Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 8933–8938, July 1998
Neurobiology

Characterization and solubilization of bitter-responsive receptors that couple to gustducin

DING MING*†, LUIS RUIZ-AVILA†‡, AND ROBERT F. MARGOLSKEE§

Howard Hughes Medical Institute, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029

Communicated by Sidney Udenfriend, Drew University, Madison, NJ, May 7, 1998 (received for review February 12, 1998)

ABSTRACT **The tastes of many bitter and sweet compounds are thought to be transduced via guanine nucleotide binding protein (G-protein)-coupled receptors, although the biochemical nature of these receptors is poorly understood at present. Gustducin, a taste-specific G-protein closely related to the transducins, is a key component in transducing the responses to compounds that humans equate with bitter and sweet. Rod transducin, which is also expressed in taste receptor cells, can be activated by the bitter compound denatonium in the presence of bovine taste membranes. In this paper, we show that gustducin is expressed in bovine taste tissue and that both gustducin and transducin, in the presence of bovine taste membranes, can be activated specifically by several bitter compounds, including denatonium, quinine, and strychnine. We also demonstrate that the activation in response to denatonium of gustducin by presumptive bitter-responsive receptors present in taste membranes depends on an interaction with the C terminus of gustducin and requires G-protein $\beta\gamma$ subunits to provide the receptor-interacting heterotrimer. The taste receptor–gustducin interaction can be competitively inhibited by peptides derived from the sites of interaction of rhodopsin and transducin. Finally, as the initial step toward purifying taste receptors, we have solubilized this bitter-responsive taste receptor and maintained its biological activity.**

Several biochemical and physiological studies implicate G-proteins and their coupled seven transmembrane-helix (heptahelical) receptors in the transduction of bitter and sweet tastes (reviewed in refs. 1 and 2). The closely related G-proteins gustducin and rod transducin are selectively expressed in taste receptor cells (3, 4). Gustducin is also expressed in apparent chemoreceptive cells of the stomach and duodenum (5). The targeted disruption of the α-gustducin gene caused a marked reduction in bitter and sweet responsiveness in α-gustducin null mice (6). Transgenic expression of the wild-type (wt) rat α-gustducin cDNA restored the responsiveness of gustducin null mice to bitter and sweet compounds (ref. 7 and G. Wong, L.R.-A., and R.F.M., unpublished observations). The residual responsiveness to bitter and sweet compounds demonstrated by α-gustducin null mice can be further reduced by transgenic expression of a dominant-negative allele of α-gustducin, suggesting that G-proteins other than gustducin may also be involved in bitter and sweet transduction (L.R.-A., G. Wong, and R.F.M., unpublished observations).

Gustducin is present in the taste cells of several vertebrate species, ranging from fish to rodents to humans (refs. 3, 6, and 8; D.M. and R.F.M., unpublished data), consistent with gustducin's fundamental role in taste transduction. However, at present there is no structural and minimal functional information regarding the bitter-responsive receptor(s) that couple to gustducin. A number of groups (9–13) have used degenerate oligonucleotide primers corresponding to conserved regions of heptahelical receptors and the PCR to amplify and then molecularly clone candidate receptors from taste tissue. In this way, olfactory- and neurokinin-like receptors were identified, some of which were also apparently expressed in taste tissue. However, none of these receptors were taste cell specific, and no functional information is presently available for these "orphan" receptors. A metabotropic glutamate receptor (mGluR4) has also been shown to be expressed in taste receptor cells and proposed to transduce the taste of glutamate (14).

We previously demonstrated that crude membrane preparations from bovine taste tissue, but not those from nontaste portions of tongue or from other control tissues, can specifically activate exogenously added transducin when stimulated by the bitter compound denatonium (4). This suggests the presence of heptahelical receptor(s) responsive to denatonium and capable of activating gustducin-like G-proteins. In this paper, we demonstrate that gustducin is present in bovine taste tissue and absent from nontaste epithelia, muscle, or brain membranes. Gustducin, like transducin, can be activated by taste membranes in response to denatonium. To characterize the biochemical nature and response profile of the taste receptor(s) that couple to gustducin and transducin, we used wt and mutated forms of recombinant gustducin and purified transducin, as reporters in *in vitro* assays of taste receptor activity. We found that the interaction of gustducin with the bitter-responsive receptor(s) can be blocked by peptides corresponding to the sites of interaction of rhodopsin and transducin, requires G-protein $\beta\gamma$ subunits, and is abolished by a point mutation in the C terminus of α-gustducin. We also found that several compounds that humans consider bitter (quinine, strychnine, sparteine, nicotine, atropine, quinacrine, naringin, epicatechin, and caffeic acid) stimulated taste receptor-containing membranes to activate transducin. Further, we report the solubilization, while maintaining biological activity, of the denatonium-responsive taste receptor.

MATERIALS AND METHODS

Materials. All chemicals were of the highest purity available and purchased from either Sigma or Boehringer Mannheim unless otherwise noted. Rhodopsin was purified in the light as 6 M urea-washed bovine rod outer segments by using pub-

Abbreviations: G-protein, guanine nucleotide binding regulatory protein; wt, wild type; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; SOA, sucrose octaacetate; GTP[γS], guanosine 5′-[γ-thio]triphosphate.

*Present address: R & D, Pepsi Cola Company, 100 Stevens Ave., Valhalla, NY 10595.
†D.M. and L.R.-A. contributed equally to this work.
‡Present address: Almirall Prodesfarma, Cardener 64, Barcelona, Spain.
§To whom reprint requests should be addressed at: Department of Physiology and Biophysics, Mount Sinai School of Medicine, Box 1677, One Gustave L. Levy Place, New York, NY 10029. e-mail: margolskee@msvax.mssm.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

lished procedures (15, 16). Bovine transducin heterotrimer and $\beta\gamma$ subunits were purified by standard procedures (17). The anti-gustducin antibody GD1 is a rabbit polyclonal antiserum directed against the keyhole limpet hemocyanin (KLH)-conjugated peptide GIDYVNPRSREDQQLLLSMANTL (amino acids 95 to 109 of the rat α-gustducin protein) (8). Monoclonal antibody TF15 (American Qualex, La Mirada, CA) was raised against transducin (18) and cross-reacted with gustducin. The rabbit polyclonal anti-transducin antibody (4) was a kind gift of Mel Simon and John Watson (California Institute of Technology, Pasadena). Bovine tissues were collected fresh from a local slaughterhouse and transported on ice to the laboratory.

Membrane Preparation. Bovine circumvallate papillae ("taste tissue") versus tongue muscle and sublingual epithelia ("nontaste tissue") were hand dissected, frozen in liquid nitrogen, and stored at −80°C until use. The collected tissues were homogenized with a Polytron homogenizer (three cycles of 20 s each at 25,000 rpm) in a buffer containing 10 mM Tris at pH 7.5, 10% (vol/vol) glycerol, 1 mM EDTA, 1 mM DTT, 10 μg/μl pepstatin A, 10 μg/μl leupeptin, 10 μg/μl aprotinin, and 100 μM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride. After particulate removal by centrifugation at 1,500 × *g* for 10 min, taste or nontaste membranes were collected by centrifugation at 45,000 × *g* for 60 min. The pelleted membranes were rinsed twice, resuspended in homogenization buffer lacking protease inhibitors, and further homogenized by 20 passages through a 25 gauge needle. Aliquots were either flash frozen or stored on ice until use. The concentration of protein in the membrane preparations was determined by the Peterson modification of the micro-Lowry method (19).

***In Vitro* Transcription/Translation.** PCR was used to eliminate the 5′-untranslated region of the full-length rat α-gustducin cDNA (3). A PCR product was generated that spanned nucleotides 160 (first nucleotide of the coding sequence) to 230 (after a unique *Hin*dIII site) and introduced an *Eco*RI site immediately upstream of the initiator ATG. This product was digested with *Eco*RI and *Hin*dIII endonucleases and subcloned in a three-piece ligation along with a *Hin*dIII–*Not*I fragment encompassing the remainder of the α-gustducin cDNA (including the 3′-untranslated region), and the *Eco*RI–*Not*I-digested pBluescript KSII vector (Stratagene). This full-length gustducin clone is referred to as plasmid KSα3. This plasmid was used as a template for mutagenesis and for *in vitro* transcription/translation using T3 RNA polymerase and the TnT reticulocyte system (Promega), following the manufacturer's suggested procedures in the presence of limiting [^{35}S]methionine (Amersham).

Mutagenesis of α-Gustducin. We substituted glycine for proline at amino acid position 352 (G352) of gustducin to generate the G352P α-gustducin mutant (L.R.-A., G. Wong, and R.F.M., unpublished data). The mutagenized regions of the KSα3–G352P plasmid were sequenced to rule out adventitious mutations. Three independent isolates of the mutated plasmid were analyzed in pilot experiments to control for secondary mutations. A detailed description of the generation and biochemical characterization of the G352P mutant and its effect on taste transduction *in vivo* will be published.

Gustducin and Transducin Activation. Activation of *in vitro* translated recombinant α-gustducin and purified native transducin was assayed as previously described for transducin (4), based on the procedure of Neer *et al.* (20). *In vitro* translated gustducin (10 μl of the transcription/translation mix) was incubated for 15 min at room temperature with $\beta\gamma$ subunits from bovine retina [10 μl at 1 mg/ml in 10 mM Tris, pH 8.0/10 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)] or with 10 μl of buffer alone ($\beta\gamma$-negative controls). All subsequent reactions were performed on ice. The gustducin mixtures, with or without $\beta\gamma$ subunits, were diluted 1:10 in incubation buffer [25 mM Tris, pH 7.5/2 mM $MgCl_2$/5 mM DTT/100 mM NaCl/100 μM GDP/0.5 μM guanosine 5′-[γ-thio]triphosphate (GTP[γS])] containing 0.25–1 mg/ml of membrane protein. To assay activation of transducin, the reaction mix was modified to contain 5 mM $MgCl_2$, 150 mM NaCl, and 3 mM DTT. Transducin was added to this mix to a final concentration of 0.4 μM.

Aliquots of 20 μl were withdrawn from the premix solution, tastant or buffer was added from 20× stocks, and the final reaction mixtures were incubated for 1–3 h on ice (gustducin) or 1 h at 30°C (transducin) and then analyzed by the trypsin digestion assay. For trypsin assays, TPCK-treated trypsin (1:25 trypsin to total protein) was added after the incubation with tastant. Trypsin digestions were performed at room temperature (15 min) and were stopped by adding soybean trypsin inhibitor (6:1 mol/mol inhibitor to trypsin). The reaction mixtures were diluted 1:3 in 2× Laemmli buffer (21), resolved by SDS/PAGE, and either transferred to poly(vinylidene difluoride) (PVDF) membranes for immunoblotting (transducin) or dried and exposed to a PhosphorImager screen (gustducin).

Immunohistochemistry and Immunofluorescence. Fresh bovine tongue tissues containing one or two circumvallate papillae were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), transferred to 20% sucrose in PBS, and then stored overnight at 4°C. Frozen sections 10 μm thick were rinsed in PBS and then blocked for 30 min to 1 h in blocking buffer (0.3% Triton X-100/1% horse serum in PBS). The GD1 antiserum was used at 1:500 dilution; the TF15 antibody was used at 25 μg/ml. After washing thoroughly with PBS, the appropriate Cy3- or fluorescein isothiocyanate-conjugated secondary antibody was added. Western blots used 0.2 μm PVDF membranes, horseradish peroxidase-conjugated antibody, and enhanced chemiluminescence (ECL; Amersham). We used the TF15 antibody to detect both bovine gustducin

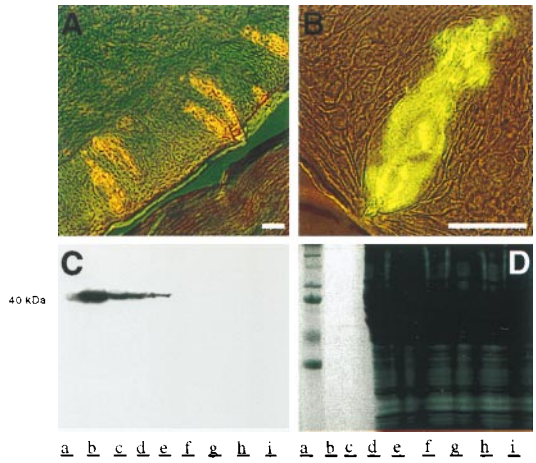


FIG. 1. Gustducin and transducin are present in bovine taste receptor cells. (*A*) Immunostaining of a bovine circumvallate papilla with anti-α-gustducin antibody and Cy3-conjugated secondary antibody only stained taste receptor cells. (*B*) Immunostaining of a single taste bud from a bovine circumvallate papilla reacted with transducin/gustducin-reactive monoclonal antibody TF15 and fluorescein-conjugated secondary antibody only stained taste receptor cells. Bars are 10 μm long. (*C*) Western blot of bovine tissues reacted with the TF15 antibody. The following samples were loaded in the gels: a, protein molecular weight markers; b, transducin (100 pg); c, transducin (10 pg); d, taste tissue membrane proteins; e, taste tissue cytosolic proteins; f, nontaste tissue membrane proteins; g, nontaste tissue cytosolic proteins; h, muscle membrane proteins; and i, brain membrane proteins. Only taste tissue protein reacted with the transducin/gustducin-reactive antibody. (*D*) Duplicate gel, stained with Coomassie Blue. For both taste and nontaste tissue preparations, 75 μg of protein was loaded in each lane.

and transducin and the polyclonal anti-transducin antibody for the routine analysis of transducin activation.

Solubilization of Bovine Taste Membranes. Bovine taste membranes were resuspended in homogenization buffer lacking protease inhibitors, and then *n*-octyl glucoside was added to a concentration of 1% (wt/vol). The sample was rocked for 15 min at 4°C. The solubilized fraction was isolated by centrifugation at 100,000 × *g* for 1 h, and the receptor activity was immediately assayed as described above.

RESULTS

Gustducin and Transducin Are Expressed in Bovine Taste Receptor Cells. Gustducin is highly expressed in rat (3), mouse (6), and human (8) taste receptor cells, although bovine gustducin has not been characterized or cloned. We have also shown that transducin is present in bovine taste tissue and rat taste receptor cells (4). Gustducin protein and mRNA are ≈25-fold more highly expressed in taste tissue than are the transducin counterparts (3, 4). Staining of bovine taste tissue with an anti-gustducin-specific antibody identified gustducin immunoreactivity in taste receptor cells of the circumvallate papillae (Fig. 1*A*), whereas staining with an anti-transducin monoclonal antibody also identified immunoreactivity in taste receptor cells (Fig. 1*B*). However, this monoclonal antibody reacts with both transducin and gustducin, so it is not possible from this experiment alone to conclude that transducin is also present in bovine taste receptor cells. Our previous results using a polyclonal anti-transducin antibody identified rod transducin in rat taste receptor cells and bovine taste tissue (4). Western blot analysis using the monoclonal antibody (Fig. 1*C*) confirmed the presence of gustducin and/or transducin in bovine taste tissue.

Bovine Taste Membranes Contain Receptors That Couple to Gustducin and Transducin. Bovine taste membranes stimu-

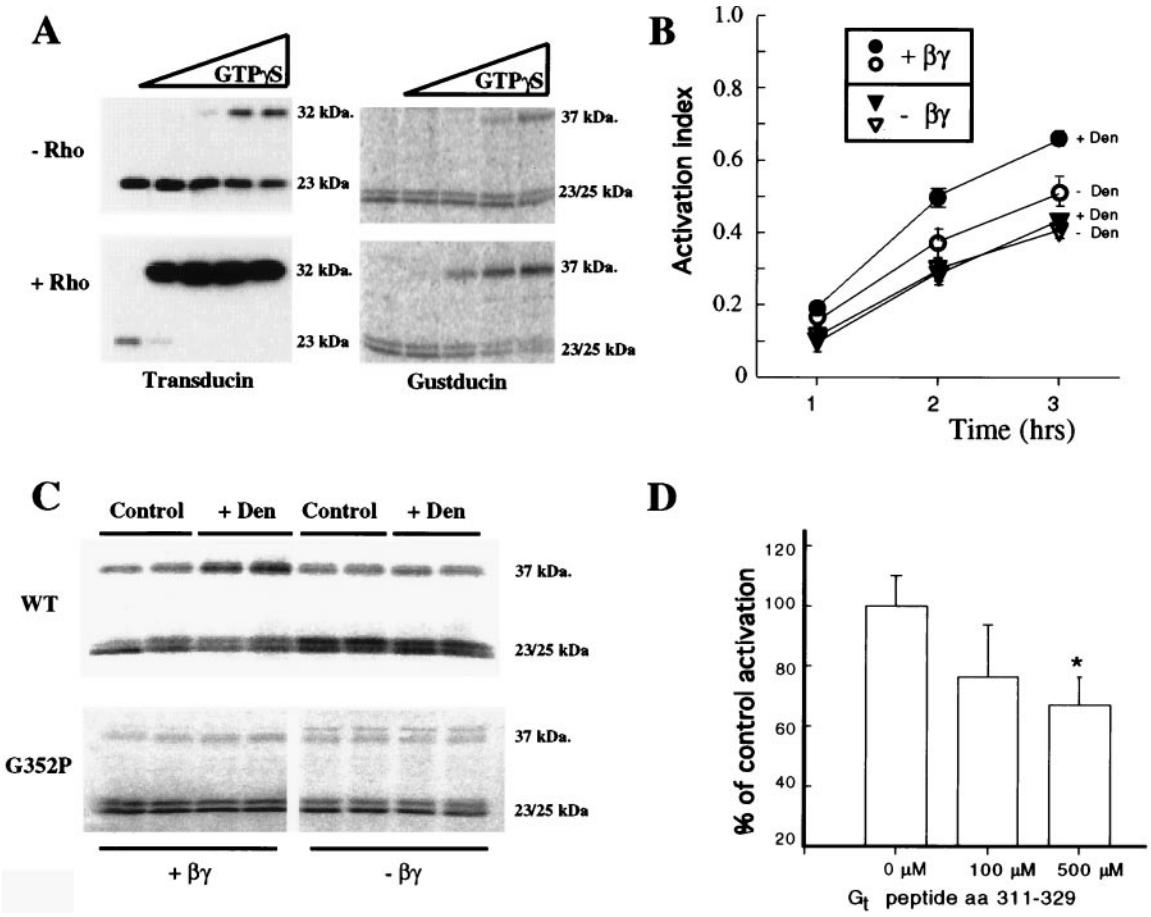


FIG. 2. Gustducin is activated by a presumptive heptahelical taste receptor. (*A*) Rhodopsin activation of transducin and gustducin was assessed by the trypsin cleavage pattern ("trypsin assay"). The nonactivated (GDP-bound) forms of native transducin (*Left*), or *in vitro* translated gustducin (*Right*) generate 23- and 23- plus 25-kDa tryptic fragments, respectively. The active (GTP[γS]-bound) conformations of the G-proteins generate 32-kDa (transducin) or 37-kDa (gustducin) fragments. Increasing concentrations of GTP[γS] (0.1, 1, 10, and 100 μM for transducin; 0.001, 0.1, 1, and 10 μM for gustducin) caused rhodopsin-independent activation of the G-proteins, but only at the highest GTP[γS] concentrations. Rhodopsin (*Lower*) enhanced this activation by ≈100- to 1000-fold. +Rho, 1 μM rhodopsin. (*B*) Activation of gustducin by denatonium-stimulated taste membranes required G-protein βγ subunits. In the absence of βγ subunits (-βγ, triangles) there was only a slight elevation, with increasing time of incubation, in the activation index (the ratio of the 37-kDa form diagnostic of activation versus the sum of the intensities of the 37- plus 23/25-kDa bands) of gustducin in the presence of taste membranes with or without the bitter tastant denatonium. When βγ subunits were added (+βγ, circles), denatonium-stimulated taste membranes increased the activation of gustducin by greater than 2-fold. Each point is the average of four independent quantitations of duplicate samples. Error bars represent the standard deviation. The displayed results are representative of three independent experiments. +Den (filled symbols), 2.5 mM denatonium. (*C*) Trypsin assays to monitor gustducin/transducin activation. *Upper*, Wild-type (WT) *in vitro* translated gustducin was activated by denatonium-stimulated taste membranes when βγ subunits were added (+βγ, *Left*) but not in their absence (-βγ, *Right*). *Lower*, G352P gustducin, a mutant defective in its interaction with rhodopsin, was not activated by taste membranes in the presence (+βγ, *Left*) or absence (-βγ, *Right*) of βγ subunits. Representative gels containing duplicate independent samples are shown. +Den, 2.5 mM denatonium. Control, water added in place of denatonium. (*D*) The Gt peptide, corresponding to amino acids 311–329 of transducin (one of transducin's primary sites of interaction with rhodopsin), inhibited by ≈50% the denatonium-dependent taste membrane activation of *in vitro* translated gustducin. The results shown are the average of six independent experiments with duplicate samples. *, $P < 0.05$, Student's *t* test.

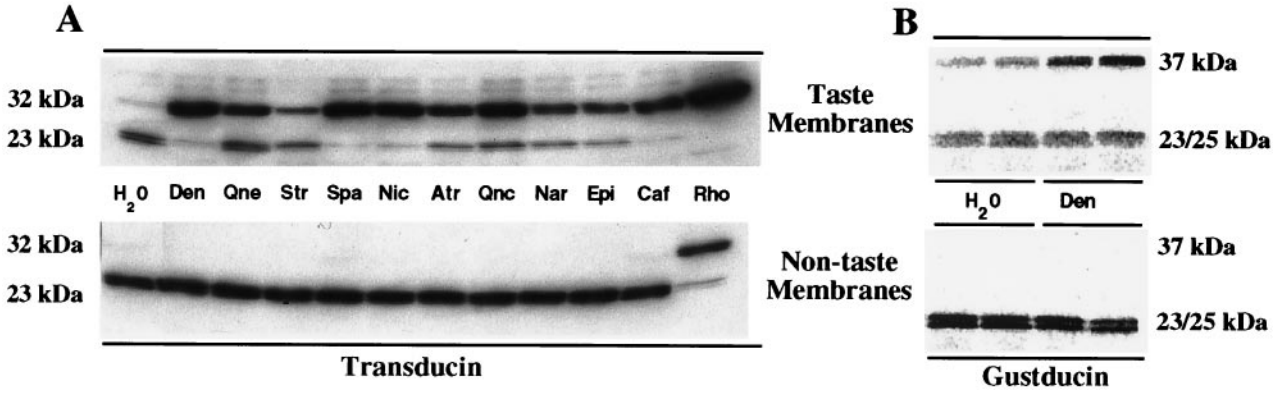
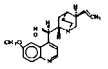
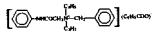
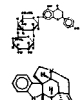
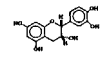
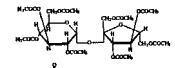


FIG. 3. In the presence of taste membranes, several bitter compounds activate transducin and/or gustducin. Trypsin assays to monitor G-protein activation. (*A*) In the presence of taste membranes (*Upper*), native transducin was activated by several bitter compounds (note the 32-kDa fragment); with nontaste membranes (*Lower*), transducin was not activated by any bitter compounds (no 32-kDa fragment). (*B*) In the presence of taste membranes (*Upper*), *in vitro* translated gustducin was activated by denatonium (note the 37-kDa fragment); with nontaste membranes (*Lower*), gustducin was not activated (no 37-kDa fragment). Den, 5 mM denatonium benzoate; Qne, 1 mM quinine; Str, 5 mM strychnine; Spa, 5 mM sparteine; Nic, 10 mM nicotine; Atr, 5 mM atropine; Qnc, 5 mM quinacrine; Nar, 5 mM naringin; Epi, 5 mM epicatechin; Caf, 5 mM caffeic acid; Rho, 1 μM rhodopsin.

lated by the bitter compound denatonium benzoate specifically activate exogenously added transducin (4). Transducin and gustducin are biochemically similar (22); each is expressed in taste cells, so we set out to test the responses of both G-proteins to a number of bitter compounds. Because α-gustducin-null mice are 50–100-fold less sensitive to the bitter compounds quinine and denatonium than are their wt littermates (6) and because gustducin is more highly expressed in taste cells than is transducin, we expected that gustducin would be the primary G-protein involved in bitter responsiveness. We used radioactively labeled, *in vitro* translated α-gustducin and conformationally sensitive proteolytic cleavage to study taste receptor-mediated activation of gustducin. We determined that the *in vitro* translated α-gustducin was properly folded and modified, because it was myristoylated by the reticulocyte lysate (data not shown), ADP-ribosylated by pertussis toxin in the presence of $\beta\gamma$ (data not shown), and underwent a conformational change upon activation (see below). The translated product of 40 kDa was of the expected size for full-length protein. As with transducin, the conformational change induced by the exchange of GDP for GTP may be monitored by the cleavage pattern after digestion with trypsin (ref. 4; L.R.-A., G. Wong, and R.F.M., unpublished observation). Trypsin digestion of native transducin and recombinant gustducin gave a major fragment of 23–25 kDa when the proteins were bound to GDP (i.e., in the nonactivated form). The size of the fragment shifted to 32 kDa (transducin) or 37 kDa (gustducin) when the G-proteins were activated by the binding of GTP[γS], a nonhydrolyzable analogue of GTP. The differences in sizes between the tryptic fragments derived from transducin versus gustducin were due to the absence of an accessible site near the C terminus of gustducin.

Table 1. Activation of transducin/gustducin by bitter compounds

Compound	mM*	M_r	Structure	Activation†
Quinine	0.1	324.43		+
Denatonium	1.0	446.60		+
Sparteine	2.5	234.37		+
Atropine	2.5	289.40		+
Caffeic acid	5.0	180.16		+
Phenylthiourea	5.0	152.22		+
Yohimbine	5.0	354.43		+
Naringin	5.0	580.53		+
Strychnine	5.0	334.42		+
(−)-Epicatechin	5.0	296.08		+
Nicotine	10	162.24		+
Sucrose octaacetate	1.0	678.58		−
Aristolochic acid	10	341.28		−
Caffeine	10	194.20		−

*Minimum concentration for "+" compounds, maximum concentration tested for "−" compounds.
†Activation of transducin/gustducin scored by trypsin sensitivity digestion assay as in Fig. 3.

We used the trypsin sensitivity assay to monitor taste receptor-mediated activation of transducin and gustducin. Rhodopsin served as a positive control, as gustducin and transducin are equally activated by rhodopsin (22). No activation of either G-protein was observed in the absence of rhodopsin and GTP[γS] (Fig. 2*A*). In the presence of GTP[γS] (above 10 μM GTP[γS] for transducin, above 1 μM GTP[γS] for gustducin), there was minimal activation of each G-protein, which was independent of rhodopsin (Fig. 2*A*). When 1 μM rhodopsin was added, transducin and gustducin were activated to a much greater extent ($\approx$100- to 1000-fold). The kinetics of activation of *in vitro* produced gustducin were similar to those with Sf9 cell-produced recombinant gustducin or with native bovine transducin (22); half-maximal activation occurred at $\approx$30 nM rhodopsin (data not shown).

Activation of *in vitro* translated gustducin by taste receptor-containing membranes (Fig. 2*B*) was similar to that previously found for transducin: denatonium-stimulated taste membranes activated α-gustducin, but only if $\beta\gamma$ subunits were added (Fig. 2 *B* and *C*), i.e., the heterotrimeric G-protein must be generated for taste receptors present in the membranes to activate the α subunit. When G352P-gustducin, a mutant with a major conformational disruption of the C terminus that

blocks rhodopsin-mediated activation of gustducin (L.R.-A., G. Wong, and R.F.M., unpublished observation), was used instead of wt gustducin, no denatonium/taste membrane activation of the mutated gustducin was observed even in the presence of $\beta\gamma$ subunits (Fig. 2*C*), suggesting that the conformation of gustducin's C terminus is critical for denatonium-responsive taste receptor activation to take place. Denatonium-responsive taste receptor activation of gustducin was competitively inhibited by a peptide derived from one of transducin's receptor-interaction regions (Fig. 2*D*). We previously showed that this peptide effectively blocked the interaction of taste receptor-containing membranes with transducin, as measured by GTP[γS] binding (4). Peptides corresponding to rhodopsin's site of interaction with transducin also blocked taste receptor-mediated activation of gustducin or transducin, whereas control scrambled peptides did not (data not shown). Taken together, these results strongly suggest that the interactions of gustducin and transducin with the denatonium-responsive receptor are physically similar and typical of other G protein–receptor interactions involving the C termini of G-proteins and cytoplasmic loops of the heptahelical receptors.

Many Bitter Compounds Activate Transducin and/or Gustducin in the Presence of Taste Membranes. We previously reported that denatonium plus taste membranes activated exogenously added transducin (4). We have now observed denatonium-responsive taste membrane-specific activation of *in vitro* translated gustducin (Fig. 2 *B* and *C* and Fig. 3*B*). We modified the trypsin assay to increase the sensitivity of the method by ≈10-fold and then tested several bitter and a number of sweet, salty, or sour compounds for their ability to activate transducin. Positive responses were obtained with denatonium, quinine, strychnine, nicotine, atropine, quinacrine, naringin, epicatechin, sparteine, and caffeic acid (Fig. 3*A*; Table 1), all of which are bitter to humans. In all cases, the responses required taste membranes, with no activation observed when nontaste membranes were used instead. Several other tastants, including the bitter compounds sucrose octaacetate (SOA), aristolochic acid, and caffeine, as well as several sugars, amino acids, and monosodium glutamate, did not activate gustducin or transducin by this assay (Table 1 and data not shown). Our results are consistent with bovine taste membranes containing multiple bitter-responsive heptahelical receptors coupled to gustducin/transducin.

Solubilization of Bitter Receptors. To determine the physical nature of the bitter-responsive activities in bovine taste membranes and to biochemically purify the molecule(s) involved, we tested a number of detergents (Triton X-100, Tween 80, Tween 20, cholic acid, CHAPS, lauryl maltoside, and *n*-octyl glucoside) for the ability to solubilize the presumptive receptor(s) while maintaining its ability to activate transducin and gustducin (Fig. 4). The 100,000 × *g* supernatant of bovine taste membranes solubilized in 1% *n*-octyl glucoside activated both transducin and wt gustducin but not the receptor interaction-defective (G352P) mutated gustducin. Bovine taste membranes solubilized in the other detergents noted above could not activate transducin or wt gustducin (i.e., receptor activity was lost). The *n*-octyl glucoside-solubilized preparation was approximately half as efficient, on a weight basis, as the crude homogenate. The results from detergent solubilization and peptide competition argue strongly that heptahelical taste receptors activate gustducin and transducin *in vivo*.

DISCUSSION

Humans perceive many chemically diverse compounds (e.g., K^+, quinine, urea, and caffeine) to be bitter. At present, the structure–activity relationships of bitter compounds are incompletely understood, as are the underlying transduction mechanisms, perhaps because of a multiplicity of different pathways. Bitter transduction has been proposed to involve disruption of the lipid bilayer (23), blockage of K^+ channels (24), direct activation of G-proteins (25), or activation of G-protein-coupled heptahelical receptors (4). Our present studies indicate that gustducin/transducin-coupled heptahelical receptors are involved in transducing responses to denatonium, quinine, and several bitter alkaloids.

Using the conformation-sensitive trypsin sensitivity assay and *in vitro* translated wt and mutant forms of gustducin, we have analyzed the nature of the receptor–G-protein interactions underlying responses to bitter compounds. Based on the following considerations, we conclude that the denatonium/taste membrane activation of transducin or gustducin involves heptahelical receptors and is mechanistically similar to rhodopsin activation of transducin. First, the heterotrimeric form of gustducin/transducin is required to interact with the putative taste receptors. Second, peptides from the rhodopsin–transducin interface inhibit the taste receptor–G-protein interaction. Third, the G352P mutation at the C terminus of gustducin blocked activation of gustducin by taste membranes. Mutations in this region of transducin are predicted to disrupt the β-turn structure of its C terminus (26), thought to be essential for effective interaction with heptahelical receptors (27–29). Substitution of the equivalent residue of transducin (G348A or G348P) blocked activation of transducin by rhodopsin (30, 31). A more detailed biochemical study of G352P gustducin and its effects *in vivo* when expressed as a transgene

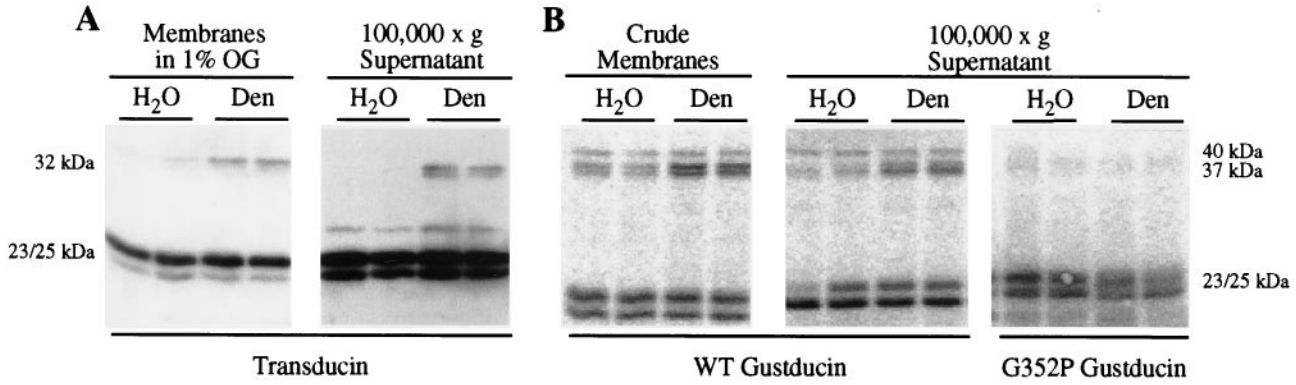


FIG. 4. Solubilization of bovine taste receptors. Trypsin assays to monitor G-protein activation. (*A*) Native transducin was activated by denatonium and bovine taste membranes in the presence of 1% *n*-octyl glucoside (OG) (*Left*); transducin was similarly activated by the OG solubilized taste membrane supernatant (*Right*). (*B*) *In vitro* translated gustducin was activated by denatonium and bovine taste membranes (*Left*); gustducin was similarly activated by the OG solubilized taste membrane supernatant (*Middle*). The receptor interaction-defective mutant of gustducin (G352P-gustducin) was not activated by denatonium plus solubilized taste membranes (*Right*). Den, 5 mM denatonium benzoate.

will be submitted elsewhere (L.R.-A., G. Wong, and R.F.M., unpublished results).

We have extended our initial observations on denatonium/taste membrane activation of transducin in three ways. First, we have substituted recombinant wt and mutant gustducin for native transducin. Second, we have demonstrated taste membrane activation of transducin by several compounds that humans find bitter: denatonium, naringin, epicatechin, caffeic acid, and several bitter alkaloids (quinine, strychnine, sparteine, nicotine, atropine, and quinacrine). Interestingly, the bitter compounds caffeine, aristolochic acid, and SOA did not activate transducin in the presence of taste membranes (D.M., L.R.-A., and R.F.M., unpublished results). Third, we have solubilized the denatonium-responsive taste receptor activity. Our results are consistent with a family of bitter compound-responsive heptahelical receptors that couple to either gustducin or transducin. Presumably, some bitter compounds (e.g., caffeine and SOA) either are not transduced by these receptors, or bovines may lack receptors for these particular compounds. Behavioral and biochemical experiments with wt versus gustducin null mice may answer this question.

Activation of transducin by quinine and several other bitter compounds required taste membranes, although the effects of quinine and denatonium were not additive (D.M., L.R.-A., and R.F.M., unpublished results), suggesting interactions with the same receptor. Psychophysical data show generalization of responses to bitter compounds, whereas electrophysiological studies of cross adaptation to bitter compounds show that most bitter compounds fall into a limited number of broad categories (M. Frank, personal communication). Bitter taste transduction may use a few broadly tuned receptors instead of the large family of receptors thought to function in olfactory transduction. We propose that most bitter compounds are transduced by a limited group of taste cell-specific, gustducin/transducin-coupled receptors with similarity to the opsins. We speculate that this group of receptors may lack high affinity binding pockets for tastants and may instead rely on displacement of a cofactor by interaction of the hydrophobic bitter compounds with the intramembrane portion of the taste receptor(s), causing a conformational change in the taste receptors similar to that produced by light-stimulated rhodopsin. The physical studies we have presented here may lead to receptor purification and molecular cloning, enabling us to test this hypothesis.

This research was supported by National Institutes of Health Grant RO1DC03055, the Howard Hughes Medical Institute, and Comissio Interdepartamental de Recerca i Innovacio Tecnologica Grant BEAI-300089 (to L.R.-A.).

1. Lindemann, B. (1996) *Curr. Biol.* **6,** 1234–1237.
2. Kinnamon, S. C. & Margolskee, R. F. (1996) *Curr. Opin. Neurobiol.* **6,** 506–513.
3. McLaughlin, S. K., McKinnon, P. J. & Margolskee, R. F. (1992) *Nature (London)* **357,** 563–569.
4. Ruiz-Avila, L., McLaughlin, S. K., Wildman, D., McKinnon, P. J., Robichon, A., Spickofsky, N. & Margolskee, R. F. (1995) *Nature (London)* **376,** 80–85.
5. Höfer, D., Puschel, B. & Drenckhahn, D. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 6631–6634.
6. Wong, G. T., Gannon, K. S. & Margolskee, R. F. (1996) *Nature (London)* **381,** 796–800.
7. Wong, G. T., Ruiz-Avila, L., Ming, D., Gannon, K. S. & Margolskee, R. F. (1996) *Cold Spring Harbor Symp. Quant. Biol.* **61**, 173–184.
8. Takami, S., Getchell, M. L., McLaughlin, S. K., Margolskee, R. F. & Getchell, T. V. (1994) *Mol. Brain Res.* **22,** 193–203.
9. Abe, K., Kusakabe, Y., Tanemura, K., Emori, Y. & Arai, S. (1993) *FEBS Lett.* **316,** 253–256.
10. Abe, K., Kusakabe, Y., Tanemura, K., Emori, Y. & Arai, S. (1993) *J. Biol. Chem.* **268,** 12033–12039.
11. Matsuoka, I., Mori, T., Aoki, J., Sato, T. & Kurihara, K. (1993) *Biochem. Biophys. Res. Commun.* **194,** 504–511.
12. Tal, M., Ammar, D. A., Karpuj, M., Krizhanovsky, V., Naim, M. & Thompson, D. A. (1995) *Biochem. Biophys. Res. Commun.* **209,** 752–759.
13. Thomas, M. B., Haines, S. L. & Akeson, R. A. (1996) *Gene* **178,** 1–5.
14. Chaudari, N., Yang, H., Lamp, C., Delay, E., Cartford, C., Than, T. & Roper, S. (1996) *J. Neurosci.* **16,** 3817–3826.
15. Mazzoni, M. R., Malinski, J. A. & Hamm, H. E. (1991) *J. Biol. Chem.* **266,** 14072–14081.
16. Willardson, B. M., Pou, B., Yoshida, T. & Bitensky, M. W. (1993) *J. Biol. Chem.* **268,** 6371–6382.
17. Fung, B. K-K., Hurley, J. B. & Stryler, L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 152–156.
18. Navon, S. E. & Fung, B. K.-K. (1988) *J. Biol. Chem.* **263,** 489–496.
19. Peterson, G. L. (1977) *Anal. Biochem.* **83,** 346–356.
20. Neer, E. J., Denker, B. M., Thomas, T. C. & Schmidt, C. J. (1994) *Methods Enzymol.* **237,** 226–239.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Hoon, M. A., Northup, J. K., Margolskee, R. F. & Ryba, N. J. (1994) *Biochem. J.* **309,** 629–636.
23. Koyama, N. & Kurihara, K. (1972) *Biochim. Biophys. Acta* **288,** 22–26.
24. Cummings, T. A. & Kinnamon, S. C. (1992) *J. Gen. Physiol.* **99,** 591–613.
25. Naim, M., Seifert, R., Nurnberg, B., Grunbaum, L. & Schultz, G. (1994) *Biochem. J.* **297,** 451–454.
26. Noel, J. P., Hamm, H. E. & Sigler, P. B. (1993) *Nature (London)* **366,** 654–663.
27. Conklin, B. R. & Bourne, H. R. (1993) *Cell* **73,** 631–641.
28. Hamm, H. E. & Rarick, H. M (1994) *Methods Enzymol.* **237,** 423–436.
29. Dratz, E. A., Furstenau, J. E., Lambert, C. G., Thireault, D. L., Rarick, H., Schepers, T., Pakhlevaniants, S. & Hamm, H. E. (1993) *Nature (London)* **363,** 276–281.
30. Garcia, P. D., Onrust, R., Bell, S. M., Sakmar, T. P. & Bourne, H. R. (1995) *EMBO J.* **14,** 4460–4469.
31. Osawa, S. & Weiss, E. R. (1995) *J. Biol. Chem.* **270,** 31052–31058.