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Taste receptor-like cells in the rat gut identified by expression of α-gustducin

(chemoreception/rat stomach/intestine/brush cells/epithelium)

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ABSTRACT **The α-subunit of the trimeric G-protein complex specific for taste receptor cells of the tongue, α-gustducin, is described here to be also expressed in the stomach and intestine. The α-gustducin-containing cells were identified as brush cells that are scattered throughout the surface epithelium of the gut and share structural features of taste receptor cells of the tongue. These findings provide clues to the long-sought molecular and cellular basis for chemoreception in the gut.**

It is generally believed that the epithelium lining the inner surface of the gut can sense chemical components of the lumenal contents. This chemosensory information appears to be important for the regulation of various aspects of gastrointestinal secretion, resorption, and motility (1, 2). Classical examples of intestinal chemosensitivity are the dependence of gastric emptying on the chemical nature of the nutrients present in the small intestine and the involvement of chemical preabsorption information in short-term regulation of food intake (2). The cellular and molecular basis for chemoreception in the gut is hitherto unknown. In this study we addressed the question of whether the epithelium of the gut might express α-gustducin, the GTP-binding α-subunit of a trimeric G-protein complex that is specific for taste receptor cells of the tongue (3). Here we show that α-gustducin is also expressed in the epithelium of the gut where it is associated with a specialized cell type long known under the names brush cell, tufted cell, or caveolated cell (4–6). The function of this cell type, which is present in humans, rats, and probably all other mammals, had been enigmatic until now.

MATERIALS AND METHODS

Antibodies and Immunostaining. A polyclonal antibody specific for α-gustducin was raised in a rabbit immunized with a synthetic peptide comprising amino acid residues 92–113 of the rat α-gustducin sequence (3). This sequence stretch is unique for α-gustducin and is not present in the sequences of any other known G-protein. Antibodies were affinity-purified to the peptide adsorbed to nitrocellulose (7, 8). Polyclonal rabbit antibodies specific for chromogranin A and serotonin (9) and mouse monoclonal antibodies to villin (Dianova, Hamburg, Germany) and cytokeratin 18 (Progen, Heidelberg) were also used in this study. Indirect immunofluorescence was applied to 1-μm thick tissue sections of quick-frozen and Epon-embedded tissues as described (8). For double-immunofluorescence sections were incubated with a mixture of the rabbit antibody against α-gustducin and mouse monoclonal antibodies either specific for villin or cytokeratin 18. Primary antibodies were diluted with PBS: anti-gustducin (1:200), anti-chromogranin (1:4,000), anti-serotonin (1:10,000), anti-villin (0.1 $\mu g/ml^{-1}$), anti-cytokeratin 18 (0.5 $\mu g/ml^{-1}$). As secondary antibodies fluorescein isothiocyanate-labeled goat anti-mouse IgG and tetramethylrhodamine isothiocyanate-labeled goat anti-rabbit IgG (Dianova) were used at concentrations of 0.1 $\mu g/ml^{-1}$.

Immunoblotting. Various tissues of the rat were subjected to SDS/10% PAGE. Proteins were subsequently transferred to nitrocellulose, blocked with 5% low fat milk powder in PBS (pH 7.4), and incubated for 24 h at 4°C with the gustducin antibody at a concentration of 0.2 $\mu g/ml^{-1}$ in PBS. As secondary antibody horseradish peroxidase-labeled goat anti-rabbit IgG was used (Sigma). Bound immunoglobulins were visualized by the chemiluminescence technique using the ECL reagent of Amersham. For inhibition studies, 1 $\mu g/ml^{-1}$ of the immunogenic peptide was added to the diluted gustducin antibody (0.2 $\mu g/ml^{-1}$ IgG) and preincubated for 2 h at 4°C before application to the blotted nitrocellulose strips (lanes 3 and 6).

PCR, Sequencing. Poly(A)$^+$ RNA was isolated using the Quick Prep Kit from Pharmacia. Poly(A)$^+$ RNA (3 μg) was reverse transcribed in a final volume of 20 μl containing 50 pmol oligo(dT) primer, 0.5 mM dNTP, 20 units RNasin, and 40 units M-MuLV reverse transcriptase (Boehringer Mannheim) in the recommended buffer. PCR primers were selected according to the published sequence (3): primer A (401–420), GATGCTAGC-CAATCCGAGAAGTAGAGAGG; primer B (complement to 850–869), CGGAGATCT-GCTGTTGAAGAGGTGAAGAC. Nine bases (underlined) were added to the 5′ ends of each primer in order to introduce a restriction endonuclease site for cloning. PCR samples contained 2 μl of the reverse transcription reaction, 25 pmol of each primer, 0.2 mM dNTP and 2 units of *Taq* DNA polymerase in a final volume of 50 μl. For DNA amplification, 40 cycles of the following profile were used: denaturation at 95°C for 30 s, primer annealing at 55°C for 1 min, and polymerization at 72°C for 1 min. The PCR products were analyzed on a 1% agarose gel. After digestion with *Nhe*I and *Bgl*II, fragments were cloned into the *Xba*I and *Bam*HI site of the pBluescript vector. After transformation of *Escherichia coli* individual colonies were used for plasmid amplification and purification. cDNA inserts of these plasmids were sequenced using T7 DNA polymerase.

RESULTS

In sections of the tongue, the α-gustducin antibody reacted selectively with epithelial cells of taste buds most strongly with their apical cell pole (Fig. 1 *A* and *B*). In the rat stomach (cardia) and duodenum, α-gustducin-like immunoreactivity was confined to individual epithelial cells scattered throughout the surface epithelium (Fig. 1 *C*, *D*, and *F*). A few gustducin-expressing epithelial cells were also observed in the colon (not shown). Like receptor cells of taste buds these cells displayed strong gustducin-like immunostaining of the lumenal (apical) cell pole and moderate labeling of the basolateral cell surface.

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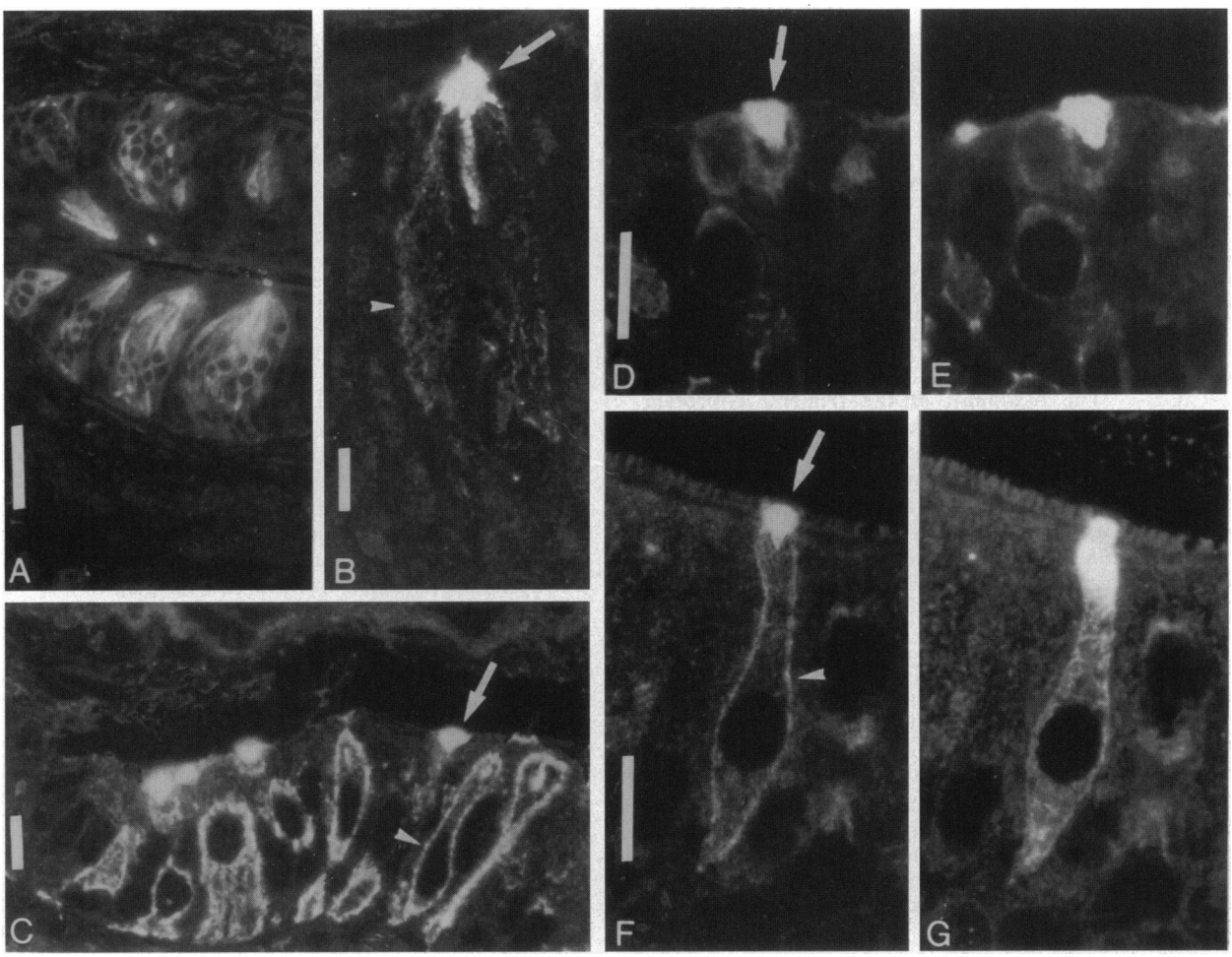

FIG. 1. Tissue sections of rat tongue papillae foliatae (*A* and *B*), and cardiac fold of the stomach (*C–E*) and duodenum (*F* and *G*). Sections were immunostained (10, 11) with an affinity-purified polyclonal antibody specific for the rat α-gustducin sequence. (*A* and *B*) Numerous cells in the taste buds are intensely labeled by the gustducin antibody. A particularly strong reaction is seen in taste pores that contain the apical microvilli of taste receptor cells (*B*, arrow). Strong immunoreactivity is associated with the apical cell pole (arrows) of epithelial cells scattered throughout the surface epithelium of the stomach (cardiac region *C* and *D* and duodenum *F*). Moderate labeling is also seen along the basolateral cell surface (arrowheads) of many brush cells (*C* and *F*) and taste receptor cells (*B*). Double-labeling of sections with antibodies against villin (*E*) and cytokeratin 18 (*G*) identify the α-gustducin-containing cells as brush cells. [Scale bars = 50 μm (*A*) and 10 μm (*B–G*)].

Preabsorption of the gustducin antibody with an excess of the immunogenic peptide abolished immunostaining of both taste buds and epithelial cells in the gut (not shown).

Double-immunolabeling of tissue sections with antibodies against α-gustducin and the actin filament-bundling protein villin, a marker molecule for brush cells (10, 12), identified these cells as brush cells (Fig. 1 *D* and *E*). Like brush cells (4, 12) the α-gustducin-expressing cells were particularly abundant in the cardiac fold of the stomach (Fig. 1*C*). Further proof for the association of α-gustducin with brush cells was obtained by double-labeling experiments with antibodies against cytokeratin 18 (Fig. 1 *F* and *G*). This intermediate filament protein is present in considerably higher concentrations in brush cells than in any other epithelial cell type of the lung and the gastrointestinal epithelium (10). At low antibody concentrations, brush cells (Fig. 1*G*), as well as receptor cells in taste buds (not shown), are distinguished from other epithelial cells by their strikingly strong cytokeratin 18-like immunoreactivity.

To determine whether α-gustducin might also be expressed in enteroendocrine cells, we performed immunostaining with antibodies against serotonin and chromogranin A. In no case did we find enteroendocrine cells (identified by these markers) reacting with the α-gustducin antibody (Fig. 2), indicating that enteroendocrine cells of the gut do not express significant amounts of α-gustducin.

Immunoblot analysis of the mucosa of the rat stomach and the taste bud-containing papillae foliatae of the rat tongue revealed selective binding of the gustducin antibody to a 42-kDa protein band (Fig. 3) that corresponds well to the calculated molecular weight deduced from the rat α-gustducin cDNA sequence of 40,163 (SwissProt Release 31.0, sequence entry GBT3_RAT) (3). No immunoreactive protein bands were detected in immunoblots of the kidney (that is devoid of brush cells) and the tip of the tongue (that contains only very few taste buds).

Further proof for the expression of α-gustducin in the gut was obtained by PCR applied to reverse transcribed mRNA extracted from the rat gastric and intestinal mucosa (Fig. 4). Two primers were used: one primer includes a sequence unique for α-gustducin (3) and the other primer was placed downstream of a possible alternative splicing site. In rat intestine and stomach a major PCR product of 487 bp was obtained. Subsequent cloning and sequencing revealed complete sequence identity to the corresponding portion of the α-gustducin sequence of the rat tongue (nt 401–869). In addition, a minor ≈350-bp PCR product was detected in the intestine. Although we have not yet sequenced this minor PCR component, it is possible that it results from an alternatively spliced gustducin mRNA reported to be expressed in the tongue (3). This splice variant in the tongue lacks nt 704–838

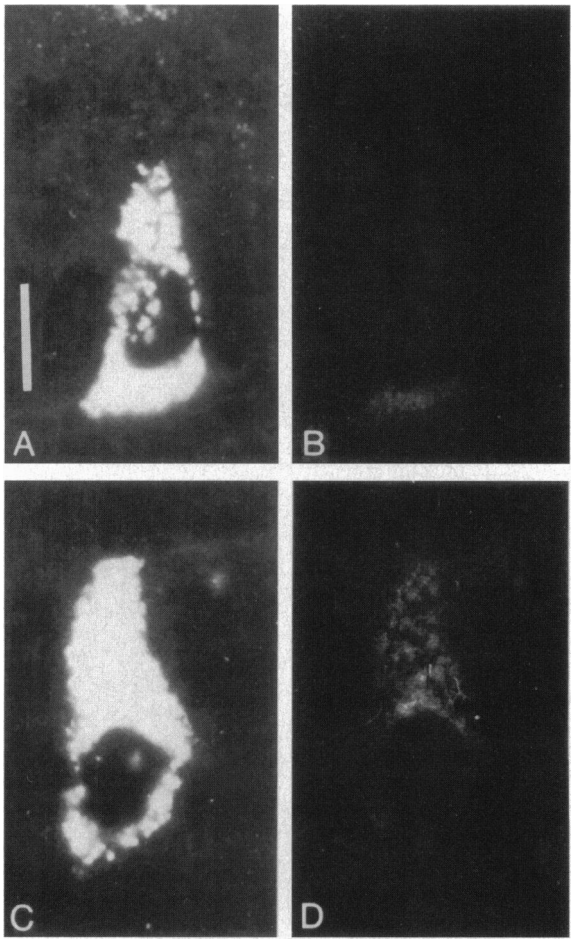

FIG. 2. Semithin tissue sections (0.5 μm in thickness) of the rat duodenal mucosa in which enteroendocrine cells were identified by antibodies against chromogranin A (*A*) and serotonin (*C*). Consecutive 0.5-μm thick sections were incubated with anti-α-gustducin (*B* and *D*). Note that the enteroendocrine cells are negative for α-gustducin. (Scale bar = 10 μm.)

and, accordingly, would result in a PCR product of 352 bp, which is in the range of the minor intestinal PCR product observed in this study (≈350 bp).

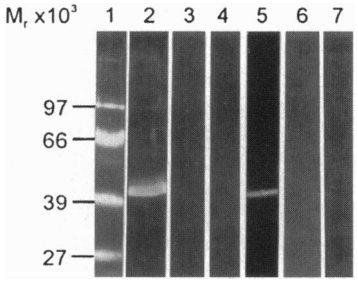

FIG. 3. Identification of α-gustducin in the rat gut. Immunoblot analysis of rat tongue papillae foliatae (lane 2), tip of the tongue (lane 4), cardiac fold of the stomach (lane 5), and kidney (lane 7) using the affinity-purified polyclonal rabbit antibody specific for α-gustducin. Lane 1 shows molecular weight standards and lanes 3 and 6 document preabsorption controls (see below). Anti-α-gustducin binds to a major ≈42-kDa protein band of the taste bud-containing papillae foliatae (lane 2) and the cardiac fold of the stomach (lane 5). No labeling is seen in immunoblots of the tip of the tongue (lane 4) and the kidney (lane 7). The former contains only very few taste buds and the latter is devoid of brush cells. Preabsorption of the gustducin antibody by addition of the immunogenic peptide inhibits binding of the antibody to the 42-kDa band in the papillae foliatae (lane 3) and stomach (lane 6).

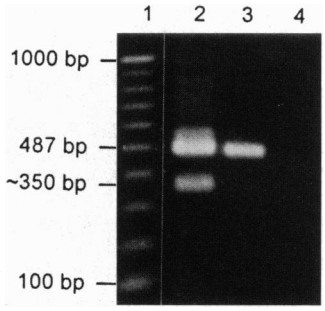

FIG. 4. Partial length amplification of α-gustducin cDNA after reverse transcription of poly(A)$^+$ RNA isolated from the mucosa of the rat small intestine (lane 2) and stomach (lane 3). Kidney tissue (lane 4) served as control. PCR primers were chosen on the basis of the cDNA sequence of rat tongue α-gustducin (3). The stomach and small intestinal mucosa contain a major PCR product of 487 bp. An additional minor product of ≈350 bp is present in the intestinal mucosa. No amplification product was detected in the rat kidney. The sequence of the 487-bp PCR product is identical to the corresponding partial length sequence of rat tongue α-gustducin (3).

DISCUSSION

The present identification of α-gustducin in brush cells of the epithelium lining the inner surface of the stomach and duodenum raises the interesting possibility that brush cells serve a chemoreceptive function. In taste cells of the tongue, α-gustducin is believed to associate with gustatory receptors of the plasma membrane (3). Binding of taste molecules to these receptors is thought to induce GTP binding to α-gustducin that, like other G_α-proteins, stimulates second messenger systems involved in signal transduction. The lumenal cell pole of both brush cells (4, 5, 6, 12) and taste receptor cells (11) contains numerous microvilli that are the most likely site for chemoreception. In support of this notion, we found that the lumenal cell pole of both cell types was the most strongly immunolabeled portion. Some immunolabel was also associated with the basolateral cell surface of taste cells as well as brush cells, revealing the possibility that these cells might also sense to a certain degree blood-borne molecules that may modify receptor cell function.

Unlike taste receptor cells of the tongue brush cells of the alimentary system do not show any synaptic contacts with nerve fibres, raising the question of how brush cells transmit their chemosensory information to other cells and tissues of the gut. In view of our recent observation that brush cells are particularly rich in nitric oxide synthase (NOS) (13, 14), it is tempting to speculate that brush cells use NO as a gaseous messenger molecule. By diffusion into the mucosa, NO might reach and stimulate nerve endings of vagal and splanchnic afferent neurons that have been shown to respond to perfusion of intestinal segments with various solutions including glucose, amino acids (e.g., glycine and histidine), or protease hydrolysates (2). In addition to acting on nerve fibres, NO might also stimulate putative target cells located in the nearer vicinity of brush cells, such as enteroendocrine cells, absorptive or secretory epithelial cells, or mucosal blood vessels. With respect to the role of NO in the stomach it has been suggested that NO released into the gastric lumen plays a role in the defense against swallowed microorganisms (15) and, furthermore, helps to protect the mucosa from acid-induced lesions (16).

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