Signal Transduction of Umami Taste: Insights from Knockout Mice

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Introduction
The sense of taste is comprised of four basic qualities: sweet, bitter, salty and sour. Umami, a Japanese term for delicious, although controversial for many years as a distinct taste is now widely accepted as a fifth taste quality. Compounds that taste umami include glutamate salts such as monosodium and monopotassium glutamate (MSG and MPG, respectively), nucleotide monophosphate (IMP, GMP), certain peptides and amino acids such as asparatate. A particular property of umami is that the taste of glutamate is enhanced by monophosphate nucleotides. Psychophysical studies and conditioned taste aversion experiments showed that humans and mice distinguish the taste of MSG from the four basic taste qualities. The umami taste may have evolved to help animals ingest food that have high protein content and is of significant importance to the food industry because of its flavor enhancement properties.

Taste signals are transduced primarily via GPCR pathways for sweet and bitter, and ion channels for salty and sour. Several taste signal transduction proteins have recently been discovered, including the T2rs, a family of bitter-responsive receptors, the T1rs which form heterodimeric sweet- and amino-acid-responsive receptors, α-gustducin a G protein α-subunit that couples these receptors to second messenger pathways, Gγ13 the γ subunit of gustducin, PLCγ2, Trpm5 a calcium activated cation channel, ENaC and ASIC two ion channels implicated in salty and sour taste, respectively (reviewed in Gilbertson et al., 2000; Lindemann, 2001).

We set out to determine if these taste signal transduction proteins contribute to the response to umami compounds. Using knockout mice, behavioral assays, electrophysiological measurements and biochemical tools, we identified several components of the umami signaling pathways.

α-Gustducin mediates responses to umami, in addition to sweet and bitter compounds
The role of α-gustducin in the transduction of sweet and bitter tastes is well established (Wong et al., 1996). To determine if α-gustducin is also involved in umami taste, we tested α-gustducin knockout (KO) mice with MSG, MPG and IMP. Two-bottle preference tests and chorda tympani (CT) and glossopharyngeal (NG) nerve recordings showed that these mice had diminished response to these umami compounds (Ruiz et al., 2003; He et al., 2004), indicating that α-gustducin plays a role in the umami taste response.

Rod α-transducin mediates responses to umami, but not to bitter or sweet compounds
Rod α-transducin is structurally and biochemically highly similar to α-gustducin and is also expressed in taste receptor cells, albeit at a much lower level than α-gustducin. To determine the role of α-transducin in taste, we compared the responses to tastants of α-gustducin/α-transducin double KO, single KO and WT mice. Two-bottle preference tests showed no difference in the response to MSG (+10 μM amiloride, to reduce the effect of the sodium ion) between α-transducin KO mice and WT controls. The α-gustducin KO mice showed a diminished preference for concentrations of MSG between 10 and 300 mM, whereas the double KO mice were indifferent to those concentrations. Thus, α-transducin plays a role in the response of mice to MSG but is less important than α-gustducin because the effect of knocking out α-transducin on the MSG response is detectable only in the absence of α-gustducin. Similar results were obtained with nerve recordings from the CT nerve. Knocking out α-transducin, in the presence or absence of α-gustducin, did not affect the responses of mice to sweet, bitter, salty or sour compounds (He et al., 2004).

α-Gustducin, but not rod α-transducin, mediates the responses to IMP and IMP enhancement of MSG
A particular characteristic of umami is that the taste response to MSG is enhanced by IMP, a compound that also tastes umami. Using behavioral tests and nerve recordings with KO mice, we showed that the response to IMP and the potentiation of the response to MSG by IMP were mediated by α-gustducin but not by α-transducin and that in the absence of α-gustducin, the potentiation by IMP was totally abolished (He et al., 2004).

T1r1 and T1r3 are involved in the transduction of preference for MSG, but other receptors and/or pathways must exist
Heterologous expression in HEK cells and calcium imaging studies showed that the combination of T1r3 plus T1r1 forms a broadly tuned l-amino acid receptor in rodents and a more narrowly tuned umami receptor in humans (Li et al., 2002; Nelson et al., 2002). In KO mice lacking T1r3 the preference for and CT response to MSG are diminished, but substantial residual responses persist (Damak et al., 2003). Thus other receptors and/or pathways must exist. Another candidate receptor is a truncated form of mGluR4 expressed in taste receptor cells that is activated by MSG and L-AP4 at concentrations that elicit umami taste (Chaudhari et al., 2000). The potentiation of MSG by IMP is abolished in the T1r3 KO mice (Damak et al., 2003). The umami response is also diminished in T1r1 KO mice (Zhao et al., 2003), consistent with the results from experiments with HEK cells.
Trpm5 and PLCβ2 mediate much of the preference for MSG, but there are residual responses to umami in Trpm5 knockout mice

Trpm5 and PLCβ2 are also involved in the response to MSG, but the extent to which there are residual responses to umami and other tastes in Trpm5 KO mice is controversial. Data from one line of KO mice showed a total lack of nerve and behavioral responses to MSG concentrations up to 100mM in Trpm5 KO mice (Zhang et al., unpublished). However, in another line of Trpm5 KO mice (Damak et al., unpublished) significant residual preferences for 100 and 300mM MSG were detected (Figure 1). Interestingly, the responses of Trpm5 KO and T1r3 KO mice were identical (Figure 1), suggesting that these two proteins are in the same pathway. The CT nerve response to MSG was diminished in the Trpm5 KO mice; in contrast, there was no significant difference in the NG responses to MSG between WT and Trpm5 KO mice (Damak et al., unpublished).

Different pathways transduce the umami responses in the front and the back of the tongue

Several lines of evidence suggest dual transduction mechanisms for umami taste. In KO mice lacking Trpm5, T1r3, α-gustducin or α-transducin the CT but not the NG nerve responses to MSG were diminished and MSG preference but not avoidance was affected. IMP potentiation of the response to glutamate occurs only in the front of the tongue and was abolished in the Trpm5, T1r3 and α-gustducin KO mice. Ex vivo stimulation by MSG of mouse fungiform papillae (located in the front of the tongue) leads to elevation of cAMP and IP3, whereas stimulation by MSG of the circumvallate papillae (located at the back of the tongue) of rats resulted in a drop of cAMP (Ninomiya et al., 2000). Taste nerve resection and conditioned taste aversion experiments in rodents showed that in the mouse, the umami-specific signals are carried by the glossopharyngeal nerve, whereas signals similar to those elicited by sweet compounds are carried by the chorda tympani (reviewed in Ninomiya et al., 2000).

In summary the cascade that transduces the response to glutamate in the front of the tongue leading to preference includes T1r1, T1r3, gustducin, transducin, PLCβ2 and Trpm5. However, presently very little is known of how the umami-specific signals that originate from the back of the tongue are transduced.

References


