



Minireview

Acid-sensing ion channels and transient-receptor potential ion channels in zebrafish taste buds



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ARTICLE INFO

Article history:

Received 16 December 2015

Received in revised form 23 June 2016

Accepted 24 June 2016

Keywords:

Taste buds

Acid-sensing ion channels

Transient receptor potential ion channels

Zebrafish

ABSTRACT

Sensory information from the environment is required for life and survival, and it is detected by specialized cells which together make up the sensory system. The fish sensory system includes specialized organs that are able to detect mechanical and chemical stimuli. In particular, taste buds are small organs located on the tongue in terrestrial vertebrates that function in the perception of taste. In fish, taste buds occur on the lips, the flanks, and the caudal (tail) fins of some species and on the barbels of others. In fish taste receptor cells, different classes of ion channels have been detected which, like in mammals, presumably participate in the detection and/or transduction of chemical gustatory signals. However, since some of these ion channels are involved in the detection of additional sensory modalities, it can be hypothesized that taste cells sense stimuli other than those specific for taste. This mini-review summarizes current knowledge on the presence of transient-receptor potential (TRP) and acid-sensing (ASIC) ion channels in the taste buds of teleosts, especially adult zebrafish. Up to now ASIC4, TRPC2, TRPA1, TRPV1 and TRPV4 ion channels have been found in the sensory cells, while ASIC2 was detected in the nerves supplying the taste buds.

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1. Introduction

Fish have well differentiated sensory organs able to transduce stimuli, like chemicals, movements or temperature, from the aquatic environment into signals that reach the central nervous system. The sensory organs in fish include the olfactory rosette, the lateral line, the inner ear and the taste buds. In addition, the skin and gills contain scattered or solitary chemosensory cells (Germanà et al., 2004b, 2006; Detrich et al., 2011).

The gustatory system in fish is a major chemosensory system devoted to the evaluation of food taste as well as to the detection of variations in the environmental chemical composition (Ishimaru et al., 2005; Abbate et al., 2006; Oike et al., 2007; Yasuoka and Abe, 2009; Abbate et al., 2012a,b; Kapsimali and Barlow, 2013; Jonz et al., 2015; Guerrero et al., 2015; Okada, 2015). In this context, taste buds are the chemosensory organs in which chemical stimuli are transduced into electrical signals conveying taste information to the brain (Abbate et al., 2008, 2010; Abe, 2008; Germanà et al.,

2009; Chaudhari and Roper, 2010; Soulika et al., 2016). The ability of fish to distinguish between nutritional versus potentially lethal food is obviously very important for survival.

In the last decades, studies in non-vertebrates and vertebrates have identified several families of ion channels that participate in the detection and transduction of a wide range of stimuli for most sensory modalities. Sensory stimuli of heterogeneous nature cause the opening and/or the closing of these channels, leading to changes in the concentrations of ions inside and outside of the sensory cell. These gradients are at the base of an electrical signaling system that ultimately causes a change in the membrane potential of recipient cells (see for a review Belmonte and Viana, 2008; Damann et al., 2008).

The identification of ion channels activated by specific classes of stimuli supported the concept that the specificity of sensory cells is determined by their expression of a particular sensor to selectively respond to stimuli of a given nature. However, it is now accepted that the molecular sensors regarded as specific transducers are not so neatly associated with the distinct types of ion channels as earlier proposed. In fact, ion channels originally associated with the transduction of one particular form of energy can be activated by stimuli of heterogeneous nature and the same types of channel

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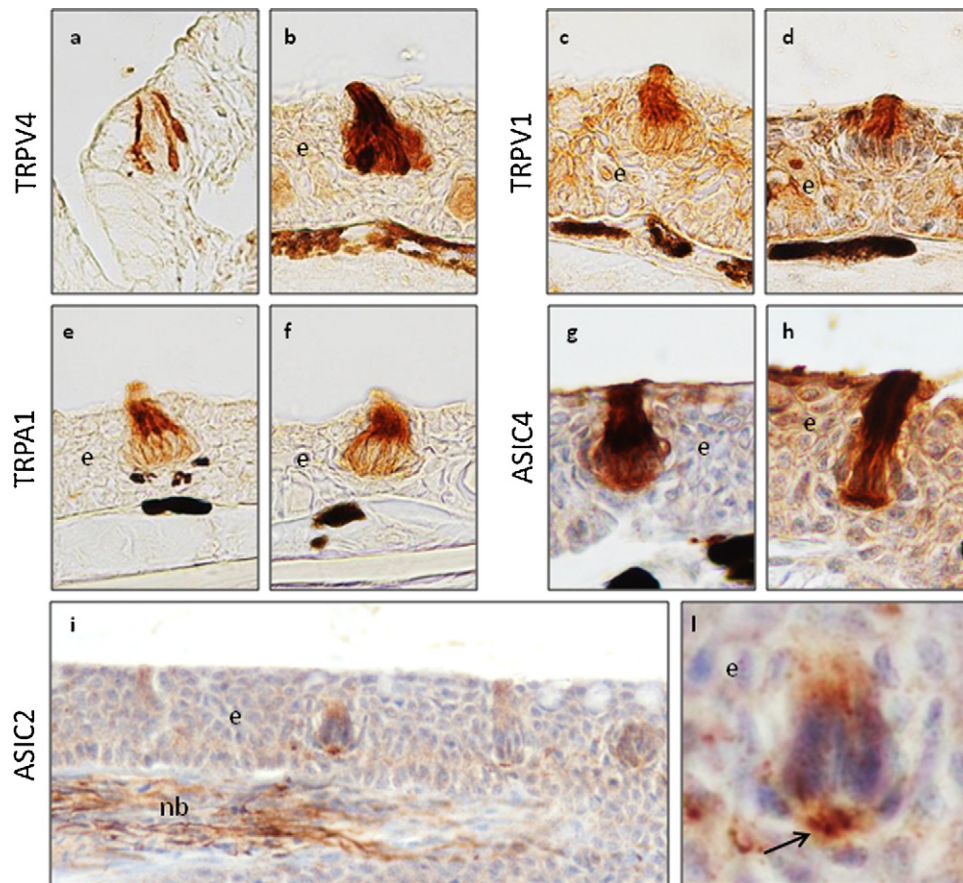


Fig. 1. Immunohistochemical detection of ASICs and TRP ion channels in cutaneous and oral taste buds of zebrafish. TRPV4 (a,b), TRPV1 (c,d), TRPA1 (e,f) and ASIC4 (g,h) were present in the sensory cells of oropharyngeal and cutaneous taste buds. Conversely, ASIC2 was never found in the sensory cells, but in the cutaneous nerves supplying taste buds (arrow in l). e: epithelial cells, nb: nerve bundle. Scale bar: 15 μm for a–h; 40 μm for i; 10 μm for l.

Table 1
Primary antibody used in the study.

Antigen	Origin	Dilution	Supplier
ASIC1	Rabbit	1:200	Abcam pcl1
ASIC2	Rabbit	1:200	Lifespan Biosciences2
ASIC3	Rabbit	1:200	Abcam pcl1
ASIC4	Rabbit	1:200	Lifespan Biosciences2
Calretinin	Mouse	1:100	Swant3
β -Tubulin	Mouse	1:100	Sigma4

can be expressed in sensory cells associated with different sensory modalities. Thus, the capacity exhibited by the different functional types of sensory cells to preferentially detect specific stimuli appears to be the result of a characteristic combinatorial expression of different ion channels (Liedtke, 2007; Belmonte and Viana, 2008). In mammalian taste buds, several different types of ion channels participate in chemodetection. These include the 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB)-sensitive chloride channels, hyperpolarization-activated channels HCN1 and HCN4, potassium (K²P) channels, leakage-type K⁺ channels, the transient receptor potential (TRP) family members, PKD1L3 (polycystin 1 like 3, transient receptor potential channel) and PKD2L1 (polycystin 2 like 1, transient receptor potential channel), and acid-sensing ionic channels (ASICs) (Ugawa et al., 1998; Miyamoto et al., 2000; Stevens et al., 2001; Lin et al., 2004; Richter et al., 2004a,b; Ishimaru et al., 2006; Shimada et al., 2006; Huang et al., 2008; Medler, 2010). In fish, different types of ion channels are similarly involved taste sensing and are present in the taste buds. They include Kv2 voltage-gated K channels (Kang et al., 2001), TRP channels (Yoshida et al.,

2007; Amato et al., 2012), calcium-activated potassium SK1 (Cabo et al., 2013), or ASICs (Viña et al., 2013).

This short review updates the current information on the occurrence and distribution of members of ASIC and TRP ion channel superfamilies in the taste buds of teleosts, in particular the widely used experimental model zebrafish.

2. Taste buds in fish

In adult teleosts the morphology of the gustatory system has been extensively studied (for review see Jakubowski and Whitear, 1990; Reutter and Witt, 1993; Yasuoka and Abe, 2009). The morpho-functional units for taste transduction are specialized chemosensory organs called taste buds, which are distributed in the external skin surface of the head, lips, and barbells as well as in the intra-oral cavity, including the anterior branchial apparatus (Hansen et al., 2002; LeClair and Topczewski, 2009, 2010). Mature taste buds are onion-shaped intraepithelial sensory organs placed on a small dermal papilla. They consist of modified epithelial sensory cells, supporting cells and basal cells; the sensory cells are fusiform in shape, oriented perpendicularly to the epithelial surface (Hansen et al., 2002). Based on their electron density, two main populations of sensory cells can be distinguished (Reutter, 1978, 1982; Jakubowski and Whitear, 1990): the so-called dark cells characterized by an apex filled with many short microvilli; and the light cells that show one single large microvillus at the apical border. In addition, the taste buds of some teleosts, including zebrafish, have a third type of sensory cells characterized by low electron density and a brush-like apical ending with several small

microvilli (Hansen et al., 2002). Between the sensory cells and the basal lamina, Merkel-like basal cells have been described (Reutter and Witt, 1993; Zachar and Jonz, 2012) where is evident the presence of the 5HT (5 hydroxy-tryptophan) serotonin precursor, so demonstrating a probable serotonergic role (Suzuki, 2007). Nerve fibers originating from the facial, glossopharyngeal or vagal nerves innervate the sensory cells (Detrich et al., 2011). These fibers make cytoneural junctions where ATP and 5-HT act as neurotransmitters to activate receptors in nerve endings (Finger et al., 2005; Miura et al., 2006).

3. Generalities on ASIC and TRP ion channels

3.1. Acid-sensing ion channels

ASICs are members of the degenerin/epithelial Na⁺ channels (DEG/ENaC). At present, six ASIC proteins encoded by four genes, have been identified as ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4 in mammals. They differ in kinetics, external pH sensitivity, tissue distribution and pharmacological properties (Krishtal, 2003, 2015; Kress and Waldmann, 2006; Benarroch, 2014). They are Na⁺-selective voltage-insensitive, amiloride-sensitive cation channels that monitor moderate deviations from the physiological values of extracellular pH (Waldmann et al., 1997; Linguaglia, 2007; Lumpkin and Caterina, 2007; Baron and Linguaglia, 2015). In addition, some ASICs may work as mechanosensors (or are required for mechanosensation), nociceptors (Reeh and Kress, 2001; Wemmie et al., 2006; Deval et al., 2008; Basbaum et al., 2009; Holzer, 2009, 2011; Delmas et al., 2011; Sherwood et al., 2012; Chen and Wong, 2013; Zha, 2013) or taste receptors (Lin et al., 2002; Ugawa et al., 2003). Structurally, ASICs consist of two transmembrane domains and a large extracellular loop (Sherwood et al., 2012).

Similarly to mammals, six ASICs have been identified in zebrafish (zASICs; zASIC1.1, zASIC1.2, zASIC1.3, zASIC2, zASIC4.1, and zASIC4.2), but they are codified by six different genes. Phylogenetic analysis demonstrated that zASIC1.1, zASIC2 and zASIC4.1 are orthologous to mammals, while zASIC1.2 and zASIC1.3 are paralogs; to date any ortholog of ASIC3 has not been identified. The proteins codified by *zasics* have similar predicted molecular masses (around 60 kDa) and share 60–75% of amino acid identity with rat and human ASICs (Paukert et al., 2004).

3.2. Transient-receptor potential ion channels

The members of the TRP superfamily are integral membrane proteins that function as ion channels. They are non-selective cation channels, few are highly Ca²⁺ selective and some are permeable for highly hydrated Mg²⁺. The TRP superfamily is subdivided into seven subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), TRPA (ankyrin) and TRPN (NOMPC-like); the latter is found only in invertebrates and fish. At least 28 different TRP proteins have been identified in mammals. Structurally, a typical TRP protein contains six putative transmembrane domains (S1–S6) with a pore-forming reentrant loop between S5 and S6. Intracellular N- and C-termini are variable in length and consist of a variety of domains (Clapham et al., 2005; Hellmich and Gaudet, 2014). Ion channels belonging to this superfamily show a variety of gating mechanisms with modes of activation ranging from ligand binding, voltage and changes in temperature to covalent modifications of nucleophilic residues (see for a review Eid and Cortright, 2009; Nilius and Owsianik, 2011; Nilius and Szallasi, 2014). TRPs have been detected in virtually all tissues (Venkatchalam and Montell, 2007).

Table 2

Occurrence (asterisk) of different ASICs and TRP in cutaneous, oro-pharyngeal taste buds and in nerve.

Protein		Cataneous taste buds	Oro-pharyngeal taste buds	Taste buds nerve
ASIC	2	*(lowpH)	*(lowpH)	*
	4	*	*	–
TRP	A1	*	*	–
	C1	*	*	–
	V1	*	*	–
	V4	*	*	–

4. ASICs and TRPs in zebrafish

The data available on the expression and distribution of ASICs and TRPs in fish, especially in the sensory organs, are scarce and have most often been obtained from experiments during larval stages. ASICs have been cloned in zebrafish and they are present at high levels in the central nervous system and the retina while their presence in peripheral organs is limited (Paukert et al., 2004). Furthermore, the ASIC2 mRNA or protein has been detected in the olfactory epithelium and retina (Levanti et al., 2011; Viña et al., 2013, 2015a,b).

The orthologs of some TRP channels of vertebrates have also been detected in zebrafish. They include TRPV1, TRPV4, one copy of TRPA1, twelve TRPC genes and transcripts, and eleven TRPM genes (Shigeru and Ryuzo, 2006; Kastenhuber et al., 2013; Von Niederhäusern et al., 2013). TRPV4 immunoreaction has been found in sensory organs, especially in the retina of adult fish (Amato et al., 2012; Sánchez-Ramos et al., 2012).

5. ASICs and TRPs in taste buds of zebrafish

The presence of ASICs in the taste buds of zebrafish was reported by Viña et al. (2013). Using immunohistochemistry these authors observed ASIC2 in the nerves supplying the taste buds and ASIC4 in the cytoplasm of the sensory cells (Figs. 1 and 2). Nevertheless, under some experimental conditions (aquatic values of pH < 7.00) ASIC2 immunostaining was also detected in the sensory cells (Fig. 2).

Mangos et al. (2007) and Amato et al. (2012) demonstrated that developing and adult zebrafish express specific TRPV4 mRNA and protein. Using immunohistochemistry, TRPV4 was detected in both oropharyngeal and cutaneous taste buds of adult fish with a variable pattern of expression. Indeed, while all the cells of cutaneous taste buds were TRPV4 positive, only a subpopulation of sensory cells in oropharyngeal taste buds expressed this ion channel (Amato et al., 2012; Figs. 1 and 2)

In addition to the cells of the dark and light type described in teleosts above, a third cell type has been described in taste buds of zebrafish. This type of cell has a brush-like apical ending that consists of thin microvilli and its electron density is lighter than the dark cell (Hansen et al., 2002; Ohmoto et al., 2011; Jackson et al., 2013). This may explain why the pattern of expression of TRPV4 is variable in different locations, as it occurs for S100 protein or calretinin (Abbate et al., 2002; Germanà et al., 2004a, 2007) (Tables 1 and 2 and Fig. 3).

6. Concluding remarks

The survival of an organism depends largely on its ability to respond and adapt to its surroundings and one of the ways of interaction is through different sensory modalities. The aquatic environment represents the vehicle for a wide range of stimuli for fish, especially signals for smell and oxygen concentration (chem-

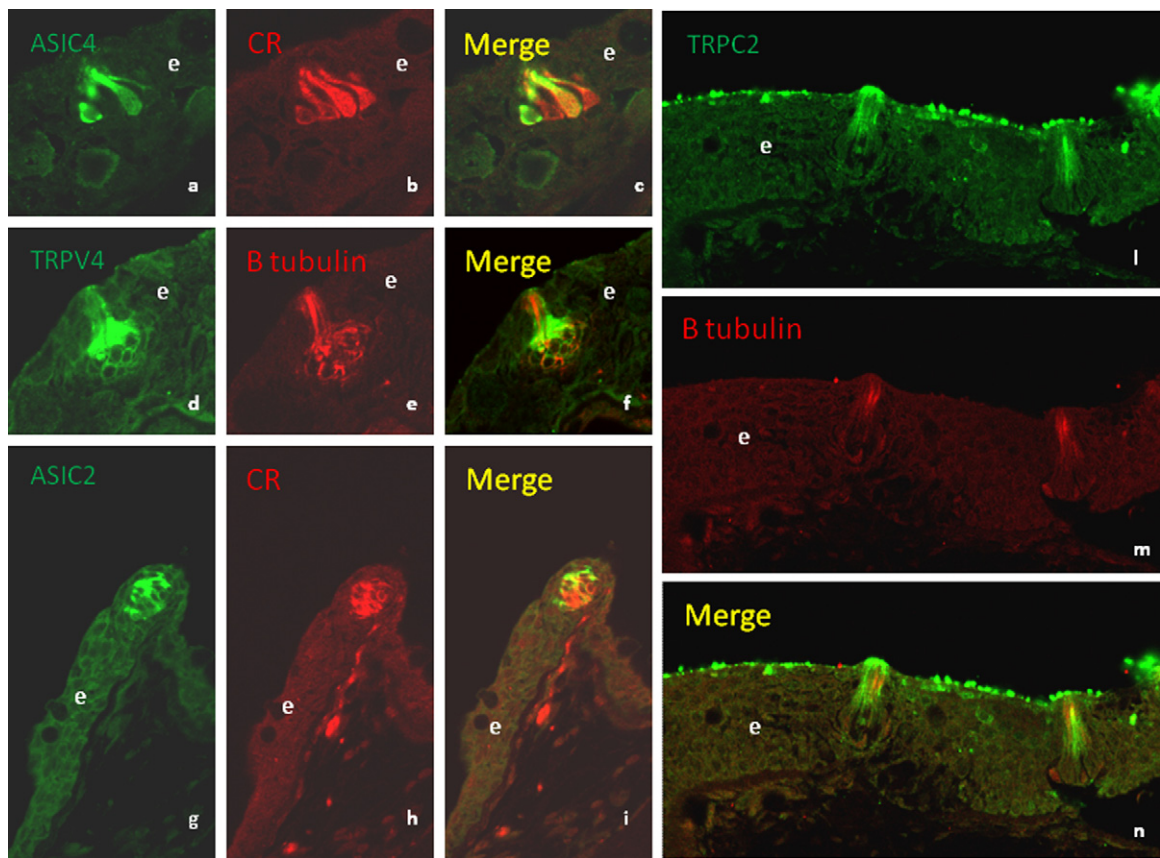


Fig. 2. Localization of ASICs and TRP, as well as some specific markers for sensory cells like β -tubulin or calretinin (CR), in the cutaneous taste buds of adult zebrafish using immunofluorescence associated with laser confocal microscopy. ASIC4- (a–c), TRPV4- (d–f), and TRPC2 (l–n) – Alexa fluor 488-conjugated goat anti-rabbit IgG (green fluorescence), and calretinin – or β -tubulin-CyTM3-conjugated donkey anti-mouse antibody (red fluorescence) label all or a population of taste sensory cells (a–c) (Germanà et al., 2007). Moreover, ASIC2 was found in cutaneous nerves and sensory cells in the taste buds of zebrafish exposed to low pH values (g–i). e: epithelial cells. Scale bar: 15 μ m for a–f, l–n; 40 μ m for g–i. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

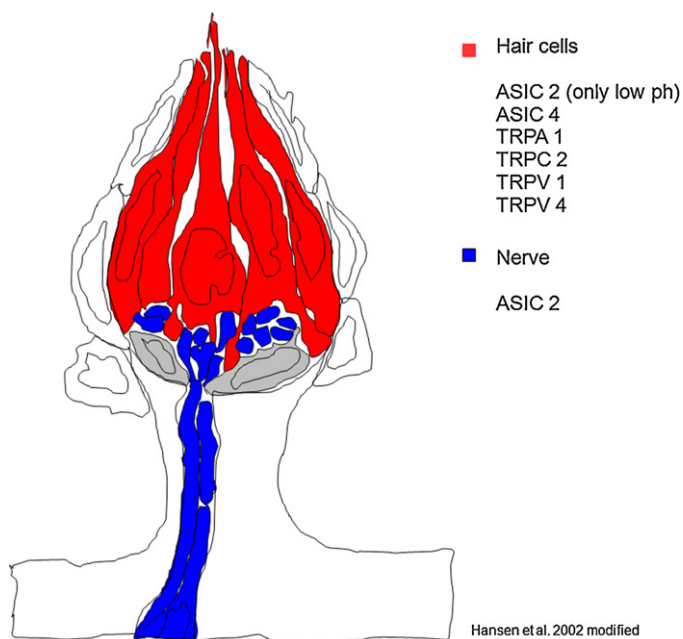


Fig. 3. Picture showing the localization of ASICs and TRP in hair cells (red) and ASIC2 in nerve (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

icals) or for temperature and movement (physical). All modalities of sensitivity depend on particular combinations of ion channels in sensory cells. In the zebrafish taste buds, the TRP and ASIC ion channels present in sensory cells can be related to signal transduction and/or the synaptic release of chemical transmitters.

The possible functions of ASICs in the taste buds of fish are unknown. In mammals it is thought that extracellular H^+ activate ASICs resulting in an influx of extracellular Ca^{2+} which, in turn, leads to neurotransmitter release (Ugawa et al., 2003; Shimada et al., 2006).

Moreover, it can be assumed that these ion channels are involved in the detection of concentration of Ca^{2+} in the water. ASIC2 has been implicated in the perception of sour taste in mammals (Lin et al., 2002, 2004; Liu and Simon, 2001; Ugawa et al., 2003). It can be activated by many different solutes, rather than from a single solute, suggesting that this and other ASICs also act as detectors of osmolarity rather than directly responding to specific ions (Frank et al., 2008; Yoshida et al., 2009).

In comparing data on zebrafish taste buds with that reported on mammals, notable differences exist (Oka and Korsching, 2011). Whereas ASIC4 was not detectable in the taste buds of rat and mouse (Shimada et al., 2006), it is regularly present in zebrafish. In contrast, ASIC1 and ASIC3 were always absent in zebrafish (Viña et al., 2013) and both have been found at the mRNA level in rat and mouse (Shimada et al., 2006). Functional experiments have demonstrated that zASIC1.1, zASIC1.2, zASIC1.3 or zASIC14.1 are acid sensitive, but zASIC2 and zASIC4.2 cannot be activated by acid test solutions (Paukert et al., 2004).

The occurrence of TRPV4 in taste buds argues for a chemosensory role of TRPV4, but it can also be activated by nociceptive and hypotonic stimuli.

Different TRP ion channels have been associated to taste in mammals. TRPM5 acts in the taste transduction pathway; the occurrence and direct role in taste of TRPV1 is controversial; TRPA1 transduces a wide variety of irritants and, if co-expressed with TRPV1, there is a broad response to noxious chemical stimuli. Other TRP channels, including TRPM8, TRPV3, and TRPV4, play less prominent roles in chemesthesis and no known role in taste. The activity of TRP channels in taste buds is modulated by the pungency of foods and beverages which is likely highly influenced by the temperature at which they are consumed (see Roper, 2014). TRPC2 (Miller, 2014) is related to behavior including altered sex discrimination and lack of male-male aggression, which are not related to taste. Electrophilic chemicals, oxygen, temperature and mechanical forces activate TRPA1 although the molecular mechanism of TRPA1 gating remains obscure (Laursen et al., 2014). Nevertheless, functions other than taste cannot be ruled out. In this case, taste cells may be regarded as multimodal sensors.

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