

Immunocytochemical evidence for co-expression of Type III IP3 receptor with signaling components of bitter taste transduction

ABSTRACT

IP3R3 is the dominant form of the IP3 receptor expressed in taste cells and our data suggest it plays an important role in bitter taste transduction.

INTRODUCTION

Background Taste receptor cells are specialized epithelial cells, which are organized into discrete endorgans called taste buds. Typical taste buds contain 50-100 polarized taste cells, which extend from the basal lamina to the taste pore, where apical microvilli protrude into the oral cavity. The basolateral membrane forms chemical synapses with primary gustatory neurons (Fig. 1A). In mammals, lingual taste buds are housed in connective tissue structures called papillae. Fungiform papillae are located on the anterior two-thirds of the tongue and typically contain 1-2 taste buds each. Vallate and foliate papillae are found on the posterior tongue and house several hundred taste buds each. Taste transduction begins when sapid stimuli interact with the apical membrane of taste cells, usually resulting in taste cell depolarization, calcium influx, and transmitter release onto gustatory afferent neurons. Simple stimuli, such as salts and acids depolarize taste cells by direct interaction with apical ion channels. In contrast, complex stimuli, such as sugars, amino acids, and most bitter compounds bind to G protein coupled receptors, initiating intracellular signaling cascades that culminate in Ca^{2+} influx or release of Ca^{2+} from intracellular stores. Inositol 1,4,5-trisphosphate (IP3) is an important second messenger in both bitter and sweet taste transduction. In both pathways, activation of taste receptors stimulates a G protein-coupled cascade resulting in activation of phospholipase C (PLC), which cleaves phosphoinositol bisphosphate (PIP2) to produce the second messengers IP3 and diacylglycerol (DAG). The soluble messenger IP3 binds to receptors located on calcium store membranes, causing release of calcium into the cytosol, while DAG remains in the membrane, where it can activate downstream effectors. While little is known about the role of IP3 in sweet taste transduction, considerable data indicate that IP3 plays an important role in bitter transduction. The first evidence for the involvement of IP3 in bitter transduction was obtained by Akabas et al., who used Ca^{2+} imaging to show that the bitter stimulus denatonium causes release of Ca^{2+} from intracellular stores. More recently, biochemical measurements have shown that several bitter compounds elevate IP3 in taste tissue. Other studies, however, suggested that a decrease in cAMP, rather than an increase in IP3, mediated bitter transduction. In 1992, a chemosensory specific G protein, α -gustducin, was identified in a subset of taste cells.

α -gustducin, which is closely related to the rod and cone transducins, activates PDE to reduce intracellular levels of cAMP. Evidence for gustducin's role in bitter taste came from knockout studies, in which a targeted deletion of α -gustducin resulted in mice with a reduced sensitivity for bitter compounds. More recently, a variety of bitter compounds have been shown to activate gustducin in biochemical assays, causing a decrease in intracellular cAMP levels via activation of PDE. It is now known that individual bitter receptors modulate both the IP3 and cAMP pathways (Fig. 1B). Bitter compounds bind to a family of G protein-coupled receptors called the T2R or TRB receptors, which activate a heterotrimeric G protein consisting of α -gustducin and a $\beta\gamma$ complex containing $\beta 3$ and $\gamma 13$. α -gustducin activates PDE to decrease intracellular levels of cAMP, while its $\beta\gamma$ partners stimulate PLC $\beta 2$ to produce IP3 and DAG. Although it

is clear that IP3 binds to receptors located on intracellular Ca²⁺ stores, the specific identity of IP3 receptors in taste cells is not known. There are at least 3 known isotypes of IP3 receptors encoded by different genes. Each protein product is about 300 Kda. Four subunits assemble to form a functional channel. Both homomultimeres and heteromultimeres have been reported. The N terminus of each subunit houses the IP3 binding domain while the C terminus anchors the protein to the membrane, is involved in the formation of the tetrameric protein, and forms the Ca²⁺ pore region. The general structure of each isoform is similar, however they differ in primary sequence, distribution, regulation, and IP3 affinity. In this study we used immunocytochemical methods to determine which IP3R isoform is expressed in taste cells and to examine the expression patterns of IP3 receptors relative to other proteins known to be important for taste transduction. We report that the Type III IP3 receptor is the dominant isotype expressed in rodent taste cells and that it is primarily found in the same subset of taste cells as other known signaling components of bitter transduction. A preliminary account of this work was published in abstract form.

CONCLUSION

Conclusions The principal finding in this study is the identification of IP3R3 as the dominant isoform of the IP3 receptor in taste cells. IP3 has been shown to be an important second messenger in both bitter and sweet taste transduction, and IP3R3 likely mediates the Ca²⁺ release from intracellular stores in response to IP3. In bitter taste transduction, many signaling components have been identified, and IP3R3 is co-expressed in the same taste cells (Fig. 6). Bitter stimuli bind to T2R/TRB taste receptors coupled to a heterotrimeric G protein complex consisting of α -gustducin and its partners, β 3 and γ 13. Alpha gustducin activates PDE, causing decreases in intracellular cAMP, while its $\beta\gamma$ partners stimulate PLC β 2 to produce IP3 and DAG. IP3 subsequently binds to IP3R3, causing increases in cytosolic Ca²⁺, due to release from intracellular stores (Fig. 1B). The unique properties of IP3R3, including its regulation by Ca²⁺ and cAMP dependent kinases, are consistent with known characteristics of bitter signaling in taste cells.