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Taste Receptor Cells Responding with Action Potentials to Taste Stimuli and their Molecular Expression of Taste Related Genes

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Introduction

Recent molecular biological studies have revealed many molecular aspects of taste transduction in receptor cells for each taste categories which are referred to as salty, sweet, sour, bitter and umami in human respectively. (Lindemann, 2001; Gilbertson and Boughter, 2003). For example, ion channels such as ENaC, ASIC and HCN mediate salty and sour taste and G-protein-coupled receptors such as T1Rs and T2Rs play a key role in sweet, bitter and umami taste. However, there is little evidence for involvements of these molecules in taste reception and transduction at the cellular level. It is necessary to examine both molecular and physiological properties in single taste receptor cell. In this study, by use of loose patch recording technique combined with single cell multiplex RT–PCR, we characterized single taste cells by their responses to four basic taste stimuli and expression of taste-related molecules.

Materials and methods

All experimental procedures were approved by the committee for Laboratory Animal Care and Use at Kyushu University, Japan. Subjects were adult C57BL/6N or C57BL/KsJ mice at >2 months of age. Animals were anesthetized and killed by cervical dislocation. The anterior part of tongue was removed and subjected to enzyme treatment. The lingual epithelium was peeled from underlying tissue. Individual taste buds of fungiform papilla were excised from the epithelial sheet and drawn into the orifice of the stimulating pipette (40–50 μm). A gentle suction on the pipette was maintained to perfuse taste solutions and to hold the taste bud in place. The bath solution [Tyrode solution containing (in mM): NaCl, 140; KCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; HEPES, 10; glucose, 10; sodium pyruvate, 10; pH = 7.40) was continuously perfused. The electrical responses of taste receptor cells in intact taste buds were recorded with a loose patch configuration from basolateral side in voltage or current clamp mode.

For data analysis, the number of spikes in unit time was counted throughout the recording. The mean spontaneous impulse discharge for each taste cells was calculated by averaging the number of spikes over the 10 s period that distilled water was applied to the taste pore. Numbers of spikes larger than the mean plus one standard deviation of the spontaneous impulse discharges was taken as the final criterion for the occurrence of a response.

For the RT–PCR experiment, a single taste cell was collected from a taste bud and transferred to a PCR tube after recording of responses. Several target sequences were simultaneously amplified by multiplex PCR with nested PCR primers and two rounds of amplification. Each pair of primers spanned at least one intron sequence to distinguish between products amplified from cDNA and genomic DNA. PCR product size for each genes was 900–1000 base pairs for RT and first round PCR and 300–400 base pairs for second round PCR.

Results

Using our experimental setup, spontaneous spike activities were recorded in some taste cells under loose patch configuration. Each spike was recorded as negative current followed by positive current in V-clamp mode and as positive potential followed by negative potential in I-clamp mode. We examined the effect of voltage gated sodium channel blocker, tetrodotoxin (TTX) on these spike activities. Bath application of 1 μM TTX completely inhibited spike activities and they recovered after wash out of TTX (Figure 1A), indicating that spike activities result from action potentials (currents) in taste receptor cells. In our experiments, action currents of taste

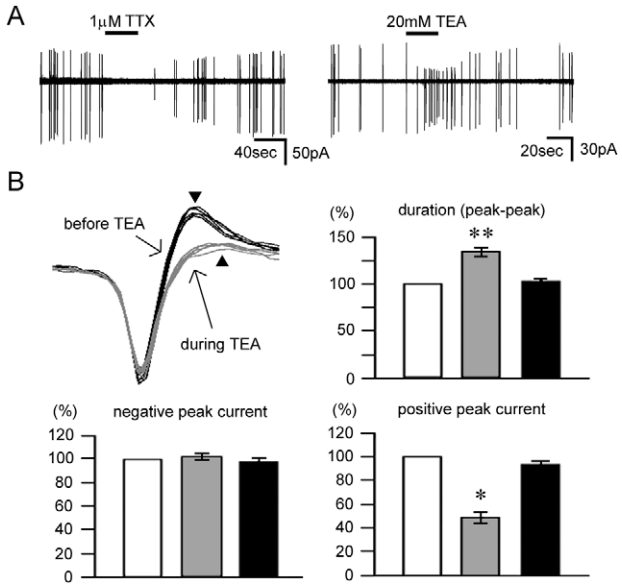

Figure 1 Effect of voltage gated channel blockers for the generation of action currents in taste cells. **(A)** Action potentials were completely inhibited by bath application of 1 μM TTX (left). Bath application of 20 mM TEA reduced magnitude of positive peak currents (right). Recordings were obtained from different preparations. **(B)** Summary of the averaged effect of 20 mM TEA ($n = 4$) on the action currents of taste cells. The inset shows superimposed action currents before (black) and during (gray) TEA application. Arrowheads indicate the averaged upper peak time. The peak values and durations between these peaks of before (white bars), during (gray bars) and after (black bars) TEA application were compared and the percentage was averaged over all preparations. Bath application of TEA affected duration between peaks and positive peak currents but not negative peak currents. Value are mean ± SEM. Asterisks indicate significant difference by Student's *t*-test, $*P < 0.001$, $**P < 0.005$.

receptor cells were also affected by potassium channel blocker, tetraethylammonium (TEA). The peak size of positive current was decreased and duration between negative and positive peak was prolonged by bath application of 20 mM TEA and recovered after wash out of TEA (Figure 1A,B). Because bath application of these blockers affected action currents of taste cells, these channels exist on the basolateral membrane of taste cells.

Next, we examined response of taste receptor cells to apical taste stimuli. NaCl, saccharin, HCl and quinine were used as taste stimulants. We recorded responses to taste stimuli as increase in firing frequency of taste receptor cells. Many taste cells responded to one of four taste stimuli and some clearly responded to multiple taste stimuli. After recording of taste response, taste receptor cells were collected from taste buds to examine their molecular expression by multiplex RT–PCR. Collection of single taste receptor cell was visually confirmed. In RT–PCR experiments, we investigated expression of sweet receptor component, T1r3 and G-protein α-subunit, gustducin in taste receptor cells responding to saccharin. Our preliminary results of these experiments were summarized in Table 1. This suggests a possibility of the existence of multiple receptor and transduction systems for sweet taste. Thus, this technique might be useful to investigate molecular aspects of taste receptor cells responding to taste stimuli.

Discussion

We recorded impulse discharges of taste cells under the loose patch configuration. Action potentials in taste receptor cells were described first in amphibian species (Kashiwayanagi *et al.*, 1983; Roper, 1983) and have been reported in mammals (Avenet and Lindemann, 1991; Gilbertson *et al.*, 1992; Cummings *et al.*, 1993). The underlying current properties of action potentials in mammalian taste cells have been well documented (Herness and Sun, 1995; Chen *et al.*, 1996). In our experiments, bath application of TTX and TEA inhibited or modulated action currents of taste cells, indicating voltage gated sodium and potassium channels contribute to generation of action potentials in taste receptor cells. It has been reported that sweet (Cummings *et al.*, 1993), salty (Avenet and Lindemann, 1991), sour (Gilbertson *et al.*, 1992) and bitter (Furue and Yoshii, 1997) stimuli elicited action potentials in taste receptor cells. In our study, responses of taste receptor cells to apical taste stimuli were recorded as increase in firing frequency. These results suggest that voltage gated Na^+ and K^+ channels might be necessary for taste transduction in taste receptor cells.

Table 1 Expression of gustducin and T1r3 in sweet responsive cells

Expression of genes			No. of cells
Gustducin	T1r3	β-Actin	
–	–	+	2
+	–	+	4
–	+	+	2
+	+	+	1

+, detected; –, not detected.

There is little evidence for molecular expression in taste receptor cells responding to taste stimuli. Recently, expression of G-protein subunit α-gustducin and Gαi was examined in taste cells responding to bitter stimuli by using Ca^{2+} imaging and immunohistochemical method (Caicedo *et al.*, 2003). In the present study, molecular expression of taste receptor cells responding to taste stimuli was examined by using loose patch recording and multiplex RT–PCR. Both techniques may be useful for investigating physiological and molecular properties of taste receptor cells; however, there are some problems in detection of molecular expression. In immunohistchemical experiment, the number of genes of which expression can be simultaneously detected is limited. In multiplex RT–PCR experiment, we cannot distinguish between PCR products amplified from cDNA or genomic DNA if mRNA is transcribed from single exon sequence such as T2Rs. In order to investigate molecular expression in taste receptor cells more in detail, further improvements are needed.

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