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Partial Rescue of Taste Responses of α-Gustducin Null Mice by Transgenic Expression of α-Transducin

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Abstract

The transduction of responses to bitter and sweet compounds utilizes guanine nucleotide binding proteins (G proteins) and their coupled receptors. α-Gustducin, a transducin-like G protein α-subunit, and rod α-transducin are expressed in taste receptor cells. α-Gustducin knockout mice have profoundly diminished behavioral and electrophysiological responses to many bitter and sweet compounds, although these mice retain residual responses to these compounds. α-Gustducin and rod α-transducin are biochemically indistinguishable in their *in vitro* interactions with retinal phosphodiesterase, rhodopsin and G protein βγ-subunits. To determine if α-transducin can function in taste receptor cells and to compare the function of α-gustducin versus α-transducin in taste transduction *in vivo*, we generated transgenic mice that express α-transducin under the control of the α-gustducin promoter in the α-gustducin null background. Immunohistochemistry showed that the α-transducin transgene was expressed in about two-thirds of the α-gustducin lineage of taste receptor cells. Two-bottle preference tests showed that transgenic expression of rod α-transducin partly rescued responses to denatonium benzoate, sucrose and the artificial sweetener SC45647, but not to quinine sulfate. Gustatory nerve recordings showed a partial rescue by the transgene of the response to sucrose, SC45647 and quinine, but not to denatonium. These results demonstrate that α-transducin can function in taste receptor cells and transduce some taste cell responses. Our results also suggest that α-transducin and α-gustducin may differ, at least in part, in their function in these cells, although this conclusion must be qualified because of the limited fidelity of the transgene expression.

Introduction

Molecular, genetic, biochemical and physiological studies indicate that there may be multiple mechanisms underlying taste transduction [reviewed in (Kinnamon and Margolskee, 1996; Lindemann, 1996)]. Several lines of evidence implicate guanine nucleotide binding proteins (G proteins) and their coupled receptors in the transduction of responses to compounds humans consider bitter or sweet [reviewed in (Kinnamon and Margolskee, 1996; Gilbertson *et al*., 2000)]. Gustducin is a taste cell-expressed G protein implicated in responses to bitter and sweet compounds (McLaughlin *et al*., 1992). Two-bottle preference tests and nerve recordings showed that α-gustducin null mice are insensitive to two bitter compounds (denatonium benzoate and quinine sulfate) and two sweet compounds (sucrose and SC45647) at low and medium concentrations (Wong *et al*., 1996). Interestingly, the α-gustducin null mice avoided bitter and preferred sweet compounds at high tastant concentrations, suggesting that other pathways and/or other G proteins may be involved in transducing the response to these compounds. Recent results with expression of a dominant-negative α-gustducin transgene support the conclusion that other G proteins are at least in part responsible for these responses (Ruiz-Avila *et al.*, 2001). Molecular biological evidence has shown that $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{14}$, $G\alpha_{15}$, $G\alpha_{q}$, $G\alpha_{s}$ and rod α-transducin are more highly expressed in taste tissue than in the surrounding nonsensory tissue (McLaughlin *et al*., 1992; Ruiz-Avila *et al*., 1995; Kusakabe *et al*., 1998; Asano-Miyoshi *et al*., 2000) and as such may be involved in taste transduction. Ribonuclease protection and immunohistochemistry showed expression of rod α-transducin mRNA and protein in taste-enriched tissue from rat tongues at levels about 1/25th of that of α-gustducin (Ruiz-Avila *et al*., 1995). *In situ* hybridization of rat vallate and foliate papillae showed expression of α-transducin in a small number of TRCs estimated at about one-fifth the number of α-gustducin expressing cells (Yang *et al*., 1999). The level of expression of α-transducin

mRNA in rat taste receptor cells (TRCs) was also weaker than that of α-gustducin mRNA. However, these authors did not report any colocalization studies. The α-subunit of cone transducin was also amplified by polymerase chain reaction (PCR) from rat taste tissue RNA, but was undetectable by RNase protection, suggesting a very low level of expression or expression in a small number of cells (Ruiz-Avila *et al*., 1995).

At the amino acid level, α-gustducin is 80% identical and 90% similar to rod α-transducin. The close relationship of these two proteins suggests that they might act similarly in taste transduction. Recombinant α-gustducin is biochemically indistinguishable from α-transducin in its interactions with rhodopsin, retinal cGMP phosphodiesterase (PDE6) and G protein βγ-subunit (Hoon *et al*., 1995). Trypsin sensitivity and GTPγS binding assays have shown that transducin is activated *in vitro* by several bitter compounds in the presence of bovine taste membranes (Ruiz-Avila *et al*., 1995; Ming *et al*., 1998). A peptide that competitively inhibits activation of transducin by rhodopsin also inhibited activation of transducin by taste membranes (Ruiz-Avila *et al*., 1995). Aluminum fluoride activated transducin or a peptide corresponding to the region of α-transducin that interacts with retinal PDE activated a taste PDE activity (Ruiz-Avila *et al*., 1995), later shown to be a PDE1 isoform (M.M. Bakre and R.F. Margolskee, unpublished results). Thus biochemical, histological and molecular biological data suggest a potential role for transducin in taste signaling.

To determine if α-transducin can function in TRCs and to compare the function of α-gustducin versus α-transducin in taste transduction *in vivo,* we introduced into α-gustducin null mice a transgene in which α-transducin was expressed under the control of the α-gustducin promoter.

Materials and methods

Generation of transgenic mice

The construct $GUS_{7.7}TD$ included (5′ to 3′) 7.7 kb of mouse α-gustducin 5′ flanking region, a rabbit β-globin intron, the 5′ untranslated region of rat α-gustducin, a bovine rod α-transducin cDNA, the 3′ untranslated region of rat α-gustducin and the SV40 polyadenylation region. The construct was made as described (Ruiz-Avila *et al*., 2001) except that a rod α-transducin cDNA was used instead of a mutant α-gustducin cDNA. The insert containing the transgene was released by digestion with *Sal*I, electrophoresed through a low melt agarose gel, then purified using Gelase (Epicentre Technologies, Madison, WI). Homozygous α-gustducin knockout male mice (gus/gus) in 99.2% C57BL/6J, 0.8% 129/svEmsJ background were bred to wild-type superovulated B6CBAF1/J females to generate zygotes for pronuclear microinjection. CD-1 female mice were used as recipients for microinjected embryos.

Transgenic mice expressing green fluorescent protein (GFP) were produced by microinjecting a similar construct to $GUS_{7.7}TD$, except that it contained GFP instead of α-transducin, into B6CBAF1/J embryos (Huang *et al*., 1999).

Production of transgenic mice was as described (Hogan *et al*., 1994). Founder animals were screened by Southern analysis using an α-transducin (or GFP) probe and PCR. Selected $GUS_{7.7}TD$ founders were mated to heterozygous α-gustducin knockout (GUS/gus) mice in 99.2% C57BL/6J, 0.8% 129/svEmsJ background.

α-Transducin/α-gustducin double-knockout mice used as negative controls for immunohistochemistry were generated by breeding α-transducin knockout mice (Calvert *et al*., 2000) with α-gustducin knockout mice (Wong *et al*., 1996) for two generations.

Immunohistochemistry

Mice were killed by carbon dioxide asphyxiation, the tongues were excised, fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 1 h at room temperature (RT), then transferred into 20% sucrose in PBS and stored at 4°C overnight. Fixed tongues were then embedded in Tissue-Tek OCT compound (Sakura, Tokyo, Japan) and 12 μm thick cryostat sections were collected. Sections were blocked in PBS containing 2% BSA, 1% horse serum, 0.3% Triton X-100 for 30 min at RT. The primary antibody used was either TD1 or GD1, which were raised in rabbits against an α-transducin-specific peptide (amino acids 91-105) and an α-gustducin-specific peptide (amino acids 95-109), respectively (Ruiz-Avila *et al.,* 1995). Primary antibodies diluted 1:500 were applied to the sections and incubated for 1 h at RT. Sections were then washed, and the secondary antibodies were applied and incubated for 30 min at RT. The secondary antibodies used were goat anti-rabbit Ig conjugated with Cy3 (Jackson Immunoresearch Laboratories, West Grove, PA). Slides were mounted in fluorescence mounting medium (Vector Laboratories, Burlingame, CA) and examined under fluorescent light.

Western blot

All chemicals were of the highest purity available and were purchased from either Sigma (St Louis, MO) or Roche Molecular Biochemicals (Indianapolis, IN). Monoclonal antibody TN15 raised against α-transducin was from American Qualex (Austin, TX).

To isolate taste papillae, an enzyme solution containing 2 mg/ml Dispase II and 1 mg/ml Collagenase B was injected underneath the lingual epithelium. After incubation at 37°C for 20 min, the lingual epithelia were peeled off from the tongues. Circumvallate (CV) and foliate papillae were then dissected and placed in cold suspension buffer (0.1 M NaCl, 0.01 M Tris–Cl, pH 7.6, 0.001 M EDTA, 1 mg/ml leupeptin, 100 mg/ml phenylmethylsulfonyl fluoride), homogenized on ice, boiled for 10 min and centrifuged at 10 000 *g* for 10 min at RT. The protein concentration was measured by the Lowry procedure. An equal volume of 2× SDS gel-loading

buffer (100 mM Tris–Cl, pH 6.8, 200 mM DTT, 4% SDS, 0.2% bromophenol blue, 20% glycerol) was added. Proteins (25 ng) were fractioned by SDS–PAGE and transferred to a nitrocellulose membrane. The primary antibody, monoclonal TN15, was applied at a dilution of 1:3000. The ECL system (Amersham, Uppsala, Sweden) was used for detection according to the manufacturer's instructions.

Two-bottle preference tests

Mice were genotyped by PCR for endogenous α-gustducin and the neomycin resistance gene (neo) to determine if they were GUG/GUS (wild-type), GUS/gus (heterozygous) or gus/gus (homozygous α-gustducin knockout) (Wong *et al.*, 1996), and for the α-transducin cDNA transgene ($GUS_{7.7}TD$). The following three groups of mice were used for the behavioral tests: (a) $GUS_{7.7}TD$ gus/gus (n = 11); (b) gus/gus (n = 10); (c) GUS/GUS or GUS/gus (n = 9). Two-bottle preference tests were performed as described previously (Wong *et al.*, 1996). Briefly, mice were individually housed, provided with food *ad libitum* and presented with two sipper bottles for 48 h. One bottle contained distilled water and the other the tastant to be tested. The bottles were switched after 24 h to account for position effects. The tastants were presented at increasing concentrations. The ratios of tastant to total liquid consumed were recorded. The data were analyzed by the general linear model repeated measures procedure using the SSPS statistical package, with a level of significance chosen as <5%. Once it was determined that differences exist among the means, Tukey's test was used to determine which means differ. All mice tested were male and were aged 6-14 weeks at the beginning of the testing.

Nerve recordings

Anesthesia was initiated with 5 μl/g body wt of a solution containing 1.75 mg/ml ketamine and 1.75 mg/ml xylazine in saline then maintained with 0.4–0.6% isoflurane. Both chorda tympani (CT) and glossopharyngeal (NG) nerves were accessed through the same incision. Responses were recorded from one nerve in some mice and from both nerves in others. Responses of the CT were recorded from nine GUS/GUS, five gus/gus and three $GUS_{7.7}TD$ gus/gus mice. Responses of the NG were recorded from eight GUS/GUS, seven gus/gus and seven $GUS_{7.7}TD$ gus/gus mice. As taste stimuli, we used 0.1 M NH_4Cl, 0.1 and 0.3 M NaCl, 10 and 20 mM citric acid, 10 and 20 mM quinine hydrochloride (QHCl), 10 and 20 mM denatonium benzoate, 0.6 M sucrose and 8 mM SC45647. All compounds except QHCl were dissolved in artificial saliva (2 mM NaCl, 5 mM KCl, 3 mM $NaHCO_3$, 3 mM $KHCO_3$, 0.25 mM $CaCl_2$, 0.25 mM $MgCl_2$, 0.12 mM K_2HPO_4, 0.12 mM KH_2PO_4, 1.8 mM HCl, pH 7) (Danilova *et al.*, 2001). QHCl was dissolved in artificial saliva diluted 1:2 to prevent precipitation. The tastants were delivered to the tongue using an open flow system, controlled by a computer (Hellekant and Roberts, 1995). This system delivers the solutions at given intervals, over a preset time period, under conditions of constant flow and temperature (33°C). Stimulation time for both nerves was 20 s. Between stimulations, the tongue was rinsed for 1 min with artificial saliva. The nerve impulses were amplified, monitored over a loudspeaker and an oscilloscope, recorded on a Gould TA11 recorder and processed by an absolute value circuit integrator. For the analysis of the data, the spontaneous activity was deducted from the responses. The responses were normalized to the responses to 0.1M NH_4Cl. One-way analysis of variance (ANOVA) and *post hoc* comparisons (Mann–Whitney test) were used to compare the responses of the three genotypes for each tastant.

Results

Expression of the α-transducin transgene in mouse taste receptor cells

Six founder transgenic mice containing the α-transducin transgene under the control of the α-gustducin promoter ($GUS_{7.7}TD$) were identified by PCR and Southern blotting (data not shown). They were all crossbred with mice heterozygous for the null allele of α-gustducin (GUS/gus), and the α-transducin transgene was transmitted to their offspring.

Analysis by immunohistochemistry showed that all six lines expressed the transgene (data not shown). The line with the highest level of expression was used for all subsequent studies. Staining with an anti-α-transducin specific antibody (TD1) showed strong immunoreactivity in TRCs of the circumvallate papillae (CVs) of α-transducin transgenic mice in the α-gustducin null background ($GUS_{7.7}TD$, gus/gus mice) (Figure 1d) but no signal above background in wild-type mice (Figure 1b). Comparable background staining was also seen in sections from α-transducin/α-gustducin double knockouts (Figure 1f) indicating that the background does not correspond to either of these two G protein α-subunits. Apparently, the level of endogenous α-transducin expression in mouse taste cells is well below that observed in rat (Ruiz-Avila *et al.*, 1995; Yang *et al.*, 1999). The α-gustducin-specific antibody GD1 showed immunoreactivity with sections from wild-type mice (Figure 1a), but not with sections from $GUS_{7.7}TD$ gus/gus mice (Figure 1c) or double knockout mice (Figure 1e), confirming the specificity of this antibody and demonstrating that it does not cross react with α-transducin.

To determine if the α-transducin transgene was expressed in the α-gustducin lineage of TRCs, we carried out immunohistochemistry with sections of CVs from double transgenic mice that expressed GFP ($GUS_{7.7}GFP$) and α-transducin from the $GUS_{7.7}$ promoter. These mice were produced by crossing $GUS_{7.7}GFP$ with $GUS_{7.7}TD$ transgenic mice. Single transgenic $GUS_{7.7}GFP$ mice were used to show that endogenous α-gustducin and transgenic GFP colocalize in ~95% of the TRCs (Figure 2a–c). Examination of four

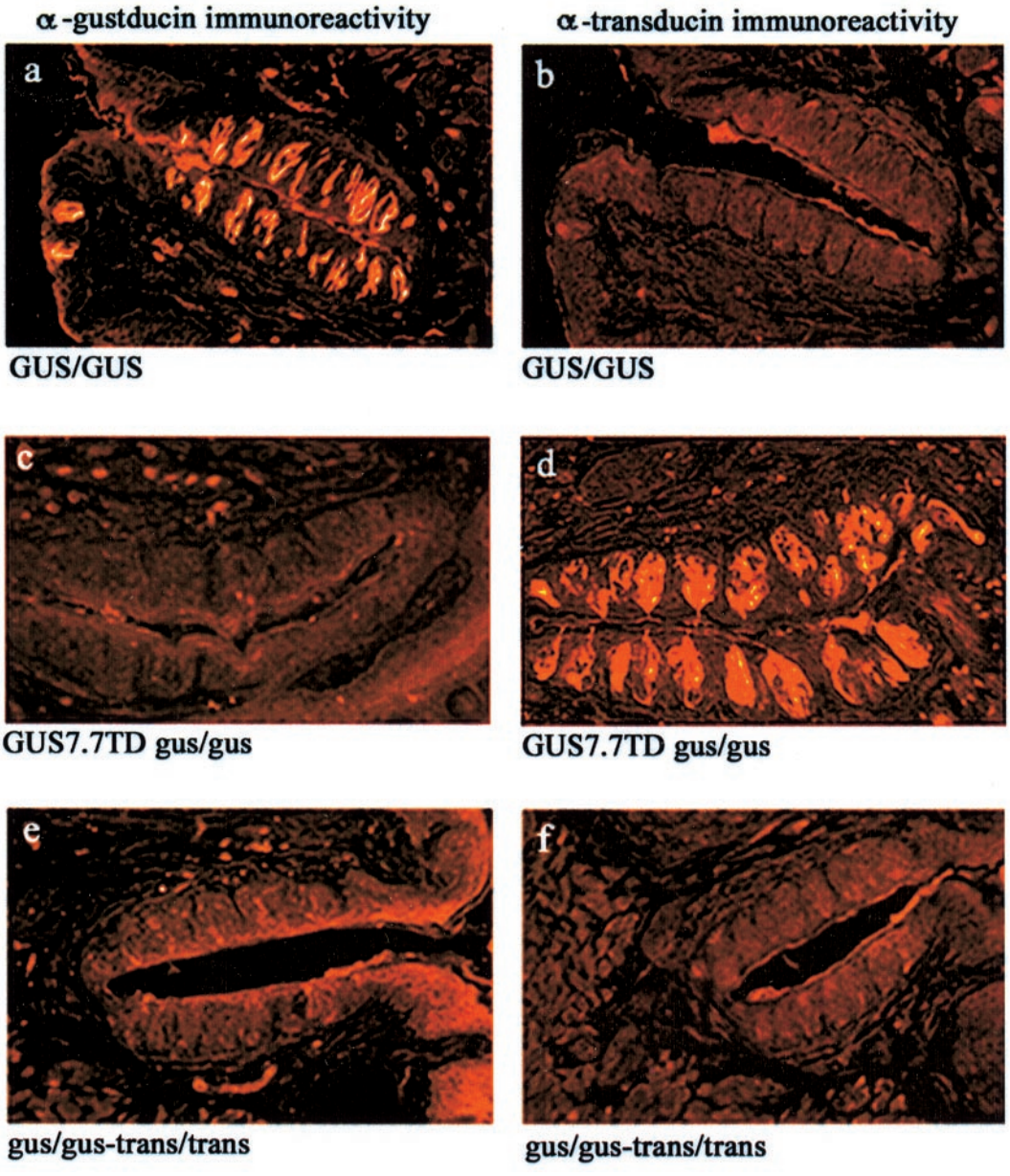

Figure 1 Immunohistochemistry of mouse CV sections with anti α-gustducin **(a,c,e)** or anti α-transducin **(b,d,f)** antibodies. a,b are from wild-type (GUS/GUS) mice; c,d are from $GUS_{7.7}$TD gus/gus mice; e,f are from α-gustducin α-transducin double knockouts (gus/gus-trans/trans). Immunostaining was obtained with the anti α-gustducin antibody applied to the wild-type mouse CV and with the anti α-transducin antibody applied to the $GUS_{7.7}$TD gus/gus mouse CV.

sections (325 GFP-positive cells) from GFP/α-transducin double-transgenic mice found expression of the α-transducin transgene in ~65% of GFP transgene-expressing (i.e. gustducin positive) cells (Figure 2d–f), indicating that most, but not all, α-gustducin positive cells also express the α-transducin transgene. No GFP-negative cells expressed the α-transducin transgene.

Expression of the α-transducin transgene was also monitored by Western blot and compared to that of endogenous α-gustducin and of a rat α-gustducin cDNA transgene driven by $GUS_{7.7}$ (Wong *et al.*, 1999). TN15, an antibody that reacts with both α-transducin and α-gustducin, was used. A band of ~40 kDa was observed in wild-type, $GUS_{7.7}$TD gus/gus and α-gustducin transgenic gus/gus mice, but not with non-transgenic gus/gus mice (Figure 3). There was a reproducible difference in apparent mobility between rat and mouse α-gustducin, presumably due to minor sequence or post-translational differences.

Behavioral tests

Forty-eight hour two-bottle preference tests were used to compare the taste responses of $GUS_{7.7}$TD gus/gus mice

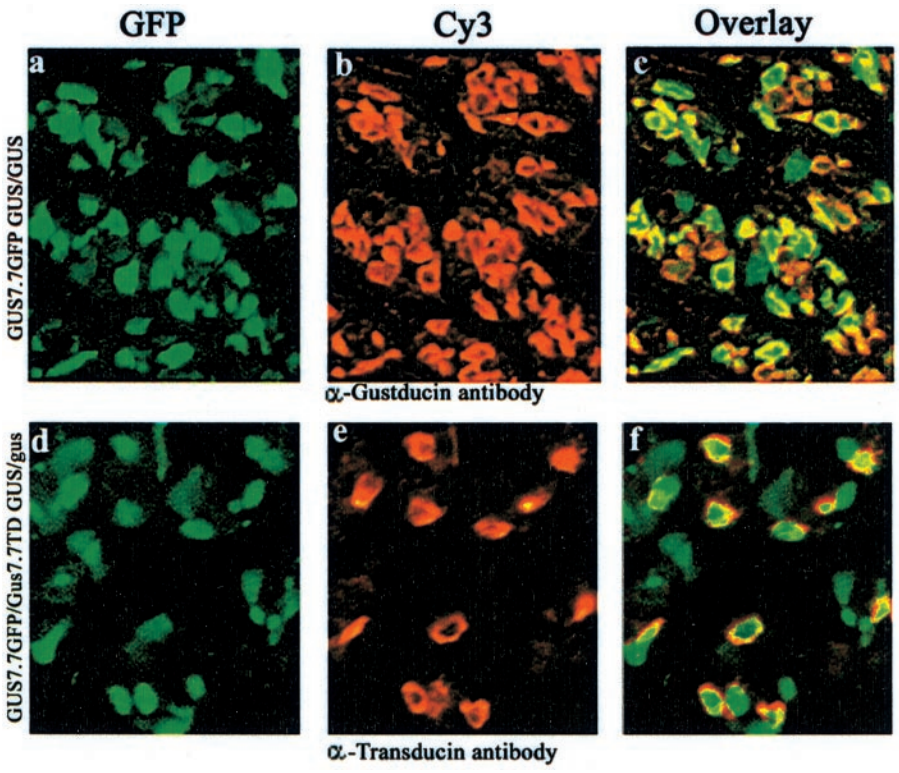

Figure 2 Colocalization of α-transducin expressed from the transgene with endogenous α-gustducin. Transgenically expressed GFP driven by $GUS_{7.7}$ was used as a marker for α-gustducin. **(a,b,c)** show colocalization of GFP and α-gustducin in ~95% of TRCs and validate the use of GFP as a marker for α-gustducin. (a) CV section from a GFP transgenic mouse examined under fluorescence microscopy with filter for GFP; (b) immunostaining of the same section with the α-gustducin specific antibody GD1 examined with a Cy3 filter; (c) overlay of (a) and (b). **(d,e,f)** show colocalization of transgenically expressed α-transducin and GFP in ~65% of TRCs. (d) CV section from a GFP/α-transducin double transgenic mouse examined under fluorescence microscopy with filter for GFP; (e) immunostaining of the same section with the α-transducin-specific antibody TD1 examined with a Cy3 filter; (f) overlay of (d) and (e).

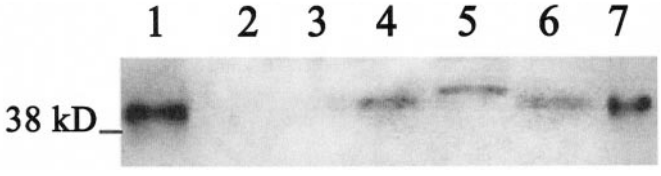

Figure 3 Western blot of mouse taste tissue homogenates. TN15, an antiserum that recognizes both α-transducin and α-gustducin was used. 1, Purified α-transducin protein (75 pg); 2, blank; 3, gus/gus; 4, gus/gus expressing the rat α-gustducin transgene; 5, wild-type; 6, $GUS_{7.7}$TD gus/gus; 7, purified α-transducin protein (25 pg).

with their α-gustducin null (gus/gus) and wild-type (GUS/GUS) or heterozygous (GUS/gus) siblings. A preference ratio (tastant solution consumed as a fraction of total liquid consumed) was calculated for each animal at each concentration. Tastants that humans consider bitter (denatonium benzoate and quinine sulfate) or sweet (SC45647 and sucrose) were tested.

Consistent with previously reported results (Wong *et al.*, 1996) non-transgenic gus/gus (knockout) mice showed markedly diminished responses to the four compounds tested. The responses of $GUS_{7.7}$TD gus/gus mice to sucrose, SC45647 and denatonium were stronger than those of the non-transgenic gus/gus mice ($P < 0.001$), but diminished compared to those of wild-type animals ($P < 0.001$), indicating that expression of the α-transducin transgene led to partial restoration of aversion to denatonium and preference for sucrose and SC45647. Interestingly, the response of $GUS_{7.7}$TD gus/gus mice to quinine was identical to that of the non-transgenic gus/gus mice, indicating that α-transducin expression in TRCs in these transgenic mice does not restore behavioral responsiveness to quinine (Figure 4).

Nerve recordings

The responses of the CT and NG nerves to six taste stimuli are shown in Figure 5. The response to denatonium was strongest in the NG, whereas those to sucrose and SC45647 were maximal in the CT. The responses to QHCl, NaCl and citric acid did not differ significantly between the two nerves.

The CT responses to both sweeteners and the NG responses to both bitter tastants were diminished in α-gustducin null mice in comparison to wild-type mice ($P < 0.005$ and $P < 0.05$, respectively). The responses to NaCl and citric acid were unchanged in the three groups of mice in both

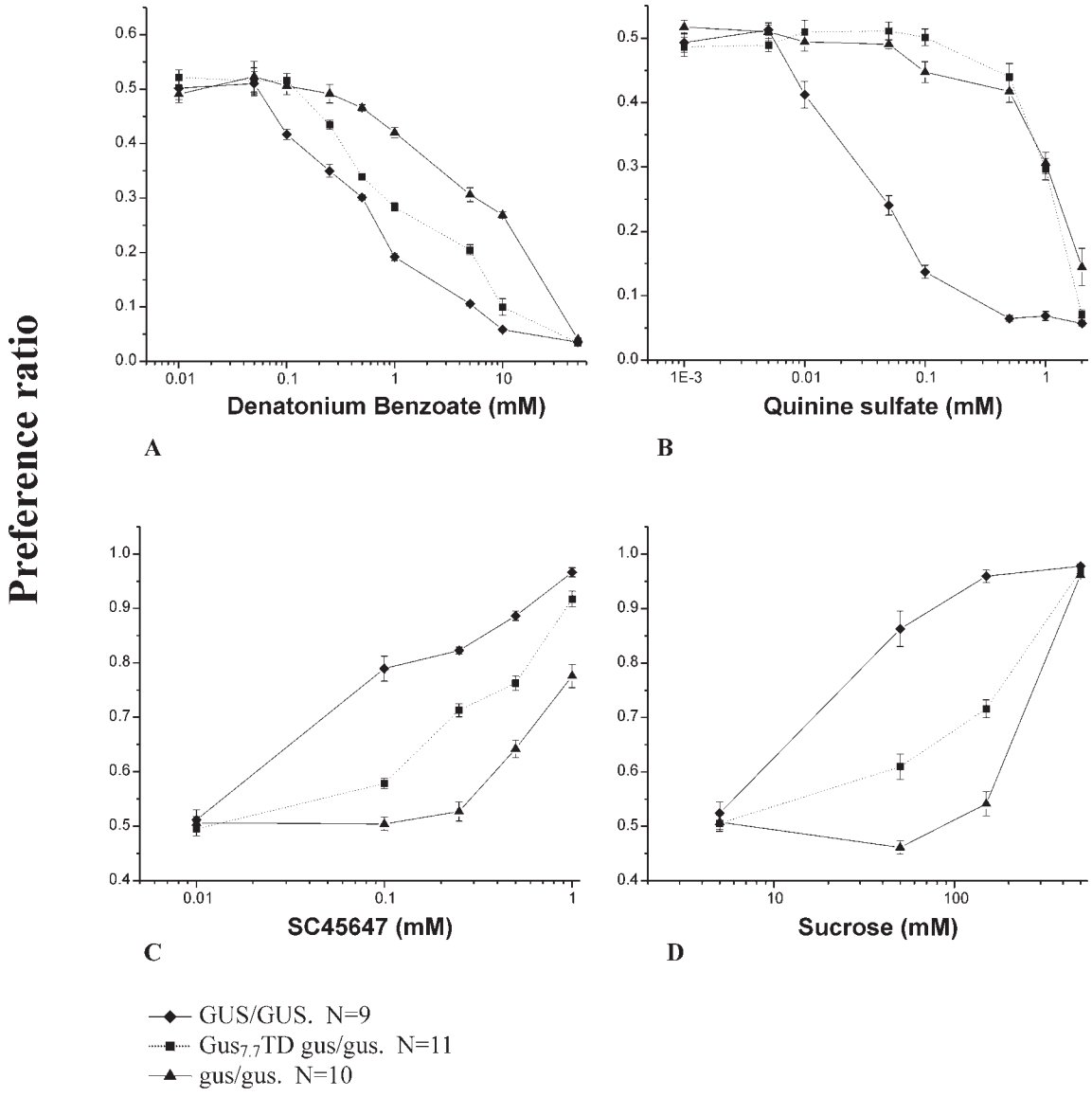

Figure 4 Mean preference ratios from 48 h two-bottle preference tests. Groups tested are GUS/GUS (n = 9, diamonds) gus/gus (n = 11, triangles) and $GUS_{7.7}$TD gus/gus (n = 10, squares, dashed line). Error bars are standard error of the mean.

nerves. The α-transducin transgene partially restored the response of the CT to sucrose ($P < 0.05$), and the response of the NG to SC45647 and QHCl ($P < 0.05$). The transgene did not affect the responses of either nerve to denatonium.

Discussion

To examine the ability of rod α-transducin to substitute for α-gustducin in taste transduction, transgenic mice expressing rod α-transducin under the control of the α-gustducin promoter were generated. Two-bottle preference tests showed that expression of the α-transducin transgene partially rescued the behavioral responses to denatonium benzoate, sucrose and the artificial sweetener SC45647 of α-gustducin null mice. However, expression of the α-transducin transgene did not restore behavioral responses to quinine sulfate. In contrast, $GUS_{7.7}$-driven expression of a wild-type rat α-gustducin transgene in the null background fully restored behavioral responses to denatonium benzoate, quinine sulfate, sucrose and SC45647 (Wong *et al.*, 1999).

The partial rescue of the α-gustducin null phenotype by α-transducin shows that transducin is capable of signaling in taste receptor cells. For most bitter and sweet compounds, the taste response is initiated by the tastant binding to G protein coupled receptors (such as the T2rs for bitter compounds) which in turn activate heterotrimeric gustducin (Chandrashekar *et al.*, 2000). The α- and βγ-subunits of gustducin then activate a variety of second messenger modulating enzymes such as PDE, PLCβ2 and adenylyl cyclase, leading to depolarization of the cell and second messenger release [reviewed in (Gilbertson *et al.*, 2000)].

While gustducin plays a key role in the signal transduction of bitter and sweet compounds, there is evidence that other pathways and G proteins are also involved. For example, quinine hydrochloride is known to directly block potassium channels (Cummings and Kinnamon, 1992). In addition, there are several lines of evidence suggesting that other G proteins are involved. First, α-gustducin null mice have profoundly diminished, but not totally abolished, responses to bitter and sweet compounds (Wong *et al.*, 1996). Second, a transgenically expressed α-gustducin mutant containing a glycine-to-proline substitution at position 352 acted as dominant negative, further reducing the responses to bitter and sweet compounds of α-gustducin null mice that expressed the transgene (Ruiz-Avila *et al.*, 2001). The dominant negative α-gustducin mutant has the ability to bind to taste receptor, Gβγ subunits and effector, but it cannot be activated by receptor. Therefore, it is believed to act as a 'βγ sink', preventing βγ-subunits from binding to other Gα-subunits, and by competing with other G protein heterotrimers for receptor binding. Third, several G protein α-subunits are expressed in TRCs, including $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{14}$, $G\alpha_{15}$, $G\alpha_{q}$, $G\alpha_{s}$ and rod α-transducin. In retina, α-transducin forms a heterotrimer with Gβ1 and Gγ1, is activated by rhodopsin and activates PDE6. Gβ1 (Huang *et al.*, 1999) and several type I PDE isoforms (Bakre and Margolskee, unpublished) that can be activated *in vitro* by α-transducin, are expressed in TRCs. Together with these data, our results suggest that a similar transducin-containing pathway exists in TRCs.

Our results suggest that transducin and gustducin differ, at least in part, in their function in TRCs and that transduction of responses to quinine may differ from those to denatonium. The lack of total restoration of responses to denatonium benzoate, SC45647 and sucrose by expression of the α-transducin transgene could be explained by one or more of the following possibilities. (i) α-transducin and α-gustducin can signal via the same pathways in the same TRCs, but α-transducin is less effective. (ii) α-Gustducin and α-transducin signal via entirely different pathways, and only a fraction of the α-gustducin expressing cells also contain the transducin pathway. (iii) The partial restoration by α-transducin of bitter and sweet responses is caused by restoration of βγ signaling. In this case α-transducin does not interact with downstream effectors, but is required to regenerate the heterotrimer which is activated by taste receptors; then all downstream signals are carried by the βγ-subunit. (iv) The level or distribution of the transgene is inadequate. Perhaps expression of the α-transducin transgene in 65% of the α-gustducin lineage TRCs is not sufficient to obtain a full response to denatonium, SC45647 and sucrose; full responses may require expression in the remaining 35% of α-gustducin lineage TRCs.

Expression of transgenes is known to vary from line to line because of the influence of the site of integration. In our previous experience with the $GUS_{7.7}$ promoter, we

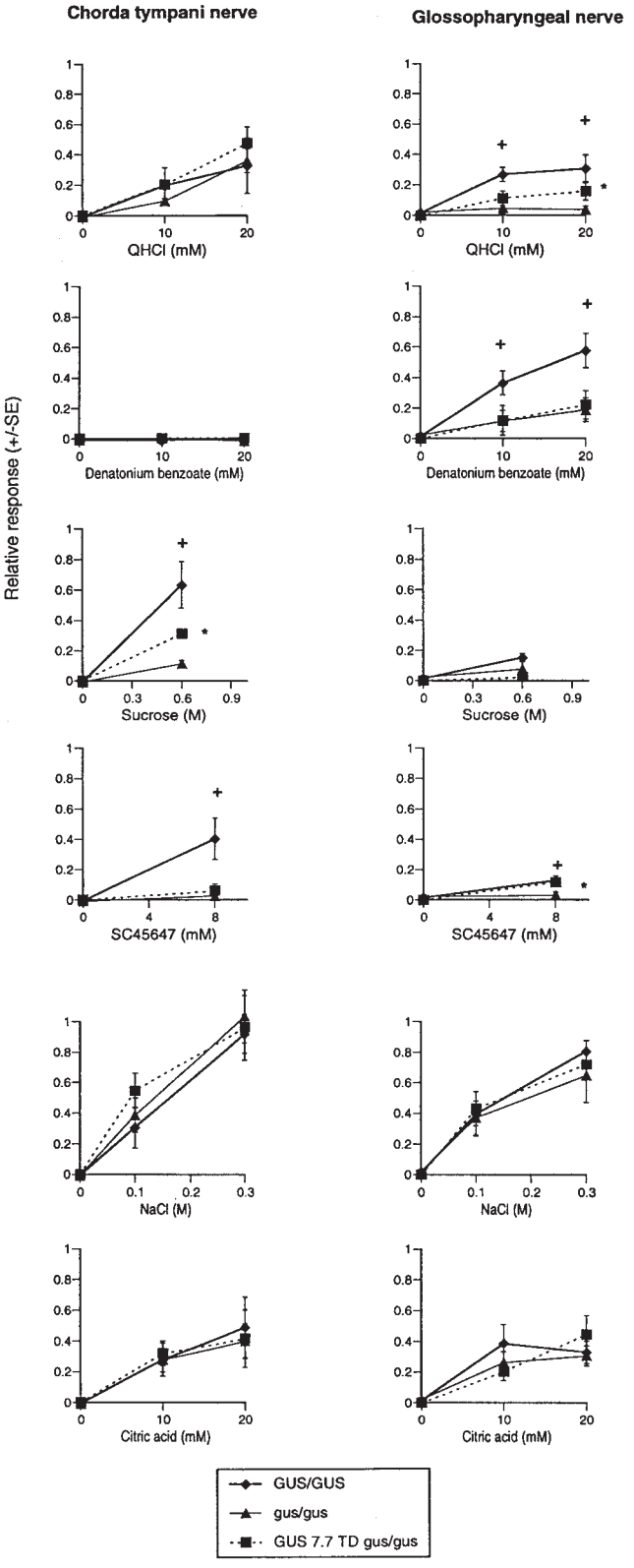

Figure 5 Recordings from the CT and NG nerves of mice. Responses of the CT were recorded from nine GUS/GUS, five gus/gus and three $GUS_{7.7}$TD gus/gus mice. Responses of the NG were recorded from eight GUS/GUS, seven gus/gus and seven $GUS_{7.7}$TD gus/gus mice. $^{+}P < 0.05$ comparing gus/gus and GUG/GUS; $^{*}P < 0.05$, comparing gus/gus and $GUS_{7.7}$TD gus/gus.

found that approximately one-third of the transgenic lines obtained with several $GUS_{7.7}$-driven transgenes showed a pattern and level of expression very similar to that of endogenous α-gustducin. In the case of α-transducin, however, in the best-expressing line the transgene was only expressed in a subset of α-gustducin-positive cells. α-Gustducin is also expressed in the gut (Hofer *et al*., 1996) and in the brain (Y.G. Shanker and R.F. Margolskee, unpublished results), in addition to taste tissue. Perhaps a wider pattern of expression of the $GUS_{7.7}$-driven α-transducin transgene that faithfully mimics that of α-gustducin is deleterious or even lethal and is therefore selected against.

Consistent with previous work, both of the sweeteners we tested elicited strong responses in the CT and the bitter stimulus denatonium elicited strong responses in the NG of wild-type mice (Shingai and Beidler, 1985; Ninomiya *et al*., 1993). Also consistent with previous work, α-gustducin null mice showed diminished responses to denatonium, quinine, SC45647 and sucrose but not to NaCl or citric acid (Wong *et al*., 1996). In the α-gustducin null background, the response of the CT to sucrose and of the NG to SC45647 were partially restored by the α-transducin transgene, consistent with the behavioral data. It is not clear why, for denatonium, the α-transducin transgene led to restoration of the behavioral responses, but not the nerve responses, and for quinine it led to the restoration of the NG responses, but not the behavioral responses. Clearly, the electrophysiological test that we used is less sensitive than the behavioral test. For example the NG responses of α-gustducin null and transgenic mice to 10 mM quinine were indistinguishable from background (Figure 5). Similarily, stimulation with 3 mM denatonium did not elicit a response above background in the NG of wild-type mice (data not shown). In contrast, the two-bottle preference test clearly showed that these mice responded to concentrations of tastants at or below the levels that did not elicit responses in nerve recordings (Figure 4). Thus, for denatonium, there may be subtle responses in the NG to low concentrations of tastants that are not distinguishable from background by whole nerve recordings, but that can signal to the brain. An alternative explanation is that there is a post-ingestive effect mediated by the α-gustducin-expressing cells of the gut that contributes to the behavioral responses of the mice. According to this scenario, both peripheral gustatory and post-ingestive effects would be abolished in the gus/gus mice. The lack of electrophysiological response in the gus/gus mice would be due to the absence of α-gustducin from the TRCs, whereas the lack of behavioral responses may be due to α-gustducin's absence from the TRCs and/or the gut cells. In the $GUS_{7.7}$TD gus/gus mice, the hypothesized post-ingestive response to denatonium would be restored by α-transducin, resulting in a behavioral, but not electrophysiological response. This is, however, unlikely because the $GUS_{7.7}$ promoter does not target expression of heterologous genes to the gut (our unpublished observations), probably because it lacks a gut-specific enhancer. Furthermore, previous work with expression of the α-gustducin transgene from the $GUS_{7.7}$ promoter in the gustducin lineage of TRCs, but not in the gut, fully restored behavioral responses to sucrose, SC45647, quinine and denatonium (Wong *et al.*, 1999).

Discrepancies between nerve recordings and behavioral tests have been reported previously. For example, recording from the CT of C57BL/6 and 129 mice did not show any response above background for 0.1 M SC45647, whereas two-bottle preference tests showed a clear preference at this concentration of SC45647 for both mouse strains. Furthermore, the CT responses to several concentrations of sorbitol were stronger in C57BL/6 than in 129 mice, whereas there were no differences in their behavioral responses to this compound. On the other hand, the CT responses to glycine were identical between those two strains, but the behavioral response of C57BL/6 mice was stronger (Bachmanov *et al*., 2001; Inoue *et al*., 2001).

In previous *in vitro* studies, α-transducin and α-gustducin were found to be biochemically indistinguishable. Here we show that α-transducin expressed in TRCs is functional, but differences may exist between these two G proteins. The precise role of endogenous α-transducin in taste signal transduction is still unclear. Whether transducin acts as a gustducin backup, is activated at high tastant concentration or transduces signals elicited by a small number of compounds remains to be determined. Behavioral and electrophysiological studies from α-transducin knockouts and α-transducin/α-gustducin double knockouts will help address these issues and are in progress.

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