Animal Cells and Systems
Vol. 15, No. 3, September 2011, 197–202

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Cell-type specific expression of vanilloid receptor 1 in the taste cells of rat circumvallate papillae

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(*Received 16 March 2011; received in revised form 21 April 2011; accepted 25 April 2011*)

The present study demonstrates the first-ever characterization of cell types that express the vanilloid receptor 1 (VR1) in the taste buds of rat circumvallate papillae. We performed electron microscopy to identify the subcellular location. The VR1 immunoreactivity was associated with the endoplasmic reticulum, cytoplasmic vesicles, and plasma membrane of taste cells. These results demonstrate the localization of the VR1 in membranous structures of the taste cells. We used double immunofluorescence histochemistry with taste cell type-specific markers to identify the cell types that express the VR1. The VR1 was detected in all functional taste cell types (Type I, Type II, and Type III cells). Together, our data suggest that the VR1 might play different roles according to the cell types within a taste bud.

Keywords: vanilloid receptor 1; GLAST; PLCβ2; SNAP25; circumvallate papillae

Introduction

Taste buds, the transducing elements of gustatory sensation, contain 50–150 heterogeneous types of cells, including taste receptor cells. Taste receptor cells are responsible for transducing chemical stimuli from the environment and relaying information to the nervous system. Several types of taste cells have been identified based on their morphological and functional properties (Yoshie et al. 1989; Smith et al. 1993, 1994). Type I cells, also known as 'dark' cells because of their high cytoplasmic electron density, account for approximately half the taste buds (Pumplin et al. 1997). They are believed to play a glial-like role within taste buds (Lawton et al. 2000). Approximately 35% of taste cells are type II cells, which are also known as 'light' cells due to the electron lucent nature of their cytoplasm, and they function as sensory receptor cells (Hoon et al. 1999; Clapp et al. 2001; Gilbertson and Boughter 2003). Type III cells, referred to as 'intermediate' cells, show synaptic conjunction and can transmit taste signals to sensory afferent nerve fibers (Crisp et al. 1975; Kinnamon et al. 1985). In addition to these three cell types, type IV cells, also known as basal cells, are found localized near the base of the taste bud without extending to the taste pore, and are considered precursor cells of the other types of cells (Yoshida and Ninomiya 2010).

The vanilloid receptor 1 (VR1), which plays a key role in the detection of noxious painful stimuli, is a ligand-gated non-selective cation channel predominantly expressed by peripheral sensory neurons (Caterina et al. 1997; Tominaga et al. 1998). Although the VR1 was found previously in neural cells, recent evidence suggests that functional VR1 is expressed in nonneuronal cells such as the epithelial cells, glial cells, skeletal muscle cells, and submandibular glands (Birder et al. 2001; Inoue et al. 2002; Doly et al. 2004; Tóth et al. 2005; Xin et al. 2005; Kim et al. 2006; Ding et al. 2010).

The VR1 is expressed in rat tongue, and application of capsaicin to the surface of the tongue increases salivation (Dunér-Engström et al. 1986). In a recent study, Gu et al. (2009) showed that the VR1 is localized in the taste receptor cells of rat circumvallate papillae. However, it is not reported whether the other types of taste cells in circumvallate papillae express the VR1. Therefore, in this study, we used immunohistochemistry to identify the types of cells that express the VR1 in rat circumvallate papillae. We also examined the subcellular location of the VR1 in taste cells by electron microscopy.

Materials and methods

Animal preparation

Adult male albino Sprague Dawley rats (200–250 g) were used for this study. All experimental animal procedures were conducted with the approval of the Catholic Ethics Committee of the Catholic University

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ISSN 1976-8354 print/ISSN 2151-2485 online

DOI: 10.1080/19768354.2011.590227
http://www.informaworld.com

of Korea, and were in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80–23, revised 1996).

Single and double immunohistochemistry

Tissues of circumvallate papillae were rapidly dissected from the rats under anesthesia with 8% chloral hydrate (0.5 ml/kg), and fixed with fixative (4% paraformaldehyde in 0.1M sodium phosphate buffer, pH 7.2) for 4–5 h. After dehydration in graded ethanols, the fixed tissues were then embedded in wax (Polyethylene glycol 400 diesterate, Polyscience, Warrington, PA, USA). Tissue sections were obtained at 8-μm thickness using a microtome (Leica RM 2135, Germany), and deposited on gelatin-coated slides. After de-waxing with xylene and hydration in graded alcohols, endogenous peroxidases were inactivated by treating the tissue sections with 1.5% hydrogen peroxide for 30 min. After blocking with 10% normal donkey serum for 1 h, the sections were incubated with a solution of a rabbit polyclonal antibody to the VR1 (Sigma-Aldrich, St Louis, MO, USA; dilution 1:100) overnight at 4°C. Primary antibody binding was visualized using peroxidase-labeled donkey anti-rabbit IgG (Jackson ImmunoResearch Labs, USA; dilution 1:100), and 0.05% 3,3′-diaminobenzidine tetrahydrochloride with 0.01% H_2O_2 as the substrate. The specificity of VR1 immunoreactivity was confirmed by the absence of immunohistochemical staining in sections from which the primary antibody was omitted.

For double-immunofluorescence immunohistochemistry, sections were incubated at 4°C overnight with the following antibodies: rabbit polyclonal antibody to the VR1, guinea pig polyclonal antibody to the GLAST (Millipore Corporation, USA & Canada; dilution 1:200), goat polyclonal antibody to the PLCβ2 (Santa Cruz Biotechnology, CA, USA; dilution 1:100), and goat polyclonal antibody to the SNAP25 (Millipore Corporation, USA & Canada; dilution 1:20). Tissue sections were washed twice in PBS, and the bound primary antibodies were visualized by incubating with Alexa Fluor 488 conjugated to donkey anti-rabbit IgG (Molecular Probes, USA; dilution 1:1000), Alexa Fluor 488 conjugated to goat anti-guinea pig IgG (Molecular Probes, USA; dilution 1:1000), Alexa Fluor 555 conjugated to donkey anti-goat IgG (Molecular Probes, USA; dilution 1:1000) or Alexa Fluor 568 conjugated to goat anti-rabbit IgG (Molecular Probes, USA; dilution 1:1000) for 1 h at room temperature. Slides were viewed with a confocal microscope (LSM 510 Meta, Carl Zeiss Co. Ltd., Germany). Images were converted to TIFF format, and the contrast levels adjusted using Adobe Photoshop v 7.0 (Adobe Systems, San Jose, CA).

Electron microscopy

Forty-micrometer-thick vibratome sections were incubated in VR1 antibody diluted 1:200 overnight at 4°C, in peroxidase-labeled donkey anti-rabbit IgG for 2 h, and in ABC solution for 1 h, and then visualized with 0.05% 3,3′-diaminobenzidine tetrahydrochloride with 0.01% H_2O_2 as the substrate. The reaction was monitored using a low-power microscope. The sections were postfixed in 1% glutaraldehyde in PB for 1 h and then with 1% OsO_4 in PB for 1 h, and the sections were flat embedded in Epon 812 mixture. Ultrathin sections (70–90 nm thick) were collected on one-hole grids coated with Formvar and examined using a transmission electron microscope (JEOL model 1200EX, Tokyo, Japan).

Results

Immunohistochemistry using anti-VR1 antibody revealed positive staining of the taste buds of the circumvallate papilla (Figure 1A). At higher magnification, we could identify some VR1-immunoreactive taste cells within a taste bud (Figure 1B). Specifically, strong labeling was observed in the cytoplasm of labeled cells. All immunoreactivity specific to the VR1 was eliminated when the primary antibody was omitted (Figure 1C).

Subcellular localization of VR1 immunoreactivity in taste cells was observed by electron microscopy. Figure 2A presents a taste bud of the circumvallate papilla, which contains VR1-immunoreactive taste cells. Electron-dense reaction products of immunohistochemistry for the VR1 were observed in the plasma membrane of microvilli of sensory receptor cells in the taste pore (Figure 2B). VR1 immunoreactivity was also detected in the endoplasmic reticulum (Figure 2C and D), cytoplasmic vesicles (Figure 2E), and plasma membrane of taste cells (Figure 2C and I).

To define the phenotype of VR1-expressing cells in the circumvallate papillae, double labeling was performed using cell type-specific markers, including the glutamate-aspartate transporter (GLAST) for Type I, PLCβ2 for Type II, and SNAP25 for Type III taste cells. Confocal images of a taste bud double immunostained for VR1 (Figure 3A) and GLAST (Figure 3B) revealed that a subset of VR1-positive cells also expressed GLAST (Figure 3C). Confocal images of a taste bud double immunostained for VR1 (Figure 3D) and PLCβ2 (Figure 3E) showed that the VR1 was colocalized with PLCβ2 (Figure 3F). Confocal images of double immunostaining for VR1 (Figure 3G) and

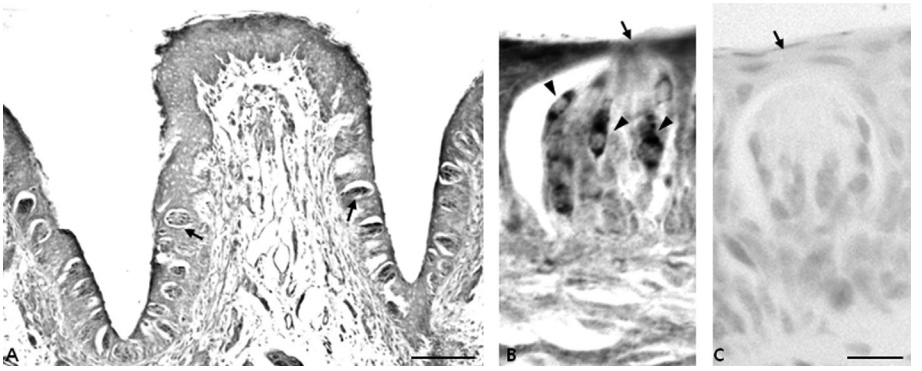

Figure 1. Immunohistochemical staining for the VR1 in the circumvallate papilla. VR1 immunoreactivity was detected in taste cells within taste buds (arrows) of the circumvallate papilla (A). (B) shows the higher-magnification view of a taste bud. Arrowheads indicate VR1-immunoreactive taste cells with intense labeling in the cytoplasm. No immunoreactivity was observed in the absence of primary antibody (C). Arrow in (B) and (C) indicates the taste pore. Scale bar = 200 μm (A), 25 μm (B, C).

SNAP25 (Figure 3H) indicated that a subset of VR1-positive cells also showed SNAP25 immunoreactivity (Figure 3I).

Discussion

The pungent sensation of hot peppers is thought to be mediated by the vanilloid receptor subtype-1 (VR1), which can be activated by capsaicin, but there is little information regarding its histological localization in the tongue. The present study revealed that the VR1 was expressed in Type I, Type II, and Type III cells of taste buds in the rat circumvallate papillae. Even though it was demonstrated originally only in neural cells, non-neural expression of the VR1 has recently become the focus of much attention. The expression of the VR1 in Type II cells agrees with previously reported data (Gu et al. 2009; Moon et al. 2010). However, this is the first description of the localization of the VR1 in Type I and Type III cells in rat circumvallate papillae. Thus, our data showed that the VR1 was localized in all kinds of functional taste cells, and suggested that it might play a different role according to the cell types within a taste bud.

Several reports have shown that Type I cells express GLAST (Lawton et al. 2000) and nucleoside triphosphate diphosphohydrolase-2 (ecto-ATPase; Bartel et al. 2006) for inactivation and uptake of transmitters and wrap around other cells (Pumplin et al. 1997), suggesting that Type I cells play supporting roles similar to glial cells in the nervous system. Moreover, Bigiani (2001) reported the existence of mouse taste bud cells with glia-like membrane properties. The VR1 is believed to function as a molecular integrator of noxious stimuli (Szallasi and Blumberg 1999). However, the recent discovery of the VR1 in both human and rat brains, where it is unlikely to be gated by noxious stimuli, has suggested additional functional roles for this cation channel in physiological processes (Mezey et al. 2000). Little is known about functional roles of the VR1 in glial cells even though the VR1 was reported to be expressed in glial cells, such as microglia in the substantia nigra (Kim et al. 2006), astrocytes around blood vessels in the brain (Tóth et al. 2005), some spinal astrocytes (Doly et al. 2004), and Müller cells in the retina (Kim et al. 2011). In a recent in vitro study, treatment of microglia with VR1 agonists induced cell death of microglia. They also demonstrated that VR1 antagonists were capable of inhibiting this toxicity, further indicating that the VR1 mediated the microglial death (Kim et al. 2006). Meanwhile, we suggested in recent data that the VR1 in retinal Müller cells played a role in protecting neurons against excitotoxicity (Kim et al. 2011). Due to their contact with the external environment, taste cells may possess both protective and regenerative mechanisms, as in the case of the olfactory epithelium. The lifespan of taste cells is thought to be approximately 10 days (Farbman 1980); therefore, continuous regeneration and cell death are expected to be necessary to maintain the shape and function of taste buds. VR1 activation is associated with robust calcium (Ca^{2+}) conductance, which has been linked to apoptotic cell death, including death of neurons and glia (Agopyan et al. 2004; Kim et al. 2006). In this study, we identified the VR1 in GLAST-immunoreactive taste cells, demonstrating that the VR1 is localized in Type I cells. Taken together, the localization of the VR1 in Type I cells suggests that they might be involved in the cytotoxic or protective glia-like roles for maintaining taste buds.

Differential morphological features of taste bud cells may correlate with their functional characteristics. Type II and Type III cells are capable of transmitting their signals to gustatory afferent nerve fibers, suggesting these types of taste cells possess taste receptors and transducing mechanisms. Several studies reported that

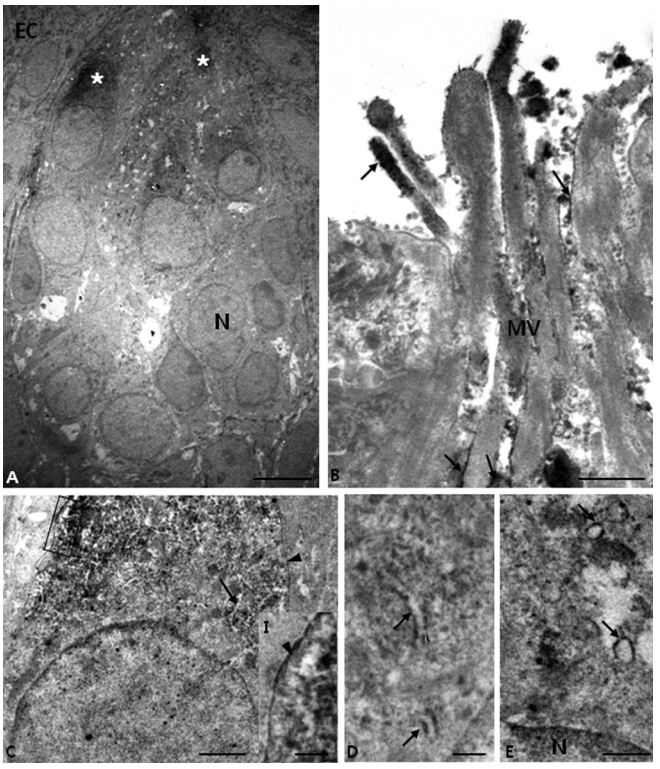

Figure 2. Electron micrographs of subcellular location of the VR1 in taste cells within a taste bud, which were processed for immunohistochemistry with anti-VR1 antibody. (A) A taste bud containing VR1-immunoreactive taste cells (asterisks). VR1 immunoreactivity is visible as electron-dense reaction products associated with microvilli in the taste pore (B, arrows). VR1 immunoreactivity is also localized in the endoplasmic reticulum (C and D, arrows), cytoplasmic vesicles (E, arrows), and plasma membrane (C and I, arrowheads) of taste cells. Inset (I) shows higher magnification of the boxed area in C. EC; epithelial cell, MV; microvilli, N; nucleus. Scale bar = 2 μm (A), 0.5 μm (B), 1 μm (C), 0.2 μm (D), 0.4 μm (E), and 0.2 μm (I).

Type II cells express T1R and T2R taste receptor proteins and downstream signaling effectors for bitter, sweet, and umami taste stimuli (Clapp et al. 2001, 2004; Miyoshi et al. 2001), and most of them did not form conventional synapses with the nervous system (Clapp et al. 2006; Yoshida and Ninomiya 2010). Contrary to the well-known roles of the VR1 in sensory nerve endings, the expression and function of the VR1 in taste receptor cells have been poorly understood. Previous studies have reported a direct interaction of capsaicin with taste receptor cells, suggesting that perception of capsaicin could be a chemesthetic response via VR1 in taste cells (Yoshii and Matui 1994; Park et al. 2003). Gu et al. (2009) reported that repeated intra-oral treatment with capsaicin increased sweet consumption in rats. Recent studies have demonstrated that the VR1 was colocalized with the sweet receptors T1R2, T2R3 or the bitter receptor T2R6 in the taste cells of rat and human circumvallate papillae (Jahng et al. 2010; Moon et al. 2010). In this study, VR1 immunoreactivity was also detected in Type II cells identified by double immunostaining with an antibody against PLCβ2, a Type II cell marker. Taken together, these data suggest that capsaicin may interact with the transduction pathway of sweet and bitter taste stimuli via the VR1 localized in taste receptor cells.

Salty and sour tastes are known to be mediated by channel-type receptors. In the case of salt tasting, the epithelial sodium ion channel (ENaC) is believed to be a receptor because amiloride, an epithelial sodium channel blocker, decreases the responses of taste cells to sodium chloride (NaCl) (Spector et al. 1996; Shigemura et al. 2008; Yoshida et al. 2009a). The VR1 was suggested as a candidate receptor for the amiloride-insensitive salt taste in mouse fungiform taste cells (Liu and Simon 2001). It was also demonstrated that the amiloride-insensitive salt taste receptor was nonfunctional in VR1-knockout mice, suggesting that the amiloride-insensitive salt taste transducer is derived from the VR1 gene (Lyall et al. 2004). Recently, Yoshida et al. (2009b) reported that 45% of amiloride-insensitive cells expressed SNAP25, suggesting that many amiloride-insensitive cells may be Type III cells in mouse fungiform taste buds. In the present study, VR1 immunoreactivity was observed in SNAP25-immunoreactive Type III cells in circumvallate papillae.

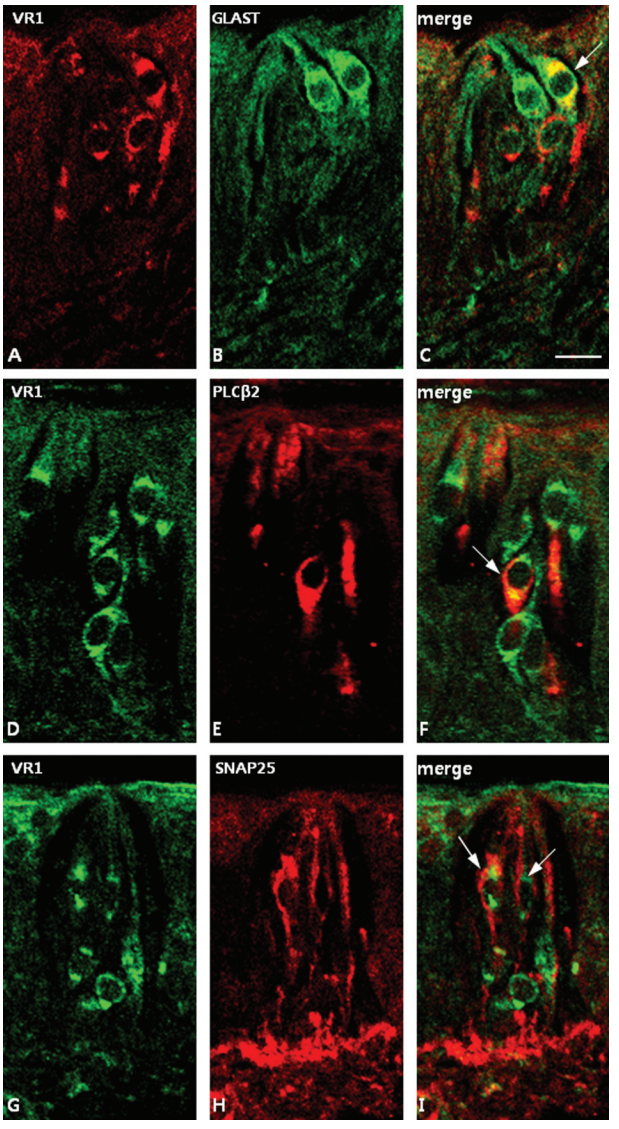

Figure 3. Identification of the phenotypes of VR1 immunoreactive cells in the taste buds of the circumvallate papilla. Double labeling for the VR1 (A), (D), (G) and GLAST (B), PLCβ2 (E) or SNAP25 (H) showed that VR1 immunoreactivity was observed in GLAST-immunoreactive Type I (C), PLCβ2-immunoreactive Type II (F), and SNAP25-immunoreactive Type III (I) cells. Arrows indicate doubly stained cells. Scale bar = 10 μm.

Therefore, the localization of the VR1 in circumvallate papillae suggests that they may function as a sensor for amiloride-insensitive salt taste.

In the present study, the VR1 is expressed throughout the cytoplasm of circumvallate taste cells. We performed electron microscopy to identify the subcellular location of the VR1. VR1 immunoreactivity was observed in the endoplasmic reticulum, cytoplasmic vesicles, and plasma membrane of taste cells. These results agree with previous published data that reported VR1 localization in the dorsal root ganglion neurons and the spinal cord (Guo et al. 1999; Morenilla-Palao et al. 2004), demonstrating the localization of the VR1 in the membranous structures of taste cells.

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