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Receptors for bitter, sweet and umami taste couple to inhibitory G protein signaling pathways

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Abstract

Taste receptors are thought to couple to the G protein Gα-gustducin to initiate signal transduction cascades leading to taste perception. To further characterize the G protein-coupling selectivity of these receptors, we expressed them in HEK293 cells and monitored the modulation of different signaling pathways upon stimulation. We found that the bitter compound cycloheximide induces phosphorylation of extracellular signal-regulated kinases1 and 2 (ERK 1/2) and inhibits cAMP accumulation in HEK293 cells expressing the mouse bitter $T2R_5$ receptor. These effects are totally abolished upon treatment with pertussis toxin. On the other hand, sweeteners and monosodium glutamate induce phosphorylation of ERK1/2 and inhibit cAMP accumulation in HEK293 cells expressing the human sweet $T1R_2/T1R_3$ receptor and the human umami $T1R_1/T1R_3$ receptor, respectively. The effects of these taste modalities are also prevented by treatment with pertussis toxin. Collectively, our results show that taste receptors can functionally couple to $G\alpha_{i/o}$ proteins to transmit intracellular signals.

Keywords: Taste; Receptor; cAMP; MAPK; G protein, inhibitory

1. Introduction

Bitter, sweet and amino acid taste (also referred to as umami taste), are triggered by activation of specific receptors located at the surface of taste receptor cells on the tongue and palate (Margolskee, 2002; Montmayeur and Matsunami, 2002). Approximately 25 to 30 genes encode receptors for bitter tasting substances in humans and rodents (Adler et al., 2000; Montmayeur and Matsunami, 2002; Matsunami et al., 2000; Bufe et al., 2002). Structurally, these receptors possess the hallmarks of G protein-coupled receptors, with seven transmembrane domains flanked by small extracellular amino-termini and intracellular carboxyl-termini. Two bitter receptors, the mouse bitter $T2R_5$ and the rat bitter $T2R_9$, recognize the protein synthesis inhibitor, cycloheximide (Bufe et al., 2002; Chandrashekar et al., 2000). It is known that rodents react aversively to the taste of cycloheximide (Lush and Holland, 1988). In humans, detection of the bitter compounds strychnine, salicin and phenylthiocarbamide is thought to be mediated by three different receptors, $TAS2R_{10}$, $TAS2R_{16}$ and $TAS2R_{38}$, respectively (Bufe et al., 2002; Kim et al., 2003). Three other taste-specific G protein-coupled receptors, $T1R_1$, $T1R_2$ and $T1R_3$ are thought to form functional heterodimers that specifically recognize sweeteners and amino acids (Li et al., 2002; Nelson et al., 2001, 2002). The rodent and human $T1R_2/T1R_3$ combinations recognize natural and artificial sweeteners (Nelson et al., 2001; Li et al., 2002) while the rodent and human $T1R_1/T1R_3$ combinations recognize several L-amino acids and monosodium glutamate, respectively (Li et al., 2002; Nelson et al., 2002). A truncated form of a metabotropic glutamate receptor, taste-$mGlu_4$ receptor, has also been suggested as a candidate G protein-coupled receptor for the sensory transduction of umami taste (Chaudhari et al., 2000).

Taste receptors use G proteins to relay intracellular signals leading to cell depolarization and, subsequently, taste perception. Deletion of the gene encoding Gα-gustducin, a taste-specific α-subunit (McLaughlin et al., 1992), produces mice that are defective in the detection of bitter and sweet substances (Wong et al., 1996). Importantly, the defect is only partial, suggesting the involvement of other G-proteins (Wong et al., 1996; Ruiz-Avila et al., 2001; He et al., 2002). However, with the exception of $G\alpha_{15/16}$ (Chandrashekar et al., 2000; Li et al., 2002; Nelson et al., 2001, 2002), it is not known which other G protein α-

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subunit(s) can functionally interact with taste receptors. Taste receptor cells are highly enriched with some of the α-subunits from the $G\alpha_{i/o}$ subfamily. $G\alpha_{i2}$ and $G\alpha_{i3}$ can be detected by in situ hybridization (Kusakabe et al., 2000; Asano-Miyoshi et al., 2000) and $G\alpha_{i2}$ can also be detected by immunostaining in taste receptor cells (Kusakabe et al., 2000). A study by Hoon et al. (1999) reports that $G\alpha_i$ proteins are expressed in almost all taste receptor cells. As a result, $G\alpha_{i2}$ positive cells are thought to be larger in number than Gα-gustducin-positive cells in rat circumvallate papillae (Kusakabe et al., 2000; Margolskee, 2002). The functional significance of this high abundance of $G\alpha_i$ subunits relative to any other α-subunit in taste receptor cells is still unclear.

As an initial effort in determining G protein-coupling selectivity of taste receptors we have examined activation of known G protein-linked signaling pathways by sweet, umami and bitter taste receptors expressed in HEK293 cells. Our results clearly demonstrate, for the first time, that taste receptors can effectively trigger two different $G\alpha_{i/o}$-signaling pathways: the activation of ERK1/2 and the inhibition of adenylyl cyclase activity. The possible physiological implications of these findings are discussed.

2. Materials and methods

Sucrose, D(−)-fructose, aspartame, cyclamate, monellin, monosodium glutamate, inosine monophosphate (IMP), isoproterenol, epidermal growth factor (EGF), denatonium benzoate, quinine sulfate, cycloheximide, rolipram and forskolin were from Sigma (St. Louis, MO). Pertussis toxin was from List Biological Laboratories (Campbell, CA). Zeocin and Dulbecco's Modified Phosphate Buffered Saline (D-PBS) were from Invitrogen (Carlsbad, CA). Puromycin was from EMD Biosciences (San Diego, CA).

2.1. Establishment of stable cell lines

An inducible expression system, GeneSwitch (Invitrogen), was used to establish a stable line expressing the human umami $T1R_1/T1R_3$ receptor. pGene derived Zeocin-resistant expression vectors that express human $T1R_1$ and human $T1R_3$ and a puromycin-resistant pSwitch-derived vector that carries the GeneSwitch protein were linearized and co-transfected in HEK293 cells expressing $G\alpha_{15}$, as previously described (Li et al., 2002). Cells were selected in 0.5 μg/ml puromycin and 100 μg/ml Zeocin in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with GlutaMAX, 10% dialyzed fetal bovine serum and 3 μg/ml blasticidin. Resistant colonies were expanded, and their responses to monosodium glutamate following induction with 100 pM Mifepristone was determined by fluorescence microscopy, as previously described (Li et al., 2002). In the present study, induction of receptor expression was initiated with 100 pM Mifepristone and was allowed to proceed for 48 h prior to the experiments. The clone used in this study did not show functional responses to monosodium glutamate without induction (data not shown and Fig. 6).

Establishment of the human sweet $T1R_2/T1R_3$-receptor expressing cell line has already been described (Li et al., 2002). Cells were maintained in low-glucose DMEM medium containing 10% heat-inactivated dialyzed fetal bovine serum, penicillin/streptomycin, 3 μg/ml blasticidin, 100 μg/ml Zeocin, and 0.5 μg/ml puromycin in Matrigel (BD Biosciences)-coated flasks.

To establish a stable cell line expressing the mouse bitter $T2R_5$ receptor, HEK293 cells were transfected with 5 μg of linearized Rhodopsin-m$T2R_5$ plasmid (Chandrashekar et al., 2000) in pEAK10 (Edge Biosystems, Gaithersburg, MD) using the TransIt-293 transfection reagent (PanVera/Invitrogen). Cells were selected in the presence of 0.5 μg/ml puromycin in high-glucose DMEM medium containing 10% heat-inactivated dialyzed fetal bovine serum and penicillin/streptomycin. Clones were isolated, expanded and analyzed by fluorescence-activated cell sorting for the presence of Rhodopsin tag immunoreactivity at the cell surface using a monoclonal antibody raised against the first 40 amino acids of Rhodopsin (Chandrashekar et al., 2000).

2.2. Transient transfection of HEK293 cells for ERK1/2 assay

Subconfluent HEK293 cells in 10-cm dishes were transfected with 4 μg of Rhodopsin-r$T2R_9$ receptor plasmid (Chandrashekar et al., 2000; Bufe et al., 2002) in pEAK10 and 2 μg pUC-18 as a carrier DNA, using the TransIt-293 transfection reagent. Cells were harvested 24 h later using Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+} and containing 1 mM EDTA (HBSS/EDTA), and plated into 6-well plates. ERK1/2 assay was performed 48-h post-transfection.

2.3. Determination of ERK1/2 phosphorylation

Cells were seeded into Matrigel (BD Biosciences)-coated 6-well plates at a density of 0.4–0.8 million cells per well 48 h prior to experiment. When necessary, umami receptor induction was initiated on the day of plating. Cells were starved for at least 16 h prior to experiment using serum-free growth media containing 1% fatty acid-free bovine serum albumin (Sigma). Cells were then stimulated with 2× agonist solutions in HBSS or D-PBS for 5 min at 37 °C. Following stimulation, cells were placed on ice and washed once with ice-cold buffer. Lysis buffer containing 150 mM NaCl, 50 mM Tris–HCl pH 8, 0.25% sodium deoxycholate, 1% igepal, 2 mM sodium orthovanadate, 1 mM sodium fluoride, and protease inhibitors was then added and cells

were scraped off the plates. Lysates were frozen immediately in liquid nitrogen and kept at −80 °C until further analysis.

Lysate protein concentration was determined using the Bradford method (Amresco, Solon, OH). Cell lysate proteins (22 μg/lane) were resolved by electrophoresis using 4–20% Tris-glycine gels (Invitrogen). Following electrophoresis, proteins were transferred to nitrocellulose membranes that were subsequently blocked with 5% fat-free milk in Tris-buffered saline containing 0.2% tween-20 (TBST). Membranes were immunoblotted with phospho-p44/42 Mitogen Activated Protein Kinase (MAPK) monoclonal antibody (Cell Signaling Technology, Beverly, MA) diluted 1:1000 in 5% milk/TBST overnight at 4 °C. The secondary antibody was a horseradish peroxidase-linked anti-mouse immunoglobulin diluted 1:2000 in 5% milk/TBST. Immunoreactive proteins were revealed using SuperSignal® West Pico Stable Peroxide Solution (Pierce Chemical, Rockford, IL). Results were quantified using Kodak Image Station 440CF. In all experiments, we also assessed the total amount of p44/42 MAPK loaded in each lane. Membranes were stripped of phospho-specific antibodies using 0.2 M glycine pH 2.5 and re-blotted with p44/42 polyclonal antibodies (Cell Signaling Technology) diluted 1:1000 in 5% milk/TBST overnight at 4 °C. Secondary antibody was horseradish peroxidase-linked anti-rabbit immunoglobulin diluted 1:2000 in 5% milk/TBST.

2.4. cAMP experiments

cAMP content of cells was determined by a commercially available chemiluminescent immunoassay kit (Applied Biosystems, Foster City, CA). Assay plates (96-well) were pre-coated with Matrigel (BD Biosciences) at a dilution of 1:400, and cells were seeded at a density of 60,000 cells/well (mouse $T2R_5$ receptor), 75,000 cells/well (human sweet $T1R_2/T1R_3$ receptor) and 50,000 cells/well (human umami $T1R_1/T1R_3$ receptor) 48 h prior to experiment. Induction of human umami $T1R_1/T1R_3$ receptor expression was also initiated 48 h prior to cAMP assays. Cell medium was aspirated and 100 μl of pre-warmed HBSS or D-PBS containing 50 μM rolipram was added to each well. Cells were incubated for 45 min at 37 °C, buffer was aspirated and 100 μl of pre-warmed agonist solutions in HBSS or D-PBS containing 50 μM rolipram and 0.7 to 5 μM forskolin was added to each of

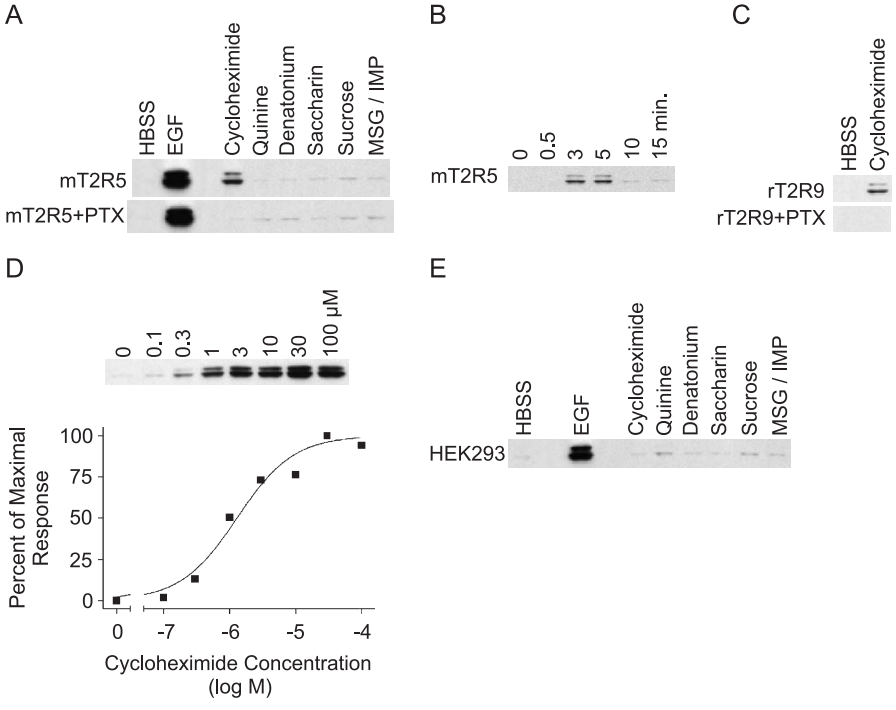

Fig. 1. The mouse bitter $T2R_5$ receptor couples to phosphorylation of ERK1/2. (A) Mouse bitter $T2R_5$ receptor-expressing HEK293 cells were incubated with buffer alone (HBSS), 100 ng/ml EGF, 40 μM cycloheximide, 250 μM quinine, 2 mM denatonium benzoate, 2 mM saccharin, 100 mM sucrose, or 5 mM monosodium glutamate/1 mM IMP in HBSS for 5 min at 37 °C. Cell lysate proteins were resolved by electrophoresis, transferred to nitrocellulose membranes and then blotted using antibodies directed against phosphorylated ERK1/2. Where indicated, cells were incubated with 100 ng/ml pertussis toxin overnight prior to experiment. (B) Time course of cycloheximide-induced ERK1/2 phosphorylation in mouse bitter $T2R_5$ receptor-expressing cells. (C) HEK293 cells transiently expressing the rat bitter $T2R_9$ receptor were treated as described in A. (D) Effect of increasing concentrations of cycloheximide on ERK1/2 phosphorylation. Mouse bitter $T2R_5$ receptor-expressing HEK293 cells were incubated with cycloheximide diluted in HBSS (0.1 to 100 μM) for 5 min at 37 °C. Cell lysate proteins were analyzed as described in A. Bands (inset) were quantified and data were normalized to maximal stimulation of phospho-ERK1/2 (at 100 μM cycloheximide). (E) Untransfected HEK293 cells were treated as described in A. Results in A, D and E are representative of at least three independent experiments. Results in B and C are representative of two independent experiments.

the corresponding wells. Plates were incubated for 15 min at 37 °C. Agonists were aspirated and reactions were terminated with addition of 60 μl of lysis buffer into each well. cAMP levels were then determined as described by the kit instructions. An independent cAMP standard curve was performed on each 96-well plate used. Chemiluminescent signals were detected using a TopCount-NXT (PerkinElmer, Wellesley, MA) set at a read-time of 2 s/well. Data were plotted using GraphPad PRISM software (San Diego, CA) and statistical analysis was performed using SigmaStat software (SPSS, Chicago, IL).

3. Results

3.1. Activation of the bitter, sweet and umami receptors induces ERK1/2 phosphorylation in a pertussis toxin sensitive fashion

To demonstrate that taste receptors can couple to the $G_{i/o}$ family of G proteins, we monitored the activation of MAPK. Several $G\alpha_{i/o}$-coupled receptors couple to MAPK activation (Liebmann, 2001; Della Rocca et al., 1997; Alderton et al., 2001a,b). The level of receptor-induced MAPK activation can be readily determined by monitoring the level of ERK1/2 phosphorylation using phospho-specific antibodies (Della Rocca et al., 1997; Alderton et al., 2001a,b). For this study, we examined the ability of the mouse bitter $T2R_5$ receptor (Chandrashekar et al., 2000), the human sweet $T1R_2/T1R_3$ receptor (Li et al., 2002) and the human monosodium glutamate $T1R_1/T1R_3$ receptor (Li et al., 2002; Nelson et al., 2002) to modulate the level of ERK1/2 phosphorylation. $T1R_2/T1R_3$ refers to co-expression of two different receptors, $T1R_2$ and $T1R_3$, that appear to be functional as dimers (Li et al., 2002; Nelson et al., 2001). Similarly, $T1R_1/T1R_3$ refers to co-expression of two different receptors, $T1R_1$ and $T1R_3$, that also appear to be functional as dimmers (Li et al., 2002; Nelson et al., 2002). A clone of HEK293 cells stably expressing the mouse bitter $T2R_5$ receptor shows robust induction of ERK1/2 phosphorylation upon exposure to cycloheximide (Fig. 1A). Induction of ERK1/2 phosphorylation by cycloheximide in mouse bitter $T2R_5$ receptor-expressing cells peaks at 3–5 min post-stimulation (Fig. 1B). This effect is specific to cycloheximide in these cells as bitter substances such as quinine and denatonium benzoate, sweeteners such as saccharin or sucrose and monosodium glutamate do not induce ERK1/2 phosphorylation (Fig. 1A). Similarly, cycloheximide induces ERK1/2 phosphorylation in cells transiently transfected with the rat bitter $T2R_9$ receptor, the receptor orthologue of the mouse $T2R_5$ receptor (Bufe et al., 2002) (Fig. 1C). On the other hand, the human sweet $T1R_2/T1R_3$ receptor cell line responds exclusively to sweeteners in the ERK1/2 assay (Figs. 2 and 3). Sweeteners such as sucrose, saccharin, cyclamate and the sweet tasting amino acid D-Tryptophan specifically induce ERK1/2 phosphorylation (Fig. 2A). Bitter substances and monosodium glutamate fail to induce phosphorylation of ERK1/2 in these cells (Fig. 2A). Similarly, the human umami $T1R_1/T1R_3$ receptor cell line responds exclusively to monosodium glutamate in the ERK1/2 assay (Fig. 2B). Sweeteners and bitter substances have no significant effect on the level of phosphorylated ERK1/2 in these cells (Fig. 2B). The effects of cycloheximide, saccharin, cyclamate, D-Tryptophan, sucrose and monosodium glutamate on ERK1/2 phosphorylation are receptor dependent.

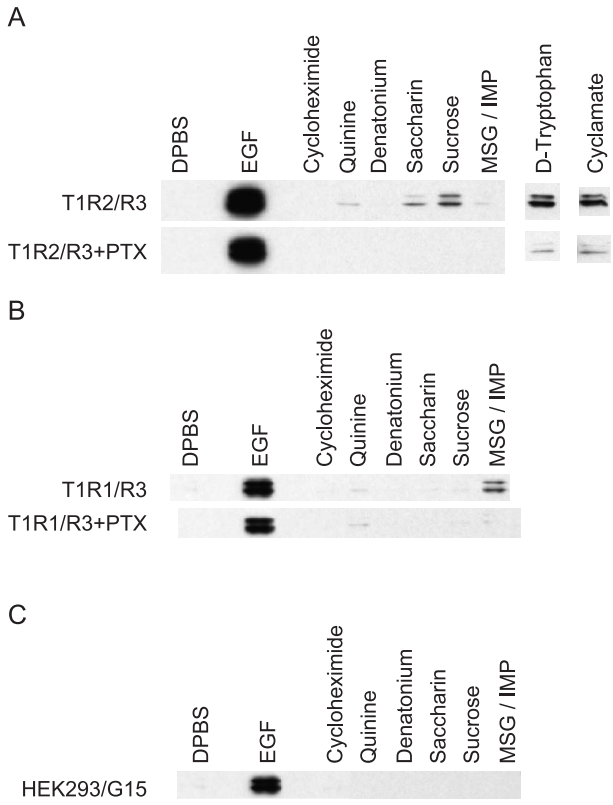

Fig. 2. The human sweet $T1R_2/T1R_3$ receptor and human umami $T1R_1/T1R_3$ receptor couple to phosphorylation of ERK1/2. (A) Human sweet $T1R_2/T1R_3$ receptor-expressing HEK293/G15 cells were incubated with buffer alone (D-PBS), 100 ng/ml EGF, 40 μM cycloheximide, 250 μM quinine, 2 mM denatonium benzoate, 2 mM saccharin, 100 mM sucrose, 5 mM monosodium glutamate/1 mM IMP, 4 mM D-Tryptophan and 10 mM cyclamate in D-PBS for 5 min at 37 °C. Cell lysate proteins were resolved by electrophoresis, transferred to nitrocellulose membranes and then blotted using antibodies directed against phosphorylated ERK1/2. Where indicated, cells were incubated with 100 ng/ml pertussis toxin overnight prior to experiment. (B) Human umami $T1R_1/T1R_3$ receptor-expressing HEK293/G15 cells were induced for receptor expression. Forty-eight hours later, cells were incubated with buffer alone (D-PBS), 100 ng/ml EGF, 40 μM cycloheximide, 250 μM quinine, 2 mM denatonium benzoate, 2 mM saccharin, 100 mM sucrose and 5 mM monosodium glutamate/1 mM IMP in D-PBS for 5 min at 37 °C. Cell lysate proteins were analyzed as described in A. Where indicated, cells were incubated with 100 ng/ml pertussis toxin overnight prior to experiment. (C) Untransfected HEK293/G15 cells were treated as described in B. Results are representative of at least three independent experiments.

Indeed, these tasting modalities do not induce significant ERK1/2 phosphorylation in untransfected HEK293 cells (Fig. 1E) and HEK293/G15 cells (Fig. 2C; and results not shown).

Cycloheximide induces ERK1/2 phosphorylation in a concentration-dependent fashion in mouse bitter $T2R_5$ receptor-expressing cells with an EC_{50} of 1.1 ± 0.4 μM (mean ± S.D. of three independent determinations) (Fig. 1D). Similarly, saccharin and sucrose also induce ERK1/2 phosphorylation in a concentration-dependent fashion in human sweet $T1R_2/T1R_3$ receptor-expressing cells (Fig. 3A and B). As expected from taste thresholds (Li et al., 2002), saccharin is much more potent with an EC_{50} of 277 ± 47 μM as compared to an EC_{50} of 73 ± 37 mM for sucrose (mean ± S.D. of three independent determinations) (Fig. 3A and B). One of the hallmarks of umami taste is its significant enhancement by IMP (Yamaguchi, 1991), an effect that can also be detected on monosodium glutamate-induced signaling in human umami $T1R_1/T1R_3$-expressing cells (Li et al., 2002). Accordingly, in the ERK1/2 assay, we observe a leftward shift of the monosodium glutamate EC_{50} of about 18-fold in the presence of 10 mM IMP (Fig. 3C) (EC_{50} monosodium glutamate: 6.7 ± 3.4 mM, EC_{50} monosodium glutamate in the presence of 10 mM IMP: 0.37 ± 0.27 mM; mean ± S.D. of three independent determinations). At maximum concentrations, cycloheximide, sucrose, and a mixture of monosodium glutamate and IMP induce a level of ERK1/2 phosphorylation corresponding to 20% to 40% of the EGF response (Figs. 1 and 2). In preliminary experiments, we found that these taste modalities induce a level of ERK1/2 phosphorylation that is two to four times greater than the response to lysophosphatidic acid receptor activation (results not shown). The lysophosphatidic acid receptor is a G protein-coupled receptor that is endogenously expressed in HEK293 cells and induces ERK1/2 phosphorylation in a $G\alpha_i$-dependent fashion (Alderton et al., 2001a,b). The greater response observed with taste G protein-coupled receptors relative to that of the lysophosphatidic acid receptor could be due to a higher receptor density and/or a more efficient coupling to the ERK1/2 signaling pathways in HEK293 cells.

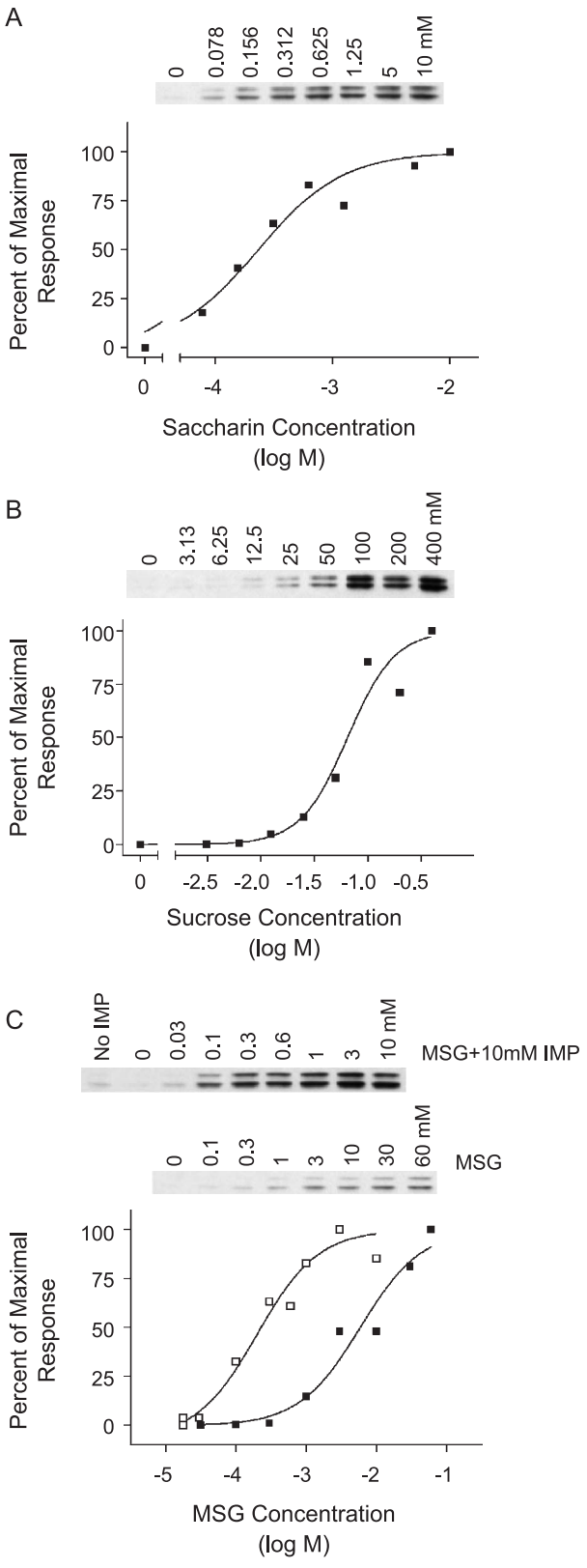

Fig. 3. Effects of increasing concentrations of sweeteners and monosodium glutamate on ERK1/2 phosphorylation. Human sweet $T1R_2/T1R_3$ receptor-expressing HEK293/G15 cells were incubated with increasing concentrations of either saccharin (panel A) (0.078 to 10 mM) or sucrose (panel B) (3.13 to 400 mM) for 5 min at 37 °C. Cell lysate proteins were resolved by electrophoresis, transferred to nitrocellulose membranes and then blotted using antibodies directed against phosphorylated ERK1/2. Bands (insets) were quantified and data were normalized to the maximal phosphorylation of ERK1/2 (at 10 mM saccharin and 400 mM sucrose respectively). (C) Human umami $T1R_1/T1R_3$ receptor-expressing HEK293/G15 cells were induced for receptor expression. Cells were then incubated with increasing concentrations of monosodium glutamate (0.03 to 60 mM) in the presence (open squares) or the absence (closed squares) of 10 mM IMP for 5 min at 37 °C. Cell lysate proteins were then analyzed as described in A. Bands (inset) were quantified and data were normalized to maximal stimulation of phospho-ERK1/2 (at 10 and 60 mM sodium glutamate). These results are representative of at least three independent experiments.

Pertussis toxin has been widely used as a powerful tool to discriminate among the different pathways used by G protein-coupled receptors to activate ERK1/2 (Liebmann, 2001). Treatment of the mouse bitter $T2R_5$ receptor, human sweet $T1R_2/T1R_3$ receptor and human umami $T1R_1/T1R_3$ receptor cell lines with pertussis toxin prevents phosphorylation of ERK1/2 by cycloheximide (Fig. 1A), by sucrose, saccharin, D-Tryptophan and cyclamate (Fig. 2A) and by monosodium glutamate (Fig. 2B), respectively. As shown in Figs. 1A and 2A,B, $G\alpha_{i/o}$-independent induction of ERK1/2 phosphorylation by EGF is not affected under these conditions, as expected. Collectively, these results indicate that taste receptors functionally couple to $G\alpha_{i/o}$ proteins to induce ERK1/2 activation in HEK293 cells.

3.2. Activation of bitter, sweet and umami taste receptors inhibits camp accumulation in HEK293 cells

Results described in Figs. 1–3 suggest that taste receptors should also inhibit adenylyl cyclase activity and reduce cAMP levels in HEK293 cells. Fig. 4A shows that cycloheximide leads to a 70% reduction of forskolin-induced cAMP accumulation in mouse $T2R_5$ receptor-expressing cells. Pertussis toxin treatment fully abolishes the inhibition (Fig. 4A), demonstrating the involvement of $G\alpha_{i/o}$ proteins. The effect of cycloheximide on cAMP accumulation is dependent on the mouse bitter $T2R_5$ receptor since cAMP levels of untransfected HEK293 cells are unchanged by cycloheximide (Fig. 4A). Cycloheximide inhibits cAMP accumulation in a concentration-dependent manner with an EC_{50} of 0.38 ± 0.22 μM (Fig. 4B) (mean ± S.D. of three independent determinations), a value similar to the EC_{50} calculated for ERK1/2 phosphorylation (Fig. 1D). Activation of the human sweet $T1R_2/T1R_3$ receptor also results in robust inhibition of cAMP accumulation in HEK293 cells. Sweeteners such as aspartame, cyclamate, saccharin and monellin (a sweet protein) decrease forskolin-induced cAMP accumulation levels by 55%, 40%, 55% and 64%, respectively (Fig. 5A). These effects are also blocked by pertussis toxin (Fig. 5A), demonstrating the direct involvement of $G\alpha_{i/o}$ proteins. Thaumatin, another sweet protein, also induces a 50% reduction in forkskolin-induced cAMP accumulation in the human sweet $T1R_2/T1R_3$ receptor cell line (results not shown). In contrast, fructose and sucrose do not inhibit cAMP accumulation in these cells. The lack of inhibitory effect of fructose and sucrose on the forskolin-induced cAMP accumulation can be explained by the fact that these two sweeteners consistently increase cAMP levels in HEK293 cells that do not express the human sweet $T1R_2/T1R_3$ receptor (Fig. 5B). Cyclamate (Fig. 5C), aspartame (Fig. 5D) and saccharin (Fig. 5E) inhibit the forskolin-induced cAMP accumulation in a concentration-dependent fashion with EC_{50}s of 1.2 ± 0.7 mM, 350 ± 60 μM and 61 ± 33 μM, respectively (Fig. 5C)

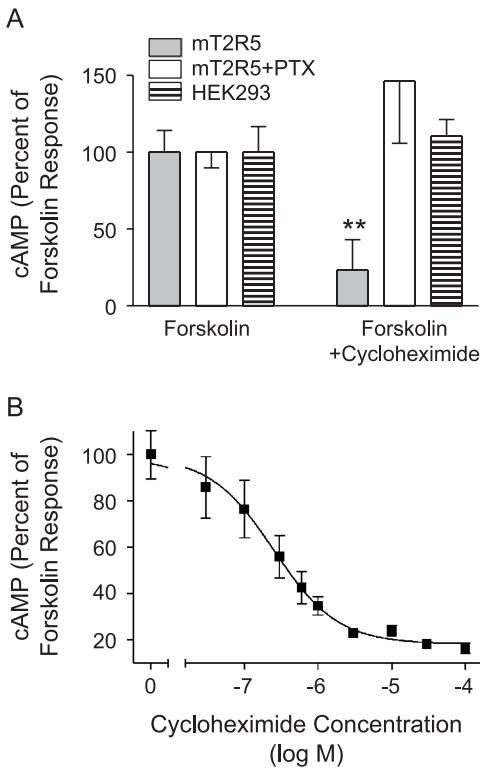

Fig. 4. Cycloheximide inhibits cAMP accumulation in mouse bitter $T2R_5$ receptor-expressing cells. (A) Mouse bitter $T2R_5$ receptor-expressing HEK293 cells and untransfected HEK293 cells were incubated with 0.7 μM forskolin and 50 μM rolipram in the presence and absence of 40 μM cycloheximide in HBSS for 15 min at 37 °C. cAMP levels were determined as described in Materials and methods. cAMP content of cells stimulated with buffer (0.525% dimethylsulfoxide (DMSO) in HBSS) was 5 pmol/well. cAMP content of cells stimulated with forskolin was 73 pmol/well. Where indicated, cells were also treated with 100 ng/ml pertussis toxin for 4 h at 37 °C and then stimulated as described above. Under these conditions, cAMP content of cells stimulated with buffer (0.525% DMSO in HBSS) was 4 pmol/well and cAMP content of cells stimulated with forskolin was 80 pmol/well. (B) Effect of increasing concentrations of cycloheximide on forskolin-induced cAMP accumulation. Mouse bitter $T2R_5$ receptor-expressing cells were incubated with 0.7 μM forskolin and 50 μM rolipram in the presence of cycloheximide diluted in HBSS (0.03 to 100 μM) for 15 min at 37 °C and cAMP levels were determined as described in Materials and methods. Results in A correspond to the mean ± S.D. of three independent experiments performed in quadruplicates. Results in B correspond to the mean ± S.D. of a quadruplicate determination and are representative of three similar experiments. Significantly different than forskolin response, $^{**}P < 0.01$ (Student's *t*-test).

(mean ± S.D. of three independent determinations). The human umami $T1R_1/T1R_3$ receptor cell line exhibits very high basal cAMP levels relative to the mouse bitter $T2R_5$ receptor and human sweet $T1R_2/T1R_3$ receptor cell lines ($T2R_5$ receptor cell line: 2.8 ± 1.9 pmol/well, $T1R_2/T1R_3$ receptor cell line: 4.5 ± 1.9 pmol/well, $T1R_1/T1R_3$ receptor cell line: 180 ± 30 pmol/well). We do not know yet if this is a result of an elevated constitutive coupling of the umami receptor to $G\alpha_s$. In any case, stimulation of the human umami $T1R_1/T1R_3$ receptor by monoso-

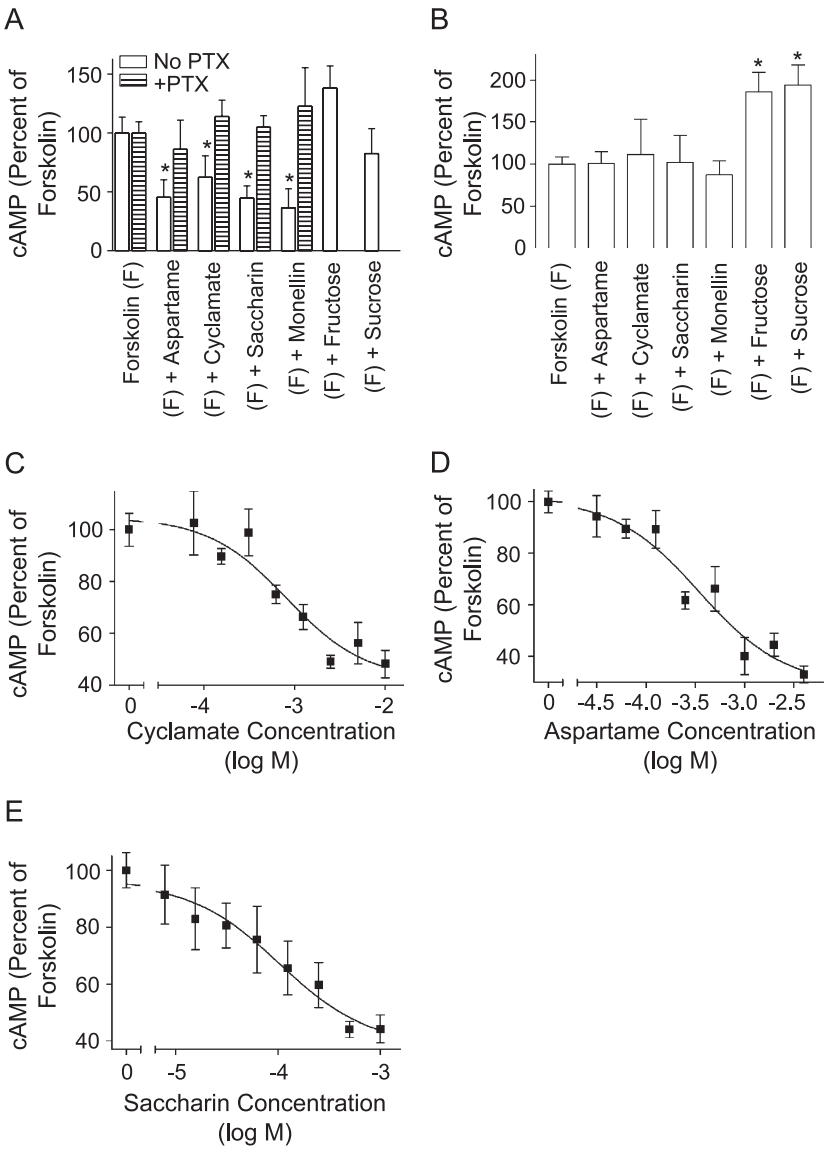

Fig. 5. Sweeteners inhibit cAMP accumulation in human sweet $T1R_2/T1R_3$ receptor expressing-cells. (A) Human sweet $T1R_2/T1R_3$ receptor-expressing HEK293/G15 cells were incubated with 5 μM forskolin and 50 μM rolipram in the presence and absence of either 200 mM fructose, 200 mM sucrose, 1 mM aspartame, 3 mM cyclamate, 2 mM saccharin or 50 μM monellin in D-PBS for 15 min at 37 °C and cAMP levels were determined as described in Materials and methods. cAMP content of cells stimulated with buffer (0.525% DMSO in D-PBS) was 3 pmol/well. cAMP content of cells stimulated with forskolin was 23 pmol/well. Where indicated, cells were also treated with 100 ng/ml pertussis toxin for 4 h at 37 °C and then stimulated as described above. Under these conditions, cAMP content of cells stimulated with buffer (0.525% DMSO in D-PBS) was 4 pmol/well and cAMP content of cells stimulated with forskolin was 149 pmol/well. (B) Untransfected HEK293/G15 cells were treated as in A. cAMP content of cells stimulated with buffer (0.525% DMSO in D-PBS) was 4 pmol/well and cAMP content of cells stimulated with forskolin was 90 pmol/well. (C) Effects of increasing concentration of cyclamate on forskolin-induced cAMP accumulation. Cells were incubated with 5 μM forskolin and 50 μM rolipram in the presence of increasing concentrations of cyclamate (0.08 to 10 mM). cAMP content of cells stimulated with forskolin alone was 11 pmol/well. (D) Effects of increasing concentration of aspartame on forskolin-induced cAMP accumulation. Cells were incubated with 5 μM forskolin and 50 μM rolipram in the presence of increasing concentrations of aspartame (0.03 to 4 mM). cAMP content of cells stimulated with forskolin alone was 14 pmol/well. (E) Effects of increasing concentration of saccharin on forskolin-induced cAMP accumulation. Cells were incubated with 5 μM forskolin and 50 μM rolipram in the presence of increasing concentrations of saccharin (0.008 to 1 mM). cAMP content of cells stimulated with forskolin alone was 24 pmol/well. Results in A and B correspond to the mean ± S.D. of three to six independent experiments performed in quadruplicates. Results in C–E correspond to the mean ± S.D. of a quadruplicate determination and are representative of three similar experiments. Statistical significance was tested by one-way ANOVA, $P = 0.001$ and by Dunn's method for multiple comparison. *$P < 0.05$.

dium glutamate reproducibly decreases basal levels of cAMP by 50% in HEK293 cells (Fig. 6), confirming that this receptor indeed couples to $G\alpha_{i/o}$ proteins. On the other hand, cAMP levels remain unchanged in the presence of monosodium glutamate if the umami receptor expression is not induced prior to the experiment (Fig. 6).

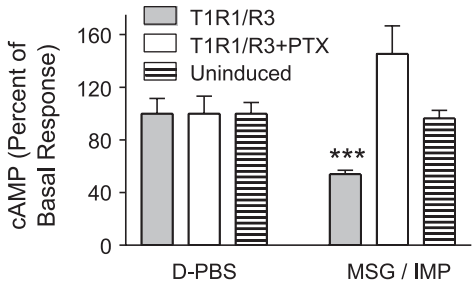

Fig. 6. Monosodium glutamate inhibits cAMP accumulation in human umami T1R1/T1R3 receptor-expressing cells. Human umami $T1R_1/T1R_3$ receptor-expressing HEK293/G15 cells were induced for receptor expression or seeded into cAMP-testing plates without induction. Forty-eight hours later, cells were incubated with 50 μM rolipram in the presence and absence of 3 mM monosodium glutamate/10 mM IMP in D-PBS for 15 min at 37 °C and cAMP levels were determined as described in Materials and methods. cAMP content of cells in the presence of rolipram was 180 pmol/well. Where indicated, cells were also treated with 100 ng/ml pertussis toxin for 4 h at 37 °C and then stimulated as described above. Results correspond to the mean ± S.D. of three independent experiments performed in quadruplicates. Significantly different than forskolin response, $***P<0.001$ (Student's *t*-test).

3.3. Sweet and bitter taste receptors do not couple to cAMP accumulation in HEK293 cells

It has been suggested that the sweet receptor may couple, in addition to Gα-gustducin, to $G\alpha_s$ and increase cAMP levels in taste receptor cells (Margolskee, 2002). Clearly, our results with ERK1/2 phosphorylation and inhibition of cAMP accumulation point to a direct coupling to $G\alpha_{i/o}$ proteins (Figs. 2, 3 and 5). However, it is still possible that this receptor could have multiple properties, coupling to both $G\alpha_{i/o}$ and $G\alpha_s$ proteins. Therefore, we sought to determine if we could detect an agonist-induced increase in cAMP levels in the human sweet $T1R_2/T1R_3$ receptor cell line. Under these experimental conditions (i.e. in the absence of forskolin), cAMP levels remain unchanged after stimulation with aspartame, cyclamate, saccharin and monellin (Fig. 7A). On the other hand, a β-adrenoceptor agonist, isoproterenol, induces a 100% increase of cAMP accumulation in human sweet $T1R_2/T1R_3$ receptor-expressing cells indicating that a functional receptor/$G\alpha_s$ interaction can be detected. The sweeteners do not induce an increase of cAMP levels even after abolishing functional coupling to $G\alpha_{i/o}$ with pertussis toxin (Fig. 7B). In contrast, the isoproterenol response increases significantly (by more than 17-fold) under these conditions, confirming that β-adrenoreceptors couple to both $G\alpha_{i/o}$ and $G\alpha_s$ proteins in HEK293 cells (Daaka et al., 1997). Similar experiments with the mouse bitter $T2R_5$ receptor cell line suggest that bitter receptors also do not functionally couple to $G\alpha_s$. Cycloheximide does not increase levels of cAMP in $T2R_5$ receptor-expressing cells, even after inhibiting coupling to $G\alpha_{i/o}$ proteins with pertussis toxin (Fig. 7C). Interestingly, inhibiting functional coupling to $G\alpha_{i/o}$ with pertussis toxin in the human umami $T1R_1/T1R_3$ receptor cell line reveals a

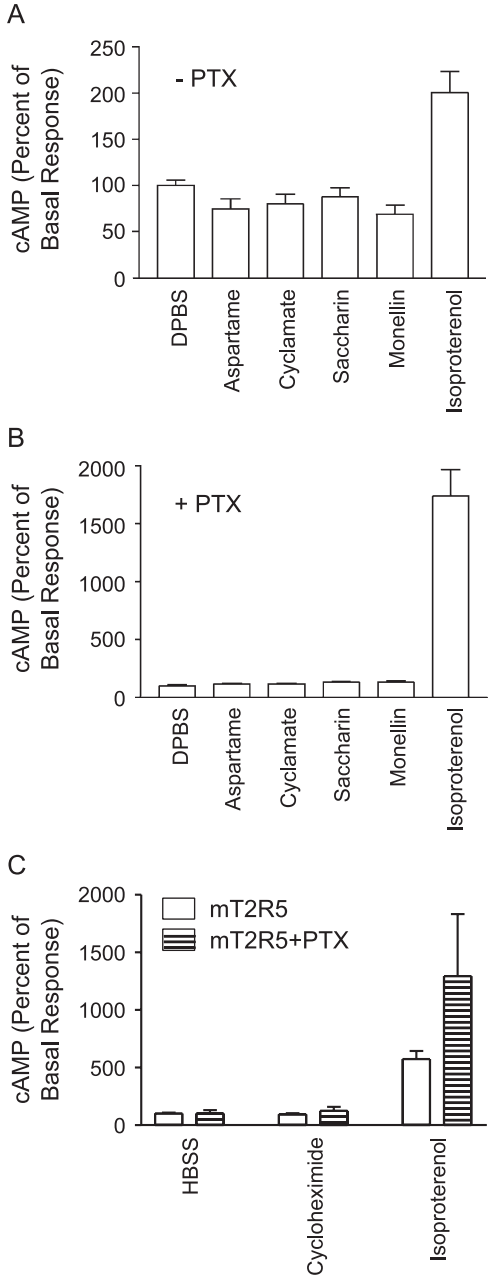

Fig. 7. The mouse bitter $T2R_5$ receptor and the human sweet $T1R_2/T1R_3$ receptor do not functionally couple to cAMP accumulation. (A) Human sweet $T1R_2/T1R_3$ receptor-expressing HEK293/G15 cells were incubated with 50 μM rolipram in the presence and absence of either 1 mM aspartame, 3 mM cyclamate, 2 mM saccharin, 50 μM monellin and 10 μM isoproterenol in D-PBS for 15 min at 37 °C and cAMP levels were determined as described in Materials and methods. Under these conditions, the basal level of cAMP was 2 pmol/well. (B) Human sweet $T1R_2/T1R_3$ receptor-expressing cells were treated with 100 ng/ml pertussis toxin for 4 h at 37 °C and then stimulated as described above. Under these conditions, the basal level of cAMP was 1.3 pmol/well. (C) Mouse bitter $T2R_5$ receptor-expressing HEK293 cells were incubated with 50 μM rolipram in the presence and absence of 40 μM cycloheximide or 10 μM isoproterenol in HBSS for 15 min at 37 °C. Under these conditions, the basal level of cAMP was 5 pmol/well. Cells were also treated with 100 ng/ml pertussis toxin for 4 h at 37 °C and then stimulated as described above. Under these conditions, the basal level of cAMP was 4 pmol/well. Results correspond to the mean ± S.D. of three independent experiments performed in quadruplicates.

modest increase of 25% in cAMP levels (Fig. 6). Further experiments are necessary to determine if the umami receptor can indeed couple to $G\alpha_s$-signaling pathways in a significant fashion.

4. Discussion

In this study, we have investigated the functional coupling of taste receptors to $G\alpha_{i/o}$ proteins by monitoring the levels of ERK1/2 phosphorylation and the levels of intracellular cAMP, two second messengers modulated by dozens of G protein-coupled receptors (Chin et al., 2002; Liebmann, 2001). cAMP is a universal second messenger used by a plethora of cell surface receptors to relay signals from the extracellular milieu to the intracellular signaling machinery such as protein kinases, transcription factors and ion channels (Chin et al., 2002). G protein-coupled receptor-mediated activation of $G\alpha_s$ typically increases while $G\alpha_{i/o}$ activation decreases intracellular cAMP levels, respectively (Hanoune and Defer, 2001). The GTP-bound form of $G\alpha_s$ directly activates the nine types of membrane-bound adenylyl cyclase (Hanoune and Defer, 2001). Conversely, the GTP-bound form of $G\alpha_i$ can directly inhibit up to six different types of adenylyl cyclases (Hanoune and Defer, 2001). ERK1/2 are activated by $G\alpha_q$, $G\alpha_s$ and $G\alpha_{i/o}$-coupled receptors (Liebmann, 2001; Gutkind, 1998) and, depending on the cellular context, several signaling pathways can be triggered to activate these MAPK. Specifically, it is thought that $G\alpha_{i/o}$-coupled receptors activate ERK1/2 mainly via the free (activated) $G\beta\gamma$ subunits (Gutkind, 1998). $G\beta\gamma$ subunits recruit and activate soluble tyrosine kinases (Gutkind, 1998), phosphatidylinositol-3 kinases (Lopez-Ilasaca et al., 1997; Murga et al., 2000; Schulte and Fredholm, 2002), or transactivate receptor tyrosine kinases at the cell surface to initiate the cascade (Liebmann, 2001).

In the current study, we report that the mouse bitter $T2R_5$ receptor, the human sweet $T1R_2/T1R_3$ receptor, and the human umami $T1R_1/T1R_3$ receptor couple to the induction of ERK1/2 phosphorylation and the inhibition of cAMP accumulation in HEK293 cells. The bitter substance cycloheximide, the sweeteners saccharin, sucrose, cyclamate, D-Tryptophan and the savory amino acid monosodium glutamate all induce ERK1/2 phosphorylation exclusively in cells expressing their respective receptors. The effects of cycloheximide, saccharin, sucrose and monosodium glutamate are all concentration-dependent and their potencies are similar to those reported for the $G\alpha_{15}$-induced mobilization of Ca^{2+} in HEK293 cells (Chandrashekar et al., 2000; Li et al., 2002). Similarly, cycloheximide, artificial sweeteners, the sweet protein monellin, and monosodium glutamate decrease intracellular cAMP levels exclusively in cells expressing their respective taste receptors. Here again, the effects are receptor dependent and the potencies of these compounds at inhibiting cAMP accumulation are in agreement with EC_{50} values reported for the $G\alpha_{15}$-mediated Ca^{2+} mobilization in transfected HEK293 cells (Chandrashekar et al., 2000; Li et al., 2002). Collectively, these results indicate that bitter compounds, sweeteners and monosodium glutamate specifically activate their taste receptors to induce ERK1/2 phosphorylation and the reduction of cAMP accumulation in heterologous cells.

$G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{o1}$ and $G\alpha_{o2}$ all contain a conserved carboxyl-terminal cysteine residue that is a site for modification by pertussis toxin, a 5′ -diphosphate-ribosyltransferase isolated from *Bortadella pertussis* (Fields and Casey, 1997). Pertussis toxin specifically and irreversibly modifies these G protein subunits with attachment of an ADP-ribose moiety and, as a result, physically uncouples the G-protein from activation by G protein-coupled receptors (Fields and Casey, 1997). An additional member of the $G\alpha_i$ family of G proteins, $G\alpha_z$, is insensitive to modification by pertussis toxin (Fields and Casey, 1997). In our assays, incubation of cells with pertussis toxin abolishes the phosphorylation of ERK1/2 by the bitter, sweet and umami taste receptors. These results indicate that one or more members of the $G\alpha_i$ family—with the exception of $G\alpha_z$—functionally link the taste receptors to this signaling pathway in HEK293 cells. Similarly, pertussis toxin prevents activation of ERK1/2 by other $G\alpha_{i/o}$-coupled receptors expressed in HEK293 cells (Della Rocca et al., 1997; Alderton et al., 2001b). Every taste G protein-coupled receptor that we studied also couples to the inhibition of cAMP accumulation in HEK293 cells and pertussis toxin treatment totally abolishes the inhibition. This result confirms that taste receptors can couple to one or more members of the $G\alpha_{i/o}$ subfamily. In this signaling pathway, activated $G\alpha_{i/o}$ proteins directly inhibit the membrane bound adenylyl cyclase. There is indeed no evidence for direct regulation of cAMP-phosphodiesterases by the $G\alpha_{i/o}$ subfamily (Francis et al., 2000).

In agreement with our data, results from independent studies support a model in which taste receptors can functionally couple to $G\alpha_i$ proteins. Application of bitter- and umami-tasting stimuli to taste tissue membranes or papillae reduces levels of cAMP (Margolskee, 2002; Yan et al., 2001; Chaudhari and Roper, 1998; Abaffy et al., 2003). This decrease in cAMP levels could be the result of a direct inhibition of adenylyl cyclases expressed in taste receptor cells (Abaffy et al., 2003). In support of this hypothesis, it has been recently estimated that 60% of taste receptor cells co-express $G\alpha_{i2}$ and adenylyl cyclase 8 (Abaffy et al., 2003). In contrast to bitter and umami-tasting stimuli, sweeteners have been reported to increase cAMP levels in taste receptor cells (Margolskee, 2002). These observations have led to the hypothesis that the sweet receptor can also couple to $G\alpha_s$, in addition to Gα-gustducin (Margolskee, 2002). In our hands, however, the human sweet $T1R_2/T1R_3$ receptor clearly couples to a reduction of intracellular cAMP levels and a phosphorylation of ERK1/2 through the direct functional coupling of the sweet receptor with $G\alpha_{i/o}$ proteins. Moreover, we cannot detect a

sweetener-induced accumulation of cAMP in human sweet $T1R_2/T1R_3$ receptor-expressing cells, even after inhibiting functional coupling to $G\alpha_{i/o}$ proteins with pertussis toxin. It is noteworthy that we can detect a fructose or sucrose-induced cAMP accumulation in untransfected HEK293/G15 cells. We suspect that this is a direct result of the high concentrations of sucrose and fructose required in these experiments. Similarly, in an independent study, application of a 1 M sucrose solution to tongue muscle membranes (a non-taste tissue) was shown to induce cAMP accumulation (Striem et al., 1989). It is therefore possible that the sweetener-induced increase in cAMP levels observed in taste tissue (Margolskee, 2002) occurs through a receptor-independent mechanism. Conversely, sweeteners could activate Ca^{2+}-sensitive adenylyl cyclases expressed in taste receptor cells leading to a net increase of cAMP concentration (Abaffy et al., 2003). In any case, our results do not support the hypothesis of a direct functional coupling of the sweet receptor to $G\alpha_s$ (Margolskee, 2002).

The receptors for human and rodent sweet and umami taste are thought to function as heterodimers with the combination of $T1R_2/T1R_3$ mediating sweet taste (Li et al., 2002; Nelson et al., 2001; Zhao et al., 2003), and $T1R_1/T1R_3$ mediating umami taste (Li et al., 2002; Nelson et al., 2002; Zhao et al., 2003). In accordance with this hypothesis, in situ hybridization studies have shown that three distinct populations of taste receptor cells exist in rodent taste tissue: those expressing both $T1R_1$ and $T1R_3$, those expressing both $T1R_2$ and $T1R_3$ and those expressing only $T1R_3$ (Nelson et al., 2001). $T1R_3$ alone is not a functional umami receptor, but it does respond to high concentrations of natural sugars (Zhao et al., 2003). At this point, we do not know which receptor between $T1R_1$, $T1R_2$ and $T1R_3$ mediates functional coupling to inhibitory G proteins in HEK293 cells or if $T1R_3$ alone is capable of activating inhibitory G protein signaling pathways. Further studies are necessary to address these issues.

Gα-subunits other than Gα-gustducin could contribute to taste transduction in taste receptor cells (Margolskee, 2002). Perhaps the most compelling evidence suggesting the involvement of other G proteins in taste perception is the residual responsiveness of Gα-gustducin-deficient mice to bitter and sweet stimuli (Wong et al., 1996; Ruiz-Avila et al., 2001; He et al., 2002). Expression of a dominant negative form of Gα-gustducin, in Gα-gustducin-deficient mice, further decreases the residual responsiveness to sweet and bitter stimuli, substantiating the idea of the involvement of another G protein (Ruiz-Avila et al., 2001). A recent study by the group of Roper shows that only about 50% of bitter-sensitive taste receptor cells from mouse taste tissue express Gα-gustducin (Caicedo et al., 2003). Moreover, the same study reports that more than 80% of bitter-sensitive cells express either $G\alpha_{i2}$ or $G\alpha_{i2}$ and Gα-gustducin (Caicedo et al., 2003), suggesting that a significant proportion of bitter-sensitive cells may use $G\alpha_{i2}$ to transmit bitter taste receptor signaling. These data and our observation that the mouse bitter $T2R_5$ receptor effectively couples to $G\alpha_{i/o}$-signaling pathways further reinforce the notion that Gα-gustducin independent signaling could occur in taste receptor cells and that $G\alpha_{i2}$ proteins would correspond to a likely candidate to perform this task. Functional coupling between taste receptors and $G\alpha_{i2}$ proteins could, technically, complement Gα-gustducin function for the activation of phospholipase-β2, an enzyme that is activated by the Gβγ subunit of the $G_{i/o}$ subfamily of G proteins and that is essential for taste transduction (Zhang et al., 2003; Li et al., 2000; Wu et al., 1993; Katan, 1998; Smrcka and Sternweis, 1993; Rhee and Bae, 1997). The interaction between taste receptor and $G\alpha_{i2}$ proteins could explain the residual responsiveness of Gα-gustducin-deficient mice to bitter and sweet stimuli (Wong et al., 1996; Ruiz-Avila et al., 2001; He et al., 2002). $G\alpha_{i2}$ is abundantly expressed in taste tissue, whereas $G\alpha_{i1}$ and $G\alpha_{i3}$ are expressed at lower levels (Asano-Miyoshi, 2000; Kusakabe, 2000; Margolskee, 2002). It is therefore possible that several members of the $G\alpha_{i/o}$ family could functionally couple to taste receptors in vivo. Further studies using pertussis toxin-insensitive $G\alpha_{i/o}$ mutants are necessary to delineate which $G\alpha_{i/o}$ subtypes can functionally interact with taste receptors.

This study clearly shows that taste G protein-coupled receptors can use $G\alpha_{i/o}$ proteins to transmit signals to downstream effectors. These results now provide investigators with additional cell based assays to study taste receptor signaling and pharmacology. Although we do not yet understand the physiological significance of the interaction between taste receptors and $G\alpha_{i/o}$ proteins, it is tempting to suggest that, in vivo, at least one member of the $G\alpha_{i/o}$ subfamily expressed in taste receptor cells may correspond to the elusive G protein complementing α-gustducin functions (Margolskee, 2002).

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References

Abaffy, T., Trubey, K.R., Chaudhari, N., 2003. Adenylyl cyclase expression and modulation of cAMP in rat taste cells. Am. J. Physiol., Cell Physiol. 284, C1420–C1428.

Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J., Zuker, C.S., 2000. A novel family of mammalian taste receptors. Cell 100, 693–702.

Alderton, F., Darroch, P., Sambi, B., McKie, A., Ahmed, I.S., Pyne, N., Pyne, S., 2001a. G-protein-coupled receptor stimulation of the p42/p44 mitogen-activated protein kinase pathway is attenuated by lipid phosphate phosphatases 1, 1a, and 2 in human embryonic kidney 293 cells. J. Biol. Chem. 276, 13452–13460.

Alderton, F., Sambi, B., Tate, R., Pyne, N.J., Pyne, S., 2001b. Assessment of agonism at G-protein coupled receptors by phosphatidic acid and lysophosphatidic acid in human embryonic kidney 293 cells. Br. J. Pharmacol. 134, 6–9.

Asano-Miyoshi, M., Abe, K., Emori, Y., 2000. Co-expression of calcium signaling components in vertebrate taste bud cells. Neurosci. Lett. 283, 61–64.

Bufe, B., Hofmann, T., Krautwurst, D., Raguse, J.D., Meyerhof, W., 2002. The human TAS2R16 receptor mediates bitter taste in response to beta-glucopyranosides. Nat. Genet. 32, 397–401.

Caicedo, A., Pereira, E., Margolskee, R.F., Roper, S.D., 2003. Role of the G-protein subunit alpha-gustducin in taste cell responses to bitter stimuli. J. Neurosci. 23, 9947–9952.

Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S., Ryba, N.J., 2000. T2Rs function as bitter taste receptors. Cell 100, 703–711.

Chaudhari, N., Roper, S.D., 1998. Molecular and physiological evidence for glutamate (umami) taste transduction via a G protein-coupled receptor. Ann. N. Y. Acad. Sci. 855, 398–406.

Chaudhari, N., Landin, A.M., Roper, S.D., 2000. A metabotropic glutamate receptor variant functions as a taste receptor. Nat. Neurosci. 3, 113–119.

Chin, K.V., Yang, W.L., Ravatn, R., Kita, T., Reitman, E., Vettori, D., Cvijic, M.E., Shin, M., Iacono, L., 2002. Reinventing the wheel of cyclic AMP: novel mechanisms of cAMP signaling. Ann. N. Y. Acad. Sci. 968, 49–64.

Daaka, Y., Luttrell, L.M., Lefkowitz, R.J., 1997. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature 390, 88–91.

Della Rocca, G.J., van Biesen, T., Daaka, Y., Luttrell, D.K., Luttrell, L.M., Lefkowitz, R.J., 1997. Ras-dependent mitogen-activated protein kinase activation by G protein-coupled receptors. Convergence of Gi- and Gq-mediated pathways on calcium/calmodulin, Pyk2, and Src kinase. J. Biol. Chem. 272, 19125–19132.

Fields, T.A., Casey, P.J., 1997. Signalling functions and biochemical properties of pertussis toxin-resistant G-proteins. Biochem. J. 321, 561–571.

Francis, S.H., Turko, I.V., Corbin, J.D., 2000. Cyclic nucleotide phosphodiesterases: relating structure and function. Prog. Nucleic Acid Res. Mol. Biol. 65, 1–52.

Gutkind, J.S., 1998. The pathways connecting G protein-coupled receptors to the nucleus through divergent mitogen-activated protein kinase cascades. J. Biol. Chem. 273, 1839–1842.

Hanoune, J., Defer, N., 2001. Regulation and role of adenylyl cyclase isoforms. Annu. Rev. Pharmacol. Toxicol. 41, 145–174.

He, W., Danilova, V., Zou, S., Hellekant, G., Max, M., Margolskee, R.F., Damak, S., 2002. Partial rescue of taste responses of alpha-gustducin null mice by transgenic expression of alpha-transducin. Chem. Senses 27, 719–727.

Hoon, M.A., Adler, E., Lindemeier, J., Battey, J.F., Ryba, N.J., Zuker, C.S., 1999. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell 96, 541–551.

Katan, M., 1998. Families of phosphoinositide-specific phospholipase C: structure and function. Biochim. Biophys. Acta 1436, 5–17.

Kim, U.K., Jorgenson, E., Coon, H., Leppert, M., Risch, N., Drayna, D., 2003. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science 299, 1221–1225.

Kusakabe, Y., Yasuoka, A., Asano-Miyoshi, M., Iwabuchi, K., Matsumoto, I., Arai, S., Emori, Y., Abe, K., 2000. Comprehensive study on G protein alpha-subunits in taste bud cells, with special reference to the occurrence of Galphai2 as a major Galpha species. Chem. Senses 25, 525–531.

Li, Z., Jiang, H., Xie, W., Zhang, Z., Smrcka, A.V., Wu, D., 2000. Roles of PLC-beta2 and -beta3 and PI3Kgamma in chemoattractant-mediated signal transduction. Science 287, 1046–1049.

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., Adler, E., 2002. Human receptors for sweet and umami taste. Proc. Natl. Acad. Sci. U. S. A. 99, 4692–4696.

Liebmann, C., 2001. Regulation of MAP kinase activity by peptide receptor signalling pathway: paradigms of multiplicity. Cell. Signal. 13, 777–785.

Lopez-Ilasaca, M., Crespo, P., Pellici, P.G., Gutkind, J.S., Wetzker, R., 1997. Linkage of G protein-coupled receptors to the MAPK signaling pathway through PI 3-kinase gamma. Science 275, 394–397.

Lush, I.E., Holland, G., 1988. The genetics of tasting in mice: V. Glycine and cycloheximide. Genet. Res. 52, 207–212.

Margolskee, R.F., 2002. Molecular mechanisms of bitter and sweet taste transduction. J. Biol. Chem. 277, 1–4.

Matsunami, H., Montmayeur, J.P., Buck, L.B., 2000. A family of candidate taste receptors in human and mouse. Nature 404, 601–604.

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

Montmayeur, J.P., Matsunami, H., 2002. Receptors for bitter and sweet taste. Curr. Opin. Neurobiol. 12, 366–371.

Murga, C., Fukuhara, S., Gutkind, J.S., 2000. A novel role for phosphatidylinositol 3-kinase beta in signaling from G protein-coupled receptors to Akt. J. Biol. Chem. 275, 12069–12073.

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Ryba, N.J., Zuker, C.S., 2001. Mammalian sweet taste receptors. Cell 106, 381–390.

Nelson, G., Chandrashekar, J., Hoon, M.A., Feng, L., Zhao, G., Ryba, N.J., Zuker, C.S., 2002. An amino-acid taste receptor. Nature 416, 199–202.

Rhee, S.G., Bae, Y.S., 1997. Regulation of phosphoinositide-specific phospholipase C isozymes. J. Biol. Chem. 272, 15045–15048.

Ruiz-Avila, L., Wong, G.T., Damak, S., Margolskee, R.F., 2001. Dominant loss of responsiveness to sweet and bitter compounds caused by a single mutation in alpha-gustducin. Proc. Natl. Acad. Sci. U. S. A. 98, 8868–8873.

Schulte, G., Fredholm, B.B., 2002. Signaling pathway from the human adenosine A(3) receptor expressed in Chinese hamster ovary cells to the extracellular signal-regulated kinase 1/2. Mol. Pharmacol. 62, 1137–1146.

Smrcka, A.V., Sternweis, P.C., 1993. Regulation of purified subtypes of phosphatidylinositol-specific phospholipase C beta by G protein alpha and beta gamma subunits. J. Biol. Chem. 268, 9667–9674.

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

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

Wu, D., Katz, A., Simon, M.I., 1993. Activation of phospholipase C beta 2 by the alpha and beta gamma subunits of trimeric GTP-binding protein. Proc. Natl. Acad. Sci. U. S. A. 90, 5297–5301.

Yamaguchi, S., 1991. Basic properties of umami and effects on humans. Physiol. Behav. 49, 833–841.

Yan, W., Sunavala, G., Rosenzweig, S., Dasso, M., Brand, J.G., Spielman, A.I., 2001. Bitter taste transduced by PLC-beta(2)-dependent rise in IP(3) and alpha-gustducin-dependent fall in cyclic nucleotides. Am. J. Physiol., Cell Physiol. 280, C742–C751.

Zhang, Y., Hoon, M.A., Chandrashekar, J., Mueller, K.L., Cook, B., Wu, D., Zuker, C.S., Ryba, N.J., 2003. Coding of sweet, bitter, and umami tastes. Different receptor cells sharing similar signaling pathways. Cell 112, 293–301.

Zhao, G.Q., Zhang, Y., Hoon, M.A., Chandrashekar, J., Erlenbach, I., Ryba, N.J., Zuker, C.S., 2003. The receptors for mammalian sweet and umami taste. Cell 115, 255–266.