ABSTRACT

L-Glutamate is known to elicit a unique taste, umami, that is distinct from the tastes of sweet, salty, sour, and bitter. Recent molecular studies have identified several candidate receptors for umami in taste cells, such as the heterodimer T1R1/T1R3 and brain-expressed and taste-expressed type 1 and 4 metabotropic glutamate receptors (brain-mGluR1, brain-mGluR4, taste-mGluR1, and taste-mGluR4). However, the relative contributions of these receptors to umami taste reception remain to be elucidated. We critically discuss data from recent studies in which mouse taste cell, nerve fiber, and behavioral responses to umami stimuli were measured to evaluate whether receptors other than T1R1/T1R3 are involved in umami responses. We particularly emphasized studies of umami responses in T1R3 knockout (KO) mice and studies of potential effects of mGluR antagonists on taste responses. The results of these studies indicate the existence of substantial residual responses to umami compounds in the T1R3-KO model and a significant reduction of umami responsiveness after administration of mGluR antagonists. These findings thus provide evidence of the involvement of mGluRs in addition to T1R1/T1R3 in umami detection in mice and suggest that umami responses, at least in mice, may be mediated by multiple receptors. Am J Clin Nutr 2009;90(suppl):747S–52S.

INTRODUCTION

L-Glutamate, typically its salt form monosodium glutamate (MSG), is widely distributed in foods, including meats, fish, and vegetables (1). In humans and probably in certain species of animals, MSG elicits a unique taste called “umami” that is distinct from the tastes of sweet, salty, sour, and bitter (2–4). Umami taste is also provoked by other amino acids, including L-aspartate, and 5'-ribonucleotides to amino acids synergistically enhance the intensity of umami taste and remarkably lowers the umami taste threshold (5, 6).

Recently, molecular studies have identified several umami receptor candidates (Figure 1). The first to be reported is a variant of brain-mGluR4—taste-mGluR4. This variant is expressed in rat circumvallate and foliate taste buds on the posterior tongue, innervated by the glossopharyngeal nerve (Figure 2); it has a truncated N-terminal to which glutamate binds, albeit with reduced affinity (7, 8) (Figure 1). The second umami receptor candidate to be discovered is a heterodimer of T1R1/T1R3 (taste receptor type 1, member 1/taste receptor type 1, member 3) (9, 10). T1R1 expression is prevalent in the fungiform taste buds on the anterior tongue, innervated by the chorda tympani nerve, but rare in the circumvallate taste buds on the posterior tongue, although T1R3 expression is comparable in all taste buds on the tongue (9). Mouse T1R1/T1R3 heterologously expressed in human embryonic kidney cells responds to many amino acids, some of which elicit taste qualities other than umami. In contrast, the human T1R1/T1R3 heterodimer preferentially responds to glutamate, and this response is enhanced by IMP (9, 10). The sequencing and functional expression of this human taste receptor for glutamate provided the first molecular basis for umami detection and perception in humans. However, with regard to mouse studies, the results obtained from mice lacking T1R3 are controversial. That is, in one study of T1R3 knockout (T1R3-KO) mice, a behavioral preference for MSG and neural responses to MSG in the chorda tympani nerve, innervating the anterior tongue, were totally absent (11), which indicated that T1R1/T1R3 is essential for MSG detection and perception in mice. In contrast, another study showed that a behavioral preference for MSG was reduced but not abolished in T1R3-KO mice (12). Analyses of the umami nerve responses to umami stimuli in T1R3-KO mice showed that the synergism between MSG and IMP was abolished. However, no large reduction was observed in the response to MSG alone in either the chorda tympani or the glossopharyngeal nerve (12). This latter study suggests the existence of more than one receptor for umami taste in mice. The other candidates proposed are brain-expressed type 1 and type 4 metabotropic glutamate receptors (brain-mGluR1 and brain-mGluR4) and a variant of mGluR1 (13–15) (Figures 1 and 2). Brain-mGluR1 and brain-mGluR4 are expressed in a subset of taste cells in fungiform and foliate and circumvallate papillae located on both the anterior and posterior tongue in rats (13, 14). Taste-mGluR4, like taste-mGluR1, is expressed in rat circumvallate and foliate papillae on the posterior tongue and has a truncated N-terminal domain with low binding affinity to glutamate (15).

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Our laboratory and others have investigated receptor and transduction pathways for umami taste using molecular, neurophysiologic, pharmacologic, and behavioral techniques. This article summarizes data obtained from recent studies, most of which provide evidence of the involvement of T1R1/T1R3-independent receptor systems (ie, through mGluRs) in umami detection and, thus, of the existence of many receptors for umami taste in mice. It is presently unknown whether such receptor variants might also be present in humans.

**TONGUE REGIONAL DIFFERENCES IN UMAMI RESPONSES**

In several mammalian species (3, 16, 17), the responsiveness to various chemical compounds differs between taste cells located on the anterior and posterior parts of the tongue, innervated by the chorda tympani and glossopharyngeal nerve, respectively. For example, in mice and rats, responses to sodium chloride in the chorda tympani nerve were suppressed by amiloride, a sodium channel blocker in various epithelial cells, whereas no such amiloride suppression of sodium chloride responses was evident in the glossopharyngeal nerve (16, 18, 19). The same was true for the suppression of responses to sweeteners by gurmarin — a peptide isolated from the plant *Gymnema sylvestre* (20–22). In the chorda tympani nerve of rats (23), mice (16, 24, 25), and hamsters (26), sodium chloride–responsive fibers are classified into 2 types. One type receives input from taste cells that are narrowly tuned to sodium chloride, and the sodium chloride response is strongly inhibited by amiloride (known as the N-type). The other type receives input from cells broadly responsive to electrolytes, including various salts and acids, and shows almost no amiloride-sensitivity (E- or H-type). Typical umami compounds, such as MSG, IMP, and a mixture of MSG and IMP, contain Na⁺. Therefore, the Na⁺ component of these umami compounds elicits responses in both N-type and E-type fibers, and the umami responses of N-type fibers are suppressed by amiloride (6). In the mouse glossopharyngeal nerve, sodium chloride–responsive fibers are mostly amiloride-insensitive E-type fibers (6).

Gurmarin is a sweet response inhibitor for rats (22) and C57BL mice; however, it has a minimal effect on sweet responses in BALB mice or sweetness perception in humans (22, 27). Recently, lactisole, a sweet taste inhibitor for humans, was shown to bind the transmembrane domain of the T1R3 subunit and to inhibit the activity not only of T1R2/T1R3 sweet receptor but also of the T1R1/T1R3 umami receptor (28). Perhaps such is also the case for gurmarin in rats and mice; if so, this peptide may also affect responses to umami compounds that are initiated by the T1R1/T1R3 receptor in these species (although this has yet to be determined). In support of this notion, single fiber response analyses in rats (29) and mice (6) indicate that most fibers...
UMAMI RESPONSES IN MICE LACKING T1R3, IP3R3, OR TRPM5

As described above, T1R3-KO mice show a greatly reduced behavioral preference for and chorda tympani nerve responses to umami compounds, including the synergism between MSG and IMP (11, 12). This suggests that T1R1/T1R3 receptors in the anterior tongue are the basis for the synergism between umami compounds and play a major role in the preference for umami taste. These findings are compatible with the data showing that T1R1 and T1R3 are co-expressed in mouse fungiform taste bud cells in the anterior tongue (34). Furthermore, the heterodimer T1R1/T1R3, when expressed in heterologous cells, responds synergistically to the mixture of glutamate (and many other amino acids) with IMP or GMP (9). However, a more important finding may be that responses to umami stimuli in the glossopharyngeal nerve were not largely affected by genetic deletion of T1R3 (12). In accordance with this finding, recent behavioral and electrophysiologic studies further showed no large reduction in responses to umami compounds in T1R3-KO mice (31, 35, 36). For example, a behavioral study (35) using psychophysical methods showed that T1R3-KO mice have detection thresholds for MSG and sucrose comparable with those of their wild-type counterparts. T1R3-KO mice were also able to discriminate between tastes of MSG and sucrose, although not as well as the wild-type mice. Also, a recent study in T1R3-KO mice showed that umami taste stimuli evoked Ca²⁺ responses in taste cells from circumvallate taste buds innervated by the glossopharyngeal nerve, albeit with a decreased amplitude (36).

Correspondingly, no large reduction in behavioral and neural responses to umami compounds has been reported in mice lacking genes controlling downstream signaling molecules, at least to T1R1/T1R3, such as Gα-gustducin and/or -transducin (37), type III inositol-1,4,5-triphosphate receptor (IP3R3) (38), or the transient receptor potential M5 cation channel (TRPM5) (39, 40). Concentration-response curves for mononutrient glutamate (MPG) and a mixture of MPG and IMP (MPG + IMP) obtained from T1R3-KO, IP3R3-KO, and TRPM5-KO mice and their wild-type counterparts with the same C57BL genetic backgrounds indicate that these 3 lines of KO mice had very similar responses to umami compounds (38, 40, 41), included reduced chorda tympani nerve responses, no synergism, and little effect on glossopharyngeal nerve responses (38, 40, 41). Gα-gustducin-KO mice were also shown to have umami responsiveness similar to that of the above-mentioned KO mice, although their genetic background was not derived from the C57BL strain (mixed background of 129 and BALB strains) (37). Together, whereas these data provide additional evidence for the involvement of T1R3, Gα-gustducin, IP3R3, and TRPM5 in umami detection on the anterior tongue, they also indicate the existence of pathways independent of these molecules, both on the anterior and posterior tongue.

Our recent studies examined single chorda tympani fiber responses to umami compounds in the wild-type C57BL mice and in T1R3-KO and TRPM5-KO mice (41–43). In wild-type mice, S- and M-type fibers were further classified into 2 subtypes, segregated by the occurrence of the synergism between MPG and IMP. S1- and M1-types exhibited the synergism, whereas S2- and M2-types did not (42, 43). The total number of impulses in response to MPG are greater in the order of E > M2 > S1 > S2 > M1 > N. This indicates that, in this classification, the largest MPG response component may be derived from the E-type, nonspecific electrolyte receptor system. Synergistic responses between MPG and IMP were much larger in S1-type than in M1-type fibers. In M1-type fibers, the total magnitude of the response to the mixture of MPG and IMP was about one-tenth that of the response in S1-type fibers. T1R3-KO and TRPM5-KO mice lack S1-type fibers, which shows the large synergism between MPG and IMP (42, 43). This is consistent with data from experiments of whole nerve responses (ie, the absence of synergism) (38, 40, 41). All other types, including S2-, M1-, M2, and E-types, still remained, although the response magnitudes to sweet substances were largely reduced in S2-type fibers (41–43). Thus, the major components of residual MPG responses of the chorda tympani nerve in these KO mice are derived from M1-type fibers with small synergism, M2-type fibers with no synergism, and E-type fibers with broad sensitivities to various electrolytes. These data suggest that T1R3-KO and TRPM5-KO mice lack the signal elicited by MPG in the anterior tongue that is not glutamate specific and may be similar to those elicited by sweet compounds (S-type cell and fibers). This lack of signal from S-type cells and fibers may lead to their greatly reduced behavioral preference for glutamate, as shown previously (11, 12, 40). The KO mice may, however, still possess glutamate-specific signals elicited by MPG, both in the anterior and posterior tongue (M-type cell and fibers); thus, these mice may be able to discriminate between the tastes of umami and...
sweet compounds, as shown in a previous study of T1R3-KO mice (35).

EFFECTS OF ANTAGONISTS FOR mGluRs ON UMAMI RESPONSES

If T1R3-independent receptor and transduction pathways for umami detection exist, mGluRs or combinations of T1R1 and mGluRs could be candidate receptors. To investigate the potential contribution of mGluRs, we examined the effects of selective antagonists for both mGluR1 [1-aminooindan-1,5-dicarboxylic acid (AIDA)] for group I mGluRs (44) and mGluR4 [(RS)-\(\alpha\)-cyclopropyl-4-phosphonophenylglycine (CPPG)] for group III mGluRs (45) on the chorda tympani and glossopharyngeal nerve responses to umami compounds in C57BL mice. The results indicated that integrated whole nerve responses of both chorda tympani and glossopharyngeal nerves to mixtures of 100 mmol MPG/L and 1–10 mmol CPPG or AIDA/L, or all 3 compounds, were significantly lower than those for the sum of responses to each compound applied separately (41–43). Inhibition of responses to umami stimuli by these antagonists was also clearly observed in particular groups of single fungiform taste cells and single chorda tympani fibers. That is, the responses to MSG + amiloride and to MPG were suppressed by the addition of 0.3–10 mmol CPPG or AIDA/L in M-type taste cells and M1- and M2-type fibers, whereas no such suppression was evident in S-type cells and fibers (43). Moreover, behavioral studies using a conditioned taste aversion paradigm showed that T1R3-KO mice were still able to learn to avoid MSG with amiloride, and the conditioned avoidance responses to MSG with amiloride were largely reduced by the addition of 0.3–1 mmol CPPG or AIDA/L (41). Conditioned taste aversion experiments using wild-type C57BL mice showed that the mGluR antagonists (at 1 mmol/L) suppressed avoidance responses to MSG at concentrations ranging from micromolar to millimolar concentrations that may overlap with functional concentration ranges for both brain-type and taste-type mGluRs (41). Taken together, these results indicate involvement of mGluR1 and mGluR4 in addition to T1R1/T1R3 in umami detection in mice.

In humans, psychophysical studies have shown that agonists of mGluR1 and mGluR4, such as ibotenate, \(DL(+)-2\)-amino-4-phosphonobutyric acid, and \((+)-1\)-aminoindanocyclopentane-\(trans\)-1,3-dicarboxylic acid, applied to the tongue elicit an umami sensation (46). However, the receptor for umami taste suggested that may overlap with functional concentration ranges for both brain-type and taste-type mGluRs may be different from that mediated by the pathway involving T1R1/T1R3 (39, 40). If mGluR receptors are involved in the residual umami responses in mice lacking each of the downstream molecules, binding of umami compounds to mGluR receptors may activate different transduction pathways from pathways activated by T1R1/T1R3.

Recent studies showed that taste cells expressing T1R and T2R receptors and the abovementioned downstream signaling molecules for sweet, bitter, and umami tastes, are type II cells. Type II cells release ATP through pannexin and connexin hemichannels dependent on membrane depolarization, generation of action potentials, and an increase in cytoplasmic Ca\(^{2+}\) (50, 51). Genetic elimination of ionotropic purinergic receptors (P2X2 and P2X3) abolished taste responses without affecting responses to mechanical and thermal stimulations, which suggests that ATP is a transmitter linking taste receptor cells to nerve fibers (52). Our recent studies confirmed that stimulation of the apical membrane with sweet, bitter, and umami compounds evokes ATP release from taste cells expressing gustducin in an action-potential-dependent manner (Y Murata, R Yoshida, Y Ninomiya, unpublished observations, 2008).

In conclusion, this article summarizes recent findings on umami taste. Most of the findings support the idea that many receptors exist for umami taste, at least in mice. The accumulating evidence indicates that the potential role of the signal mediated by the transduction pathway involving T1R1/T1R3 may be different from that mediated by the pathway involving mGluRs. The former signal occurs mainly in the anterior tongue and plays a major role in preference behavior, whereas the latter occurs mainly in the posterior tongue and is active in mice lacking T1R3, Gz-gustducin, IP\(_3\), and TRPM5, and contributes to behavioral discrimination between umami and other taste compounds.

In humans, unlike in mice, T1R1/T1R3 acts as an umami-specific receptor that can discriminate between umami and other tastes, and thus accounts for umami-linked preferences or discrimination. Future studies will hopefully determine whether other taste glutamate receptors, such as those in mammals other than humans, also function in humans. (Other articles in this supplement to the Journal include references 53–81.)

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