T1R receptors mediate mammalian sweet and umami taste

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ABSTRACT

The T1R family of taste receptors mediates 2 taste qualities: T1R2/T1R3 for sweet taste and T1R1/T1R3 for umami taste. Functional expression in heterologous system and gene knockout studies has shown their functions as taste receptors. Structure-function relation studies on T1R2/T1R3 showed multiple ligand binding sites on both subunits. The umami taste of L-glutamate can be drastically enhanced by 5′ ribonucleotides, and the synergy is a hallmark of this taste quality. On the basis of chimeric T1R receptors, site-directed mutagenesis, and molecular modeling data, we recently proposed a cooperative ligand binding model that involved the Venus flytrap region and 5′ ribonucleotides bind to an adjacent site close to the opening of the flytrap to further stabilize the closed conformation. This novel mechanism may apply to other class C, G protein–coupled receptors. Am J Clin Nutr 2009;90(suppl):733S–7S.

INTRODUCTION

Humans can detect \( \geq 5 \) basic taste qualities, including sweet, umami, bitter, salty, and sour. Because of the limited amount of taste tissue, identification of mammalian taste receptors has lagged behind other sensory modalities, such as vision and olfaction. Great progress has been made over the past decade, and the molecular identity of many taste signaling components have been elucidated. The breakthrough started with a family of G protein–coupled receptors (GPCRs) named T1R. This review focuses on the discovery and functional characterization of T1R.

DISCOVERY OF T1R RECEPTORS

There are 3 genes in the T1R family. The first 2 (T1R1 and T1R2) were identified in 1999 by sequencing a subtracted cDNA library derived from rat taste tissue (1). The 2 receptors belong to the family of class C GPCRs, distantly related to calcium sensing receptor, V2R pheromone receptors, and metabotropic glutamate receptors (mGluRs). In situ hybridization has shown selective expression in taste tissue and a distinct expression pattern of the 2 receptors, with T1R1 more enriched in the fungiform taste buds and T1R2 in the circumvallate taste buds. The 2 T1Rs were proposed to be taste receptors but were not linked to any specific taste quality.

In 2001, the third member of the T1R family, T1R3, was identified in the human genome (2–7). Importantly, mouse T1R3 was mapped to a genomic interval containing Sac, a locus that influences sweet taste sensitivity in mice. Different inbred strains of mice are known to have different sweet taste sensitivities. The difference was found to be dependent solely on the Sac locus (8). The dominant Sac allele (taster) is associated with higher taste sensitivity than the recessive allele (nontaster). To prove that the Sac locus does encode T1R3, transgenic mice were generated to introduce the T1R3 gene from a taster into a nontaster strain. As a result, the taste deficiency of nontaster mice was fully rescued, indicating that T1R3 was indeed the Sac gene (2).

T1R3 was also found to be selectively expressed in a subset of taste receptor cells. Although T1R1 and T1R2 are expressed in different subsets of taste receptor cells, they are each coexpressed with T1R3 (2, 4, 5), which suggests a heterodimeric receptor formation similar to the \( \gamma \)-aminobutyric acid type B receptor. Besides the T1R1/T1R3 and T1R2/T1R3 cells, a fraction of T1R3-expressing cells do not express either T1R1 or T1R2 (Figure 1).

FUNCTIONAL EXPRESSION OF T1Rs

Functional expression of T1Rs in heterologous cells provided the most direct evidence for the taste qualities associated with these receptors. Following their in vivo expression pattern, T1R2 and T1R1 were each coexpressed with T1R3 in human embryonic kidney (HEK) cells and stimulated with different taste stimuli (2, 9). The heteromeric human T1R2/T1R3 receptor selectively responded to all of the \( \sim 20 \) known sweeteners tested at physiologically relevant concentrations, and the responses were inhibited by lactisole, a human sweet taste inhibitor (9). Similarly, rat T1R2/T1R3 also responded to all of the dozen molecules that generate similar behavioral responses as sucrose (Figure 2A).

The observation that T1R2/T1R3 recognizes all sweeteners suggested that T1R1/T1R3 mediates a different taste quality. Indeed, human T1R1/T1R3 receptor expressed HEK cells selectively responded to L-glutamate (Figure 2B) (9), the primary umami taste stimulus. Discovered in 1908 by Ikeda, umami taste was only recently accepted as a basic taste quality. Synergism is the most unique characteristic of umami taste. Purinic ribonucleotides such as inosine-5′-monophosphate (IMP) and

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First published online August 5, 2009; doi: 10.3945/ajcn.2009.27462G.
guanosine-5′-monophosphate strongly potentiate umami taste (10). Before the discovery of T1Rs, a splicing variant of mGluR4 (taste-mGluR4) had been proposed to mediate umami taste (11). Taste-mGluR4 was detected in taste tissue by reverse transcription–polymerase chain reaction and responded to glutamate and 2-amino-4-phosphonobutyrate when expressed in Chinese hamster ovary cells. Several lines of evidence argue against the idea. First, the truncated receptor is missing a large portion of the Venus flytrap (VFT) domain, which is essential for glutamate binding. It is hard to imagine that such a truncated form of mGluR4 can still form a binding site for glutamate. Second, the activities of the receptor are inconsistent with the hallmark characteristic of umami taste: No synergy was observed between glutamate and ribonucleotides. Third, one would expect diminished glutamate preference in mGluR4 knockout mice if it is involved in umami taste, but the mice displayed enhanced glutamate preference.

In contrast, the activities of human T1R1/T1R3 in heterologous cells correlate nicely with umami taste (9). The heteromeric receptor recognized glutamate with an effective concentration for 50% inhibition closely matching umami detection threshold, and, more importantly, the glutamate-induced activity was strongly potentiated by IMP and guanosine-5′-monophosphate. The human receptor is highly selective for umami stimuli, responding to only glutamate, aspartate, and L-2-amino-4-phosphonobutyrate. In contrast, mouse T1R1/T1R3 was found to be far more promiscuous, responding to virtually all L-amino acids in heterologous cells (2).

The same functional assays for human T1R1/T1R3 and T1R2/T1R3 were adopted for high-throughput screening of synthetic libraries at Senomyx Inc (San Diego, CA). To date, multiple novel chemical classes of umami tasting compounds, sweeteners, and sweet taste enhancers (G Servant et al, unpublished data, 2009) have been identified, further validating the role of T1Rs as human sweet and umami taste receptors.

**TIR KNOCKOUT MICE**

Behavioral and physiologic studies with the use of knockout mice confirmed that T1Rs mediate sweet and umami taste. Zhao et al (12) generated systematic T1R1, T1R2, T1R3 knockouts, and T1R2/T1R3 double knockouts and analyzed the animals with the use of brief access taste tests and chorda tympani (CT) nerve recording. As expected, T1R1-null mice exhibited complete loss in preference for umami tastants; T1R2-null mice exhibited complete loss in preference for artificial sweeteners and greatly diminished preference for sugars; and T1R3-null mice lost preferences for both umami stimuli and artificial sweeteners completely and displayed greatly diminished responses to sugars. The CT nerve responses were consistent with the behavioral data. Interestingly, both T1R2- and T1R3-null mice retained a certain level of behavioral and CT nerve responses to high concentration of sugars. Only the T1R2/T1R3 double knockout mice completely lost the residual responses. It is possible that T1R2 or T1R3 alone can function as low-affinity sweet taste receptor without their partner. Indeed, in situ hybridization experiments in mice showed that a significant number of taste receptor cells express T1R3 without T1R1 or T1R2, and HEK cells expressing mouse T1R3 responded to high concentrations of sucrose, which led to the hypothesis that T1R3 homodimer could be a second sweet taste receptor in addition to T1R2/T1R3. However, the mouse T1R3 response to high concentration of sugars was not observed with human T1R3 (X Li and H Tang, unpublished data, 2005).

Independently, Damak et al (13) also generated T1R3 knockout mice and reported different results. With the use of

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**FIGURE 1. TIR expression patterns define 3 cell types.** Double-label fluorescent in situ hybridization was used to directly examine the overlap in cellular expression of T1Rs. A: Fungiform papillae showing coexpression (yellow) of T1R1 (red) and T1R3 (green). At least 90% of the cells expressing T1R1 also express T1R3; similar results were observed in the palate. Note the presence of some T1R3-positive but T1R1-negative cells. B: Circumvallate papillae showing coexpression (yellow) of T1R2 (green) and T1R3 (red). Every T1R2-positive cell expresses T1R3. Reproduced with permission from reference 2.
T1R2, T1R3, and G1 receptors respond to glutamate dose dependently, and the activities are greatly potentiated by IMP. Reproduced with permission from reference 9.

FIGURE 2. Functional expression of T1R taste receptors in human embryonic kidney (HEK) cells. A: Gx15 cells transiently transfected with human T1R2 and T1R3 and HEK-293T cells transiently transfected with rat T1R2, T1R3, and Gx15/i1 were assayed for intracellular calcium increases in response to 300 mmol sucrose/L in the presence (+) and absence (−) of 1.25 mmol lactisole/L. Human and rat T1R2/T1R3 heteromeric receptors respond to sucrose. Lactisole inhibits the human but not the rat receptor activities. B: Gx15 cells transiently transfected with human T1R1 and T1R3 were assayed for intracellular calcium increases in response to increasing concentrations of L-glutamate in the presence or absence of 1 mmol inosine-5′-monophosphate (IMP)/L. Human T1R1/T1R3 heteromeric receptor responds to glutamate dose dependently, and the activities are greatly potentiated by IMP. Reproduced with permission from reference 9.

traditional 2-bottle preference tests, the investigators found that their T1R3-null mice displayed no preference for artificial sweeteners, diminished preference for sucrose, but essentially the same preferences as the wild-type mice for glucose and maltose. The preference for monosodium glutamate (MSG) in T1R3-null mice was reduced but not abolished. Whole-nerve recording experiments showed that the CT nerve responses were largely consistent with the behavioral data. However, the glossopharyngeal nerve responses were largely unchanged in the T1R3-null mice. The investigators concluded from this study that mice can still taste sweet and umami in the absence of T1R3 and proposed other receptors exist for the 2 taste qualities.

The conflicting results from behavioral studies in these 2 reports are likely due to the different taste test protocols. The brief access taste tests were carried out within 30 min, and stimuli were presented in 5-s trials, whereas in 2-bottle tests, the animals were exposed to the tastants for 48 h. By now, it is well established that postigestive effects can greatly distort the outcome of 2-bottle preference tests. Recently, de Araujo et al (14) showed that TRPM5-null mice, which lack the cellular machinery required for sweet taste transduction, can develop a robust preference for sucrose solutions based solely on caloric content. The postigestive effect of MSG is also documented in the literature (12, 15). Therefore, data from the brief access tests are more relevant to taste physiology. The different results from nerve recording experiments are hard to reconcile. However, one might find some of the nerve responses reported in Damak et al (13) inconsistent with the behavioral data. For example, there is a clear behavioral difference between T1R3-null and wild-type mice in detecting 30–100 mmol MSG/L, yet no difference was detected in either CT or glossopharyngeal nerve responses. In general, it is not easy to correlate whole-nerve recording data with taste, because many tastants trigger responses of one or more taste modality. At high concentrations, MSG induces both umami and salty taste. The nerve responses to the salty tastants can only be partially blocked by amiloride in CT nerves (16). Therefore, a significant proportion of the whole-nerve activities induced by MSG >100 mmol/L could be due to its salty taste, especially in the glossopharyngeal nerves.

With the use of the same T1R3-null mice as reported in Damak et al (13), Delay et al (17) later reported that the knockout mice had essentially the same detection thresholds as did the wild-type mice for sucrose and MSG and could differentiate MSG from sucrose as well as the wild-type mice. The data were used to argue for the existence of additional receptors for both sweet and umami tastes. However, the experimental procedures gave the animals repeated exposure to sucrose and MSG during the training trials, and the reward and punishment scheme probably provided the animals a much stronger learning motivation than did the postigestive effect. The animals could be using sensory cues other than sweet and umami tastes to detect the tastants.

FUNCTIONAL DOMAINS OF T1R2/T1R3

T1R2/T1R3 recognizes all sweeteners, including sugars, D-amino acids, sweet proteins, and various artificial sweeteners. How does a single receptor interact with so many structurally diverse molecules? There are likely multiple ligand binding sites on both T1R2 and T1R3 subunits, which are all capable of activating the receptor. Human and rodent sweet receptors have different ligand specificities. A number of artificial sweeteners, sweet proteins, and the sweet taste inhibitor lactisole work on the human but not the rodent receptor. T1R2 appeared to confer recognition of a large proportion of such sweeteners, because the “humanized mice” (T1R2-null mice expressing human T1R2 transgene) exhibited preference for aspartame, glycyrrhizic acid, and sweet proteins monellin and thumatin (12). We exploited these species differences to ask which domains of the human receptor enable the recognition of these ligands (18). With the use of human-rat chimeric T1R receptors, we first showed the different roles of functional domains on human T1R2/T1R3: the T1R2 VFT domain is required for recognizing aspartame and neotame, whereas the T1R3 transmembrane domain is required for responding to cyclamate and lactisole. The T1R2 transmembrane domain is required for G protein coupling. S819, a novel synthetic sweetener identified at Senomyx, targeted the T1R2 transmembrane domain (19). Because cyclamate and lactisole probably bind to T1R3, the shared subunit of the sweet and the umami taste receptors, we tested the effects of these compounds on the umami taste receptor. We found that both compounds modulated umami taste receptor activity, indicating the 2 compounds most likely do bind to T1R3. Lactisole inhibited the human T1R1/T1R3 receptor in the receptor assay and human umami taste in sensory tests (18). Lactisole had not been known as an umami taste inhibitor until...
our finding, which further validates T1R1/T1R3 as the human umami taste receptor. Cyclamate did not activate the umami taste receptor but did potentiate the activity induced by glutamate. The interaction sites for lactisole and cyclamate on the T1R3 transmembrane domain were later confirmed and further defined by other groups with the use of human-rodent chimeric T1Rs and site-directed mutagenesis studies (20–22).

Similar chimeric receptor approaches were used to determine potential interaction sites for other sweeteners. The cysteine-rich region of human T1R3 was shown to be required for responding to the sweet protein brazzein (23). Neohesperidin dihydrochalcone was shown to require the human T1R3 transmembrane domain (24), whereas neocolin appeared to require human T1R3 VFT domain (25). With the use of purified recombinant proteins, Nie et al (26) observed conformation changes in both T1R2 and T1R3 N-terminal domains induced by sucralose, sucrose, and glucose, suggesting both subunits are involved in ligand binding for these sweeteners. Each T1R protein can be roughly divided into the N-terminal extracellular and the transmembrane domains. To date, all 4 domains from both subunits of the T1R2/T1R3 sweet taste receptor have been implicated in binding different ligands (Figure 3).

SYNERGISTIC EFFECT IN UMAMI TASTE

In contrast to the sweet taste receptor, the functional domains of the umami taste receptor remain largely unknown. The synergistic effect between glutamate and purinic ribonucleotides represents the only naturally occurring example of such. With the use of chimeric T1R receptors, mutagenesis analysis, and molecular modeling, we collected data that showed a novel molecular mechanism for the synergy (19): both glutamate and IMP interact with the T1R1 VFT domain (Figure 3). Glutamate binds near the hinge region and induces the closure of the VFT domain, whereas IMP binds near the opening and further stabilizes the closed conformation. The same mechanism could also apply to other class C GPCRs. In fact, sweet taste enhancer molecules identified at Senomyx work similarly to IMP on the human sweet taste receptor (X Li et al, unpublished data, 2009).

CONCLUSIONS

Recent identification and functional studies on T1R taste receptors have provided significant insights into the molecular basis of taste signal transduction. Functional expression and mouse gene knockout studies firmly established T1R2/T1R3 as the principal sweet taste receptor and T1R1/T1R3 as the principal umami taste receptor. Mammalian sweet and umami taste receptors are closely related and share the same evolutionary origin.

Scientific breakthroughs in taste receptor biology have found immediate applications in the food and beverage industry. On the basis of the activities of T1R taste receptors in functional assays, novel flavor ingredients have been developed to modulate sweet and umami taste and will soon generate positive effects on public health. (Other articles in this supplement to the Journal include references 27–55.)

I thank B Moyer, A Pronin, H Liu, and H Xu for comments on the manuscript.

The author is an employee of Senomyx Inc, a company that develops novel flavors, flavor enhancers, and bitter blockers for the food, beverage, and ingredient supply industries, including umami tastants and enhancers. The author’s expenses and an honorarium associated with participation in the symposium were paid by the conference sponsor, the International Glutamate Technical Committee, a nongovernmental organization funded by industrial producers and users of glutamate in food.

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