Cellular/Molecular

Umami Taste Responses Are Mediated by α-Transducin and α-Gustducin

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The sense of taste comprises at least five distinct qualities: sweet, bitter, sour, salty, and *umami*, the taste of glutamate. For bitter, sweet, and *umami* compounds, taste signaling is initiated by binding of tastants to G-protein-coupled receptors in specialized epithelial cells located in the taste buds, leading to the activation of signal transduction cascades. α-Gustducin, a taste cell-expressed G-protein α subunit closely related to the α-transducins, is a key mediator of sweet and bitter tastes. α-Gustducin knock-out (KO) mice have greatly diminished, but not entirely abolished, responses to many bitter and sweet compounds. We set out to determine whether α-gustducin also mediates *umami* taste and whether rod α-transducin ($\alpha_{t\text{-}rod}$), which is also expressed in taste receptor cells, plays a role in any of the taste responses that remain in α-gustducin KO mice. Behavioral tests and taste nerve recordings of single and double KO mice lacking α-gustducin and/or $\alpha_{t\text{-}rod}$ confirmed the involvement of α-gustducin in bitter (quinine and denatonium) and sweet (sucrose and SC45647) taste and demonstrated the involvement of α-gustducin in *umami* [monosodium glutamate (MSG), monopotassium glutamate (MPG), and inosine monophosphate (IMP)] taste as well. We found that $\alpha_{t\text{-}rod}$ played no role in taste responses to the salty, bitter, and sweet compounds tested or to IMP but was involved in the *umami* taste of MSG and MPG. *Umami* detection involving α-gustducin and $\alpha_{t\text{-}rod}$ occurs in anteriorly placed taste buds, however taste cells at the back of the tongue respond to *umami* compounds independently of these two G-protein subunits.

Key words: **taste; *umami*; knock-out mice; gustducin; transducin; signal transduction**

Introduction

Bitter, sweet, salty, and sour are four widely accepted basic taste qualities (for review, see Lindemann, 1996). *Umami* (a Japanese word meaning delicious) taste is elicited by glutamate, aspartate, some peptides, derivatives of ribonucleotides such as inosine monophosphate (IMP) and GMP, and the metabotropic glutamate receptor agonist L-AP-4 (Sato et al., 1970; Maga, 1983; Monastyrskaia et al., 1999; Stapleton et al., 1999). Many investigators consider *umami* as a unique fifth taste quality, based on psychophysical experiments in humans, conditioned taste aversion tests, and genetic studies in mice, which indicate that *umami* is distinct from sweet, salty, or other taste qualities (Yoshida and Saito, 1969; Ohara et al., 1979; Ninomiya and Funakoshi, 1989a; Bachmanov et al., 2000). However, others argue that *umami* is not unique because in rats, conditioned taste aversion to monosodium glutamate (MSG) generalizes to NaCl or sucrose in the absence or presence of amiloride, respectively (Yamamoto et al., 1985, 1991; Stapleton et al., 1999).

Taste responses to bitter, sweet, and *umami* compounds are initiated by G-protein-coupled receptors (GPCRs) and transduced via G-protein signaling cascades (for review, see Chaudhari and Roper, 1998; Gilbertson et al., 2000; Lindemann, 2001). During the past few years, several GPCRs have been identified in taste cells and implicated in taste signal transduction (Adler et al., 2000; Chandrashekar et al., 2000; Chaudhari et al., 2000; Max et al., 2001; Nelson et al., 2001, 2002; Li et al., 2002). T1r3 plus T1r1 and a truncated type 4 metabotropic glutamate receptor missing most of the N-terminal extracellular domain (taste mGluR4) have been implicated in the transduction of *umami* signals in taste receptor cells (TRCs) (Chaudhari et al., 2000; Li et al., 2002; Nelson et al., 2002). T1r1 and T1r3 are coexpressed in taste buds in the anterior part of the tongue (Nelson et al., 2001). Taste mGluR4 is expressed in taste buds of circumvallate and foliate papillae (Yang et al., 1999). Human embryonic kidney (HEK) 293 cells heterologously expressing T1r1 plus T1r3 and a promiscuous G-protein responded to glutamate, and this response was potentiated by IMP (Adler et al., 2000; Li et al., 2002; Nelson et al., 2002). At concentrations of MSG and L-AP-4 similar to those that produce the *umami* sensa-

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tion in humans, Chinese hamster ovary cells expressing taste mGluR4 responded by lowering cellular cAMP concentrations (Chaudhari et al., 2000). The G-proteins that couple these potential *umami* receptors to intracellular signaling pathways have not been identified.

Among the G-protein α subunits known to be selectively expressed in TRCs is α-gustducin (McLaughlin et al., 1992). α-Gustducin shares 80% identity with cone and rod α-transducins (McLaughlin et al., 1992). α-Gustducin knockout (KO) mice showed strongly reduced, but not completely abolished, behavioral and nerve responses to several bitter and sweet compounds (Wong et al., 1996), indicating that α-gustducin plays a key role in the transduction of these tastants. Rod α-transducin ($\alpha_{t\text{-}rod}$) also is expressed in TRCs, albeit at much lower levels than is α-gustducin (Ruiz-Avila et al., 1995; Yang et al., 1999). *In vitro*, $\alpha_{t\text{-}rod}$, like α-gustducin, binds Gγ13, can be activated by bitter-responsive taste receptors, and can activate taste-expressed phosphodiesterase isoforms (Ruiz-Avila et al., 1995). α-Gustducin null mice expressing $\alpha_{t\text{-}rod}$ as a transgene driven by the α-gustducin promoter partially recovered responses to sweet and bitter compounds, indicating that the functions of these two G-protein subunits in taste may overlap (He et al., 2002). Despite the presence of $\alpha_{t\text{-}rod}$ in taste cells and its biochemical similarity to α-gustducin, it has never been shown directly that endogenous $\alpha_{t\text{-}rod}$ plays a role in taste. To determine the role of $\alpha_{t\text{-}rod}$ *in vivo* in taste responses, we performed behavioral and electrophysiological tests with KO mice lacking α-gustducin and/or $\alpha_{t\text{-}rod}$. We determined that $\alpha_{t\text{-}rod}$ is not involved in responses to bitter, salty, or sweet compounds but is involved in responses to two *umami* compounds [MSG and monopotassium glutamate (MPG)]. We determined also that α-gustducin is a key mediator of *umami* taste responses.

Materials and Methods

KO mice. The design and production of α-gustducin and $\alpha_{t\text{-}rod}$ KO mice have been described (Wong et al., 1996; Calvert et al., 2000). Double KOs (gus−/− trans−/−), $\alpha_{t\text{-}rod}$ KOs (trans−/−), α-gustducin KOs (gus−/−), and doubly heterozygous wild-type (WT) littermate controls (gus+/− trans+/−) were bred by crossing α-gustducin KO mice in a 129S1/SvImJ background with $\alpha_{t\text{-}rod}$ KO mice in a 50% BALB/c 50% 129S1/SvImJ background. The resulting gus+/− trans+/− G1 offspring were intercrossed. Gus−/− trans+/− and gus+/− trans−/− G2 offspring were also intercrossed. G2 and G3 single and double KOs and gus+/− trans+/− (WT) littermate controls were used for behavioral and electrophysiological studies (we see no difference between gus+/+ and gus+/− in their behavioral responses to several sweet and bitter compounds) (Wong et al., 1996; Ruiz-Avila et al., 2001; our unpublished results).

Genotyping of mice was performed with PCR of tail DNA. The primers used were (5′to 3′) GAGCAAATCAACTGCCCAGC and CCAACTCTGCCAGCTTGTTCC, specific for the region deleted in α-gustducin KO mice; TGCCTGTTGTAGCGAGCACCG and GCCAAGCTCTTCAGCAATATCAC, specific for a sequence immediately upstream from the region deleted in the α-gustducin KO mice and for neo, respectively; CTTGAAGGAGAATTGAGTCTCGA and CTCGAGTTCATTGCCATCATCTA, specific for the region deleted in $\alpha_{t\text{-}rod}$ KO mice; and TGAGTGTTCCCTGCCCATC and GCTGTCCATCTGCACGAGAC, specific for a sequence immediately upstream from the region deleted in the $\alpha_{t\text{-}rod}$ KO mice and for neo, respectively.

Two-bottle preference tests. Male mice were caged individually and given access to food *ad libitum*. They were presented for 48 hr with two 25 ml bottles, one containing water and the other a tastant solution. The bottles were switched after 24 hr to account for position preference. The tastant solutions were presented at ascending concentrations. Where indicated, MSG and IMP (sodium salt) solutions contained 10 μM amiloride to reduce the taste of sodium. Between tastant trials, the mice were kept on water for 7 d. The volume of liquid consumed was recorded, and a ratio of tastant to total fluid consumed was determined. The data were analyzed using the general linear model repeated measures of the statistics package SPSS with concentrations as dependant variables and genotype as a fixed factor. When a statistically significant difference among the means was found, the Tukey's test was used to determine which means differed. For additional details of the two-bottle preference tests, see the report by Wong et al. (1996).

Nerve recordings. Recordings from the chorda tympani (CT) and the glossopharyngeal nerves (NGs) were performed as described previously (Kawai et al., 2000). Tastants were applied to the tongue for 30 sec (CT) or 60 sec (NG) at a regular flow rate. Integrated whole-nerve response magnitudes (time constant, 1 sec) were measured 5, 10, 15, 20, and 25 sec (for the CT) and 5, 10, 20, 30, and 40 sec (for the NG) after stimulus onset and averaged. These averages were normalized to the responses to NH_4Cl and analyzed with the general linear model multiple measures of SPSS with concentrations as within-subjects variables and genotypes as between-subjects factors.

Results

In comparison with WT mice, α-gustducin KO mice have diminished, but not abolished, responses to bitter and sweet compounds (Wong et al., 1996). Furthermore, expression of a dominant-negative form of α-gustducin from the α-gustducin promoter in α-gustducin KO mice further reduces their responses to bitter and sweet compounds, indicating that other taste-expressed G-proteins couple with bitter and sweet-responsive taste receptors (Ruiz-Avila et al., 2001).

α-Gustducin, but not $\alpha_{t\text{-}rod}$, mediates bitter and sweet taste

To determine whether the residual responses of the α-gustducin KO mice are mediated by $\alpha_{t\text{-}rod}$, we performed two-bottle preference tests with two bitter compounds, denatonium benzoate and quinine sulfate, and two sweet compounds, sucrose and the artificial sweetener SC45647 (Fig. 1). We compared responses of α-gustducin KO (gus−/−), $\alpha_{t\text{-}rod}$ KO (trans−/−), double KO (gus−/− trans−/−), and doubly heterozygous littermate WT control (gus+/− trans+/−) mice. The responses of the gus−/− single KO mice to all four compounds tested were diminished compared with control mice, confirming previous results (Wong et al., 1996). With these four compounds, we found no significant difference between the responses of gus−/− trans−/− double KO and gus−/− single KO mice, or between those of trans−/− single KO mice and WT control mice. Thus, $\alpha_{t\text{-}rod}$ does not play a role in the taste responses to these sweet and bitter compounds.

α-Gustducin and $\alpha_{t\text{-}rod}$ mediate *umami* taste

Because *umami* taste seems to involve GPCRs, but the G-protein involved is unknown, we set out to determine whether α-gustducin and/or $\alpha_{t\text{-}rod}$ might be involved in *umami* taste signal transduction. We performed behavioral tests comparing each single KO, the double KO, and WT littermate controls (Fig. 2). Forty-eight-hour two-bottle preference tests showed that the WT mice preferred MSG at concentrations between 10 and 300 mM and avoided it at 1000 mM (Fig. 2*a*). The gus−/− mice showed less preference for MSG than did the control mice ($p < 0.05$), whereas gus−/− trans−/− double KO mice were indifferent to the concentrations between 10 and 300 mM preferred by WT and gus−/− mice ($p < 0.05$, comparing gus−/− with gus−/− trans−/− for these concentrations). There was no difference between trans−/− and WT controls and no difference between all four groups of mice at the aversive concentration (1000 mM). These data demonstrate that both $\alpha_{t\text{-}rod}$ and α-gustducin are involved in the taste of and preference for MSG. α-Gustducin plays a more prominent role in these behavioral re-

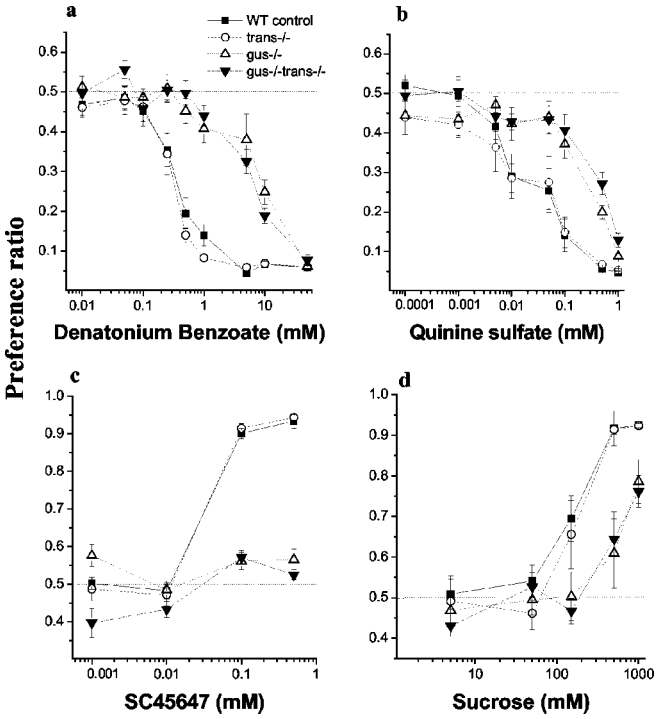

Figure 1. Mean preference ratios for bitter (denatonium benzoate and quinine sulfate) and sweet (SC45647 and sucrose) compounds from 48 hr two-bottle preference tests (tastant vs water). Four strains were compared: gus−/− single KO (△; $n = 8$), trans−/− single KO (○; $n = 12$), gus−/− trans −/− double KO (▼; $n = 11$), and doubly heterozygous littermates (WT controls; ■; $n = 10$). gus−/− single KO and gus−/− trans −/− double KO mice showed diminished response for all four compounds; trans−/− single KO mice did not differ from WT littermate controls for any of these compounds. Error bars are SEM.

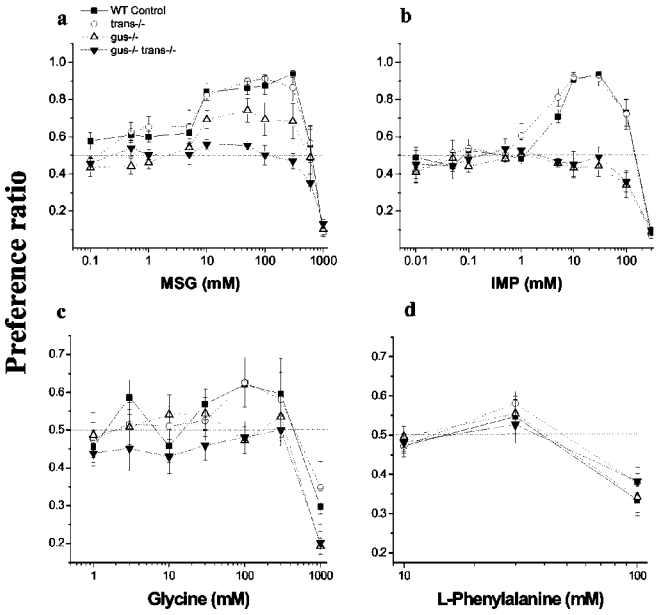

Figure 2. Mean preference ratios for two *umami* compounds (MSG and IMP) and two non-*umami* amino acids (L-phenylalanine and glycine) from 48 hr two-bottle preference tests (tastant vs water). The same four strains were analyzed as in Figure 1 ($n = 8$–12). gus−/− single KO mice showed diminished preference for MSG and no preference for IMP; gus−/− trans−/− double KO mice showed no preference for MSG or IMP; trans−/− single KO mice did not differ from WT littermate controls in their responses to MSG, IMP, L-phenylalanine, or glycine. MSG and IMP (sodium salt) solutions contained 10 μm amiloride to reduce the taste of sodium. Error bars are SEM.

sponses to MSG, whereas the involvement of $\alpha_{t\text{-}rod}$ is only apparent in the absence of α-gustducin.

IMP, another *umami* compound, was also tested (Fig. 2*b*). gus−/− and gus−/− trans−/− mice showed no preference for IMP concentrations between 5 and 100 mM, whereas controls and trans−/− mice strongly preferred IMP in this range. IMP (300 mM) elicited strong aversion in all four groups of mice, with no difference between gus−/− and gus−/− trans−/− or between WT controls and trans−/− mice. Thus, α-gustducin, but not $\alpha_{t\text{-}rod}$, is required for IMP preference. However, neither of these G-protein α subunits affects avoidance of IMP.

To determine whether α-gustducin and $\alpha_{t\text{-}rod}$ are involved in taste responses to amino acids in general, we performed two-bottle preference tests with glycine, D-tryptophan, L-phenylalanine, and L-proline. There was no difference in the response to glycine between trans−/− and the WT control or between gus−/− trans−/− and gus−/− at any of the concentrations tested (Fig. 2*c*). When we grouped the mice into gus−/− and gus+/− groups, regardless of the status of the $\alpha_{t\text{-}rod}$ locus, we found a significant difference between the two groups in their responses to 100 and 1000 mM glycine ($p < 0.005$), consistent with the preference for this sweet amino acid being mediated, at least in part, by α-gustducin but not by $\alpha_{t\text{-}rod}$ (data not shown). With the three other amino acids tested, there were no significant differences between the four groups of mice in their preference responses (Fig. 2*d*, L-phenylalanine; data not shown for L-proline and D-tryptophan).

α-Gustducin and $\alpha_{t\text{-}rod}$ mediate *umami* signals at the front of the tongue

One limit of the two-bottle preference test is that the behavioral response of mice integrates peripheral, central, and post-ingestive effects of the compounds tested. To determine whether the differences in behavioral responses to *umami* compounds between genotypes were caused by peripheral factors, we recorded from the taste nerves. In mice, both the CT and the NG respond to *umami* compounds (Ninomiya and Funakoshi, 1989a,b). To also determine whether there are regional differences in taste responses to umami compounds mediated by $\alpha_{t\text{-}rod}$ and α-gustducin, we performed whole-nerve recordings from the CT, which innervates taste buds in the anterior part of the tongue, and from the NG, which innervates taste buds in the posterior part of the tongue (Figs. 3, 4). In comparison with the WT controls, we found that the gus−/− trans−/− double KO mice had markedly diminished CT responses to MSG (with or without 10 μM amiloride; $p < 0.05$ and $p < 0.001$, respectively), to MSG plus 0.5 mM IMP ($p < 0.05$), to MPG ($p < 0.001$), and to MPG plus IMP ($p < 0.005$) (Figs. 3*a–d*, 4*a*,*d*). The responses of either of the single KOs were significantly stronger than the responses of the double KOs to MSG (with or without amiloride; $p < 0.05$) (Fig. 3*a*,*b*) and to MPG ($p < 0.05$) (Fig. 3*c*). The responses of trans−/− mice were stronger than those of double KO mice to MPG plus IMP ($p < 0.05$) (Fig. 3*d*). The responses of the gus−/− mice were significantly weaker than those of WT control mice for MPG plus IMP ($p < 0.005$) (Fig. 3*d*) and for 30 mM MSG without amiloride ($p < 0.05$) (Fig. 3*a*). The responses of trans−/− mice to 1000 mM MSG were significantly weaker than those of the WT controls (Fig. 3*a*). For other concentrations of *umami* compounds, when the differences were not statistically significant, there was a trend for the responses of either single KO to be weaker than those of the WT control mice, with the difference more marked for the gus−/− mice (Fig. 3*a–d*). These data implicate both $\alpha_{t\text{-}rod}$ and α-gustducin in anterior tongue responses to MSG and MPG. In contrast to the CT data,

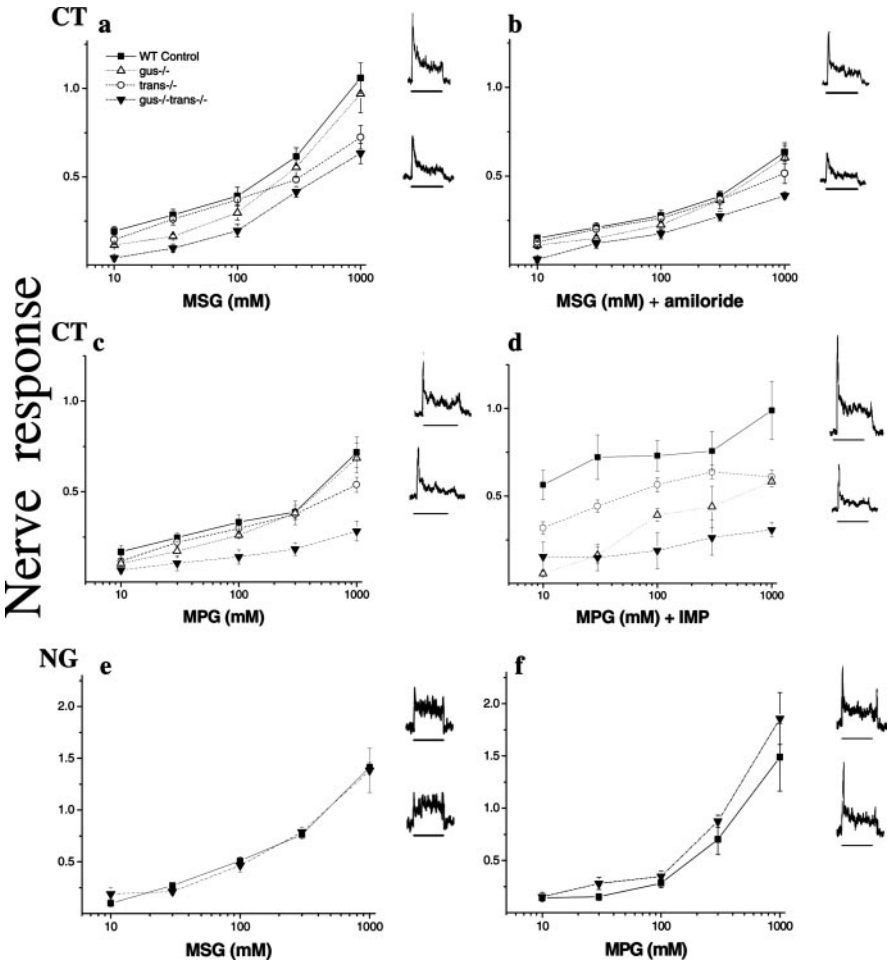

Figure 3. Whole-nerve recordings from CT and NG taste nerves stimulated by lingual application of taste stimuli. Integrated responses to glutamate by the CT nerve (*a–d*) and NG nerve (*e*, *f*) were collected from the same four strains as in Figure 1. MSG alone (*a*, *e*), MSG plus 10 μM amiloride (to reduce sodium taste; *b*), MPG alone (*c*, *f*), and MPG plus 0.5 mM IMP (to potentiate *umami*; *d*) were tested. Nerve responses were normalized to responses to NH_4Cl. For every compound, each genotype group included six to eight mice, except for MPG plus IMP ($n = 3–5$). All concentrations of a given compound were tested on the same mice. The mice were typically tested with several, but not all, compounds. Error bars are SEM. Typical recording traces are shown in the inset beside each graph for WT control (top) and gus−/− trans−/− (bottom) mice. The lines below the traces represent the duration of tastant application (30 sec for CT, 60 sec for NG).

there was no difference between double KOs and WT controls in their NG responses to MSG or MPG (Fig. 3*e*,*f*), indicating that neither $\alpha_{t\text{-rod}}$ nor α-gustducin are involved in posterior tongue responses to these *umami* compounds.

A particular property of *umami* taste is that the response to glutamate is potentiated by IMP in the anterior part of the tongue (Sato et al., 1970; Yamamoto et al., 1991). To examine the potential involvement of $\alpha_{t\text{-rod}}$ and α-gustducin in IMP potentiation, we examined the CT responses of WT, single, and double KO mice. IMP potentiated the CT responses to MSG and MPG in the WT control and trans−/− mice ($p < 0.05$, compared with mice of the given genotype with MSG or MPG alone) (Figs. 3*c*,*d*, 4*a*,*b*), indicating that $\alpha_{t\text{-rod}}$ does not affect IMP potentiation of MSG. IMP did not potentiate the CT responses to MSG and MPG of α-gustducin single KO or gus−/− trans−/− double KO mice (Fig. 4*c*,*d*), indicating that IMP potentiation requires α-gustducin.

α-Gustducin and $\alpha_{t\text{-rod}}$ do not mediate NaCl taste

Both IMP and MSG are sodium salts. To determine the contribution of the Na^+ ion to the responses of the mice to these umami compounds, we performed two-bottle preference tests and nerve recordings with NaCl in the presence or absence of amiloride (Fig. 5). Statistical analysis of the behavioral responses of the four groups of mice to NaCl indicates that they do not differ ($0.46 < p < 0.99$, comparing any two of the four genotypes) (Fig. 5*a*). Likewise, the behavioral responses of the four groups of mice to NaCl plus amiloride were indistinguishable ($0.48 < p < 1$, comparing any two of the four genotypes) (Fig. 5*b*). The CT and NG nerve responses to NaCl of gus−/− trans−/− double KO mice were indistinguishable from those of WT controls ($p = 0.40$ and $p = 0.61$, respectively) (Fig. 5*c*,*e*). The CT and NG nerve responses to NaCl plus amiloride of gus−/− trans−/− double KO mice were indistinguishable from those of WT controls ($p = 0.29$ and $p = 0.51$, respectively) (Fig. 5*d*,*f*). We had previously shown that there are no statistically significant differences in WT versus gus KO mice in behavioral or CT nerve responses to NaCl (Wong et al., 1996). Amiloride decreased the CT response to NaCl of both types of mice (Fig. 5, compare *c*, *d*) but had no effect on NG responses to NaCl (Fig. 5, compare *e*, *f*).

Discussion

α-Gustducin, $\alpha_{t\text{-rod}}$, and $\alpha_{t\text{-cone}}$ are three closely related G-protein α subunits, each of which is expressed in TRCs (McLaughlin et al., 1992; Ruiz-Avila et al., 1995). The role of α-gustducin in taste transduction of bitter and sweet is well known (Wong et al., 1996) and was recently extended to *umami* taste (Ruiz et al., 2003). Although the roles of $\alpha_{t\text{-rod}}$ and $\alpha_{t\text{-cone}}$ in retinal phototransduction have been studied intensively for decades, nothing was known of their involvement in taste responses *in vivo*. That $\alpha_{t\text{-rod}}$ and α-gustducin show identical biochemical properties *in vitro* (Ruiz-Avila et al., 1995) and that transgenic expression of $\alpha_{t\text{-rod}}$ from the α-gustducin promoter partially restores function in α-gustducin KO mice (He et al., 2002) suggests that endogenous $\alpha_{t\text{-rod}}$ may function *in vivo* in taste very much like α-gustducin does. Our findings that $\alpha_{t\text{-rod}}$/α-gustducin double KO mice have reduced behavioral and electrophysiological responses to glutamate compared with α-gustducin single KO mice demonstrates that $\alpha_{t\text{-rod}}$ is indeed involved in taste, specifically in *umami* taste. Additional support for the involvement of α-gustducin and $\alpha_{t\text{-rod}}$ in *umami* taste comes from our observation that CT responses to *umami* compounds of $\alpha_{t\text{-rod}}$ KO and α-gustducin KO mice were similarly reduced versus those of WT, but not as much as were the CT responses in the $\alpha_{t\text{-rod}}$/α-gustducin double KO mice. Unlike α-gustducin, however, $\alpha_{t\text{-rod}}$ apparently plays no role in bitter or sweet. Furthermore, $\alpha_{t\text{-rod}}$ plays a lesser role in *umami* than does α-gustducin based on the following two observations. First, behavioral responses to MSG of α-gustducin KO mice were very much diminished versus those of WT mice, whereas the behav-

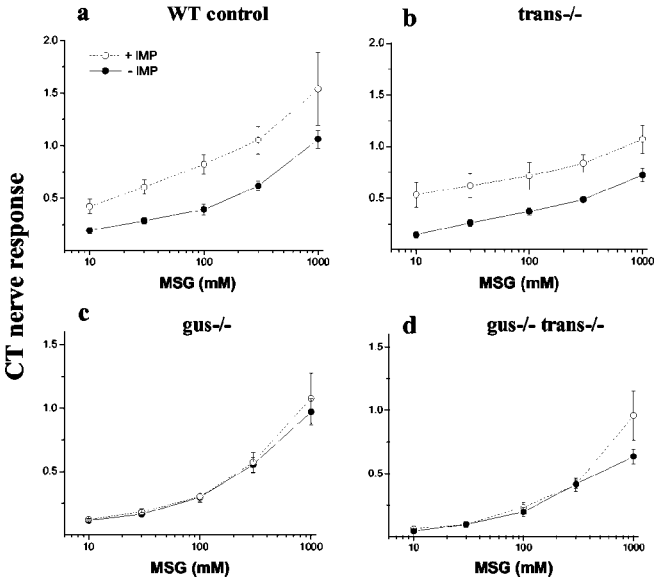

Figure 4. Integrated CT responses to MSG (●) and MSG plus 0.5 mM IMP (○). CT recordings were from the same four strains as in Figure 1: WT control (*a*), trans−/− (*b*), gue−/− (*c*), and gus −/− trans−/− double KO (*d*). Nerve responses were normalized to responses to NH_4C1. For every compound, each genotype group included 5–10 mice. All concentrations of a given compound were tested on the same mice. Most mice were tested with both MSG and MSG plaus IMP. MSG and MSG plus IMP solutions contained 10 μM amiloride. Error bars are SEM.

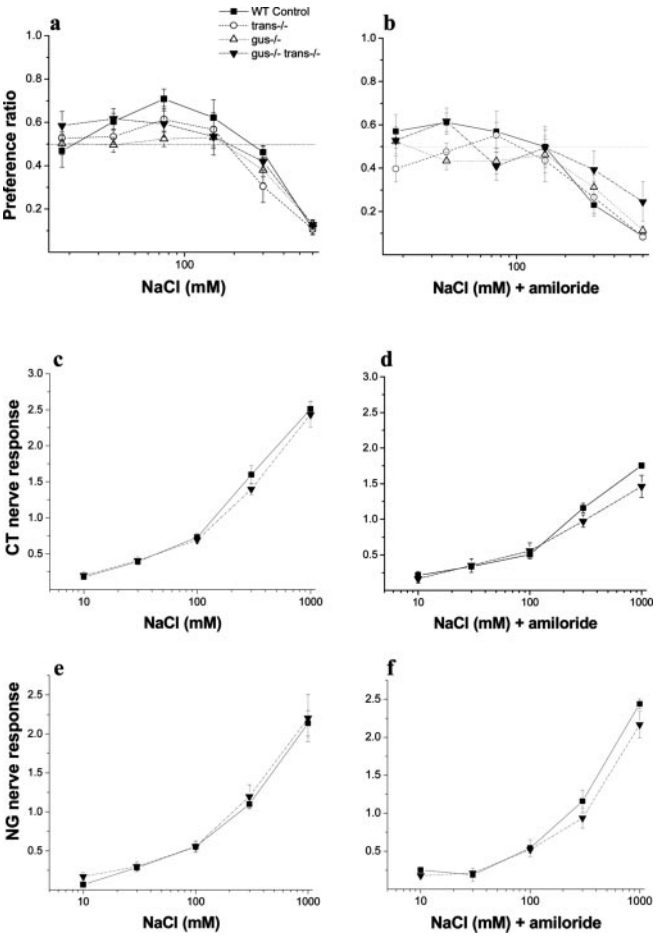

Figure 5. Mean preference ratios for NaCl (with and without 10 μM amiloride) from 48 hr two-bottle preference tests (tastant vs water; *a*, *b*) and whole-nerve recordings from CT (*c*, *d*) and NG (*e*, *f*) taste nerves stimulated by lingual application of NaCl. For behavioral tests, each genotype group included 9–12 animals. For nerve recordings, each genotype group included six to nine animals. Error bars are SEM. No difference was found between the responses of mice from the four genotypes (behavioral tests) or between those of WT controls and double KOs (nerve recordings).

ioral responses of $\alpha_{t\text{-}rod}$ KO mice to MSG were indistinguishable from those of WT mice. Second, α-gustducin KO mice, but not $\alpha_{t\text{-}rod}$ KO mice, showed decreased behavioral responses to IMP. Nevertheless, it is clear that $\alpha_{t\text{-}rod}$ acts in *umami* as can be seen when comparing responses to MSG of $\alpha_{t\text{-}rod}$/α-gustducin double KO mice versus those of α-gustducin single KO mice: $\alpha_{t\text{-}rod}$ may serve as a "backup" for α-gustducin in mediating MSG signals.

In general, our behavioral and electrophysiological data were qualitatively consistent, however an apparent discordance was the observation that the double KO mice showed residual CT responses to concentrations of MSG between 30 and 300 mM but were behaviorally indifferent in this range. One explanation could be that the electrophysiological signals are below the threshold needed to elicit a behavioral response. Alternatively, at these concentrations of MSG, the taste nerves of the double KO mice carry a mixture of signals, of which some lead to aversion, others to preference, with the net being indifference. This may also underlie the indifference of WT mice to 600 mM MSG, in which the mice go from preference for 300 mM to avoidance of 1000 mM: 600 mM may be the concentration at which avoidance and preference signals cancel each other. We chose the two-bottle preference test over short access tests because it is very robust and can detect subtle differences that may be missed by the short access test. Nevertheless, it is possible that some differences between behavior and nerve responses are attributable to post-ingestive effects.

Umami is not universally accepted as a unique taste quality. Some argue, based mainly on behavioral data from rats (Yamamoto et al., 1985, 1991; Stapleton et al., 1999), that the taste of MSG is a combination of salty and sweet taste. Although we included amiloride when the mice were tested with MSG and/or IMP, the responses obtained may include a sodium component that is insensitive to amiloride. In most mammals, including mice, there is a residual taste response to NaCl in the presence of amiloride that is believed to be mediated by a vanilloid receptor-1 variant (Lyall et al., 2004). The differences in behavioral and electrophysiological responses to glutamate between our four groups of mice cannot be accounted for merely by the amiloride-sensitive or -insensitive taste of Na^+ because all four groups showed similar responses to NaCl (and to NaCl with amiloride). Furthermore, the α-gustducin KO and double KO mice responded differently to MSG but identically to IMP (another sodium salt). That α-gustducin KO and the double KO mice responded identically to NaCl and to the sweet compounds sucrose and SC45647, but differently to MSG and MPG, argues in favor of the uniqueness of the taste of glutamate, at least in mice. This is consistent with genetic studies in mice that showed that the strain differences in MSG acceptance were not related to the strain differences in salt or sweet preference (Bachmanov et al., 2000).

Previous behavioral (Ninomiya and Funakoshi, 1989a; Sako et al., 2000) and electrophysiological (Ninomiya and Funakoshi, 1989a,b; Sako et al., 2000) studies suggest that there are at least two *umami* response mechanisms. Nerve recordings in mice and rats showed that *umami* signals are carried by the CT, NG, and greater superficial petrosal (GSP) nerves (Ninomiya and Funakoshi, 1989a,b; Sako et al., 2000). In mice, both the CT and NG

nerves responded to *umami* compounds; bilateral sectioning of both NG nerves abolished the ability of mice to discriminate between MSG and NaCl (Ninomiya and Funakoshi, 1989a). This result suggests that in mice the NG carries the *umami* signals that can be discriminated from NaCl and other taste stimuli. The predominance of the NG in some species in carrying *umami*-specific signals is also suggested by the presence of MSG-best fibers in the NG of mice and rhesus monkeys (Ninomiya and Funakoshi, 1989b; Hellekant et al., 1997), and by psychophysical studies in humans that have shown that the back of the tongue is more sensitive to *umami* substances than is the front (Yamaguchi, 1998). In rats, however, the CT and GSP nerves were most responsive to mixtures of IMP and MSG, whereas the NG responded only minimally to these compounds (Sako et al., 2000). When both the CT and GSP nerves were resected, the rats were no longer able to acquire a conditioned taste aversion to *umami* compounds (Sako et al., 2000). Rats could not discriminate MSG plus amiloride from sucrose (Yamamoto et al., 1991; Stapleton et al., 1999). Gurmarin, a sweet response inhibitor, reduced the response to mixtures of MSG and IMP in C57BL mice of the CT, but not of the NG. Gurmarin did not affect the response of either nerve to L-AP-4 (Ninomiya et al., 2000). In summary, these data suggest that the signals elicited by MSG in the anterior part of the tongue are not *umami* specific and may be similar to those elicited by sweet compounds.

Recent biochemical data also argue for multiple response mechanisms to *umami*. TRCs from different parts of the tongue were shown to elicit different second messenger changes in response to MSG stimulation. *Ex vivo* stimulation with MSG and/or IMP of fungiform papillae from C57BL mice increased the concentrations of both cAMP and IP_3 (Ninomiya et al., 2000), suggesting that downstream effectors may be adenylyl cyclase and phospholipase C, as has been inferred for sweet compounds (Bernhardt et al., 1996). In contrast, rat circumvallate papillae taste tissues responded to MSG and L-AP-4 by decreasing cAMP (Abaffy et al., 2003), as is the case with bitter compounds (Lindemann, 1996; Yan et al., 2001).

Our results also suggest dual mechanisms underlying *umami* taste responses. Knocking out both α-gustducin and $\alpha_{t\text{-}rod}$ in mice led to a reduction in their CT responses to glutamate and abolished their preference for MSG but did not affect their NG response to glutamate, nor their aversion to high concentrations of MSG. Thus, α-gustducin and $\alpha_{t\text{-}rod}$ contribute to the preference for MSG dependent on taste cells at the front of the tongue but do not play a role in the aversive response to MSG that depends on taste cells at the back of the tongue. Furthermore, the response to IMP occurs only in the anterior part of the tongue and only if α-gustducin is present, suggesting that the *umami* taste of IMP is mediated by only one of the mechanisms.

Based on the following *in vitro* and *in vivo* studies, it seems likely that α-gustducin and $\alpha_{t\text{-}rod}$ are involved in the transduction of *umami* taste. First, HEK cells heterologously expressing T1r1 and T1r3 respond to *umami* compounds (Li et al., 2002; Nelson et al., 2002). KO mice lacking T1r1 and/or T1r3 have greatly reduced responses to *umami* compounds (Damak et al., 2003; Zhao et al., 2003). Together, these data argue that T1r1 plus T1r3 acts *in vivo* as an *umami*-responsive receptor. Second, in HEK cells, T1r receptors have been shown to couple best with chimeric G-proteins with C termini (the major determinant for G-protein receptor coupling) from gustducin, transducin, or Gi (Zhao et al., 2003; our unpublished results). *In vivo*, T1r1, T1r3, and α-gustducin have been found to be coexpressed in fungiform papillae TRCs (Max et al., 2001; Nelson et al., 2001; Kim et al., 2003; our unpublished results). These observations suggest that *in vivo*, gustducin (and possibly transducin and/or Gi) may couple with the *umami*-responsive T1r1 plus T1r3 taste receptor. The downstream second messengers are probably cAMP and IP_3, based on biochemical data (see above) and results from KO mice that show little or no response to umami compounds in the absence of Trpm5 or PLCβ2 (Zhang et al., 2003; our unpublished results). In the α-gustducin and α-transducin KOs, regulation of both of these second messengers is likely to be disrupted: directly, by the lack of α-gustducin and α-transducin regulation of phosphodiesterase (cAMP), and indirectly, by loss of heterotrimers and the disruption of βγ regulation of PLCβ2 (IP_3).

We propose that in the front of the tongue, glutamate activates the T1r1 plus T1r3 receptor, which couples with α-gustducin and/or $\alpha_{t\text{-}rod}$ to elicit preference for this compound. Glutamate responses from the back of the tongue may involve a different receptor (possibly taste mGluR4) and G-proteins other than $\alpha_{t\text{-}rod}$ and α-gustducin (possibly Gi).

References

Abaffy T, Trubey KR, 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 MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS (2000) A novel family of mammalian taste receptors. Cell 100:693–702.

Bachmanov AA, Tordoff MG, Beauchamp GK (2000) Intake of umami-tasting solutions by mice: a genetic analysis. J Nutr 130:935S–941S.

Bernhardt SJ, Naim M, Zehavi U, Lindemann B (1996) Changes in IP3 and cytosolic Ca2+ in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat. J Physiol (Lond) 490:325–336.

Calvert PD, Krasnoperova NV, Lyubarsky AL, Isayama T, Nicolo M, Kosaras B, Wong G, Gannon KS, Margolskee RF, Sidman RL, Pugh Jr EN, Makino CL, Lem J (2000) Phototransduction in transgenic mice after targeted deletion of the rod transducin alpha-subunit. Proc Natl Acad Sci USA 97:13913–13918.

Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ (2000) T2Rs function as bitter taste receptors. Cell 100:703–711.

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

Chaudhari N, Landin AM, Roper SD (2000) A metabotropic glutamate receptor variant functions as a taste receptor. Nat Neurosci 3:113–119.

Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Varadarajan V, Zou S, Jiang P, Ninomiya Y, Margolskee RF (2003) Detection of sweet and umami taste in the absence of taste receptor T1r3. Science 301:850–853.

Gilbertson TA, Damak S, Margolskee RF (2000) The molecular physiology of taste transduction. Curr Opin Neurobiol 10:519–527.

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

Hellekant G, Danilova V, Ninomiya Y (1997) Primate sense of taste: behavioral and single chorda tympani and glossopharyngeal nerve fiber recordings in the rhesus monkey, *Macaca mulatta*. J Neurophysiol 77:978–993.

Kawai K, Sugimoto K, Nakashima K, Miura H, Ninomiya Y (2000) Leptin as a modulator of sweet taste sensitivities in mice. Proc Natl Acad Sci USA 97:11044–11049.

Kim MR, Kusakabe Y, Miura H, Shindo Y, Ninomiya Y, Hino A (2003) Regional expression patterns of taste receptors and gustducin in the mouse tongue. Biochem Biophys Res Commun 312:500–506.

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

Lindemann B (1996) Taste reception. Physiol Rev 76:718–766.

Lindemann B (2001) Receptors and transduction in taste. Nature 413:219–225.

Lyall V, Heck GL, Vinnikova AK, Ghosh S, Phan T-HT, Alam RI, Russell OF, Malik SA, Bigbee JW, DeSimone JA (2004) The mammalian amiloride-insensitive non-specific salt taste receptor is a vanilloid receptor-1 variant. J Physiol (Lond) 558:147–159.

Maga JA (1983) Flavor potentiators. Crit Rev Food Sci Nutr 18:231–312.

Max M, Shanker YG, Huang L, Rong M, Liu Z, Campagne F, Weinstein H,

Damak S, Margolskee RF (2001) Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. Nat Genet 28:58–63.

McLaughlin SK, McKinnon PJ, Margolskee RF (1992) Gustducin is a taste-cell-specific G protein closely related to the transducins. Nature 357:563–569.

Monastyrskaia K, Lundstrom K, Plahl D, Acuna G, Schweitzer C, Malherbe P, Mutel V (1999) Effect of the umami peptides on the ligand binding and function of rat mGlu4a receptor might implicate this receptor in the monosodium glutamate taste transduction. Br J Pharmacol 128:1027–1034.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS (2001) Mammalian sweet taste receptors. Cell 106:381–390.

Nelson G, Chandrashekar J, Hoon M, Feng L, Zhao G, Ryba NJP, Zuker CS (2002) An amino-acid taste receptor. Nature 416:199–202.

Ninomiya Y, Funakoshi M (1989a) Behavioral discrimination between glutamate and the four basic taste substances in mice. Comp Biochem Physiol [A] 92:365–370.

Ninomiya Y, Funakoshi M (1989b) Peripheral neural basis for behavioural discrimination between glutamate and the four basic taste substances in mice. Comp Biochem Physiol [A] 92:371–376.

Ninomiya Y, Nakashima K, Fukuda A, Nishino H, Sugimura T, Hino A, Danilova V, Hellekant G (2000) Responses to umami substances in taste bud cells innervated by the chorda tympani and glossopharyngeal nerves. J Nutr 130:950S–953S.

Ohara I, Tanaka Y, Otsuka SI (1979) Discrimination of monosodium glutamate and sodium chloride solutions by rats. Physiol Behav 22:877–882.

Ruiz CJ, Wray K, Delay E, Margolskee RF, Kinnamon SC (2003) Behavioral evidence for a role of α-gustducin in glutamate taste. Chem Senses 28:573–579.

Ruiz-Avila L, McLaughlin SK, Wildman D, McKinnon PJ, Robichon A, Spickofsky N, Margolskee RF (1995) Coupling of bitter receptor to phosphodiesterase through transducin in taste receptor cells. Nature 376:80–85.

Ruiz-Avila L, Wong GT, Damak S, Margolskee RF (2001) Dominant loss of responsiveness to sweet and bitter compounds caused by a single mutation in alpha-gustducin. Proc Natl Acad Sci USA 98:8868–8873.

Sako N, Harada S, Yamamoto T (2000) Gustatory information of umami substances in three major taste nerves. Physiol Behav 71:193–198.

Sato M, Yamashita S, Ogawa H (1970) Potentiation of gustatory response to monosodium glutamate in rat chorda tympani fibers by addition of 5′-ribonucleotides. Jpn J Physiol 20:444–464.

Stapleton JR, Roper SD, Delay ER (1999) The taste of monosodium glutamate (MSG), L-aspartic acid, and *N*-methyl-D-aspartate (NMDA) in rats: are NMDA receptors involved in MSG taste? Chem Senses 24:449–457.

Wong GT, Gannon KS, Margolskee RF (1996) Transduction of bitter and sweet taste by gustducin. Nature 381:796–800.

Yamaguchi S (1998) Basic properties of umami and its effects on food flavor. Food Rev Int 14:139–176.

Yamamoto T, Yuyama N, Kato T, Kawamura Y (1985) Gustatory responses of cortical neurons in rats. III. Neural and behavioral measures compared. J Neurophysiol 53:1370–1386.

Yamamoto T, Matsuo R, Fujimoto Y, Fukunaga I, Miyasaka A, Imoto T (1991) Electrophysiological and behavioral studies on the taste of umami substances in the rat. Physiol Behav 49:919–925.

Yan W, Sunavala G, Rosenzweig S, Dasso M, Brand JG, Spielman AI (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.

Yang H, Wanner IB, Roper SD, Chaudhari N (1999) An optimized method for in situ hybridization with signal amplification that allows the detection of rare mRNAs. J Histochem Cytochem 47:431–446.

Yoshida M, Saito S (1969) Multidimentional scaling of taste amino acids. Jpn Psychol Res 11:149–166.

Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112:293–301.

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, Zuker CS (2003) The receptors for mammalian sweet and umami taste. Cell 115:255–266.