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ABSTRACTS

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1. Signal transduction in bitter taste

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The structural diversity of compounds which impart a bitter taste suggests that multiple and specific receptor/transduction sequences exist for the modality of bitterness. These sequences may involve both extra- and intracellular targets, with or without modulation of second messenger levels. Earlier studies noted that bitter stimuli could induce the inactivation of K^+ channel conductances, could release intracellular Ca^{2+}, and could activate G proteins, phosphodiesterase and phospolipase C. Most likely additional bitter taste signal transduction pathways will be identified. Our recent results indicate that several bitter tasting stimuli induce the production of the second messenger, inositol 1,4,5-trisphosphate (IP_3) in a G protein dependent manner. We have used a quench flow system to measure the production of IP_3 in the millisecond time frame in taste tissue homogenates from rodents in response to such bitter stimuli as sucrose octaacetate (SOA), strychnine, denatonium benzoate and caffeine. IP_3 production reached a maximum between 50 and 150 ms, depending upon the stimulus, then declined toward basal levels by 200–500 ms. The rapid and transient nature of the IP_3 formation is consistent with its role as a second messenger in bitter taste signal transduction. Previous work suggested that SOA-induced IP_3 production is mediated by a pertussis toxin-sensitive, cholera toxin-insensitive G protein. These studies implicate a receptor mediated transduction sequence where the receptor activates a G protein which, in turn, stimulates the activity of a phospholipase C to metabolize the membrane lipid, phosphatidylinositol 4,5-bisphosphate, into the two potential second messengers, IP_3 and diacylglycerol.

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2. Specific inhibitor for bitter taste

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The development of a specific inhibitor for bitter substances has been widely required in the fields of taste physiology and pharmaceutical sciences, but no inhibitor has been available. We found that a lipoprotein, PA-LG composed of phosphatidic acid (PA) and β-lactoglobulin (LG) reversibly suppressed the responses of the frog glossopharyngeal nerve to the bitter substances. The frog tongue was treated with PA-LG solution for 10 min and then stimulated by a stimulus dissolved in water. The responses to all the hydrophobic bitter substances examined were completely suppressed by PA-LG, while those to the salt type bitter substances such as CsCl, $MgCl_2$ and tetraethylammonium chloride were not suppressed. The responses to NaCl, galactose, acetic acid and *L*-alanine were unchanged or only slightly increased. It was found that PA-LG is strongly bound to the frog tongue surface and the hydrophobic model membranes coated on the quartz-crystal microbalance. The results suggested that binding of PA-LG to the hydrophobic sites on the taste receptor membranes leads to suppression of the responses to the bitter substances. PA-LG also suppressed bitterness of various substances in humans. PA-LG is useful not only for elucidating the receptor mechanisms of bitter substances, but also can be safely used to mask the bitter taste of foods and drugs, since both PA and LG are prepared from foods (soybean and milk).

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3. Quinine-activated cationic conductance in bullfrog taste receptor cells

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Quinine is known to be a bitter substance for various vertebrates. Direct blockage of K^+ channel and Cl^- transport have been implicated in the transduction mechanisms for this compound. We report here an additional mechanism; quinine-activated cationic conductance. Whole-cell recordings under voltage-clamp were made from isolated taste receptor cells of bullfrog (*Rana catesbeiana*). Quinine stimulation elicited an inward current at the resting potential. This response showed a high cooperativity in the concentration dependence. When the blocking effect of quinine on K^+ currents was eliminated by Cs^+ in the patch pipette, the current-voltage relation of the response was linear (the reversal potential; +16 mV), and this showed a clear increase in membrane conductance by quinine stimulation. The quinine-activated conductance was cation selective, and the relative permeability ratio was $P_{Na}:P_{Cs}:P_K$ = 1:0.42:0.5. This cationic conductance was suppressed by external Ca^{2+}, sharing a feature known in the taste nerve responses to quinine. The application of 8-Br-cGMP or 8-Br-cAMP decreased the quinine-induced current. This result suggests that cyclic nucleotides may act as mediators or modulators of the quinine-activated conductance.

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4. Patch-clamp and optical studies of bitter transduction in isolated taste cells

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Several mechanisms for bitter taste transduction have been proposed, including (1) release of Ca^{2+} from internal stores (Akabas *et al.*, 1988; Spielman *et al.*, 1994); (2) activation of phosphodiesterase (Ruiz-Avila *et al.*, 1995); and (3) direct block of apical K^+ channels (Cummings and Kinnamon, 1992). We reported previously that the bitter compound denatonium benzoate (DN) increased $[Ca^{2+}]_i$ in a small subset of isolated hamster taste cells (Ogura *et al.*, in press). In this study, we have used Ca^{2+}-imaging analysis with fura-2 and tight-seal whole-cell recording to study the responses of isolated *Necturus* taste cells to DN. DN (5 mM) increased $[Ca^{2+}]_i$ by 50–150% in most taste cells. The rise in $[Ca^{2+}]_i$ began in the apical tip of the taste cell. The response to DN persisted in Ca^{2+}-free extracellular medium and was blocked after depletion of internal Ca^{2+} stores with thapsigargin (1 μM), a Ca^{2+}/ATPase inhibitor, and by U73122 (5 μM), a phospholipase C inhibitor. Ryanodine (10–200 μM) had no direct effect on $[Ca^{2+}]_i$ and failed to block the DN response. These data suggest that DN increases $[Ca^{2+}]_i$ by causing Ca^{2+} release from ryanodine-insensitive internal stores. Whole-cell recording showed that DN (1–5 mM) increased Ca^{2+}-dependent K^+ and Cl^- currents in most taste cells. The response to DN was blocked when the pipette contained GDP-βS (1 mM), suggesting the involvement of a G protein. The phosphodiesterase inhibitor IBMX (100–200 μM) reduced the response of intracellular Ca^{2+} as well as the Ca^{2+}-dependent currents. These data suggest that the bitter taste of denatonium in *Necturus* is due primarily to an IP_3-dependent increase of $[Ca^{2+}]_i$, which is modulated by phosphodiesterase activity.

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5. Sweetener-induced responses of mammalian taste bud cells

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We investigated signal transduction of sweet stimuli. Taste buds were isolated from the fungiform papillae of the gergils. Ion channel currents were recorded with the whole cell patch clamp technique. Changes in intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, in the taste bud of the excised lingual epithelium were measured with the fura-2 fluorescence imaging.

Outward K^+ currents induced by depolarizing pulses from a holding potential of −60 mV were suppressed by 10 mM Na-saccharin. On the other hand, the K^+ currents were potentiated by 10 mM D-phenylalanine and D-tryptophan (a sweet amino acid). The outward K^+ currents were potentiated by intracellular application of 5 μM IP_3.

Outward K^+ currents were no longer suppressed when 20 μM gurmarin of an inhibitor of sugar response was added to 10 mM Na-saccharin, while the K^+ currents were still potentiated by 10 mM D-tryptophan containing the gurmarin.

Taste cells exhibited a rise in $[Ca^{2+}]_i$ in response to 10 mM D-phenylalanine in a Ca^{2+} free Tyrode solution with 2 mM EGTA.

These facts suggest that sweet taste stimulation of the taste cell with D-phenylalanine may increase an intracellular level of IP_3 which releases Ca^{2+} from the internal store. We propose that one group of sweetener such as Na-saccharin binds to gurmarin-sensitive receptor sites, produces cAMP and closes K^+ channels, but the other group of sweeteners such as D-tryptophan binds to gurmarin-insensitive receptor sites, produces IP_3 and opens K^+ channels. The second messenger systems may play a role in signal transduction.

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6. The amplitude of Ca^{2+} transients elicited in taste receptor cells responding to sweet stimuli

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Fura-2 imaging of taste buds was used by several investigators to study effects of tastants on the cytosolic Ca concentration (Ca_c) of

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receptor cells. In such experiments, isolated or in-situ taste buds were exposed to fura-2 acetoxylmethyl ester, such that most cells became loaded with the dye. It was typically found that few cells of a bud responded to a given stimulus. Thus, the method was well suited to find among 60 or more receptor cells the one or two which were responsive. The increase in Ca_c observed in responsive cells was usually small and seldom exceeded 200 nM. This concentration cannot be considered sufficient for synaptic activation. The question arises, therefore, whether the method used caused a systematic underestimation of the Ca signal. The problem was studied with deblurring algorithms applied to sweet-responding taste buds of the rat and by model considerations.

In a loaded taste bud, a responding cell is surrounded by non-responding, but fura-containing neighbors which contribute light to the image of the in-focus responding cell. The application of deblurring algorithms was found to hardly change the magnitude of the ambient low-Ca signal, but to drastically magnify a Ca_c increase which was limited to a small sample volume. For instance, the response of a rat taste cell to the sweetener SC-45647 was originally described as going from 30 nM to a peak value of 200 nM Ca_c. However, deblurring of the images revealed a response reading 700 nM or larger values.

Model considerations showed that the underestimation is mainly due to the poor vertical resolution of microscope objectives of large working distance, which have a low numerical aperture. In fura experiments, the vertical averaging occurs especially at 380 nm, where the small volume responding with a Ca_c increase has a lower fluorescence than the ambient, non-responding volume. Vertical averaging, which is not corrected by the use of ratio-dyes, can be recognized by the fact that the relative changes occuring at 340 and 380 nm do not correspond to each other. The use of objectives of high numerical aperture and, where large working distances are required, the use of deblurring algorithms is recommended.

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7. Structure and function of sweet taste suppressing peptide, gurmarin

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Gurmarin is a polypeptide composed of 35 amino acids, with a molecular weight of 4209 found in leaves and roots of the Indian plant, *Gymnema sylvestre*. It is in sharp contrast to the well known sweet taste inhibitor, gymnemic acid, which is also found in the same plant. Gurmarin shows a prominent inhibitory effect on the sweet taste responses recorded from the chorda tympani of rats (Miyasaka and Imoto, 1995) and some mouse strains (Ninomiya and Imoto, 1995), but not humans. Recently, a three-dimensional structure of the polypeptide was elucidated from a two dimensional nuclear magnetic resonance study (Arai *et al.*, 1995). In addition to having a rigid and stable structure due to three intramolecular disulfide bonds, a unique feature of the molecule was revealed such that a hydrophobic pocket, rich in aromatic residues, is formed by two Trp's, two Tyr's and one His closely sterically positioned with each other. Taking results of chemical modifications into consideration, this region may be responsible for the specific interaction of gurmarin with sweet taste receptor proteins. The inhibitory effect of gurmarin is so strong that the effect lasts as long as several hours even after continuous rinsing of the tongue. By utilizing such strong interaction, proteins in the rat taste papilla that bind gurmarin could be identified as strong candidates of the sweet taste receptors. Preliminary results along this line will be presented in brief.

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8. Amino acid-induced responses of isolated taste bud cells in mice

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In order to investigate the taste transduction mechanisms for amino acid stimuli, the responses of solitary taste bud cells to them were examined using whole-cell patch-clamp technique in mice. The tongue epithelium containing circumvallate and foliate papillae was peeled off 30 min after an injection of collegenase into subepithelial region. Taste bud cells were finally dissociated by pipetting, following successive 10 min treatments with collagenase and EDTA. Taste cells were identified by their long cell process and spindle or flask shape.

Some isolated taste bud cells demonstrated a slow inward current in response to 40–85 nM Monosodium L-glutamate (MSG) applied by puff at a holding potential of −80 mV, while others exhibited either outward currents or no current. This inward current was reversed at +10–+20 mV, indicating that MSG may have activated cation-selective channels. The membrane potential change of taste bud cells in the slice preparation was also optically recorded using voltage-sensitive dye. MSG elicited a slow depolarization in small population of taste bud cells. Sweet tastant, 100 mM D-phenylalanine (Phe) reduced outward K^+ currents by about 15%. Phe applied with 2 mM Na-saccharin, which is a synergist, reduced the outward currents by about 25%, suggesting that the synergistic effect of these sweetners may be produced by enhanced blocking of K^+ channels.

9. Arginine-induced responses in catfish taste cells

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Responses of catfish taste receptor cells to amino acids are mediated by several distinct transduction pathways. For example, L-arginine and L-proline appear to act directly on ion channels in the receptive membranes of taste cells, while L-alanine interacts with G-protein coupled receptors that are indirectly linked to changes in membrane conductance by second messenger cascades. L-arginine elicits depolarizing (excitatory) responses in about half of the responding taste cells and hyperpolarizing (inhibitory) responses in the remaining

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cells. The depolarizing responses (inward currents under voltage-clamp at negative potentials) reversed near 0 mV, consistent with direct stimulus activation of nonselective cation channels characterized in earlier reconstitution studies. The hyperpolarizing responses (outward currents at −40 mV) reversed near −60 mV, consistent with activation of one or more classes of K^+ channels in the apical membrane of the cell. Both increases and decreases in $[Ca^{2+}]_i$ were observed in response to L-arginine in isolated catfish taste cells using the Ca^{2+} indicator, fura-2 and digital imaging techniques. In 15 of 21 cells that responded, L-arginine produced increases in $[Ca^{2+}]_i$. These increases were usually dependent upon the presence of external Ca^{2+} and were antagonized by equimolar concentrations of D-arginine, consistent with activation of L-arginine-gated cation channels, which are permeable to Ca^{2+} and blocked by D-arginine. In the remaining taste cells, L-arginine produced decreases in $[Ca^{2+}]_i$. In addition, D-arginine elicited both increases and decreases in $[Ca^{2+}]_i$, indicating independent receptors for both enantiomers, each coupled to pathways leading to increases or decreases in $[Ca^{2+}]_i$. The heterogeneous nature of the responses of catfish taste cells to L-arginine, in particular the apparent simultaneous excitation and inhibition of different cells with a taste bud, suggest that significant local processing of taste signals may occur within the taste bud.

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10. G-proteins specific in fish taste cells

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The gustatory system of carp is extremely sensitive to amino acids, and is endowed with a large number of taste buds on its barbels and lips. So, the carp suites for studies on taste signal transduction and also on phylogenetic development of vertebrate taste system. It has been shown that vertebrate taste cells specifically express a G-protein α-subunit, α-gustducin, which closely resembles a visual G-protein, α-transducin. Recently, in addition to the α-gustducin, α-transducin was also shown to be involved in gustatory transduction mechanism. We attempted to detect cDNA of G-protein α-subunit in the carp taste cells. Poly $(A)^+$ mRNAs were obtained from the barbels and lips, and cDNAs were synthesized as templates for PCR. PCR was carried out using primers having sequences of conserved regions of G-proteins. The PCR resulted to amplify DNA fragments of about 300 bp which was the length expected from the primers used. Nucleic acid sequence of the DNAs and deduced amino acid sequences indicated that they are related to partial sequences of two types of G-protein, G_i and G_s. The sequence of the G_i type DNA fragment resembled that of α-gustducin. *In situ* hybridization with RNA probe synthesized with the G_i type DNA showed reactions exclusively within taste bud cells. These results strongly suggest that the G_i type clone can be a partial nucleic acid sequence of a gustducin-like G-protein of the carp taste cells.

11. Stimulant-binding proteins in the taste system

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We found the two types of stimulant-binding proteins in the taste organ of the fly; the lipophilic ligand-binding proteins and the sugar-binding proteins.

The fly has the taste organ in the shape of sensillum, which possess four functionally different receptor cells; the sugar, the salt, the water and the fifth cell.

The lipophilic ligand-binding protein is water-soluble and extracellularly distributes around the receptor membranes as the major component of the receptor lymph. This protein functions as a carrier for the fragrant components of natural foods. With the help of this protein, such lipophilic stimulants can reach the receptor membrane of the fifth cell across the receptor lymph.

On the other hand, the water-soluble stimulants like sugars do not need any help of carrier proteins. They can reach the receptor membrane of the sugar receptor cell by themselves and are received by the sugar receptor proteins. Two kinds of sugar-binding protein which we show as the sugar receptor molecule candidates are the membraneous proteins found in the isolated taste sensilla. Their binding abilities for sugars are consistent with the electrophysiological data for the two kinds of sugar receptor molecule.

12. Inositol 1,4,5-trisphosphate depolarizes frog taste cells by releasing Ca^{2+} from the internal stores

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The biochemical experiments have shown that taste cells increase the level of either adenosine-3′,5′-cyclic monophosphate (cAMP) or inositol 1,4,5-trisphosphate (IP_3) in response to gustatory stimuli. In the present experiment, the effect of intracellular application of 1,4,5-IP_3 from the patch pipette was analysed in isolated frog taste cells under whole-cell patch clamp. Intracellular dialysis of 50 μM 1,4,5-IP_3 in K^+-internal solution induced sustained inward current of −196 ± 37 pA (mean ± SE, n = 6) at the membrane potential of −50 mV. The IP_3-induced inward current was observed in 37% of the cells. Steady state I−V relationships of IP_3-gated currents with K^+-internal solution were almost linear. The reversal potential was −0.2 ± 4.6 mV (n = 6). The IP_3-induced currents were inhibited by external Cd^{2+} (2 mM), but not by amiloride (0.1 mM). The dialysis of 1 mM Ca^{2+} in K^+-internal solution elicited similar inward currents which could be inhibited by Cd^{2+}. The I−V relationships of Ca^{2+}-activated currents also were linear. The reversal potential was −8.8 ± 3.3 mV (n = 5). Bath application of acetic acid (sour stimulus) also elicited inward currents in the cells whose membrane voltages were clamped using perforated patch-clamp technique. The results suggest that IP_3 can depolarize frog taste cells with mediation by intracellular Ca^{2+}.

13. Whole-cell recording of salt response from non-dissociated taste cells in mouse taste bud

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The chorda tympani nerve responses induced by salt show a high amiloride-sensitivity in an inbred mouse strain, C57Bl/6, but little

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sensitivity in another inbred strain, BALB/c mouse. We examined whether other mechanisms other than amiloride-sensitive pathway exist in the salt-signal tranduction process using whole-cell recording method. Using preparations consisting of a taste bud attached to a small piece of the lingual epithelium, we might simultaneously perform patch-clamping, visualization of taste cell morphology, localized taste stimulation and maintenance of microenvironment around the taste organ. The results suggest that activity of non-selective cation channel and block of K^+ channels mediated by second messenger as well as amiloride-sensitive pathway are involved in the salt-signal transduction process of mouse taste cells.

This study was supported in part by a grant from the Human Frontier Science Program Organisation and Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

14. Differential distribution of two types of K^+ channels and non-selective cation channel in bullfrog taste cells

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With single channel recordings of the patch clamp method, we could identify 80 pS and 40 pS K^+ channels and 30 pS non-selective cation channel in the bullfrog taste cell membrane. 80 pS K^+ channels were dependent on voltage and Ca^{2+}, but 40 pS K^+ channels were not. The open probability of 40 pS K^+ channels was decreased by the simultaneous presence of cyclic adenosine monophosphate (cAMP) and adenosine triphosphate (ATP), and the suppressive effect was antagonized by protein kinase inhibitor (PKI). Thirty pS non-selective cation channels were permeable to various mono- and divalent cations. These three ion channels showed differential distributions throughout the taste cell membrane. The functions in gustatory transduction of these ion channels are under investigation.

15. Transduction mechanisms of insect taste cells

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Transduction mechanisms of the sugar, salt and water receptor cells in the labellar taste hair of the flesh fly, *Boettcherisca peregrina*, were investigated by the electrophysiological methods, including receptor current fluctuation analysis and patch clamp study. In both salt and sugar receptor cells, stimulant application caused the increase of receptor current fluctuation, measured as the potential difference between the sensory hair tip and the microelectrode inserted into the outer lumen of the TTX-treated taste hair. Magnitudes of fluctuation and time constants of autocorrelation were different, dependent on the stimulant species, which were known to be specific to four different receptor sites. This result and very short latency (about 1 ms) suggest existence of ionotropic stimulant receptors. Existence of mM order Ca^{2+} ion in the stimulant solution caused more rapid decrease of current fluctuation compared with Ca^{2+} free stimulant solution. This is consistent with the observation that the sugar response was more rapidly adapted when the simulant solution contains mM order Ca^{2+}, suggesting that Ca^{2+} flows into the taste cell upon stimulation and causes adaptation. Ozaki and Amakawa (1992) showed that introduction of Ca^{2+} into the taste cell making use of a detergent caused rapid adaptation of sugar response. Whole cell clamp study both on the isolated taste cells from the pupa and on the sensory process grown out from the cut end of the labellar sensory hair of the pupa just before eclosion, showed inward current response upon stimulation which had reversal potential of about 10 mV, suggesting existence of non-specific transduction cation channels. Introduction of cGTP into the taste cells from the whole cell patch pipette, caused transient inward current on about one-quarter of the taste cells, suggesting that cGMP mediated transduction process may exist in addition to direct ionotropic receptors. Dose–response curves of the water receptor to alkali metal cations, LiCl, NaCl, KCl, RbCl and CsCl, were quite different depending on the cation species, suggesting that specific cation receptor sites may exist.

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16. Neurogenetic analysis of taste receptor mechanisms in mice

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Mouse taste responses to a sweet tasting amino acid, D-phenylalanine (D-phe) is controlled by a single gene, *dpa*, on chromosome 4. We developed a *dpa* sweet taster congenic line using standard techniques (donor sweet taster strain; C57BL/6, and congenic partner nonsweet taster strain; BALB/c), and compared their taste responses of the chorda tympani nerve with those of the donor and congenic partner strains. In both congenic and C57BL mice, some of single fibers responding to D-phe and other sweeteners were suppressed by a new sweet inhibitor, gurmarin, whereas no such gurmarin-sensitive fiber was found in BALB mice. The results suggest that congenic and its donor C57BL strains possess both gurmarin-sensitive and -insensitive components of sweet receptors and their specific neural coding channels, whereas BALB mice possess only a gurmarin-insensitive one. The *dpa* gene probably influences the expression of the gurmarin-sensitive sweet receptor in the taste cell which is responsible for taste responses to not only D-phe, but other sweeteners as well.

17. Candidates for neurotransmitters in *Necturus* taste buds

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Chemosensory information is transduced by taste cells and transmitted to sensory axons. Transmission is believed to be mediated by neurotransmitters. Serotonin (5HT), glutamate (Glu) and GABA have

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been implicated, but little is known about these or other transmitters at synapses in taste buds. In amphibia, 5HT is present in Merkel-like basal taste cells; Glu and GABA occur in sensory axons. We studied the uptake and release of 5HT, Glu and GABA in *Necturus* taste buds. Lingual tissue was incubated in 0.4 to 6 μM ^{3}H-5HT, -Glu, or -GABA and processed for autoradiography. Merkel-like basal cells selectively accumulated ^{3}H-5HT. If tissues were depolarized with 40 mM K^+, Merkel-like basal cells released ^{3}H-5HT. ^{3}H-5HT release was Ca^{2+}-dependent and was blocked by reducing Ca^{2+} (0.4 mM) and elevating Mg^{2+} (20 mM). In contrast, ^{3}H-Glu was taken up by epithelium cells, glial cells and some taste cells. ^{3}H-Glu was not released by depolarizing the tissue with elevated K^+. No taste cells accumulated ^{3}H-GABA, but cells lying outside the taste buds, including glial cells, did. Sensory axons did not accumulate ^{3}H-Glu or ^{3}H-GABA. Of the three neuroactive compounds tested, 5HT is the most likely neurotransmitter candidate in *Necturus* taste buds.

18. Morphology and morphogenesis of vertebrate taste buds

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Vertebrate taste buds are composed of several distinct cell types. However, ultrastuctural investigators of taste buds in both mammalian and non-mammalian species have failed to reach an agreement concerning the labelling cell types and which is the gustatory transducer cell. In order to expand the limited knowledge of taste buds at the ultrastructural level, we have used computer-assisted, three-dimensional reconstructions from electron micrographs of serial ultra-thin sections for analysis of cell types in the taste buds of mouse fungiform and vallate papillae.

Only the type III cells (of which there are 1–3 in a fungiform bud and 5–10 in a vallate bud) make synaptic contacts with nerve terminals and contain both synaptic-type vesicles and dense-cored granules in their cytoplasm, indicating that these cells have a sensory function. Each type III cell in the fungiform bud makes 6–8 synapses with 6–12 nerve terminals. In contrast, each type III cell in the vallate bud makes 3–5 synapses with 5–8 nerve terminals. The type III gustatory cells show immunoreactivity for both serotonin and neuron-specific enolase (NSE) antisera.

The taste buds in teleosts and amphibians have another distinct cell type located exclusively in the basal portion. Similar to mammalian type III cells, these basal cells also show immunoreactivity for both serotonin and NSE antisera. Our results suggest that these basal cells are a type of Merkel cells, and that they may function as producers of trophic substances that attract axon terminals necessary for the development and maintenance of the taste bud.

19. Comparison of gustatory transduction mechanisms in vertebrate taste cells

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Gustatory transduction mechanisms for basic taste stimuli are compared among taste cells in frog, salamander and mammal. The receptor potentials of taste cells in response to NaCl occur by activities of cation and anion channels at the receptive membrane, as well as cation channels at the basolateral membrane in frog and occur by activities of amiloride-sensitive Na^+ channels at the apical membrane in salamander and mammal. The receptor potentials in response to acid stimuli are induced by activities of apical proton-gated cation channels and proton-pumps in frog, by block of apical K^+ channels in salamander and by activities of apical amiloride-sensitive Na^+ channels in mammal. The receptor potentials in response to bitter stimuli are induced by a secretion of intracellular Cl^- through the apical membrane in frog and by block of apical K^+ channels in salamander. Gustatory transduction for bitter stimuli in mammal is concerned with a second messenger IP_3. Gustatory transduction for sweet stimuli is related to apical H^+ channels in frog and to second messengers of cAMP and IP_3 in mammal.

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20. Gustducin's role in taste transduction: insights from knock out mice

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We have used gene targeting by homologous recombination to generate gustducin deficient mice. We used a positive/negative selection strategy to increase the frequency of isolating homologous recombination events over non-homologous integrations. The targeting vector included 1.4 and 8.5 kb of gustducin genomic sequence flanking both sides of the *neo* gene. These fragments were chosen such that the recombined gustducin allele will have a *neo* insertion while deleting the ATG^{met} initiation codon and the first protein-coding exon. The targeting construct was electroporated into the W9.5 male ES cell line, after transfection and selection on G418 and FIAU, individual colonies were picked and screened by genomic Southern analysis for homologous vs. non-homologous recombination events. We detected 19 homologous recombination events out of 280 ES cell clones screened (a targeting frequency of 7%). Five of seven clones demonstrating homologous recombination were confirmed to be karyotypically normal. Chimeric mice were generated by blastocyst injection with three different clones. Chimeric animals were scored for the relative contribution of the ES cells into the embryo by the amount of agouti coat colour along with sex conversion due to male ES cell contribution to the germline. Founder chimeras were bred back to C57Bl/6 mice, then agouti offspring and founders were screened by genomic Southern analysis to determine if they carried the targeted gustducin allele. Heterozygotes were bred to each other to generate mice homozygous for the gustducin null allele. Heterozygotes and homozygotes were viable and phenotypically normal as to gross appearance and behavior. Taste buds from the knock outs were qualitatively normal in appearance and apparently normal as to the number of taste cells per taste bud and the number of taste buds per papilla. *In situ* hybridization with a murine gustducin probe showed that the gustducin knock out mice fail to express gustducin; they are, in fact, gustducin null animals. Behavioral and electrophysiological experiments with the knock out mice are being carried out to determine if they are altered in their responses to particular classes of tastants.

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