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Cell communication in taste buds

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Abstract. Taste bud cells communicate with sensory afferent fibers and may also exchange information with adjacent cells. Indeed, communication between taste cells via conventional and/or novel synaptic interactions may occur prior to signal output to primary afferent fibers. This review discusses synaptic processing in taste buds and summarizes results showing that it is now possible to measure real-time release of synaptic transmitters during taste stimulation using cellular biosensors. There is strong evidence that serotonin and ATP play a role in cell-to-cell signaling and sensory output in the gustatory end organs.

Keywords. Synapses, serotonin, ATP, paracrine, sensory afferent fibers.

Introduction

Taste buds are peripheral sensory organs that respond to a wide variety of sapid chemicals. These gustatory end organs transmit signals to afferent nerves by way of synapses between taste cells and primary afferent sensory fibers, and perhaps also from cell to cell within a taste bud [1, 2, reviewed in 3].

Historically, mammalian taste bud cells have been classified into four categories on the basis of their cytological and ultrastructural features: Type I, II, III, and basal or Type IV cells [4–6, reviewed in 7]. Basal (Type IV) cells are progenitor cells that restock the taste bud during its normal course of cell turnover. Type I cells are believed to be supporting cells and may have glial-like properties such as those described by Bigiani [8]. Basal and Type I cells, although undoubtedly important players in the taste bud, will not be discussed further in this review because their roles in signal processing are presently unknown. In contrast, Types II and III cells appear to represent taste receptor cells and synaptic output cells, respectively, for taste signaling (see below). The original categorization of taste cells based on cytological and ultrastructural features is being eclipsed by more recent classifications based on the expression of certain molecules. These categories may be more revealing regarding taste bud function [e.g., 6, 9, 10]. Presently, the field is in the awkward stage of transition from a historical nomenclature based on electron micrographs to molecular and functional profiles based on immunostaining, *in situ* hybridization and single cell reverse transcription-polymerase chain reaction (RT-PCR).

Gustatory signal processing and synapses in taste buds

Type II cells are likely to be the primary sensory receptor cells in the taste bud. This conclusion stems from recent observations that the molecular machinery for taste transduction, including G-protein-coupled taste receptors (GPCRs) and downstream effectors, is primarily expressed in Type II cells [6, 9–12]. Paradoxically, although sensory afferent fibers come into close contact with Type II cells, there are no classical ultrastructural specializations indicating the presence of synapses between Type II cells and gustatory afferents [13]. Instead, only Type III cells form recognizable synapses with nerve processes [14]. As a consequence, Type III cells are considered by many investigators to generate the final output signal from gustatory end organs. Type III cells ('synaptic output cells') have also been shown to express SNAP25, a vital member of the SNARE complex of vesicle release machinery associated with synapses [10]. Furthermore, a voltage-gated Ca^{2+} channel found in presynaptic terminals, α1A, and an extracellular adhesion molecule found at synapses, NCAM, are also expressed specifically in cells of this category (i.e. synaptic output cells) [10]. In

short, the molecular machinery of transmitter release and other components of synapses are found in synaptic output cells. This raises the conundrum that taste transduction takes place in one type of cell (receptor, Type II) but output signals from taste buds appear to arise from another cell type (synaptic output, Type III). If this is the case, cell-cell communication between receptor and synaptic output cells must occur within the peripheral gustatory sensory organs during taste stimulation.

Chemical synapses and gap junctions between taste cells have been identified and might be routes for cell-cell signaling [3, 15, 16], but little is known about how they operate. One possibility is that groups of two to five taste cells are united into a 'gustatory processing unit' by gap junctions [15, 17]. Cells within the processing unit might share electrical signals and/or second messengers such as inositol 1,4,5-trisphosphate (IP_3) via the gap junctions. Paracrine secretion of norepinephrine, glutamate, serotonin, CCK, VIP and other transmitters within the taste bud may be another route of cell-cell signaling [2, 18–23]. Lastly, taste cells conceivably communicate directly with each other and with closely apposed sensory afferent fibers via novel mechanisms not involving conventional synapses.

The above findings give rise to a heuristic model for taste bud signal processing (Fig. 1). A taste bud with Types I, II, III and basal cells is shown in Figure 1a. Nerve fibers come into close apposition with Type II and III cells, but only form conventional synapses with the latter. Groups of Type II and III cells may form a taste 'processing unit' (Fig. 1b). According to the model, tastants act on Type II (receptor) cells which (i) directly excite primary afferent fibers via unconventional synaptic contacts in a manner not yet understood (Fig. 1b, circle), or (ii) secrete paracrine transmitter(s) that stimulate Type III cells and thus indirectly lead to afferent nerve output (Fig. 1c), or (iii) some combination of both these possibilities. Not shown, but also postulated, are gap junctions that transmit generator potentials or second messengers from cell to cell within the processing unit.

What neurotransmitters do taste cells release?

If cell-cell interactions take place within mammalian taste buds, identifying the transmitters would be paramount to sort out the synaptic logic of signal processing in these gustatory end organs. To date, serotonin (5-hydroxytryptamine; 5HT) has been one of the best-studied transmitter candidates of taste buds, although whether it is a paracrine secretion, a conventional synaptic neurotransmitter, or both, is not yet clear. 5HT has been identified with high-performance liquid chromatography (HPLC) in mammalian taste tissues [24]. Histochemical and immunocytochemical techniques have demonstrated that 5HT is present in a subset of Type III taste cells in circumvallate and foliate papillae of mouse, rat, rabbit and monkey [6, 25–28] and in basal-like Merkel cells in amphibian taste buds [29–31]. By using autoradiographic techniques with H^3-labelled 5HT and exploiting the large taste cells in *Necturus* taste buds, researchers demonstrated that certain taste cells selectively take up 5HT and release it in a Ca^{2+}-dependent manner when depolarized [32]. Ren et al. [33] reported that the Na-dependent 5HT transporter SET was expressed in rat taste cells. These workers suggested that the actions of 5HT released from one cell and acting on other cells within the taste bud was terminated by this transporter. Early reports with patch clamp recordings indicated that bath-applied 5HT modulates Ca^{2+} currents in amphibian taste cells: Ca^{2+} current in some cells was upregulated and in other cells it was downregulated [34]. These actions were believed to be mediated by 5HT1a-like receptors. Later studies by Herness and his colleagues, also using patch clamp electrophysiology, showed that 5HT decreased K^+ and Na^+ currents in mammalian taste cells [35]. Ewald and Roper [19] impaled adjacent taste cells in the large taste buds of *Necturus* and found that depolarizing one cell led to a hyperpolarization in a subset of adjacent cells. This hyperpolarization was mimicked by bath-applying 5HT. Finally, RT-PCR and immunostaining have indicated that mammalian taste buds express certain subtypes of 5HT recep-

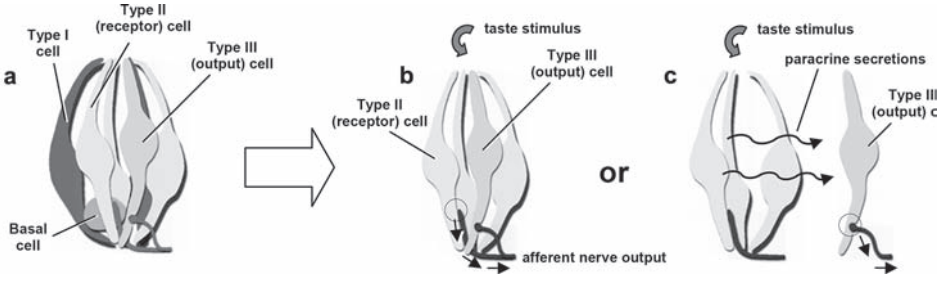

Figure 1. Schematic diagram of a mammalian taste bud (*a*) and hypothesized signal processing units (*b, c*). Type I cells are believed to be glial-like cells. Basal cells are progenitor cells. Type II cells are likely to be the sensory receptor cells, at least for taste stimuli transduced by G-protein-coupled receptors. Type III cells synapse with sensory afferent fibers and may be synaptic output cells for the taste bud. Not shown are gap junctions that may join cells within the processing unit and that may also participate in cell-cell communication. In *b*, taste signals are transmitted from receptor cells directly to gustatory afferent fibers. In *c*, taste signals are transmitted to Type III synaptic output cells via paracrine secretions. See text for further description.

tors, with 5HT1a receptors occurring on cells within taste buds and other 5HT receptors occurring on nerve fibers innervating taste buds [2]. The original report that gustatory afferent nerve fibers express 5HT3 receptors and the suggestion that those receptors mediate taste sensations [2] may need to be refined. Recent findings indicate that mutant mice lacking 5HT3 receptors show no obvious taste behavior deficits [36].

Taken collectively, the above findings suggest the following scenario: in response to gustatory stimulation, (i) certain taste cells secrete 5HT onto adjacent taste cells (paracrine secretion) to modulate surrounding taste cell activity via metabotropic receptors (5HT1a); (ii) taste cells (specifically, serotonergic Type III) also release 5HT onto primary afferent fibers to excite them (synaptic transmission). A crucial missing link in this scenario is whether 5HT is released during gustatory stimulation. This link is provided by a recent publication, as described later [37].

There is also excellent evidence that ATP is one of the taste bud neurotransmitters acting on sensory afferent fibers. Bo et al. [38] had originally reported the presence of purinergic fibers innervating taste buds and specifically that P2X2 and P2X3 purinoceptors were expressed on gustatory afferent fibers. Finger and associates [36] tremendously advanced this notion by studying taste buds and taste nerve responses in mutant mice lacking P2X2 and P2X3 purinoceptors. Mice lacking P2X2 and P2X3 receptors fail to respond to many, but not all, sapid stimuli [36]. Furthermore, epithelial sheets removed from the lingual surface from mice secrete ATP when stimulated with tastants. This evidence vindicates ATP as one of the neurotransmitters between taste cells and afferent fibers. Conceivably, ATP and 5HT may be co-released from Type III synaptic output cells. ATP may also be employed as a cell-to-cell messenger within taste buds. Taste cells express P2Y purinoceptors and respond to exogenously applied ATP in a manner consistent with P2Y-mediated mechanisms [39, 40].

Other candidates for paracrine secretions and synaptic neurotransmitters in taste buds have also been proposed, including norepinephrine, acetylcholine, glutamate and peptides [reviewed by 41–43]. Supporting data include immunocytochemical localization of norepinephrine, cholecystokinin (CCK), vasoactive intestinal peptide (VIP), glutamate and glutamate transporters in taste buds [18, 22, 23, 44, 45]; expression of cholinergic (mAChR1) and cholecystokinin (CCK-A) receptors on taste cells [23, 46]; and patch clamp and Ca^{2+} imaging recordings demonstrating physiological actions of norepinephrine, CCK, ACh and glutamate on taste cells [21, 22, 23, 46]. Substance P and leptin receptors have also been identified in taste buds, further suggesting peptidergic modulation of the gustatory end organ [47, 48]. Thus, while these observations suggest that a large number of different transmitters and receptors are found in taste buds, apart from 5HT and ATP there is no firm evidence for taste-evoked release of any of them and little understanding of how they might function in taste buds.

Taste buds in different regions of the oral cavity might utilize different synaptic strategies to process gustatory signals, and different species may use different taste transmitters. These possibilities are raised by the noticeable differences in sensitivities between taste buds in different regions of the oral cavity and marked interspecies differences in taste preferences [e.g. 49–52]. One explanation for the differences is the demonstrated variations in the regional expression of taste receptors (e.g. T1Rs, T2Rs, ion channels such as ENaC and ASIC) among the taste regions of the oral cavity and between species [9, 53–57]. Further, there are important polymorphisms in the T1R and T2R proteins between species and among strains that contribute to the differences in taste sensitivities [54, 58, 59]. Nonetheless, one cannot exclude possible differences in synaptic processing between species and from region to region within a species. Indeed, evidence for this latter notion was presented by Finger et al. [36] who showed that gustatory transmission from solitary taste receptor cells in the larynx was unaffected in P2X2/P2X3-null mice, contrary to the lack of gustatory transmission for taste buds on the tongue in these mutant mice.

Is transmitter release triggered by Ca^{2+} influx or by Ca^{2+} from intracellular stores?

This question is important because Huang et al. [37] reported that the release of serotonin from stimulated taste buds depends on multiple sources of Ca^{2+}, including influx and store-release. Nearly all synapses investigated to date depend on Ca^{2+} to trigger the release of synaptic vesicles that store neurotransmitters. Ca^{2+} activates a complex of synaptic proteins (SNARE), most likely by interacting with synaptotagmin, and initiates the fusion of transmitter-laden vesicles with the presynaptic membrane [reviewed by 60]. For nearly all synapses, the source of the Ca^{2+} is a localized influx across the presynaptic membrane via voltage-dependent Ca channels. Omitting Ca^{2+} from the bath and/or substituting Mg^{2+} for Ca^{2+} shuts down vesicular release. These are among the canonical features of conventional synaptic transmission. Accordingly, Huang et al. [37] found that depolarization-stimulated serotonin release from taste buds was indeed dependent on extracellular Ca^{2+}.

Yet, certain forms of synaptic transmission persist in low extracellular [Ca^{2+}], leading some investigators to conclude that there are release mechanisms independent of Ca^{2+} influx [61, reviewed by 62]. One possible explanation for these observations is that even under nominally 0 mM Ca^{2+} conditions, there remains residual extracel-

lular Ca^{2+} whose influx is enhanced by the reduction of an adsorbed layer (Gouy layer) of divalent cations (i.e. decrease in the zeta potential, [63]). In short, merely removing Ca^{2+} from the bath may or may not be a sign of transmitter release independent of Ca^{2+} influx.

Alternatively, studies have shown that Ca^{2+} derived from presynaptic intracellular stores can augment synaptic transmission otherwise initiated by Ca^{2+} influx. Ca^{2+} from intracellular stores may even on its own trigger transmitter release from neurons [e.g. 64–72; but see 73]. Release elicited by Ca^{2+} from intracellular stores is a significant departure from conventional neuronal synaptic mechanisms but may be a common feature of exocytosis from neuroendocrine cells [74]. This is an emerging new theme in the physiology of synapses. Transmitter release evoked by taste stimulation (but not by depolarization) was triggered by intracellular Ca^{2+} stores in taste buds [37].

Lastly, some synapses appear to be truly Ca-independent. They do not rely on Ca^{2+} influx or release from intracellular stores. These atypical synapses utilize voltage-dependent transporters to control the release of transmitters [reviewed by 75]. These synapses are quite unique and were first reported for noradrenergic release in the rabbit heart [76] and γ-aminobutyric acid-(GABA)-ergic synapses between horizontal cells and photoreceptors in the toad retina [77]. An important characteristic of transport-mediated synapses is the absence of key ultrastructural features of more conventional synapses, namely synaptic vesicles and synaptic membrane thickenings. Rather, transmitter is shuttled across the presynaptic membrane directly from the cytosol into the synaptic cleft (and vice versa) via Na^{+}-dependent transporters. The close apposition between Type II (receptor) taste cells and primary afferent nerve terminals in the taste bud might represent sites of transporter-mediated synaptic transmission. Indeed, taste buds are reported to express 5HT and glutamate transporters [33, 45].

Are there other, non-synaptic, forms of information transmission?

All the synapses reviewed above are characterized by close, focal proximity of pre- and postsynaptic sites. Transmitters are released, diffuse across a narrow synaptic cleft, and act on the opposing face of a receptor-studded chemosensitive postsynaptic membrane surface. There are other, less conventional routes for information transmission. These unconventional synapses might play important roles in taste buds. Paracrine secretion, for example, is part way between synaptic transmission and endocrine interactions. A potent neuroactive substance secreted from one cell can diffuse a short distance (~ up to several cell diameters) to activate a target cell in the nearby vicinity. Paracrine interactions do not have typical ultrastructural pre- or postsynaptic specializations characteristic of conventional synapses. Paracrine secretion is believed to underlie communication between glial cells, and between glial cells and neurons [reviewed by 78]. For example, paracrine secretion of glutamate and ATP explains how mechanical perturbation of astrocytes *in vitro* results in a Ca^{2+} wave that propagates throughout the cell culture. Stimulated astrocytes secrete glutamate and ATP which evoke successive Ca^{2+} transients in adjacent astrocytes. Other transmitters in addition to, or instead of glutamate and ATP, also participate in the extracellular pathway for this type of long-range communication [79]. Paracrine secretion is independent of extracellular Ca^{2+} and does not appear to involve vesicular release. Instead, paracrine mediators may be released from activated cells through gap junction hemichannels in the plasma membrane that are opened by intracellular Ca^{2+} transients [80].

In addition to paracrine secretion, cell-cell communication can be mediated by diffusion of second messengers, particularly IP_3, through gap junctions or gap junction hemichannels. IP_3 releases intracellular Ca^{2+} from stores in the follower cells and propagates the Ca^{2+} wave. Glutamate, ATP and IP_3 act in concert to spread the wave of excitation [78].

Paracrine secretion and gap junctional transmission may also occur in the mouse taste bud and may, in fact, explain how excitation of Type II gustatory receptor cells leads to signal output (Type III cells) in stimulated taste buds.

Using cellular biosensors to detect neurotransmitters

Membrane patches have long been used as biosensors to detect second messengers and neurotransmitters. Hume et al. [81] used excised patches of membrane from cultured chick myotubes and electrophysiological recording techniques to detect ACh release from growth cones of embryonic neurons. Patches of receptor-rich membranes have subsequently been employed to measure ACh, ATP, glutamate, GABA, cyclic nucleotides and IP_3 in a number of cells and tissues [reviewed by 82]. Sometimes the patch probe has even been inserted into the interior of the cell ('patch cramming', [83]). Among the first investigators to employ intact living cells as biosensors to detect transmitters were Tachibana and Okada [84] who used freshly isolated catfish horizontal cells to study glutamate release from goldfish retinal bipolar cells. Morimoto et al. [85] measured ACh release by bringing a myocyte into contact with CHO cells that had been pre-loaded with that transmitter.

The above studies used electrophysiological recordings and measured electrical currents generated by biosensor receptor channels as the readout. More recently, Peti-Peterdi et al. [86] used Fura 2-loaded HEK 293 cells expressing the E-prostanoid receptor EP1 to monitor the release of PGE2 from macula densa cells isolated from rabbit kidneys. This group subsequently used cultured

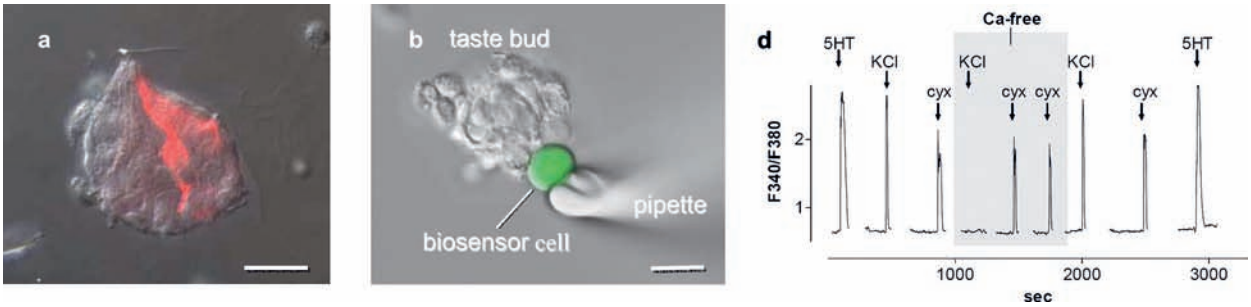

Figure 2. Isolating taste buds and testing with biosensor cells. (*a*), A fixed, isolated taste bud immunostained for serotonin (5HT). Two immunopositive taste cells (red) are visible. (*b*), A Fura 2-loaded (green) biosensor cell abutted against an isolated taste bud in a living preparation. In both *a* and *b*, Nomarski differential interference contrast and fluorescence microscopy images were merged. Calibration, 20 µm. (*c*), 5HT release from taste buds depends on Ca^{2+} influx for KCl depolarization but not for taste stimulation with a bitter (cycloheximide) or sweet (saccharin, not shown) compound. Sequential biosensor responses (increase in intracellular Ca^{2+}, as measured by Fura 2) from an isolated taste bud stimulated with 50 mM KCl (↓) or 100 µM cycloheximide (cyx, ↓). The 5HT biosensor was calibrated at the beginning and end of the recording with 3 nM serotonin (5HT, ↓). During the shaded region, Ca^{2+} in the bath (2 mM) was exchanged for 8 mM Mg^{2+}. The data indicate that 5HT release evoked by KCl depolarization depends on Ca^{2+} influx, whereas taste-evoked responses do not require extracellular Ca^{2+} (modified from [37]).

mouse mesangial cells to detect ATP released from macula densa cells [87]. ATP release from several tissues and cells has also been shown using PC12 or HEK 293 cells transfected with P2X2 receptors [88–90].

The advantages of using imaging methods to detect Ca^{2+} increases in cellular biosensors expressing specific receptors, such as employed by Peti-Peterdi et al. [87], include the following: (i) HEK 293 and CHO cells are easily grown and maintained in the laboratory; (ii) cells stably expressing high-affinity receptors for a number of different transmitters have already been produced or can readily be generated; and (iii) the readout (ratiometric Ca^{2+} imaging) is rapid and reliable. A possible disadvantage to Ca^{2+} imaging with biosensors is that the transfected receptors must be coupled downstream to Ca^{2+} signalling in the biosensor cell.

CHO cells expressing highly sensitive 5HT2c receptors recently were used as biosensors to detect serotonin release from taste bud cells. By positioning CHO biosensor cells against single taste buds isolated from mouse vallate papillae, Huang et al. [37] showed that sweet, bitter and sour stimulation, as well as potassium depolarization, evoked 5HT release (Fig. 2). Importantly, depolarization-mediated release was dependent on Ca^{2+} in the extracellular bath, but taste-mediated release was independent of extracellular Ca^{2+}, as discussed earlier. Work in progress also indicates taste stimulation releases ATP from isolated taste buds, as detected by CHO cells expressing sensitive P2X receptors (cf. [90]). This release, like that of 5HT, is also independent of Ca^{2+} in the bath and may be triggered by Ca^{2+} from intracellular stores.

Interpretations and speculation

Unconventional mechanisms exist in taste buds for tastant-evoked transmitter release. These mechanisms do not appear to depend on Ca^{2+} influx in taste buds. These results, combined with the model for taste processing presented in this review, may resolve a troubling dilemma confronting the field. Namely, gustatory sensory cells appear to express only a limited set of taste receptors (i.e. either bitter or sweet or umami GPCRs) and would therefore expect to be narrowly 'tuned' to these specific taste qualities. Yet, in functional tests many taste cells respond to multiple taste stimuli [91, 92]. They are broadly tuned to taste stimulation. The working hypothesis presented in this review, and specifically of taste-processing units (see Fig. 1), may resolve this conundrum. Namely, taste signals might well be generated in dedicated receptor cells which are narrowly tuned to specific taste qualities. Signals from these narrowly tuned cells could then be transmitted via paracrine secretions to synaptic output cells (i.e. Type III). Synaptic output cells would thus receive convergent information from several receptor cells, not necessarily all alike. Hence, synaptic output cells would comprise the more broadly tuned cells observed in functional studies of taste buds. Clearly, studies specifically testing this working model are needed. Biosensor cells may be well-suited for these future tests.

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