Mouse Taste Buds Release Serotonin in Response to Taste Stimuli

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Introduction

Cell-to-cell interactions and synaptic integration may occur within taste buds prior to signals being exported from these peripheral sensory organs to the CNS (Roper, 1992; Kaya et al., 2004). In principle, these interactions could include chemical and electrical synapses between taste cells and synapses from taste cells to sensory afferent fibers. Knowing which transmitters are released at synapses in taste buds would help clarify how signals are processed in taste buds but to date this information is lacking.

A number of neurotransmitter candidates have been proposed for taste bud synapses, including serotonin (5 hydroxytryptamine, 5HT), glutamate, acetylcholine, ATP, peptides and others, but none has been unambiguously identified (reviewed by Nagai et al., 1996). On balance, the preponderance of evidence suggests that 5HT is one of the transmitters. For example, 5HT is found in a subset of taste cells in a wide variety of species (Kim and Roper, 1995). Taste bud cells that possess synapses with nerve fibers take up the 5HT precursor (Takeda, 1977; Yee et al., 2001), and tryptophan hydroxylase, the enzyme that converts tryptophan to 5 hydroxytryptophan (5HTP), is found in taste buds (Cao et al., 2004). There is indirect evidence from autoradiographic studies that amphibian taste cells release 5HT when they are depolarized (Nagai et al., 1998). Pharmacological and molecular biological studies suggest that taste cells express 5HT1A-like receptors and primary afferent fibers possess 5HT3 receptors (Delay et al., 1997; Herness and Chen, 1997; Kaya et al., 2004). Yet despite all the above evidence, one of the canonical criteria for identifying synaptic neurotransmitters, namely detecting its release from stimulated synapses, has yet to be established for 5HT in taste buds. We have addressed this question by using biosensor cells that are sensitive to 5HT to reveal transmitter release from mouse taste buds during sensory stimulation. The results indicate that depolarizing mouse taste cells with KCl or stimulating them with sweet and bitter tastants elicits 5HT release. Collectively, these data firmly identify 5HT as a taste cell neurotransmitter.

Identifying stimulus-evoked release of serotonin from taste buds

Biosensor cells for detecting 5HT release consisted of Chinese hamster ovary (CHO) cells that stably express 5HT3 receptors (Berg et al., 1994). These cells, when loaded with the calcium-sensing dye Fura 2AM and imaged with fluorescent excitation, responded with a robust increase in [Ca2+] when stimulated with 5HT at concentrations ≤3 nM. Responses to 5HT were reversibly and reliably blocked by mianserin but stimulation of endogenous purinergic receptors with ATP was unaffected by mianserin. Biosensor CHO/5HT3e cells on their own did not generate a Ca2+ response when they were depolarized with KCl (50 mM), or stimulated with cycloheximide (a well-established aversive taste compound for rodents) or saccharin (a sweet tasting compound). Lastly, CHO/5HT3e cells maintained a response to 5HT even if Ca2+ in the medium was replaced with Mg2+, consistent with the coupling of 5HT3e receptors to intracellular Ca2+ release.

We removed taste buds from vallate papillae of the mouse tongue, transferred them to a recording chamber and manipulated individual CHO/5HT3e biosensor cells, preloaded with Fura 2, up against an isolated taste bud. Mere physical contact between a biosensor cell and a taste bud did not elicit a response. Nor did perfusion with Tyrode solution generate a biosensor response. However, perfusing the chamber with KCl, cycloheximide, or saccharin evoked rapid and repeatable responses from biosensor cells when they were apposed to a taste bud. Responses to these bath-applied stimuli were abolished if the biosensor cell was withdrawn even a few microns from an isolated taste bud, indicating that the taste bud was releasing a compound that triggered biosensor cell activity. Furthermore, biosensor cell responses to KCl, cycloheximide, and saccharin were reversibly blocked by mianserin, verifying 5HT as the compound released from taste buds.

Ca2+-dependence of serotonin release from stimulated taste bud cells

We tested whether the release of 5HT from taste buds was Ca2+-dependent. In the case of KCl depolarization, replacing bath Ca2+ with Mg2+ rapidly and reversely blocked 5HT release from taste buds, as detected with the CHO/5HT3e biosensor. Surprisingly, however, 5HT release elicited by cycloheximide or saccharin was not affected by replacing bath Ca2+ with Mg2+. Cycloheximide and saccharin are known to stimulate intracellular Ca2+ release in taste cells via a cascade of PLCb2 and IP3. Thus, a likely source of Ca2+ for transmitter release elicited by these compounds was an intracellular store. To test this, we isolated taste buds from PLCb2-null mutant mice (Jiang et al., 1997) and tested their ability to release 5HT following taste stimulation. Taste buds from PLCb2-null mice responded to bath-applied KCl, showing normal release of 5HT as above. However, we were unable to detect 5HT release evoked by cycloheximide or saccharin from taste buds of mutant mice.

Summary and conclusions

Collectively, our findings indicate that 5HT is one of the neurotransmitters released by taste cells in response to gustatory stimulation and to depolarization. The results suggest that whereas depolarization elicits Ca2+-dependent transmitter release from taste cells via Ca2+ influx, certain taste stimuli (namely, cycloheximide and saccharin) evoke transmitter release in response to Ca2+ from intracellular stores.

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References


