Characterization of a Newly Identified Signaling Pathway in Taste Cells

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Abstract

Our sense of taste depends on taste receptor cells located in the oral cavity that detect chemical stimuli and transduce them into electrical signals. There are two distinct signaling pathways that are known to transmit different taste qualities. In some taste cells, sour and salty stimuli cause a calcium influx through voltage-gated calcium channels (see Figure 1a). In other taste cells, bitter, sweet, and umami taste stimuli activate a G-protein coupled receptor (GPCR) pathway the depends on phospholipase Cβ2 (PLCβ2) (see Figure 1b). We recently identified third group of cells that express a unique GPCR signaling pathway (Figure 1c), however, not all of the components have been identified. We are using immunocytochemistry and RT-PCR analysis to identify these components. Characterizing this pathway will increase our understanding of how the peripheral taste system transmits taste signals to the brain.

Background & Objective

- To identify signaling components of the dual-responsive pathway which uses both GPCRs and voltage-gated calcium channels to transmit taste signals.
- We hypothesize that the TRPM4 channel, which has similar characteristics to TRPM5, is a critical protein in the dual responsive taste cells (1c).

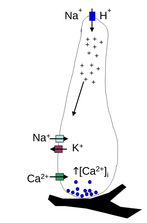


Figure1a. Ionic stimuli transduction pathway. Type III cells express voltage gated calcium channels

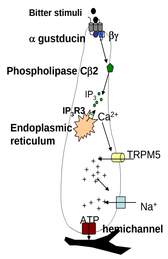


Figure1b. Complex stimuli transduction pathway. Type II cells express IP_3R3, PLCβ2, and TRPM5 which are required to cause neurotransmitter release through a hemi-channel

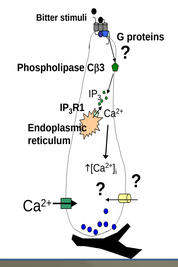


Figure1c. Schematic of the signaling components in the dual-responsive cells.

Methods & Materials

Immunocytochemistry

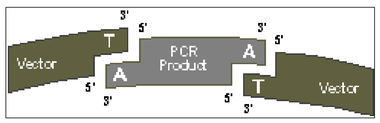
- A GAD-67 GFP mouse was euthanized in compliance to University at Buffalo Animal Care and Use Committee procedure and perfused with 4% paraformaldyde/0.1MPB, pH7. Tongue was isolated and cyroprotected ON at 4C.
- 40μM sections were cut on a cryostat, washed with PBS, and blocked to prevent nonspecific binding.
- Sections were incubated with anti-TRPM4 through a dilution series ON at 4C.
- The next day, sections were washed and then incubated with a rhodamine-tagged, goat anti-rabbit secondary antibody for 2 hrs at RT.
- After a final wash, sections were mounted on slides using Fluoromount G and coverslipped. Sections were viewed using a Zeiss LSM 510 Meta Confocal Microscope.

Reverse Transcription-Polymerase Chain Reaction

- RNA was isolated from foliate and circumvallate taste papillae for analysis.
- RNA is reverse transcribed into a cDNA template using Superscript III Reverse Transcriptase (Invitrogen). Samples were tested for genomic DNA contamination using GAPDH as a genomic control.
- cDNA was then used to optimize a PCR reaction for TRPM4 to determine if this channel is expressed in taste receptor cells.

Subcloning and Blue/White Screening

- PCR products amplified from the CV papillae were ligated into a TA cloning vector that had ampicillin resistance.
- Plasmids are transformed into *E. coli* and plated onto LB plates containing ampicillin and X-gal for ON at 37C.
- Blue/white screening was used to identify cells with the PCR inserts. DNA from the white colonies was isolated for sequence analysis.
- Sequencing of our products confirmed that TRPM4 was being amplified from the taste cells.



Concept behind TA cloning method

Results

MDSSINGAGPPGTVEPSAKVALERRQRRRPGRALCCGKFSKRWSDFWGAPVTAFLGNV
VSYLLFLLLFAHVLLVDFQPTKPSVSELLLYFWAFTLCEELRQGLGGGWGSLASGGRG
PDRAPLRHRLHLYLSDTWNQCDLLALTCFLLGVGCRLTPGLFDLGRTVLCLDFMIFTL
RLLHIFTVNKQLGPKIVIVSKMMKDVFFFLFFLCVWLVAYGVATEGILRPQDRSLPSI
LRRVFYRPYLQIFGQIPQEEMDVALMIPGNCSMERSGWAHPEGPVAGSCVSQYANWLL
LLIVFLLVANILLLNLLIAMFSYTFSKVHGNSDLYWKAQRYSLIREFHSRPALAPPLI
IISHVRLLIKWLRRCRRCRRANLPASPVFEHFRVCLSKEAERKLLTWESVHKENFLLA
QARDKRDSDSERLKRTSQKVDTALKQLGQIREYDRRLRGLEREVQHCSRVLTWMAEAL
SHSALLPPGAPPPPSPTGSKD

Figure 2. Presumed amino acid sequence of TRPM4. Underlined letters indicate the transmembrane domains while bold letters indicate designated PCR primer locations.

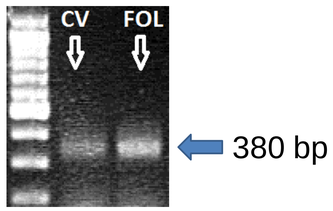


Figure 3. Gel electrophoresis of PCR products for TRPM4 that was amplified from circumvallate papillae (CV) and foliate papillae (FOL) that contain taste receptor cells. PCR products were approximately 380bp.

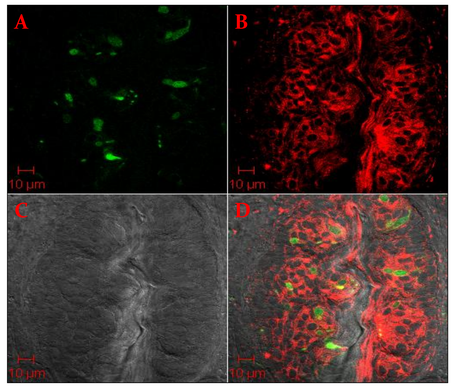
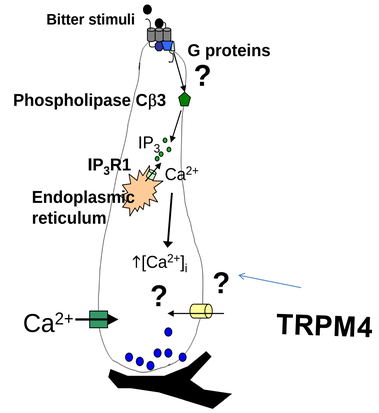


Figure 4. (A) GAD67-GFP expression which labels many taste cells with chemical synapses. (B) Anti-TRPM4 labeling (C) The DIC image of the taste buds is shown. (D) Overlay of taste buds, GFP expression and TRPM4 immunoreactivity. Co-localization is shown in yellow.

Data Analysis

- DNA sequencing confirmed that mRNA transcripts for TRPM4 are expressed in the mouse taste receptor cells.
- The immunocytochemical analysis also verifies that TRPM4 is expressed in taste cells. Further, there is co-localization of TRPM4 with the GAD67-GFP expression which indicates that TRPM4 is expressed in dual responsive cells.
- More immunocytochemistry analyses are needed to verify these initial results.



Conclusions

- TRPM4 is widely expressed in mouse taste cells.
- TRPM4 co-localizes with GAD67-GFP expression which indicates TRPM4 is found in dual-responsive taste cells, though its expression is not restricted to these cells.

Future Research

- To determine the functional role of TRPM4 in the dual-responsive taste cells. These experiments will rely on calcium imaging and electrophysiological analyses.

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