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Multiple Receptor Systems for Glutamate Detection in the Taste Organ

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L-Glutamate and 5'-ribonucleotides such as guanosine-5'-monophosphate (GMP) and inosine-5'-monophosphate (IMP) elicit a unique taste called 'umami' that is distinct from the tastes of sweet, salty, sour, and bitter. For umami, like sweet and bitter compounds, taste signaling is initiated by binding of tastants to G-protein-coupled receptors (GPCR) in taste bud cells. To date, several GPCRs for umami compounds have been identified in taste cells, including the heterodimer T1R1/T1R3, and truncated type 1 and 4 metabotropic glutamate receptors missing most of the N-terminal extracellular domain (taste-mGluR4 and truncated-mGluR1). Apparently contradictory data in T1R3 knock-out (KO) mouse models have been reported. One study showed that behavioral preference and taste nerve responses to umami stimuli in T1R3-KO mice were totally abolished, suggesting that T1R1/T1R3 is a sole receptor for umami taste. The other reported reduced but not abolished responses to umami in T1R3-KO mice, suggesting existence of multiple receptors for umami taste. In this paper, we summarized the data from recent studies that further addressed this issue by using different experimental techniques. Some of the studies provided additional evidence for the existence of umami receptor systems mediated by mGluR1 and mGluR4 in addition to T1R1/T1R3. It is proposed that the signal mediated by the pathway involving T1R1/T1R3 may play a different role from that derived from the mGluRs. The former occurs mainly in the anterior tongue, and plays a major role in preference behavior, whereas the latter occurs mainly in the posterior tongue and contributes to behavioral discrimination between umami and other taste compounds.

Key words umami taste; receptor; transduction pathway; downstream signaling molecule

1. INTRODUCTION

Monosodium L-glutamate (MSG) is widely distributed in many foods such as meat, fish, 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)} The characteristic feature of umami is synergism, the taste intensity of MSG is enhanced by 5'-ribonucleotide monophosphates such as inosine-5'-monophosphate (IMP) and guanosine-5'-monophosphate (GMP).^{5,6)}

Recent progress in molecular biology has identified candidates for umami receptor. The candidate molecule first discovered is a variant of brain-expressed metabotropic glutamate receptor 4 (taste-mGluR4). This variant is expressed in rat circumvallate taste buds on the posterior tongue innervated by the glossopharyngeal (GL) nerve and has a truncated N-terminal to which glutamate still binds, albeit with reduced affinity.^{7,8)} The secondly discovered candidate is a heterodimer of T1R1/T1R3.^{9,10)} The mouse T1R1/T1R3 heterologously expressed in human embryonic kidney (HEK) cells responds to many amino acids present in food, whereas the human type heteromer preferentially responds to glutamate and this response is enhanced by IMP.^{9,10)} The sequencing and functional expression of the human taste receptor for glutamate determined by these studies provided the first molecular basis for umami detection and perception in humans. However, with regards to animal 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,¹¹⁾ indicating that the T1R1/T1R3 solely contributes to MSG detection and perception in mice. In contrast, the other study revealed that behavioral preference for MSG was reduced but not abolished in the T1R3-KO mice. Analyses of taste nerve responses to umami stimuli demonstrated that in T1R3-KO mice the synergism between MSG and IMP was abolished in the CT responses but no large reduction was observed in responses to MSG alone in both CT and GL nerve responses.¹²⁾ These results suggest the existence of multiple receptors for umami taste. The other candidate is a variant of type I metabotropic glutamate receptor. This variant, like taste-mGluR4, is expressed in rat circumvallate taste buds on the posterior tongue and has a truncated N-terminal domain (truncated mGluR1) with low binding affinity to glutamate.¹³⁾

Our laboratory and others have further investigated receptor and transduction pathways for umami taste by using molecular, neurophysiological, pharmacological, and behavioral techniques. This paper summarizes the data obtained from recent studies, most of which provided evidence for involvement of T1R1/T1R3-independent receptor systems (*i.e.*, through mGluRs) in umami detection, and thus for the existence of multiple receptors and transduction pathways for umami taste.

2. TONGUE REGIONAL DIFFERENCES IN SENSITIVITY TO UMAMI COMPOUNDS

Previous electrophysiological studies in several mam-

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malian species^{14–16)} demonstrated differences in sensitivities of taste bud cells located on the anterior and posterior parts of the tongue, innervated by the CT and GL nerve, respectively. The most clear-cut difference may be sensitivity to amiloride,^{14,17,18)} a sodium channel blocker in various epithelial cells and an inhibitor of NaCl responses in taste cells, and gurmarin, a peptide isolated from the plant, *Gymnema sylvestre*, and a sweet response inhibitor, in some strains of mice^{19,20)} and rats.²¹⁾ The two compounds selectively suppress responses of the CT nerve to NaCl or sweeteners by about 50% of control, but do not inhibit responses of the GL nerve to any stimulus. Typical umami substances (US), such as MSG, IMP and mixture of MSG and IMP, contain Na⁺, which explains why responses of the CT nerve to these US are partly suppressed by amiloride. Gurmarin is a sweet response inhibitor for rats²¹⁾ and C57BL mice; however, it has a minimal effect on sweet responses in BALB mice or sweetness perception in humans.^{21,22)} In the CT nerve of several mammalian species, single fiber response analysis indicated that most fibers showing large synergism between MSG and IMP are sensitive predominantly to sucrose (S-type fibers).²³⁾ In C57BL mice, gurmarin almost completely abolished responses of this particular group of fibers not only to sucrose but also to mixture of MSG and IMP. However, CT fibers predominantly sensitive to US including the metabotropic glutamate agonist 2-amino-4-phosphonobutyrate (L-AP4) (M-type fibers) exhibited no such suppression of responses to US by gurmarin.^{23,24)} As compared to the CT nerve, the GL nerve of the mouse contains a much greater number of M-type fibers and showed greater responses to US.²³⁾ Consistently, behavioral studies using a conditioned taste aversion paradigm demonstrated that normal mice could discriminate MSG from the four basic taste stimuli.^{4,25)} However, mice that had undergone bilateral sectioning of the GL nerve could not discriminate MSG from NaCl, suggesting that the GL nerve conveys taste information for US, 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 monkeys⁵⁾ 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.²⁶⁾ Thus the posterior part of the tongue may be the dominant site for glutamate taste perception in several mammals.

3. RESIDUAL RESPONSES TO UMAMI STIMULI IN MICE LACKING T1R3, IP₃R3 OR TRPM5

As mentioned above, two previous studies^{11,12)} demonstrated that in T1R3-KO mice behavioral preference and CT nerve responses to US including the synergism between MSG and IMP were greatly reduced. This suggests that T1R1/T1R3 receptors play a major role in preference for umami taste and occurrence of the synergism between US in the anterior tongue. These findings are comparable with *in vivo* data showing that T1R1 and T1R3 are coexpressed in mouse fungiform taste bud cells in the anterior tongue,²⁷⁾ and the heterodimer T1R1/T1R3, when expressed in heterologous cells, synergistically responds 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 US

in the GL nerve were not largely affected by genetic deletion of T1R3 reported by Damak *et al.*¹²⁾ In accordance with this finding, recent behavioral and electrophysiological studies further revealed no large reduction of responses to US in T1R3-KO mice.^{24,28,29)} For example, a behavioral study²⁸⁾ 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. Maruyama *et al.*,²⁹⁾ investigated umami taste responses in mouse circumvallate taste buds innervated by the GL nerve, and found that taste cells in T1R3-KO mice exhibited umami-evoked Ca²⁺ responses that still persist, albeit with a decreased amplitude. Finally, our laboratory examined taste nerve responses to umami compounds at single fiber levels, and found that umami-responsive single CT fibers in wild type mice could be classified into sucrose-best responsive S-type and umami-best responsive M-type fibers. S-type showed a large synergistic effect on responses to MSG or monopotassium glutamate (MPG) when IMP was added. In T1R3-KO mice, S-type fibers showing the large synergism were absent, but M-type fibers with slight or no synergism still remained.²⁴⁾ These data suggest that T1R3-KO mice lack the signal elicited by MSG in the anterior tongue that are not umami specific and may be similar to those elicited by sweet compounds (S-type cell and fibers). This signal may play a major role in behavioral preference. But they still possess umami specific signals elicited by MSG both in the anterior and posterior tongue (M-type cell and fibers), and thereby mice are able to discriminate between tastes of umami and sweet compounds.

Correspondingly, no large reduction of behavioral and neural responses to US has also been reported in mice lacking gene(s) controlling downstream signaling molecules, such as G protein α subunit (G α) gustducin and/or transducin,³⁰⁾ type III inositol-1-4,5-triphosphate receptor (IP₃R3)³¹⁾ and a cation channel, transient receptor potential M5 channel (TRPM5).^{32,33)} Figure 1 shows concentration-response curves for MPG and mixture of MPG and IMP (MPG+IMP) obtained from T1R3-, IP₃R3-, TRPM5-KO mice and their wild type counterparts with the same C57BL genetic backgrounds. It is noted that these three lines of KO mice exhibited almost identical responsiveness to US with reduced CT nerve responses, no synergism and almost unaffected GL nerve responses. G α -gustducin-KO mice were also reported to show similar umami responsiveness, although their genetic background was not derived from the C57BL strain (mixed background of 129 and BALB strains).³⁰⁾ Together, these data provide additional evidence for the involvement of T1R3, G α -gustducin, IP₃R3 and TRPM5 in umami detection on the anterior tongue, and again existence of another pathways independent upon these molecules both on the anterior and posterior tongue.

4. EFFECTS OF ANTAGONISTS FOR mGluRs ON UMAMI RESPONSES

If there exist T1R3-independent receptor and transduction pathways for umami detection, mGluRs or combinations of T1R1 and mGluRs could be candidate receptors. To investi-

Fig. 1. Concentration-Response Curves for MPG and Mixture of MPG and IMP (MPG+IMP) Obtained from the Chorda Tympani (CT) and Glossopharyngeal (GL) Nerve of T1R3- (Open Circles), IP₃R3- (Filled Triangles), TRPM5- (Open Triangles) KO Mice and Their Wild Type Counterparts (Filled Circles) with the Same C57BL Genetic Backgrounds

It is noted that these three lines of KO mice exhibited almost identical responsiveness to US with reduced CT nerve responses, no synergism and almost unaffected GL nerve responses.

gate potential contribution of mGluRs, we examined the effects of selective antagonists for both mGluR1 [1-aminoindan-1,5-dicarboxylic acid (AIDA)] for group I mGluRs³⁴ and mGluR4 [(*RS*)- α -cyclopropyl-4-phosphonophenylglycine (CPPG)] for group III mGluRs³⁵ on taste nerve responses to US. Figure 2A shows sample recordings of integrated whole nerve responses of the GL nerve in C57BL mice to mixture of 100 mM MPG and 10 mM CPPG and to each component alone. These three stimulants produced similar magnitudes of responses, indicating no additive increase in the integrated nerve response by addition of CPPG to MPG. As shown in Fig. 2B, concentration-response curves for mixture of MPG with CPPG or AIDA are significantly lower than that for the sum of responses to each component alone. This indicates suppressive effects of CPPG and AIDA on MPG responses. Inhibition of responses to US by these antagonists was more clearly observed in the measurements of responses of particular groups of single fungiform taste cells and single CT fibers. That is, the responses to MSG with amiloride and MPG were suppressed by the addition of CPPG or AIDA in M-type taste cells (Yoshida *et al.*, unpublished observation) and fibers (Yasumatsu *et al.*, unpublished observation), whereas no such suppression was evident in S-type cells and fibers. Moreover, behavioral studies using a conditioned taste aversion paradigm revealed that T1R3-KO mice were still able to learn to avoid US and the conditioned avoidance to US measured by short-term lick responses. The conditioned avoidance responses to US were largely reduced by the addition of CPPG or AIDA to US. (Shirosaki *et al.*, unpublished observation). Behavioral experiments using the wild type C57BL mice demonstrated that the mGluRs antagonists at 1 mM suppressed 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. Brain-expressed mGluR1 and

Fig. 2. (A) Sample Recordings of Integrated Whole Nerve Responses from the Glossopharyngeal Nerve of C57BL Mice to 0.1 M NH₄Cl, 0.1 M MPG, 10 mM CPPG and Mixture of 0.1 M MPG and 10 mM CPPG

The response to 0.1 M NH₄Cl was used as a standard.

(B) Concentration-Response Curves for Mixture of MPG with CPPG or AIDA (Filled Triangles), MPG (Filled Circles) and the Sum of Responses to Each Component Alone (Open Circles)

It is noted that responses to the mixture are significantly lower than that for the sum of responses to each component alone.

mGluR4 are shown to be expressed in rat taste cells in the posterior tongue.^{36,37} Taken together, all these results indicate involvement of mGluR1 and mGluR4 in addition to T1R1/T1R3 in umami detection.

5. PROPOSED RECEPTOR AND TRANSDUCTION PATHWAYS FOR UMAMI TASTE

Accumulating evidence suggests existence of multiple receptor systems involving T1R1/T1R3 heterodimers, brain- and taste-types of mGluR1 and mGluR4 for umami taste. Also signal transduction pathway for umami taste may involve G protein G α -gustducin, (and transducin) the phospholipase C (PLC) isoform, PLC β 2, IP₃R3 and TRPM5. This interpretation is based on the above-mentioned findings that taste nerve responses and behavioral responses to US 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. As shown in Fig. 3, binding of umami compounds to T1R1/T1R3 and/or other receptors (mGluRs) activates the heterodimeric G proteins gustducin³⁰ or G α i³⁸ leading to the release of the G $\beta\gamma$ subunits and the subsequent stimulation of PLC β 2.³⁹ Activation of PLC β 2 hydrolyses phosphatidylinositol-4,5-bisphosphate to produce the second messengers IP₃ and diacylglycerol.⁴⁰ Binding of IP₃ to IP₃R3 leads to Ca²⁺ release from intracellular stores which activates TRPM5 channels.³¹ Activation of TRPM5 leads to Na⁺ entry, membrane depolarization and generation of action potential of taste cells.³² Recent studies demon-

Fig. 3. Models for Signal Transduction Pathways for Umami Taste

One major pathway proposed is as follows: binding of umami compounds to T1R1/T1R3 and/or other receptors (brain- and taste-mGluRs) activates the heterodimeric G proteins gustducin or $G\alpha_i$ leading to the release of the $G\beta\gamma$ subunits and the subsequent stimulation of phospholipase ($PLC\beta_2$), and reduction of cAMP level through the activation of phosphodiesterase (PDE). Activation of $PLC\beta_2$ hydrolyses phosphatidylinositol-4,5-bisphosphate (PIP_2) to produce the second messengers IP_3 and diacylglycerol (DAG). Binding of IP_3 to IP_3R3 leads to Ca^{2+} release from intracellular stores which activates TRPM5 channels. Activation of TRPM5 leads to Na^+ entry, membrane depolarization and generation of action potential of taste cells. DAG may activate protein kinase C (PKC) which leads to closure of potassium channel and depolarization of the cell membrane. Umami-responsive cells are proposed to release adenosine 5'-triphosphate (ATP) through pannexin and connexin hemichannels dependent upon membrane depolarization, generation of action potential, and increase of cytoplasmic Ca^{2+} . Ionotropic purinergic receptors (P2X2 and P2X3) on the taste axons detect the ATP signal and convey information from taste cells to the brain.

strated that taste cells expressing T1R and T2R receptors and the above-mentioned downstream signaling molecules for sweet, bitter and umami tastes (receptor cells: Type II cells) release adenosine 5'-triphosphate (ATP) through pannexin and connexin hemichannels dependent upon membrane depolarization, generation of action potential, and increase of cytoplasmic Ca^{2+} .^{41,42)} 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.⁴³⁾

In conclusion, this paper summarized recent findings on umami taste. Most of the findings support the idea that there exist multiple receptors and transduction pathways 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 from 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 still active in mice lacking T1R3, $G\alpha$ -gustducin, IP_3R3 , and TRPM5, and contributes to behavioral discrimination between umami and other taste com-

pounds.

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