Abstract: The oral cavity is the first line of defense, sensation, and secretion of the alimentary canal. Oral perception contributes to the enjoyment of food and beverages and to avoiding consumption of poisonous or harmful substances. Oral sensation is served by somatosensory nervous systems distributed to the oral membrane. Recent studies reported that oral epithelial cells may transduce temperature and touch through membranous sensors, which comprise ion channels with multimodal properties, and nerves. Here, we describe the possible role of oral epithelial cells in oral perception.

Keywords: oral epithelium; nerve; ion channel; sensor; TRP channel.

Introduction

The pleasant sensations of food and the painful feelings of hot liquids in the mouth are transferred by somatosensory nerves that innervate the oral mucosa—the moist mucous membrane that lines the entire oral cavity. The surface lining is the oral epithelium, which is exposed to various stimuli from foods and beverages. Oral sensation is responsible for the highly sensitive evaluation of whether to ingest or expel items in the oral cavity. Food palatability mainly depends on the coding of taste (as sweet, bitter, sour, salty, or umami) by taste cells through specific taste receptors. In addition to the five basic tastes, perception of oral environmental stimuli includes temperature, texture, pungency, starchiness, and stickiness, and other qualities. Oral sensations other than the basic tastes are considered to be initiated by somatosensory neurons that innervate the oral mucosa. Sensory stimuli activate the peripheral terminals of trigeminal nerves and evoke action potentials, which conduct toward the central nervous system.

The remarkably diverse sensory afferents are broadly classified as Aβ-, Aδ-, or C-fibers and then further classified by sensory modality. Under microscopic observation, sensory nerves are distributed in the oral mucosa as thick nerve bundles in the lamina propria. A dense plexus gives off branches as fine nerve fibers with varicosities, and then individual nerve fibers separate into the epithelium or form specialized structures (1). Most fibers appear to end as free nerve endings in the lamina propria. A small number of nerves also seem to penetrate epithelial layers running among oral epithelial cells. Several specialized regions within the oral epithelium, such as the junctional epithelium (2,3) and the tip of the palatal rugae (4), are supplied by rich intraepithelial nerves, which suggests that these regions serve as sentinels. We wondered how this restricted distribution of nerves could provide the finely tuned sensitivity observed in the oral mucosa. Here, we review new findings on the cellular and molecular events underlying oral somatosensation. Recent studies
have revealed a group of ion channels that are candidate transducers of temperature in the oral mucosa. These studies suggest that non-neuronal oral epithelial cells directly sense oral environmental changes and adapt and maintain responses to these changes.

**Transient receptor potential channels in the oral epithelium**

Transient receptor potential (TRP) channels were first discovered in photoreceptor cells of the fly eye (5,6). In 1997, Caterina et al. reported that mammalian transient receptor potential vanilloid subfamily, member 1 (TRPV1; also known as VR1) was the molecular target of capsaicin, the main pungent ingredient of hot chili peppers (7). This non-selective cation channel was activated not only by capsaicin, but also by temperatures above 42°C and acid (8). Genetic ablation of TRPV1 in mice eliminates the capsaicin response and prolongs the latency of heat-evoked paw withdrawal behavior (9,10).

TRPV1 is expressed in a population of neurons with unmyelinated and thinly myelinated nerve fibers within the rodent dorsal root ganglia (8,11) and trigeminal ganglion (12). Accumulating evidence indicates that TRPV1 is one of the main pharmacological targets for pain (13,14). We previously reported that peripheral nerves distributed in the oral epithelium show sensitivity to capsaicin (15) and thus explored TRPV1 expression in oral mucosa.

Reverse-transcriptase-polymerase-chain-reaction (RT-PCR) analyses showed traces of rat TRPV1 mRNA in the oral mucosa. We also found a rich TRPV1-immunoreactive nerve supply, especially in taste buds in the tongue epithelium of rats (4). In the circumvallate, foliate, and fungiform papillae, TRPV1 nerve bundles were abundantly distributed in the lamina propria, and their branches penetrated the taste buds. TRPV1 nerve fibers gave out branches around the taste buds, and some ran into the buds through the intercellular space around taste cells. Immunoreactivity was limited in taste cells but was abundant in the epithelium around taste buds. In addition, we unexpectedly observed strong expression of TRPV1 in the summit of the palatal rugae, which protrudes into the oral cavity. We speculated this TRPV1 functions as a sensor, because the location of the rugae is assumed to be the route for food or drink passage in the oral cavity toward the throat. We confirmed that numerous nerves in the area contained TRPV1-immunoreactive epithelial cells. These topological features on histological observation suggest that the oral epithelium functions as an environmental sensor by means of specific sensor molecules.

The family of TRP channels is fundamentally involved in the perception of various environmental stimuli, including temperature, mechanical stretching, and chemicals (16,17). After the early discovery of TRPV1 during heat transduction in mammals, another heat-gated channel, TRPV2, was found to be a homologue of TRPV1 (18). TRPV2 is expressed in somatosensory neurons with Aδ- and Aβ-fiber branches and is activated by temperatures above 52°C, membrane stretching (19), and growth factors (20). We noted strong expression of TRPV2 in the junctional epithelium, which is attached to tooth enamel (21). Epithelial TRPV2 expression was uniquely observed in the junctional epithelium, in addition to non-neuronal cells such as macrophages and T cells with TRPV2. The junctional epithelium is served by abundant free nerve endings, while the oral sulcular epithelium or oral epithelium continuous to the junctional epithelium is devoid of TRPV2 expression and nerves. Specific topographical features in the oral mucosal epithelium are related to heat-sensitive TRPV channels.

Temperature sensation has a substantial effect on nutrient intake. In rat oral epithelium with peeled-off connective tissues, we detected expressions of TRPV1, TRPV2, TRPV3, TRPV4, TRPV6, transient receptor potential melastatin 2 (TRPM2), TRPM5, TRPM8, and transient receptor potential ankyrin 1 (TRPA1) (22). We isolated rat oral epithelial cells from the rat palatal mucosa, buccal mucosa, and tongue. Calcium imaging showed that these cells were activated by stimulation with camphor, menthol, and several TRPV3 or TRPV4 chemical compounds (22). Furthermore, isolated oral epithelial cells responded to more than one herb ingredient, such as capsaicin, camphor, or menthol. We also observed that expression profiles and amounts differed among the regions of the oral cavity, which suggests that each region of the epithelium has its own functional roles. Calcium imaging experiments showed that almost all isolated oral epithelial cells responded to camphor and 2-aminoethoxydiphenyl borate (2-APB) and that the magnitudes of these reactions were rather large. Although camphor and 2-APB are not specific agonists for TRPV3 or TRPV4, our findings suggest that TRPV3 and TRPV4 have prominent functional significance. TRPV3 and TRPV4 were reported to be activated by ambient temperatures approximating body temperature (23-26). TRPV3 was initially thought to be expressed only in skin keratinocytes and to be activated by plant-derived components. Xu et al. showed that TRPV3 was expressed in the nasal and tongue mucosa and that epithelial cells responded to carvacrol and camphor—ingredients of the herbs oregano and clove (27). Sherkhali et al. examined
human HaCaT skin keratinocytes and electrophysiologically demonstrated their temperature and camphor sensitivity through TRPV3 (28). Oral epithelial TRPV3 and TRPV4 may contribute to appreciation of herb taste in the oral cavity.

To identify the molecular targets of temperature sensitivity in oral epithelial cells we used quantitative RT-PCR and detected expressions of TRPV1, TRPV2, TRPV3, TRPV4, and TRPM8, along with small traces of TRPA1, in mouse oral epithelium (29). Among them, TRPV3 and TRPV4 were most strongly expressed in buccal epithelial cells. Temperature changes in the bath solution raised the intracellular calcium level in buccal epithelial cells, which suggests that these oral epithelial cells are temperature-sensitive. In experiments using whole-cell patch-clamp recording, temperatures from 25 to 40°C evoked inward currents in rapidly isolated oral epithelial cells (Fig. 1). These heat-induced currents were suppressed in cells from TRPV3 or TRPV4 gene-deleted mice (29). Interestingly, electrophysiological analyses revealed that TRPV3 contributed to oral sensitization after repeated heat stimulation under a condition of TRPV4 ablation, which was consistent with the findings of a report on TRPV3-transfected cells (23,25,30). These findings suggested that the oral epithelium can perceive temperature changes through the temperature-sensitive ion channels TRPV3 and TRPV4. Nevertheless, the reaction was not totally suppressed, which suggests that there are other unknown mechanisms for temperature sensation.

Some TRP channels are expressed in epithelial cells of the skin (23) and urinary bladder (31); thus, we propose that, in addition to somatosensory neurons, epithelial cells themselves may contribute to sensing temperature and touch (for a review, see 32,33).

Physiological functions of TRP channels in oral mucosa

We found that TRPV3 mRNA expression was higher in oral epithelial cells than in skin keratinocytes (29). The oral mucosa is continuous with the skin, and the oral epithelium is more susceptible to injury because it is continuously exposed to diverse stimuli. In general, we noted that wounds in the oral cavity recovered faster than those in the skin and with less scar formation (34,35). Therefore, we hypothesized that TRPV3 was more strongly activated by the higher temperatures of the oral cavity than in skin, thus resulting in active proliferation or migration of oral epithelial cells. We found that wound closure after tooth extraction was slower in TRPV3 gene-deleted mice than in wild-type mice. These results suggest that TRPV3 affects wound healing and are supported by the finding of significant upregulation of TRPV3 mRNA expression at wound sites at 3 and 5 days after extraction. However, a study of a dorsal skin full-thickness wound model did not show clear association of TRPV3 to wound healing (36). We speculate that this difference between dorsal skin and oral mucosa may depend on higher TRPV3 activation with temperature changes in oral epithelial cells.
in the oral cavity than the dorsal skin and that higher TRPV3 expression in the oral epithelia. Wound healing involves finely tuned dynamic and complex processes. We identified epidermal growth factor receptor (EGFR) signaling as a possible downstream mechanism. In the oral epithelium of TRPV3 gene-deleted mice, EGFR phosphorylation was inhibited as compared with that in wild-type mice. The signaling molecules from the oral epithelium under temperature stimulation associated with calcium signaling have not yet been identified. Possible candidate molecules are adenosine triphosphate (37), nitric oxide (36), and transforming growth factor α (38). These molecules are claimed to stimulate proliferation, inflammation, and/or differentiation in keratinocytes.

The temperature sensitivities of TRPV3 and TRPV4 were reported to be similar. TRPV4 expression in the oral epithelium was particularly strong in the basal layer (Fig. 2). In our previous study, wound healing was not impaired in TRPV4-deficient mice. This suggests that TRPV3 and TRPV4 have similar temperature sensation but play distinct roles in epithelial maintenance. Future studies should investigate the relationship between these roles of temperature sensation and epithelial maintenance.

Hereditary channelopathy is a disease caused by mutations in ion channel genes. The first cutaneous TRP channelopathy identified was Olmsted syndrome, which is characterized by congenital palmoplantar and periorificial keratoderma, diffuse alopecia, and immune dysfunction. Genetic analyses revealed multiple gain-of-function mutations in TRPV3 (39-41). TRPV3 is also involved in the development of several acquired skin diseases that result in pruritus or inflammation (42). Furthermore, accumulating evidence indicates that changes in expression of TRP channels are associated with resistance to cancer chemotherapy (43,44).

Recent studies indicate that many ion channels are in the correct place and possess suitable properties to have roles in oral perception and functions. Research advances have increased our understanding of the complexity of somatosensory transduction and suggested recurring themes. In addition, studies of TRP channels have revealed novel epithelial properties and yielded new hypotheses. These fresh insights on the oral epithelium are an exciting starting point for future studies of epithelial integrity, particularly the roles of temperature and ion channels, which will improve understanding of the related diseases.

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Conflict of interest
The authors declare no conflict of interest.

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