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Review Article

Cellular Mechanisms in Taste Buds

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Abstract

In the soft palate, tongue, pharynx and larynx surrounding the oral region, taste buds are present, allowing the sensation of taste. On the tongue surface, 3 kinds of papillae are present: fungiform, foliate, and circumvallate. Approximately 5,000 taste buds cover the surface of the human tongue, with about 30% fungiform, 30% foliate and 40% circumvallate papillae. Each taste bud comprises 4 kinds of cells, namely high dark (type I), low light (type II), and intermediate (type III) cells in electron density and Merkel-like taste basal cells (type IV) located at a distance from taste pores. Type II cells sense taste stimuli and type III cells transmit taste signals to sensory afferent nerve fibers. However, type I and type IV cells are not considered to possess obvious taste functions. Synaptic interactions that mediate communication in taste cells provide signal outputs to primary afferent fibers. In the study of taste bud cells, molecular functional techniques using single cells have recently been applied. Serotonin (5-HT) plays a role in cell-to-cell transmission of taste signals. ATP fills the criterion of a neurotransmitter that activates receptors of taste nerve fibers. Findings on 5-HT and ATP suggest that various different transmitters and receptors are present in taste buds. However, no firm evidence for taste-evoked release from type III cells has been identified, except for 5-HT and ATP. These results suggest that different transmitters and receptors may not be present in taste buds. Accordingly, an understanding of how transmitters might function remains elusive.

Key words: Taste bud cells—Cell communication—Second messenger—Serotonin—ATP

Introduction

Taste buds are peripheral sensory organs that respond to various taste stimuli. Signals from taste are transmitted via synaptic pathways between gustatory cells and afferent nerves, using these gustatory end organs. Afferent nerve fibers send signals to the central nervous system (CNS) via 3 kinds of neurons. Morphologically, mammalian taste cells can be classified into 4 types: type I, type II, type III and type IV (Merkel-like basal). Type IV cells are progenitor cells that appear during the normal course of cell turnover. Bigiani[4] noted that these cells possess properties of glial-like cells. Properties of type I and type IV cells are not well known, because receptive functions for carrying signals are currently unknown. On the other hand, type II and III cells seem to function as taste receptor cells and synaptic

outputs for taste signaling, respectively. According to a recent classification based on cytology, ultrastructure, and expression of certain molecules, the functions of conventional taste bud cells have been lost[1,4,65]. At present, a historical nomenclature based on electron micrography, molecular and functional features based on electron micrography, molecular and functional features based on immunostaining, and *in situ* hybridization and RT-PCR in single cells are suitable for these taste bud cells.

General Characterization of Taste Receptive Mechanism

The transduction of both sweet- and bitter-tasting substances is thought to involve membrane-bound receptors coupled to second-messenger systems[38]. Gustducin is an α-subunit of a G protein closely related to these transductions that is expressed in taste tissue[42]. Studies with gustducin-knockout mice have implicated gustducin in the detection of both sweet- and bitter-tasting compounds. Taste buds are specialized epithelial structures containing gustatory receptor cells. Taste buds in rat are grouped into several populations: fungiform papillae on the anterior portion of the tongue, and foliate and circumvallate papillae on the posterior tongue, in the epithelia of the nasoincisor ducts and geschmacksstreifen of the palate, and on the laryngeal surface of the epiglottis[19,43,56,58]. These populations differ in gustatory sensitivities, as shown by electrophysiological studies on peripheral taste fibers. In rat, chorda tympani nerve fibers innervating the fungiform papillae are the most sensitive to salts and acids[20,21]. The greater superficial petrosal nerve, which innervates taste buds on the palate, is most responsive to sweet-tasting stimuli[48,59]. Glossopharyngeal nerve fibers innervating the foliate and circumvallate taste buds respond well to acids and bitter-tasting stimuli[22,23]. In contrast, fungiform taste buds of the hamster are much more sensitive to sugars than those of the rat, but like the rat are relatively insensitive to bitter stimuli[19,21]. Single fibers in the rat and hamster superior laryngeal nerve, which innervates taste buds of the epiglottis, respond well to water[53,55], but little to sweet- or bitter-tasting compounds.

Although taste bud populations possess different sensitivities, the morphology of taste buds is similar throughout the oral cavity. Taste buds contain at least two morphological cell types, dark (type I), and low light (type II)[17,37,38,52]. Light cells are larger than dark cells, have electron-lucent cytoplasm, and are circular or oval in cross-section, with apical processes extending toward the taste pore. Dark cells display electron-dense cytoplasm and an irregular outline, with sheet-like cytoplasmic projections separating adjacent light cells[50]. The relationship among these cell types and the mechanism of gustatory transduction is unknown.

If α-gustducin is present in cells that transduce bitter and sweet stimuli, the number of rat taste cells expressing gustducin should be greater in both palatal and posterior tongue taste buds than in those of the anterior tongue. Fungiform taste buds of hamsters should likewise contain more gustducin-expressing cells than those of rats. Boughter *et al.*[6] used immunofluorescence to quantify the distribution of gustducin-immunoreactive cells among taste buds of various regions in both rats and hamsters.

Function of Type I Cell on Taste Buds

Type I cells are believed to be supporting, glial-like cells controlling extracellular ion concentrations. Using the patch-clamp technique, Bigiani[4] identified a new cell type in the taste buds of the mouse circumvallate papilla. These cells represented about 30% of total cells and were characterized by a large leakage current. The leakage current was carried by K^+, and was blocked by Ba^{2+}. Other taste cells, such as those possessing voltage-gated Na^+ currents and thought to be chemosensory in function, did not express any sizeable leakage current. Consistent with the presence of a leakage conductance, leaky cells

had a low input resistance (~25 GΩ). Resting potentials were close to the equilibrium potential for potassium ions. Electrophysiological analysis of membrane currents remaining after pharmacological block by Ba^{2+} revealed that leaky cells also possessed Cl^- conductance. In resting conditions, the membrane of these cells was about 60-fold more permeable to K^+ than to Cl^-. Resting potassium conductance in leaky cells could be involved in rapidly dissipating the increase in extracellular K^+ during action potential discharge in chemosensory cells. Leaky cells might thus represent glial-like elements in taste buds. These findings support a model in which specific cells control the chemical composition of intracellular fluid in taste buds. Guanylyl cyclase activity has been associated with type I cells[2]. Accordingly, the concept that type I cells represent supporting cells remains unchanged. Type I cells are not involved in signal processing for taste sensation.

Function of Type II and Type III Cells of Taste Buds

Synaptic interactions, both electrical and chemical, are produced in taste cells. Type II cells seem to represent the primary sensory receptive cells. Type II cells are probably sensory receptive cells for taste stimuli transduced by G-protein coupled-receptors and down-stream effectors, which are expressed primarily in type II cells[1,4,6]. Although type II cells and the sensory afferent nerve are in closely contact with type III cells, no ultrastructural specializations indicate a synaptic presence between type II cells and gustatory afferents[11]. Murray[44] reported that only type III cells form synaptic contacts with nerve endings. Consequently, type III cells seem to generate the terminal output in gustatory organs.

Gilbertson *et al.*[26] recorded the whole-cell responses of 120 taste cells in rat fungiform papillae and soft palate maintained within intact epithelium. Taste stimuli were: 0.1 M sucrose, 0.1 M KCl, 0.1 M NH_4Cl, 0.032 M NaCl, 3.2 mM HCl, and 3.2 mM quinine hydrochloride (QHCl). When the cells were held at resting potentials, taste stimulation resulted in conductance changes. Sucrose and QHCl produced a decrease in outward current and membrane conductance, whereas NaCl, KCl, NH_4Cl, and HCl elicited inward currents accompanied by increased conductance.

1. Serotonin receptors

Taste receptor protein (TR) and G protein (gustducin) form a coupled unit. RT-PCR was performed on RNA extracted from rat posterior taste buds with 14 primer sets representing 5-HT_1 through 5-HT_7 receptor subtype families. Data suggest that 5-HT_{1A} and 5-HT_3 receptors are expressed in taste buds. Immunocytochemistry with a 5-HT_{1A}-specific antibody demonstrated that subsets of TRCs were immunopositive for 5-HT_{1A}. Kaya *et al.*[35] hypothesized that 5-HT released from TRCs activates postsynaptic 5-HT_3 receptors on afferent nerve fibers and, via a paracrine route, inhibits neighboring TRCs via 5-HT_{1A} receptors.

In mammals, the transmitter of most taste cells making synapse with the gustatory nerve is 5-HT. These taste cells uptake 5-HT precursor, and are immunopositive for 5-HT. RT-PCR data indicate that 5-HT_{1A} and 5-HT_3 receptors are present in the terminals of primary afferent fibers[12,35].

Huang *et al.*[32] used Chinese hamster ovary cells stably expressing 5-HT_{2C} receptors as biodetectors to monitor 5-HT release from taste buds. When taste buds were depolarized with KCl or stimulated with bitter, sweet or sour (acid) tastants, 5-HT was released. KCl^- and acid-induced 5-HT release, but no release attributable to sweet or bitter stimulations, which require Ca^{2+} influx. In contrast, 5-HT release evoked by sweet and bitter stimulation seemed to be triggered by intracellular Ca^{2+}-release. These experiments strongly implicate 5-HT as a taste bud neurotransmitter and reveal unexpected transmitter release mechanisms.

2. Taste receptors

Using expression patterns of taste receptors

and double-labeled *in situ* hybridization[38], gustducin on foliate and circumvallate papillae of mice was investigated. The T1r family is part of the receptor family belonging to class C type of G protein-coupled receptors, and comprises 3 taste bud-specific receptors, T1r1, T1r2 and T1r3. T1r1 and T1r2 are known to display distinctive patterns of regional expression. T1r1 is expressed in taste buds of fungiform papillae, but is rare in taste buds of circumvallate papillae. T1r2 is rarely expressed in fungiform papillae, but is expressed in all taste buds of circumvallate papillae. T1r3 is strongly expressed in both fungiform and circumvallate papillae and forms an aminoacid (umami) receptor and a sweet receptor in combination with T1r1 and T1r2, respectively. These patterns suggest that taste cells in circumvallate papillae receive sweet taste substances through the heterodimer of T1r2 and T1r3 (T1r2/T1r3), while those in fungiform papillae receive umami substances through the heterodimer of T1R1 and T1r3 (T1r1/T1r3). Kusakabe *et al.*[39] observed different expression patterns of T1rs and gustducin in fungiform and circumvallate papillae.

Detection of sweet-tasting compounds is mediated in large part by a hetrodimeric receptor comprising T1r2+T1r3. Lactisol, a broad-acting sweet antagonist, suppresses the sweet taste of sugars, protein sweetener, and artificial sweeteners in human T1r3[33].

3. Gustducin

Gustducin is a transducin-like G protein (guanine nucleotide-binding protein) that is expressed in taste cells. Gustducin is believed to be involved in bitter, and possibly sweet taste transduction. Type II cells are spindle-shaped and lack the apical dense granules characteristic of type I cells. Subsets of taste cells may possess different functional properties. Protein gene product (PGP, ubiquitin carboxylhydolase) 9.5 immunoreactivity[64] is only present in type III cells of rats. Antisera show the presence of gustducin in taste buds of rat circumvallate papillae. Gustducin is present in both the microvilli and cytoplasm of immunoreactive cells[63]. Gustducin is also involved in umami taste[39,64].

4. Second messengers

Rat taste buds are candidates for taste transduction. However, the physiological roles of these cells are unclear. Inositol 1,4,5-triphosphate (IP_3) has been implicated as an important second messenger in bitter, sweet, and umami taste transductions. Previously, Clapp *et al.*[11] identified the type III IP_3 receptor (IP_3R3) as the dominant isoform in taste receptor cells. In addition, a recent study showed that phospholipase Cβ_2 (PLCβ_2) is essential in the transduction of bitter, sweet, and umami stimuli. IP_3R3 and PLCβ_2 are expressed in the same subset of cells. To identify taste cell types that express proteins involved in PLC signal transduction, Clapp *et al.*[11] used 3,3′-diaminobenzidine tetrahydrochloride immunoelectron microscopy and fluorescence microscopy to identify cells with IP_3R3. Confocal microscopy was used to compare IP_3R3 or PLCβ_2 immunoreactivity with that of some known cell type markers such as serotonin, PGP 9.5 and neural cell adhesion molecule. Clapp *et al.*[11] showed that a large subset of type II cells and a small subset of type III cells display IP_3R3 immunoreactivity within the cytoplasm. These data suggest that type II cells are the principal transducers of bitter, sweet, and umami tastes. However, no synapses between type II cells and nerve fibers were identified[11]. Clapp *et al.*[11] speculated that some type II cells may communicate with the nervous system via subsurface cisternae of smooth endoplasmic reticulum in lieu of conventional synapses.

5. Electrical coupling

Cell-to-cell communication through low-resistance pathways (electrical or electrotonic coupling) has been observed between excitable and non-excitable cells alike in a variety of tissues, including many epithelia. In taste buds also, both electrophysiological and morphological observations have also demonstrated the existence of electrical couplings between taste cells. The junction resistance between taste receptor cells is around 200–300 MΩ[3].

6. Gap junction

Type II and type III cells possess gap junctions. Type III cell function involves connection with gustatory nerves. Using a recording technique with whole cell patch clamping, Ca^{2+}-activated K^+ current and voltage-dependent Na^+ channels with taste receptor cells were investigated. Serotonergic effects in rat taste receptor cells are mediated by 5-HT_{1A} receptors. It is confirmed that this observation is serotonergic. Ca^{2+}-activated K^+ current in rat posterior taste receptor cells is reportedly inhibited by 5-HT[28].

In mouse fungiform papillae, only type III cells show synaptic contacts with taste nerves. Type II cells appear to transmit taste information to type III cells through gap junctions[16]. When type III cells possess neurotransmitter receptors, paracrine systems in addition to gap junctions can transmit taste information. Gap junctions, together with paracrine systems, thus contribute to the formation of (taste bud cell (TBC)) networks to process taste information[65].

7. Chemical synapse

The suggestion has been made that 5-HT functions as a neurotransmitter. In CNS, 5-HT in the synaptic cleft is transported into synaptic terminals by selective Na^+-dependent 5-HT transporters, then the serotonergic transmission is terminated. The 5-HT transporter or other termination mechanisms should be present in taste buds if 5-HT acts as a transmitter in taste transduction[62]. This suggests that the 5-HT-induced sensation of taste is terminated by 5-HT uptake through 5-HT transporters[51].

Function of Type IV (Merkel-like Taste Basal) Cells

The function of type IV basal cells is not understood. One type of basal cell is an undifferentiated cell, presumably a stem cell. By combining light microscopic immunocytochemistry with electron microscopy, Delay *et al.*[14] showed that the other type of basal cell is positive for 5-HT-like immunoreactivity and that these cells exhibit ultrastructural features similar to those found in cutaneous Merkel cells. Based on these findings, and the facts that Merkel-like cell taste cells (type IV cells) have been shown to make synaptic contacts with adjacent taste cells and innervating nerve fibers, Delay *et al.*[14] concluded that these Merkel-like basal taste cells represent serotonergic interneurons.

1. Serotonergic interneurons

Pairs of taste cells were impaled with intracellular recording microelectrodes in intact taste buds in slices of Necturus lingual epithelium. Applying short pulses of 140 mM KCl or 200 mM $CaCl_2$ solutions to the apical pore elicited receptor potentials in taste receptor cells. Chemostimulation of receptor cells elicited postsynaptic responses in basal cells in the taste buds. Douglas *et al.*[15] directly depolarized individual receptor cells and tested whether this would evoke postsynaptic responses in basal cells. A comparison between responses in basal cells evoked by depolarizing single receptor cells, and responses evoked by stimulating the entire receptor cell population with KCl, suggests extensive synaptic convergence from receptor cells onto each basal cell. Douglas *et al.*[15] also tested whether electrical excitation of basal cells would elicit (retrograde) synaptic responses in receptor cells. Basal cells release 5-HT, which mediates modulatory effects on receptor cells. Merkel-like basal cells were concluded to release 5-HT onto adjacent taste receptor cells, enhancing electrotonic propagation of receptor potentials from the apical (chemosensitive) tip to the Merkel-like basal (synaptic) processes of receptor cells[15]. Activation of basal cells increases the chemosensitivity of taste receptor cells[16].

Merkel-like basal cells are sets of basal cells that form chemical synapses with taste receptor cells and innervating nerve fibers. Although Merkel-like basal cells can not interact directly with taste stimuli, recent studies have shown that Merkel-like base cells contain 5-HT, which may be released onto taste recep-

tor cells in response to taste stimulation. With the use of whole-cell voltage clamps, Delay *et al.*[13] examined whether focal applications of 5-HT to isolated taste receptor cells affects voltage calcium current (I_{Ca}). Two different effects were observed, with 5-HT at 100 μM increasing I_{Ca} in 33% of taste receptor cells, and decreasing I_{Ca} in 67%. Both responses used a 5-HT receptor subtype with a pharmacological profile similar to that of the 5-HT_{1A} receptor, but potentiation and inhibition of I_{Ca} by 5-HT were mediated by two different second-messenger cascades. These results indicate that functional subtypes of taste receptor may also be defined by response to the neurotransmitter 5-HT, and suggest that 5-HT released by Merkel-like basal cells could modulate taste receptor function[13].

Histochemical and immunocytochemical evidence suggests that 5-HT is present in basal-like Merkel cells in Necturus taste buds[14]. Nagai *et al.*[47] demonstrated that taste cells selectively uptake and release 5-HT in a Ca^{2+}-dependent manner when depolarized. These findings support the hypothesis that 5-HT is a neurotransmitter or neuromodulator released by Merkel-like taste buds in Necturus taste buds. Merkel-like cells may thus release 5-HT onto adjacent cells, and 5-HT probably enhances the electrotonic propagation of receptor potentials in type III cells from the apical tip to Merkel-like basal cells. A paracrine system may be involved in the release of 5-HT.

Neurotransmitter Functions of Taste Bud Cells

Identification of transmitters is important in the discussing of the signal processes of these gustatory end organs, if cell-cell interactions take place within mammalian taste buds.

1. Serotonin

Whether 5-HT represents a paracrine secretion, conventional synaptic neurotransmitter or both, is not yet clear, but Zancanaro *et al.*[66] described 5-HT as one of the best-studied transmitter candidates for taste buds to date. The presence of 5-HT has been identified in mammalian gustatory organs. Histochemical and immunocytochemical techniques have demonstrated that 5-HT is present in a subset of type III taste cells in foliate and circumvallate papillae of mice, rats, rabbits and monkeys[23,36,45,60,66] and in Merkel-like basal cells in amphibian taste buds[14,57,61]. Using autoradiographic techniques with ^{3}H-labeled 5-HT and exploiting large taste cells in Necturus, Nagai *et al.*[47] demonstrated that certain taste cells selectively uptake 5-HT and then release it in a Ca^{2+}-dependent manner when depolarized. Na-dependent 5-HT transporter (serotonin transporter (SET)) is expressed in rat taste cells. Ren *et al.*[51] reported that Na-dependent SET is expressed in taste cells, and suggested that the actions of 5-HT released from taste cells and actions on other cells within the taste bud were terminated by this transporter. Delay *et al.*[13] stated that early reports with patch clamp recordings indicated that bath-applied 5-HT modulates Ca^{2+} currents in amphibian taste cells. Ca^{2+} current was up-regulated in some cells and down-regulated in other cells. These actions were believed to be mediated by 5-HT_{1A}-like receptors. Using patch clamp electrophysiology, 5-HT decreased K^+ and Na^+ currents in mammalian taste cells[28]. Reseachers[16] impaled adjacent taste cells in large taste buds of Necturus and found that depolarizing one cell led to hyperpolarization in a subset of adjacent cells. This hyperpolarization was mimicked by bath-application of 5-HT.

2. Purinergic substances

ATP may be one transmitter from type III cells to gustatory afferent nerves. Bo *et al.*[5] originally reported the presence of purinergic fibers innervating taste buds and specifically noted that P2X2 and P2X3 purinoceptors were expressed on gustatory afferent fibers. This notion was greatly advanced by studying taste buds and taste nerve responses in mutant mice lacking P2X2 and P2X3 purinoceptors[18].

Recently, taste cells have been found to express P2Y purinoceptors and respond to exogenously applied ATP in a manner consis-

tent with P2Y-mediated mechanisms[7,34]. P2Y of mouse taste cell receptors is coupled to IP_3 production and Ca^{2+} mobilization. Studies[7] on the expression profile of particular P2Y isoforms in the mouse taste tissue have revealed that ATP and UTP equipotently mobilize intracellular Ca^{2+} at saturating concentrations, suggesting that common receptors for both nucleotides, *i.e.*, P2Y2 and P2Y4 subtypes, might be involved. RT-PCR and immunohistochemistry have confirmed the presence of P2Y2 and P2Y4 receptors in a population of taste bud cells from foliate and circumvallate papillae. Transcripts for P2Y1 and P2Y6 isoforms have also been detected in taste tissue preparations. These data suggest that P2Y2 and P2Y4 receptors play predominant roles in mediating taste cell responses to ATP and UTP[7].

Other candidates for paracrine secretions and synaptic neurotransmitters in taste buds have also been proposed, including noradrenaline, acetylcholine, glutamate and peptides[29,31,46,64]. Supporting data include immunocytochemical localization of noradrenaline, cholecystokinin (CCK), vasoactive intestinal peptide, glutamate and glutamate transporters in taste buds[27,29, 30,40,41].

3. Catecholamine

A communication has reported[29] the novel observation that taste receptor cells respond to adrenergic stimulation, with noradrenaline application inhibiting outward K^+ currents in a dose-dependent manner[29]. This inhibition was mimicked by the β agonist isoproterenol and blocked by the β antagonist propranolol. The α agonists clonidine and phenylephrine both inhibited K^+ currents and elevated intracellular Ca^{2+} levels. Inwardly rectifying K^+ currents were unaffected by adrenergic stimulation. Experiments using the RT-PCR technique demonstrate that lingual epithelium expresses multiple α (α1a, α1b, α1c, α1d, α2a, α2b, α2c) and β (β1, and β2) subtypes of adrenergic receptors, and immunocytochemistry localized noradrenaline to a subset of taste receptor cells. These data strongly imply that adrenergic transmission within the taste bud may play a paracrine role in taste physiology[29].

4. Glutamate

Glutamate, a major excitatory neurotransmitter in other sensory organs, might act at synapses in taste buds. Caicedo *et al.*[8] used a Co^{2+} staining technique to detect Ca^{2+}-permeable glutamate receptors in taste buds, to establish whether glutaminergic synapses might be present in gustatory organs. When 500-μm slices of foliate and circumvallate papillae were briefly exposed to 1 mM glutamate in the presence of $CoCl_2$, a set of spindle-shaped taste cells accumulated Co^{2+}. Co^{2+} uptake indicated concentration-dependency in the range from 10 μM to 1 mM of glutamate. Higher glutamate concentrations depressed Co^{2+} uptake. This concentration-response relationship for Co^{2+} uptake suggests that synaptic glutamate receptors, not receptors for glutamate taste, were activated. Glutamate-stimulated Co^{2+} uptake in taste cells was antagonized by the non-NMDA receptor antagonist CNQX. Depolarization with 50 mM K^+ and application of NMDA (300 μM) did not increase the number of stained taste cells. This pharmacological characterization of Co^{2+} uptake suggests that non-NMDA receptors are present in taste cells. These receptors might be autoreceptors at afferent synapses, postsynaptic receptors of a putative efferent system, or postsynaptic receptors at synapses with other taste cells.

5. Acetylcholine

Previous studies have suggested that acetylcholine (ACh) is a transmitter released from taste cells, as well as a transmitter in cholinergic efferent neurons innervating taste buds. However, the physiological effects on taste cells have not been established. Ogura[49] examined effects of ACh on taste-receptor cells by monitoring intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). ACh increased $[Ca^{2+}]_i$ in both rat and mudpuppy taste cells Atropine blocked ACh response, but D-tubocurarine did not. U73122, a phospholipase C inhibitor, and thapsigargin, a Ca^{2+}-ATPase inhibitor that depletes intracellular Ca^{2+} stores, blocked

ACh response. These results suggest that ACh binds to $M_1/M_3/M_5$-like subtypes of muscarinic ACh receptors, causing increases in IP_3 and subsequent release of Ca^{2+} from intracellular stores. These findings[49] suggest that Ca^{2+} store-operated channels may be present in taste cells and may participate in the stained phase of $[Ca^{2+}]_i$ increase. Immunocytochemical experiments have indicated that the M_1 subtype of muscarinic receptors is present in both rat and mudpuppy taste cells.

6. Other aminoacids

Taste cells of rat foliate papillae were loaded with calcium green dextran[8]. Lingual slices were perfused with glutamate, kainate, AMPA, or NMDA. Responses to glutamate were localized to basal processes and cell bodies, which were synaptic regions of taste cells. Glutamate responses were dose-dependent and induced by concentrations as low as 30 μM. The non-NMDA receptor antagonists CNQX and GYKI 52466 reversibly blocked responses to glutamate. Kainate, but not AMPA, also elicited Ca^{2+} responses. NMDA stimulated increases in $[Ca^{2+}]_i$ when the bath medium was modified to optimize NMDA receptor activation. Presumably, 2 populations of glutamate-sensitive taste cells exist: one with NMDA receptors, and another without. The function of GluRs in taste buds is not yet known, but the data suggest that glutamate acts as a neurotransimitter. GluRs in taste cells might be presynaptic autoreceptors or postsynaptic receptors at afferent or efferent synapses[9].

Subsequently, cloning and characterization have revealed that the novel mGiuR1 variant, found in circumvallate papillae, functions as an umami for L-glutamate stimuli in rat[24].

7. Neuropeptides

Peripheral CCK receptors may be involved at both peripheral and central sites. Peripheral CCK receptors are mostly CCK-A receptors, whereas the brain contains both CCK-A and CCK-B receptors. Selective antagonists of both types of receptors inhibit satiety. However, CCK-B antagonists are 100-fold more potent in inhibiting satiety than CCK-A antagonists[25]. Central CCK receptors thus seem to be more important.

Novel data have been presented demonstrating that brain-gut peptide CCK is expressed in subsets of taste receptor cells, and may play a previously unknown signaling role within the taste bud. Immunocytochemistry has revealed positively stained subsets of cells within taste buds throughout the oral cavity. These cells are classified as type II (light) cells. Peptide expression was verified using nested PCR on template cDNA derived from mRNA extracted from isolated posterior taste buds. An outward K^+ current, recorded with the patch-clamp technique, was inhibited by exogenous application of sulfated CCK octapeptide in a reversible and concentration-dependent manner. Pharmacological analysis suggests that this inhibition is mediated by CCK-A receptors and involves PKC phosphorylation. An inwardly rectifying K^+ current was also inhibited by CCK. Furthermore, exogenous CCK was effective in elevating intracellular Ca^{2+} as measured by ratometric techniques with the Ca^{2+}-sensitive dye fura-2. These Ca^{2+} elevations were mediated by CCK-A receptors and were dependent on intracellular Ca^{2+} stores[30].

Substance P and leptin receptors have also been identified in taste buds, further suggesting peptidergic modulation of the gustatory end organ[10,54]. While these observations suggest that a large number of different transmitters and receptors are found in taste buds, no firm evidence indicates taste-evoked release of any of them, except 5-HT and ATP. Accordingly, a true understanding of how these substances function in taste buds has yet to be achieved.

Conclusion

1. Type I cells have been considered as supporting cells. In taste buds, glial-like cell control in extracelluar ion concentration has not been identified. Bigiani[4] identified and named leaky cells in the taste buds of mouse

circumvallate papillae. Type I cells do not seem to be involved in signal processing for taste sensation. Accordingly, the idea that these are supporting cell remains unchanged.

2. Type II cells are primary sensory receptive cells. Type II cells and the sensory afferent nerve are in close contact with type III cells. In mammalian papillae, only type III cells have synaptic contact with taste nerves. Primary afferent fibers display 5-HT_{1A} and 5-HT_3 receptors in the terminals. Type II cells appear to transmit taste information to type III cells through gap junctions. When type II cells possess receptors of neurotransmitters, paracrine systems, in addition to gap junctions, can transmit taste information. Gap junctions thus contribute, together with paracrine systems, in forming TBC networks that process taste information. The 5-HT[35] released from TBCs activates postsynaptic 5-HT_3 receptors and, via paracrine routes, inhibits neighboring TBCs via 5-HT_{1A} receptors. In mammals, the transmitter of most taste cells forming synapses with gustatory nerves is 5-HT[35].

3. Delay *et al.*[13] indicated that Merkel-like cell taste cells (type IV cells) make synapse contacts with adjacent taste cells, and innervating nerve fibers. They concluded that one type of Merkel-like basal taste cells is serotonergic interneurons. Merkel-like basal cells release 5-HT onto adjacent cells, and this 5-HT probably enhances electrotonic propagation of receptor potentials in type III cells from the apical tip to Merkel-like basal cells. Release of 5-HT may be induced by paracrine systems.

4. ATP meets the criterion as a neurotransmitter that operates receptors of taste nerve fibers. The functions of a number of different transmitters (5-HT, catecholamine, ACh, aminoacids), are unknown. However, no firm evidence has been found for these transmitters, except for 5-HT and ATP. Accordingly, the function of these neurotransmitters is currently difficult to understand.

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