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Identification of two α-subunit species of GTP-binding proteins, Gα15 and Gαq, expressed in rat taste buds

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Abstract

We cloned cDNAs for two G protein α-subunits belonging to the Gαq family, each capable of activating PLCβ, from rat tongue. One is a Gαq in the narrow sense, and the other, termed rat Gα15, is a rat counterpart of mouse Gα15, sharing an amino acid sequence similarity of 94%. RT-PCR and Northern blot analysis demonstrated that rat Gα15 and Gαq were distinctly expressed in tongue epithelia containing taste buds. Immunostaining also showed that rat Gα15, together with the Gαq, was localized mainly in taste buds. These studies suggest the possibility that these two Gα proteins function for taste signal transduction in sensory cells. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: G protein; Gαq family; PLCβ; Taste bud; Taste signal transduction

1. Introduction

GTP-binding proteins (G proteins) are known to be involved in receptor-mediated signal transduction across the plasma membrane. Each G protein is made up of α, β, and γ subunits, and there are many α-subunit species; the Gαs, Gαi, Gαq, and Gα12 families [1]. Gαs family members activate adenylyl cyclase (AC) to increase the intracellular level of cAMP as a second messenger [2,3], whereas Gαi family members inhibit AC [4]. On the other hand, Gαq family members activate phospholipase C β (PLCβ) to generate inositol trisphosphate (IP_3) [5,6] and diacylglycerol.

For taste signal transduction, some tastants are believed to bind to G protein-coupled, seven-transmembrane-domain (7TMD) receptors to activate coupling G proteins. It is suggested that the intracellular transduction triggered by sugar-type sweet tastants involves a Gαs family protein that activate AC to generate cAMP as a second messenger [7]. The cAMP in turn activates protein kinase A (PKA) [8,9] causing a PKA-sensitive potassium channel on a taste cell to be phosphorylated and close [8]. Another mode of intracellular signaling is expected for bitter and non-sugar sweet tastes. In this signaling, it is thought that phosphatidylinositol (PI) turnover occurs and IP_3 is produced to elevate intracellular Ca^{2+} concentrations [10,11]. This process requires PLC-activating G protein(s).

We have identified many 7TMD receptors ex-

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pressed in tongue epithelia [12], and succeeded in cloning a full-length cDNA for representative 7TMD receptor (GUST27) expressed in rat tongue epithelial tissues containing taste buds [13,14]. We have also found that these receptors show high degrees of structural homology to olfactory receptors [15]. Other groups have cloned similar receptors from bovine and rat circumvallate papillae [16,17]. Concerning taste-specific Gα proteins, one paper has reported that a member of the Gαi family, gustducin, is expressed specifically in taste buds [18]. Another recent paper reports the in vivo function of gustducin, showing that gustducin knock-out mice actually lose their response to several bitter and sweet tastants [19]. Gustducin, however, belongs to the Gαi family, whose members are unable to activate AC and PLCβ. Therefore, other G proteins probably exist to cause PI turnover, and these may be Gαq family members or the βγ subunits of trimeric Go and Gi proteins. Among them, the Gαq family has been generally shown to play an essential role in many PI-turnover signalings [20,21]. This family comprises various molecular species including Gαq (narrow sense), Gα11, Gα14, Gα15 and Gα16. In taste cells, however, little is known about the molecular entity of Gαq family members other than Gα14 [18].

We carried out experiments to obtain clues as to the involvement of Gαq family members in taste signal transduction. We report the molecular cloning of a ubiquitous Gαq (narrow sense) and a mouse Gα15-related protein, rat Gα15, and describe their expression and function in enhancing PLCβ activity. The possibility that both play roles in mammalian taste signal transduction is discussed.

2. Materials and methods

2.1. Reverse transcription-polymerase chain reaction (RT-PCR)

Poly(A)$^+$ RNA was prepared from the tongue epithelia of Wistar rats using oligo(dT)-cellulose. Single-stranded cDNA was synthesized by the standard method [22]. The two oligonucleotide primers, 5′-TT (TC) GA (AG) (CA) A(TC)C(AC)ITA(TC)GTI (AG)A(TC)GCIAT(TCA)AA-3′ and 5′-TCIACIA (AG) (AG) TGIGA (AG) T (AG) IA(TGA) (AGT)AT (TC)TT, were synthesized according to the amino acid sequences FE(HN)(QP)YV(ND)AIK and KI (ML)(YH)SHLVD, respectively, that are generally conserved among Gαq family members [23]. PCR was conducted with rat tongue epithelial cDNA under the following conditions of 45 s at 96°C for denaturation, 2 min at 45°C for annealing, and 3 min at 72°C for extension for a total of 30 cycles. The amplified DNA fragments, ca. 500 bp, were excised from the gel and cloned into pUC18 vector.

To examine the expression of the mRNAs of Gαq and rat Gα15 in circumvallate papillal epithelia, two pairs of specific oligonucleotide primers were prepared, each completely matching the partial nucleotide sequences of Gαq [23] or rat Gα15, respectively. These were 5′-GCCAAGGAAGCCCGGAGG-3′ and 5′-TAGGTGGGAATACATGAT-3′ for the Gαq, and 5′-TGGCGGCCAGAAGTCAGA-3′ and 5′-CCTTGGCTGCAGTTCCGA-3′ for rat Gα15. Circumvallate papillal epithelia were peeled from rat tongues after treatment with collagenase (2 mg/ml) for 30 min. cDNA was synthesized by the same method as above, and PCR was carried out at 94°C for 30 s, 50°C for 1 min, and 72°C for 1 min for 30 cycles.

2.2. Construction and screening of a tongue epithelial cDNA library

Using the poly(A)$^+$ RNA prepared from the epithelia, we synthesized double-stranded cDNA according to Gubler and Hoffman [24] and ligated it to λgt10 phage vector. After in vitro packaging (Stratagene), the phages were plated with *Escherichia coli* C600hfl. Recombinant plaques were transferred onto nylon filters (Hybond-N) prehybridized for 2 h at 60°C, and hybridized for 24 h at 60°C with probes labeled with [α-^{32}P]dCTP as described previously [22]. The filters were finally washed at 60°C in 2×SSC containing 0.1% SDS.

2.3. Nucleotide sequencing

DNA was extracted from the recombinant phages, digested with *Eco*RI, and subcloned into pUC18 vector. Nucleotide sequences were determined with a model 373A sequencer (Perkin Elmer).

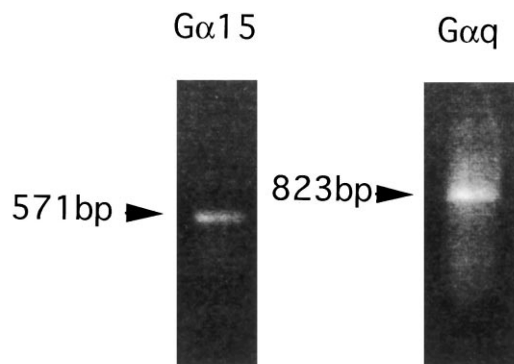

Fig. 1. Identification of rat Gα15 and Gαq transcripts in circumvallate papillae. RT-PCR products after 30 cycles were separated on a 0.8% agarose gel. The sizes of the expected PCR products are 823 bp for Gαq and 571 bp for rat Gα15.

2.4. RNA blot hybridization

Total RNA was extracted from rat tongue epithelia, denatured, and electrophoresed in formaldehyde-containing agarose gels. After electrophoresis, the RNA was transferred onto nylon membranes (Hybond-N) and hybridized with ^{32}P-labeled cDNA for rat Gα15 or Gαq at 65°C in rapid hybridization solution (Amersham). The filters were finally washed at 65°C in 0.1×SSC containing 0.1% SDS.

2.5. Preparation of an anti-rat Gα15 antiserum

A peptide, H·Ala-Arg-Tyr-Leu-Asp-Glu-Ile-Asn-Leu-Leu·OH, was synthesized in accordance with the COOH-terminal sequence of rat Gα15. A cysteine residue was attached to the N-terminus of the peptide to allow it to conjugate with keyhole limpet hemocyanine. A male rabbit (New Zealand) was immunized with this conjugate at 2-week intervals. An anti-Gαq antibody was purchased from Santa Cruz Biotechnology.

2.6. Immunohistochemical detection of Gαq and rat Gα15

The tongue of a 5-week-old rat (Wistar) was frozen and cut into 5 μm sections. The sections were fixed with 3.8% formaldehyde in phosphate buffer and then incubated for 2 h at room temperature with the anti-Gαq antibody (1:100) or anti-rat Gα15 antiserum (1:200). In the anti-rat Gα15 antiserum experiment, the sections were rinsed in PBS and then incubated for 1 h at room temperature with FITC-labeled anti-rabbit IgG at a dilution of 1:400. The signals were observed under a fluorescence microscope (Olympus LSM-GB200). On the other hand, in the experiment with anti-Gαq antibody, the sections were incubated with biotin-labeled anti-rabbit IgG at a dilution of 1:1000, then treated 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 (Olympus BX60).

2.7. Construction of active Gα protein mutants

A constitutively active rat Gα15 mutant in which Gln^{212} is replaced with Leu (rat Gα15 Q212L) was obtained by site-directed mutagenesis of a double stranded DNA by overlap extension based on PCR methodology [25]. The mutagenic oligonucleotides had the sequences 5′-TGGCGGCC<u>T</u>GAAGTCA-GA-3′ (+627 to +644) and 5′-TCTGACTT-C<u>A</u>GGCCGCCA-3′ (+644 to +627) (Fig. 2), where the modified nucleotides are underlined. A DNA fragment containing the mutagenized site was amplified by the primers 5′-AGGCGTGAATTCCACCT-3′ (+451 to +467) and 5′-CCTTGCCTGCAGTTCC-GA-3′ (+1198 to +1181) (Fig. 2). Initially, a non-mutation full-length cDNA for rat Gα15 was inserted into pSRD vector, and pSRD-rGα15 was constructed. The *Eco*RI–*Pst*I fragment of pSRD-rGα15 was replaced with the above mutated DNA fragment digested with *Eco*RI and *Pst*I, and termed pSRD-rGα15Q212L. An expression plasmid encoding an active Gαq mutant [26], pGαqQ209L, was gift from Dr. Z. Honda.

2.8. Cell culture and DNA transfection

COS-1 cells were propagated in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS. Plasmid pSRD-rGα15, pSRD-rGα15Q212L, pGqQ209L, or pSRD (1 μg/ml) was transfected into 10^6 COS-1 cells per 100 mm dish in the presence of 250 μg/ml DEAE-dextran and 100 μM chloroquine for 2 h. Cells were shocked with 10% DMSO

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CTTAT                                                                        -226
GAGTATTTCTTCCAGGGTGCTGGGTAGTCCCCACCTTTTGAGCAAGTTCAGCCTGGTTAAGTCCAAGCTGGGGCT -151
AGCTGGGGCTCCCACAGGCCCTGGGCAGGGACACGGGGGGTCCTGGAGGGACCTCCACCCCCCACTCCATCCAGA  -76
GAAGAAAGAATCCGGTAGCTGGGGCTGTGCAGGCTGCAGGAGCAATGTCGCCTGGTGGCCTGTGAGGCGCCCACC   -1
               *
ATGGCCCGGTCCCTGACTTGGGGCTGCTGTCCCTGGTGCCTGACGGAAGAGGAGAAGACTGCCGCCAGAATCGAC   75
M  A  R  S  L  T  W  G  C  C  P  W  C  L  T  E  E  E  K  T  A  A  R  I  D     25

CAGGAGATCAACAAGATTTTGTTGGAACAGAAGAAACAAGAGCGCGGGGAATTGAAACTCCTGCTGTTGGGGCCC  150
Q  E  I  N  K  I  L  L  E  Q  K  K  Q  E  R  G  E  L  K  L  L  L  L  G  P     50

GGTGAGAGCGGGAAAAGCACGTTCATCAAGCAAATGCGCATCATTCACGGCGCCGGCTACTCTGAGGAGGACCGC  225
G  E  S  G  K  S  T  F  I  K  Q  M  R  I  I  H  G  A  G  Y  S  E  E  D  R     75

AGAGCCTTCCGGCTGCTCGTCTACCAGAACATCTTCGTCTCCATGCAGGCCATGATTGAAGCAATGGACAGGCTG  300
R  A  F  R  L  L  V  Y  Q  N  I  F  V  S  M  Q  A  M  I  E  A  M  D  R  L    100

CAGATCCCCTTCAGCAGGCCGGACAGCAAACAGCACGCCAGCCTGGTGATGACCCAGGACCCCTATAAAGTGAGC  375
Q  I  P  F  S  R  P  D  S  K  Q  H  A  S  L  V  M  T  Q  D  P  Y  K  V  S    125

TCGTTCGAGAAGCCATATGCAGTGGCCATGCAGTACCTGTGGCGGGACGCGGGCATCCGCGCATGCTACGAGCGG  450
S  F  E  K  P  Y  A  V  A  M  Q  Y  L  W  R  D  A  G  I  R  A  C  Y  E  R    150

AGGCGTGAATTCCACCTGCTGGACTCCGCGGTGTACTACCTGTCACACCTGGAGCGCATCGCCGAGGACGACTAC  525
R  R  E  F  H  L  L  D  S  A  V  Y  Y  L  S  H  L  E  R  I  A  E  D  D  Y    175

ATCCCCACTGCGCAGGACGTGCTGCGCAGTCGCATGCCCACCACTGGCATCAATGAGTACTGCTTTTCCGTGCAG  600
I  P  T  A  Q  D  V  L  R  S  R  M  P  T  T  G  I  N  E  Y  C  F  S  V  Q    200

AAAACCAAACTGCGCATCGTGGATGCTGGCGGCCAGAAGTCAGAACGTAAGAAATGGATCCACTGTTTCGAGAAC  675
K  T  K  L  R  I  V  D  A  G  G  Q  K  S  E  R  K  K  W  I  H  C  F  E  N    225

GTGATTGCCCTCATCTACCTGGCGTCTCTGAGCGAGTATGACCAGTGTCTGGAGGAGAACAGTCAGGAGAACCGT  750
V  I  A  L  I  Y  L  A  S  L  S  E  Y  D  Q  C  L  E  E  N  S  Q  E  N  R    250

ATGAAGGAGAGTCTCGCTCTGTTTAGCACGATCCTAGAGCTGCCCTGGTTCAAGAGCACCTCGGTCATCCTCTTC  825
M  K  E  S  L  A  L  F  S  T  I  L  E  L  P  W  F  K  S  T  S  V  I  L  F    275

CTCAACAAGACAGACATCCTGGAGGATAAAATCCACACCTCCCACCTAGCCTCATACTTCCCCAGCTTCCAGGGA  900
L  N  K  T  D  I  L  E  D  K  I  H  T  S  H  L  A  S  Y  F  P  S  F  Q  G    300

CCCCGGAGGGACGCAGAGGCCGCCAAGCGCTTCATCTTGGACATGTACGCGCGCGTGTACGCGAGCTGTGCAGAG  975
P  R  R  D  A  E  A  A  K  R  F  I  L  D  M  Y  A  R  V  Y  A  S  C  A  E    325

CCCCACGACGGTGGCAGGAAGGGATCCCGCGCGCGCCGCCTCTTCGCACACTTCACCTGTGCCACGGACACGCAC 1050
P  H  D  G  G  R  K  G  S  R  A  R  R  L  F  A  H  F  T  C  A  T  D  T  H    350

AGCGTCCGCAGCGTGTTCAAGGACGTGCGGGACTCAGTGCTGGCCCGGTACCTGGACGAGATCAACCTGTTGTGA 1125
S  V  R  S  V  F  K  D  V  R  D  S  V  L  A  R  Y  L  D  E  I  N  L  L  *    374

CACGGGGAACCCGAAGCGGTGGTGGCGAGGACACTGCGCCCCCTGGTGGCCACTCTCGGAACTGCAGGCAAGGGA 1200
AGTCAAGTCCAAGATCTGCTCCCCAGGGGCCCTCCAAGCCCAGCCCCTCTGTAATTCATCCCCTCACTCTCCTAG 1275
CTGTTAGAGAAAGGACCTTTATCCCCAGCGCTCGGAGGCAGAGGGAGGAGGATCCTCTGTGAGTACCGGGATCCT 1350
GGGCCAAAAACTAAACAAACAAAACCGGATAGAACTGTCCGGGGCTTGTGACTTCCCAGGGATCCTGTCCAAGTC 1425
CTCCTGTGGGCCTCATGCTGGGTAAGTGAAATGTGCAAGGAAGGAAGTTCTACTTGCTGTCTACCCATTGGTTGA 1500
ACTGAGGTAGACTCCAATTAAAGATTGACTTTTGATAAAAAAAAAAAAAA                          1550
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Fig. 2. Nucleotide and deduced amino acid sequences of rat Gα15 cDNA. In-frame termination codons in the 5′-non-coding sequence are marked by asterisks.

in PBS for 2 min, washed once with PBS, and added to DMEM containing 10% FCS. After transfection, the cells were cultured for 2 days. The cells were detached by treatment with 0.05% trypsin/0.53 mM EDTA at 37°C for 2 min, and transferred into 15 ml tubes. The cells were collected by centrifugation (5 min at 800 rpm), suspended in PBS, transferred into a 1.5 ml microtube, and washed again with PBS. The cells were used for Western blot analysis and measurement of IP_3.

2.9. Western blot analysis

The membrane fraction of cells transfected with plasmid pSRD-rGα15 or pSRD were obtained by sonication and centrifugation (18 000×g, 15 min), subjected to 10% SDS-PAGE, and blotted onto a PVDF membrane (Millipore). The membrane was incubated with anti-Gα15 antiserum at 1:500 dilution overnight and treated with alkaline phosphatase-conjugated anti-rabbit IgG as a second antibody for 1 h. The signals were developed by treatment with nitroblue tetrazolium (340 μg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (170 μg/ml) as substrates.

2.10. Measurement of IP_3

Cells transfected with pSRD-rGα15Q212L, pGαqQ209L, or pSRD were used for the measurement of IP_3. 1×10^6 cells in 150 μl of PBS were added to 50 μl of 10% perchloric acid. The samples were centrifuged at 10 000×g for 15 min at 4°C, and the supernatant was neutralized with 10 N KOH. Precipitated materials were removed by centrifugation, and the IP_3 content of the supernatant was measured by a competitive radioreceptor assay using an IP_3 ^3H Radioreceptor Assay Kit (NEN).

3. Results

3.1. Cloning and characterization of cDNAs for Gαq family proteins

Initially, we conducted PCR using primers corresponding to amino acid sequences conserved among Gαq family members. As a result, we obtained two sequences of RT-PCR clones coding for Gαq family proteins; one coded for a Gαq (in the narrow sense) and the other for a protein closely related to mouse Gα15 [27] (see below, Fig. 1). The latter was named rat Gα15. We next carried out RT-PCR using primers specific for the two Gαq clones and a cDNA synthesized from circumvallate papillal epithelium. The result, shown in Fig. 1, clearly demonstrates the presence of both Gαq family mRNA species in circumvallate papillae. We next screened a cDNA library constructed from rat tongue epithelial tissue containing circumvallate papillae using the RT-PCR fragments as probes. Two cDNA clones obtained covered the entire coding sequences of Gαq and Gα15. Amino acid sequence comparison showed that rat Gα15 has 94.9% similarity to mouse Gα15 and 85.6% similarity to human Gα16, the human counterpart of mouse Gα15 [28]. The similarities between rat Gα15 and other Gαq family members were in the range of 53–56%, with much lower similarities to other G protein α subunits, 34.9% to Gαs [29], 42% to Gαi2 [30], 42% to Gαo1 [31], and 41% to Gαt [32].

3.2. Expression of Gαq and rat Gα15 mRNAs in tongue epithelium

Northern blot analysis was conducted to confirm the expression of rat Gα15 and Gαq in tongue epithelia. A strong band of ca. 1.8 kb and a faint band of ca. 3 kb were detected when 20 μg of total RNA from tongue epithelium was probed with rat Gα15 cDNA. On the other hand, a single band of 4.0 kb was observed using a Gαq cDNA insert as a probe

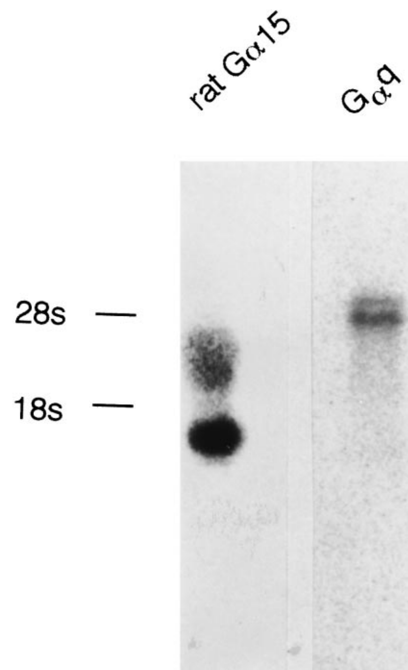

Fig. 3. Expression of Gαq and rat Gα15 mRNAs in rat tongue epithelia. A 20 μg amount of total RNA was electrophoresed. Blotted membranes were hybridized with ^{32}P-labeled cDNA inserts of Gαq and rat Gα15. The positions of 28S and 18S ribosomal RNAs are indicated.

(Fig. 3). The results indicate that the two Gαq family members, rat Gα15 and Gαq, are expressed at substantial levels in rat tongue epithelia containing taste buds.

3.3. Localization of Gαq and rat Gα15 proteins in rat tongue epithelium

In order to study the expression of Gαq and rat Gα15 more precisely, we raised antiserum against a synthetic peptide corresponding to the C-terminus of rat Gα15. The reactivity and specificity of the antiserum obtained were examined as follows. The total proteins from COS-1 cells transfected with pSRD-rGα15 or pSRD were subjected to Western blotting

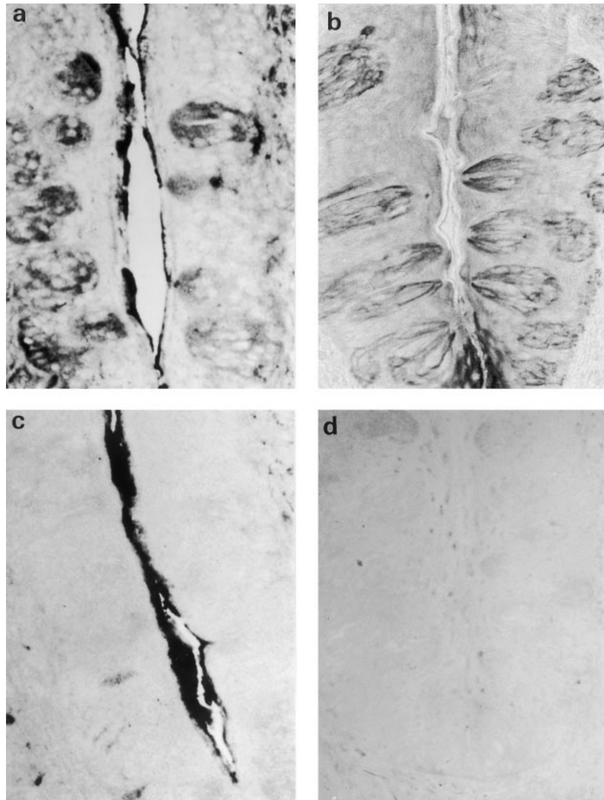

Fig. 4. Localization of Gαq and rat Gα15 proteins in circumvallate papillae. Rat circumvallate papillae were cryosectioned, fixed, and immunoreacted with the following antisera. (a) Staining with anti-rat Gα15 antiserum. (b) Staining with anti-Gαq antibody. (c) Staining with anti-rat Gα15 antiserum preabsorbed with the synthetic peptide. (d) Staining with control serum (×340). Results (a), (c) and (d), obtained by an immunofluorescence technique. Result (b), obtained by an immunoenzymatic technique.

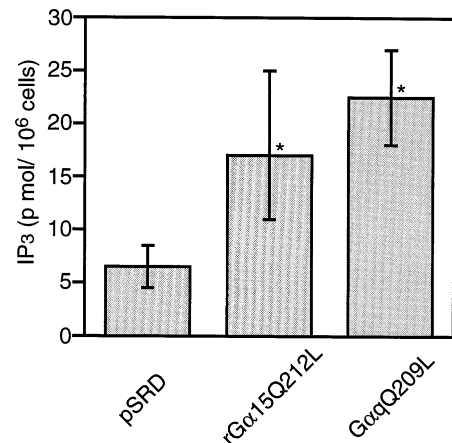

Fig. 5. Enhancement of IP_3 by the constitutive active forms of Gαq and rat Gα15. COS-1 cells were transfected with pSRD (control), pSRD-rGα15Q212L (active form of rat Gα15), and pGαqQ209L (active form of Gαq). The amounts of IP_3 per 10^6 cells are represented as means ± S.E. *Significantly different from control at $P < 0.05$.

using the antiserum. As a result, a single band of 43 kDa was specifically observed from cells expressing rat Gα15 (data not shown). The antiserum was thus found to be useful as an anti-rat Gα15 antiserum.

Using this anti-rat Gα15 antiserum, as well as the commercially available anti-Gαq antibody (Santa Cruz Biotechnology), we conducted immunostaining. When the anti-rat Gα15 antiserum was used, strong fluorescence signals were detected at the positions of taste buds (Fig. 4a), while no signal was observed when control serum was applied or when anti-rat Gα15 antiserum was used after preabsorption with the antigen peptide (Fig. 4c,d). Also, strong signals were observed at the positions of taste buds when the anti-Gαq antibody was applied (Fig. 4b). In these cases, the signals were observed distinctly in all taste buds.

3.4. Increased IP_3 levels observed in COS-1 cells expressing active Gαq and rat Gα15 proteins

Since mouse Gα15 has been shown to activate PLCβ [33], rat Gα15 was expected to show the same activity. We then confirmed rat Gα15 activity by investigating whether the intracellular IP_3 level increases when a constitutively active rat Gα15 is expressed in COS-1 cells. As shown in Fig. 5, an approximately three-fold increase in the amount of IP_3 was observed in cells expressing active rat Gα15,

a level almost the same as in the case of Gαq expression.

4. Discussion

In the present study, we identified rat Gα15 and a Gαq in tongue epithelia. Our results thus add two new proteins to the known G protein, Gα14, that has already been shown to exist in this tissue [18]. On immunohistochemical examination, both Gαq and rat Gα15 are widely expressed in taste buds, suggesting that these Gαq members play some fundamental roles in taste cells. In particular, the expression of rat Gα15 at the positions of taste buds suggests it may make a significant contribution to taste signal transduction, since it is generally known that this type of G protein is capable of coupling with various 7TMD receptors [33]. Actually, many cDNA clones encoding 7TMD receptors of lingual origin have been described [13,16,17,34].

In addition to the Gαq members, five types of Gαi are expressed in tongue epithelia [18,35], including Gαgust, Gαt-rod, Gαt-cone, Gαi2, and Gαi3. Extremely interesting among these is Gαgust, which shows taste cell-specific expression and is reported, from a gene targeting experiment, to be essential for signal transduction involving sugar and denatonium [19]. However, knock-out mice still respond strongly to other sweet tastants (such as saccharin) (personal communication), and to denatonium [19] to a lesser extent. This implies the possible presence of some Gαgust-independent pathways of sweet taste signal transduction. For bitter taste signal transduction there may be a bypathway in which Gαgust and another Gα protein function alternately. Considering that PI turnover is deeply involved in the taste signal transduction induced by saccharin and bitter tastants [10,11], it is unlikely that the above-mentioned five Gαi family members participate positively in this process. The possibility thus exists that there is another Gα-mediated system that induces Ca-influx. The population of taste cells that express Gαgust is as small as 10 out of about 100 cells constituting a taste bud [36]. Such a small population means the presence of taste cells that lack Gαgust and contain other Gα proteins instead. The rat Gα15 and Gαq found in the present study are ubiquitously expressed as shown above, and might exist in taste cells that lack Gαgust. This supports the possibility of a Gαgust-independent pathway in which rat Gα15 and/or Gαq may act as auxiliary signal transducers to complement the action of Gαgust.

This hypothesis suggests the need for more extensive studies on the interaction between 7TMD receptors and Gα proteins in taste cells. Such studies will contribute to a better understanding of mammalian taste signal transduction.

Acknowledgements

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References

[1] M.I. Simon, M.P. Strathmann, N. Gautam, Diversity of G proteins in signal transduction, Science 252 (1991) 802–808.

[2] D.T. Jones, R.R. Reed, Golf: an olfactory neuron specific-G protein involved in odorant signal transduction, Science 244 (1989) 790–795.

[3] F. Quan, L. Thomas, M. Forte, *Drosophila* stimulatory G-protein α subunit activates mammalian adenylyl cyclase but interacts poorly with mammalian receptors: implications for receptor-G protein interaction, Proc. Natl. Acad. Sci. USA 88 (1991) 1898–1902.

[4] T. Katada, G.M. Bokoch, J.K. Northup, M. Ui, A.G. Gilman, The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase, J. Biol. Chem. 259 (1984) 3568–3577.

[5] A.V. Smrcka, J.R. Hepler, K.O. Brown, P.C. Sternweis, Regulation of polyphosphoinositide-specific phospholipase C activity by purified Gq, Science 251 (1991) 804–807.

[6] S.J. Taylor, H.Z. Chae, S.G. Rhee, J.H. Exton, Activation of the β1 isozyme of phospholipase C by α subunits of the Gq class of G proteins, Nature 350 (1991) 516–518.

[7] B.J. Striem, U. Pace, U. Zehavi, M. Naim, D. Lancet, Sweet tastants stimulate adenylate cyclase coupled to GTP-binding protein in rat tongue membranes, Biochem. J. 260 (1989) 121–126.

[8] P. Avenet, F. Hofmann, B. Lindemann, Transduction in taste receptor cells requires cAMP-dependent protein kinase, Nature 331 (1988) 351–354.

[9] K. Tonosaki, M. Funakoshi, Cyclic nucleotides may mediate taste transduction, Nature 331 (1988) 354–356.

[10] M.H. Akabas, J. Dodd, Q. Al-Awqati, A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells, Science 242 (1988) 1047–1050.

[11] S.J. Bernhardt, M. Naim, U. Zehavi, B. Lindemann, Changes in IP_3 and cytosolic Ca^{2+} in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat, J. Physiol. (Lond.) 490 (1996) 325–336.

[12] K. Abe, Y. Kusakabe, K. Tanemura, Y. Emori, S. Arai, Multiple genes for G protein-coupled receptors and their expression in lingual epithelia, FEBS Lett. 316 (1993) 253–256.

[13] K. Abe, Y. Kusakabe, K. Tanemura, Y. Emori, S. Arai, Primary structure and cell-type specific expression of a gustatory G protein-coupled receptor related to olfactory receptors, J. Biol. Chem. 268 (1993) 12033–12039.

[14] Y. Kusakabe, K. Abe, K. Tanemura, Y. Emori, S. Arai, GUST27 and closely related G-protein-coupled receptors are localized in taste buds together with Gi-protein α-subunit, Chem. Senses 21 (1996) 335–340.

[15] L. Buck, R. Axel, A novel multigene family may encode odorant receptors: a molecular basis for odor recognition, Cell 65 (1991) 175–187.

[16] I. Matsuoka, T. Mori, J. Aoki, T. Sato, K. Kurihara, Identification of novel members of G-protein coupled receptor superfamily expressed in bovine taste tissue, Biochem. Biophys. Res. Commun. 194 (1993) 504–511.

[17] M. Tal, D.A. Ammar, M. Karpuj, V. Krizhanovsky, M. Naim, D.A. Thompson, A novel putative neuropeptide receptor expressed in neural tissue, including sensory epithelia, Biochem. Biophys. Res. Commun. 209 (1995) 752–759.

[18] S.K. McLaughlin, P.J. McKinnon, R.F. Margolskee, Gustducin is a taste-cell-specific G protein closely related to the transducins, Nature 357 (1992) 563–569.

[19] G.T. Wong, K.S. Gannon, R.F. Margolskee, Transduction of bitter and sweet taste by gustducin, Nature 381 (1996) 796–800.

[20] G. Berstein, J.L. Blank, A.V. Smrcka, T. Higashijima, P.C. Sternweis, J.H. Exton, E.M. Ross, Reconstitution of agonist-stimulated phosphatidylinositol 4,5-bisphosphate hydrolysis using purified m1 muscarinic receptor, Gq/11, and phospholipase C-β1, J. Biol. Chem. 267 (1992) 8081–8088.

[21] Z. Honda, T. Takano, N. Hirose, T. Suzuki, A. Muto, S. Kume, K. Mikoshiba, K. Itoh, T. Shimizu, Gq pathway desensitizes chemotactic receptor-induced calcium signaling via inositol triphosphate receptor down-regulation, J. Biol. Chem. 270 (1995) 4840–4844.

[22] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

[23] M. Strathmann, M.I. Simon, G protein diversity: a distinct class of α subunits is present in vertebrates and invertebrates, Proc. Natl. Acad. Sci. USA 87 (1990) 9113–9117.

[24] U. Gubler, B.J. Hoffman, A simple and very efficient method for generating cDNA libraries, Gene 25 (1983) 263–269.

[25] S.N. Ho, H.D. Hunt, R.M. Horton, J.K. Pullen, L.R. Pease, Site-directed mutagenesis by overlap extension using the polymerase chain reaction, Gene 77 (1989) 51–59.

[26] T. Watanabe, I. Waga, Z. Honda, K. Kurokawa, T. Shimizu, Prostaglandin F2α stimulates formation of $p21^{ras}$-GTP complex and mitogen-activated protein kinase in NIH-3T3 cells via Gq-protein-coupled pathway, J. Biol. Chem. 270 (1995) 8984–8990.

[27] T.M. Wilkie, P.A. Scherle, M.P. Strathmann, V.Z. Slepak, M.I. Simon, Characterization of G-protein α subunits in the Gq class: expression in murine tissues and in stromal and hematopoietic cell lines, Proc. Natl. Acad. Sci. USA 88 (1991) 10049–10053.

[28] T.T. Amatruda III, D.A. Steele, V.Z. Slepak, M.I. Simon, Gα16, a G protein α subunit specifically expressed in hematopoietic cells, Proc. Natl. Acad. Sci. USA 88 (1991) 5587–5591.

[29] T. Nukada, T. Tanabe, H. Takahashi, M. Noda, T. Hirose, S. Inayama, S. Numa, Primary structure of the α-subunit of bovine adenylate cyclase-stimulating G-protein deduced from the cDNA sequence, FEBS Lett. 195 (1986) 220–223.

[30] D.T. Jones, R.R. Reed, Molecular cloning of five GTP-binding protein cDNA species from rat olfactory neuroepithelium, J. Biol. Chem. 262 (1987) 14241–14249.

[31] M. Strathmann, T.M. Wilkie, M.I. Simon, Alternative splicing produces transcripts encoding two forms of the alpha subunit of GTP-binding protein Go, Proc. Natl. Acad. Sci. USA 87 (1990) 6477–6481.

[32] C.J. Raport, B. Dere, J.B. Hurley, Characterization of the mouse rod transducin α subunit gene, J. Biol. Chem. 264 (1989) 7122–7128.

[33] S. Offermanns, M.I. Simon, Gα15 and Gα16 couple a wide variety of receptors to phospholipase C, J. Biol. Chem. 270 (1995) 15175–15180.

[34] M.B. Thomas, S.L. Haines, R.A. Akeson, Chemoreceptors expressed in taste, olfactory and male reproductive tissues, Gene 178 (1996) 1–5.

[35] S.K. McLaughlin, P.J. McKinnon, N. Spickofsky, W. Danho, R.F. Margolskee, Molecular cloning of G proteins and phosphodiesterases from rat taste cells, Physiol. Behav. 56 (1994) 1157–1164.

[36] J.D. Boughter Jr., D.W. Pumplin, C. Yu, R.C. Christy, D.V. Smith, Differential expression of α-gustducin in taste bud populations of the rat and hamster, J. Neurosci. 17 (1997) 2852–2858.