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RESEARCH PAPER

Bitter Taste Responses of Gustducin-positive Taste Cells in Mouse Fungiform and Circumvallate Papillae

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Abstract—Bitter taste serves as an important signal for potentially poisonous compounds in foods to avoid their ingestion. Thousands of compounds are estimated to taste bitter and presumed to activate taste receptor cells expressing bitter taste receptors (Tas2rs) and coupled transduction components including gustducin, phospholipase Cβ2 (PLCβ2) and transient receptor potential channel M5 (TRPM5). Indeed, some gustducin-positive taste cells have been shown to respond to bitter compounds. However, there has been no systematic characterization of their response properties to multiple bitter compounds and the role of transduction molecules in these cells. In this study, we investigated bitter taste responses of gustducin-positive taste cells *in situ* in mouse fungiform (anterior tongue) and circumvallate (posterior tongue) papillae using transgenic mice expressing green fluorescent protein in gustducin-positive cells. The overall response profile of gustducin-positive taste cells to multiple bitter compounds (quinine, denatonium, cyclohexamide, caffeine, sucrose octaacetate, tetraethylammonium, phenylthiourea, L-phenylalanine, $MgSO_4$, and high concentration of saccharin) was not significantly different between fungiform and circumvallate papillae. These bitter-sensitive taste cells were classified into several groups according to their responsiveness to multiple bitter compounds. Bitter responses of gustducin-positive taste cells were significantly suppressed by inhibitors of TRPM5 or PLCβ2. In contrast, several bitter inhibitors did not show any effect on bitter responses of taste cells. These results indicate that bitter-sensitive taste cells display heterogeneous responses and that TRPM5 and PLCβ2 are indispensable for eliciting bitter taste responses of gustducin-positive taste cells.

Key words: bitter receptor, breadth of responsiveness, taste coding, transgenic mouse, bitter antagonists.

INTRODUCTION

Bitter taste protects animals from the ingestion of poisonous compounds. Many structurally diverse compounds such as alkaloids, terpenoids, flavonoids, phenylpropanes and thiols elicit bitter taste (Wiener et al., 2012). Bitter compounds are detected by the type 2 taste receptors (Tas2rs), which comprise a large G protein-coupled receptor family encoded by *Tas2r* genes (Adler et al., 2000; Chandrashekar et al., 2000; Matsunami et al., 2000; Mueller et al., 2005). The number of functional *Tas2r* genes varies depending on the species with 25 in humans and 35 in mice (Go et al., 2005). Many of Tas2rs have had their cognate ligands identified in heterologous expression assays (Meyerhof et al., 2010; Lossow et al., 2016). These Tas2rs vary greatly in their

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Abbreviations: BCML, Nα,Nα-bis(carboxymethyl)-L-Lysine; Caf, caffeine; Chx, cyclohexamide; CV, circumvallate papillae; Den, denatonium benzoate; FP, fungiform papillae; GABA, γ-aminobutylic acid; GFP, green fluorescent protein; GIV 3727, 4-(2,2,3-trimethylcyclopentyl) butanoic acid; IP_3R3, inositol-1,4,5-triophosphate receptor type 3; KO, knockout; L-Phe, L-phenylalanine; PLCβ2, phospholipase Cβ2; PTU, phenylthiourea; QHCl, quinine-HCl; Sac, saccharin-Na; SOA, sucrose octaacetate; Tas2rs, the type 2 taste receptors; TEA, tetraethylammonium; TPPO, triphenylphosphosphine oxide; TRPM5, transient receptor potential channel M5.

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breadth of tuning, ranging from very broadly to extremely narrowly tuned receptors.

In taste cells, binding of bitter compounds to Tas2rs activates the following signaling molecules: the heteromeric G-protein gustducin (Wong et al., 1996), phospholipase Cβ2 (PLCβ2, Zhang et al., 2003), inositol-1,4,5-triophosphate receptor type 3 (IP_3R3, Hisatsune et al., 2007) and transient receptor potential channel M5 (TRPM5, Zhang et al., 2003, 2007). Bitter-activated taste cells generate action potentials (Yoshida et al., 2006) and release neurotransmitters (Huang et al., 2007; Murata et al., 2010). Mice lacking these signaling molecules also showed diminished behavioral and neural responses to multiple bitter compounds (Wong et al., 1996; Zhang et al., 2003; Dotson et al., 2005; Damak et al., 2006; Hisatsune et al., 2007), suggesting that these signaling molecules are required for bitter taste responses. However, there is little evidence showing the contribution of these signaling molecules to bitter responses at the taste cell level *in situ*.

In taste buds, there are 4 types of taste cells (Type I–IV cells), which are characterized by their morphology and expression pattern (Iwata et al., 2014). Bitter receptors and coupled transduction molecules are expressed in Type II cells (Yang et al., 2000; Pérez et al., 2002; Clapp et al., 2004). Indeed, a subset of Type II cells in mouse fungiform (FP) and circumvallate (CV) papillae respond to bitter taste stimuli consistent with the expression pattern of receptors and transduction components for bitter taste (Tomchik et al., 2007; Yoshida et al., 2009a). However, several reports showed different response properties of bitter-sensitive taste cells. Our previous study demonstrated that the majority of bitter-sensitive cells in mouse FP responded to multiple bitter compounds (Yoshida et al., 2009a) whereas another study demonstrated that most bitter taste cells in rat CV respond to one or two of five bitter stimuli (Caicedo and Roper, 2001). Such discrepancy may be derived from methodological differences or from different locations of taste buds examined (FP vs CV) since gustatory nerve recordings showed different sensitivities to bitter compounds between the chorda tympani and the glossopharyngeal nerve (Ninomiya and Funakoshi, 1989; Ninomiya et al., 1991). The responsiveness of bitter-sensitive taste cells has not been systematically compared between FP and CV using the same animal species and same experimental method.

Recent studies reported some bitter blockers for human Tas2rs. For example, 4-(2,2,3-trimethylcyclopentyl) butanoic acid (GIV 3727) inhibits six bitter taste receptors (Slack et al., 2010). Probenecid inhibits hTAS2R16, 38, and 43 (Greene et al., 2011). Sesquiterpene lactones and 6-methoxyflavanones block hTAS2R46 and hTAS2R39, respectively (Brockhoff et al., 2011; Roland et al., 2014). Some amino acid derivatives such as γ-aminobutylic acid (GABA) and Nα,Nα-bis(carboxymethyl)-L-Lysine (BCML) block hTAS2R4 (Pydi et al., 2014). These bitter blockers could be used to avoid unpleasant bitter taste of some medicines and health foods. These compounds were tested in heterologous expression system and some human psychophysics. But it is not clear whether these blockers inhibit activation of bitter-sensitive taste cells.

In this study, we focused on gustducin-positive mouse taste cells from both FP and CV and compared their responses to multiple bitter compounds. We also investigate the effect of pharmacological inhibitors for signaling molecules (PLCβ2 and TRPM5) in bitter-sensitive taste cells and the effect of several bitter antagonists on activation of gustducin-positive taste cells.

EXPERIMENTAL PROCEDURES

Animals

All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the committee for Laboratory Animal Care and Use at Kyushu University, Japan. Subjects were adult (> 8 weeks old) male and female transgenic mice expressing green fluorescent protein (GFP) under control of the gustducin promoter (gustducin-GFP mice, $n = 53$) (Wong et al., 1999). All mice were housed under a 12:12-h light–dark cycle (lights on 0800–2000 h) and had ad libitum access to tap water and food pellets (CE-2, CLEA Japan, Tokyo, Japan).

Taste cell recording

Recording procedures were similar to those used previously (Yoshida et al., 2006, 2009a, 2015) with some modifications to record responses from CV taste cells. Animals were sacrificed by cervical dislocation. The anterior (for FP preparation) and the posterior parts (for CV preparation) of the tongue were removed and injected with 50–100 μl of Tyrode solution containing 0.5–2 mg/ml elastase (Elastin Products, Owensville, MO). After incubation for 10–20 min at room temperature (25 °C), the lingual epithelium was peeled and pinned out in a Sylgard coated culture dish. Individual FP or CV taste buds with a piece of surrounding epithelium were excised from this sheet and the mucosal side was drawn into the orifice of the stimulating pipette. The residual epithelial sheet was stored at 4 °C for another series of experiments. A gentle suction on the stimulating pipette was maintained to perfuse taste solutions and to hold the taste bud in place. Bath solution (Tyrode solution) was continuously flowed into the recording chamber with a peristaltic pump at approximately 2 ml/min. The receptor membrane was rinsed with distilled water at least 30 s before and after taste stimulation (15 s). Taste stimuli were applied to taste cells in randomized order. Taste bud cells containing GFP were identified by confocal laser scanning microscopy (FV-1000; Olympus, Tokyo, Japan) and were approached by a recording electrode (inner diameter ~1–3 μm, pipette resistances 1.5–3.5 MΩ). Seal resistances were typically 3–10 times the pipette resistances. Electrical signals were recorded by a high-impedance patch-clamp amplifier (Axopatch 200B; Axon Instruments, Foster City, CA) interfaced to a computer (Windows XP or 7) by an analog-to-digital board (Digidata 1320A; Axon Instruments). Signals were filtered at 1 kHz, sampled at 10

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