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Acetylcholine and Acetylcholine Receptors in Taste Receptor Cells

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Neuroactive substances play important roles as transmitters and neuromodulators. Although many of these substances and/or their receptors are known to be present in taste receptor cells and nerve fibers innervating taste buds, physiological functions of these substances are not well understood (Nagai *et al.*, 1996). Using Ca^{2+} imaging and immunocytochemical techniques, we have examined the physiological responses of taste receptor cells to acetylcholine (ACh), classified the types of ACh receptors, and determined the underlying signaling mechanisms in taste receptor cells. Our results suggest that ACh may be involved in cell-to-cell communication within the taste bud and in neuromodulation of taste transduction mechanisms.

In Ca^{2+}-imaging study, freshly isolated taste receptor cells were loaded with Ca^{2+}-sensitive fluorescent dye Fura-2 and intracellular Ca^{2+} levels were measured ratiometrically. ACh induced increases in intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) in taste receptor cells of mouse, rat and mudpuppy. The magnitude of the peak Ca^{2+} response to ACh was concentration-dependent with half-maximum responses around 1 μM. To determine which subtypes of ACh receptors and signaling pathway were involved, we examined the effect of receptor antagonists and inhibitors for selective pathway. Atropine (0.5 μM), a muscarinic ACh receptor antagonist, blocked the ACh response, while D-tubocurarine (250 μM), a nicotinic ACh receptor antagonist, had no effect. In addition, the phospholipase C (PLC) inhibitor U73122 (5 μM) and the Ca^{2+}-ATPase inhibitor thapsigargin (1 μM), which depletes intracellular Ca^{2+} stores, blocked the ACh responses. These results suggest that ACh binds to muscarinic ACh receptors, which activates PLC, resulting in the production of IP_3 and the subsequent release of Ca^{2+} from the IP_3-sensitive-intracellular stores. Since it is known that binding of ACh to muscarinic receptor subtypes M1/M3/M5 activates the PLC signaling pathways (Caulfield, 1993), our data indicates the presence of at least one of these receptors in taste receptor cells.

Additionally, we found that prolonged stimulation (>1 min) with ACh (10 μM) induced a biphasic response with a transient followed by a sustained $[Ca^{2+}]_i$ increase. The sustained phase of the $[Ca^{2+}]_i$ increase was dependent on Ca^{2+} influx as removal of extracellular Ca^{2+} eliminated the response. Subsequently adding external Ca^{2+} induced increases in $[Ca^{2+}]_i$, suggesting Ca^{2+} entry through Ca^{2+}-permeable channels. This is consistent with our previous studies showing presence of Ca^{2+} store-operated channels (SOC) in taste cells (Ogura, 2002; Ogura *et al.*, 2002). SOCs are activated solely by store depletion without requirement of a receptor-mediated mechanism, a mechanism also known as 'capacitative calcium entry'. Thus it is possible that the sustained part of the ACh-induced calcium response is mediated by Ca^{2+} influx through SOCs.

In immunocytochemical study, sections containing rat circumvallate and foliate papillae were immunoreacted with an antiserum against the M1 subtype of muscarinic ACh receptors. Positive reaction was observed in many taste cells of each taste bud. In cross-sections of rat circumvallate papillae, roughly half of the taste cells were immunolabeled. No selective labeling was observed in control sections, in which primary antibody was omitted. Preabsorption with antigen significantly reduced the labeling. This result suggests that taste receptor cells express M1 subtype of ACh receptor. Thus ACh can bind to the M1 subtype of the muscarinic receptors and activate the PLC/IP3 pathway.

To study whether ACh is stored in synaptic vesicles in taste receptor cells and/or adjacent nerve fibers, we immunolabeled the vesicular ACh transporter (VAChT), a key element of ACh-containing vesicle in mouse taste tissue. A subset of taste receptor cells exhibited positive immunoreactivity to the antibody against VAChT. In addition, certain nerve fibers surrounding or within taste buds are positively reacted to antibodies against VAChT. These results suggest that taste receptor cells could release ACh for cell-to-cell communications among taste receptor cells and/or synaptic transmission from taste receptor cells to taste sensory fibers. The presence of VAChT in adjacent nerve fibers also reveals a possibility of cholinergic modulations of taste receptor cells via the muscarinic receptors.

Taken together, our results demonstrate ACh responses and its signaling pathway in taste receptor cells. Since ACh increases $[Ca^{2+}]_i$ via PLC-mediated pathway, ACh may regulate taste responses by means of changing $[Ca^{2+}]_i$ levels or PLC signaling. It is known that the PLC pathway mediates taste responses to bitter, sweet and umami substances (Ogura *et al.*, 1997, 2002; Zhang *et al.*, 2003). Further experiments are needed to demonstrate these modulatory effects.

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