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Receptors that mediate sweetness: Inferences from biochemical, electrophysiological and psychophysical data

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Abstract: Identification, isolation, and characterization of taste receptors for sweet compounds have not yet been accomplished due to inadequate biochemical techniques appropriate for studying receptor binding in gustation. However, a series of biochemical, electrophysiological, and psychophysical studies suggest that proteinaceous receptors coupled to the G-protein/adenylate cyclase second messenger cascade mediate sweet taste for some compounds. Other second messenger systems (e.g. the phosphatidyl inositol system) as well as ion channels and non-receptor mechanisms may also be involved. There is ample evidence that multiple types of sweet receptors are required to transduce signals for the many chemical classes of compounds that taste sweet: e.g. low molecular weight carbohydrates, aminoacyl sugars, amino acids, peptides, proteins, terpenoids, chlorinated hydrocarbons, halogenated sugars, *N*-sulfonyl amides, sulfamates, polyketides, anilines, and ureas. Evidence for multiple receptors comes from a variety of studies including: 1) use of sweetness inhibitors (e.g. gymnemic acid or phenoxyalkanoic acid compounds), 2) electrophysiological recordings using modifiers of second messenger systems, 3) cross-adaptation studies, 4) sweetener mixtures that produce synergy, and 5) structure-activity studies combined with molecular modeling. When adequate biochemical techniques are finally achieved for isolating and characterizing sweet receptor proteins, the rational and systematic design of sweeteners by computer will replace serendipity in the discovery of new sweetener compounds.

SWEET-TASTE RECEPTORS AND THE ADENYLATE CYCLASE SYSTEM

The search for taste receptors that bind sweeteners has not been as successful as the pursuit of neurotransmitter and hormone receptors. In fact, no receptor for sweet taste has yet been isolated and characterized. Isolation of sweet taste receptors is limited by the low affinity of tastants for receptors as well as the physiology of the gustatory system. Although the isolation of sweetener receptors has proven difficult, current consensus is that the sweet taste response is mediated by taste cell surface receptors that utilize the adenylate cyclase system as a second messenger system. The adenylate cyclase system, which is also the cellular signaling system for many hormones, involves the cascade of events shown in Figure 1. The sweetener molecule (agonist) binds to a receptor which transmits a signal via the guanine nucleotide-binding protein (G protein) resulting in activation of adenylate cyclase. Adenylate cyclase then induces hydrolysis of ATP to cAMP which leads to activation of the phosphorylating enzyme known as protein kinase A. The activated kinase then phosphorylates an ion channel in the taste cell membrane leading to depolarization of the taste cell. The validity of the model in Figure 1 for sweet taste transduction is supported by biochemical investigations that have established the existence and activation of components of the adenylate system in taste buds including adenylate cyclase (ref. 1-4), cAMP phosphodiesterase (ref. 3,5), cAMP (6), and cAMP-dependent kinase (7). Certain sweeteners including sucrose and saccharin cause a stimulation in adenylate cyclase activity leading to elevated levels of cAMP (6).

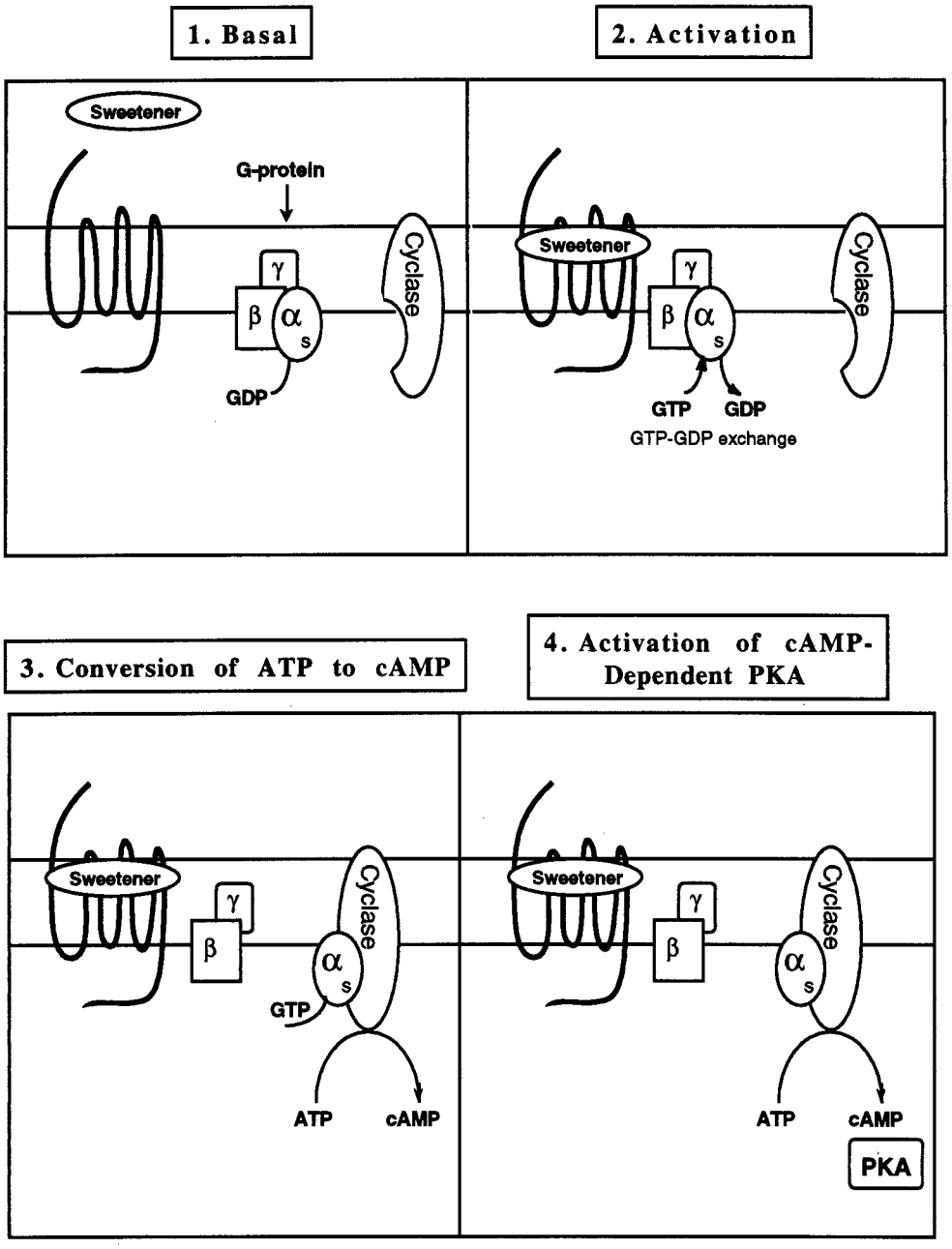

Figure 1.

Sweet taste receptors also probably undergo desensitization in a manner similar to other receptors coupled to the adenylate cyclase cascade (see ref. 8). Desensitization explains the phenomenon of adaptation. First, sweeteners bind with cell surface receptors on taste cells leading to activation of the G_S protein. Next, activation of the sweetener receptor probably initiates translocation of a protein found in the cytoplasm called βark-2 (β-adrenergic receptor kinase) to the plasma membrane. βark then phosphorylates the stimulated receptor which initiates the process of receptor inactivation. Desensitization by βark is a general molecular mechanism operative in many G-protein-coupled receptor systems including synaptic neurotransmitter receptors. Phosphorylation of the sweetener receptor by βark allows a second protein called β-arrestin to bind to the receptor. Binding of β-arrestin to the stimulated

receptor phosphorylated by βark inactivates the receptor by quenching phosphorylated receptor coupling to G_s. The receptor is then sequestered and finally returns to the cell surface. βark enzymes are ubiquitous throughout the body. There are numerous types of βark that have been found to desensitize a broad range of cell receptors including those for odorants (ref. 9,10).

The βark mechanism can be used to explain the differential decrements in adaptation and cross-adaptation found for the different sweeteners. Cross-adaptation studies suggest that sugars and sugar alcohols bind to more receptor types than high potency sweeteners (ref. 8,11). Thus, sugars and sugar alcohols would be expected to be least affected by the βark mechanism. For high potency sweeteners, a large proportion of the limited number of receptors may be inactivated on the first taste so that few receptors are available for binding on subsequent tastes resulting in extensive adaptation. When a sugar binds to sweet receptors, βark would inactivate those stimulated taste cells but many receptors would still be available for binding sugars.

SWEET-TASTE RECEPTORS AND OTHER TRANSDUCTION MECHANISMS

Transduction of sweet taste signals across plasma membranes in numerous cell types may also be induced (or modulated) by pathways involving a variety of lipid-derived second messengers produced from membrane phospholipids, including the signal-activated phospholipase pathways of the phosphatidylinositol system. In the phosphatidylinositol system, a ligand binds to a receptor, activates a G protein or a tyrosine kinase, which subsequently activates specific isoforms of phospholipase C resulting in the hydrolysis of the phospholipid phosphatidyl-inositol 4,5-biphosphate (PIP_2). The hydrolysis of PIP_2 generates two products, the sugar phosphate inositol-triphosphate IP_3 as well as diacylglycerol (DAG). IP_3 and DAG influence intracellular functioning and can lead to the subsequent depolarization of a cell. IP_3 binds to a receptor on the endoplasmic reticulum which triggers the mobilization of calcium from intracellular stores. Calcium in concert with DAG activates protein kinase C, and protein kinase C phosphorylates key proteins that regulate the response of the target cell to the ligand. Calcium may also activate other cellular enzymes as well as ion channels. DAG can also be derived from membrane phospholipids in addition to PIP_2. For example DAG can be produced directly from phosphatidylcholine (PC) by phospholipase C or produced indirectly from PC via another phospholipase called phospholipase D. Calcium mobilization occurs in the phosphatidylinositol system but has not been shown in PC pathways.

Schiffman et al. (ref. 12) found that three modulators of lipid-derived second messenger systems alter sweet taste responses: two membrane permeable analogs of DAG, 1-oleoyl-2-acetyl glycerol (OAG) and dioctanoyl glycerol (DiC8) as well as thapsigargin, which releases Ca^{2+} from intracellular stores. OAG (125 μM) and DiC8 (100 μM) enhanced the taste response to several sweeteners. Thapsigargin suppressed several sweet taste responses. The finding that OAG and DiC8 significantly enhanced sweet responses suggests that there may be cross-talk between lipid-derived second messengers and the adenylate cyclase system. This is not surprising since stimulation of receptors coupled to the phosphatidylinositol system can enhance cAMP production mediated by stimulation of the β-adrenergic receptor. Cross-talk has previously been reported in the olfactory system via a calmodulin, found in olfactory neurons, that potently activates olfactory adenylate cyclase. Thus, mechanisms analogous to those in olfaction also appear to exist in the taste system since DiC8 enhances sweet taste (ref. 12). Cross-talk in the taste system explains the efficacy of the empirical use of sugar or sweeteners to suppress bitterness in foods, beverages, and drugs.

Amiloride-sensitive sodium channels (ref. 13-15) and receptor-independent activation of G proteins (ref. 16) also appear to play a role in sweet taste transduction. Naim et al.'s finding

(ref. 16) that several amphiphilic sweeteners may stimulate cellular events through direct activation of G-proteins rather than by binding to receptors on the cell surface is intriguing. Several amphiphilic sweeteners were found to activate transducin and Gi/Go-proteins. Na saccharin, neohesperidin dihydrochalcone, aspartame, Na cyclamate, and monellin significantly stimulated the GTPase activity of Gi/Go-proteins. The concentrations of sweeteners required to activate G-proteins *in vitro* was highly correlated with concentrations that elicit taste in humans.

MULTIPLICITY OF TASTE RECEPTORS FOR SWEETENERS

Many lines of evidence suggest that there are multiple sweet receptors types (and/or transduction types if one considers Naim et al.'s data) involved in sweet taste transduction. Data that suggest the existence of multiple types of sweetener receptors include: 1) use of sweetness inhibitors (e.g. gymnemic acid or phenoxyalkanoic acid compounds), 2) cross-adaptation studies, 3) qualitative differences among sweeteners 4) different shapes of dose-response curves, 5) cooling studies, 7) effects of caffeine on sweet taste, 8) age-related losses in sweet taste, 9) electrophysiological recordings using modifiers of second messenger systems, 10) sweetener mixtures that produce synergy, and 11) structure-activity studies combined with molecular modeling.

Sweetener Inhibitors

+2-(4-methoxyphenoxy) propanoic acid. Substituted phenoxyalkanoic acid compounds have been reported to block sweet taste (ref. 17-19). The sodium salt of ±2-(4-methoxyphenoxy) propanoic acid (Na-PMP) has been found to selectively block the sweetness intensity for 12 of 15 sweeteners at both the 250 ppm and the 500 ppm levels (ref. 20). These include 3 sugars (fructose, glucose, sucrose), 2 terpenoid glycosides (rebaudioside-A, stevioside), 2 dipeptide derivatives (alitame, aspartame), 2 *N*-sulfonylamides (acesulfame-K, sodium saccharin), 2 polyhydric alcohols (mannitol, sorbitol), and 1 sulfamate (sodium cyclamate). However, when the same concentrations of Na-PMP were mixed with 3 of the 15 sweeteners (monoammonium glycyrrhizinate, neohesperidin dihydrochalcone, and thaumatin), there was little reduction in sweetness intensity. These data suggest that Na-PMP is a selective competitive inhibitor of sweet taste and provide evidence for multiple sweet receptor types. Interestingly, Na-PMP is almost tasteless (faint bitter or metallic taste), but it is structurally similar to the sweetener dulcin (ref. 19).

Gymnemic acid (from *Gymnema sylvestre*). Gymnemic acid (GA) is a mixture of 20 distinct oleanane-type glycosides (ref. 21). An overview of studies on gymnemic acid provides evidence for multiple sweet receptor types. Kurihara (ref. 22) found that GA suppressed the taste of sucrose, Na Cyclamate, D-tryptophan, D-leucine, beryllium chloride, and Pb Acetate in humans. However, it did not suppress the sweet taste of chloroform. Faurion et al. (ref. 23) found that the degree of suppression of various sweeteners varied with chemical structure, and like Kurihara, they found little suppression of the sweetness of chloroform. Hellekant and Gopal (ref. 24) reported species differences in the effect of GA, further suggesting multiple sweet receptor types. They found that GA suppressed the taste of sucrose in hamsters with no effect on saccharin. The opposite was found in rats, i.e. there was suppression of saccharin with no effect on sucrose. In general, Hellekant and Roberts (ref. 25) did not find any dramatic decrease of responses to either sweet or non-sweet substance in hamster after treatment with GA. In chimpanzee, Hellekant et al. (ref. 26), like Faurion et al., found that the degree of suppression of the sweet response varied with the structure of the sweetener. GA completely abolished responses to acesulfame-K, aspartame, D-tryptophan, monellin, and thaumatin. However, there was 75% suppression of sucrose and only 50% suppression of xylitol. There is disagreement whether GA is an inhibitor for taste qualities other than sweetness (ref. 22,27-29), and the bulk of the evidence suggests that GA inhibition of sweetness is not due to competition for receptor sites (ref. 30).

Gurmarin (from *Gymnema sylvestre*). Pretreatment of the tongue with gurmarin, a peptide consisting of 35 amino acids isolated from the leaves of *Gymnema sylvestre* (ref. 31) suppressed responses to sucrose without affecting responses to NaCl, HCl, and quinine in C57BL mice; however, gurmarin did not significantly suppress sucrose responses in BALB

mice (ref. 32). These findings provide evidence for multiple sweet receptor types in mice. In rats, gurmarin blocked responses to sugars, sweet amino acids and saccharin (ref. 33).

Ziziphins (from *Ziziphus jujuba*). Ziziphins are triterpenoid glycosides (ref. 21) that provide further evidence for multiple sweet receptors. In rat and man, ziziphins suppress responses to various sugars (glucose, fructose) and artificial sweeteners (Na saccharin) but have no effect on some sweet-tasting amino acids (glycine, L-alanine) (ref. 34). Ziziphins are effective as sweet-taste inhibitors used both in mixtures (ref. 35) or as a pretreatment (ref. 35-37).

Proteases. An overview of studies using proteases provides evidence for multiple sweet receptor types. Faurion et al. (ref. 23) found that Pronase E blocked the taste of some sweeteners but had no effect on glycyrrhizzic acid. In addition, Faurion (ref. 38) reported that the degree of suppression of sweetness by Pronase E was unique to each subject suggesting individual differences in sweet receptor populations on human tongues. There is disagreement whether proteases are specific for sweet taste. Hiji (ref. 39) found that Pronase E suppressed the sweetness of sucrose with no effect on bitter, salty, or sour stimuli. Giroux and Henkin (ref. 40), however, tested a variety of proteases and found that while some, but not all, produced elevated thresholds for sucrose, these effects were not specific for sweet taste but affected other taste qualities as well.

Heavy metals. Iwasaki and Sato (ref. 41) treated the tongue of mice with 7 heavy metal salts and found that pretreatment with $CuCl_2$ and $ZnCl_2$ at 0.01 mM inhibited responses to sucrose and Na saccharin with no effect on responses to bitter, salty, or sour stimuli. Subsequent studies by Iwasaki and Sato (ref. 42) which employed a range of sweet-tasting stimuli provide evidence for multiple sweet receptor types. Iwasaki and Sato (ref. 42) found that $CuCl_2$ and $ZnCl_2$ at 0.1 mM blocked responses to sugars (sucrose, maltose, fructose, glucose) as well as Na saccharin but had little effect on sweet-tasting amino acids including glycine, L-alanine, L-serine, L-proline, and D-tryptophan. Yamamoto and Kawamura (ref. 43) found that pretreatment with higher concentrations of cupric and zinc salts produced irreversible reductions in responses to sucrose and quinine HCl.

Amiloride. The diuretic amiloride, a potent inhibitor of sodium transport, blocks the tastes of both sweet and salty (sodium salts) stimuli (ref. 13, 14) depending on the species. Pretreatment of the human tongue with 500 mM amiloride reduced the intensity of all ten sweeteners tested but to varying degrees (ref. 13). The greatest suppression was for stevioside which was blocked by 81%; fructose was the least affected and was reduced by 44%. These data showing differences in degree of suppression are consistent with multiple sweet receptors. Mixtures of amiloride and sucrose also reduced sweet responses (ref. 15).

Sweetener derivatives. Several sweetener derivatives have been found to block the taste of sucrose when the inhibitor was combined with the sweetener. These include: p-nitrophenyl α-D-glucopyranoside and chloramphenicol (ref. 45), methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside (ref 46, 47), and *N*-(4-cyanophenyl)-*N'*-[(sodiosulfo)methyl]urea (ref. 48). Human data with methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside provide support for multiple sweet receptors since it has variable effects on sweeteners with different structures. Sodium saccharin was reduced by 21%, acesulfame-K was reduced by 30%, sucrose was reduced by 32%, and stevioside by 54%. Aqueous solutions of *N*-(4-cyanophenyl)-*N'*-[(sodiosulfo)methyl]urea and sweeteners also show a range of suppression for different sweeteners. Monoammonium glycyrrhizzate was inhibited by 24%; thaumatin, by 30%; Na cyclamate, by 40%; neohesperidin dihydrochalcone, by 58%; rebaudioside A, by 59%; aspartame, by 61%; sucrose, by 66%; acesulfame-K, by 82%; and sucralose, by 83%.

Tannic acid. The astringent compound tannic acid inhibits the intensity of a variety of sweet compounds to varying degrees providing further support for multiple sweet receptors. In mixtures with tannic acid, the greatest suppression was found for acesulfame-K, Na saccharin, rebaudioside-A, and stevioside; the least adaptation occurred with the sugars, polyhydric alcohols, and neohesperidin dihydrochalcone (ref. 49).

Cross-adaptation

Sweet-tasting compounds do not equally cross-adapt which suggests that there are multiple receptor site types. Schiffman et al. (ref. 11) found that the degree of cross-adaptation among sweet-tasting compounds was related to the types of hydrogen bonds that a stimulus molecule could form with the taste cell membrane. Stimuli that showed the greatest cross-adaptation such as sodium saccharin and acesulfame-K have chemical structures that would form similar hydrogen bonds with the taste cell membrane (and thus presumably bind to the same receptor sites). Neohesperidin dihydrochalcone and acesulfame-K which do not cross adapt have totally different ways in which they would form hydrogen bonds with the taste cell membrane (and thus bind to different receptor types).

Qualitative differences among sweeteners

Multidimensional scaling experiments in which sweeteners are arranged in a space based on similarity of perceived quality also suggest that there are multiple sweet receptors (ref. 50). Sugars were located distant from large proteins such as thaumatin in the three-dimensional space based on sweet quality. Sweeteners were found to vary widely in the type or nature of the sweet sensation they impart which suggests "sweetness" itself is not a single or unitary quality mediated by a single receptor. In addition, sweeteners vary in the area of the tongue that they activate. For example, sweet tasting proteins such as thaumatin produce more intense sweet sensations at the edges of the tongue while sucrose is more intense at the tip of the tongue (ref. 51). In addition, the sweetness for the proteins thaumatin and monellin develops more slowly and has a longer duration than the sweet sensation of sucrose. These differences in sensory characteristics between sweet proteins and sucrose suggest that they interact with different taste receptors on the tongue.

Different shapes of dose-response curves.

The shapes of the dose-response curves are dramatically different for different sweeteners. DuBois et al. (ref. 52) constructed dose-response curves for a wide range of sweeteners from suprathreshold intensity judgments made by a trained taste panel. Thaumatin never got sweeter than a 9% sucrose equivalent. Aspartame and alitame never got much sweeter than 15-16% sucrose even at maximum solubility. However, the dose-response curves for sucrose, fructose, and sugar alcohols continued to increase in intensity far beyond the equivalent sweetness of 15% sucrose.

Cooling studies

Green and Frankmann (ref. 53) found that when the tongue was cooled to 20 degrees centigrade, the sweetness of sucrose and the bitterness of caffeine were reduced in intensity; the sourness of citric acid and the saltiness of sodium chloride, however, were unaffected by cooling. A subsequent study showed that cooling the tongue did not reduce the sweetness of all sweet compounds (ref. 54). While fructose and glucose had temperature sensitivities similar to sucrose, saccharin did not. This suggests that multiple rather than a single receptor mechanism underlies the perception of sweet taste.

Caffeine studies

Schiffman et al. (ref. 55) found that pretreatment of the tongue with caffeine enhances the taste of some sweeteners including thaumatin, stevioside, sodium saccharin, acesulfame-K, neohesperidin dihydrochalcone, and D-tryptophan with no effect on other sweeteners such as aspartame, sucrose, fructose, and calcium cyclamate. This finding again emphasizes that multiple mechanisms for sweetness must occur in the taste cell.

Age-related losses in sweet taste

Evidence for multiple receptors comes from suprathreshold intensity data on sweeteners in young and elderly subjects (ref. 56). Age-related loss in perceived intensity was not uniform

across sweeteners; rather, the greatest loss was for large molecules such as thaumatin, rebaudioside, and neohesperidin dihydrochalcone.

Electrophysiological recordings using modifiers of second messenger systems

Integrated chorda tympani (CT) recordings have been made to sweet tastants before and after application of modulators of the adenylate cyclase system (ref. 57) and lipid-derived second messenger systems (ref. 12). These modulators have produced different responses on sweeteners depending upon their chemical structures.

Sweetener mixtures that produce synergy

Binary mixtures of sweeteners varying in chemical structure display different degrees of synergy (58). This variability in synergy further suggests the existence of multiple sweet receptor types.

Structure-activity studies combined with molecular modeling

There is ample evidence that multiple types of sweet receptors are required to transduce signals for the many chemical classes of compounds that taste sweet: e.g. low molecular weight carbohydrates, aminoacyl sugars, amino acids, peptides, proteins, terpenoids, chlorinated hydrocarbons, halogenated sugars, *N*-sulfonyl amides, sulfamates, polyketides, anilines, and ureas.

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