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# 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 $5HT_{1A}$-like receptors and primary afferent fibers possess $5HT_3$ 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 $5HT_{2c}$ 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 $[Ca^{2+}]_i$ 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/$5HT_{2c}$ cells on their own did not generate a $Ca^{2+}$ 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/$5HT_{2c}$ cells maintained a response to 5HT even if $Ca^{2+}$ in the medium was replaced with $Mg^{2+}$, consistent with the coupling of $5HT_{2c}$ receptors to intracellular $Ca^{2+}$ release.

We removed taste buds from vallate papillae of the mouse tongue, transferred them to a recording chamber and manipulated individual CHO/$5HT_{2c}$ 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.

## $Ca^{2+}$-dependence of serotonin release from stimulated taste bud cells

We tested whether the release of 5HT from taste buds was $Ca^{2+}$-dependent. In the case of KCl depolarization, replacing bath $Ca^{2+}$ with $Mg^{2+}$ rapidly and reversibly blocked 5HT release from taste buds, as detected with the CHO/$5HT_{2c}$ biosensor. Surprisingly, however, 5HT release elicited by cycloheximide or saccharin was *not* affected by replacing bath $Ca^{2+}$ with $Mg^{2+}$. Cycloheximide and saccharin are known to stimulate intracellular $Ca^{2+}$ release in taste cells via a cascade of PLCb2 and $IP_3$. Thus, a likely source of $Ca^{2+}$ for transmitter release elicited by these compounds was an intracellular store. To test this, we isolated taste buds from PLCβ2-null mutant mice (Jiang *et al.*, 1997) and tested their ability to release 5HT following taste stimulation. Taste buds from PLCβ2-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 $Ca^{2+}$-dependent transmitter release from taste cells via $Ca^{2+}$ influx, certain taste stimuli (namely, cycloheximide and saccharin) evoke transmitter release in response to $Ca^{2+}$ from intracellular stores.

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## References


**Berg, K.A., Clarke, W.P., Sailstad, C., Saltzman, A.** and **Maayani, S.** (1994) *Signal transduction differences between 5-hydroxytryptamine type 2A and type 2C receptor systems.* Mol. Pharmacol., 46, 477–484.

**Cao, J., Huang, L.** and **Brand, J.** (2004) *The neural isoform of tryptophan hydroxylase is localized to taste bud cells.* Abstract presented at 2004 Association of Chemoreception Sciences Conference, Sarasota.

**Delay, R.J., Kinnamon, S.C.** and **Roper, S.D.** (1997) *Serotonin modulates voltage-dependent calcium current in Necturus taste cells.* J. Neurophysiol., 77, 2515–2524.

**Herness, S.** and **Chen, Y.** (1997) *Serotonin inhibits calcium-activated $K^+$ current in rat taste receptor cells*. Neuroreport, 8, 3257–3261.

**Jiang, H., Kuang, Y., Wu, Y., Xie, W. Simon, M.I.** and **Wu, D.** (1997) *Roles of phospholipase C beta2 in chemoattractant-elicited responses.* Proc. Natl Acad. Sci. USA, 94, 7971–7975.

**Kaya, N., Shen, T., Lu, S.G., Zhao, F.L** and **Herness, S.** (2004) *A paracrine signaling role for serotonin in rat taste buds: expression and localization of serotonin receptor subtypes*. Am. J. Physiol. Regul. Integr. Comp. Physiol., 64, R649–R658.

**Kim, D.J.** and **Roper, S.D.** (1995) *Localization of serotonin in taste buds: a comparative study in four vertebrates*. J. Comp. Neurol., 353, 364–370.

**Nagai, T., Kim, D.J., Delay, R.J.** and **Roper, S.D.** (1996) *Neuromodulation of transduction and signal processing in the end organs of taste.* Chem. Senses., 21, 353–365.

**Nagai, T., Delay, R.J., Welton, J.** and **Roper, S.D.** (1998) *Uptake and release of neurotransmitter candidates, [$^3$H]serotonin, [$^3$H]glutamate, and [$^3$H]gamma-aminobutyric acid, in taste buds of the mudpuppy,* Necturus maculosus. J. Comp. Neurol., 392, 199–208.

**Roper, S.D.** (1992) *The microphysiology of peripheral taste organs.* J. Neurosci., 12, 1127–1134.

**Takeda, M.** (1977) *Uptake of 5-hydroxytryptophan by gustatory cells in the mouse taste bud*. Arch. Histol. Jpn., 40, 243–250.

**Yee, C.L., Yang, R., Bottger, B., Finger, T.E.** and **Kinnamon, J.C.** (2001) *'Type III' cells of rat taste buds: immunohistochemical and ultrastructural studies of neuron-specific enolase, protein gene product 9.5, and serotonin*. J. Comp. Neurol., 440, 97–108.