Taste receptors for umami: the case for multiple receptors

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ABSTRACT
Umami taste is elicited by many small molecules, including amino acids (glutamate and aspartate) and nucleotides (monophosphates of inosinate or guanylate, inosine 5'-monophosphate and guanosine-5'-monophosphate). Mammalian taste buds respond to these diverse compounds via membrane receptors that bind the umami tastants. Over the past 15 y, several receptors have been proposed to underlie umami detection in taste buds. These receptors include 2 glutamate-selective G protein–coupled receptors, mGluR4 and mGluR1, and the taste bud–expressed heterodimer T1R1+T1R3. Each of these receptors is expressed in small numbers of cells in anterior and posterior taste buds. The mGluRs are activated by glutamate and certain analogs but are not reported to be sensitive to nucleotides. In contrast, T1R1+T1R3 is activated by a broad range of amino acids and displays a strongly potentiated response in the presence of nucleotides. Mice in which the Grm4 gene is knocked out show a greatly enhanced preference for umami tastants. Loss of the Tas1r1 or Tas1R3 genes is reported to depress but not eliminate neural and behavioral responses to umami. When intact mammalian taste buds are apically stimulated with umami tastants, their functional responses to umami tastants do not fully resemble the responses of a single proposed umami receptor. Furthermore, the responses to umami tastants persist in the taste cells of T1R3-knockout mice. Thus, umami taste detection may involve multiple receptors expressed in different subsets of taste cells. This receptor diversity may underlie the complex perception of umami, with different mixtures of amino acids, peptides, and nucleotides yielding subtly distinct taste qualities. Am J Clin Nutr 2009;90(suppl):738S–42S.

INTRODUCTION
Umami is the meaty, mouth-filling, rich taste found in many types of seafood, seaweed, fish, meats, and mushrooms. The last few years have witnessed substantial growth in our understanding of umami taste. Starting from Ikeda’s initial characterization of monosodium glutamate (MSG) as the prototypic umami stimulus, we now recognize that umami is also elicited by a few other amino acids (primarily aspartate), many short peptides, some organic acids (eg, lactic, succinic, and propionic acids) (1), and possibly other compounds. A key feature of umami taste is the synergistic enhancement of potency when glutamate or aspartate is combined with monophosphate esters of guanosine or inosine nucleosides [guanosine-5’-monophosphate (GMP) and inosine 5’-monophosphate (IMP)].

UMAMI—A COMPLEX TASTE
Natural and processed foods contain diverse types and combinations of umami compounds. The titers of various umami compounds (amino acids and nucleotides) vary dramatically across many seafoods. These varying combinations of simple umami compounds are reported to elicit subtle differences in perceived umami taste (2). Novel taste compounds continue to be discovered that are potent umami stimuli or that enhance the umami taste of known compounds (3). When foods are supplemented with umami compounds, interactions with food components occur. For instance, MSG is most effective at enhancing the palatability of some foods, IMP or GMP is more effective at enhancing the palatability of other foods, and nucleotides may even decrease the palatability of some foods (4). These observations suggest that umami is much more complex than just the taste of MSG.

The natural ligands that elicit bitter taste are chemically diverse. Detection of such a wide array of compounds in foods is believed to require multiple taste receptors—a need met by a large family of bitter taste receptors expressed in small but overlapping subsets of bitter-sensing taste cells (5). Given the chemical and combinatorial diversity of umami tastants, it is reasonable to consider that the perceptual complexity of umami may be similarly encoded by multiple taste receptors.

SEVERAL RECEPTORS HAVE BEEN PROPOSED FOR UMAMI TASTE
Taste buds are aggregates of specialized neuroepithelial cells embedded in the stratified epithelia of the oral cavity. The apical tips of taste cells protrude into a taste pore, which make contact with saliva and food substances. The assumption is that membrane receptors that detect umami (and other taste) stimuli are located in the plasma membrane at these apical tips.

Early studies in fish and amphibians showed that the taste system in these species detects certain amino acids with remarkable specificity (6). Biochemical and biophysical measure-
ments further suggested that, in fish, glutamate and other amino acids may be detected via ionotropic receptor proteins, i.e., ion channels that are gated open after binding of amino acids (7). In mammals, however, taste detection of glutamate (and presumably other amino acids) seems primarily to involve G protein–coupled receptors. During the past decade, several G protein–coupled receptors have been proposed as detectors of umami tastants and meet the above essential criteria to various extents. These receptors include mGluR4 (8), T1R1+T1R3 (9, 10), and mGluR1 (11, 12).

A METABOTROPIC GLUTAMATE RECEPTOR FOR DETECTING UMAMI TASTE

Using reverse transcriptase polymerase chain reaction, in situ hybridization, and a RNase protection assay, we identified mRNA for a variant metabotropic glutamate receptor (taste-mGluR4) that is expressed in rat taste cells (8, 13, 14). To confirm protein expression, we generated a polyclonal antibody against an extracellular epitope in taste-mGluR4. When applied to cryosections of tongue, this antibody showed immunofluorescence in subsets of taste cells in both rats and mice. Taste buds comprise ≥3 morphologically and functionally distinct classes of mature cells (15, 16). To determine which of these 3 cell types express mGluR4, we used cryosections from phospholipase C β2 mice, in which type II receptor cells of taste buds are illuminated by green fluorescent protein (GFP) (15, 17). In vallate (Figure 1, A and B), fungiform (Figure 1C) and palatal taste buds (data not shown), mGluR4-immunoreactivity was clearly limited to a subset of GFP-expressing taste cells. That is, the putative mGluR4 taste receptor is expressed in receptor cells that possess the established canonical pathway for transduction downstream of G protein–coupled taste receptors (15, 16, 19, 20). The mGluR4 protein appeared to be expressed on the surface of cells, and, especially in the case of vallate taste buds, was concentrated in the apical process (Figure 1, B and C). In summary, mGluR4-immunoreactivity in taste cells is restricted to an appropriate cell type and in a subcellular location that would be expected of a chemosensory receptor for umami taste.

The truncated taste-mGluR4 as well as full-length mGluR4, when expressed in CHO cells in culture, produces functional responses to glutamate (8). We showed that the full-length protein detects glutamate with a half maximal effective concentration of ~2 μM, consistent with the full-length protein being a neuronal glutamate receptor. In marked contrast, the half maximal effective concentration for the truncated form is ≥2 orders of magnitude higher—0.3 mmol/L. The effective concentration of glutamate needed to activate truncated mGluR4 is thus in a range that is effective as a taste stimulus. As previously reported for mGluR4 (21), taste-mGluR4 couples negatively to a cAMP cascade (8), as expected from their shared cytoplasmic C-termini. Interestingly, glutamate stimulation of vallate taste buds also produces a consistent and strong decrease in cAMP concentrations (22, 23).

The glutamate analog, L-((+)-2-amino-4-phosphonobutryic acid (L-AP4), activates all group III mGluRs (types 4, 6, 7, and 8). Thus, we asked whether it also generates an umami-like taste. Using a conditioned taste aversion assay, we tested the hypothesis in rats and considered if agonists for other types of glutamate receptors also elicit an umami-like taste. We found that rats generalize (i.e., find similar) the tastes of glutamate and L-AP4 (13, 24, 25). In contrast, agonists of ionotropic glutamate receptors did not appear to taste similar to glutamate. This similarity in the taste of glutamate and L-AP4 was subsequently also shown to apply to humans (26) and mice (27).

FIGURE 1. Mouse taste cells express mGluR4 and/or other group III mGluR proteins on their apical processes. A: Cryosections of vallate papilla from phospholipase C β2 (PLC β2) mice illuminated by green fluorescent protein (GFP) were incubated with anti-mGluR4 and visualized with Alexa 594-labeled secondary antibody (red). Immunoreactivity to mGluR4 and/or other group III mGluRs is seen in many cells within taste buds and appears to be limited to GFP-expressing (green) cells. B: Higher magnification of a vallate taste bud as in A, showing mGluR4-like immunofluorescence, especially in apical processes of some GFP-expressing (type II receptor) taste cells. C: mGluR4-like immunofluorescence in fungiform taste buds. Scale bars for all taste fields, 20 μm. D and E: Validation of anti-mGluR4 antibody. Cryosections of cerebellum from wild-type (WT crbl) (D) and mGluR4-knockout (KO crbl) (E) mice were incubated in parallel with anti-mGluR4 antibody. Cryosections of cerebellum from wild-type (WT crbl) (D) and mGluR4-knockout (KO crbl) (E) mice were incubated in parallel with anti-mGluR4 antibody. Cryosections of cerebellum from wild-type (WT crbl) (D) and mGluR4-knockout (KO crbl) (E) mice were incubated in parallel with anti-mGluR4 antibody. Cryosections of cerebellum from wild-type (WT crbl) (D) and mGluR4-knockout (KO crbl) (E) mice were incubated in parallel with anti-mGluR4 antibody.

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We examined the taste behavioral consequence of ablating mGluR4. In a 2-bottle preference test, mutant mice lacking mGluR4 expression showed a significantly increased preference for MSG, with or without GMP, relative to their wild-type counterparts (28). The mutant mice had normal responses to sweet, salty, and sour tastants, but were more sensitive to bitter stimuli than were wild-type mice. Thus, the loss of mGluR4 appeared to significantly alter the detection of umami taste. However, the interpretation of these results is complicated by the fact that mGluR4 is also a neurotransmitter receptor at central synapses. Thus, the knockout would likely affect central pathways involved in taste. We are not aware of any mouse strains in which the truncated, taste-selective form is selectively ablated and in which peripheral taste effects could be separated from other sites of action.

In summary, many aspects of umami taste are displayed by mGluR4, heterologously expressed in cultured cells. Nevertheless, other aspects of umami are not modeled by mGluR4. Principal among these is the well-known synergy between glutamate and the nucleotides GMP and IMP.

**WILL THE REAL UMAMI RECEPTOR PLEASE STAND UP?**

In 2002, the demonstration that heterodimers of T1R3 and T1R1, when expressed in cultured cells, produce a Ca\(^{2+}\) response to umami compounds introduced much controversy. Other investigators (9, 10) have argued that because the responses of the heterodimer to glutamate and IMP were synergistic, T1R1+T1R3 must be the only taste receptor for umami. A subsequent study from the same group reported that genetic ablation of T1R3 or T1R1 eliminated behavioral responses to umami. This further emphasized the interpretation of a unitary receptor for umami (29). Yet, soon after this, the laboratory of Margolskee (30) reported that their independently generated T1R3-knockout mouse retained very significant taste sensitivity to MSG, both in a behavioral (2-bottle preference) assay and in afferent nerve recordings. While maintaining umami sensitivity, this latter T1R3-knockout had substantial deficiencies in responses to sweet stimuli, as expected. The most prominent effect for umami was that the synergistic enhancement of glutamate responses by nucleotides was lost when T1R3 was ablated (30). Thus, independent studies from 2 laboratories confirmed that amino acid–nucleotide synergy is dependent on taste receptors that include T1R3.

The distinct strategies of genetic manipulation used to produce the 2 strains of mice may eventually reveal insights on how taste G protein–coupled receptors dimerize and function in taste cells. At the very least, it now appears clear that responses to umami are G protein–coupled receptors dimerize and function in taste cells. For example, mouse T1R1+T1R3, when expressed in HEK293 cells, was reported to be activated by a broad range of amino acids, many of which were much more effective than glutamate (10). Furthermore, the response to each amino acid was enhanced by the presence of IMP. In contrast, in native taste cells, glutamate was by far the most effective of the amino acids. Many amino acids that robustly activate T1R1+T1R3 did not stimulate taste cells at all (31).

We also found that Ca\(^{2+}\) responses to glutamate and to L-AP4 were mostly detected by separate cells in vallate taste buds (31). This result was unexpected given that both mGluR4 and T1R1+T1R3 are reportedly stimulated by L-AP4. These data suggest that the response profiles of taste cells may be modified by the presence of other receptors or signaling components. Interestingly, although glutamate and L-AP4 have similar tastes, rodents are easily able to distinguish them (24). A possible mechanism for this discrimination could be that, at least in some taste fields, the 2 compounds are detected by separate cells, as shown in our functional imaging data. Finally, we also confirmed that umami responses in many vallate taste cells persist in the absence of T1R3, again confirming that umami compounds can be detected in the complete absence of T1R3 (31). Whereas our data were obtained in rodents (mice and rats), it has been suggested that a diversity of mechanisms for detecting umami may apply in humans as well. Humans do appear to vary in their ability to detect umami taste (32), and recent data suggest that polymorphisms in more than one gene may be responsible for some of this diversity across populations (33).

Umami taste is sometimes defined narrowly as the nucleotide-potentiated taste of glutamate. Yet, diverse responses to glutamate with and without potentiation by nucleotides are also seen in electrical recordings of taste cells and taste nerve fibers (34). Thus, it appears that there are distinct responses to umami compounds, potentially mediated by different molecular receptors in separate taste cells. How these separate detection events contribute to the perception of umami taste quality will be an important area for future investigation.

**CONCLUSIONS**

In summary, some of the functional responses to umami stimuli in native taste cells are similar to those elicited in heterologous expression systems by either mGluR4 or T1R1/T1R3 or both. Yet, other characteristics of native umami responses do not correspond well to any expressed umami taste receptor reported to date. This may suggest the presence of additional receptors for different umami stimuli, unexplored interactions among known receptors, or both. It is also possible that taste cell responses to different umami stimuli interact or converge on afferent fibers or centrally to produce the integrated recognition of umami. (Other articles in this supplement to the Journal include references 35–63.)
The authors’ responsibilities were as follows—NC and SDR: responsible for the conceptual design; and EP: carried out the immunohistochemistry. All authors were involved in writing the manuscript and approved the final version.

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