Unraveling the biochemistry of sweet and umami tastes

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Taste is an important part of everyone's daily life. Sweet taste is particularly important as evidenced by the fact that wars have been fought and people have been enslaved over sugar, the prototypical sweet stimulus (1). Savory taste, often referred to as umami (delicious in Japanese), is also important, its prototypical stimulus being monosodium glutamate (MSG). Despite the importance of these taste sensations to our daily enjoyment of life, until the late 1980s, the biochemical pathways that mediate them were largely unknown. Then, for sweet taste, evidence began to accumulate that it must be by G protein-coupled receptor (GPCR)-mediated. More specifically, it was generally believed to be mediated by several GPCRs because the findings of biochemical, electrophysiological, and psychophysical experiments could only be easily explained by a plurality of receptors (2, 3). And this expectation was supported by the fact that multiple subtypes of GPCRs commonly exist for other important signal molecules (e.g., acetylcholine, norepinephrine, dopamine, serotonin, etc.). Then, in the early 2000s, a breakthrough occurred, dramatically increasing the understanding of both sweet and umami tastes. Nelson *et al.* (4) reported the discovery of the rat sweetener receptor. In a functional assay, they showed that all substances that rats generalize to sucrose taste are mediated by a single receptor, which is a heterodimer of two GPCRs, T_1R_2 and T_1R_3. Further, they showed that umami taste is also mediated by a heterodimer of the two GPCRs T_1R_1 and T_1R_3. T_1R_1, T_1R_2, and T_1R_3 are members of the small family of class C GPCRs. The most studied members of the class C GPCRs are the homodimeric metabotrophic glutamate (mGluR), heterodimeric γ-aminobutyric acid type B ($GABA_BR$), and homodimeric extracellular calcium receptors, which have recently been reviewed (5). Interestingly, the umami and sweetener receptors are 50% identical in that they share the common subunit T_1R_3. This rat receptor discovery was quickly followed by a report of parallel findings on the human system by Li *et al.* (6). Again, it was surprising to see that the single human heterodimeric sweetener receptor, often written as T_1R_2/T_1R_3, responded to all structural types of sweeteners tested and did so in a manner consistent with expectation from sensory experiments.

Class C GPCRs are unique in that they possess very large N-terminal Venus flytrap-like domains (VFDs). In the case of metabotrophic glutamate receptor 1, it has been demonstrated that its VFD closes on binding glutamate just as expected (7). This precedent, and the fact that the sweetener and umami receptors contain the common subunit T_1R_3, leads to the expectation that sweeteners likely bind in the VFD of T_1R_2 and glutamate likely binds in the VFD of T_1R_1. The work of Xu *et al.* (8) in this issue of PNAS probes the fundamental question of sweetener binding locus with the finding that, although some sweeteners do bind in the VFD of T_1R_2 (i.e., aspartame and neotame), at least one sweetener (i.e., cyclamate) does not, but rather it binds within the seven-transmembrane domain (TMD) of T_1R_3. The sweetener receptor is the first class C GPCR demonstrated to have more than one locus of agonist binding (orthosteric site).

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What Is the Sweetener Pharmacophore?

During the 1980s and before, the taste literature was dominated by discoveries of novel synthetic sweeteners and structure elucidations of natural sweeteners. By the 1980s, chemists had discovered at least 50 structural classes of sweet-tasting organic compounds, and many models had been developed to rationalize their activities. These were pharmacophore models, and the most publicized of them was the so-called A-H/B model of Shallenberger and Acree (9). They hypothesized that all sweeteners contain H-bond donor and H-bond acceptor groups separated by not <2.5 or >4.0 Å. This model was later elaborated by Kier (10) and, more recently, Tinti and Nofre (11) to rationalize the activities of sweeteners more potent than simple carbohydrates. An assumption implicit in all of these models is that sweetness is mediated by a single receptor with a single orthosteric site. Xu *et al.* (8) demonstrate that the human sweetener receptor contains a minimum of two orthosteric sites, and thus the Shallenberger/Acree model and its improvements, although perhaps correct for some sweeteners, are not correct in the general sense.

What Do Sweeteners Bind to the Sweetener Receptor?

Many more questions remain to be answered about the sweetener receptor. Xu *et al.* (8) have demonstrated that aspartame and neotame bind to the VFD of T_1R_2. Is this the orthosteric site for sucrose and other carbohydrate sweeteners? Kniazeff *et al.* (12) have demonstrated that both VFDs of the homodimeric metabotrophic glutamate 5 must be populated by glutamate to give a maximal response. And for the heterodimeric γ-aminobutyric acid (GABA) type B receptor, Knaizeff *et al.* demonstrated that binding of GABA to only the GABA type B1 receptor provides a maximal response (13). If sucrose and other carbohydrate sweeteners bind to the VFD of T_1R_2, do they also bind to the VFD of T_1R_3 as might be expected from glutamate binding in metabotrophic glutamate 5, or, because the VFD of T_1R_3 is different from that of T_1R_2, do they bind only in T_1R_2? It has been demonstrated in psychophysical experiments that carbohydrate sweeteners invariably give higher, and apparently equivalent, maximal responses, whereas other structural types of sweeteners give lower, and variable, maximal responses (14). Could this be because the sweetener receptor binds two carbohydrate sweetener molecules, one in each VFD, thus leading to a high state of activation, while it responds to other structural types of sweeteners binding only a single molecule in T_1R_2 with a lower state of activation? Another curiosity on the sweetener receptor relates to its enantioselectivity. I (unpublished work) and others (15) have observed that some enantiomeric pairs of simple carbohydrates (e.g., D- and L-glucose) are identical in taste, whereas others are quite different (e.g., D- and L-fructose). How can this be explained?

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What Is the Basis for Sweetness Synergy?

The biochemical basis for the phenomenon of sweetness synergy is also not known. It has long been known that aspartame and cyclamate are synergistic in sensory experiments (16). Xu *et al*. (8) have demonstrated that the sweetener receptor has separate orthosteric sites for aspartame and cyclamate, and thus it seems reasonable that a cooperative binding effect may explain aspartame/cyclamate synergy. It is noteworthy that there are many other synergistic sweetener combinations. Exemplary are saccharin and cyclamate. Does saccharin/cyclamate synergism require different orthosteric sites? Saccharin and aspartame are also synergistic, however, and if binding cooperativity is the mechanism mediating sweetness synergy, then the sweetener receptor must have at least three orthosteric sites to explain the synergism observed with just these three sweeteners. Sweetness synergy has been observed in other combinations of sweeteners as well, which suggests that the number of orthosteric sites on the sweetener receptor may be more than three. Much more work must be done to map the many known sweeteners to their orthosteric sites. Another anomaly is that, although significant synergism is common among pairs of noncarbohydrate sweeteners, synergism is weak or nonexistent in carbohydrate/noncarbohydrate sweetener pairs (e.g., aspartame/sucrose). Why should synergy be limited to noncarbohydrate sweetener pairs?

Are Selective Sweetener Antagonists Known?

A number of antagonists of the sweetener receptor are known, of which the most studied is lactisole. Only one antagonist has been reported that is selective in inhibition of sweetness of the various structural types of sweeteners: Zn^{2+} ion (17). In a study of 15 sweeteners from eight different structural classes, Zn salts inhibited all of them, except cyclamate. The observations on sweetness antagonism now can be rationalized with the new information provided by Xu *et al*. (8). It seems most plausible that lactisole, binding in the seven-TMD of T_1R_3, is a negative allosteric modulator and thus is able to allosterically inhibit the activities of sweeteners that bind in the VFD and perhaps competitively inhibit cyclamate because it also binds in the T_1R_3 seven-TMD. And it seems quite plausible that Zn^{2+} ion may bind in the VFD of T_1R_2, thereby competitively inhibiting activities of sweeteners acting at this orthosteric site, while having no effect on cyclamate that acts in the T_1R_3 seven-TMD.

How Can Sweet Water Aftertaste Be Explained?

A curious observation for several sweetener receptor antagonists is that they exhibit "sweet water aftertaste."[†] This effect is particularly dramatic for lactisole. If one tastes a solution of lactisole at a concentration known to give strong sweetness inhibition, expectorates the solution, and then rinses the mouth with water, the water tastes strikingly sweet. It is tempting to rationalize this phenomenon by a mechanism involving inactivation of a constitutively active sweetener receptor by an inverse agonist. Is this the operative mechanism?

A final curiosity about sweetener receptor function relates to findings in cross-adaptation psychophysical experiments. For example, if one adapts the sensory system to the sweetness of aspartame, sweetness perceived from D-tryptophan is strongly suppressed, while at the same time, the sweetness of many other sweeteners including saccharin and cyclamate are unaffected (18). How can this be understood in terms of a single sweetener receptor?

What Is the Mechanism of Action for Enhancers of the Umami Receptor?

The work of Xu *et al*. (8) also provides insight into the biochemistry of umami taste. A longstanding mystery in the sensory field is the striking synergistic effect of IMP on the taste of MSG. Xu *et al.* report that cyclamate, although exhibiting no activation of the umami receptor by itself, enhances the activity of MSG. And because they have established the locus of binding for cyclamate to be in the seven-TMD of T_1R_3 and because MSG's orthosteric site is presumed to be in the VFD of T_1R_1, it seems that cyclamate may be a positive allosteric modulator of the umami receptor. But still, questions remain. How does IMP enhance the activity of MSG? Does IMP also act in the seven-TMD of T_1R_3? And, of course, is the VFD of T_1R_1 the orthosteric site for MSG? And finally, must MSG populate the VFDs of both T_1R_1 and T_1R_3 to provide a maximal response?

Great progress has been made over the last 2 years in understanding the biochemical pathways that mediate sweet and umami tastes. Xu *et al.* (8), using chimeric rat/human receptors, have shed light on several important questions. However, there is no shortage of additional questions to be resolved before we really understand sweet and umami tastes.

[†]DuBois, G. E. & D'Angelo, L., XXIth Association of Chemoreception Sciences Meeting, April 16, 1999, Sarasota, FL, poster.

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