Colocalization of the α-subunit of gustducin with PYY and GLP-1 in L cells of human colon

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Rozengurt, Nora, S. Vincent Wu, Monica C. Chen, Carlos Huang, Catia Sternini, and Enrique Rozengurt. Colocalization of the α-subunit of gustducin with PYY and GLP-1 in L cells of human colon. Am J Physiol Gastrointest Liver Physiol 291: G792–G802, 2006. First published May 25, 2006; doi:10.1152/ajpgi.00074.2006.—In view of the importance of molecular sensing in the function of the gastrointestinal (GI) tract, we assessed whether signal transduction proteins that mediate taste signaling are expressed in cells of the human gut. Here, we demonstrated that the α-subunit of the taste-specific G protein gustducin (Goαgust) is expressed prominently in cells of the human colon that also contain chromogranin A, an established marker of endocrine cells. Double-labeling immunofluorescence and staining of serial sections demonstrated that Goαgust localized to enteroendocrine L cells that express peptide YY and glucagon-like peptide-1 in the human colonic mucosa. We also found expression of transcripts encoding human type 2 receptor (hT2R) family members, hT1R3, and Goαgust in the human colon and in the human intestinal endocrine cell lines (HuTu-80 and NCI-H716 cells). Stimulation of HuTu-80 or NCI-H716 cells with the bitter-tasting compound phenylthiocarbamamide, which binds hT2R38, induced a rapid increase in the intracellular Ca2+ concentration in these cells. The identification of Goαgust and chemosensory receptors that perceive chemical components of ingested substances, including drugs and toxins, in open enteroendocrine L cells has important implications for understanding molecular sensing in the human GI tract and for developing novel therapeutic compounds that modify the function of these receptors in the gut.

Type 2 receptor family; gastrointestinal peptides; peptide YY; glucagon-like peptide-1; chromogranin A; serotonin; phenylthiocarbamamide

The gastrointestinal (GI) tract responds to a large array of signals originating in the lumen, including nutrient and non-nutrient chemicals, mechanical factors, and microorganisms (6, 12, 17, 45). Molecular sensing by GI cells plays a critical role in the control of multiple fundamental functions, including digestion, food intake, pancreatic insulin secretion, and metabolic regulation. Although these fundamental control systems have been known for considerable time, the initial molecular recognition events that sense the chemical composition of the luminal contents have remained elusive.

The gustatory system has been selected during evolution to detect nonvolatile nutritive and beneficial (sweet) compounds as well as potentially harmful (bitter) substances (22, 26). 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 (19, 54, 58). A family of bitter taste receptors [referred as type 2 receptors (T2Rs)] expressed in specialized neuroepithelial taste receptor cells organized within taste buds in the lingual epithelium has been identified in humans and rodents (1, 9, 34). These putative taste receptors belong to the G protein-coupled receptor (GPCR) superfamily (1), which are characterized by seven transmembrane α-helices (25). Similarly, the GPCRs of the T1R family, namely, T1R1, T1R2, and T1R3, have been identified as the receptors that perceive sweet substances, including l-amino acids (32, 40). Extensive genetic and biochemical evidence has indicated that the specific G protein gustducin mediates bitter and sweet gustatory signals in the taste buds of the lingual epithelium (35, 36, 50, 51, 57).

Outside the tongue, expression of the α-subunit of gustducin (Goαgust) has been also localized to gastric (24, 60) and pancreatic (23) cells, suggesting that a taste-sensing mechanism may also exist in the GI tract (59, 60). Indeed, we (59, 60) have demonstrated the expression of members of the bitter taste receptors of the T2R family in the mouse and rat GI tract and in enteroendocrine cells in culture. More recently, these results have been confirmed by other laboratories (33) and extended to the expression of T1Rs (13). Collectively, these findings have demonstrated the expression of taste signal transduction pathways in cells of the GI tract of mice and rats.

The identity of the GI cells involved in Goαgust-dependent signaling remains incompletely understood. A previous report (24) has indicated that in rodents, Goαgust is expressed by a distinct population of GI epithelial cells, called brush or caveolae cells. These cells are characterized by the apical and basolateral expression of villin and the lack of intracellular secretory vesicles typical of enteroendocrine cells. Interestingly, brush or caveolated cells are much less abundant in the GI and respiratory tract of normal humans than in animals (47).

It is conceivable that Goαgust signaling does not operate in the GI tract of humans or, alternatively, that Goαgust is expressed by a different population of specialized GI cells. Therefore, it is important to determine whether Goαgust and receptors that mediate taste signaling are expressed by cells implicated in molecular sensing in the human GI tract.

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 >20 identified hormones (46). In the context of molecular sensing, open enteroendocrine cells have been regarded as specialized transducers of luminal factors that respond by releasing GI peptides at the basolateral side. For instance, enteroendocrine L cells, localized in the distal region of the small intestine and in
the colon, are characterized by the production of peptide YY (PYY), a peptide that is attracting considerable attention because the peripheral administration of the PYY(3-36) form of PYY (27) reduced food intake in mice, rats, and humans (4, 5) and, like bitter stimuli, evoked an aversive food response in mice (20). A population of L cells also expresses peptides derived from proglucagon (14), including glucagon-like peptide (GLP)-1, an incretin that mediates food-stimulated, glucose-dependent insulin secretion from pancreatic β-cells (18, 21). Despite intense interest in understanding the mechanisms leading to PYY and GLP-1 release from human endocrine cells, it is not known whether GoGust-dependent signaling operates in these cells.

In view of the importance of molecular sensing in regulatory functions of the GI tract, it was of major importance to assess whether transducers that mediate taste signaling are expressed in open endocrine cells of the human gut. Here, we demonstrate, for the first time, that taste-specific GoGust is expressed in chromogranin A (CgA)-containing enteroendocrine cells in the human colonic mucosa. In particular, our results demonstrated that GoGust colocalizes with PYY and GLP-1. We also found the expression of transcripts encoding T2Rs family members, T1R3, and GoGust in the human colon and in the human intestinal endocrine cell line HuTu-80. Stimulation of either HuTu-80 or NCI-H716 cells with the bitter-tasting compound phenylthiocarbamide (PTC), the agonist of human (h)T2R38, induced a rapid increase in the intracellular Ca2+ concentration ([Ca2+]i) in these cells. The identification of chemosensory G proteins and receptors that perceive chemical components of ingested substances, including drugs and toxins, in GI peptide-producing enteroendocrine cells has important implications for understanding molecular sensing in the human GI tract and for developing novel therapeutic compounds that modify the function of these receptors in the gut.

MATERIALS AND METHODS

Bioinformatics. Human T2R genes and related expressed sequence tag sequences were retrieved by a BLAST search against human genome databases [GenBank and Ensembl based on National Center for Biotechnology Information (NCBI) build 35]. A total of 33 T2R members including 25 true genes and 8 pseudogenes were obtained with their specific chromosomal locations verified. Phylogenetic analysis was performed on nucleic acid sequences of all T2R genes/pseudogenes and on the predicted protein sequences of 25 intact T2R members. Dendrograms were constructed by ClustalW alignment and TreeView.

Cell culture. Human intestinal HuTu-80 and NCI-H716 cell lines were obtained from the American Type Culture Collection and maintained under conditions recommended by the supplier. HuTu-80 cells were grown in minimum essential Eagle’s medium containing 10% FBS and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B) in plastic or collagen I-coated plates. NCI-H716 cells, originated from an adenocarcinoma of the colon (41), were grown in RPMI-1640 supplemented with 10% FBS, 2 mM t-glutamine, and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml gentamycin) in a humidified atmosphere with 5% CO2-95% air at 37°C.

Genomic DNA and cDNA. Human genomic DNA was isolated from the human cell line HuTu-80. Poly A+ RNA isolated from the pooled human colon was purchased from two independent commercial sources (BD Sciences and Stratagene). First-strand cDNA was synthesized from 1 μg of poly A+ RNA as a template primed by oligo dT20 using thermostable reverse transcriptase at 60°C for 1 h (ThermoScript cDNA Synthesis Kit, Invitrogen).

RT-PCR analysis. Human T2R, bitter taste signaling molecules, and GI hormone markers were amplified using the specific primer pairs shown in Table 1. Most of the PCR primers were designed for amplifying full-length hT2R coding sequences (with a few exceptions for partial length) with special consideration to avoid single-nucleotide polymorphism ambiguity by MacVector (version 7.2, Accelerex). Genomic DNA extracted from the HuTu-80 cell line was used as the template in genomic PCR to validate the primer specificity and to optimize the reaction conditions for RT-PCR. Reaction conditions were as follows: predenaturation at 94°C for 2 min, denaturation at 94°C for 40 s, annealing at 59°C for 45 s; extension at 72°C for 1.5 min (32–35 cycles), and final extension at 75°C for 5 min. PCR products were separated on agarose gels and stained with ethidium bromide. Gel images were recorded by a digital camera using a Kodak image-analysis system. Predicted T2R cDNA fragments were excised from the gel and extracted by a Qiagen quick-spin column. Each T2R cDNA was then cloned into the pCRII-TOPO vector, and at least three positive clones were selected. DNA sequencing was performed either directly on purified PCR products or from plasmid clones.

Immunohistochemistry and double-labeling immunofluorescence. Histological sections of the human colon, including the ascending, transverse, descending, and sigmoid colon, were provided by the Tissue Procurement Core Laboratory of the Department of Pathology and Laboratory Medicine of the University of California (Los Angeles, CA). Four-micrometer histological sections were produced from archive paraffin blocks of uninvolved proximal and distal resection margins from surgical specimens. All tissue specimens were fixed in 4% paraformaldehyde and embedded in paraffin. All the training, certifications, and approvals required for the handling of human specimens were obtained according to University of California Institutional Review Board regulations.

We used an affinity-purified rabbit polyclonal antibody raised against a peptide corresponding to a highly amino acid sequence of GoGust (I-20, sc-395, Santa Cruz Biotechnology, Santa Cruz, CA). This antibody was used at a dilution of 1:250 in either single- or double-labeling immunohistochemistry. Other affinity-purified polyclonal antibodies used included the following: goat polyclonal against CgA (1:500, sc-1488, Santa Cruz Biotechnology); goat polyclonal against villin (1:200, sc-7672, Santa Cruz Biotechnology); rabbit polyclonal against PYY (1:1,000, 13-B52-1, Alpco Diagnostics, Salem, NH); rabbit polyclonal against serotonin (1:250, PU686-UP, Biogenex, San Ramon, CA); and goat polyclonal against GLP-1 (1:50, sc-26637, Santa Cruz Biotechnology).

Tissue sections were deparaffinized in xylene and rehydrated in graded alcohols followed by water. All primary antibodies raised against peptide antigens required antigen retrieval to unmask immunogenic epitopes. This was performed by steaming the slides in 1 mM EDTA (pH 8) for 25 min at 97–98°C. Slides were allowed to gradually cool in the steamer for an additional 15–20 min, removed from the steamer, and cooled to room temperature. Slides were then rinsed with double distilled H2O and incubated in 3% H2O2 in PBS for 15 min to block endogenous peroxidase activity. Sections were then washed once with PBS for 5 min followed by three more washes with PBS containing 0.1% Tween (PBST). After being blocked with 6% normal donkey serum (NDS) for 60 min, sections were incubated overnight at 4°C with mixtures of the appropriate primary antibodies diluted to the indicated concentration in 3% NDS and PBST.

For the nonfluorescent labeling technique, tissue sections were washed and incubated with biotinylated donkey anti-rabbit (1:300) or anti-goat (1:300) secondary antibodies for 90 min. All secondary antibodies were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). After being washed three times with PBST, sections were then incubated with avidin/biotinylated horseradish peroxidase (HRP). Development was done using the 3,3’-diaminobenzidine (DAB) Substrate Kit for Peroxidase (Vector Laboratories,.
Burlingame, CA). Sections were then counterstained with hematoxylin and mounted routinely.

For fluorescent labeling, after being incubated with the primary antibody and washed three times with PBST, sections were incubated for 180 min at room temperature with the appropriate secondary antibodies diluted in 3% NDS and PBST (e.g., rhodamine-conjugated donkey anti-rabbit IgG antibody, when used at a 1:75,000 dilution, was detected with FITC-labeled tyramide but not with rhodamine-conjugated donkey anti-rabbit IgG antibody. This was confirmed for each primary antibody by omitting the primary antibody and being washed, sections were incubated overnight at 4°C with the protocol labeled PYY cells with FITC (green fluorescence). After being washed, tissue sections were grown on 9 mm glass coverslips in 35-mm dishes.

For the addition of 1 μM of the immunogenic peptide to the antibodies before the immunohistochemical staining. In these studies, the immunogenic peptides prevented subsequent immunostaining. The primary antibodies combined for double immunostaining detected Gαq and PYY, GLP-1, serotonin, CgA, or villin.

Because the antibodies to Gαq and PYY were raised in the same species, we used a two-step procedure for these double-label studies (55). First, tyramide signal amplification (TSA) was performed using the TSA-Plus Fluorescein System (Perkin-Elmer Life Sciences, Boston, MA) to detect PYY. After antigen retrieval, tissue sections were deparaffinized, rehydrated, treated with H2O2, and blocked with 6% NDS. The first rabbit primary antibody (PYY) was incubated overnight at 4°C with the optimal dilution for TSA, as previously defined by titration (1:250). After being washed with buffer, tissue sections were incubated with HRP-conjugated donkey anti-rabbit IgG antibodies (30 min at room temperature), washed with buffer, and incubated with FITC-conjugated tyramide (10 min at room temperature). This protocol labeled PYY cells with FITC (green fluorescence). After being washed, sections were incubated overnight at 4°C with the second rabbit primary antibody directed against Gαq and PYY at the standard dilution (1:250). Tissue sections were then washed and incubated with rhodamine-conjugated donkey anti-rabbit IgG (red fluorescence) for 180 min at room temperature. Because TSA amplified the signal from the highly diluted first primary antibody to such a great extent, the first primary antibody was not detected by the rhodamine-conjugated donkey anti-rabbit IgG antibody used to detect the second primary antibody. This was confirmed for each primary antibody by omitting the second primary antibody. For example, the rabbit anti-PYY antibody, when used at a 1:75,000 dilution, was detected with FITC-labeled tyramide but not with rhodamine-conjugated donkey anti-rabbit IgG.

Sections were examined with an epifluorescence microscope (Axioskop 2, Carl Zeiss, Thornwood, NY) using filter sets for fluorescein and rhodamine (Chroma Technology, Brattleboro, VT) and Zeiss lenses (×63/1.4 Plan-APoCHROMAT and ×40/1.0 Plan-APoCHROMAT). Digital images of fluorescence were obtained with a cooled charge-coupled device camera (SPOT 2, Diagnostic Instruments, Sterling Heights, MI) and associated software (SPOT 4.5, Diagnostic Instruments) and stored on computer disk as 24-bit uncompressed TIFF files for later analysis.

**Assay of [Ca2+]i.** [Ca2+]i was measured by Ca2+ fluorometry using fura 2-AM as previously described (56). Briefly, HuTu-80 cells were grown on 9 by 22-mm glass coverslips in 35-mm dishes. Similarly, NCI-H716 cells were grown on coverslips coated with Matrigel (Becton Dickinson, Bedford, MA) in RPMI medium. Cells
were washed twice with Hanks’ balanced salt solution (GIBCO-BRL) supplemented with HEPEs (pH 7.4), 1.26 mM CaCl₂, 0.5 mM MgCl₂, 0.4 mM MgSO₄, and 0.1% BSA (referred to as Ca²⁺ buffer) and were incubated at 37°C for 15 min in 1 ml of the same buffer with 1.0 μM fura 2-AM. Cultures were then washed three times with Ca²⁺ buffer, and the coverslips were inserted into a quartz cuvette containing 2 ml of Ca²⁺ buffer. The cuvette with the coverslip was placed into a Hitachi F-2000 fluorospectrophotometer. The incubation medium was continuously stirred at 37°C. The excitation wavelengths were set at 340 and 380 nm, and the emission wavelength was set at 510 nm. Maximum fluorescence was determined by injecting 100 μl of 5 mM digitonin into the cuvette, and minimum fluorescence was measured after the injection of 100 μl of 0.5 M EGTA (pH 8.0). A K_d of 224 nM was used for the Ca²⁺ dissociation constant from fura 2 in the cells at 37°C. [Ca²⁺], was determined automatically by the cation measurement software of the F-2000 fluorospectrophotometer.

RESULTS

Identification of Ga₉gust-positive cells in the human colonic mucosa. As the first step to assess whether Ga₉gust is expressed in the human gut, we labeled sections of the human colon, duodenum, and stomach with a specific antibody directed against a unique amino acid sequence of Ga₉gust. We found Ga₉gust-positive cells throughout the human GI tract, but Ga₉gust expression was prominent in the lining of the proximal colonic mucosa, as shown in Fig. 1A. Ga₉gust was localized to both elongated cells present in the surface epithelium (Fig. 1B) as well as in round cells located in the glandular epithelium (Fig. 1C). Ga₉gust-positive cells were detected in 12 independent donors and were evident in the ascending, transverse, descending, and sigmoid colon. In all cases, omission of the primary antibody or exposure of the Ga₉gust antibody to the corresponding immunogenic peptide completely abolished immunostaining of human colonic cells (results not shown).

To determine whether Ga₉gust mRNA is expressed in the human colon, we performed RT-PCR using specific primers based on the human Ga₉gust sequence (Table 1). A major PCR product of the predicted size for the full-length of Ga₉gust was detected (Fig. 1D). The sequence of this PCR product was identical to the predicted sequence of human Ga₉gust in the NCBI database (Accession No. XM 294370). The deduced amino acid sequence of human Ga₉gust showed 98% identity to the sequence of rat gustducin. These results demonstrate, for the first time, the expression of Ga₉gust in the human gut.

Human Ga₉gust-positive cells contain CgA but not cytoplasmic villin. We next attempted to identify specific cells that express Ga₉gust in the human gut. In view of previous studies (23, 24) in rodents demonstrating that Ga₉gust cells were identified as villin-positive brush cells, we used double-labeling immunofluorescence to determine whether Ga₉gust and villin are coexpressed in human colonic cells. Brush cells are characterized by the apical and basolateral expression of villin and lack of intracellular secretory vesicles typical of enteroendocrine cells (24). As shown in Fig. 2, top, we did not detect any cytoplasmic colocalization of villin with Ga₉gust in human cells located in either the glandular or surface epithelium of the colon (Fig. 2, insets). As expected, the villin antibody stained the luminal border of epithelial cells in the colon (Fig. 2, top middle and top right). These results demonstrate that human Ga₉gust-positive cells do not express basolateral villin in the colon.

To determine whether Ga₉gust is expressed in enteroendocrine cells, sections of the human colon were labeled with antibodies directed against Ga₉gust and CgA, a well-established marker of endocrine cells (16, 42, 43). Consistent with previously published results (16), we detected high levels of CgA immunoreactivity in cells scattered throughout the glandular and surface epithelium of the human colon (Fig. 2, middle and bottom, respectively). A salient feature of the results shown in Fig. 2 was that most Ga₉gust-positive cells in either the glands or surface epithelium also contain CgA. Interestingly, Ga₉gust was expressed in both the basal and apical regions of elongated cells located in the surface epithelium, whereas CgA was predominantly localized to the basal and apical regions of the same cells. Consequently, strong colocalization of these proteins was evident in the basal region of the cell. The results shown in Fig. 2 demonstrate that Ga₉gust is expressed in endocrine cells of the colonic mucosa.

Identification of the enteroendocrine cells that express Ga₉gust in the human colon. GI endocrine cells produce multiple peptide hormones (as well as histamine and serotonin) that...
serve as markers to identify different subtypes of cells. In the mouse colon, PYY, GLP-1, CCK, and neurotensin are coexpressed in some cells. Likewise, serotonin-containing cells coexpress substance P but not PYY or GLP-1, leading to the hypothesis that there are two major branches for enteroendocrine differentiation in the colon (49, 53). This prompted us to determine whether Gαsust localized to PYY-containing L cells or to serotonin-containing enterochromaffin cells. In these experiments, we used consecutive sections of the human colonic mucosa that were stained with affinity-purified rabbit antibodies that detected Gαsust and PYY (Fig. 3, top) or Gαsust and serotonin (Fig. 3, bottom). As revealed by the examination of serial sections, Gαsust-positive cells coincided with PYY-positive cells, as indicated by the arrows, suggesting that Gαsust is expressed by endocrine cells of the human colon that contain PYY. In contrast, the distribution of Gαsust-positive cells was clearly different from that of serotonin-expressing cells. The findings presented in Fig. 3 indicate that Gαsust and serotonin are localized to different cell types in the colonic mucosa and imply that Gαsust expression is specific to PYY-containing enteroendocrine cells.

To substantiate that Gαsust localizes to PYY-containing cells, we performed double-label immunofluorescence studies. Because both primary antibodies used in these studies were raised in rabbits, we conducted a two-step procedure using TSA (55). Our results, illustrated in Fig. 4, top, demonstrate striking colocalization of Gαsust and PYY in cells scattered in the human colonic mucosa. Interestingly, coexpression of Gαsust and PYY was seen in elongated cells present in the surface epithelium (see Fig. 4, insets) as well as in cells present in the glandular epithelium.

![Image](http://ajpgi.physiology.org/ by 10.220.33.4 on November 7, 2017)
Given that L cells located in the distal ileum and colon often coexpress PYY and GLP-1 (14, 15, 37), we used double-label immunofluorescence to determine whether Go(gust) also colocalizes with GLP-1. The results, shown in Fig. 4, demonstrate the presence of Go(gust)-positive cells in the surface and glandular epithelium of the human colonic mucosa that also contain immunoreactive GLP-1.

Expression of hT2Rs and hT1Rs in human colonic tissue. Having established that Go(gust) is expressed in enteroendocrine cells of the human colon, our next step was to determine whether members of the T1R and T2R families are also expressed in the human colon. To identify all the members of the hT2R family in the human genome, we undertook a bioinformatics homology-based screen of the human genome for sequences related to the T2R family of bitter taste receptors. We searched the database at the NCBI (human build 35.1) using published sequences of taste receptors. Our analysis identified a total of 25 T2Rs in the human genome, which are presented in the dendogram shown in Fig. 5. One hT2R (hT2R1) 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. Analyses by other investigators have produced a similar number of intact human hT2R genes (8, 10, 44).

To determine the presence of transcripts encoding for hT2Rs in the human colon, we used RT-PCR with human subtype-specific primers for all receptors (Table 1). As illustrated in Fig. 5, multiple hT2R transcripts were detected in the human colon, including hT2R3, hT2R4, hT2R5, hT2R10, hT2R13, hT2R38, hT2R39, hT2R40, hT2R42, hT2R43, hT2R44, hT2R45, hT2R46, hT2R47, hT2R49, hT2R50, and hT2R60. Interestingly, some of the hT2Rs detected have defined ligands, including hT2R10, the strychnine receptor (8); hT2R38, the PTC receptor (7, 28); and hT2R43 and hT2R44, the saccharin and acesulfame K receptors (29). All amplified products were cloned and sequenced, confirming that they were identical to known human taste receptor sequences.

Heterodimers of the T1R family, namely, T1R1/T1R3 and T1R2/T1R3, have been identified as the receptors that perceive sweet substances (32, 40). In view of the common role of hT1R3 in the heterodimers that sense sugars and amino acids, we determined whether this member of the hT1R family is expressed in the human colon. RT-PCR using specific primers for hT1R3 produced a product that corresponded to hT1R3 in the human colon. The results presented in Figs. 1–5 demon-
strate the expression of Gα₁₁/II₅₁ gust, multiple hT2Rs, and hT1R3 in the human GI tract. However, the examination of taste receptor expression at the protein level as well as the colocalization of these receptors with Gα₁₁/II₅₁ gust in enteroendocrine cells must await the development of specific antibodies directed against T1Rs and T2Rs.

Expression of Gα₁₁/II₅₁ gust, members of the hT2R family, and GI peptides in HuTu-80 and NCI-H716 Cells. We next attempted to determine whether human intestinal cell lines that produce GI peptides also coexpress Gα₁₁/II₅₁ gust and receptors implicated in intracellular taste signal transduction. Recently, HuTu-80 cells have been identified as secretin-producing intestinal endocrine cells (31). As shown in Fig. 6A, RT-PCR revealed that these cells expressed transcripts encoding for Gα₁₁/II₅₁ gust. We confirmed that the sequence of the PCR product of Gα₁₁/II₅₁ gust was identical to the predicted sequence of human Gα₁₁/II₅₁ gust in the NCBI database (Accession No. XM 294370). The expression of Gα₁₁/II₅₁ gust was also demonstrated by immunostaining fixed cultures of HuTu-80 cells with affinity-purified Gα₁₁/II₅₁ gust antibody (results not shown). In addition, RT-PCR and sequencing also demonstrated the expression of glucagon/GLP-1, PYY, gastric insulino tropeptide (GIP), and the differentiation marker NeuroD1. Collectively, these results suggested that HuTu-80 cells could provide a model of human endocrine cells that coexpress Gα₁₁/II₅₁ gust and GI peptides and thus prompted us to examine whether these cells also express bitter and sweet taste receptors.

As illustrated in Fig. 6B, RT-PCR and sequencing revealed the presence of hT1R3 and multiple hT2Rs, including hT2R38, the receptor that recognizes the bitter compound PTC (7, 28). We verified that hT2R38 expressed by HuTu-80 cells was identical to the PTC receptor obtained from human taste cells, as shown by sequencing the cDNA encoding full-length hT2R38 isolated from HuTu-80 cells.

Having demonstrated that HuTu-80 cells express Gα₁₁/II₅₁ gust and hT2R38, we next determined whether the addition of PTC, an agonist of hT2R38, induces a functional response in these cells. We monitored responses in [Ca²⁺]ᵢ by using HuTu-80 cells loaded with the fluorescence Ca²⁺ indicator fura 2-AM. We found that the addition of PTC to cultures of HuTu-80 cells induced a rapid elevation in [Ca²⁺]ᵢ measured in either cell populations (Fig. 6C) or individual cells by microscopic [Ca²⁺]ᵢ imaging (results not shown). At 10 mM, PTC induced a marked increase in [Ca²⁺]ᵢ in ~75% of the cells examined. PTC elicited an increase in [Ca²⁺]ᵢ in HuTu-80 cells in a dose-dependent manner (Fig. 6C, inset).

NCI-H716 cells have been identified as intestinal endocrine cells that produce GLP-1 (2, 3, 48). To substantiate the results obtained with HuTu-80 cells, we also determined whether NCI-H716 express Gα₁₁/II₅₁ gust and receptors for bitter substances. RT-PCR and DNA sequence analysis revealed that NCI-H716 cells expressed Gα₁₁/II₅₁ gust and hT2R38. The full-length PCR products are shown in Fig. 7, inset, and were similar to the results obtained with HuTu-80. As shown in Fig. 7, the sequential
addition of 7.5 mM PTC and bombesin to cultures of NCI-H716 cells induced rapid elevations in \([\text{Ca}^{2+}]_i\). It is worth noting that both HuTu-80 and NCI-H716 cells express haplo-
type G (A49, V262, and I296) of hT2R38, which is associated with a lower responsiveness to PTC (28) and thus could explain the relative lower apparent affinity for PTC showed by these cell lines in \([\text{Ca}^{2+}]_i\) assays. It is noteworthy that PTC, at similar concentrations, did not induce any detectable change in \([\text{Ca}^{2+}]_i\) in a variety of other mouse, rat, and human cell lines that do not express T2Rs, including human colonic T84 cells, pancreatic BxPC3 or Panc-1 cells, and kidney HEK-293 cells [Refs. 59 and 60 and results not shown]. These results indicate...
that PTC selectively stimulates second messenger production in human enteroendocrine HuTu-80 and NCI-H716 cells.

DISCUSSION

In the present study, we demonstrate the expression of Go\textsubscript{gust} in cells of the human colonic mucosa. Our results produced several lines of evidence indicating that Go\textsubscript{gust} localizes to enteroendocrine cells. Specifically, we demonstrated that Go\textsubscript{gust}-positive cells in the surface and glandular epithelium of human colonic mucosa coexpress CgA, an established marker of endocrine cells in the GI tract. To distinguish the subtypes of enteroendocrine cells that express taste-specific signal transducing proteins, we stained consecutive sections and performed double-label immunofluorescence studies with antibodies directed against Go\textsubscript{gust} and markers specific for GI endocrine cells. Because our results demonstrated abundant Go\textsubscript{gust}-positive cells in the lining of the colonic mucosa and because PYY and GLP-1 are peptides expressed predominantly in L cells located in the distal ileum and colon (14, 15, 37), we determined whether Go\textsubscript{gust} is expressed in cells that contain these peptides. We found colocalization of Go\textsubscript{gust} with PYY and GLP-1, which are produced by open enteroendocrine L cells. These results raise the attractive possibility that Go\textsubscript{gust}-dependent signaling in open enteroendocrine cells plays a role in sensing the chemical composition of the luminal contents.

Although the differentiation pathways leading to specific enteroendocrine cells remain incompletely understood (53), it appears that there are, at least, two major branches for enteroendocrine differentiation in the colon (49, 53). One leads to cells that express PYY, GLP-1, CCK, and neuropeptide, whereas the other corresponds to cells that express serotonin and substance P (49, 53). Interestingly, our results demonstrating that Go\textsubscript{gust} is expressed by enteroendocrine cells that contain PYY or GLP-1 but not serotonin suggest that Go\textsubscript{gust}-dependent signaling may operate in a specific lineage of enteroendocrine cells of the human colon.

Using RT-PCR with primers designed to each member of hT2R family and to hT1R3, we detected the presence of transcripts corresponding to members of the hT2R and hT1R families of bitter and sweet taste receptors in the human colon.

To support the notion that taste receptors are expressed by human enteroendocrine cells, we explored whether human enteroendocrine cell lines that produce GI peptides also express Go\textsubscript{gust} as well as members of these taste receptor families. We focused on human intestinal cell lines HuTu-80 and NCI-H716. HuTu-80 cells have been recently demonstrated to show enteroendocrine properties (31), and our present results demonstrate that these cells also express transcripts encoding the GI peptides PYY, GIP, and the precursor for glucagon/ GLP-1. Interestingly, we showed that HuTu-80 cells express Go\textsubscript{gust}, hT1R3, and multiple bitter taste receptors, including hT2R38, which is the PTC receptor (7, 28). Accordingly, the addition of PTC to cultures of HuTu-80 cells promoted a rapid increase in [Ca\textsuperscript{2+}]\textsubscript{i} in these cells. Furthermore, our results also showed that NCI-H716 cells, identified as intestinal endocrine cells that produce GLP-1 (2, 3, 48), express Go\textsubscript{gust} and hT2R38 and respond to PTC with a rapid elevation in [Ca\textsuperscript{2+}]\textsubscript{i}. In contrast, PTC did not produce any effect on [Ca\textsuperscript{2+}]\textsubscript{i} in many other cell types that do not express Go\textsubscript{gust} or bitter taste receptors (59, 60). The results obtained with HuTu-80 and NCI-H716 cells provide additional evidence supporting the hypothesis that taste signal transduction mechanisms operate in human enteroendocrine cells.

The localization of Go\textsubscript{gust} in GI cells that contain PYY and GLP-1 has a number of important implications. Administration of the PYY\textsubscript{3-36}, form of PYY (27) has been reported to reduce food intake in mice, rats, and humans (4, 5). Although these effects might require pharmacological rather than physiological doses in humans (11), PYY is attracting intense interest as a possible approach in the treatment of obesity (30). The incretin GLP-1 exerts physiologically relevant actions critical for glucose homeostasis (38), and GLP-1 signaling is increasingly regarded as a pharmacologically attractive target for the development of agents for the treatment of Type 2 diabetes (21). Interestingly, recent results have indicated that the two peptides released by enteroendocrine L cells, PYY and GLP-1, cooperate in reducing food intake in mice (56) and humans (39), and, like bitter stimuli, both peptides mediate an aversive food response in mice (20, 52). 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. The results presented in this study, suggesting that Go\textsubscript{gust} dependent signaling mechanisms may participate in the regulation of the release of PYY and GLP-1 from open enteroendocrine L cells of the human colon, raise the attractive possibility of exploiting tastant-induced endogenous release of these GI peptides as a novel approach for therapeutic intervention in obesity and Type 2 diabetes.

GRANTS

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