Assaying Activity of the Ion Channel TRPM5 in Identified Taste Bud Cells

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TRPM5 is a poorly-selective cation channel gated by intracellular Ca^{2+} . As suggested by immunofluorescent staining, TRPM5 channels are exclusively present in taste cells of type II, which are specialized in transduction of taste stimuli of the bitter, sweet and umami categories. Notably, TRPM5-null mice exhibit dramatically diminished neuronal and behavior responses to tastants of the above-mentioned taste modalities, thus indicating that TRPM5 is a key signaling protein in transduction machinery. Meanwhile, activity of TRPM5 channels in taste cells was not examined yet with electrophysiological methods.

The heterogeneous population of mammalian taste cells includes three cellular subtypes, type I to type III. In previous studies, we demonstrated that individual taste cells of the particular type can unequivocally be identified based on their electrophysiological properties. In the present work, we were trying to elucidate a subpopulation of taste cells, wherein TRPM5 channels would be functional, and then to analyze certain characteristics of these ion channels. Given that specific blockers of the TRPM5 channels are not available, their identification in taste cells was based on the functional properties of recombinant TRPM5 channels. Particu-

larly, TRPM5 studied in heterologous systems has been shown to be gated by intracellular Ca^{2+} , to be comparably permeable to Na^+ , K^+ , and Cs^+ ions and impermeable to NMDG⁺, and to exhibit high thermosensitivity ($Q_{10} \sim 10$) and inward rectification. By using Ca^{2+} ionophore ionomycin to elevate intracellular Ca^{2+} and stimulate Ca^{2+} -gated channels in taste cells, we demonstrated that highly thermosensitive ($Q_{10} = 6.7 \pm 0.5$) TRPM5-like channels were specific for the taste cells of type II. In type I cells, ionomycin stimulated Ca^{2+} -gated anion currents, while this compound affected negligibly resting currents in type III cells. To validate physiological data clearly indicating that *Trpm5* gene is functionally expressed in type II cells, *Trpm5* transcripts were searched for in individual identified taste cells with single-cell RT-PCR. Consistently with our electrophysiological data and in agreement with immunohistochemical evidence of others, the single-cell RT-PCR assay revealed mRNA encoding TRPM5 exclusively in taste cells of type II.

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