Bitter Taste for Flavor and Health*

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The evolution of and individual variation in bitter taste and speculation on the future directions and applications of fundamental taste research

Humans are generally thought to perceive five basic taste qualities: salty, sour, bitter, sweet and umami/savory. Unlike other sensory systems such as audition and vision, which detect and perceive the external physical world of sound frequencies or light wavelengths, the taste system enables humans and animals to explore the chemical nature of the environment and subserves the internal physiological needs of the organism. For example, salty, sweet and umami tastes allow humans and animals to seek out essential minerals and energy- or nutrient-rich foods, while sour and bitter tastes help the organism avoid ingesting putrefied foods, unripe fruits, potentially harmful plant alkaloids and other toxins.

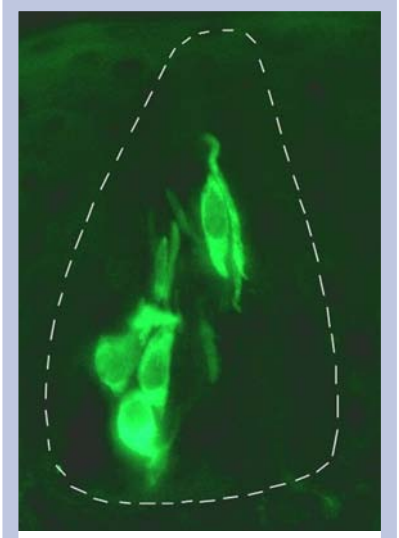
This column will provide a general overview of recent progress in studies on human taste, with a focus on bitter taste. Topics covered include: the location and activation of taste receptors; the evolution of and individual variation in bitter taste; and speculation on the future directions and applications of fundamental taste research.

Taste Buds: the Peripheral Organs

Taste sensation is initiated by the interaction of sapid molecules in foodstuff with receptor cells located in taste buds. A taste bud (**F-1**) is an onion-shaped structure consisting of 50 to 100 cells embedded in the stratified epithelial layer of the oral cavity. Taste buds on the tongue's surface are aggregated into three types of papillae that are visible on the surface of the tongue: mushroom-shaped *fungiform* on the front, leaf-shaped *foliate* on the sides, and round-shaped *circumvallate* papillae in the back. In addition, some individual taste buds are also found in the soft palate, pharynx, larynx, esophagus and epiglottis. The taste buds located in these different areas are innervated by one of three cranial nerves: the facial, glossopharyngeal and vagus nerves, which convey gustatory information to the brainstem where it is combined with other ascending and descending information before being relayed on to thalamus, amygdala and cortical areas.

The density of taste papillae and the total number of taste buds vary from person to person. People with denser papillae and a greater number of taste buds tend to be more sensitive to taste stimuli. In addition to variation in the periphery, how the brain processes gustatory input can also contribute to one's taste sensitivity and acuity. The wide variation in taste sensitivity is part of Mother Nature's diversity, somewhat similar to the variability found in visual or hearing abilities within the general population. Differences in taste perception, however, may have important health and nutritional consequences; by influencing food selection, what you eat, and how much you eat, individual differences in taste sensitivity can directly affect your health and well-being.

A human taste bud **F-1**



This is a fluorescent image of a human fungiform taste bud with the taste pore located at the top. This longitudinal section was stained with an antibody against phospholipase C β2, an enzyme involved in sweet, bitter and umami taste transduction, followed by a fluorescein isothiocyanate (FITC)-conjugated secondary antibody staining. The green fluorescent cells shown here are bitter, sweet or umami taste receptor cells.

Receptor Activation Triggers Signaling Cascades

The mechanisms for sour and salty taste detection remain incompletely understood. Protons and sodium ions, the putative stimuli for these tastes, most likely pass through channel receptors to directly enter taste cells, where they

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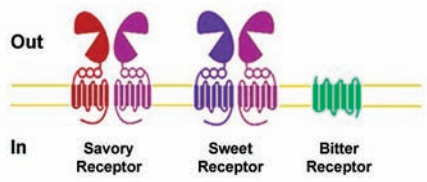
Umami (savory), sweet and bitter receptors **F-2**



Dimerized umami (T1R1/T1R3) and sweet (T1R2/T1R3) receptors share a common monomer T1R3 and a similar 3-D structure. Both receptors have bulky extracellular domains that form multiple binding sites for sweet compounds, enhancers as well as inhibitors. In contrast, bitter receptors (T2Rs) have much smaller extracellular domains and fewer ligand binding sites.

initiate a signaling cascade that ultimately causes the cell to fire. In contrast, sweet, bitter and umami compounds in foodstuff interact with G-protein coupled receptors (GPCRs) on the cell surface (see **F-2**). These polypeptide transmembrane receptors resemble beads on a string that thread through the cell membrane seven times, with one end exposed on the cell's exterior and the other end located inside the membrane.

It is certainly possible that genetic variation in bitter genes will result in individuals having very personalized bitter taste profiles.

Sweet and umami tastes each have only one receptor. Both receptors are dimerized, meaning that they are comprised of two intertwined polypeptide components. Interestingly, umami and sweet receptors, designated by their components as TAS1R1/TAS1R3 and TAS1R2/TAS1R3, respectively, share the TAS1R3 monomer. Another common characteristic of these receptors is their bulky extracellular domains, which can bind to many different sweet or umami molecules, inhibitors and enhancers. Mutations in these domains have resulted in species variation in the ability to detect various taste stimuli. For example, New World monkeys cannot taste certain sweet proteins, such as monellin, or artificial sweeteners that humans and Old World monkeys do detect and perceive as sweet. Similarly, sensitivity to sweet tastes has been reduced or eliminated in cats through disruption of the gene for TAS1R2.

In contrast to the single receptors for sweet and umami tastes, the number of bitter taste receptors in vertebrate animals is much greater, and scientists have identified approximately 25 different bitter taste receptors in humans. Unlike the sweet and umami receptors, bitter receptors have a much smaller extracellular structure and they typically form a ligand-binding pocket that spans from the extracellular space deep into the transmembrane region. Due to this structural difference, bitter receptors possess fewer binding sites and thus display greater specificity for compounds than their sweet and umami counterparts.

When taste substances bind to a GPCR receptor, the binding changes the receptor's shape or conformation, causing the receptor to become activated and altering the conformation of intracellular mediators known as *G-proteins*. One receptor can stimulate thousands of G-proteins, and each G-protein in turn activates thousands of subsequent downstream signaling proteins. Therefore, this G-protein-mediated signaling cascade can amplify the signal from an original single-taste molecule by millions-fold, which eventually leads to the opening of a transient receptor potential channel named *TRPM5*. The open channel allows sodium and other positively charged ions outside the receptor cell to rush into the cell, which changes the voltage difference across the cell membrane. This change in voltage differential triggers the receptor cell to release *transmitter molecules* into the extracellular space around the taste bud. The transmitters are in turn detected by receptors on fibers of cranial nerves VII, IX and X, which activate the nerves to send electrical signals to the brain. The brain processes the taste quality and intensity information as well as the pleasantness or unpleasantness of the taste. The innate response of newborns to sweet taste is one of liking while the innate response to bitterness is dislike. Mild bitterness, however, can actually be attractive to adults, most likely due to learning.

Interestingly, the brain perceives taste quality via the taste receptor cell that is activated rather than the taste receptor itself. With the exception of salty, taste qualities are normally transduced by discrete taste receptor cells such that non-native expression of receptors on taste

bud cells can cause misattributions of taste quality. For instance, animal experiments have shown that when the sweet receptor is expressed in bitter receptor cells, sweet substances can taste bitter, evoking aversive responses. This finding suggests that under some extreme clinical conditions, inappropriate expression of receptors on taste receptor cells may contribute to taste disorders such as dysguesia, a taste disorder characterized by distorted taste sensations.

Human's Dietary Change Relaxes Constraints Against Bitter Receptors

Most humans have approximately 25 functional bitter receptors that are encoded by ~25 different genes. Most of these genes are clustered on two loci of chromosomes 7 and 12, with a single bitter receptor gene located on chromosome 5. This organization implies that the clustered genes may have been generated from an ancestral gene by tandem duplication. Both rodents and primates have dozens of bitter receptors and share a similar clustering organization of bitter genes, suggesting that the bitter receptor repertoire expanded before these different species embarked on divergent evolutionary routes.

Early humans and other primates mostly lived in tropical forests and were largely plant-eaters, i.e., herbivores, gathering most of their food from leaves, bark, roots, flowers, fruits and other plant parts. Plants, however, contain many potentially toxic bitter-tasting substances, such as tannins, phenols and terpenes. Mutations in taste receptors that enabled more efficient detection of these dangerous compounds may have been positively selected during the establishment of new habitats. Throughout time, the diets of your human ancestors have diversified. The shift from herbivore to omnivore was critical for supporting the ever-enlarging human brain, since animal source foods such as meat and milk are not only richer in nutrients but also generally less toxic. More recently, with the advent of cooking, farming, animal domestication and most recently, with the creation of the food processing industry, the human diet has become more digestible, more nutritious and safer. In concert with the diet changes, approximately one third of the human bitter receptors have degenerated and their genes have become non-functional pseudogenes. Modern human's bitter receptor repertoire has shrunk to approximately 25 functional receptors.

How Many Substances Taste Bitter to Humans?

Despite the reduction of their bitter taste receptor repertoire, humans are still able to taste many naturally occurring poisonous compounds. However, there appears to be little correlation between a compound's bitterness and its toxicity. Small amounts of some bitter substances, such as those found in tea and coffee, are not toxic. In fact, humans have come to accept and even prize the tastes of tea and coffee and have consumed these beverages in large quantities for thousands of years.

It is not yet known how many different bitter compounds humans can taste. The relatively recent identification of human bitter receptor genes has led to

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the use of high-throughput screening methods to identify bitter compounds; these methods will ultimately lead to a more thorough understanding of the repertoire of bitter compounds perceived by humans. Instead of using human subjects to test various natural and artificial compounds, artificial "tongues" can be grown in a dish by culturing cells that express bitter receptors. These cells can be loaded into 96-, 384- or 1,536-well plates and compounds of interest can be applied onto the cells in each well. Activation or inhibition of bitter receptors by any compound can be detected by monitoring the change in calcium concentrations inside the cells; following the cascade described above, an increase in intracellular calcium indicates that the test compound has activated a bitter receptor. Using this large-scale screening methodology, the response profiles to all existing compounds can be defined for each bitter receptor, as well as for the tongue as a whole.

Currently available data reveals that a number of compounds extracted from plants activate one or more human bitter receptors. For example, glucopyranosides found in almonds and willow bark, isothiocyanates from broccoli, brussel sprouts and other cruciferous vegetables, and picrotoxinin from fishberries all have been found to be ligands for bitter receptors. Human bitter receptors are also activated by bitter peptides that are produced during food processing and found in many fermented foods such as beer, wine and cheese.

Based on response profiles, human bitter receptors can be classified into two categories: narrowly tuned and broadly tuned. The former are activated by single substances or a group of structurally related compounds, while the latter class of receptors respond to a variety of structurally unrelated compounds. This dichotomy may have enabled you to specifically recognize special compounds that are crucial to your physiological needs and even survival, while at the same time allowing you to detect a wide range of toxic substances with broadly tuned receptors.

Bitter to You, but Not to Me

Although humans have about 25 functional bitter taste receptor genes, there are at least 151 variations or polymorphisms that have been discovered thus far among these genes. Many of these genetic polymorphisms result in changes in the amino acid residues of the affected bitter receptors, suggesting that the different variants of a receptor may lead to markedly altered activation profiles. One well-known example is the variation observed in taste sensitivity to PROP (6-*n*-propylthiouracil) and PTC (phenylthiocarbamide). Genetic variation in the TAS2R38 bitter receptor causes different individuals either to taste PROP or PTC as completely tasteless or to perceive these compounds as tasting moderately to strongly bitter.

This is a striking example of taste blindness; other examples will likely be discovered, along with marked individual differences in the perceived intensity of various bitter compounds. It is certainly possible that genetic variation in bitter genes will result in individuals having very personalized bitter taste profiles.

Future Perspectives

One important remaining question is whether people can discriminate one bitter taste from another: that is, does every bitter compound have the same bitter taste? Experiments on rats and monkeys demonstrate that these animals cannot differentiate among various bitter compounds. Cellular and molecular studies indicate that one taste receptor cell can express multiple bitter receptors. The brain presumably perceives the same bitter quality when a given cell is stimulated by different bitter receptors on that cell. However, not all bitter receptors are expressed in one cell and some bitter receptors are found preferentially in one class of taste papillae. Further, the three cranial nerves that innervate taste buds respond differently to the same bitter substances. Therefore humans may still retain some discriminatory power among bitter compounds.

The future also presents the opportunity to create effective bitter blockers. The excess bitterness of medicines or foods such as broccoli and certain types of cheese makes it difficult for many individuals, especially children, to take these medicines or consume these beneficial foods. As knowledge grows about how various bitter substances stimulate the repertoire of bitter receptors, it becomes possible to search for receptor-based specific bitter inhibitors or blockers. It should be possible to specifically eliminate or reduce a particular compound's bitterness without affecting an individual's general sensitivity to desirable bitter tastes, such as those present in cheese, beer or coffee. Since each person may have a unique combination of bitter receptor variants, it should also be possible in the future to design personalized bitter blockers, thus making bitter a better taste for all.

Finally, bitter receptors have recently been found in other organs, including the gut and pancreas, suggesting that these receptors' function may extend beyond traditional concepts of taste. Further studies will reveal their role in digestion and absorption and may provide innovative strategies to prevent and treat the diseases of the 21st century: obesity and diabetes.

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Suggested Reading

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