



Neuromodulation of Transduction and Signal Processing in the End Organs of Taste

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Abstract

Chemical synapses transmit gustatory signals from taste receptor cells to sensory afferent axons. Chemical (and electrical) synapses also provide a lateral pathway for cells within the taste bud to communicate. Lateral synaptic pathways may represent some form of signal processing in the peripheral end organs of taste. Efferent synaptic input may also regulate sensory transduction in taste buds. To date, the synaptic neurotransmitter(s) or neuromodulator(s) released at chemical synapses in taste buds have not been identified unambiguously. This paper summarizes the attempts that have been made over the past 40 years to identify the neuroactive substances acting at taste bud synapses. We review the four traditional criteria for identifying chemical transmitters elsewhere in the nervous system—localization, uptake/degradation, release and physiological actions—and apply these criteria to neuroactive substances in taste buds. The most complete evidence to date implicates serotonin as a neuromodulator of taste transduction in the end organs. However, studies also suggest that adrenergic, cholinergic and peptidergic neurotransmission may be involved in taste buds. **Chem. Senses** 21: 353–365, 1996.

Introduction

Chemosensory reception in gustatory end organs, i.e. taste buds, involves several steps. These steps include:

- (i) transduction of a sapid stimulus into receptor currents, usually at the apical, chemosensitive membrane tips of receptor cells;
- (ii) propagation of receptor currents throughout the taste cell;
- (iii) synaptic transmission of the electrical signal to other taste cells and to sensory afferent axons.

Neuroactive substances found in taste buds can potentially influence each of these steps. The last step, synaptic transmis-

sion, most certainly is mediated, in part, by neuroactive compounds, i.e. by chemical transmitters. Neuromodulation of any of these three steps raises the possibility for peripheral signal processing in taste buds. The topic of this review is what is currently known about neuroactive substances in taste buds—which substances are present; how do they modulate taste transduction; and where do they act.

First, it is important to understand what is intended by the term, neuroactive substances. For our purposes, these are endogenous chemicals that alter the electrical properties of taste cells and thus regulate signalling in taste buds. These

substances include neurotransmitters and neuromodulators.

The distinction between a neurotransmitter and a neuromodulator is difficult to define precisely. Neurotransmitters act over a period of milliseconds. Neurotransmitters are released at chemical synapses, usually in a Ca-dependent manner, reach their peak post-synaptic actions in a few ms and then subsequently disappear from the synaptic cleft within 10's of ms. The action of a neurotransmitter consequently is phasic or pulsatile. Neurotransmitters elicit a direct effect on the post-synaptic membrane conductance. Under most conditions, neurotransmitters produce post-synaptic potentials and transmembrane currents in the target cell. (If the reversal potential for the post-synaptic response is at or near the resting potential of the cell, there will be no post-synaptic potential change.) In contrast, neuromodulators act more sluggishly over a period of 100's of ms to s and their actions may be somewhat indirect. For example, neuromodulators may alter the properties of ion channels that are activated by neurotransmitters, such as channel open probabilities or receptor desensitization rates. Thus, the actions of a neuromodulator may be invisible until and unless chemical neurotransmission is in progress. Neuromodulators often act through second messenger systems. The prolonged time course of their action reflects the activation of a cascade of intracellular enzymes and biochemical changes inside the target cell. Neuromodulators may be released by presynaptic terminals, perhaps even co-released with neurotransmitters. Alternatively, neuromodulators may be released by nearby cells and diffuse to local target cells (parahormones) or by sources at some distance, and reach the post-synaptic, reactive sites via the circulatory system (hormones).

The identification of a particular neuroactive substance as a neurotransmitter traditionally has depended upon certain obligatory criteria. These criteria include:

- (i) **localization**: the substance is present in the presynaptic cell;
- (ii) **uptake/degradation**: a specific mechanism exists for removing the substance from its site of action (e.g. synaptic cleft) and terminating transmission;
- (iii) **release**: the substance is released in amounts sufficient to exert post-synaptic actions;
- (iv) **physiological actions**: when applied exogenously at a physiologically meaningful concentration, the neuroactive substance mimics action of endogenously released neurotransmitter.

Another criterion, that the substance is synthesized by the presynaptic cells, is often included. These criteria can also be applied to identifying neuromodulators.

In the case of taste buds, no neurotransmitters have yet been identified with confidence. The best evidence to date, reviewed below, is for serotonin as a neuromodulator of taste reception. Data supporting other candidate neurotransmitters and neuromodulators are less compelling. The findings for serotonin, catecholamines, amino acids, neuropeptides and acetylcholine are summarized in the following paragraphs, following the criteria for identifying neurotransmitters, listed above. Figure 1 summarizes sites for synaptic transmitters in taste buds.

Serotonin in taste buds

Localization

Early studies on potential neuroactive substances in taste buds quickly identified biogenic amines in these peripheral gustatory organs. Initial histofluorescence techniques implicated catecholamines and monoamines. Subsequent refinements of the histofluorescence methodology in taste buds focused attention on serotonin (see references in Roper, 1992; Kim and Roper, 1995). Highly selective immunocytochemical detection methods have confirmed the presence of serotonergic cells in taste buds in amphibia and mammals

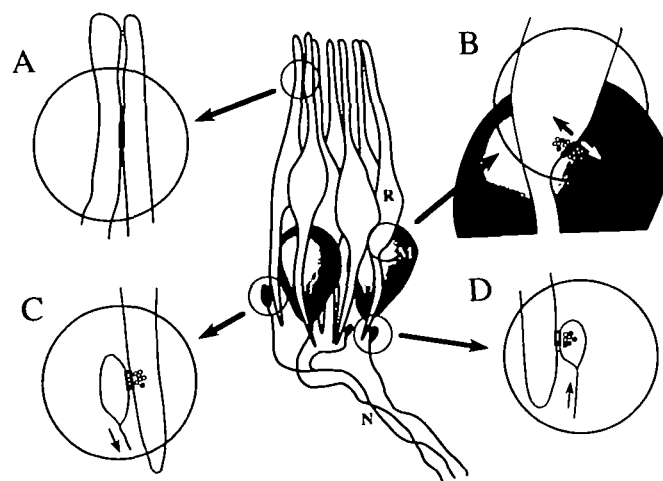


Figure 1 Schematic drawing of sites for electrical and chemical synaptic connections in taste buds. This diagram depicts taste cells from the amphibian, *Necturus maculosus*. A group of receptor cells (open, R) and Merkel-like basal cells (shaded, M) are shown. (A) Adjacent taste cells are electrically coupled, presumably through gap junctions. (B) Receptor cells have been shown to form synapses with serotonergic Merkel-like basal cells. These may be reciprocal synapses, as shown here. Merkel-like basal cells do not occur in mammalian taste buds. Other cell types may perform their function in taste buds from mammals. (C) Receptor cells form chemical synapses with afferent sensory axons. (D) Putative efferent fibers may form synapses with taste bud cells. Evidence for these synapses is reviewed in the text and in Roper (1992). Abbreviations: R, receptor cells; M, Merkel-like basal cells; N, nerve fibers.

(Uchida, 1985; Fujimoto *et al.*, 1987; Kuramoto, 1988; Kim and Roper, 1995). In amphibian species, Merkel-like basal cells are immunopositive for serotonin. Six to 20 serotonergic Merkel-like cells form a peripheral ring around the base of taste buds (Kuramoto, 1988; Sbarbati, *et al.*, 1989; Kim and Roper, 1995). In taste buds from mammals, serotonergic cells in the taste bud resemble elongate receptor cells instead of basal cells. Serotonin-rich cells in mammalian taste buds tend to predominate at the periphery, like staves of a barrel, although this localization is not as distinctive as in amphibian taste buds (Kim and Roper, 1995). The serotonin-rich taste cells in mammalian taste buds may be Type III cells (Takeda and Kitao, 1980; Takeda and Suzuki, 1983; Uchida, 1985; Kim and Roper, 1995). Biochemical studies, using HPLC, have confirmed the presence of serotonin in lingual tissues bearing taste buds (Zancanaro *et al.*, 1995). Figure 2 summarizes the localization of serotonin in taste bud cells in amphibian and mammalian species.

Uptake/degradation

Serotonin is a well-established neurotransmitter in the central nervous system. Powerful high-affinity uptake mechanisms clear the synaptic cleft of the monoamine to help terminate its actions, as also happens for catecholamine and amino acid neurotransmitters (Fuller and Wong, 1977, 1990; Nicholls and Attwell, 1990; Trendelenburg, 1991). We have tested for high affinity serotonin uptake mechanisms in taste buds to determine whether analogous transporters exist there, as well (Nagai *et al.*, 1994, in preparation). We bathed lingual epithelium dissected from the amphibian, *Necturus*

maculosus, in Ringer solution containing ^3H -serotonin for brief periods. Subsequently, we fixed the tissues and used autoradiographic techniques to determine which, if any, cells in the epithelium transported serotonin. Our data were definitive. Merkel-like basal cells in the taste buds selectively took up the monoamine. These cells were heavily labeled with silver grains in the autoradiographic preparations. No other cells in the epithelium were similarly labeled in non-taste epithelium except for cutaneous Merkel cells. Monoamine uptake into *Necturus* Merkel-like taste cells was prevented if low concentrations of imipramine, a potent blocker of high affinity serotonergic uptake in the CNS, were present in the bath along with ^3H -serotonin.

Release

The uptake of serotonin into Merkel-like basal cells in *Necturus* taste buds allowed us to investigate whether this monoamine was released by depolarization, an important criterion for identifying neurotransmitters or neuromodulators. We modeled our experiments on transmitter studies in other nervous tissues (e.g. O'Malley and Masland, 1989). Namely, bathing a preparation in a solution that contains elevated potassium depolarizes cells and stimulates them to release accumulated neurotransmitter. In our study, we incubated pieces of freshly dissected lingual epithelium in ^3H -serotonin, as before. Then, before fixing the tissue for autoradiography, we briefly rinsed the living tissue in amphibian Ringer that contained 40 mM KCl. After this treatment, Merkel-like basal cells were not as heavily labeled as before. Quantitative autoradiography indicated that the accumulation of ^3H -serotonin was reduced by about half that of control samples. That is, depolarizing the tissue with KCl released ^3H -serotonin.

Furthermore, this depolarization-induced release of ^3H -serotonin was dependent on calcium in the bathing medium. Replacing [Ca] with [Mg] in the incubation solutions blocked K-stimulated release. Ca-dependent transmitter release is a *sine qua non* of conventional chemical synapses. The release of serotonin from Merkel-like basal taste cells appears to be no exception.

Physiological actions

Researchers have tested the physiological actions of serotonin in taste buds in a variety of experimental situations. An early study by Morimoto and Sato (1977) reported that perfusing the frog tongue with serotonin increased spontaneous activity in the glossopharyngeal nerve. However, these same treatments depressed the responses to

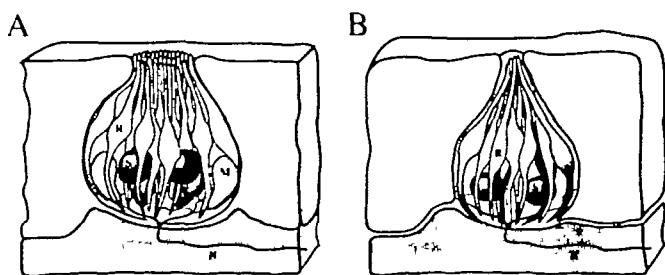


Figure 2 Illustration of serotonergic and other cells in taste buds. **(A)** Taste bud from the amphibian, *Necturus maculosus*. In amphibian species, Merkel-like basal cells (light shading, M) contain serotonin and are distributed towards the periphery at the base of the taste bud. Synapses have not been seen on stem cells (dark shading, S). **(B)** Taste bud from a mammal. Mammalian taste buds do not possess Merkel-like basal cells. Instead, taste cells that contain serotonin (light shading) in the mammalian taste bud closely resemble receptor cells (R). Serotonergic cells in mammalian taste buds tend to be distributed towards the periphery, as shown here. Abbreviations: R, receptor cells; M, Merkel-like basal cells; S, stem cells; N, nerve fibers

taste stimuli. Conflicting results emerged when serotonergic antagonists and 5HT-depleting agents were perfused through the lingual vasculature. Namely, when the 5HT content of taste buds was depleted, taste responses were hardly affected. Morimoto and Sato (1977) concluded that a 'catecholamine is a more likely candidate for a chemical neurotransmitter in frog taste organ than 5HT'. Esakov and his colleagues (Esakov *et al.*, 1983) conducted similar experiments, but observed a different outcome. Namely, they showed that injecting 5HT subepithelially into the tongue in frogs also increased spontaneous activity from the glossopharyngeal nerve. However in their experiments, this produced heightened taste responses, especially to glucose, but also to NaCl, quinine and HCl. Esakov *et al.* (1983) were aware that their findings conflicted with those of Morimoto and Sato (1977). Esakov *et al.* (1983) stated that a long delay (20–60 min) was required between injecting 5HT and observing an enhanced taste response. This may have prevented Morimoto and Sato from recording any effects, i.e. the Japanese workers had not waited long enough. Esakov *et al.* (1983) interpreted this delay as representing the time for taste bud cells to take up 5HT from the injection site, store it in vesicles and then release it during taste stimulation. Thus, according to the Russian group, exogenous 5HT is effective by virtue of its uptake and presumed subsequent release from serotonergic taste bud cells.

More direct tests of the actions of 5HT at the cellular level have recently been performed on isolated tissues, including lingual slices and isolated taste cells from *Necturus*. These studies show that serotonin has multiple actions on taste bud cells. When applied directly onto taste receptor cells by bathing lingual slices in 100 mM 5HT, the monoamine increases input resistance and hyperpolarizes the membrane (Ewald and Roper, 1994a, b). Serotonin also acts on voltage-gated Ca^{2+} currents in taste cells (Delay *et al.*, 1994, submitted). These currents are believed to be involved in transmitter release. In about half of the taste cells that were tested, serotonin depressed I_{Ca} . In the other half, serotonin augmented I_{Ca} . Two different classes of 5HT receptors appeared to mediate these opposing actions. The dual actions of serotonin on I_{Ca} involved second messenger mechanisms, implying that 5HT₃ receptors were not involved. The evidence pointed to 5HT₁-like receptor mechanisms.

Some of the aforementioned responses to exogenously applied serotonin may mimic the actions of endogenously-released transmitter. Ewald and Roper (1994a) stimulated *Necturus* Merkel-like basal taste cells by passing current through intracellular micro-electrodes, while simultaneously

recording responses elicited in neighboring receptor cells. Excitation of Merkel-like basal cells elicited a slow hyperpolarization and increased input resistance in receptor cells in the taste bud immediately adjacent to the stimulated cell. These responses closely resembled the effects obtained by perfusing the tissue with serotonin.

In summary, serotonin is found in certain cells in vertebrate taste buds. In amphibia, Merkel-like basal taste cells are serotonergic. In mammals, elongate cells, possibly Type III cells, contain serotonin. Merkel-like basal taste cells selectively take up serotonin by way of a high affinity transporter and release the monoamine in a Ca-dependent fashion when depolarized. Serotonin acts on taste cells at physiological concentrations and some of these actions mimic the effects produced by endogenous transmitter. 5HT has a dual effect on Ca currents in taste receptor cells. Collectively, these findings strongly implicate serotonin as a neuromodulator of taste cell function. Presumably, serotonin augments responses to chemical stimulation in some cells and depresses it in other cells.

What remains to be determined is how does serotonin affect taste cell responses in the intact tissue. Does 5HT modulate taste reception? What triggers serotonergic taste bud cells to release this neuromodulator?

Several therapeutic drugs have side-effects that include taste disturbances. Interestingly, many of these drugs have one fact in common: they act on serotonergic mechanisms. For example, antidepressants such as imipramine, clomipramine and fluoxetine (Prozac) are potent blockers of serotonin reuptake. These antidepressants are often associated with dysgeusia and nausea (Deems *et al.*, 1991; Finley, 1994; Henkin, 1994; Arky, 1995, pp. 596–599, 943–947). Lithium salts, used to treat mania, have a side-effect of producing unpleasant taste (reviewed by Mott and Leopold, 1991). Lithium is also known to enhance serotonergic neurotransmission and alter the concentration of serotonin in the brain (e.g. Otero and Rubio, 1992; Power *et al.*, 1993; Aulakh *et al.*, 1994; Williams and Jope, 1994). Sumatriptan, an antimigraine medication, has an adverse side-effect of bad or bitter taste (reviewed by Brown *et al.*, 1991; Mott and Leopold, 1991; Salonen *et al.*, 1994). Sumatriptan is a 5HT₁ receptor agonist (Saxena and Ferrari, 1992). Similarly, buspirone, an anti-anxiety drug and another 5HT₁ receptor agonist, alters taste and palatability in humans and animals (Treit and Berridge, 1990; Arky, 1995). The well-known β -adrenergic antagonist, propranolol, is used as an anti-hypertensive agent and is associated with adverse taste responses (Griffin, 1992). Propranolol, curiously, is also a

potent 5HT₁ receptor antagonist (Pierson *et al.*, 1989). Even the antidiuretic-diuretic drug amiloride, a well-established Na-channel blocker that is associated with bad taste or a loss of salt taste (Mott and Leopold, 1991; Arky, 1995, pp. 1591–1593) is also a monoamine oxidase (MAO) blocker (Palaty, 1985). MAO is the catabolic enzyme for serotonin. Another, unrelated agent, levamisole, an immunomodulator, produces a metallic taste (Mutch and Hutson, 1991; reviewed by Griffin, 1992; Arky, 1995). Levamisole also blocks monoamine oxidase (Vanhoutte *et al.*, 1977) and thus might be expected to alter serotonergic mechanisms where are present.

Admittedly, the above correlation between certain serotonergic drugs and adverse side-effects on taste may be forced and artificial. The taste disturbances might well be epiphenomena and secondary to xerostomia (dry mouth), dysphagia, gastroesophageal reflux, or nausea, all of which are well-known adverse effects of many of these drugs. Alternatively, the alteration in taste may be secondary to the drugs' CNS actions. Moreover, the taste alterations may be caused by non-specific actions of the drugs on receptor mechanisms other than serotonergic ones, such as cholinergic neurotransmission. Nonetheless, it is intriguing that such a diverse array of drugs with adverse taste effects do have a property in common, namely altering serotonergic mechanisms. It may not be so far-fetched that the side-effects in taste are related to these drugs' peripheral actions on serotonergic synapses in taste buds.

Apart from acting as a neuromodulator, as discussed above, serotonin has been suggested as functioning during morphogenesis of the taste bud (Toyoshima, 1994). This would not preclude serotonin from also acting as a *bona fide* neurotransmitter/neuromodulator, of course.

Norepinephrine, epinephrine and dopamine in taste buds

Localization

Biogenic amines other than serotonin are present in taste buds and may play some role in neurotransmission or neuromodulation. However, the evidence for such roles is somewhat limited at present. Histofluorescence studies initially led investigators to believe that norepinephrine was present in certain taste cells and axons innervating taste buds in rabbits and frogs (Gabella, 1969; DeHan and Graziadei, 1973; Savushkina *et al.*, 1974). Later studies that used a refined histofluorescence technique implicated the

presence of serotonin rather than norepinephrine (at least for cells in taste buds from rabbits; Nada and Hirata, 1975). However, more recent immunocytochemical data confirm that norepinephrine and dopamine are also found in taste cells from rats and mice (D-J. Kim and S.D. Roper, submitted). There is general consensus that adrenergic nerve fibers surround taste buds (perigemmal fibers) (e.g. references in Paparelli *et al.*, 1986; Welton *et al.*, 1992). The density of these fibers varies considerably from species to species. The adrenergic nerve fibers appear to be branches from perivascular axons.

Uptake/degradation and release

No specific information is available on these biological processes for catecholamines in taste buds.

Physiological actions

There are few physiological studies at the cellular level on the role of catecholamines in taste buds. Stimulating the sympathetic nerve supply to the tongue was shown to enhance taste responses recorded in the chorda tympani nerve in rats (Kimura, 1961) and glossopharyngeal nerve in frogs (Chernetski, 1964). Furthermore, systemic administration of epinephrine (intravenous injections) in rats was claimed to increase taste responses in the chorda tympani nerve (Kimura, 1961). Findings from Esakov's laboratory in Russia appear to contradict these observations. Esakov and Serova (1980) reported that in rats, propranolol blocks the putative efferent inhibition of taste buds that gastric distension induces (Esakov, 1961). Sympathectomy decreased the efferent inhibition in rats. Additionally, they found that injecting the sympathomimetic, isoproterenol, subepithelially into the tongue suppressed salt and sour responses in the rat. Esakov and Serova (1980) interpreted these findings as suggesting the existence of an inhibitory adrenergic (efferent) supply to taste buds. To date, there has been no explanation or resolution of these contradictory findings.

In an attempt to apply drugs more focally onto taste buds, Morimoto and Sato (1982), and Nagahama and Kurihara (1985) perfused the lingual artery of frog tongues with 50–100 mM norepinephrine. Both groups reported that this treatment enhanced glossopharyngeal nerve taste responses elicited by a variety of chemical stimuli. Perfusing adrenaline also enhanced taste responses, but dopamine was without effect. All these studies concluded that taste cells had taken up norepinephrine and released it as a neurotransmitter. Delay *et al.* (1994, submitted) reported that serotonin, but

not norepinephrine and dopamine, modulated ion conductances in *Necturus* taste buds. These findings speak against adrenergic neuromodulation of receptor cells, themselves, but do not rule out the possibility that norepinephrine is released by taste cells as a transmitter onto sensory axons.

An alternate physiological role for catecholamines has been proposed. Paparelli *et al.* (1986) suggested that adrenergic influences on taste responses may be secondary to changes in the papillary blood circulation. For example, noradrenaline could stimulate the local vasculature and influence the turgescence of the papillae that contain taste buds.

All the above findings are consistent with a role for norepinephrine in taste buds, but the detailed sites and mechanisms of action remain to be elucidated. Considering all the criteria for identifying neurotransmitters/neuromodulators (see Introduction), the catecholamines fall short. Nonetheless, the physiological actions of noradrenaline and adrenaline are intriguing, and warrant future, more detailed and exhaustive studies on the roles of these putative neurotransmitters/neuromodulators in taste buds.

Amino acids: GABA and glutamate in taste buds

Localization

γ -Amino butyric acid (GABA) and glutamate are potent neurotransmitters in the vertebrate CNS. Glutamate is a ubiquitous excitatory transmitter in the brain and underlies rapid synaptic transmission, long-term plasticity and excitatory neurotoxicity. GABA is an inhibitory neurotransmitter. GABA and glutamate both are found in taste buds. Immunocytochemical studies by Jain and Roper (1991) identified nerve fibers innervating *Necturus* taste buds contained GABA and glutamate.

Uptake/degradation

We have conducted uptake studies on radiolabeled glutamate and GABA in an attempt to test this criterion for neurotransmitter identification (Nagai *et al.*, 1994, in preparation). These studies conclusively showed that a broad variety of cells in the lingual epithelium of *Necturus* transported ^3H -GABA and ^3H -glutamate. The most intense uptake appeared in glial cells that accompanied nerve fibers in the lamina propria. This is consistent with reports that glial cells in the brain also avidly transport GABA and glutamate (e.g. Krnjevic, 1984; Reynolds and Herschkowitz, 1984, 1986; Radian, *et al.*, 1990; Torgner and Kvamme, 1990). Some

taste bud cells that resembled receptor cells accumulated ^3H -glutamate, but not ^3H -GABA. However, experiments failed to demonstrate that these taste cells released ^3H -glutamate when stimulated (see below). Epithelial cells, especially those in the stratum basale, also transported ^3H -GABA and ^3H -glutamate. Curiously, goblet cells in the lingual epithelium and taste bud cells were specifically devoid of ^3H -GABA or ^3H -glutamate accumulation. This pattern of radiolabeled GABA and glutamate uptake was not particularly revealing. The pattern of glutamate and GABA uptake in the lingual epithelium was more consistent with transport of metabolically important amino acids into a wide variety of cells and not of a neurotransmitter into taste bud cells.

Release

Using the same protocol as had been used to demonstrate serotonin release from taste cells, we found that depolarizing the tissue with 40 mM KCl had no effect on GABA or glutamate uptake. Depolarization did not release ^3H -glutamate or ^3H -GABA that had been accumulated. This was in marked contrast to serotonin uptake/release from Merkel-like basal taste cells.

Physiological actions

The effects of amino acids on taste bud cells have mainly focused on glutamate and other essential amino acids as taste stimuli. Tateda and Beidler (1964) only briefly mention the effects of GABA on taste buds. These workers reported that 100 mM GABA, applied topically to the frog tongue, inhibited the glossopharyngeal nerve response to NaCl. These investigators concluded that GABA had abolished synaptic transmission between taste receptor cells and sensory axons. No follow-up studies on the effects of GABA on taste bud function have been reported, to our knowledge.

The actions of glutamate when applied to exposed taste buds do not mimic any known synaptic actions in taste buds. Namely, high concentrations (1–100 mM) are required to see any effect and the effects are not yet well-defined (Sugimoto, 1994; Bigiani *et al.*, 1995; Hayashi *et al.*, 1995). When bath-applied to taste cells in a recording chamber, glutamate elicits membrane potential changes. This could be due to a reduction in Cl conductance, a decrease of a non-selective cation conductance, an increase in intracellular Ca^{2+} or some combination of these actions. Known glutamatergic synapses are activated by μM concentrations of glutamate, not mM. The effects of exogenously applied glutamate are more likely explained by its action as a taste

stimulus, not as a neurotransmitter. Behavioral thresholds for glutamate taste are 1–10 mM for a number of animal species, including humans. This is consistent with the preliminary reports of glutamate effects in taste cells, cited above.

One might argue that glutamate is not a good candidate for neurotransmission in taste buds in any case. Gustatory end organs are relatively well exposed to plasma constituents, including circulating levels of free glutamate. At sites where glutamatergic synaptic transmission occurs, for example, in the brain and the retina, there are powerful blood/tissue barriers for this amino acid (Hutchison *et al.*, 1985; Salceda and Saldana, 1993; Fernstrom, 1994). These barriers protect the sensitive synapses from glutamate circulating in the bloodstream. Free glutamate in the circulation can reach 250 μM under normal conditions (e.g. Table 18–2 in Henry *et al.*, 1974). Blood/tissue barriers in the brain and retina keep the interstitial concentration for this potent neuroactive substance at about 1 μM near the synapses. Known glutamatergic synapses have EC₅₀'s for glutamate from 20–50 μM and concentrations near 100 μM are neurotoxic. In contrast to the brain and retina, taste buds are freely accessible to substances in the bloodstream. This is evidenced, for example, by the phenomenon of intravascular taste (Bradley, 1973); namely, chemicals injected in the bloodstream are readily tasted by virtue of their access to the basolateral membranes of taste bud cells. Consequently, it is reasonable that glutamatergic synapses in taste buds probably do not exist. They would be exposed to considerable concentrations of glutamate in the bloodstream.

Taken together, the existing data do not especially support a role for GABA or glutamate in synaptic transmission in taste buds. Although both amino acids are present in some axons, exogenous application (at least in the case of glutamate) does not evoke responses at concentrations consistent with glutamatergic transmission elsewhere. Furthermore, uptake and release studies did not implicate either of the amino acids as transmitters. The presence of glutamate and GABA in axons that innervate taste buds may reflect that these axons are the peripheral endings of sensory cells that use glutamate and GABA at their central synapses.

Peptides in taste buds

Localization

Using immunocytochemical techniques, investigators have shown that a host of peptides are present in taste bud cells and axons. These include substance P, VIP, CGRP, CCK

and several other peptides (e.g. see Table 3 in Welton *et al.*, 1992). Of these, only CCK and VIP appear to be found in taste bud cells. Peptides are mainly present in axons that innervate taste buds.

Uptake/degradation and release

No information is available for uptake, release or catabolism of neuropeptides in taste bud cells to our knowledge.

Physiological actions

Researchers have studied peptidergic neurotransmission in taste buds by using animal behavioral experiments and by recording afferent nerve activity. Silver *et al.* (1985) reported that depleting the peptidergic innervation of taste buds in rats caused a marginal increase on the rats' intake of quinine solutions. The loss of peptidergic axons to taste buds did not seem to affect the rats' intake of salty or sour solutions. Serova and Esakov (1985) injected CCK i.p. in rats and noted that taste responses (recorded in the chorda tympani nerve) to sweet, salty and bitter stimuli, but not sour, were augmented over the subsequent 30 s to 5 min. These investigators later tested the effects of another neuropeptide, substance P. Injecting substance P subepithelially into the rat tongue increased chorda tympani responses to NaCl and citric acid. Substance P did not affect responses to sucrose nor quinine (Esakov and Serova, 1988). In this study they showed that water-deprived rats injected subepithelially (tongue) with substance P drank less NaCl solution than control rats. No changes on the consumption of sucrose solutions were noted after injecting substance P. More recently, Wang *et al.* (1995) reported that stimulating a peptidergic nerve supply to taste buds in rats decreased chorda tympani responses to 100 mM NaCl stimulation.

It is difficult to identify a common theme in the above regarding the physiological actions of neuropeptides on taste transduction. At best, the data suggest some form of peptidergic modulation of taste sensitivity. However, which peptide(s) are responsible and what are the membrane mechanisms at the level of the receptor cell remain unanswered questions. Finger (1986) speculated that capsaicin (e.g. during consumption of spicy foods) stimulates peptidergic mucosal sensory fibers that arborize in the lingual epithelium near taste buds. Excitation of these fibers would transmit action potentials orthodromically into the CNS, as well as release peptides locally onto taste buds via synaptic release from antidromic excitation of axon collaterals. The peptides would modulate taste transduction.

Alternative explanations for the role of neuropeptides

include that they are involved in neurotrophic influences instead of (or in addition to) neuromodulation. This has been suggested for substance P and CGRP in taste buds (e.g. Lundberg *et al.*, 1979; Yamasaki *et al.*, 1984; Solovyeva and Esakov, 1984, 1986; Silverman and Kruger, 1989; Ichikawa *et al.*, 1990; Kinnman and Aldskogius, 1991). Alternatively, peptides may only indirectly influence taste reception through their modulation of salivary secretions (Silverman and Kruger, 1989).

Lastly, one might speculate that peptidergic transmission, if it occurs in taste buds, modulates the actions of other neurotransmitters. For example, one or more neuropeptides could be co-released with acetylcholine at synapses in taste buds (see below). VIP and CGRP regulate cholinergic transmission at nicotinic and muscarinic cholinergic synapses in other tissues (e.g. Miles *et al.*, 1989; Haganir and Greengard, 1990; Kim, 1991; Lu *et al.*, 1993; Gurantz *et al.*, 1994).

Acetylcholine in taste buds

Localization

Acetylcholine (ACh) has been the focus of several studies on neurotransmission in taste buds. Early experiments were aimed at biochemical localization of ACh in lingual epithelium-bearing taste buds, compared with non-taste epithelium. These studies indicated that ACh was present at about three-fold higher concentration in foliate papillae of rabbits relative to other, non-taste regions of lingual epithelium (Brucke *et al.*, 1948). The ACh content of foliate papillae decreased by about one-third after sectioning the glossopharyngeal nerves with no changes in ACh concentration occurring in non-taste epithelium after this denervation. Indirect localization of cholinergic mechanisms in taste buds was furthered by observations that acetylcholinesterase (AChase), the enzyme responsible for degrading the transmitter at known cholinergic synapses, is present in high concentration in taste buds (see references in Welton *et al.*, 1992). This evidence, however, is not compelling for ACh being a neurotransmitter at these sites; the mere presence of AChase is not a reliable indicator of cholinergic neurotransmission. However, more recent studies, using immunocytochemical localization of choline acetyltransferase (ChAT), a key biosynthetic enzyme for ACh, have revealed the transmitter in taste bud cells and axons innervating taste buds in rats and mice (Kim and Roper, 1994). Cholinergic axons appear to originate, in part, from nearby autonomic neurons that form intrinsic ganglia at the base of the vallate

papillae (Graziadei and Graziadei, 1978; Ferrell and Tsuetaki, 1983). If so, this ganglion may be a source of putative efferent autonomic regulation, as suggested by certain physiological actions of ACh (see below).

Uptake/degradation

Cholinergic neurotransmission, unlike signalling at adrenergic or monoaminergic synapses, is terminated by extracellular degradation of the transmitter by a potent enzyme. As mentioned above, the enzyme responsible for degrading and terminating synaptic actions of ACh at synapses, AChase, is abundant in taste buds. However, blocking ACh degradation with anticholinesterases has variable effects on taste reception, summarized below.

Release

Rapuzzi and his colleagues published an abstract stating that they were able to detect ACh (10^{-10} to 10^{-7} M) in a perfusate from frog tongues when they stimulated the tongue with calcium salts (Rapuzzi and Ricagno, 1965). They did not detect ACh in the perfusate from unstimulated tongues. These experiments have not been repeated to the best of our knowledge and few details were presented in the abstract. Nonetheless, the data are very intriguing.

Physiological actions

A rich literature exists on the physiological actions of ACh in taste buds. However, the findings are divided, especially in the older literature. The earliest attempt to study neurotransmission in taste buds is that of Zotterman (1944) who reported that injecting cholinergic agonists into the lingual artery in cats did not stimulate gustatory sensory afferents. Subsequently, Zotterman and his colleagues tested the effects of cholinergic agents applied topically onto the frog tongue (Landgren *et al.*, 1952, 1954; see also Ozeki and Noma, 1972). These results showed that topical ACh enhanced spontaneous activity recorded in the glossopharyngeal nerve, nerve activity elicited by chemosensory stimulation and receptor potentials recorded intracellularly from taste receptor cells. Similar results were obtained by topically applying drugs that inhibit cholinesterases in an attempt to increase ACh at synaptic sites in the taste bud. Conversely, topically applied curare inhibited glossopharyngeal nerve responses. To achieve better penetration into taste buds, Sakai (1965b) applied ACh dissolved in 10% propylene glycol onto frog tongues. The same results were obtained, namely, topically-applied ACh (in propylene glycol) increased glossopharyngeal nerve responses to a variety of taste stimuli. Sakai

(1964) also applied AChE, itself, directly onto the frog tongue, to reduce ACh in the taste bud. He reported that this pretreatment reduced glossopharyngeal responses to subsequent chemosensory stimulation with bitter-tasting substances. Sakai's findings corroborated those of Landgren *et al.* (1954). Similar studies have also been conducted on rats. Pretreating the rat tongue with AChE inhibitors selectively doubled the amplitude of chorda tympani nerve responses elicited by sour and salty taste stimuli, without affecting sweet or bitter taste (Sakai, 1965a). Topical application of ACh or of AChase inhibitors even onto human tongues was reported to decrease the threshold for salty and acid taste (Sakai, 1965a) or of acid taste sensations elicited by anodal stimulation (Skouby and Zilstorff-Pedersen, 1955). Yet, other studies found that applying either ACh or methacholine, a muscarinic agonist, to the tongue in humans did not normally alter taste sensitivity (Henkin and Kopin, 1964). [These agonists did, however, improve taste responses when injected intravenously or subcutaneously to patients suffering from familial dysautonomia, a congenital disorder characterized by decreased taste (Henkin and Kopin, 1964).] In summary, the above data suggest that ACh (and agents like anticholinesterases that would increase ACh at synaptic sites) enhances taste responses. The implication, drawn by most of these investigators, is that ACh is a neurotransmitter at synapses between taste receptor cells and sensory axons.

A critical problem with the above investigations, of course, is that the route of drug administration (mostly topical applications) was very indirect. Furthermore, the taste of the agent, itself, may confound the results from topical drug application. The data may have little bearing on the putative role of ACh as a neurotransmitter at synapses buried deep in the tissue. Nevertheless, other routes of drug administration have been tried. Intravenous injection (femoral vein) of acetylcholine in rats was claimed to increase taste responses recorded from the chorda tympani nerve when gustatory stimuli were subsequently applied to the tongue (Kimura, 1961). Neither intravenous eserine nor atropine affected taste responses in these experiments. In an attempt to achieve more localized application, Duncan (1964) injected anticholinesterases directly into the lingual artery in frogs and monitored glossopharyngeal nerve activity during gustatory stimulation. He was unable to replicate any of the enhancing effects on taste claimed by earlier investigators. Furthermore, perfusing curare, hexamethonium or decamethonium also failed to affect taste sensitivity. Duncan (1964) concluded that cholinergic neurotransmission in taste buds was unlikely. Rappuzzi and his co-workers in

Pavia obtained different results. These investigators also perfused the arterial supply of frog tongues with cholinergic agents and measured glossopharyngeal nerve responses. For example, Rappuzzi and Ricagno (1964) reported that low (10–100 μM) concentrations of ACh, when perfused into the lingual artery, stimulated responses in the glossopharyngeal nerve. ACh at concentrations of 0.5 mM and above, however, depressed glossopharyngeal nerve activity elicited by stimulating the tongue with sapid solutions. Atropine blocked the action of ACh to stimulate spontaneous glossopharyngeal nerve activity. However, these experiments were complicated by the addition of ATP to the test solutions (Rappuzzi and Violante, 1968; Rappuzzi *et al.*, 1969).

In summary, no clear and convincing picture emerges from the early literature on the putative role of ACh as a neurotransmitter in taste buds. It is difficult to find a logical thread that binds all the results together and findings from one laboratory conflict with those from another.

More recently, using a biochemical approach, Hwang *et al.* (1990) reported that carbachol enhanced phosphatidylinositol turnover in rat taste cells. They suggested that efferent muscarinic synaptic inputs regulate taste transmission via second messenger mechanisms involving inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) production. Ewald and Roper (1994b) published an abstract describing preliminary findings that focally-applied ACh, and oxotremorine hyperpolarized *Necturus* receptor cells and decreased membrane Cl conductance. Focal application of nicotine had no similar effects. These results would be consistent with a muscarinic activation of taste cells, as suggested by the biochemical data and by the actions of atropine that were described in some of the earlier literature (above).

Summary

Based on well-accepted criteria for identifying synaptic transmitters and neuromodulators in nervous tissues, the best evidence to date is that serotonin is a neuromodulator. That is, 5HT is released from serotonergic neurons embedded in the taste bud and regulates the properties of adjacent receptor cells. Certain side-effects of drugs, especially the tricyclic antidepressants that block monoamine uptake, may be attributable, in part, to serotonergic mechanisms in taste buds. Detailed examinations of the localization and characterization of 5HT receptors in taste buds; what triggers serotonin release from taste cells and how serotonin influences peripheral mechanisms of taste in intact animals, would greatly strengthen this conclusion.

Acetylcholine remains a viable candidate for neurotransmission or neuromodulation in taste buds, especially at putative efferent muscarinic synapses. Major gaps in the characterization of cholinergic mechanisms remain, however, including information about uptake, release and physiological actions of this candidate transmitter.

Much the same can be said about adrenergic and peptidergic neurotransmission in taste buds, namely, existing data suggest adrenergic and peptidergic mechanisms. Yet, whether these substances act at synapses onto afferent sensory axons, at synapses between cells within the taste bud or at putative efferent synapses from sources outside

the taste bud is not known with any certainty. Additionally, peptides may be co-released with other neurotransmitters at synapses in taste buds and modulate synaptic transmission in this fashion.

Present data tend to exclude glutamate and GABA as viable candidates for neurotransmission or neuromodulation in taste buds.

Finally, although this essay has focused on the participation of neuroactive substances in synaptic transmission, *per se*, the role of any of the above candidates in neurotrophic processes within the taste bud cannot be ruled out (e.g. Lauder, 1993).

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