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BIOCHEMISTRY OF VERTEBRATE OLFACTION AND TASTE

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INTRODUCTION

The chemical senses of smell (olfaction) and taste (gustation), together with visual and somatosensory inputs, subserve a variety of behavioral and physiological responses associated with nutrient acquisition, identification, and ingestion. A recent symposium volume documents the increasing appreciation of the importance of the chemical senses in nutrition (57). The role of the chemical senses in the series of physiological events collectively referred to as

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cephalic phase responses has also been reviewed previously in this series (14). Ultimately, however, the interactions of nutrition and the chemical senses depend on detection and identification of appropriate chemical stimuli by specialized peripheral cells that transduce chemical information into neuronal activity. In olfaction, primary chemosensory neurons detect odorants, transduce these signals into electrical activity, and propagate this information directly to the central nervous system. In taste, specialized multicellular structures (taste buds) transduce stimulus signals into cellular activity that subsequently activates primary sensory neurons.

This review summarizes recent progress in our understanding of the molecular basis of chemosensory detection and signal transduction by vertebrate olfactory neurons and taste cells. Historically, biochemical and cellular studies of the chemical senses have not been as intensively pursued as those in other sensory systems such as vision (110). Unlike vision and audition, no well-defined quantitative spectrum exists for the diverse range of chemosensory stimuli to which vertebrates respond. Uncertainties regarding stimulus delivery and the cellular specificity of stimulus-evoked responses have also contributed historically to an incomplete, sometimes ambiguous, description of the molecular basis of chemoreception.

Through the application of modern biochemical, neurophysiological, and genetic techniques, substantial progress in this area has been achieved in the last decade. This review summarizes this recent progress and emphasizes advances reported since about 1980. At that time, much of the earlier biochemical literature relevant to the chemical senses was reviewed (25). The review is constrained to consideration of the peripheral mechanisms underlying stimulus detection and transduction in the primary chemosensory systems of olfaction and taste. Other aspects of chemosensation, such as trigeminal, terminal nerve, and vomeronasal chemoreception, as well as central decoding and processing of primary sensory information, are not considered. Introductory treatments of these aspects of chemosensation and their relationship to olfaction and taste were published recently (39).

OLFACTION

Cellular Organization of Olfactory Epithelium

The olfactory epithelium (olfactory mucosa) is the specialized peripheral end-organ subserving olfaction in vertebrates. In mature animals, the epithelium is composed of two morphologically distinct areas, the sensory neuroepithelium that is proximal to the environment and an underlying lamina propria layer. The pseudostratified columnar neuroepithelium is mitotically active even in mature animals and undergoes continual cellular degeneration and replacement. It is composed of three major cell types: olfactory receptor neurons, sustentacular cells, and basal cells (40, 41). The receptor cells are

primary chemosensory neurons, specialized for odorant detection. As described below, these cells have many unique properties in addition to their chemical detection capability. The receptor cells are separated by sustentacular cells that are believed to function primarily as secretory supporting cells for the receptor cells. Basal cells provide a pool of progenitor cells that subsequently differentiate and replace senescent receptor cells. The vascularized lamina propria contains a variety of components, including receptor cell axons, melanocytes, connective tissue, and secretory glands. Secretions from these glands, believed to be under adrenergic and cholinergic regulation, together with sustentacular cell secretions, provide the mucus layer covering the neuroepithelium.

While a considerable amount of anatomical and morphological data on vertebrate olfactory systems is now available at the macroscopic, light microscopic, and ultrastructural levels, isolation of the individual cell types and description of their biochemical and electrophysiological properties have been more limited. Mixed cell suspensions have been exploited by application of single-cell electrophysiological techniques to morphologically identified cells (40, 80, 86, 119). However, dissociated receptor cells often have not exhibited reproducible odorant-elicited electrophysiological responses and may also lose their distinctive morphology following dissociation (50, 64). Fractionation of cell suspensions from the olfactory epithelium to obtain purified preparations of individual cell types has not been extensively investigated. The intrinsic cellular heterogeneity of the neuroepithelium has complicated attempts to isolate purified cell preparations by methods based on cell size and density (50). The continuous and differential rates of maturation, senescence, and turnover of each cell type in the neuroepithelium (33, 40) would also lead one to expect that a particular cell population would exhibit a range of biochemical, electrophysiological, and morphological properties.

A persistent lack of cell-specific markers that are accepted, readily available, and widely applicable across species has also complicated the development of purified or cultured cell preparations from the olfactory epithelium. Recently, monoclonal antibodies have been developed that recognize cell-surface glycoprotein antigens of rat sustentacular and olfactory neurons (2, 47). Monoclonal antibodies that recognize carbohydrate epitopes also cross-react with a subpopulation of receptor cells in rabbit (84). Lectins have been reported specifically to label individual cell types in the neuroepithelium (48, 59), although the applicability of these reagents may be limited by carbohydrate structural differences across species.

Properties of Olfactory Neurons

Olfactory receptor cells are primary bipolar neurons with a single dendrite projecting toward the external environment. The single, unbranched, and nonmyelinated axons project through the lamina propria and collectively form

the olfactory nerve (cranial nerve I), which synapses directly with the central nervous system at the glomeruli of the olfactory bulb (40, 41, 72). Bulbectomy, transection of the olfactory nerve, or topical application of some chemicals on the neuroepithelium leads to selective degeneration of the receptor cells (33). Because the receptor cells continuously turnover and selective techniques for manipulating their maturation cycle have been developed, the olfactory epithelium represents a unique model system for neurogenesis studies. The apical end of the receptor cell dendrites are somewhat enlarged (olfactory knob) and have specialized extensions of the dendritic membrane that emanate from them. Most receptor cells terminate in cilia, which form a dense network in the mucus layer overlying the epithelium. An additional receptor cell type terminates in microvilli. Microvillous receptor cells are particularly abundant in fish (124), but have also been identified at the ultrastructural level in humans (83). The functional significance of the two receptor cell types is currently unresolved.

Olfactory neurons can be selectively labeled in situ with fluorescent dyes (112). The receptor cells also contain several distinctive biochemical markers, including carnosine (β-ala-L-his) and carnosine synthetase. The degradative enzyme carnosinase is nonneuronal and presumably localized in sustentacular cells (50). Monoclonal antibodies to carnosine synthetase from rabbit have been developed that cross-react with the enzyme in several mammalian species. An improved assay for the enzyme in soluble tissue extracts has been described that is based on immunoadsorption of the unstable enzyme on a monoclonal-antibody-linked insoluble support and subsequent assay of the enzyme activity bound to the antibody (78). The functional roles of carnosine and its biosynthetic enzyme in olfactory neurons remain unresolved, although levels of the dipeptide and the enzyme decrease following olfactory nerve transection and increase in parallel with subsequent regeneration of the neurons (76). Mature receptor cells also express a unique cytosolic protein of unknown function, olfactory marker protein (77). The amino acid sequence of the protein has been determined and shows virtually no homology with previously sequenced proteins (79). Nerve-specific enolase immunoreactivity has also been detected in human olfactory neurons (113).

Intermediate filaments have also been identified in the neuroepithelium by immunochemical methods. In addition to neurofilaments, olfactory receptor cells also express vimentin, which is localized in the axons (103, 113). The astrocytic marker, glial fibrillary acidic protein, was identified in Schwann cells surrounding the receptor cell axons (7). In adult rats, neurofilament expression is apparently confined to a small population of receptor cells, while vimentin is more widely distributed throughout the neuronal population (103). The retention of the "juvenile" marker vimentin in olfactory neurons emphasizes another unique property of these cells, since vimentin expression

is generally associated with immature neurons that cease vimentin expression and accumlate neurofilaments as maturation proceeds. In contrast to these reports, no immunoreactive vimentin or neurofilaments were detected in another study of the neuroepithelium in adult rats (122). Thus, the utility of intermediate filaments and related proteins as cell-specific markers in the olfactory system remains to be determined.

The unique dendritic cilia of the receptor cells have attracted attention for many years. A variety of evidence indicates that these organelles are the site of the initial interaction of odorants with the receptor cells (99). The cilia are readily detached from the neuroepithelium by calcium treatment of the tissue. The detached cilia are then isolated by differential centrifugation and further purified on discontinuous sucrose gradients (3, 11, 31, 99). The cilia are enriched in tubulin, the major axonemal structural protein. In cross section, the cilia exhibit the characteristic "9 × 2 + 2" microtubule morphology. The ciliary membrane is readily solubilized in Triton X-100 and contains a variety of glycoproteins (3, 11, 29, 31). One of these, gp95, has been shown to be exclusively localized in frog olfactory cilia (30, 32). This transmembrane glycoprotein appears to be widely distributed since putative homologs of frog gp95 have been identified in several species (3, 11, 32). Although the function of gp95 is not known, it may be a useful marker for olfactory cilia because of its unique localization in these membranes.

Molecular Characteristics of Olfactory Receptors

Their proximity to the external environment and the evidence implicating them in chemoreception (99) point to the olfactory cilia as the site of the initial events in olfactory reception and signal transduction. The apical dendritic knob may also provide additional sites by which odorants activate the receptor cells (40, 41). Odorant access to the cilia and apical surface of the neuroepithelium is generally considered to be diffusion controlled. Several factors contribute to the rate of odorant access to the chemosensory membranes, particularly the partition and diffusion characteristics of individual odorants in the mucus. Odorant access, as well as subsequent clearance from the neuroepithelium surface, also depends on the rate of mucociliary transport, uptake and metabolism of some odorants, and odorant interactions with components in the mucus.

Two hypotheses have dominated the literature regarding the nature of odorant interactions with the ciliary membrane. On the one hand, it has been proposed that receptor cell activation is mediated by the interaction of odorants with specific membrane-associated receptor proteins (72, 99). In contrast, alternative mechanisms, independent of specific ligand-receptor interaction, have been proposed to account for olfactory responses (69). Biochemical support for the receptor hypothesis was first provided by the pioneering work

of Cagan and coworkers (99, 100), who showed that specific binding sites for amino acids, olfactory stimuli for many fish, were present in isolated cilia preparations from trout. The interaction of odorant amino acids with the binding sites was specific, saturable, and reversible, satisfying several expected properties of membrane-associated receptor proteins. In addition, the amino acid binding data were consistent with neurophysiological specificity and efficacy properties of these stimuli. Similar results were obtained by Brown & Hara (16), although these workers concluded that the binding data represented amino acid transport and accumulation, rather than specific ligand-receptor interaction. Amino acid binding studies have also been reported in skate (89), salmon (98), and catfish (17, 55). In most fish species, the affinity of the interaction of amino acids with the binding sites was characterized by dissociation constants of 10^{-7} to 10^{-5} M. In most cases, the biochemical binding data paralleled stimulus specificity and potency characteristics determined in behavioral and electrophysiological assays.

Odorant binding studies in species that detect volatile odorants have been complicated by low affinity and by nonspecific interactions of hydrophobic stimuli with biological membranes. However, chemical modification experiments with protein-selective reagents are also consistent with the likely participation of receptor proteins in olfactory reception (40). Recently, amino acid binding in isolated cilia from catfish was shown to be selectively and differentially inhibited by lectins (55). These results were consistent with a glycoprotein nature for the ciliary binding sites, an expected characteristic of a membrane-associated receptor protein. The lectin inhibition results were also consistent with the differential inhibition by concanavalin A of electrophysiological responses to some odorants in rat (104, 105).

While substantial evidence is now available that supports the hypothesis of odorant interaction with specific, probably glycoprotein, receptor proteins in the ciliary membrane, the receptors have not been identified at the molecular level or isolated. Several odorant-binding proteins have, however, been identified in and/or isolated from the olfactory epithelium. An odorant-binding protein that exhibits high affinity binding of pyrazine and its derivatives was isolated and purified to homogeneity from bovine nasal epithelium (93, 94). Although originally suggested to represent an olfactory receptor protein, the pyrazine-binding protein is soluble and has been localized by immunohistochemical methods to the secretory glands in the neuroepithelium (92, 94). The protein is found in mucus and tears and has a molecular weight of about 20,000. Although the pyrazine-binding protein is similar in molecular weight to olfactory marker protein, it does not cross-react with antisera to the marker protein (94). The complementary DNA sequence for the same or similar protein from frog has been determined; it shows some homology to serum transport proteins, but no homology with the olfactory marker protein

(74). The functional role of the pyrazine-binding protein in olfactory reception and sensory transduction remains unresolved, although it has been proposed that it may participate in odorant transport and solubilization in the mucus (93). Binding sites for camphoraceous odorants were detected in rat olfactory epithelium, and anisole- and benzaldehyde-binding proteins were isolated by affinity chromatography from canine neuroepithelium (95, 97). That these isolated odorant-binding proteins represent olfactory receptor proteins has not been confirmed.

It has also been proposed that olfactory responses are mediated by mechanisms independent of specific ligand-receptor interaction (69). Kurihara and coworkers showed that several odorants elicited membrane depolarization responses in a neuroblastoma cell line that is devoid of olfactory receptors. The odorant concentrations required to depolarize the membrane coincided with those that altered membrane fluidity (58). Thus, a model of olfactory reception was proposed in which cell-to-cell differences in membrane lipid composition provided selective adsorption sites for odorants with no requirement for receptor protein. It is not known if the cell-to-cell membrane lipid composition is heterogeneous in the olfactory epithelium as suggested in this model, since lipid composition analyses have not been reported. However, it is also not unexpected that many odorants, particularly those that are hydrophobic, would perturb membrane properties. Thus, it is likely that some odorants may contribute nonspecific components to the overall mechanism that activates olfactory neurons.

Molecular Mechanisms of Olfactory Signal Transduction

Although not rigorously established, it is likely that the initial events of olfactory detection and discrimination are mediated by odorant interaction with membrane protein receptors. It has been known for many years that odorants elicit depolarization of the chemosensory membrane and that membrane depolarization is intimately associated with action potential generation and synaptic transmission (40, 41, 72). However, the sequence of molecular events between the initial interaction of the odorant with the chemoreceptive membrane and subsequent electrical events remained unknown for many years. Recent progress in elucidation of cellular signal transduction in many cells, including visual cells (110), has encouraged renewed interest in, and efforts to describe, the biochemical basis of chemosensory transduction. In olfaction, biochemical and neurophysiological evidence supports the hypothesis that olfactory receptor activation initiates a series of intracellular events involving "second messenger" molecules. The documented ability of cyclic nucleotide and lipid-derived second messengers to regulate protein phosphorylation and intracellular ion concentrations suggests that these

messengers may function in olfactory neurons to couple receptor activation and subsequent membrane electrical events.

A major advance in understanding cellular signal transduction mechanisms was the discovery of a family of integral membrane, GTP-binding regulatory proteins (G-proteins) that function to couple cell surface receptors to intracellular enzymes that catalyze second messenger formation (43). Members of the G-protein family have also been identified in the olfactory epithelium. G_s, the G-protein that mediates stimulation of adenylate cyclase, was detected in isolated cilia preparations from frog (4, 90, 91) and catfish (17). G_i, the G-protein that mediates inhibition of adenylate cyclase, was also identified in isolated frog cilia (4, 90). An additional pertussis toxin substrate that cross-reacted with antisera to G_o, a G-protein of unknown function, was identified in frog cilia (4). In contrast to frog, immunoreactive G_o in catfish was not detected in the cilia, although it was readily identified in brain membranes (17) and in mouse olfactory epithelium. A unique pertussis toxin substrate of 40,000 daltons was also identified in catfish, but not frog, cilia (17). This pertussis toxin substrate cross-reacted with antisera to a G-protein common amino acid sequence, but did not cross-react with antisera to G_o. The function of this unique G-protein has not been determined. It is also not known whether the catfish is exceptional in G-protein composition in the olfactory system or whether fish in general exhibit G-protein profiles in this tissue that are distinct from frog and rodents. It should also be noted that, as in many other tissues, initial gene cloning experiments indicate that as many as five distinct G-proteins may be present in rat olfactory epithelium (54).

The localization of G-proteins in olfactory cilia, but not in respiratory cilia (4), suggests that these proteins participate in olfactory signal transduction. By analogy to their documented role in mediating transmembrane signalling in many cells, olfactory receptor stimulation by odorant would activate G-proteins that subsequently mediated regulation of intracellular events such as second messenger formation. Olfactory receptor interaction with G-proteins was shown in isolated cilia from catfish by ligand binding experiments (17). In the presence of GTP or a nonhydrolyzable analogue, the affinities of two L-amino acid olfactory receptors for their stimuli were decreased, which indicates that these receptors were functionally coupled to G-proteins in the expected classical manner described for hormone and neurotransmitter receptors (42, 43). Additional evidence, described below, has accumulated implicating G-proteins in olfactory transduction. However, the guanine nucleotide-induced shift in receptor affinity documented, at the level of the initial binding event, functional interactions between the binding sites and G-proteins and further supported the hypothesis that these sites represented physiologically relevant receptor proteins.

The participation of adenylate cyclase in olfactory transduction has been

suspected for many years since high basal levels of the enzyme were observed in the olfactory epithelium (68). Early electrophysiological studies showed that membrane-permeable cyclic AMP analogues and cyclic nucleotide phosphodiesterase inhibitors reversibly reduced the amplitude of odorant-evoked responses (81). These initial observations were not pursued further for several years until Lancet and coworkers investigated odorant regulation of adenylate cyclase in isolated cilia from frog (72, 90). These workers confirmed the previous observation of high basal cyclase activity in the olfactory epithelium and showed that nearly half of the activity was associated with the cilia. The enzyme exhibited several properties expected of the classical hormone-sensitive adenylate cyclase (42). In addition, a mixture of odorants elicited modest increases in cAMP levels in the isolated cilia, but not in brain or liver membranes. Odorant-stimulated cAMP formation was GTP dependent, which strongly suggests that a G-protein, presumably G_s, participates in mediating activation of the olfactory adenylate cyclase.

The observations of Lancet and associates were subsequently confirmed and extended by Sklar et al (108), who tested a large number of odorants for the ability to stimulate cAMP formation in isolated cilia from frog and rat. Most fruity, floral, minty, and herbaceous odorants stimulated cAMP formation 30–65% over basal levels, while putrid odorants and odorous chemical solvents were ineffective. These investigators also showed that Ca^{2+} inhibited both basal and GTP-stimulated adenylate cyclase activity in isolated cilia. This observation was confirmed in rat olfactory epithelium by Shirley et al, who also showed that Ca^{2+} inhibited odorant-stimulated cAMP formation (106). Sklar et al also showed in homologous series of pyrazine, thiazole, and pyridine compounds that those with the longest hydrocarbon chains elicited the largest enhancement of cyclic AMP accumulation; thus hydrophobicity may be important in the activation mechanism (108). Since some odorants failed to activate adenylate cyclase, Sklar et al concluded that additional transduction mechanisms were involved for these odorants (108).

A major second messenger function of cAMP in many cells is activation of cyclic nucleotide-dependent protein kinase, which phosphorylates a variety of tissue-specific proteins involved in cellular responses to external stimuli. Cyclic nucleotide-dependent protein kinase activity was identified in isolated cilia from frog (46). The kinase was stimulated by cyclic AMP and cyclic GMP, although the latter was about 10-fold less potent than cAMP. Endogenous protein substrates for the kinase were also identified in the isolated cilia. A phosphoprotein, pp 24, exhibited enhanced phosphorylation in response to cAMP. The identity of this phosphoprotein and its functional role in olfactory transduction have not been established. Activation of the protein kinase by odorant stimulation of adenylate cyclase was attempted in this study, but consistent results were not obtained (46). Neurophysiological

evidence also suggests a role for cyclic nucleotides in mediating cation channel activity in olfactory neurons. Incorporation of rat olfactory epithelium into planar phospholipid bilayers conferred odorant sensitivity to the bilayers that was mimicked by cAMP (121). A cyclic nucleotide-gated conductance, sensitive to cyclic AMP and cyclic GMP, was also observed in excised membrane patches from individual cilia (86). It was proposed that this conductance was directly gated by cyclic nucleotide, in contrast to a mechanism dependent on protein phosphorylation. However, stimulus-gated channels may also be present in the ciliary membrane that do not require second messengers for regulation (71).

Odorant regulation of adenylate cyclase was also investigated in isolated cilia from catfish (19; R. C. Bruch and J. H. Teeter, submitted for publication). In this model system, electrophysiological studies (28) and ligand binding experiments (55; R. C. Bruch and R. D. Rulli, submitted for publication) indicate the presence of distinct olfactory amino acid receptor classes that recognize acidic, basic, and neutral ligands. Ten amino acids, representative of each receptor class and covering a wide range of electrophysiological efficacy were tested for the ability to stimulate cyclic AMP formation. Amino acid stimulation of adenylate cyclase was GTP dependent, and cAMP levels were increased 2- to 3-fold over basal levels with all tested stimuli. Little correlation was obtained between receptor specificity or electrophysiological potency and the ability to stimulate cAMP formation. The stereoselectivity exhibited by the enantiomers of alanine in neurophysiological (27) and ligand binding assays was not reflected in their ability to stimulate adenylate cyclase. Dose-response and time course studies indicated that significant enhancement of cAMP formation was obtained only at high receptor occupancy levels, which suggests that adenylate cyclase may participate in olfactory transduction under conditions of high odorant concentrations or prolonged (several minutes) exposure to stimulus.

A role for G_s-mediated activation of adenylate cyclase in human olfaction was suggested by Weinstock et al (123). These investigators found reduced ability to identify ten common odorants in type 1a pseudohypoparathyroidism patients. These patients exhibit G_s deficiency and are resistant to the cAMP-mediated actions of several hormones. In contrast, type 1b patients exhibited normal G_s activity and olfactory ability.

A second receptor-mediated transduction pathway has also been identified in olfactory cilia that involves the stimulated catabolism of phosphoinositide lipids. The participation of phosphoinositide metabolism in transmembrane signalling has been demonstrated in a large number of tissues (10). Agonist occupation of appropriate receptors activates phospholipase C (phosphatidylinositol-4,5-bisphosphate phosphodiesterase) by a mechanism that is probably mediated by a G-protein. The identity of the G-protein(s) that mediate phos-

pholipase C activation has not been established. Phospholipase C rapidly hydrolyzes phosphatidylinositol-4,5-bisphosphate to form two second messengers, inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. Diacylglycerol operates within the membrane to activate protein kinase C, which phosphorylates a variety of tissue-specific proteins. IP_3 is released into the cytoplasm and subsequently interacts with a receptor in the endoplasmic reticulum to release internal stores of Ca^{2+}.

Initially, the possible involvement of phosphoinositide hydrolysis in olfaction was suggested because phospholipase C was activated by odorant amino acids in isolated cilia from catfish (18, 52). This initial study also indicated that the enzyme was activated by guanine nucleotides, which makes it likely that a G-protein participates in mediating phosphoinositide hydrolysis in the cilia. The enzyme activity was also identified in mouse and rat olfactory epithelium. Subcellular fractionation studies in all species examined showed that, as in many other tissues, the majority of the enzyme was soluble. Since these original observations were reported, subsequent time course studies showed that 2- to 3-fold increases in IP_3 levels were obtained within 10 seconds of exposure to stimulus (19). Odorant-stimulated release of IP_3 was also GTP dependent, which confirms the earlier suggestion of G-protein involvement (18, 52). The ability of IP_3 to release Ca^{2+} from isolated microsomes from the olfactory epithelium was also confirmed (R. C. Bruch, unpublished). In these preparations, IP_3 mediated the rapid and transient release of sequestered Ca^{2+}, which suggested that intracellular Ca^{2+} flux may also be important in olfactory transduction. Protein kinase C has also been identified in frog olfactory epithelium by immunoreactivity and phorbol ester binding (4). However, initial attempts to activate the enzyme with Ca^{2+} and phospholipid, required cofactors for the enzyme, were unsuccessful (46).

TASTE

Organization of the Gustatory Epithelium

Taste buds in the oral cavity are the specialized peripheral receptive structures for soluble (taste) stimuli. In humans, taste buds are located in discrete receptive fields in the oral cavity (49). Fungiform papillae, innervated by the chorda tympani branch of the VIIth or lingual nerve, cover the anterior two thirds of the tongue. The 20–60 fungiform papillae may each contain between 0 and 8 taste buds along with specialized pressure, tactile, and temperature receptors. Filiform papillae that do not contain taste buds surround the fungiform papillae. Circumvallate (vallate) papillae are located on the posterior third of the tongue. These papillae usually number between 7 and 20, contain 10–100 taste buds, and are innervated by a branch of the glossopharyngeal (IXth cranial) nerve. Palatal papillae usually contain a single taste

bud, are localized primarily near the junction of the hard and soft palate, and are innervated by the IXth and Xth cranial nerves. Taste buds and papillae may also appear in other oral and pharyngeal structures in humans, including the lips, the inner surface of the lingual or mucous membrane, the epiglottis, and various areas of the pharynx.

Properties of Taste Buds

The taste buds (gemmae) located in each type of taste papillae can be distinguished by their characteristic appearance. In general, taste buds are slender to globular in shape and are made up of 20–50 neuroepithelial cells extending from the basal laminae of the epithelial layer to the surface of the tongue (49, 60). Perigemmal epithelial cells and filamentous material distinguish the outer edge of the taste bud. Sensory nerve fibers enter the base of the bud through the basal lamina and course throughout the proximal two thirds of the bud to form afferent synaptic contacts with taste bud cells (60). The apical portion of the human taste bud is characterized by a constriction through which pass the microvillar processes of the taste cells.

Occluding junctions join the apical ends of the cells in the taste bud dividing the taste cell membrane into an apical portion (facing the external solution) and a basolateral portion (facing the interstitial fluid). The occluding junctions are thought to restrict diffusion of substances across the sensory epithelium. Consequently, the apical region is presumed to be the site where chemical stimuli interact with the microvillus of the taste cells and thereby initiate the transduction processes. However, this primary site of action does not exclude the existence of receptor sites proximal to the occluding junction.

The number of cell types in taste buds and their role in chemoreception remain areas of continuing investigation. Using light and electron microscopy and a variety of staining techniques, several different types of cells in mammalian taste buds have been classified. These cells have been characterized as dark (type I), light (type II), gustatory (type III), and basal (type IV) cells (36, 60, 85). In addition, neurosecretory cells have been identified within the taste bud (101). The cells of the mammalian taste bud are under continual renewal (9, 36), possessing a life cycle of ten days to two weeks. The constant turnover of the cells within the taste bud has complicated the identification and characterization of the properties of the chemosensory cells. Identification of gustatory receptor cells has also been complicated by the observation that light, dark, and basal cells all appear to be suitably innervated (60, 115). Moreover, nearly all of the cells in the taste bud (as well as surface epithelial cells not associated with the taste buds of amphibians and fish) respond to common taste stimuli (114). Because of their small size, taste cells of most commonly used animal models are not readily amenable to intracellular recordings. However, significant progress in understanding the

ionic bases of receptor potentials has recently been achieved by using favorable models such as mudpuppy (61, 62), frog (6), and catfish (116). Intracellular recordings of membrane potential indicate that taste cells are electrically excitable and contain several types of ion-selective channels, although the role of these channels in signal transduction and their regulation have not been firmly established.

Single taste cells usually respond to a variety of different stimuli, including representatives of more than one of four taste qualities (NaCl, salty; quinine, bitter; sucrose, sweet; HCl, sour). Some of these responses appear to be mediated by fundamentally different mechanisms, so more than one transduction mechanism may be available to each taste cell. In addition, since a particular stimulus may evoke responses attributable to different mechanisms in different cells, functional categories of cells may also exist (115).

Molecular Characteristics of Taste Receptors

The intracellular processes that underlie recognition of taste stimuli at the membrane level have received increasing attention. As in olfaction, taste receptor cell activation is presumably mediated by the specific interactions of, at least some, stimuli with receptor proteins in the apical chemosensory membrane. Since the putative receptor proteins have not been identified at the molecular level or isolated, alternative mechanisms that do not require the existence of specific receptor sites have also been proposed to account for taste cell responses to some stimuli such as small ions and lipophilic compounds. There is also evidence that a chemical need not touch the apical chemoreceptive membrane for taste perception, since some compounds, such as sodium dehydrocholate and saccharin, are tasted as they circulate through the tongue following intravenous injection (12).

It is presumed that the receptor site(s) for salty stimuli are localized at the apical membrane of taste receptor cells. Topically applied proteolytic enzymes (63) and protein-modifying agents (82) suppress chorda tympani nerve responses to some, but not all, salty stimuli. Stimulation by NaCl is very rapid and readily reversible (8). The molecular events that may underlie salty taste reception include specific adsorption sites (8), nonelectrostatic interactions (35, 69), and sites or channels of selective ion transport (15, 45). Several lines of evidence indicate that amiloride-sensitive Na^+ channels in the apical membranes of taste cells in mammals and frogs play a primary role in responses to Na^+ and Li^+, but not K^+ or Rb^+, salts (15, 45, 107). Inhibition of salt responses by amiloride applied to the dorsal surface of the tongue is rapid and reversible but not complete (15), which suggests that more than one receptor for salts may be localized in the apical plasma membrane.

The limited number of stimuli that impart a sweet taste and the structural similarity among them has led to the hypothesis that sweet taste recognition is

mediated by one or more specific receptor proteins. In humans, competition for binding sites of the sweet tasting protein monellin suggested that the sweet receptor is broadly tuned (26), while psychophysical studies (38) and studies using inhibitors both support the hypothesis that more than one receptor site for sweet compounds exists (21, 53). Electrophysiological studies in the gerbil with compounds that taste sweet to humans yield evidence for at least two sweet receptor mechanisms (120). There are numerous artificial sweeteners that presumably bind tightly to sweet receptor proteins and hence would be expected to be useful tools for characterization of the interaction of sweet stimuli with receptor sites. However, there is species specificity for certain sweet substances. Few of the known artificial sweeteners are active in animals such as the steer, where the generous amounts of tissue required for biochemical studies are available. Biochemical studies have, however, localized binding sites for biologically active sugars, such as sucrose, in plasma membrane fractions from bovine taste epithelium, but not in lingual epithelium lacking taste buds (75). Putative receptor sites for the sweet tasting protein thaumatin have also been identified at the ultrastructural level in the microvilli of monkey taste buds (37).

The large number of compounds that taste bitter and the diversity of their structures suggest that unique membrane-associated receptors may not exist for bitterness. Many bitter stimuli are lipophilic (65) and a correlation between the threshold concentrations of bitter stimuli required for depolarization of N-18 neuroblastoma cells and taste thresholds in humans has been reported (66). Since N-18 cells presumably lack taste receptor proteins, and since many bitter stimuli carry a positive charge at neutral pH, these observations led Kurihara and coworkers (69) to propose that bitter compounds activate receptor cells through an electrostatic interaction at the cell membrane. However, these investigators and others have reported that many bitter compounds inhibit cyclic AMP phosphodiesterase (67, 73). Thus, intracellular phosphodiesterase may act as the "receptor" protein to permeant stimuli and mediate taste cell function by transient accumulation of cAMP. This may explain the correlation between bitterness and lipophilicity since stimuli would have to penetrate the plasma membrane to interact with phosphodiesterase to evoke a response.

More recently, Akabas and coworkers (1) presented results suggesting that bitter transduction occurs by an alternative pathway. They demonstrated that taste receptor cells of rat responded to the bitter stimulus denatonium by a rise in intracellular Ca^{2+} and this was not accompanied by opening of voltage-gated Ca^{2+} channels. They proposed that the initial event in bitter reception involves binding of a bitter substance to an apical cell surface receptor followed by generation of an intracellular second messenger. In the absence of binding data, however, a rise in intracellular Ca^{2+} could also occur after

intracellular accumulation of a stimulus such as the very penetrating quaternary amines, if the stimulus also gives rise to, for example, an accumulation of IP_3 or some other calcium-releasing messenger.

Two problems have generally hampered biochemical characterization of stimulus binding to taste receptors: the lack of stimuli that bind tightly to the presumed receptors, and difficulties in obtaining the large quantities of tissue necessary for biochemical studies. Because of these problems, many of the studies upon which current concepts of taste receptor mechanisms are based have been performed in aquatic animals. The catfish has an extraordinary number of taste buds on its barbels and body surface (5, 34) that are highly sensitive to various amino acids as taste stimuli, and it has thus proved to be an excellent model for the study of taste receptor-stimulus interactions. L-[^{3}H]-alanine and L-[^{3}H]-arginine have been shown to bind specifically, reversibly, and in a saturable manner (criteria expected for binding to specific receptor sites) to a sedimentable fraction from cutaneous epithelium of the catfish with dissociation constants in the micromolar range (13, 23, 24, 115; D. L. Kalinoski, et al, submitted for publication). In addition, competition and kinetic ligand binding studies indicated the presence of at least two different classes of receptors for L-arginine. Whether these classes of arginine sites represent two separate macromolecular receptor proteins or two different affinity states of the same receptor is yet to be determined.

Biochemical and neurophysiological studies in the catfish taste system yield comparable results. Electrophysiological studies indicate that certain L-amino acids are effective at low concentrations and that there may be at least two types of taste receptors, one of which is maximally responsive to L-alanine, the other to L-arginine. The L-alanine receptor would be expected to be the more broadly tuned, responding to a variety of amino acids including L-alanine, L-arginine, L-serine, L-threonine, and glycine. The arginine receptor would be expected to be more specific, responding to arginine and lysine. In accordance with the electrophysiological results, biochemical studies have demonstrated that the L-alanine binding site is less selective than that for L-arginine (13, 23, 115). Recently, two types of electrophysiological responses to L-arginine have been recorded from catfish taste cells (J. H. Teeter, submitted for publication). It is not known, however, whether these responses correspond to stimulation of the two classes of receptor sites suggested by L-[^{3}H]arginine binding studies. Consistent with the hypothesis that the amino acid binding sites in purified plasma membranes from catfish taste epithelium represent physiologically relevant receptor proteins, a monoclonal antibody has been developed that inhibits L-alanine binding (44). The antigen or putative receptor recognized by the antibody has been identified by immunohistochemical methods (125) and shown to be a membrane-associated glycoprotein by lectin reactivity and immunoblotting analysis (20).

Molecular Mechanisms of Taste Signal Transduction

Because of the small size of taste receptor cells and the fundamental conceptual problems in interpreting the available information, there is limited consensus regarding taste receptor mechanisms (69, 96, 115, 117). The role of ion channels and membrane depolarization in taste signal transduction have often been inferred from recordings obtained from the nerves innervating taste cells. There is presumptive evidence that changes in membrane potential are related to changes in firing in the sensory nerves, but there is no direct evidence that membrane depolarization leads to a release of neurotransmitter and subsequent impulses in taste responsive nerves. Depending upon which animal and stimulus are chosen, the correlation between depolarization of receptor cell and nerve impulse generation can vary from quite good to moderate at best. However, recent evidence from single-cell electrophysiological studies indicate that ion channels play a considerable, although not completely defined, role in taste transduction.

The simplest mechanism for taste signal transduction involves alteration of the membrane potential by entrance of ionic stimuli through channels in the apical membrane against their electrochemical gradients. Chorda tympani recordings indicate that at least part of the response to NaCl is through amiloride-sensitive sodium channels (15, 45). Intracellular recordings from taste cells exposed to various extracellular ions indicated that, for frogs, 50% of the cell membrane depolarization was due to amiloride-sensitive channels in the apical membrane (102). Another 40% of the NaCl response was attributed to channels located in the basolateral membrane and 10% to phase boundary potential.

Several investigators have recently reported voltage-dependent ionic currents in taste cells of salamanders (111), mudpuppies (62), and frogs (6). Voltage-dependent Na^+, Ca^{2+}, and two types of K^+ currents have been identified by patch-clamp recordings. Using calcium channel blockers, two types of K^+ channels were characterized. Individual receptor cells were shown to possess different proportions of these channels. In addition, some K^+ channels close in response to bitter (1) and acid (61) stimuli, which could lead to receptor cell depolarization. Opening (or closing) of ion channels in taste cell membranes could result in either depolarization or hyperpolarization of the cell, depending upon the nature of the ions involved. Influx of cations (Na^+ or Ca^{2+}) or efflux of anions or stimulus-evoked closure of open K^+ channels could result in depolarization, while influx of anions or efflux of cations as well as closure of Ca^{2+} or Na^+ channels could hyperpolarize the cell. All combinations of changes in membrane potential and resistance that could result from these possibilities have been recorded from taste cells when stimulated with various types or concentrations of stimuli. Thus, ambiguity remains regarding the role of ion channel activity in taste transduction and the

molecular mechanisms that underlie stimulus regulation of the electrical events mediated by ion channels.

The possible involvement of second messengers in taste transduction is beginning to be characterized. Cyclic AMP may contribute to taste signal transduction (68), although early studies failed to demonstrate stimulus-enhanced accumulation of labeled cyclic AMP in tissues prelabeled with [^{3}H]-adenosine (22, 88). However, adenylate cyclase, cAMP phosphodiesterase, and guanine nucleotide-binding regulatory proteins, all of which are components of the adenylate cyclase system, have been identified in taste epithelium (17, 87). Avenet & Lindemann (6) have also demonstrated with patch-clamp recordings from isolated frog taste cells that cAMP caused reversible depolarization of taste membranes due to partial blockage of one class of K^+ channels. Since the cAMP-mediated effect was observed only in the presence of ATP, these results suggested that cAMP initiated phosphorylation of one set of K^+ channels leading to a nonconducting conformation. However, evidence that adenylate cyclase activity is regulated by taste stimuli remains limited. Two recent studies demonstrated that cAMP formation is increased in the presence of stimuli in taste epithelium from rats (109) and catfish (56). Thus, while cAMP may act as a second messenger for some taste stimuli, its role in transduction remains to be firmly established.

Other second messengers may also participate in taste transduction, but little direct evidence has been reported. Guanylate cyclase has been localized to taste cells in mammals by histochemical methods (88). Cyclic AMP and cyclic GMP when perfused in the lingual artery altered taste responses of the glossopharyngeal nerve of frog to some stimuli applied to the dorsal surface of the tongue (69). However, since the cyclic nucleotides were not shown to cross the plasma membrane of taste cells (or to reach the interstitial fluid in the taste buds) interpretation of these studies is limited. In a recent study, injection of cyclic GMP and a Ca^{2+} chelator into taste cells altered stimulus-induced membrane depolarization of mouse taste receptor cells, which suggests a role for Ca^{2+} and cyclic GMP in taste transduction (118). In addition, inositol phospholipids have also been suggested as second messengers for some taste stimuli. L-alanine and guanine nucleotides increased phospholipase C activity in homogenates of catfish taste epithelium (51). The catalytic activity of phospholipase C was shown to be calcium-ion dependent, but not Ca^{2+} regulated. There is also evidence that some K^+ channels in mudpuppy taste cells are Ca^{2+} dependent (62). Thus, there appear to be a variety of transduction mechanisms by which stimulus interaction with receptor at the apical membrane may be coupled to modulation of neurotransmitter release at the basolateral synapse. The type of coupling may depend not only upon the nature of the stimulus, but also the cell type.

CONCLUSIONS

The biochemical and neurophysiological evidence summarized in this review documents many recent advances in our understanding of stimulus-response coupling in chemosensory cells. While substantial progress has been achieved, the molecular basis of chemoreception and signal transduction remains incompletely described. Further progress in elucidation of the molecular and cellular mechanisms of chemosensation will require rigorous confirmation of the receptor hypothesis by identification and isolation of the relevant molecular species. Additional evidence is necessary to establish firmly the roles of identified G-proteins and second messengers in mediating receptor cell activation, adaptation, and recovery. It may be anticipated that integration of biochemical and single-cell electrophysiological and biophysical techniques will lead to elucidation of the molecular mechanisms underlying regulation of ion channel activity and neurotransmitter release. A clearer understanding of the peripheral events of chemosensation will be the inevitable result.

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