fluorescence and is absent in the wild-type mouse. This population was sorted as GFP+ cells. The P6 population was sorted as the GFP− control cells. **(d)** RT-PCR of reverse transcribed mRNA from unsorted cells, GFP+, and GFP− cells. The values shown are amount of RNA relative to GAPDH RNA.

Which cells express taste-signaling proteins in the mouse GI?

To study the Trpm5-expressing cells in the GI, transgenic mice were generated with a construct containing eGFP under the control of the Trpm5 promoter. We used immunohistochemistry to show that the transgene was expressed in the taste cells that express Trpm5. Available antibodies against Trpm5 did not give satisfactory results so we used a Gγ13 antibody, as it has been previously shown that Gγ13 and Trpm5 colocalize in the circumvallate papilla (Perez et al. 2002). We found that there was >90% colocalization of eGFP and Gγ13 in TRCs (Figure 2b). We also made a single cell preparation of the small intestine epithelium, sorted the eGFP-expressing cells by FACS (Figure 2c), and measured Trpm5 mRNA levels by RT-PCR in the sorted cells (Figure 2d). We found that, compared with the unsorted cells, Trpm5 mRNA was enriched 70 times in the eGFP cell

population and reduced 120 times in the depleted cells. These results validate the use of eGFP as a surrogate for Trpm5.

By examining sections from the GI of these transgenic mice, we found eGFP fluorescence in several cells of the antrum, duodenum, jejunum, ileum, and colon. They were isolated cells disseminated throughout the lining epithelium of the villi and the glands (Figure 3). The eGFP-expressing cells of the villi are pear shaped with a tuft of microvilli in the apical end and a narrow basal process extending to the lamina propria (Figure 4). The shape and distribution of these cells suggest that they are tufted cells (also known as caveolated or brush cells), a population of solitary epithelial cells found in the gastrointestinal and respiratory epithelia (reviewed in Sbarbati and Osculati 2005a). There are also eGFP-expressing cells in the intestinal glands that are less intensely fluorescent. These cells have a triangular shape, with a process extending toward the lumen of the gland, suggesting that they are enteroendocrine cells (Figure 4).

Do taste-signaling proteins colocalize in the GI?

To determine if the Trpm5-expressing cells of the GI use the same signal transduction pathways as TRCs, we carried out colocalization studies of duodenum and colon tissues, using immunohistochemistry. We used eGFP as a surrogate for Trpm5 in the Trp-eGFP transgenic mice. We found marked differences in expression pattern between duodenum and colon and between villi and glands in the duodenum (Figure 4 and Table 1). In the duodenal villi, there is a large degree of colocalization of Trpm5, α-gustducin, T1r1, and T1r3, but fewer cells coexpress Trpm5 and PLCβ2. In the duodenal glands, 43%, 33%, and 38% of Trpm5-expressing cells also express PLCβ2, T1r3, or α-gustducin, respectively, but the majority of T1r1, α-gustducin, or PLCβ2-expressing cells do not express eGFP (56%, 70%, and 70%, respectively). The exception is T1r3, with 36% of T1r3-expressing cells not expressing eGFP. In the colon, the eGFP-expressing cells were found mostly at the surface epithelium. There is a large degree of colocalization between eGFP and α-gustducin but no or very little colocalization of PLCβ2 or T1r1 and eGFP. No T1r3 immunoreactivity was found in the colon. No T1r2 immunoreactivity was found in any part of the GI.

Are the putative gut chemoreceptor cells involved in food intake control?

A possible role for the cells expressing taste-signaling proteins, especially those expressing taste receptors, is the analysis of the chemical nature of the content of the GI in order to modulate appetite in response to the food ingested. Therefore, we looked at possible coexpression of taste signal transduction proteins with hormones and peptides that have been implicated in food intake control. Immunoreactivity for cholecystokinin (CCK), peptide YY (PYY), Ghrelin, orexin A, and glucagon-like peptide (GLP)1 was detected

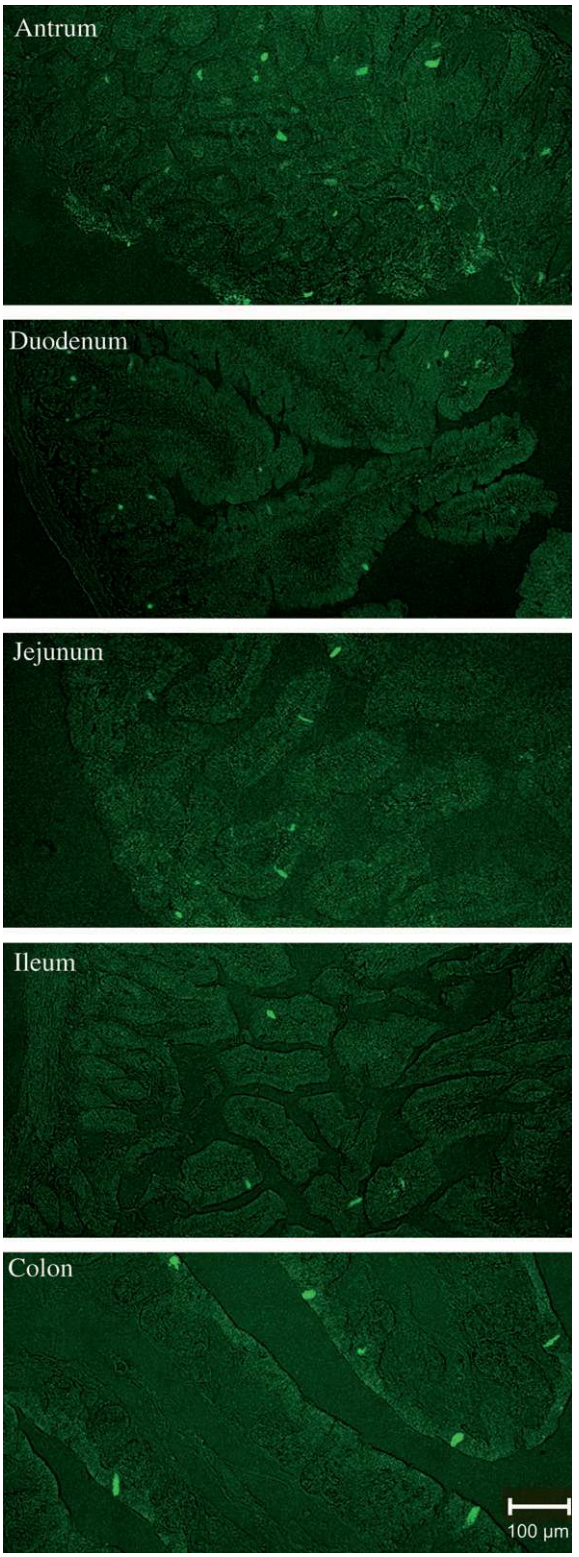

Figure 3 Sections from the antrum, duodenum, jejunum, ileum, and colon of a Trp-eGFP transgenic mouse examined under confocal microscopy, showing expression of eGFP in several isolated cells disseminated throughout the epithelium of the villi and glands of the GI.

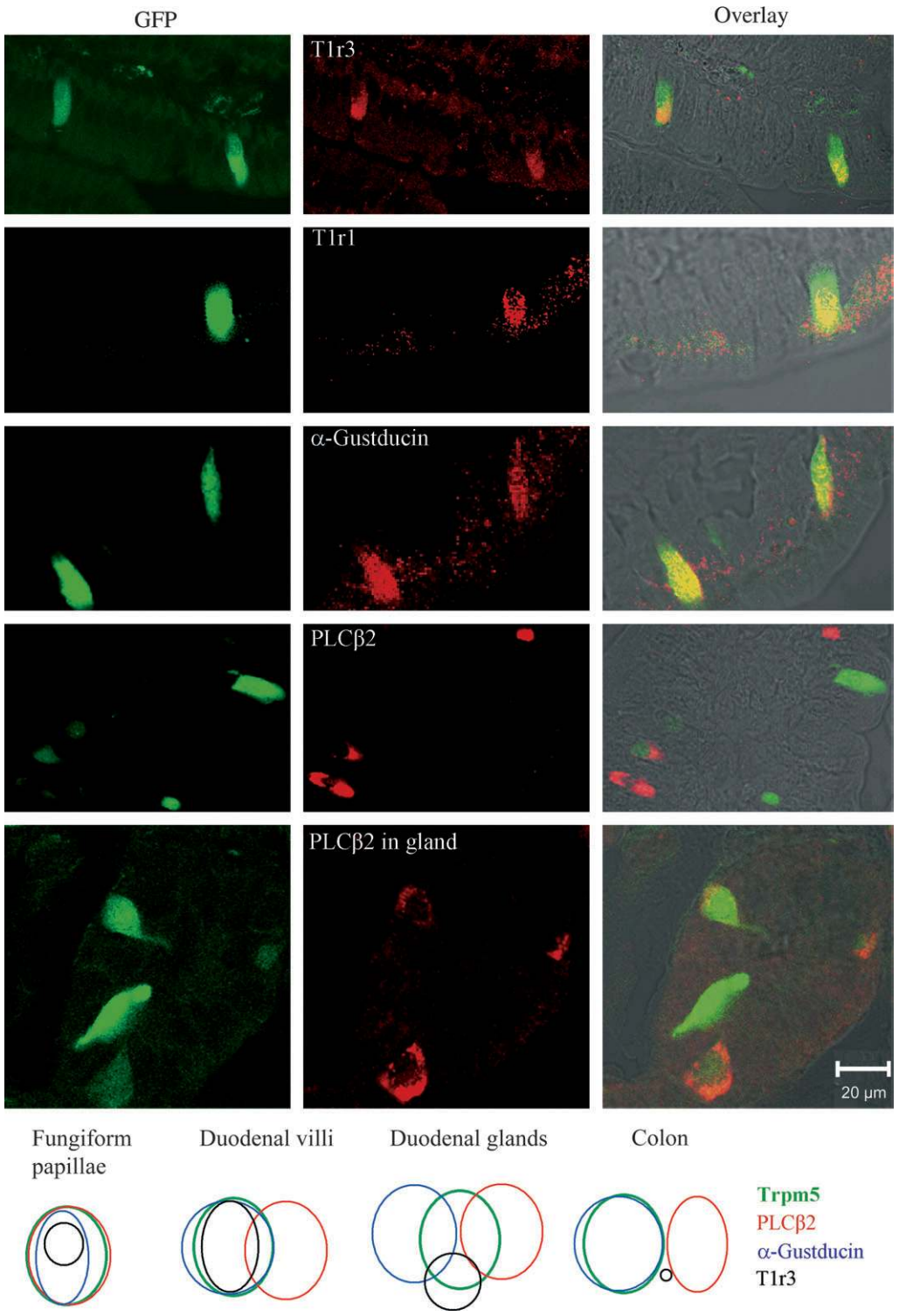

Figure 4 Sections from the duodenum of a Trp-eGFP transgenic mouse stained with antibodies against T1r3, T1r1, α-gustducin, and PLCβ2. All panels show parts of the duodenal villi, except the bottom row, which shows a section of a duodenal gland. The Venn diagram at the bottom of the figure shows the relationships between subsets of taste or intestinal cells expressing eGFP and subsets of cells expressing other taste signal transduction proteins. The relationship between subsets of intestinal cells expressing taste signal transduction proteins other than Trpm5 have not been investigated. The diagram relating the taste cells is based on data from the literature. To avoid crowding the figure, T1r1 is not included in the Venn diagram. Its pattern of expression is similar to that of T1r3, except that there are cells in the duodenal villi and colon that express T1r1 but not eGFP.

Table 1 Colocalization of taste-signaling proteins with eGFP

	Immunoreactivity only (%)	Colocalization (%)	eGFP only (%)	Cells counted
Duodenal villi				
T1r3	0	44 ± 8.1	56 ± 8.1	810
T1r1	21 ± 6.6	27 ± 7.6	52 ± 3.5	1015
α-Gustducin	22 ± 3.8	78 ± 3.8	0	1010
PLCβ2	55 ± 3.0	15 ± 2.6	30 ± 4.9	1781
Duodenal glands				
T1r3	16 ± 1.8	28 ± 5.0	56 ± 4.1	974
T1r1	41 ± 11.3	32 ± 3.5	27 ± 12.9	1136
α-Gustducin	48 ± 2.1	20 ± 4.7	32 ± 6.8	1197
PLCβ2	50 ± 13.8	21 ± 12.8	28 ± 0.9	1262
Colon				
T1r3	0	0	100	1011
T1r1	66 ± 3.2	6 ± 1.5	28 ± 4.8	2841
α-Gustducin	16 ± 2.7	84 ± 2.7	0	1217
PLCβ2	59 ± 9.8	0	41 ± 9.8	2502

Number of cells in the duodenal villi, duodenal glands, and colon that show colocalization of T1r3, T1r1, α-gustducin, or PLCβ2 with eGFP. All fluorescent cells from 9 sections (3 mice, 3 sections per mouse) from each tissue/antibody combination were counted. Values are mean percentages ± standard error of the mean (n = 3 mice).

in the GI but did not colocalize with eGFP (Figure 5 for PYY, not shown for CCK, Ghrelin, orexin A, and GLP1).

Discussion

It is believed that the GI mucosa is capable of sensing the chemical nature of its content, suggesting the existence of chemoreceptor cells in the gut. Several experiments demonstrated that infusion of nutrients into the lumen of the GI leads to nutrient-specific physiological changes such as gastric emptying, intestinal motility, and appetite modulation (Mei 1985). These physiological responses to nutrients are mediated at least in part through a neural circuit carried by the vagal nerve and by gastrointestinal hormone release. Nerve fiber and sensory ganglion recordings showed a specific response to the infusion of different nutrients in the intestinal lumen, showing that this response is not a postabsorptive effect (Mei 1985). Furthermore, tracing studies showed that the intestinal nerves do not enter the duodenal epithelium excluding the possibility that receptors located at the nerve endings act as sensors of the intestinal lumen contents (Berthoud et al. 1995). Therefore, it is more likely that excitable cells respond to chemical changes in the lumen of the GI by secreting hormones and neurotransmitters. Newson et al. (1982) found cells morphologically similar to taste cells in the rat ileal mucosa and proposed that they may carry a chemosensory function.

The discovery that α-gustducin and α-transducin are expressed in the rodent stomach and duodenum (Hofer et al. 1996; Wu et al. 2002) suggested that the gut chemoreceptor cells might sense the content of the GI using the same signaling pathways that TRCs use. It was later shown that Trpm5, T1r1, T1r2, T1r3, and the T2rs are also expressed in the mouse GI (Perez et al. 2002; Wu et al. 2002; Dyer et al. 2005). Our results show that T1r1, T1r3, Trpm5, and α-gustducin are also expressed in the human GI. In addition, we showed that PLCβ2 is expressed in both human and mouse GI. T1r2 was hardly detectable by PCR in the human GI and was not detectable by immunohistochemistry or RT-PCR in the mouse GI, with the exception of the ileum where it was amplified by RT-PCR (data not shown). Dyer et al. (2005) showed expression of T1r2 in the mouse duodenum, jejunum, and ileum by RT-PCR and western blot. The level of expression (~1/10th of that of T1r3) was found to be very weak. This apparent discrepancy between Dyer et al. and our results may be due to methodological differences, and it is possible that the antibody we used is not suitable for detection of very small amounts of T1r2. Together, Dyer et al. and our results suggest that T1r2 may be very weakly expressed in the GI.

Are these taste signal transduction proteins expressed in the same cells in the GI? Our colocalization studies carried out on the duodenum and the colon showed that this is partly the case, but there is heterogeneity in the cell population expressing these genes. The population of intestinal cells that most resembles taste cells are tufted cells in the duodenal villi with an elongated shape, a tuft of microvilli at the apical end, and a narrow basal process. These cells are found in several hollow organs of mammals, including the GI and are thought to be involved in chemodetection (for review, see Sbarbati and Osculati 2005b). In the duodenal villi, tufted cells coexpress T1r1, T1r3, α-gustducin, and Trpm5, suggesting that they are implicated in L-amino acid detection. Unlike TRCs, most of these cells do not express PLCβ2, suggesting that although they use the same receptors and G-protein as TRCs, they may use a different downstream transduction pathway.

The variation of the pattern of expression of mouse T1r1 and T1r2 along the GI may reflect different functions in the different parts of the GI. That T1r2 is expressed only in the ileum suggests that it might be implicated in the ileal brake, a physiological mechanism by which the presence of nutrients in the ileum leads to inhibition of gastric emptying, thereby reducing the delivery of nutrients to the small intestine. Expression of T1r1 in the duodenum but not in jejunum argues that T1r1 is implicated in nutrient detection, which takes place mainly in the duodenum.

Because cells expressing taste signal transduction proteins are probably gut chemoreceptor cells, it is important to determine how they communicate the information they detect

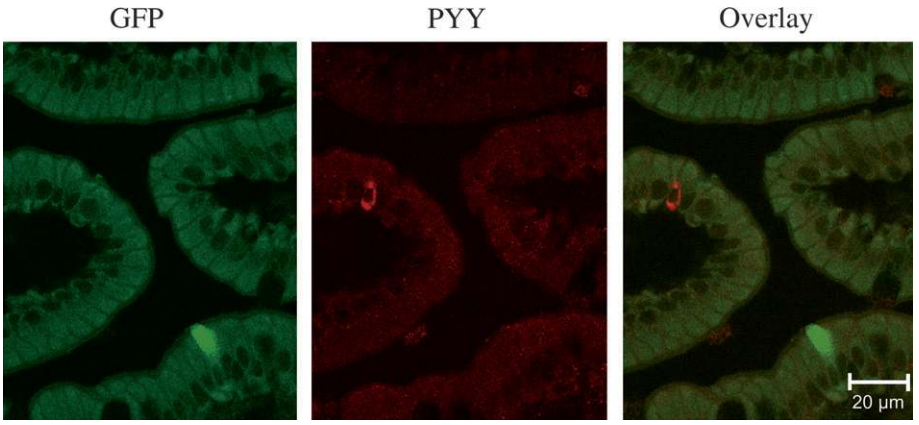

Figure 5 Sections of the duodenum of a Trp-eGFP transgenic mouse immunostained with an antibody against PYY. The secondary antibody is labeled with Cy3. There is no colocalization of eGFP with PYY.

and transduce. We investigated the possibility that they are neuroepithelial cells that have synapses, like some TRCs. This turned out to be unlikely because, using immunohistochemistry, we could not detect in these cells the presynaptic marker SNAP25 (not shown). We also tested the hypothesis that these cells are implicated in appetite control and that they respond to the presence of specific nutrients in the GI by modulating the levels of hormones known to control food intake. Although we did not find colocalization of eGFP and ghrelin, orexin A, PYY, GLP-1, or CCK, we cannot exclude a role in the modulation of food intake for these cells, as they may modulate the activity of hormone secreting intestinal cells or nerve termini through an as yet unidentified messenger.

Other candidate gut chemoreceptor cells include tufted cells of the colon that coexpress α-gustducin and Trpm5 but do not express T1rs and enteroendocrine cells in the duodenal glands that coexpress PLCβ2 and Trpm5 but do not express T1rs or α-gustducin. The latter are likely excitable cells as PLCβ2 and Trpm5 are part of a pathway that leads to increased intracellular calcium concentration and entry of cations into the cell. Furthermore, they are open enteroendocrine cells because they have a thin cellular process that reaches the intestinal lumen. Therefore, they likely respond to the content of the intestinal gland lumen to modulate hormonal secretion. The identity of the receptors expressed in these cells is unknown as is their function.

There is evidence indicating that members of the sodium/glucose cotransporter SGLT are required for the glucose-induced inhibition of gastric emptying (Freeman et al. 2006). The di- and tripeptide transporter PepT1 was shown to play a role in the ability of the intestine to activate the vagal nerve in response to the presence of proteins in the intestinal lumen. The detection of fat in the gut was proposed to occur via formation of chylomicrons and expression of ApoA-IV (Raybould et al. 2006). It is not known whether these molecules are expressed in the Trpm5-expressing cells. Some of these molecules may be the primary signaling proteins with which macronutrients interact, and Trpm5 and/or PLCβ2 may be part of the downstream signaling cascade. Alternatively, they may signal independently of these taste-signaling molecules. In that case, the taste-like pathway would constitute an alternative pathway, which might mediate different physiological responses to the presence of macronutrients in the intestine.

The next challenge is to determine which receptors and other genes implicated in the regulation of gut function are expressed in these cells, and to determine the function of these cells, and in particular the role that they may play in food intake control and intestinal motility.

Our data give more ground to the existence of a diffuse taste system in the GI by showing that several taste-signaling proteins are coexpressed in solitary epithelial cells of the intestine. Furthermore, the heterogeneity of these cells is consistent with the multiple functions this system is likely to carry out in response to the variety of molecules ingested during a meal.

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