Monosodium glutamate (MSG) elicits a unique taste called umami. A characteristic feature of umami taste is its potentiation by 5'-ribonucleotides (primarily GMP and IMP), which also have an umami taste of their own. Based on recent molecular studies, two putative umami receptors have been identified: a truncated variant of the metabotropic glutamate receptor mGluR4 (taste-mGluR4) (Chaudhuri et al., 1996, 2000) and the heterodimer, T1R1 + T1R3 (Li et al., 2002; Zhao et al., 2003). Both of these receptors are expressed in taste cells and both receptors have been expressed in heterologous cells, where they respond to glutamate at taste effective concentrations. The TIR1 + TIR3 heterodimer is potentiated strongly by IMP, when MSG is presented together with the nucleotide. Targeted gene deletion of TIR1 or TIR3 abolishes the synergistic responses of MSG and IMP (Damak et al., 2003; Zhao et al., 2003), suggesting that TIR1 + TIR3 is required for the nucleotide potentiation. Responses to MSG alone vary, depending on the study. While Zhao et al. (2003) found that responses were completely abolished, Damak et al. (2003) reported only a slight reduction, even in the presence of amiloride to reduce the effects of Na+. Thus, these results open the possibility for a role of additional receptors, particularly for MSG.

Umami receptors are coupled to a signaling pathway involving activation of PLCβ2, production of IP3 and diacylglycerol, release of Ca2+ from intracellular stores and activation of a transient receptor potential channel, TRPM5. Evidence for this hypothesis comes primarily from targeted gene knockouts, showing that both PLCβ2 and TRPM5 are necessary for umami transduction (Zhang et al., 2003). Several important questions remain, however, including whether additional receptors contribute to umami taste, what G proteins couple umami receptors to their downstream signaling effectors and how TRPM5 contributes to transduction. In this paper, we review our studies of whole-cell recording and Ca2+ imaging of rat fungiform taste buds in response to umami stimuli. Collectively, these studies provide insights into umami receptors and their downstream signaling effectors. Whole cell recording from isolated taste cells showed that bath applied glutamate (1 mM MSG) elicits three types of responses: a depolarizing inward current with an increase in conductance, mimicked by the ionotropic glutamate agonist NMDA; a hyperpolarizing outward current with a decrease in conductance, mimicked by the metabotropic glutamate agonist L-AP4; and a biphasic response, consisting of both types of responses (Lin and Kinnamon, 1999). These data are consistent with earlier studies, showing that NMDA and/or L-AP4 elicit responses in subsets of taste cells (Hayashi et al., 1996; Bigiani et al., 1997) or activate channels isolated from taste cell membranes (Teeter et al., 1992). In a small subset of taste cells, L-AP4 elicited a depolarizing inward current with an increase in conductance, suggesting the presence of an additional metabotropic receptor for glutamate. Since NMDA receptors have been localized to the basolateral membrane of taste cells (Caicedo et al., 2000), it is likely that they represent neurotransmitter receptors rather than taste receptors.

To elucidate the role of GMP in umami signaling, MSG, GMP and MSG + GMP were bath-applied to single rat fungiform taste cells during voltage-clamp recording or Ca2+ imaging (Lin et al., 2003). GMP elicited responses similar to those of MSG, although not always in the same taste cells, involving increases in membrane conductance that resemble the effects of the metabotropic receptor for GMP. Further, although most cells that responded to MSG or GMP usually responded to both compounds, only a small subset (27%) exhibited synergy when nucleotides were applied together with MSG. These data provide evidence that nucleotide receptors independent of the TIR1 + TIR3 heterodimer are expressed in taste buds and may contribute to umami taste. To determine whether responses resulted in changes in intracellular Ca2+, fura-2-loaded taste cells were examined in response to umami stimuli. All cells showed increases in intracellular Ca2+, including cells showing synergism when MSG and GMP were presented together. These data indicate that even hyperpolarizing responses involve increases in intracellular Ca2+, suggesting release from intracellular stores. This was confirmed by examining responses in Ca2+ free Tyrodes, showing that a portion of the response was independent of intracellular Ca2+ (Lin et al., 2003). These data are consistent with gene knockout data showing that PLCβ2 is necessary for umami transduction.

TRPM5 is presumed to be the target ion channel for umami transduction. TRPM5 knockouts are insensitive to umami stimuli, as well as to sweet and bitter stimuli, (Zhang et al., 2003) and the channel is co-expressed with PLCβ2 signaling components (Perez et al., 2002, 2003). The properties of TRPM5 vary according to the heterologous expression system used. When expressed in oocytes, TRPM5 behaves as a store-operated Ca2+ channel (Perez et al., 2002, 2003), but when expressed in HEK293 cells TRPM5 behaves as a Ca2+-activated, monovalent-selective cation channel (Hofmann et al., 2003; Liu and Liman, 2003; Prawitt et al., 2003). Our electrophysiological studies do not support a role of TRPM5 as a monovalent cation channel in taste cells. In only a small subset of taste cells do umami stimuli activate G protein-coupled pathways that result in an increase in membrane conductance (Lin and Kinnamon, 1999). In most cells responses to umami stimuli, including synergistic responses to MSG and GMP, involve decreases in membrane conductance and hyperpolarization, likely due to inhibition of a Cl channel (Lin and Kinnamon, 1999; Lin et al., 2003). We hypothesize that in taste cells, TRPM5 may associate with other TRP channels or particular scaffolding proteins to confer Ca2+ permeability on the channel. Thus, the resulting Ca2+ influx would contribute directly to transmitter release without the need for membrane depolarization and voltage-gated Ca2+ influx. Indeed, at least in vallate taste buds of mice, taste cells expressing PLC signaling components generally lack voltage-dependent Ca2+ channels (Medler et al., 2003). Further studies will be required to determine if all umami-responsive taste cells lack voltage-gated Ca2+ channels.
What G proteins couple umami taste receptors to downstream signaling effectors? A recent study shows that T1R3 and T1R1 are co-expressed with α-gustducin in fungiform taste buds (Kim et al., 2003) and we have found that a similar relationship exists in palatal taste buds (L. Stone, T. Finger and S. Kinnamon, unpublished data). Thus, gustducin may be the α-subunit activated by T1R1 + T1R3. We hypothesized that since gustducin decreases intracellular cAMP levels, membrane permeant analogs of cAMP should antagonize responses to umami stimuli. This was indeed the case: 8cpt-cAMP and 8-bromo cAMP completely abolished the electrophysiological responses to umami stimuli. This was indeed the case; 8cpt-cAMP levels, membrane permeant analogs of cAMP should antagonize signaling effectors? A recent study shows that T1R3 and T1R1 are essential component of the umami signaling pathway. Finally, we have conducted two-bottle preference tests on α-gustducin knockout mice and have found, as predicted, that they are insensitive to umami stimuli (Ruiz et al., 2003). These data, coupled with those of Damak et al. (this symposium) provide strong support that umami receptors couple to α-gustducin.

The role of the decreased cAMP in transduction is unclear. Membrane permeant analogs of cAMP do not activate or block any conductances in umami-sensitive taste cells (Lin et al., 2003), suggesting that cAMP may target upstream signaling effectors, possibly by altering their phosphorylation state.

A model for the transduction of umami stimuli is illustrated in Figure 1. The heterodimer T1R1 + T1R3 binds both MSG and IMP/GMP, resulting in a synergistic response. Other receptors, possibly taste-mGluR4 (Chaudhari et al., 2000) and taste-mGluR1 (Gabriel, this symposium) likely bind GMP and/or MSG. These receptors couple to a heterotrimeric G protein consisting of α-gustducin and its βγ partners. Gustducin activates a phosphodiesterase to decrease intracellular cAMP, while its βγ partners activate PLCβ2 to produce IP3 and diacylglycerol. IP3 elicits release of Ca2+ from intracellular stores, which subsequently activates TRPM5, although its precise role is unclear. The increase in intracellular Ca2+ presumably causes release of transmitter and activation of gustatory afferents. What is still unclear is the mechanism involved in the inhibition of chloride channels and whether these channels play any significant role in the transduction process. Also unclear are the role of TRPM5 in the transduction cascade and the precise target of the decreased cAMP. Further studies will be required to elucidate these questions.

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References