GUST27 and Closely Related G-protein-coupled Receptors are Localized in Taste Buds Together with Gi-protein α-Subunit

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Abstract

Gustatory, like olfactory signalling is probably mediated by seven-transmembrane receptors and coupling GTP-binding proteins (G proteins). We investigated the expression of a subset of these receptors and the Gi protein α-subunit by using their specific antibodies. Based on our previous finding that the mRNA for GUST27, one of these receptors, is expressed in rat lingual epithelia, we first prepared an antibody to the synthetic nonapeptide, H·Ser-Tyr-Ser-Gln-Ile-Ala-Ser-Ser-Leu·OH, which corresponds to the third intracellular domain of GUST27 and also to those of a subset of related receptors whose occurrence can be predicted by PCR. Immunohistochemical studies with rat circumvallate papillae indicated that the anti-GUST27 antibody reacted with many of the taste buds examined, with strong signals appearing in particular taste cells. We then carried out a similar immunohistochemical experiment with an antibody to the Gi protein α-subunit and found that this subunit is also expressed in taste buds as demonstrated in the case of gustducin and transducin. Taken together, these results strongly suggest that GUST27 and closely related receptors, as well as Giα proteins, are involved in intracellular taste signal transduction. **Chem. Senses 21: 335–340, 1996.**

Introduction

There are a variety of receptors in sensory organs that function as triggers to transmit the senses of sight, smell and taste to the brain. Photoreceptor (opsin) (Nathans and Hogness, 1983) and olfactory receptor (Buck and Axel, 1991) have been extensively studied and found to be seven-transmembrane receptors that function by coupling with GTP-binding proteins (G proteins).

It is physiologically demonstrated that the sense of taste is triggered at the sensory cells that constitute taste buds existing on the surface of the tongue. We have cloned several G protein-coupled receptors from rat tongue epithelia (Abe *et al.*, 1993a) and found by Northern blot analysis that one of these clones, GUST27, is expressed specifically in this epithelial tissue (Abe *et al.*, 1993b). Our *in situ* hybridization analysis has also shown that the GUST27 mRNA is expressed at and near the taste bud sites of fungiform and circumvallate papillae.

Taste signal transduction proceeds though molecular events that activated G proteins to act on effectors such as adenylyl cyclase and phospholipase C β (PLC β) to produce

second messengers, such as cAMP (Avenet *et al.*, 1988; Striem *et al.*, 1989) and inositol triphosphate (IP_3) (Akabas *et al.*, 1988; Hwang *et al.*, 1990). Recently, Ruiz-Avila *et al.* (1995) reported that gustducin and transducin, classed as Gi protein α-subunits (Giα), are present specifically in taste buds. They also found that transducin activates phosphodiesterase when taste cells are stimulated with denatonium.

The present study reports the results of immunohistochemical observations using a specific antibody in order to define the localization of the possible taste receptors in taste buds together with Giα.

Materials and methods

Preparation of antibodies

An antibody specific to GUST27 was prepared by using the synthetic peptide, H·Ser-Tyr-Ser-Gln-Ile-Ala-Ser-Ser-Leu·OH, which corresponded to the sequence from Ser^{198} to Leu^{206} (intracellular domain 3) of GUST27 (Figure 1; Abe *et al.*, 1993b). This synthetic nonapeptide was added a cysteine residue at the NH_2-terminus and the resulting decapeptide was conjugated to keyhole limpet haemocyanin. A rabbit was immunized with the haemocyanine-conjugated decapeptide to prepare antiserum containing an anti-GUST27 antibody. An antibody specific to Giα raised against the peptide H·Lys-Gln-Gln-Leu-Lys-Asp-Cys-Gly-Leu-Phe·OH, which corresponds to the sequence from Lys^{344} to Phe^{353} of Giα (Jones and Reed, 1987), was a kind gift from Dr T. Haga, Laboratory of Biochemistry, Institute for Brain Research, the University of Tokyo. These antisera were used as antibodies for western blot analysis and immunostaining.

Immunohistochemical observations

The tongue of a 5-week-old male rat (Fischer) was perfused through the heart first with Ringer solution and then with Bouin solution fixative (Sigma). The tongue was dehydrated and embedded in paraffin, and cross-sections, 4 μm in thickness, were immunoreacted with anti-GUST27 antibody in PBS at 1:20 dilution or with anti-Giα antibody in PBS at 1:50 dilution for 1 h at 37°C. The sections were rinsed with PBS and then incubated for 30 min at 37°C with rhodamine-conjugated anti-rabbit IgG (Molecular probes, Eugene, OR, USA) at a dilution of 1:50. The sections were again rinsed with PBS, mounted in glycerol-Tris buffer and observed with a light microscope. A negative control experiment was performed by omitting the primary antiserum from the protocol described above. For a pre-absorption control experiment, anti-GUST27 antibody was incubated with the synthetic peptide overnight, centrifuged and the resultant supernatant was used as a first antibody. Biotinylated anti-rabbit IgG was then used as a second antibody at a dilution of 1:100 and incubated with avidin-biotin-peroxidase complex (Vector Laboratories). The signals were developed by treatment with 0.05% 3,3'-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 10 mM sodium phosphate (pH 7.4), and observed under a light microscope.

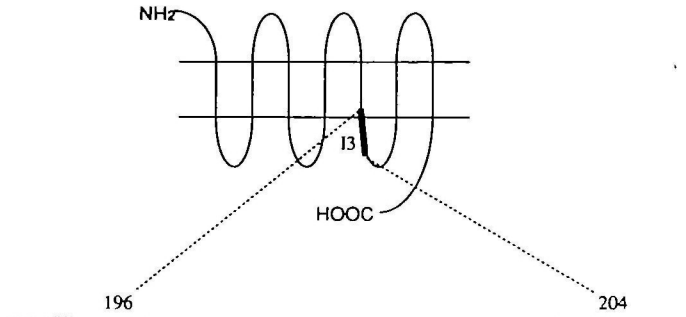
Western blot analysis

Since the GUST27 content of the tongue was considered to be low, a membrane fraction rich in taste buds was prepared as follows. Collagenase was injected beneath the circumvallate papilla tissue of a rat tongue. The membrane was stripped off and the circumvallate papilla was cut with a pair of scissors under a microscope. The papillal fraction was homogenized in 20 mM Tris-HCl buffer, pH 7, containing 1 mM phenylmethanesulphonyl fluoride. The homogenate was subjected to 10% SDS-PAGE and blotted onto a PVDF membrane (Millipore corp.). The membrane was incubated with anti-GUST27 antibody at 1:30 dilution overnight and immunological staining was carried out using peroxidase-conjugating anti-rabbit IgG (second antibody) using the ECL™ western blotting detection system (Amersham Corp.). Pre-absorption control experiment was performed as immunohistochemical observation.

Results

Immunoblot analysis using anti-GUST27 antibody

The peptide antigen used to raise anti-GUST27 antibody corresponds to the third intracellular domain of GUST27 and must be specific not only for GUST27, but also for a subset of multiple gustatory receptors (Figure 1). Thus, the anti-GUST27 antibody will recognize other closely related receptors, e.g. PTE33, as well as GUST27, but the recognition may be greatly limited because the antigenic peptide sequence is found only in GUST27 and closely related receptors (Figure 1). First, we carried out western blot analysis using a total extract from tongue epithelium, but no significant signal was detected (data not shown). This indicates that the quantity of immunoreactive proteins is small, a finding consistent with the fact that the population of taste receptor cells is very small in tongue tissues (Mistretta and Baum, 1984). Then, we collected epithelium enriched in taste buds as described in Materials and methods,

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GUST27    SYS--------------------------------QIASSL
PTE33     SYS--------------------------------QIVSSL  8/9
PTE45     SYS--------------------------------KIVSSI  6/9
OLFF3     SYF--------------------------------KIVSSI  5/9
PTE38     SYI--------------------------------YTVSSV  4/9
HGMP07J   SYV--------------------------------LIISTI  4/9
OLFF5     SYI--------------------------------HITCAV  3/9
TXR       TVSVATLCHVYHG----------------------QEAA-Q  2/9
BR        CYGQLVFTV--------------------------KEAAAQ  1/9
MM1       LYWRIYRETENRA--(122 residues)--KPRGKEQMAK  1/9
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Figure 1 Design of a peptide for use in the preparation of anti-GUST27 antibody The location of the amino acid sequence in GUST27 is shown at the top. The amino acid sequence of the designed peptide and those in the corresponding positions of GUST27 and various G protein-coupled receptors are aligned. PTE33, 38 and 45, RT-PCR clones of tongue epithelium (Abe *et al.*, 1993a, b), OLFF3 and F5, olfactory receptors (Buck and Axel, 1991); HGMP07J, human germ cell receptor (Parmentier *et al.*, 1992); BR, bovine rhodopsin (Nathans and Hogness, 1983); TXR, human thromboxane A2 receptor (Hirata *et al.*, 1991), MM1, mouse M1 muscarinic acetylcholine receptor (Shapiro *et al*, 1988) Similarities among these proteins are represented by the numbers of identical amino acids divided by nine which is the number of amino acids in the third intracellular domain (I3) of GUST27

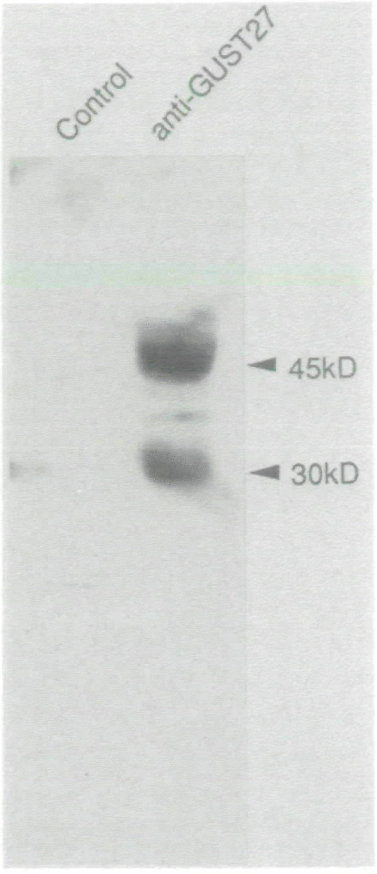

Figure 2 Western blot analysis of GUST27 Taste bud tissue was obtained from an epithelial membrane fraction of circumvallate papillae and its total protein (40 μg) was subjected to electrophoresis and western blotting. The resulting filter was treated with anti-GUST27 antibody (anti-GUST27) or pre-absorption control serum (control) as described in 'Materials and methods'.

and used them for western blot analysis. As shown in Figure 2, the circumvallate papillae contain two proteins, 45 and 30 kDa, that cross-react with anti-GUST27 antibody. Both bands disappeared when the anti-GUST27 antiserum had been absorbed by the synthetic nonapeptide. Since a potential N-glycosylation site is situated in the third extracellular domain of GUST27, there is the possibility, though not experimentally shown yet, that the 45 kDa band may correspond to a glycosylated species of GUST27 and/or related receptors. The possibility also exists that the 30 kDa band corresponds to a non-glycosylated species of GUST27 or to other related receptors, such as PTE45 (Figure 1) which have no N-glycosylation site.

Immunohistochemical observation using anti-GUST27 antibody

Using the anti-GUST27 antibody, we performed immunostaining of tissue sections of circumvallate papillae. As shown in Figure 3a, strong fluorescence signals were detected at taste bud positions, while no signal was observed when control serum (Figure 3b) or antibody pre-absorbed with the antigen peptide (Figure 3c) was used. A signal was also detected when the papillal surface was investigated (Figure 3a). This result is consistent with our previous *in situ* hybridization studies (Abe *et al.*, 1993b). At ×250 magnification (Figure 3e), it was clearly observed that different taste buds show different fluorescence intensities.

Immunostaining using anti-Giα antibody

Next, the distribution of Giα was examined by immunostaining tongue tissue sections with the anti-Giα antibody raised against H·Lys-Gln-Gln-Leu-Lys-Asp-Cys-Gly-Leu-Phe·OH, which corresponds to the C-terminus of the α-subunits of most members of the Gi family. The antibody is thus expected to detect the α-subunit of gustducin and transducin existing in tongue tissues (Ruiz-Avila *et al.*, 1995). As shown in Figure 3d, taste buds were strongly stained, together with a marginal section between the connective tissue and papillae, as well as the apical surface of the epithelium.

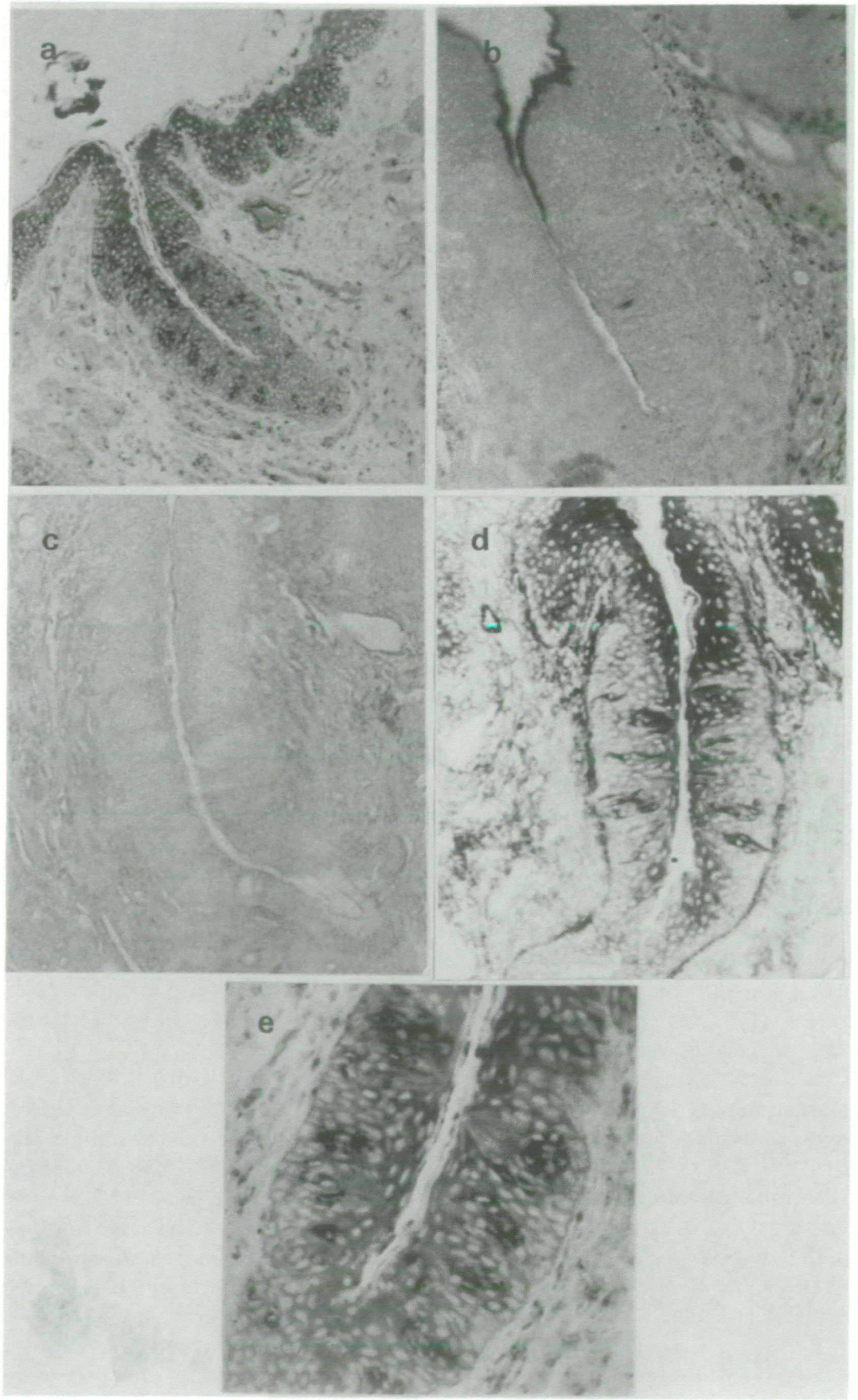

Figure 3 Immunostaining of circumvallate papillae with anti-GUST27 antibody and anti-Giα antibody. **(a)** Staining with anti-GUST27 antibody (×100). **(b)** Staining without primary anti-GUST27 antibody (×150). **(c)** Staining with anti-GUST27 antibody pre-absorbed with the synthetic peptide (×150). **(d)** Staining with anti-Giα antibody (×150) **(e)** Staining with anti-GUST27 antibody observed at high magnification (×250). Results a, b, d and e were obtained by an immunofluorescence technique. Result c was obtained by an immunoenzymatic technique.

Discussion

In the present study, we demonstrate that GUST27, a probable taste receptor, is distinctly expressed in the taste bud area (Abe *et al.*, 1993b) where gustducin and transducin, Gi class α subunits, exist (Ruiz-Avila *et al.*, 1995). Furthermore, a weak signal was detected in the epithelium bordering the taste buds. This observation is consistent with the fact that taste bud cells and surrounding epithelial cells are cognate (Stone *et al.*, 1994), and that taste bud cells have a rapid rate of turnover and are regenerated instantly from these epithelial cells (Beidler and Smallman, 1965). This suggests that GUST27 is already expressed as a protein in epithelial tissues surrounding taste buds. However, it is still uncertain whether GUST27 and/or related receptors in the taste buds are directly coupled through gustducin or transducin expressed in the same site, although the structure of GUST27, i.e. a seven-transmembrane conformation, suggests that it is coupled to some G protein for taste signal transduction. Meanwhile, as shown in Figure 3, staining by the antibody is positive only in some taste buds, indicating that the expression of GUST27 is region-specific. The results also suggest that each taste cell contains one or only a small number of receptor molecules, as reported for olfactory epithelium (Resslar *et al.*, 1993). In terms of intracellular signalling coupled with GUST27 and related receptors, it is noteworthy that Gi-protein subunits in whole or in part co-localize with GUST27, although the subtypes of Gi proteins involved are not defined. Further biochemical and physiological studies may show that α and/or βγ subunits of Gi proteins are involved in cAMP and/or Ca^{2+} signalling of GUST27, or related taste receptors. Further information on cytological localization of less closely related receptors, such as PTE01 and PTE38 (Abe *et al.* 1993a, b), as well as G protein subunits of other classes will provide significant further insight into the physiological roles and intracellular signal pathways of taste transduction.

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