Chem. Senses 30 (suppl 1): i29–i30, 2005 doi:10.1093/chemse/bjh097

Multiple Pathways for Signaling Glutamate Taste in Rodents

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Key words: cAMP, functional imaging, taste receptor, umami

Umami: a complex taste

L-glutamate, typically as its Na salt (MSG), elicits a taste termed umami. A characteristic feature of umami taste is the synergistic potentiation of glutamate taste by purine nucleotide (inosine, guanosine) monophosphates. This is manifested as an enhanced electrophysiological response from taste receptor cells, as an increase in nerve firing rate, or as increased preference in behavioral assays. Apart from this enhanced intensity, it is not clear whether the addition of nucleotides also leads to a change in the perceived quality of glutamate in animals and humans.

The magnitude of nucleotide-potentiation in nerve recordings varies considerably between the chorda tympani (CT) and glossopharyngeal (GL) nerves (Ninomiya *et al.*, 1993). Single-unit recordings further highlight the heterogeneity of umami responses in that nucleotide-potentiated signals are seen in distinct fiber-types (sucrose-best or glutamate-best) in the CT and GL nerves (Ninomiya and Funakoshi, 1989; Yamamoto *et al.*, 1991; Formaker *et al.*, 2004). Gurmarin, a peptide that inhibits sweet taste in rodents, inhibits umami signals differentially across the CT and GL nerves (Ninomiya *et al.*, 1993; Sako and Yamamoto, 1999). Collectively, the nerve recording data suggest that responses to MSG differ significantly between the anterior (CT innervation) and posterior (GL innervation) lingual taste fields. The implication is that umami responses may originate from more than a single type of receptor or receptor combination.

Identifying taste receptors

In the last few years, several strategies have been successfully used for identifying taste receptors. The genetic approach employed naturally occurring phenotypic variations in taste sensitivity. Mapping taste loci has revealed candidate receptors for bitter (e.g. T2R5) and sweet (T1R3) receptors. Molecular cloning approaches, on the other hand, begin with identifying cDNAs selectively expressed in taste tissue, confirming the presence of corresponding mRNA and/or protein in taste cells and examining the functional properties of cloned receptors when they are expressed in heterologous cells. An essential final step of this approach should be to determine how closely the functional properties of cloned receptors approximate those of native taste cells.

At least two distinct G protein-coupled receptors (GPCRs) have been proposed to underlie the detection of glutamate in mammalian taste buds. A taste-specific variant of a metabotropic glutamate receptor, taste-mGluR4, was cloned from rat circumvallate papillae (Chaudhari *et al.*, 2000) and mGluR4 mRNA was localized to taste cells by *in situ* hybridization (Chaudhari *et al.*, 1996; Yang *et al.*, 1999).

When expressed in transfected cells, taste-mGluR4 responds to MSG and L-AP4 (a glutamate analog) at taste-effective concentrations (Chaudhari *et al.*, 2000). To further explore the significance of mGluR4 in taste buds, we carried out immunoblots on taste papillae using antibodies specific for mGluR4. Extracts from circumvallate and foliate papillae from rats and mice contained immuno-reactive bands of molecular weight predicted for both taste- and brain-mGluR4. These bands were not apparent in extracts from non-taste samples. In immunocytochemical experiments also, circumvallate, foliate and palatal taste buds were immuno-reactive with anti-mGluR4 antibodies. Only a subset of spindle-shaped cells were labeled in each taste bud (Chaudhari *et al.*, 2003). Using double label immunocytochemistry, we further determined that mGluR4 expression is principally in cells that also express phospholipase Cβ2 (PLCβ2), an effector implicated in taste responses.

Another candidate umami receptor, the T1R1/T1R3 heterodimer, when expressed in heterologous cells along with promiscuous G proteins, also confers the ability to respond to glutamate (Nelson *et al.*, 2002). T1R1/T1R3 dimers display nucleotide potentiation of glutamate responses and are activated by a broad range of non-umami amino acids. When either T1R1 or T1R3 were genetically ablated, the chorda tympani (CT) response to glutamate was eliminated and mice entirely lost taste preference for umami stimuli in brief-access tests (Zhao *et al.*, 2003). The stated interpretation was that the T1R1/T1R3 dimer is uniquely necessary and sufficient for umami taste. However, much data exists to the contrary. A knockout of T1R3, produced by another group, showed decreased CT responses to MSG, while GL responses to MSG were hardly changed (Damak *et al.*, 2003). It should be noted that T1R1 is expressed independently of T1R3 in substantial numbers of cells, especially in the vallate taste buds (Max *et al.*, 2001; Kim *et al.*, 2003). These findings suggest that T1R1/T1R3 pairing is not obligatory in native cells, that other partners are likely for T1R1, and that umami receptors are likely to be different in vallate versus fungiform taste buds.

Umami responses of taste cells

To understand the significance of the various receptors discussed above, it is essential to compare their functional properties against the characteristics of umami responses in native taste cells. Hence, we employed two semi-intact preparations of taste tissue. First, we measured cAMP levels in intact rat and mouse taste buds stimulated with umami tastants. As we reported previously, circumvallate taste buds respond to glutamate with a concentration-dependent decrease in cAMP concentration (Abaffy *et al.*, 2003). This cAMP modulation is seen in both rats and mice, and is only detected at glutamate concentrations >1 mM, indicating that this second messenger is related to umami taste. The cAMP responses to MSG were distinct in circumvallate taste buds relative to palatal or fungiform taste buds. In rat (but not mouse) vallate taste buds, the addition of IMP to MSG yielded an enhanced response. Although the significance of the cAMP signal in the overall transduction process remains to be established, these results suggested that taste buds exhibit more than a single type of response to glutamate.

We have also used a slice preparation of circumvallate taste papillae, loaded with calcium green–dextran and confocal imaging to examine physiological responses (Ca^{2+} transients) to umami stimuli. In this preparation, taste buds are focally stimulated just at the taste pore, emulating *in vivo* stimulation (Caicedo *et al.*, 2000). The basolateral membrane of taste cells is protected from exposure to the stimulus and the measured Ca^{2+} signals likely represent primary taste responses. In mouse vallate papillae, Ca^{2+} responses to glutamate were detected in ≈5% of taste cells and could be elicited by other Na and K salts. Both Na and K salts of glutamate are known to activate taste nerves. As expected for umami responses, low concentrations of IMP enhanced the glutamate-elicited Ca^{2+} responses. We also determined that the Ca^{2+} responses of vallate taste cells to monopotassium glutamate (MPG) stimulation represents release of Ca^{2+} from intracellular stores. This is in keeping with the loss of umami sensitivity in mice that are genetically deficient in PLCβ2, a key mediator of Ca^{2+} release in taste cells (Zhang *et al.*, 2003; Dotson *et al.*, 2004).

Some taste cells in vallate slices responded to MPG, some to a sweet tastant (SC45647) and some to both. Interestingly, cells that responded to L-AP4 (an umami agonist) did not respond to MPG. No cells were observed to respond to both. Taste cells that responded to MPG also responded to varying numbers of other amino acids. Overall, the Ca^{2+} responses of individual taste cells are consistent with single-unit taste nerve recordings, but do not correspond well with the functional properties of any single heterologously expressed receptor, neither T1R1/T1R3 (Nelson *et al.*, 2002), nor mGluR4 (Chaudhari *et al.*, 2000).

Our data suggest that detection of umami stimuli by native taste cells is neither as simple nor as monotonic as the hypothesis of a unique T1R1/T1R3 umami receptor. In the taste slice preparation, umami responses were significantly different than those reported for mouse T1R1/T1R3 dimers, heterologously expressed (Li *et al.*, 2002; Nelson *et al.*, 2002). For instance, individual glutamate-sensitive cells in vallate papillae responded to different combinations of amino acids, as expected given the varied behavioral responses of rodents to different amino acids (Iwasaki *et al.*, 1985). In contrast, the expressed T1R1/R3 dimer responded to all of these amino acids (Nelson *et al.*, 2002). Taste generalization between L-AP4 (an umami compound) and MSG indicated that these compounds taste similar to rodents, the inference being they activate a common receptor (Chaudhari *et al.*, 1996). Yet, rats also easily discriminate between the tastes of MSG and L-AP4 (Delay *et al.*, 2004), suggesting that some taste receptors may exist that are activated by one but not the other ligand. Collectively, these observations suggest that umami responses are complex and may be generated by more than a single type of receptor (Sako *et al.*, 2003).

Conclusion

Our observations highlight possible distinctions among native glutamate-taste receptors, and suggest the presence of additional receptors for different umami stimuli or unexplored interactions among known receptors. Indeed, taste would not be unique in possessing such redundancy of receptors. Most mammalian sensory systems include more than a single receptor capable of responding to a given stimulus, whether these are multiple opsins (responding to a given wavelength of light), multiple ORs (responding to a single odorant) or multiple receptors in peripheral nociceptors (responding to metabolites arising from tissue damage).

Acknowledgements

Supported by grants from IGTC and NIH (5P01-DC3013, 1R01-DC6021 and 2R01-DC374).

References

Abaffy, T., Trubey, K. and **Chaudhari, N.** (2003) *Adenylyl cyclase expression and modulation of cAMP in rat taste cells*. Am. J. Physiol. Cell. Physiol., 284, C1420–C1428.

Caicedo, A., Jafri, M.S. and **Roper, S.D.** (2000) In situ *Ca^{2+} imaging reveals neurotransmitter receptors for glutamate in taste receptor cells*. J. Neurosci., 20, 7978–7985.

Chaudhari, N., Yang, H., Lamp, C., Delay, E., Cartford, C., Than, T. and **Roper, S.** (1996) *The taste of monosodium glutamate: membrane receptors in taste buds*. J. Neurosci., 16, 3817–3826.

Chaudhari, N., Landin, A.M. and **Roper, S.D.** (2000) *A metabotropic glutamate receptor variant functions as a taste receptor*. Nat. Neurosci., 3, 113–119.

Chaudhari, N., Pereira, E., Landin, A.M. and **Roper, S.D.** (2003) *Immunodetecting a candidate umami receptor, taste-mGluR4, in taste cells*. Chem. Senses., 28, 559.

Damak, S., Rong, M., Yasumatsu, K., Kokrashvili, Z., Varadarajan, V., Zou, S., Jiang, P., Ninomiya, Y. and **Margolskee, R.F.** (2003) *Detection of sweet and umami taste in the absence of taste recetptor T1R3*. Science, 301, 850–853.

Delay, E.R., Sewczak, G.M., Stapleton, J.R. and **Roper, S.D.** (2004) *Glutamate taste: discrimination between the tastes of gutamate agonists and monosodium glutamate in rats*. Chem. Senses, 29, 291–299.

Dotson, C.D., Richter, T.A., Roper, S.D. and **Spector, A.** (2004) *PLCβ2 knock-out mice display lick avoidance to high concentrations of quinine and denatonium*. Chem. Senses, 29, 000.

Formaker, B.K., Stapleton, J.R., Roper, S.D. and **Frank, M.E.** (2004) *Responses of the rat chorda tympani nerve to glutamate–sucrose mixtures*. Chem. Senses, 29, 473–482.

Iwasaki, K., Kasahara, T. and **Sato, M.** (1985) *Gustatory effectiveness of amino acids in mice: behavioral and neurophysiological studies*. Physiol. Behav., 34, 531–542.

Kim, M.-R., Kusakabe, Y., Miura, H., Shindo, Y., Ninomiya, Y. and **Hino, A.** (2003) *Regional expression patterns of taste receptors and gustducin in the mouse tongue*. Biochem. Biophys. Res. Commun., 312, 500–506.

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M. and **Adler, E.** (2002) *Human receptors for sweet and umami taste*. Proc. Natl Acad. Sci. USA, 99, 4692–4696.

Max, M., Shanker, Y.G., Huang, L., Rong, M., Liu, Z., Campagne, F., Weinstein, H., Damak, S. and **Margolskee, R.F.** (2001) *Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac*. Nat. Genet., 28, 58–63.

Nelson, G., Chandrashekar, J., Hoon, M.A., Feng, J., Zhao, G., Ryba, N.J. and **Zuker, C.S.** (2002) *An amino-acid taste receptor*. Nature, 416, 199–202.

Ninomiya, Y. and **Funakoshi, M.** (1989) *Peripheral neural basis for behavioural discrimination between glutamate and the four basic taste substances in mice.* Comp. Biochem. Physiol. B, 92, 371–376.

Ninomiya, Y., Kajiura, H. and **Mochizuki, K.** (1993) *Differential taste responses of mouse chorda tympani and glossopharyngeal nerves to sugars and amino acids*. Neurosci. Lett., 163, 197–200.

Sako, N. and **Yamamoto, T.** (1999) *Analyses of taste nerve responses with special reference to possible receptor mechanisms of umami taste in the rat*. Neurosci. Lett., 261, 109–112.

Sako, N., Tokita, K., Sugimura, T. and **Yamamoto, T.** (2003) *Synergistic responses of the chorda tympani to mixtures of umami and sweet substances in rats*. Chem. Senses, 28, 261–266.

Yamamoto, T., Matsuo, R., Fujimoto, Y., Fukunaga, I., Miyasaka, A. and **Imoto, T.** (1991) *Electro-physiological and behavioural studies on the taste of umami substances in the rat*. Physiol. Behav., 49, 919–925

Yang, H., Wanner, I.B., Roper, S.D. and **Chaudhari, N.** (1999) *An optimized method for* in situ *hybridization with signal amplification that allows the detection of rare mRNAs*. J. Histochem. Cytochem., 47, 431–446.

Zhang,Y., Hoon, M.A., Chandrashekar, J., Mueller, K.L., Cook, B., Wu, D., Zuker, C.S. and **Ryba, N.J.** (2003) *Coding of sweet, bitter and umami tastes: different receptor cells sharing similar signaling pathways*. Cell, 112, 293–230.

Zhao, G.Q., Zhang, Y., Hoon, M.A., Chandrashekar, J., Erlenbach, I., Ryba, N.J. and **Zuker, C.S.** (2003) *The receptors for mammalian sweet and umami taste*. Cell, 115, 255–266.