

## Communication Routes within the Taste Bud by Neurotransmitters and Neuropeptides

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### Introduction

Taste receptor cells (TRCs) are located throughout the oral cavity, sequestered into morphological structures known as taste buds. These differentiated epithelial cells relay the presence of tastants to the central nervous system via sensory afferent nerves. Only a minority of TRCs within the bud synapse with these nerve fibers. This anatomical finding has led to the dichotomization of TRCs into ‘true’ TRCs and supporting cells. However, this original conception—that a single TRC is excited by a tastant molecule and releases neurotransmitter onto its postsynaptic afferent nerve fiber—has been undermined by more recent physiological and molecular findings. For example, a single neurotransmitter, as might be expected from this scheme, has not been observed. Rather, there is evidence for at least five neurotransmitters within the mammalian taste bud: glutamate, serotonin, norepinephrine, acetyl choline and GABA. Additionally, TRCs expressing essential transduction molecules such as gustducin and members of the T2R family are not synaptically connected to the CNS. How then do TRCs equipped to respond to stimuli communicate with the central nervous system? The cloistering of TRCs into the precisely arranged structure of the bud may provide a clue. The bud, a highly conserved morphology of the vertebrate gustatory system, is an obvious substrate for cell to cell communication. Hence, alternative signaling pathways, such as paracrine communication, could explain this paradox. Rather than acting as arbitrarily collected detectors, TRCs within the bud may operate as a unit. Our investigations of neurotransmitters and neuropeptides in rat circumvallate and foliate TRCs have elucidated new pathways of communication among TRCs. Two, serotonin and cholecystokinin, are described here.

### Serotonin as a paracrine signaling agent in taste buds

One of the best studied neurotransmitters in the vertebrate taste bud is serotonin (5HT). Anatomical studies localize serotonergic TRCs to a subset of type III cells (the TRCs which form synapses with the afferent nerve); however, less is known of its physiological role(s). Since it is assumed to be one of the transmitters between the TRC and the afferent nerve, it was somewhat surprising when TRCs (rather than the afferent fiber) were demonstrated to be responsive to serotonin. In particular, calcium-activated potassium current and voltage-dependent sodium current were both inhibited by micromolar concentrations of 5HT (Herness and Chen, 1997, 2000). Pharmacological exploration suggested this effect to be mediated by 5HT<sub>1A</sub> receptors; 8OH-DPAT, a specific 5HT<sub>1A</sub> agonist, mimicked this effect, whereas phenylbiguanide, a 5HT<sub>3</sub> agonist, was without effect. To further explore the expression of serotonergic receptors in taste buds, an RT-PCR survey was conducted using gene specific primers against fourteen receptor subtypes (5HT<sub>1A</sub>, 5HT<sub>1B</sub>, 5HT<sub>1C</sub>, 5HT<sub>1D</sub>, 5HT<sub>1F</sub>, 5HT<sub>2A</sub>, 5HT<sub>2B</sub>, 5HT<sub>2C</sub>, 5HT<sub>3</sub>, 5HT<sub>4</sub>, 5HT<sub>5A</sub>, 5HT<sub>5B</sub>, 5HT<sub>6</sub>, 5HT<sub>7</sub>). The mRNA of two—5HT<sub>1A</sub> and 5HT<sub>3</sub>—was observed to be expressed in taste buds (Kaya *et al.*, 2004). Immunocytochemistry using 5HT, 5HT<sub>1A</sub>, or 5HT<sub>3</sub> specific antibodies was employed to examine the pattern of their cellular expression.

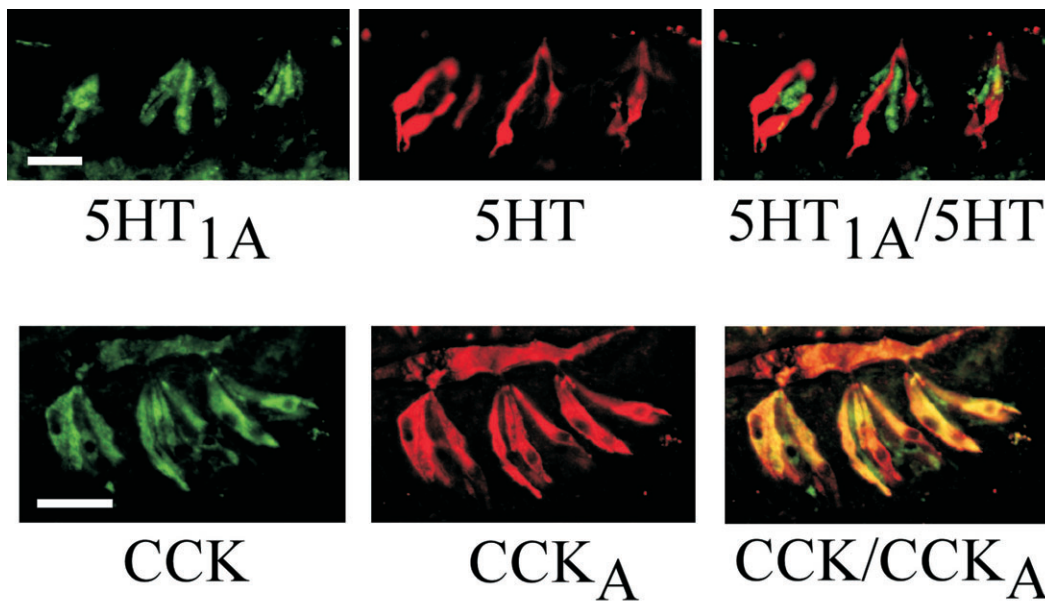
Whereas 5HT and 5HT<sub>1A</sub> immunoreactivity was observed in TRCs, 5HT<sub>3</sub> immunoreactivity was not. Instead, 5HT<sub>3</sub> immunoreactivity was observed in neural elements with the dermal core of the papillae, but not within the taste bud. These data are in agreement within other studies which have demonstrated a subset of 5HT<sub>3</sub> positive neurons in the petrosal ganglion, where the cell bodies of these afferent fibers are located (Wang *et al.*, 2002). On the other hand, using an antibody specific for 5HT<sub>1A</sub> receptors, a distinct subset of TRCs were labeled. Immunocytochemical double labeling experiments subsequently demonstrated that 5HT-expressing and 5HT<sub>1A</sub> receptor-expressing TRCs are non-overlapping populations (Figure 1, top panel), hence establishing a serotonergic paracrine pathway among TRCs of the bud.

Collectively, these observations suggest that serotonin plays a more complex role than originally imagined. We suggest there is local circuitry within the bud; release of serotonin may simultaneously act to excite the afferent nerve via 5HT<sub>3</sub> receptors while inhibiting a subpopulation of TRCs via the 5HT<sub>1A</sub> receptors.

### Cholecystokinin as a peptide signaling agent in the taste bud

In addition to classic ‘small molecule’ neurotransmitters, TRCs have more recently been demonstrated to express neuropeptides. These include vasoactive intestinal peptide (VIP; Herness, 1989), cholecystokinin (CCK; Herness *et al.*, 2002), neuropeptide Y (NPY; personal observations) and somatostatin (SST; personal observations). Peptides often co-localize with classic small molecule neurotransmitters and act, through specific peptide receptors, as neuromodulators that accentuate the effect of the classic neurotransmitter at higher stimulus intensities.

CCK, the best studied peptide in TRCs, is distributed in a subset of TRCs throughout the oral cavity (Herness *et al.*, 2002). Its expression, evidenced by immunocytochemistry, was verified with mRNA expression using RT-PCR on isolated taste buds. These results suggest that CCK in TRCs may be regulated differently than other cells of the body, with robust peptide expression but low levels of mRNA expression. To examine function, physiology experiments were conducted using patch clamp analysis and fura-2 calcium imaging techniques on dissociated rat posterior TRCs. Three major physiological actions in response to micromolar concentrations of CCK (CCK-8, sulfated) were observed—inhibitions of two types of potassium current (delayed-rectifier and inward-rectifier) and increases of intracellular calcium. These physiological effects would act in concert to place the cell into a more excitatory state. They were blocked by proglumide, a non-specific CCK-receptor antagonist, lorglumide, a specific antagonist of the CCK-A receptor, but were unaffected by L-365,260, a specific CCK-B blocker, pharmacologically demonstrating their mediation by a specific peptide receptor. In a separate physiology study using calcium imaging, CCK-responsive TRCs were demonstrated to be bitter responsive and respond to ACh (Lu *et al.*, 2003). These physiological investigations were followed up by immunocytochemical studies. In one, a



**Figure 1** Taste receptor cells express signaling agents and their receptors in unique patterns. These images illustrate immunocytochemical double labeling experiments that simultaneously use two antibodies, one directed against the signaling agent and one against its receptor. Each antibody is presented singly (left and middle columns) and then superimposed in an overlay (right column). Single labeled cells retain their original color whereas double labeled cells are yellow. In the top panel, serotonin and the serotonin receptor 5HT<sub>1A</sub> are expressed in non-overlapping patterns in rat circumvallate taste buds. This establishes a paracrine route of serotonergic processing. The bottom panel illustrates double labeling results from experiments using antibodies for CCK and the CCK-A receptor on tissue from a rat foliate papillae. Note that these two cell populations are completely overlapping, establishing an autocrine route for this peptide.

significant number of CCK-expressing TRCs (~60%) co-expressed gustducin. In another study, CCK-A receptor expression was observed in a subset of individual TRCs within the taste bud. In double labeling experiments using CCK and CCK-A receptor antibodies, the surprising result was obtained that there is almost complete overlap of these two subpopulations of cells (Figure 1, bottom panel). This co-distribution pattern indicates that CCK operates in an autocrine manner in the taste bud.

Taken together, a unifying picture of the action of cholecystokinin in the taste bud is emerging. We hypothesize that CCK acts as an autocrine agent to potentiate the excitatory actions of tastants on TRCs. When sufficiently excited by a tastant, an individual TRC releases CCK which, via CCK-A receptors, intensifies the underlying physiological response via changes in electrical excitability and increases of intracellular calcium. It is possible that CCK may be involved in bitter transduction since CCK-expressing TRCs are bitter sensitive and also significantly co-express gustducin.

## Conclusions

Although the possibility of cell-to-cell communication in the taste bud has been often raised, it is only recently that discreet signaling pathways have been elucidated. One question—how TRCs expressing tastant receptors yet lacking neural innervation communicate with the central nervous system—is being addressed by advances in our understanding of expression patterns of neurotransmitters and neuropeptides within the taste bud. Two examined in this communication, serotonin and cholecystokinin, represent previously unrecog-

nized paracrine and autocrine routes, respectively. Other neurotransmitters—norepinephrine, acetyl choline, glutamate—will likely have similar though still undiscovered roles. Ultimately, elucidating the expression patterns of neurotransmitter and neuropeptide receptors will be required to understand the pathways of information processing within the taste bud.

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