— Current Topics —

Taste and Health: New Frontiers in Oral Physiology and Rehabilitation

Multiple Umami Receptors and Their Variants in Human and Mice

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L-Glutamate and 5′-ribonucleotides are known to elicit a unique taste, "umami," that is distinct from the tastes of sweet, salt, sour and bitter. Recent progress in molecular biology has identified several umami receptor candidates, such as the heterodimer T1R1/T1R3, and brain-expressed and taste-expressed type 1 and 4 metabotropic glutamate receptors (brain- and taste-mGluR1 and mGluR4). This paper summarizes recent findings on the receptor system for umami taste. Most of the findings support the idea that multiple 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, is active in mice lacking T1R3, Gα-gustducin, IP_3R3 or 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 account for umami-linked preferences or discrimination.

Key words —— umami taste, receptor, transduction pathway, downstream signaling molecule

INTRODUCTION

L-glutamate, typically its salt form, monosodium glutamate (MSG), is widely distributed in many foods such as meats, fishes, and some 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] It is the characteristic feature of umami that the taste intensity of MSG is synergistically enhanced by 5′-ribonucleotides, such as inosine-5′-monophosphate (IMP) and guanosine-5′-monophosphate (GMP).[5,6] Recent progress in molecular biology has identified several umami receptors, such as a heterodimer of T1R1 (official gene name and symbol: taste receptor type1, member 1, *Tas1r1*) and T1R3 (taste receptor type1, member 3, *Tas1r3*), and mGluR1 (glutamate receptor, metabotropic 1, *Grm1*) and mGluR4 (glutamate receptor, metabotropic 4, *Grm4*).

In mice and humans, there is substantial variation in umami taste sensitivity.[7–12] However, little is known about the relative contribution of these receptors to umami taste reception and the genetic basis of this variation in umami taste perception. Understanding the nature of the variation in umami taste sensitivity, the genetic variation in its receptors, and how this variation influences diet selection may have important implications for human health.

In this paper, we summarize recent findings on glutamate taste responses obtained from molecular-genetic, electrophysiological and behavioral studies in mice and genetic studies in humans and discuss about potential existence of multiple receptor sys-

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tems for umami taste in mice and humans.

MULTIPLE UMAMI RECEPTOR CANDIDATES IN MICE

Recent progress in molecular biology has identified several umami receptor candidates. The first discovered candidate is a variant of brain-expressed metabotropic glutamate receptor 4 (taste-mGluR4). This variant is expressed in rat circumvallate and foliate taste buds on the posterior tongue innervated by the glossopharyngeal (GL) nerve and has a truncated N-terminal to which glutamate binds, albeit with reduced affinity.[13,14] The second discovered candidate is a heterodimer of T1R1/T1R3.[15,16] Mouse T1R1/T1R3 heterologously expressed in human embryonic kidney (HEK) cells responds to many amino acids, some of which elicit taste qualities other than umami. In contrast, the human T1R1/T1R3 heteromer preferentially responds to glutamate, and this response is enhanced by IMP.[15,16] With regards to mouse genetic studies, results obtained from mice lacking T1R3 are controversial. That is, one study showed that behavioral preference for MSG and neural responses to MSG in the chorda tympani (CT) nerve, innervating the anterior tongue, were totally abolished,[17] indicating that the T1R1/T1R3 solely contributes to MSG detection and perception in mice. In contrast, another study revealed that behavioral preference for MSG was reduced but not abolished in T1R3 knockout (KO) mice. Analyses of CT nerve responses to umami stimuli in T1R3-KO mice demonstrated that no large reduction was observed in responses to MSG alone in either the CT or GL nerve, innervating the posterior tongue, but synergism between MSG and IMP was abolished.[18] The latter study indicates the existence of multiple receptors for umami taste responses in mice. The other candidates proposed are brain-expressed type 1 and type 4 metabotropic glutamate receptors (mGluR1 and mGluR4) and a variant of mGluR1.[19–21] Brain-expressed mGluR1 and mGluR4 are expressed in a subset of taste cells located on both the anterior and posterior tongue.[19,20] The variant of mGluR1, like taste-mGluR4, is expressed in a subset of taste cells located on the posterior tongue and has a truncated N-terminal domain (truncated mGluR1) with low binding affinity to glutamate.[21]

MULTIPLE NEURAL PATHWAYS FOR UMAMI RESPONSES IN MICE

In wild type mice, CT and GL nerve convey taste information for umami stimuli, which can be discriminated from that of the other basic tastes. In previous studies, MSG-responsive single fibers in the two taste nerves were classified into 4 groups based on a cluster analysis, S-type,[6,22] M-type,[23,24] N-type and E-type (or H-type).[25–29] S-type fibers are characterized by their best response to sucrose and large synergism between MSG and IMP.[6,22] M-type fibers exhibited best responses to umami stimuli and showed small synergism. N-type fibers responded best to NaCl and E-type fibers responded to various electrolytes. Since typical umami compounds, such as MSG, IMP and the mixture of MSG and IMP, contain Na^+, the Na^+ component of these umami compounds elicits responses in N- and E-type fibers.[25–29]

Our recent studies showed that in wild type mice S- and M-type fibers were further classified into two subtypes, according to the occurrence of the synergism between monopotassium glutamate (MPG) and IMP. S1- and M1-types exhibited the synergism, whereas S2- and M2-types did not.[30,31] 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 E-type, non-specific 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.

As compared to the CT nerve, the GL nerve of the mouse contains a much greater number of M-type fibers and shows greater responses to umami stimuli.[23] Behavioral studies using a conditioned taste aversion paradigm demonstrated that normal mice could discriminate MSG from the four basic taste stimuli.[4,32] However, mice that had undergone bilateral sectioning of the GL nerve could not discriminate MSG from NaCl, suggesting that the GL nerve transmits taste information for umami stimuli, which can be discriminated from that of the other basic tastes.[4,23] The dominance of the GL in carrying umami-specific signals is also suggested by the presence of M-type fibers in the GL nerve of rhesus monkeys[3] and by psychophysical

studies in humans, showing that the back of the tongue is more sensitive to umami substances than the front.[33] Thus the posterior tongue may play an important role in the dominant site for glutamate taste perception in several mammals.

UMAMI RESPONSES IN MICE LACKING T1R3, IP_3R3, Gα-GUSTDUCIN OR TRPM5

In previous studies, behavioral preference and CT nerve responses to umami compounds including the synergism between MSG and IMP were greatly reduced in T1R3-KO mice.[17, 18] However, GL nerve responses to umami stimuli were not largely affected by genetic deletion of T1R3.[18] In accordance with this important finding, recent behavioral and electrophysiological studies further revealed no large reduction of responses to umami compounds in T1R3-KO mice.[30, 34, 35] For example, a behavioral study[34] using psychophysical methods demonstrated that T1R3-KO mice have detection thresholds for MSG and sucrose comparable to 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.

Correspondingly, no large reduction of behavioral and neural responses to umami compounds has been reported in mice lacking gene(s) controlling downstream signaling molecules, such as Gα-gustducin and/or transducin,[36] type III inositol-1-4,5-triphosphate receptor (IP_3R3)[37] or the transient receptor potential M5 cation channel (TRPM5).[38, 39] Concentration-response curves for MPG and the mixture of MPG and IMP (MPG + IMP) obtained from T1R3-, IP_3R3- or TRPM5-KO mice and their wild type counterparts with the same C57BL genetic backgrounds indicate that these three lines of KO mice exhibited almost identical responsiveness to umami compounds with reduced CT nerve responses, no synergism, and little effect on GL nerve responses.[37, 39, 40] Gα-gustducin-KO mice were also reported to show umami responsiveness similar to these three lines of KO mice, although their genetic background was not derived from the C57BL strain (mixed background of 129 and BALB strains).[36] Thus, these data provide additional evidence for the involvement of T1R3, Gα-gustducin, IP_3R3 and TRPM5 in umami detection on the anterior tongue, indicating the existence of pathways independent of these molecules on the anterior and posterior tongue.

Our recent studies examined taste nerve responses to umami compounds in the wild type C57BL mice and in T1R3-KO and TRPM5-KO mice.[30, 31, 40] T1R3-KO and TRPM5-KO mice lacked S1-type fibers.[30, 31] To exhibit the large synergism between MPG and IMP by S1-type fibers, this is consistent with data from whole nerve response experiments (*i.e.*, the absence of synergism).[37, 39, 40] All other types including S2-, M1-, M2, and E-types still remained, although response magnitudes to sweet substances were largely reduced in S2-type fibers.[30, 31, 40] Thus, the major components of residual MPG responses of the CT nerve in these KO mice are derived from M1-type fibers with small synergism and M2-type fibers with no synergism and E-type fibers with broad sensitivities to various electrolytes. These data suggest that T1R3- 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). Losing this signal may greatly reduce behavioral preference for glutamate as shown previously.[17, 18, 39] 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), and thus these mice may be able to discriminate between the tastes of umami and sweet compounds.[34]

INVOLVEMENT OF mGluRs IN UMAMI RESPONSES

If there exist umami receptors and transduction pathways that are independent of T1R3, 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-aminoindan-1,5-dicarboxylic acid (AIDA)] for group I mGluRs[40] and mGluR4 [(RS)-alpha-cyclopropyl-4-phosphonophenylglycine (CPPG)] for group III mGluRs[41] on the CT and GL nerve responses to umami substances in C57BL mice. The results indicated that integrated whole nerve responses of both CT and GL nerves to mixtures of 100 mM MPG and 1–10 mM CPPG or AIDA, or all three compounds, were significantly lower than

those for the sum of responses to each compound applied separately.[30, 31, 40] Moreover, a behavioral study using a conditioned taste aversion paradigm revealed 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 mM CPPG or AIDA.[40] A conditioned taste aversion experiment using wild type C57BL mice demonstrated that the mGluR antagonists at 1 mM suppressed avoidance responses to MSG at concentrations ranging from micromolar to millimolar levels that may overlap with functional concentration ranges for both brain-type and taste-type mGluRs.[40] Brain-expressed mGluR1 and mGluR4 are expressed in a subset of taste cells in both the anterior and the posterior tongue.[19, 20] Taken together, all these results suggest that mGluR1 and GluR4 may be related to umami detection in addition to T1R1/T1R3.

VARIATION OF UMAMI RECEPTOR GENES IN MICE

To understand the association of genetic variation with umami taste responses in both human and rodents, we investigated possible relationships of individual differences in umami sensitivity with single nucleotide polymorphisms (SNPs) of *Tas1r1*, *Tas1r3*, *Grm1* and *Grm4*. In a recent study, there are 3 SNPs with an amino acid substitution in mouse *Tas1r1* (M347T, K443N and K626E) between C57BL/6J and 129P3/J,[43] and no amino acid changes in both mouse *Grm1* and *Grm4* among C57BL/6J, 129S1/SvImJ, 129X1/SvJ and C3H/HeJ (unpublished observation). Moreover the B6/129 sequence variants do not affect its sensitivity to umami compounds, although the T1R3 receptor is involved in transduction of umami taste.

It has been speculated that MSG and IMP each bind to the T1R1, because neither has any effect on the T1R2/T1R3 sweet taste receptor.[44] These results suggest that the differences in umami sensitivity among inbred strains may be related to these amino acid mutations in *Tas1r1*, not to ones in *Tas1r3*, *Grm1* and *Grm4*.

INTER-INDIVIDUAL DIFFERENCES OF UMAMI SENSITIVITY IN HUMANS

Lugaz *et al.* reported individuals with a specific ageusia to MSG.[45] In that study, the sample distribution of individual MSG detection thresholds showed a bi-modal distribution curve, with taste thresholds of MSG differing about 5-fold between taster (mean = 0.08 mM, ranging from 0.03 to 0.18 mM) and hypotaster (mean = 0.39 mM, ranging from 0.14 to 1.07 mM). They also reported that subjects could be classified into the taster [81% (47/58) of subjects], hypotaster [At least 10% (6/58)] and non-taster [3.5% (2/58)] categories by four tests [1. detection threshold, 2. isointensity (reference = 29 mM NaCl), 3. time-intensity MSG > NaCl and 4. discrimination test].[45]

VARIATION OF UMAMI RECEPTORS IN HUMANS

A recent molecular study indicated that complete DNA sequences of *TAS1R1*- and *TAS1R3*-coding regions revealed 14 and 6 non-synonymous SNPs in *TAS1R1* and *TAS1R3*, respectively. In the dbSNP in NCBI database analysis, we found 7 SNPs in *TAS1R1*, 5 SNPs in *TAS1R3*, 8 SNPs in *GRM1*, and 1 SNP in *GRM4* for a total of 21 variant amino acid sites (unpublished observation). Of these, V110A, E347 K, T372A and C603R in *TAS1R1*, R757C in *TAS1R3* have been reported by Kim *et al.*[46]

Examination of the distribution of polymorphisms across the different domains of the protein shows that 61.1%, (22/36) of the variant amino acid positions reside in the predicted N-terminal extracellular ligand-binding domain, 22.2% (8/36) in the transmembrane domain, 13.9% (5/36) in the C-terminal intracellular domain, and 2.8% (1/36) in the cysteine rich domain which intervenes between the N-terminal ligand-binding and transmembrane regions. One SNP, which substitutes an A for the normal G at position 2318 in the *TAS1R1* cDNA sequence introduces a premature stop codon.[46]

Therefore, we investigated possible relationships of individual differences in umami sensitivity with SNPs of *TAS1R1*, *TAS1R3*, *GRM1* and *GRM4* in human. The results indicated that distributions of MSG and IMP taste recognition thresholds showed a normal distribution curve and ones

of MSG with IMP showed a tri-modal distribution curve. In a sequencing analysis, 5 SNPs (amino acid positions: E12 H, T139 M, N191S, E347 K, T372A) in *TAS1R1*, 3 SNPs (T716 K, R757C, R825S) in *TAS1R3* and 1SNP (S993P) in *GRM1* with amino acid substitutions were identified. A statistical study showed significant association of recognition thresholds for umami substances with *TAS1R1* (E12 H, T372A), *TAS1R3* (R757C) and *GRM1* (S993P) (Shigemura *et al.*, unpublished observation). Moreover, a recent report suggested that T1R1/T1R3 receptor was likely to be the umami receptor and that this receptor variants underlay inter-individual differences of umami sensitivity in humans.[47)]

RECEPTORS AND TRANSDUCTION PATHWAYS FOR UMAMI TASTE

Accumulating evidence suggests the existence of multiple receptor systems involving T1R1/T1R3 heterodimers, and brain- and taste-types of mGluR1 and mGluR4 for umami taste. Also, the signal transduction pathway for umami taste may involve the G protein Gα-gustducin, (and transducin), the phospholipase C (PLC) isoform, PLCβ2, IP_3R3 and TRPM5. This interpretation is based on the above-mentioned findings that taste nerve responses and behavioral responses to umami stimuli were largely reduced or absent in mice genetically lacking each of these molecules in taste cells. One major transduction pathway for umami taste, which is common to sweet and bitter tastes, has been proposed. That is, binding of umami compounds preferentially to T1R1/T1R3 receptors activates the heterodimeric G proteins α-gustducin[36)] or Gαi,[48)] leading to the release of the G$\beta\gamma$ subunits and the subsequent stimulation of PLCβ2.[49)] Activation of PLCβ2 hydrolyses phosphatidylinositol-4,5-bisphosphate to produce the second messengers IP_3 and diacylglycerol.[50)] Binding of IP_3 to IP_3R3 leads to Ca^{2+} release from intracellular stores, which activates TRPM5 channels.[37)] Activation of TRPM5 leads to Na^+ entry, membrane depolarization and generation of action potentials in taste cells.[38,39)] If mGluR receptors are related to the residual umami responses in mice lacking each of the downstream molecules, transduction pathways activated by mGluR receptors may be different from ones activated by T1R1/T1R3.

Recent studies demonstrated that taste cells expressing T1R and T2R receptors and the above-mentioned downstream signaling molecules for sweet, bitter and umami tastes (Type II cells) release adenosine 5′-triphosphate (ATP) through pannexin and connexin hemichannels dependent upon membrane depolarization, generation of action potentials, and the increase of cytoplasmic Ca^{2+}.[51,52)] Genetic elimination of ionotropic purinergic receptors (P2X2 and P2X3) abolished taste responses without affecting responses to mechanical and thermal stimulations, suggesting that ATP is a transmitter linking taste receptor cells to nerve fibers.[53)]

In conclusion, this paper summarizes recent findings on umami taste. Most of the findings support the idea that multiple receptors exist for umami taste. 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, is active in mice lacking T1R3, Gα-gustducin, IP_3R3, 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 account for umami-linked preferences or discrimination.

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