

Introductory Remarks on Umami Research: Candidate Receptors and Signal Transduction Mechanisms on Umami

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Umami as a unique taste

Nearly a century ago, Ikeda insisted that there existed one other taste which is distinct from the four basic tastes of sweet, sour, salty and bitter, and tried to isolate a unique taste substance from a major ingredient of Japanese broth, seaweed *Laminaria japonica*. He identified glutamic acid as the taste substance and named the taste of glutamate umami. His paper in old-style Japanese was recently translated into English and published in this journal (Ikeda, 2002). His discovery and assertion, however, had remained unnoticed for decades because the taste of glutamate is subtle and perceived more clearly at moderate concentrations than at high concentrations.

Since about 1980, research on umami taste has proceeded on a larger scale. In 1985, the first international symposium on umami was held in Hawaii, the most basic issue discussed being whether umami was a unique taste quality. A multidimensional scaling analysis of similarity judgement among various taste substances in humans showed that monosodium glutamate (MSG) is located outside the taste tetrahedron (Schiffman and Gill, 1987). The same result was obtained from the analysis of generalization patterns among chemicals in mice conditioned to avoid MSG (Ninomiya and Funakoshi, 1987). These results suggested that the taste of MSG cannot be reproduced by mixing the four basic tastes and is independent of them.

A big breakthrough was made by the recent discovery of a variant of brain-expressed metabotropic glutamate receptor 4 (mGluR4) with a truncated N-terminal (Chaudhari *et al.*, 2000) and the T1R1 + T1R3 heterodimer receptor (Li *et al.*, 2002; Zhao *et al.*, 2003), which are expressed in heterologous taste cells and respond to glutamate at taste-effective concentrations. These findings clearly demonstrated the presence of umami-specific receptors and strongly supported the idea that umami is a unique taste.

Tongue regional differences in umami sensitivity

Tongue regional difference was clearly shown in gustatory neural responses to umami substances. Ninomiya and colleagues (Ninomiya and Funakoshi, 1987; Ninomiya *et al.*, 2000) reported that MSG-best (M-type) fibers were exclusively found in the glossopharyngeal (GL) nerve innervating the posterior tongue and some of M-type fibers displayed marked synergism between MSG and guanosine 5'-monophosphate (GMP), which is a typical nature of umami response. In contrast, in the chorda tympani (CT) nerve innervating the anterior tongue, amiloride-sensitive NaCl-best fibers exhibited robust responses to MSG, which may be due to high concentration of Na⁺ dissociated from MSG. Some sucrose-best fibers in the CT also responded to MSG and showed the synergism between MSG and GMP. These phenomena suggest that the umami component of the MSG response in the CT may be masked by greater salty or sweet components, while that in the GL is more

dominant than salty or sweet components. Thus, for the umami-specific information, the M-type fibers in the GL nerve may play a crucial role in mice.

Greater sensitivities to umami substances in the posterior than the anterior part of the tongue were confirmed in primates. The electrophysiological studies in rhesus monkeys (Hellekant *et al.*, 1997) revealed the presence of M-type fibers in the GL but not in the CT. The human psychophysical study (Yamaguchi and Ninomiya, 2000) demonstrated that taste sensitivities to MSG and a mixture of MSG and inosine 5'-monophosphate (IMP) were much higher at the back than at the front of the tongue. Thus, most investigators consider that the information from the posterior tongue is important for umami taste.

Unlike other species, rat GL nerves displayed very poor responses to MSG and IMP (Sako *et al.*, 2000), suggesting that umami-specific information may be poor in rats. This feature may relate to the result that rats could not distinguish MSG from sucrose or NaCl (Yamamoto *et al.*, 1991).

Recent investigations of candidate umami receptors

The first candidate umami receptor, taste-mGluR4 (Chaudhari *et al.*, 2000), was reported to be dominantly expressed in taste buds on the posterior tongue. The low binding affinity of this receptor was consistent with the GL nerve sensitivity to MSG. In contrast, Toyono *et al.* (2002, 2003) found more intense expression of brain-mGluR4 at the apical end of taste buds in all taste papillae by using immunohistochemistry and subsequently revealed the expression of brain-mGluR1 in the circumvallate papillae by using *in situ* hybridization. Their results imply that the glutamate receptors with much higher sensitivities are also expressed in taste cells. Furthermore, Gabriel *et al.* (this symposium) have shown the expression of a variant of brain-mGluR1 with a short amino-terminal extracellular domain in circumvallate taste cells.

Another stream of research on candidate umami receptors proceeded independently. In 1999, two novel families of G protein-coupled receptors (GPCRs) expressed in taste receptor cells were identified (Hoon *et al.*, 1999), one is T1Rs and the other is T2Rs. Concerning the function of T1R family, Nelson *et al.* (2002) reported that T1R3 responds to sweet substances in combination with T1R2, while it responds to a wide range of amino acids including glutamate in combination with T1R1 in mice. Li *et al.* (2002) revealed that human T1R1 + T1R3 heterodimer receptors respond specifically to glutamate, indicating that they are umami specific receptors. Kim *et al.* (2003) and Kusakabe *et al.* (this symposium) investigated the regional expression patterns of T1R family and gustducin in the mouse tongue and found that T1R1 and T1R3 are coexpressed in a subset of taste cells both in the fungiform and circumvallate papillae,

whereas T1R3 is coexpressed with gustducin only in fungiform taste cells, suggesting that T1R1 + T1R3 receptor may couple to gustducin in the anterior tongue.

Possible transduction mechanisms for umami taste

As described above, five candidate umami receptors and involvement of gustducin in transduction have been proposed. Evidence for involvement of T1R3 and gustducin was provided by the behavioral analyses and neural recordings in gustducin- or T1R3-knockout mice, where their preference and neural responses to umami substances were almost abolished (Ruiz *et al.*, 2003; Damak *et al.*, this symposium). Collectively, it is suggested that T1R1 + T1R3 receptor coupled to gustducin in the fungiform papilla may be essential for preference behavior for MSG.

Biochemical and Ca²⁺-imaging studies have been conducted to investigate intracellular signaling pathway for umami taste. MSG, mixtures of MSG and 5'-ribonucleotides, or L-AP4 induced cAMP decrease in circumvallate taste buds (Chaudhari *et al.*, this symposium). IP₃ increase in taste tissue and [Ca²⁺]_i mobilization in isolated taste cells (Ninomiya *et al.*, 2000; Sugimoto *et al.*, 2001) and in a subset of taste cells in a slice preparation of circumvallate papillae (Chaudhari *et al.*, this symposium) were generated by umami stimulation, suggesting that the downstream responses may be mediated by phospholipase C (PLC).

The recent investigation with knockout mice has demonstrated PLC-β2 and a transient receptor potential channel, TRPM5, are essential molecules for umami taste as well as sweet and bitter tastes (Zhang *et al.*, 2003). Toyono *et al.* (this symposium) further revealed coexpression of Gα_q, PLC-β2 and PLC-β4, as downstream molecules for brain-mGluR1 in circumvallate papillae. Damak *et al.* (this symposium) also suggested that TRPM5 mediates much of the preference for MSG, though TRPM5-knockout mice had residual umami responses. Involvement of TRPM5, however, is not consistent with the data of patch-clamp analysis, where most cells displayed hyperpolarization in response to umami stimuli (Kinnamon, *et al.*, this symposium).

In conclusion, to date it has been clarified that there are multiple umami receptors, and glutamate information from anterior tongue is predominantly mediated by T1R1 + T1R3 heterodimer receptors coupled to gustducin and play an important role in preference behavior. However, many issues such as regional expression patterns of other candidate umami receptors, especially in the posterior tongue, and the downstream signaling mechanisms and functional roles of them are left to be elucidated.

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